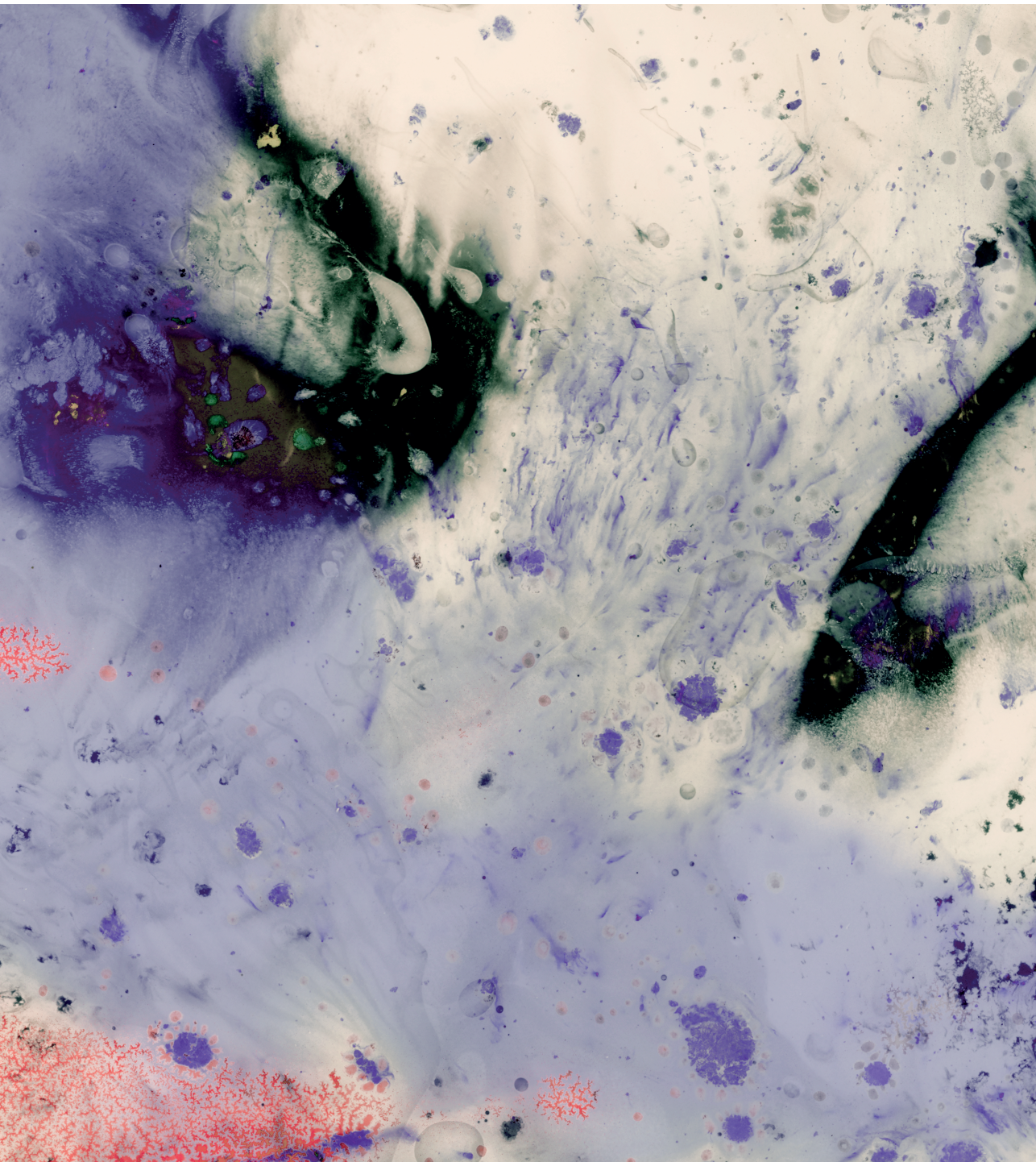
Early-pregnancy Glucose Metabolism and Childhood Health

From associations to prediction

RAMA WAHAB





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Early-pregnancy Glucose Metabolism and Childhood Health

From associations to prediction

RAMA JOSEPHINE WAHAB

Early-pregnancy Glucose Metabolism and Childhood Health

From associations to prediction

Glucosemetabolisme in de vroege zwangerschap en gezondheid op de kinderleeftijd
Van associaties naar predictie

Proefschrift

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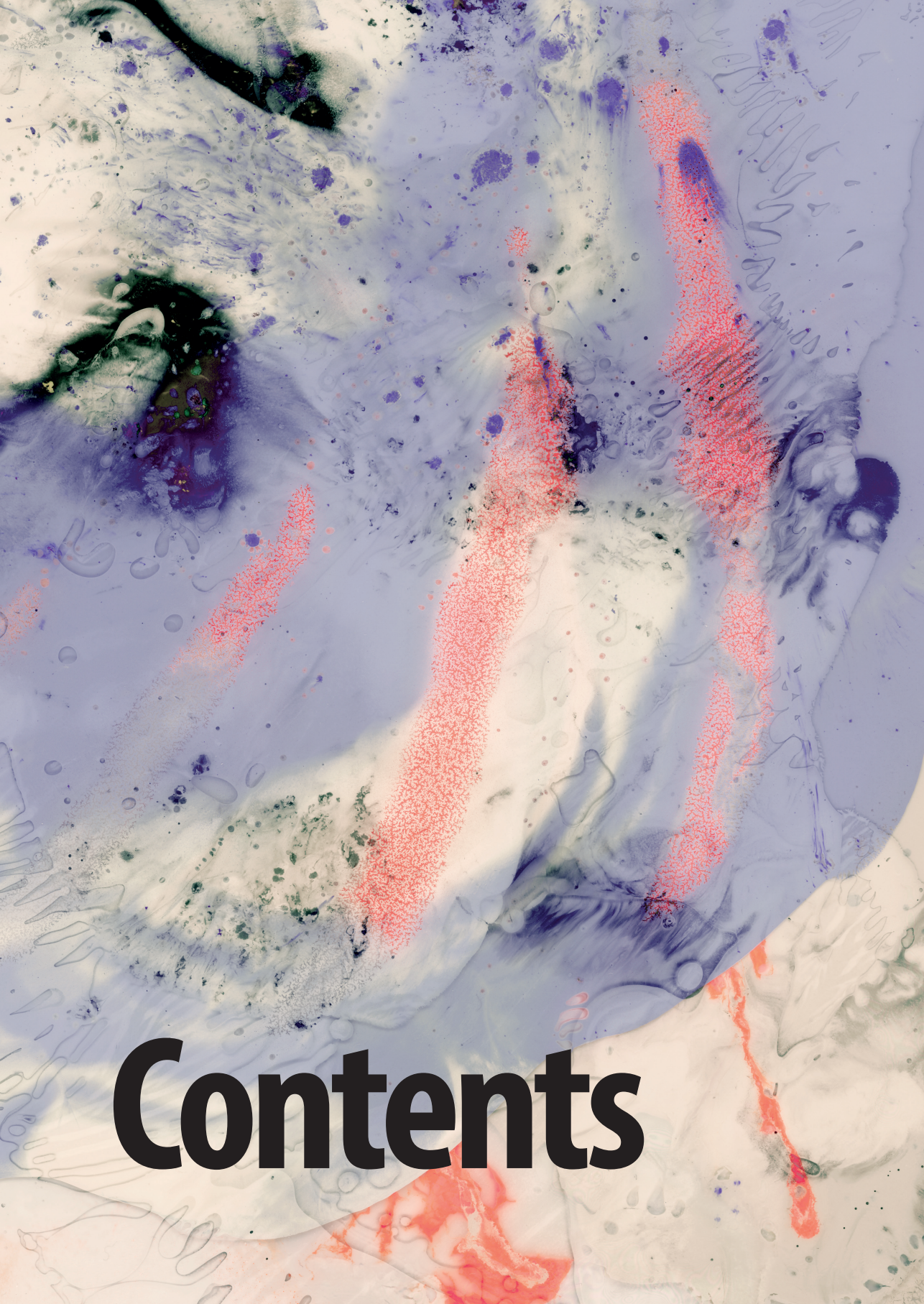
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Chapter 2.2 - Geurtsen ML, **Wahab RJ**, Felix JF, Gaillard R, Jaddoe VWV. Maternal early pregnancy glucose concentrations and liver fat among school age children. Hepatology, 2021 May 18. doi: 10.1002/hep.31910

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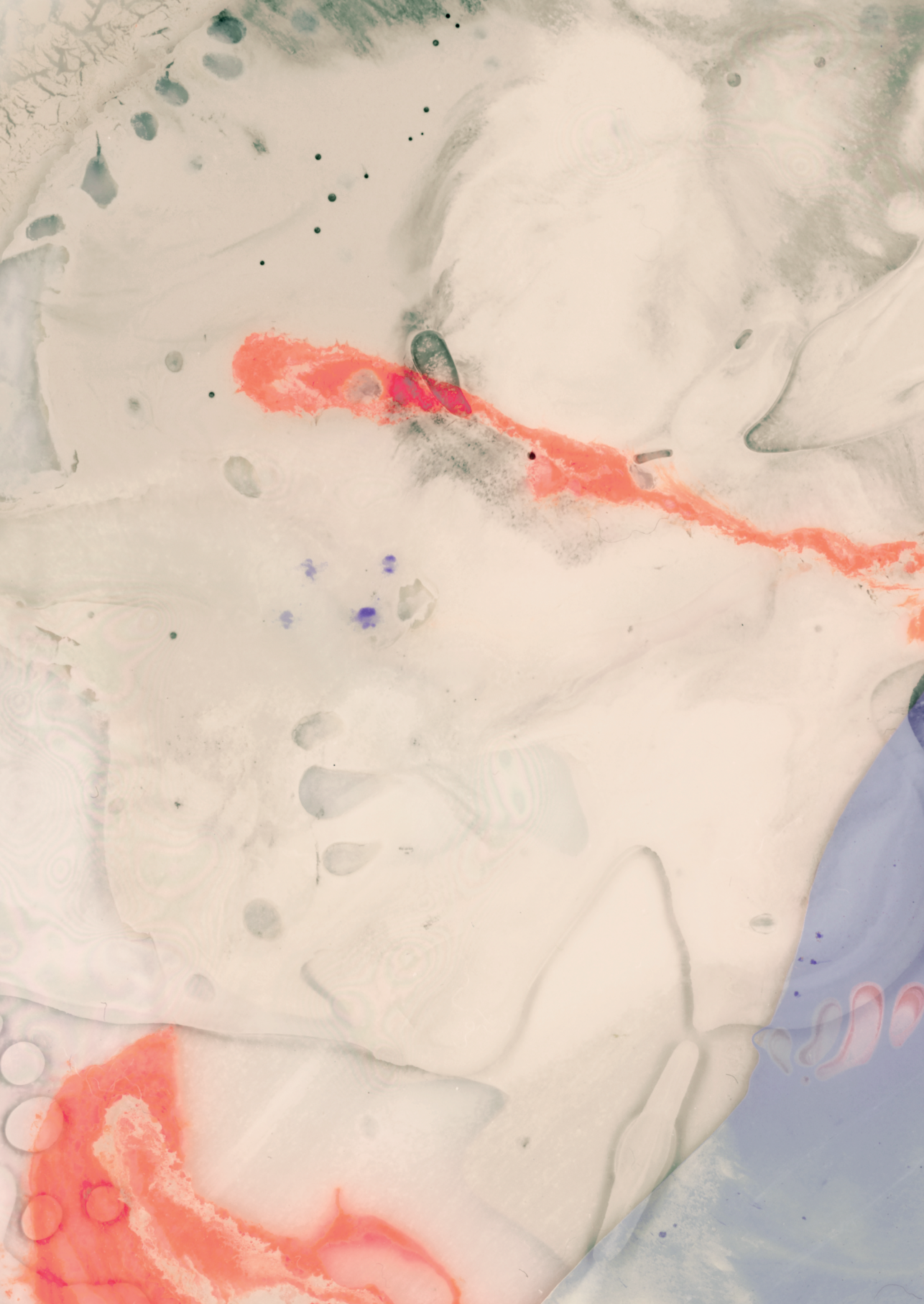
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Chapter 4.2 - **Wahab RJ,** Jaddoe VWV, Steegers EAP, Gaillard R. Prediction of healthy pregnancy outcomes in women with overweight and obesity. Submitted

Chapter 4.3 - Wahab RJ, Jaddoe VWV, Van Klaveren D, Vermeulen MJ, Reiss IKM, Steegers EAP, Gaillard R. Risk prediction of common birth complications in preconception and early pregnancy: development of a prediction tool within a population-based prospective cohort. Submitted



01

General introduction

RATIONALE

Aristotle stated that all individuals are born as a *tabula rasa,* a blank slate. Throughout time, scientific evidence has started to contradict Aristotle's proposition: when it comes to health, no one starts with a blank slate. Factors already prior to birth contribute to individuals' health throughout the life course. Genetics and place and time of birth have already been addressed as major determinants of development of disease (1, 2). A more recent focus of interest has become the intrauterine life as an important period for health throughout life (3). It has been proposed that intrauterine life is a critical period for adverse exposures to increase susceptibility of disease and mortality later in life. Especially maternal health around conception and during pregnancy could be a key determinant for a good start in life (4).

Maternal metabolism around conception and during pregnancy is an essential health factor for a successful pregnancy. As a physiological process, a woman's metabolism goes through major changes during pregnancy (5). Glucose, lipid and amino acid homeostasis adapt to the pregnancy state to enable fetal growth and development, placentation and to counter increased maternal energy expenditure (6). An important adaptation is the decrease of maternal insulin resistance in early pregnancy and the insulin resistance increase later in pregnancy. This change in insulin resistance throughout pregnancy affects maternal glucose concentrations, which are the main fuels for fetal growth and organogenesis (5). The increase in insulin resistance later in pregnancy not only causes higher glucose concentrations, but also contributes to the increase of lipids and protein synthesis as sources for fetal growth and development (6). Suboptimal maternal characteristics, such as obesity and an unhealthy diet, lead to disturbances in maternal metabolism during pregnancy (6, 7). As an adequate maternal metabolism during pregnancy is crucial for fetal growth and development, disturbances can have irreversible consequences for embryonic and fetal development, increasing offspring risk of disease over their lifetime (**Figure 1.1**).

Because of the adverse effects of maternal metabolism on fetal development, antenatal care aims to focus on women with metabolic diseases during pregnancy, for example by screening for gestational diabetes, which is defined as a disorder of glucose metabolism first recognized during pregnancy. However, also among healthy pregnant women a suboptimal metabolism already within the normal range may have an adverse effect of fetal development (8). Multiple studies have shown that moderately elevated maternal glucose concentrations below diagnostic thresholds of diabetes are associated with an increased risk of offspring birth complications (9). These findings may imply that while the majority of women are considered healthy during pregnancy, a substantial number

of women may still have increased risks of these offspring adverse outcomes. More than nine out of ten women at a reproductive age have at least one risk factor that may influence metabolism during pregnancy which may subsequently increase the risk of birth complications or offspring cardio-metabolic risk factors (4). Especially in municipal areas, pregnant women and their fetal offspring are exposed to clustering of lifestyle and environmental risk factors that negatively influence circumstances for an optimal pregnancy (10). For the improvement of health on a population level, more insight into the effects of a maternal suboptimal metabolism below thresholds of disease on offspring birth and long-term cardio-metabolic health is needed. Insight into modifiable dietary targets for intervention could provide further insight into how maternal health during pregnancy can be improved on a population level. After obtaining insight into markers and targets of maternal metabolism during pregnancy, these maternal characteristics could be translated into screening tools to enable personalized risk prediction and early-identification on a population level of women at risk of birth complications and adverse offspring cardio-metabolic health.

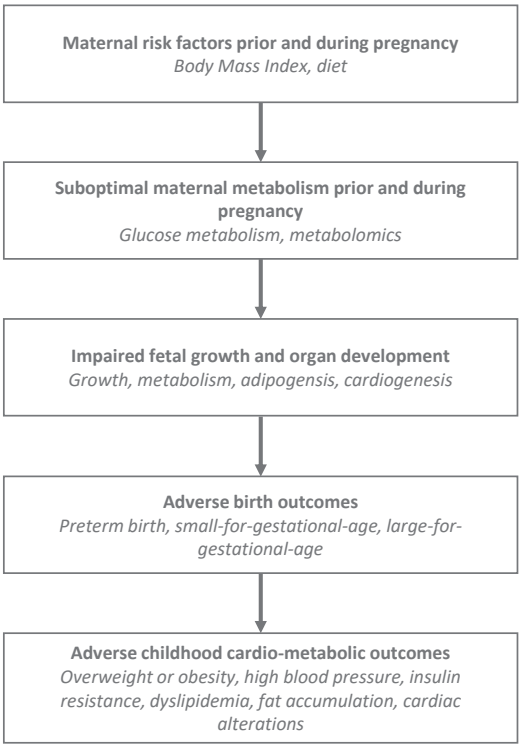


FIGURE 1.1 Hypothesis for studies in this thesis

Therefore, studies presented in this thesis were designed to examine the effects of maternal glucose metabolism during pregnancy on offspring birth and cardio-metabolic outcomes, to identify maternal dietary characteristics that can influence maternal metabolism and subsequent offspring health outcomes and to assess whether these maternal characteristics can be used to develop screening tools for identification of those who are at increased risk of adverse offspring health outcomes (**Figure 1.2**).

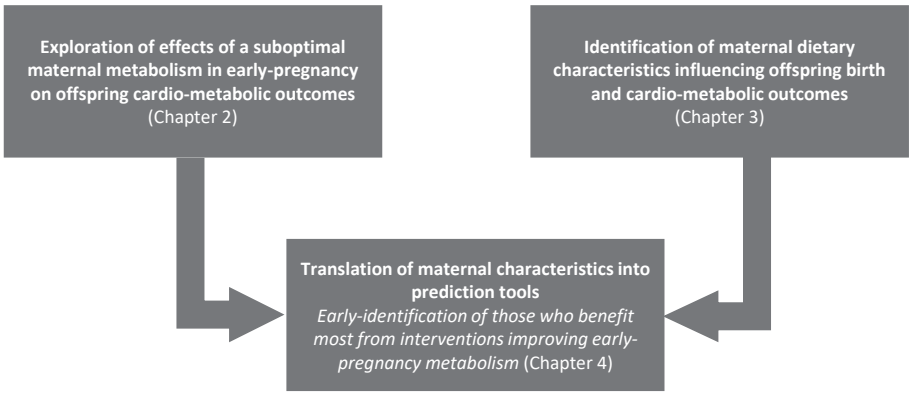


FIGURE 1.2 Outline of aim of this thesis

MATERNAL EARLY-PREGNANCY GLUCOSE METABOLISM

Gestational diabetes affects 4 to 8% of all pregnancies and is a well-known risk factor for maternal and offspring morbidity (11). Offspring of mothers with gestational diabetes have an increased risk of being large-for-gestational-age at birth and developing obesity and insulin resistance in childhood (12). An increasing body of evidence indicates that these offspring risks are not confined to women diagnosed with gestational diabetes (8, 13). The Hyperglycemia and Adverse Pregnancy Outcomes Study including 25,505 pregnant women from nine countries reported conclusive results on this subject (13). The results from this study indicated strong, continuous associations of maternal glucose concentrations measured between 24 and 32 weeks of gestation below those diagnostic threshold of gestational diabetes with an increased birthweight. Based on these results new criteria for diagnosis of diabetes in pregnancy have been proposed by the Internal Association of Diabetes and Pregnancy Study Groups using lower thresholds for glucose concentrations (8, 13, 14). These criteria are based on studies focused on the associations of maternal glucose concentrations in mid- and late pregnancy with

offspring risk of birth complications, obesity and insulin resistance. As embryonic and fetal cardio-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 offspring cardio-metabolic systems. The effects of a suboptimal maternal glucose metabolism in early-pregnancy may also affect development of other organ systems, which are crucial for development of disease in later life. Especially cardiac development may be irreversibly altered by maternal early-pregnancy glucose concentrations, as the human heart is the first functional organ to develop (17). More insight into the continuous effects of maternal glucose concentrations already from early-pregnancy onwards on offspring adiposity, glucose metabolism and cardiac development is needed to enable more accurate identification of women at risk of adverse offspring outcomes. We hypothesized that higher maternal glucose concentrations already below the diagnostic thresholds of diabetes from early-pregnancy onwards are associated with persistent offspring cardio-metabolic adaptations.

MATERNAL DIETARY INFLUENCES DURING PREGNANCY

The maternal diet is inseparable linked to maternal metabolism. During pregnancy, maternal nutritional needs increase to enable maternal metabolic changes to pregnancy and to provide sufficient fetal nutrient availability (18). The maternal diet may therefore have a major potential in women with a suboptimal metabolism to improve fetal nutrient availability and subsequent growth and development.

Maternal glucose concentrations are the main fuel for fetal growth and development. However, too high concentrations of maternal glucose during pregnancy may contribute in the etiology of offspring birth complications and development of cardio-metabolic diseases. Therefore, there is a need for identification of modifiable factors during pregnancy that can optimize maternal glucose concentrations in pregnancy. The main determinant of glucose concentrations in blood is the dietary carbohydrate intake. The dietary glycemic index and glycemic load are measures that can be used to qualify and quantify the maternal postprandial glycemic response to the maternal dietary carbohydrate intake (19). These measures are indicative for the postprandial glucose available for maternal energy, storage and transfer to the fetus (19, 20). Consumption of high glycemic index products cause peak increases in postprandial glucose concentrations, whereas consumption of low glycemic index products cause lower and more stable glucose concentrations (19). In non-pregnant normal weight and overweight populations, a diet with a low glycemic index has been associated with

decreased risk of cardio-metabolic disease and mortality (21). In pregnant women with obesity or gestational diabetes lowering the dietary glycemic index and load has shown potential beneficial effects on offspring birth outcomes (22, 23). In the general pregnant population, not much is known about the effects of the maternal dietary glycemic index and load on fetal development and subsequent birth outcomes. Also, the long-term effects of the dietary glycemic index and load during pregnancy on offspring growth and cardio-metabolic development remain to be established. More insight into the direct effects of the maternal dietary glycemic index and load during pregnancy on offspring outcomes is needed to assess the feasibility of a low glycemic index diet within the general population. The maternal dietary glycemic index and load during pregnancy may offer a new target for intervention strategies aiming improvement of offspring health, already in women without an impaired glucose metabolism.

Besides the effects of maternal carbohydrate metabolism on maternal glucose concentrations in pregnancy, a suboptimal maternal dietary polyunsaturated fatty acid (PUFA) intake could also influence maternal glucose metabolism and fetal development (24, 25). PUFA, in particular omega-3 (n-3) and omega-6 (n-6) PUFAs, cannot be synthesized by the human body (26). Nevertheless, through fatty fish consumption or fish oil supplementation, these essential nutrients can easily be obtained from the diet. As the fetus fully depends on maternal polyunsaturated fatty acids (PUFA) intake and subsequent placental transfer, maternal dietary intake of n-3 and n-6 PUFAs, is essential for fetal growth and development (27-30). Whereas it previously has been thought that adequate maternal PUFA intake during pregnancy only improved offspring neurological outcomes, there is an increasing body of evidence that maternal dietary PUFA intake may also have a role in the development of maternal insulin resistance during pregnancy and fetal hepatic adipocyte differentiation (23, 24, 31). Optimization of maternal PUFA concentrations may therefore also have beneficial effects offspring liver fat development (23, 31).

We hypothesized the maternal diet, in particular the maternal dietary glycemic index and PUFA intake, may be modifiable factors for the prevention of an adverse offspring body fat distribution, in particular by reducing ectopic fat accumulation.

RISK PREDICTION OF OFFSPRING HEALTH OUTCOMES

New markers of maternal metabolism during pregnancy

Maternal hyperglycemia during pregnancy is an established marker of an impaired metabolism during pregnancy (6). Maternal hyperglycemia during pregnancy is strongly

related to maternal overweight or obesity prior and during pregnancy and is suspected to be highly involved in pathways underlying the associations of a higher prepregnancy body mass index (BMI) with adverse offspring outcomes. However, maternal glucose concentrations do not seem to fully explain these associations (15, 32). There is a need for new markers of metabolism to further explore unknown metabolic mechanisms leading to adverse offspring outcomes and to enable more accurate identification of those at risk of adverse offspring outcomes. Novel metabolomics techniques can provide more insight into alterations of maternal metabolism during pregnancy by enabling a detailed characterization of maternal metabolite profiles (33). These maternal metabolite profiles could be useful in unraveling the detailed alterations in maternal metabolism during pregnancy in response to a higher prepregnancy BMI and in exploring how these alterations impact fetal growth and development. After identification of metabolic profiles for the understanding of metabolic mechanisms underlying the associations of a higher maternal prepregnancy BMI with a birth complications, these metabolite profiles may provide as novel biomarkers for the early-identification of women at increased risk of adverse offspring outcomes.

Risk prediction in high risk and general populations

Maternal metabolic and lifestyle characteristics are associated with the risk of offspring birth complications and adverse cardio-metabolic health outcomes. This knowledge led to an increasing interest in preventive strategies improving metabolic health and lifestyle already from preconception onwards (3, 4). However, identification of women who are likely to benefit from these intervention strategies remains a major challenge (34, 35). Currently, preventive interventions and intensified monitoring in health care are focused on high risk populations and risk selection is performed during pregnancy, when it is often too late to intervene (35-37). Definition of high risk populations is mainly based on a single maternal characteristics, such as obesity or smoking (36, 37). However, these approaches bring limitations and may not select the women most prone of adverse offspring outcomes (38). First, not all women with overweight or obesity develop adverse offspring outcomes. Women with overweight or obesity during pregnancy are subject to a larger extend of characteristics that may influence their risk of adverse offspring outcomes (39). Women with overweight or obesity who are likely to have an uncomplicated pregnancy may have redundant intensified monitoring during pregnancy. These women may have an unnecessary and maybe even harmful medicalization of pregnancy. Differences in risk should lead to different approaches of antenatal care. Screening tools including a larger extent of maternal characteristics could enable personalized risk prediction of adverse pregnancy outcomes within this high risk population to allow for tailored antenatal care in pregnant women with

overweight or obesity. On the other hand, among a general population not classified as high risk, women could also develop adverse offspring outcomes. These women should be identified for preventive interventions to target those who will benefit most from lifestyle programs and to allow for intensified antenatal monitoring of those prone for birth complications. Especially among this group, early-identification already within the preconception period offers a great opportunity for interventions to improve maternal health and lifestyle during pregnancy. Last, novel markers of maternal metabolism and health may have an incremental value for more accurate risk prediction prior and during pregnancy. Screening tools enabling personalized risk prediction are needed to offer a tailored approach for those who need it the most. These tailored approaches may offer an opportunity to prevent from adverse offspring birth and subsequent adverse long-term health outcomes and may contribute to a good start at life.

GENERAL AIM OF THIS THESIS

The main aims of studies presented in this thesis were to identify maternal risk factors and influences of maternal metabolism during pregnancy on maternal and offspring outcomes, and to subsequently develop screening tools for risk prediction of adverse offspring health outcome (**Figure 1.2**).

Therefore, we first aimed to explore the impact of maternal metabolism during pregnancy on offspring cardio-metabolic outcomes. Second, we identified maternal characteristics that can influence maternal metabolism and subsequent offspring health outcomes. Third, we assessed whether these maternal characteristics can be used to develop screening tools for risk prediction of adverse offspring health outcomes.

GENERAL DESIGN

Studies in this thesis were embedded in the Generation R study. The Generation R study is a population-based cohort study from fetal life until adulthood in Rotterdam, The Netherlands (32). The Generation R Study aims to identify early environmental and genetic determinants of growth, development and health. Written consent was obtained from all participating women. All pregnancy women were enrolled between 2001 and 2005. Response rate at birth was 61%, which was calculated by dividing the number of participating live born children by the total number of live born children born in the study area during the inclusion period. Enrollment was possible in pregnancy and at birth but aimed at early pregnancy (total n=9,778, participants were enrolled during pregnancy n=8,879). In early, mid and late pregnancy, ultrasounds, physical

examinations, body sample collections and questionnaires were planned. From birth onwards, data collection was performed using information form municipality health centers, questionnaires and visits to a dedicated research center in Erasmus MC – Sophia's Children's Hospital at the ages of 6 and 9 years. A subgroup of the cohort participated in magnetic resonance imaging (MRI) scans at 9 years.

OUTLINE OF THIS THESIS

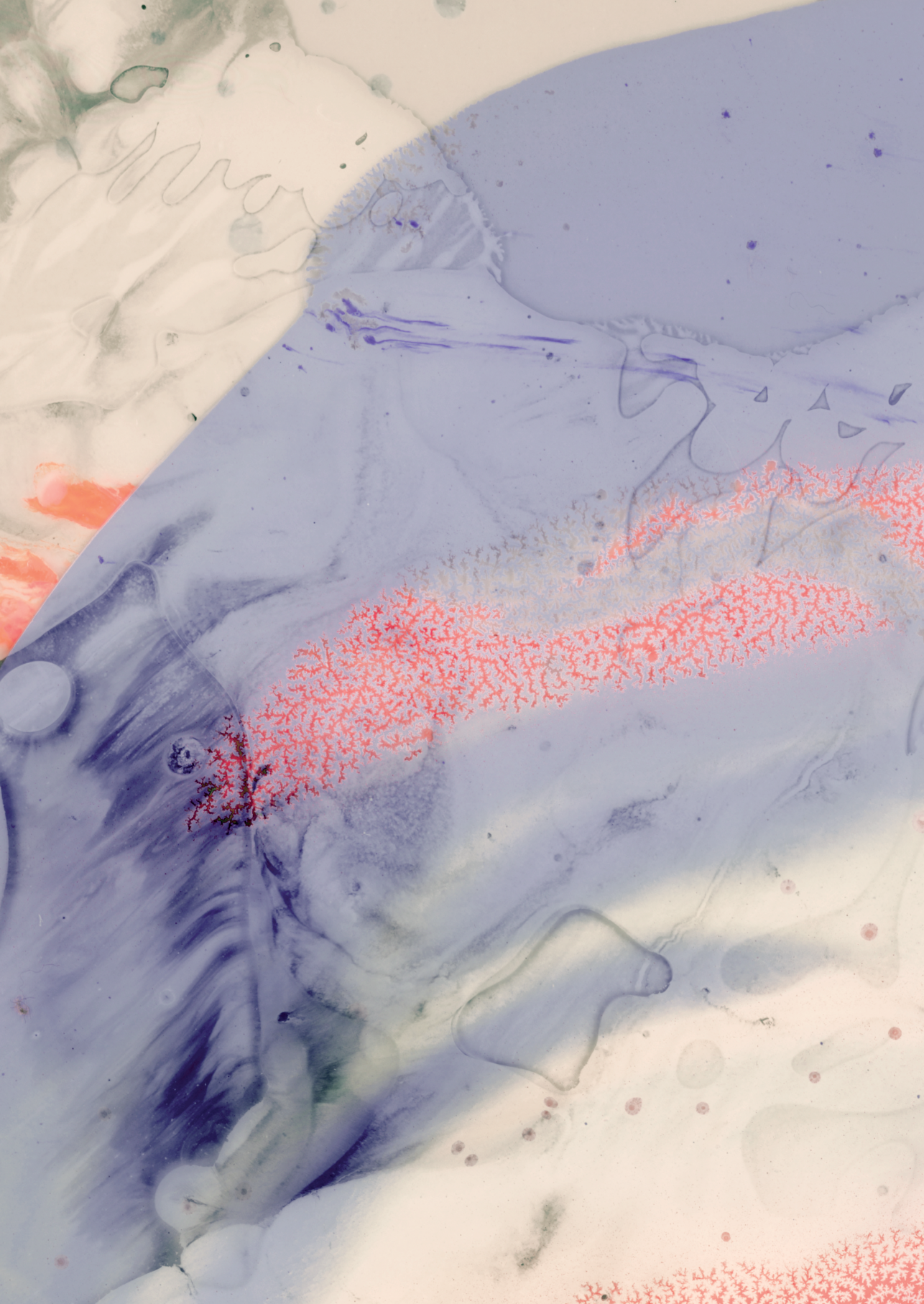
The objectives of the studies in this thesis are addressed in various chapters. **Chapter 2** describes association studies of maternal early-pregnancy glucose metabolism with childhood cardio-metabolic outcomes. First, we examined the associations of maternal early-pregnancy glucose concentrations with childhood blood pressure, lipid and glucose metabolism and fat distribution in **Chapter 2.1**, and the associations of maternal early-pregnancy glucose concentrations with childhood liver fat accumulation (**Chapter 2.2)** and with childhood cardiac outcomes (**Chapter 2.3)**. **Chapter 3** describes association studies of maternal dietary factors during pregnancy with offspring fetal, birth and childhood outcomes. We examined in **Chapter 3.1** the associations of maternal dietary glycemic index with fetal growth patterns and birth outcomes, in **Chapter 3.2** the associations of maternal dietary glycemic index with childhood general, abdominal and ectopic fat accumulation and in **Chapter 3.3** the associations of maternal PUFA concentrations during pregnancy with childhood liver fat accumulation. In **Chapter 4**, we assessed the role of maternal characteristics prior and during pregnancy in the risk prediction of adverse offspring outcomes. In **Chapter 4.1** we assessed whether maternal early-pregnancy metabolites could be useful in the prediction of a higher birthweight in women with a higher prepregnancy BMI. In **Chapter 4.2** we developed a prediction model based on maternal clinical and experimental characteristics for the risk prediction of healthy pregnancies in women with overweight or obesity. In **Chapter 4.3** we developed a prediction model for the risk prediction of birth complications among a general population.

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02

Maternal early-pregnancy glucose metabolism

2.1

Maternal early-pregnancy glucose metabolism and childhood cardio-metabolic outcomes

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ABSTRACT

Objective: This study aimed to examine the associations of maternal early-pregnancy glucose and insulin concentrations with offspring cardio-metabolic 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 to 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 cardio-metabolic 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% Confidence Interval (CI): 0.04 to 0.11) and insulin concentrations (0.07 SDS, 95% CI: 0.03 to 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 cardio-metabolic 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 cardio-metabolic 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 cardio-metabolic 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 cardio-metabolic disease in later life (4, 8-12). Altered childhood body fat development may especially be involved in the associations of maternal glycemia with offspring cardio-metabolic 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 cardio-metabolic disease (14, 15). We hypothesized that higher maternal early-pregnancy glucose concentrations are associated with an unfavorable offspring cardio-metabolic 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 cardio-metabolic 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. Cardio-metabolic follow-up measurements at the age of 10 years were available for 3,737 of their children (**Figure 2.1.1**). Main reasons for missing data were participants lost to follow-up and no consent or failure of venous punctures (16).

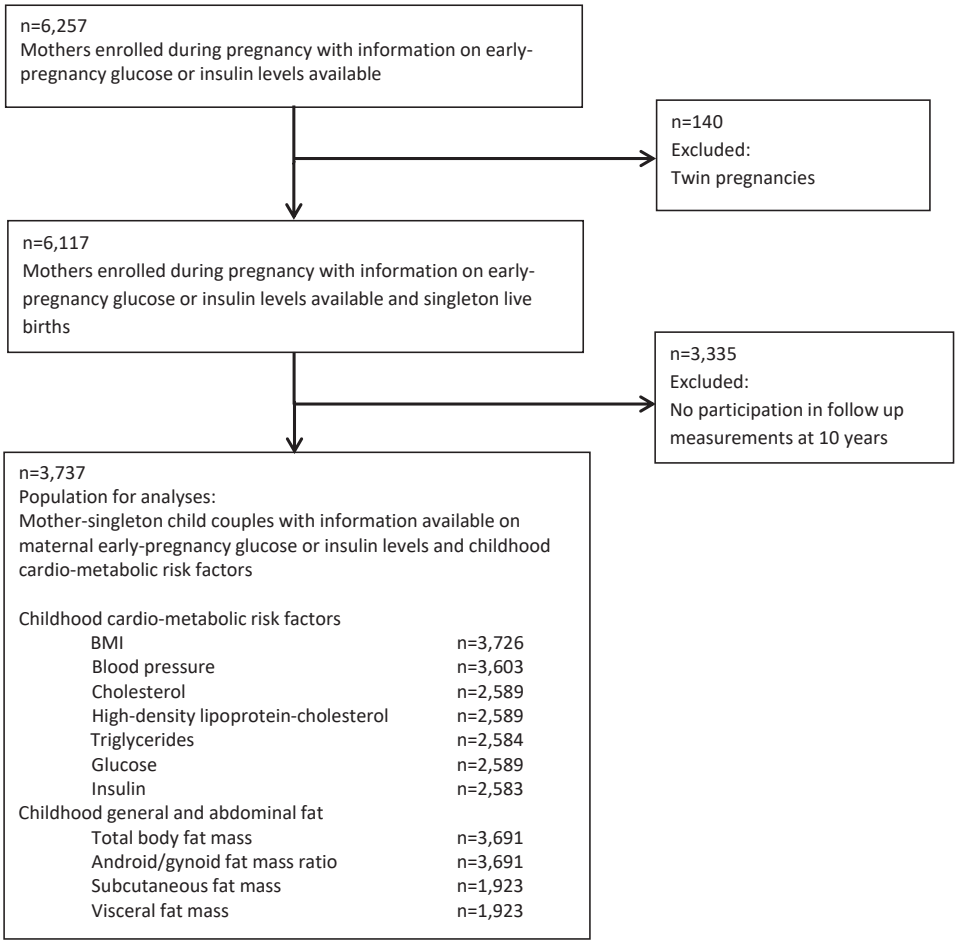


FIGURE 2.1.1. Flow chart of the study participants

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 to 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 cardio-metabolic 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 **Supplementary Methods S2.1.1**) (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 cardio-metabolic 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) during 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 birthweight 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 birthweight 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 birthweight, 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 cardio-metabolic 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, birthweight, 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 α of 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 2.1.1 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 birthweight. No differences in early-pregnancy glucose and insulin concentrations were present (**Supplementary Table 2.1.1**).

TABLE 2.1.1. 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% range), kg	65.0 (50.3 to 90.0)
Prepregnancy BMI, median (95% range), kg/m^2	22.6 (18.8 to 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)
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% range), weeks	13.2 (10.5 to 17.1)
Glucose concentration, mean (SD), mmol/l	4.4 (0.9)
Insulin concentration, median (95% range), pmol/l	114.0 (24.1 to 491.8)
Gestational diabetes, n (%)	34 (0.9)
Infant characteristics	
Sex, n female (%)	1894 (50.7)
Gestational age at birth, median (95% range), weeks	40.3 (37.14 to 42.14)
Birthweight, 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% range), kg	33.8 (26.4 to 49.7)
BMI, median (95% range), kg/m^2	16.9 (14.4 to 23.3)
Fat	

TABLE 2.1.1. Continued

	Total group (n=3,737)
Total fat mass, median (95% range)	8417 (4905 to 19116)
Android/gynoid fat mass ratio, median (95% range)	0.24 (0.16 to 0.44)
Subcutaneous fat mass, median (95% range), g	1294 (642 to 4271)
Visceral fat mass, median (95% range), g	369 (187 to 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% range), mmol/l	0.98 (0.47 to 2.28)
Glucose, mean (SD), mmol/l	5.20 (0.94)
Insulin, median (95% range), pmol/l	174.60 (45.87 to 512.40)
Overweight/obese, n (%)	643 (17.2)
Clustering of cardio-metabolic risk factors, n (%)	261 (7)

Childhood cardio-metabolic risk factors

Figure 2.1.2 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% Confidence Interval (CI): 1.04 to 1.24 and OR 1.20, 95% CI: 1.10 to 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 cardio-metabolic risk factors in childhood (OR 1.20, 95% CI: 1.04 to 1.38 per SDS increase in maternal insulin concentration). All of these associations attenuated to nonsignificance after adjustment for maternal prepregnancy BMI. **Table 2.1.2** shows the associations of maternal glucose and insulin concentrations with each of the childhood cardio-metabolic 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 to 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 to 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, birthweight, infant breastfeeding, and childhood BMI did not materially affect the associations (**Supplementary Table S2.1.2**).

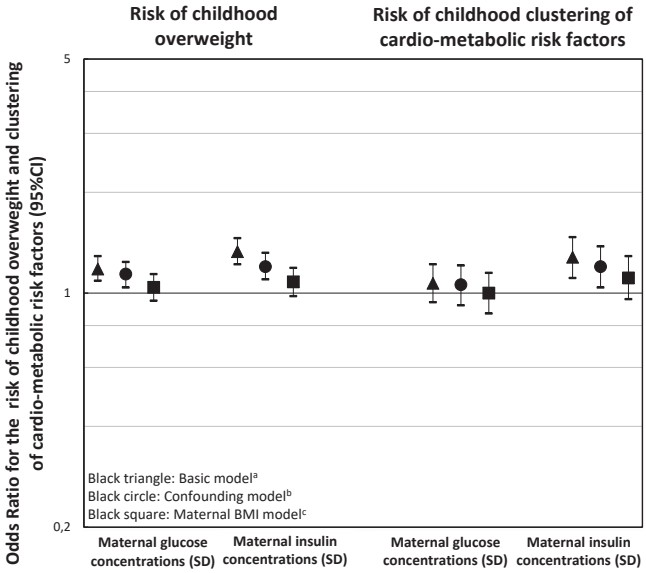


FIGURE 2.1.2. Associations of maternal early-pregnancy glucose and insulin concentrations and childhood risks of overweight and clustering of cardio-metabolic risk factors.

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 2.1.2. 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 to 0.07)	0.03 (0.00 to 0.06)	0.04 (0.01 to 0.07)*	-0.01 (-0.05 to 0.03)	-0.05 (-0.08 to -0.01)*	-0.02 (-0.06 to 0.02)	0.08 (0.04 to 0.11)*	0.04 (0.00 to 0.08)
Confounding model[b]	0.02 (-0.01 to 0.06)	0.02 (-0.01 to 0.06)	0.03 (0.00 to 0.07)	-0.01 (-0.05 to 0.03)	-0.04 (-0.08 to -0.01)*	-0.03 (-0.06 to 0.01)	0.08 (0.04 to 0.11)*	0.04 (0.00 to 0.07)
Maternal BMI model[c]	N.A.	N.A.	0.02 (-0.01 to 0.06)	N.A.	-0.03 (-0.07 to 0.01)	N.A.	0.08 (0.04 to 0.12)*	0.03 (-0.01 to 0.06)
Maternal insulin concentrations (SDS)								
Basic model[a]	0.08 (0.05 to 0.12)*	0.06 (0.03 to 0.09)*	0.05 (0.01 to 0.08)*	0.00 (-0.04 to 0.04)	-0.06 (-0.10 to -0.02)*	0.01 (-0.03 to 0.05)	0.02 (-0.02 to 0.06)	0.08 (0.04 to 0.12)*
Confounding model[b]	0.05 (0.02 to 0.08)*	0.04 (0.01 to 0.07)*	0.03 (-0.01 to 0.06)	-0.01 (-0.04 to 0.03)	-0.05 (-0.09 to -0.01)	0.00 (-0.04 to 0.04)	0.02 (-0.02 to 0.06)	0.07 (0.03 to 0.10)*
Maternal BMI model[c]	-0.01 (-0.05 to 0.02)	0.01 (-0.02 to 0.05)	N.A.	N.A.	N.A.	N.A.	N.A.	0.05 (0.02 to 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)*

Childhood general and abdominal fat

Table 2.1.3 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 to 0.09 per SDS increase in insulin concentration), android/gynoid fat mass ratio (0.05 SDS, 95% CI: 0.02 to 0.08 per SDS increase in insulin concentration), and subcutaneous fat mass index (0.07 SDS, 95% CI: 0.03 to 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.

TABLE 2.1.3. 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 to 0.08)*	0.04 (0.00 to 0.07)	0.04 (-0.01 to 0.08)	-0.01 (-0.05 to 0.04)
Confounding model[b]	0.03 (0.00 to 0.06)	0.02 (-0.01 to 0.05)	0.03 (-0.02 to 0.07)	-0.01 (-0.06 to 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 to 0.14)*	0.09 (0.06 to 0.12)*	0.11 (0.06 to 0.15)*	0.03 (-0.01 to 0.08)
Confounding model[b]	0.06 (0.03 to 0.09)*	0.05 (0.02 to 0.08)*	0.07 (0.02 to 0.11)*	0.02 (-0.02 to 0.07)
Maternal BMI model[c]	0.01 (-0.02 to 0.04)	0.01 (-0.02 to 0.04)	0.02 (-0.02 to 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)*

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 (**Supplementary Tables S2.1.3-S2.1.6**).

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 cardio-metabolic 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 cardio-metabolic 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 cardio-metabolic 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 prepregnancy 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 cardio-metabolic 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 cardio-metabolic 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 cardio-metabolic 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 cardio-metabolic risk factors in childhood, explains the associations of maternal early-pregnancy glucose and insulin concentrations with childhood overweight and cardio-metabolic risk factors (9). When we further explored the associations of maternal early-pregnancy glucose and insulin concentrations with individual cardio-metabolic 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, birthweight, 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 cardio-metabolic 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 cardio-metabolic 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). 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 cardio-metabolic 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 (36). 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, 37, 38). Furthermore, higher maternal glucose concentrations may also be involved in gene expression through DNA methylation, leading to altered insulin secretion in the offspring (39). 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 (40). 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 (41). Maternal prepregnancy BMI seems to explain the associations of maternal glucose and insulin concentrations with other childhood cardio-metabolic risk factors and childhood body fat development. This suggests that preventive strategies, aimed at improving offspring cardio-metabolic 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 (42, 43). 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 (44). 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 cardio-metabolic risk factors were present.

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SUPPLEMENTARY MATERIAL

Supplementary Methods S2.1.1: 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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SUPPLEMENTARY TABLE S2.1.1. Non-response analysis for loss to follow-up at the age of 10 years (n=6,117)

	Follow up at 10 years (n=3,737)	No follow up at 10 years (n=2,380)	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% range), kg	65.0 (50.3 to 90.0)	63 (50.0 to 92.0)	<0.01
Pre-pregnancy BMI, median (95% range), kg/m^2	22.6 (18.8 to 31.9)	22.6 (18.6 to 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% range), weeks	13.2 (10.5 to 17.1)	13.4 (10.4 to 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% range), 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 (%)	1,894 (50.7)	1,123 (47.2)	0.01
Birthweight, mean (SD), grams	3,437 (550)	3,386 (583)	<0.01
Gestational age at birth, median (95% range), weeks	40.3 (37.1 to 42.1)	40.0 (36.4 to 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.*

SUPPLEMENTARY TABLE S2.1.2. 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=2,589)	Insulin Concentrations (SDS) (n=2,583)
Maternal glucose concentrations (SDS)		
Gestational weight gain model[a]	0.07 (0.03 to 0.12)*	0.03 (-0.02 to 0.07)
Birthweight model[b]	0.08 (0.04 to 0.12)*	0.03 (-0.01 to 0.07)
Infant model[c]	0.07 (0.03 to 0.11)*	0.03 (-0.01 to 0.06)
Child BMI model[d]	0.07 (0.03 to 0.11)*	0.03 (-0.01 to 0.07)
Maternal insulin concentrations (SDS)		
Gestational weight gain model[a]	0.02 (-0.02 to 0.07)	0.05 (0.01 to 0.10)*
Birthweight model[b]	0.03 (-0.01 to 0.07)	0.06 (0.02 to 0.10)*
Infant model[c]	0.02 (-0.02 to 0.07)	0.06 (0.02 to 0.10)*
Child BMI model[d]	0.02 (-0.02 to 0.07)	0.06 (0.02 to 0.09)*

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

[a]Gestational weight gain model includes the maternal BMI model additionally adjusted for gestational weight gain

[b]Birthweight model includes the maternal BMI model additionally adjusted for gestational-age-adjusted birthweight

[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

SUPPLEMENTARY TABLE S2.1.3. 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=3,571)	Systolic blood Pressure (SDS) (n=3,454)	Diastolic blood Pressure (SDS) (n=3,454)	Total Cholesterol Concentrations (SDS) (n=2,485)	HDL-cholesterol Concentrations (SDS) (n=2,485)	Triglyceride Concentrations (SDS) (n=2,480)	Glucose Concentrations (SDS) (n=2,485)	Insulin Concentrations (SDS) (n=2,480)
Maternal glucose concentrations (SDS)								
Basic model[a]	0.04 (0.00 to 0.07)	0.03 (0.00 to 0.06)	0.04 (0.01 to 0.07)	-0.01 (-0.03 to 0.02)	-0.05 (-0.08 to -0.01)*	-0.01 (-0.03 to 0.01)	0.08 (0.04 to 0.12)*	0.04 (0.00 to 0.08)
Confounding model[b]	0.02 (-0.01 to 0.06)	0.03 (-0.01 to 0.06)	0.03 (0.00 to 0.06)	-0.01 (-0.03 to 0.02)	-0.04 (-0.08 to -0.01)*	-0.02 (-0.03 to 0.00)	0.08 (0.04 to 0.12)*	0.04 (0.00 to 0.07)
Maternal BMI model[c]	N.A.	N.A.	0.02 (-0.01 to 0.05)	N.A.	-0.03 (-0.07 to 0.01)	N.A.	0.08 (0.04 to 0.12)*	0.03 (-0.01 to 0.06)
Maternal insulin concentrations (SDS)								
Basic model[a]	0.09 (0.05 to 0.12)*	0.06 (0.03 to 0.09)*	0.05 (0.01 to 0.08)*	0.00 (-0.03 to 0.02)	-0.02 (-0.03 to 0.00)	0.00 (-0.02 to 0.02)	0.04 (0.00 to 0.08)	0.08 (0.04 to 0.11)*
Confounding model[b]	0.05 (0.02 to 0.09)*	0.04 (0.01 to 0.08)	0.03 (-0.01 to 0.06)	-0.01 (-0.03 to 0.02)	-0.01 (-0.03 to 0.00)	0.00 (-0.02 to 0.02)	0.04 (0.00 to 0.08)	0.06 (0.02 to 0.10)*
Maternal BMI model[c]	-0.01 (-0.04 to 0.03)	0.02 (-0.02 to 0.05)	N.A.	N.A.	N.A.	N.A.	N.A.	0.05 (0.01 to 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)*

SUPPLEMENTARY TABLE S2.1.4. 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=2,977)	Systolic blood Pressure (SDS) (n=2,884)	Diastolic blood Pressure (SDS) (n=2,884)	Total Cholesterol Concentrations (SDS) (n=2,068)	HDL-cholesterol Concentrations (SDS) (n=2,069)	Triglyceride Concentrations (SDS) (n=2,064)	Glucose Concentrations (SDS) (n=2,070)	Insulin Concentrations (SDS) (n=2,064)
Maternal glucose concentrations (SDS)								
Basic model[a]	0.04 (0.00 to 0.07)	0.03 (-0.01 to 0.07)	0.04 (0.00 to 0.07)	-0.01 (-0.03 to 0.02)	-0.05 (-0.08 to -0.01)*	0.00 (-0.03 to 0.02)	0.06 (0.01 to 0.10)*	0.04 (0.00 to 0.08)
Confounding model[b]	0.02 (-0.02 to 0.06)	0.02 (-0.01 to 0.06)	0.03 (-0.01 to 0.07)	-0.01 (-0.03 to 0.02)	-0.04 (-0.08 to -0.01)*	-0.01 (-0.03 to 0.01)	0.06 (0.01 to 0.10)*	0.04 (-0.01 to 0.08)
Maternal BMI model[c]	N.A.	N.A.	0.02 (-0.02 to 0.06)	N.A.	-0.01 (-0.02 to 0.01)	N.A.	0.06 (0.01 to 0.10)*	0.02 (-0.02 to 0.07)
Maternal insulin concentrations (SDS)								
Basic model[a]	0.09 (0.05 to 0.12)*	0.07 (0.03 to 0.10)*	0.05 (0.01 to 0.08)*	0.00 (-0.03 to 0.03)	-0.02 (-0.03 to 0.00)	0.01 (-0.01 to 0.03)	0.01 (-0.03 to 0.06)	0.08 (0.04 to 0.12)*
Confounding model[b]	0.05 (0.01 to 0.09)*	0.05 (0.01 to 0.08)*	0.03 (-0.01 to 0.06)	0.00 (-0.03 to 0.03)	-0.02 (-0.03 to 0.00)	0.00 (-0.02 to 0.03)	0.02 (-0.03 to 0.06)	0.07 (0.03 to 0.11)*
Maternal BMI model[c]	-0.01 (-0.05 to 0.03)	0.02 (-0.01 to 0.06)	N.A.	N.A.	-0.01 (-0.02 to 0.01)	N.A.	N.A.	0.05 (0.01 to 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)*

SUPPLEMENTARY TABLE S2.1.5. 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=3,540)	Android/gynoid fat mass ratio (SDS) (n=3,540)	Subcutaneous fat mass index (SDS) (n=1,846)[d]	Visceral fat mass index (SDS) (n=1,846)[d]
Maternal glucose concentrations (SDS)				
Basic model[a]	0.05 (0.02 to 0.08)*	0.04 (0.01 to 0.07)	0.03 (-0.01 to 0.08)	-0.01 (-0.06 to 0.03)
Confounding model[b]	0.03 (0.00 to 0.06)	0.02 (-0.01 to 0.06)	0.02 (-0.02 to 0.06)	-0.02 (-0.06 to 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 to 0.14)*	0.09 (0.06 to 0.12)*	0.10 (0.06 to 0.15)*	0.03 (-0.02 to 0.07)
Confounding model[b]	0.07 (0.04 to 0.10)*	0.05 (0.02 to 0.08)*	0.07 (0.03 to 0.11)*	0.02 (-0.03 to 0.06)
Maternal BMI model[c]	0.02 (-0.01 to 0.05)	0.01 (-0.02 to 0.05)	0.02 (-0.02 to 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)*

SUPPLEMENTARY TABLE S2.1.6. 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 to 0.08)	0.04 (0.00 to 0.07)	0.04 (-0.01 to 0.09)	-0.01 (-0.06 to 0.04)
Confounding model[b]	0.03 (-0.01 to 0.06)	0.02 (-0.01 to 0.05)	0.03 (-0.02 to 0.07)	-0.02 (-0.07 to 0.03)
Maternal BMI model[c]	N.A.	N.A.	N.A.	-0.06 (-0.11 to -0.01)
Maternal insulin concentrations (SDS)				
Basic model[a]	0.11 (0.08 to 0.14)*	0.10 (0.06 to 0.13)*	0.11 (0.06 to 0.15)*	0.03 (-0.02 to 0.08)
Confounding model[b]	0.06 (0.03 to 0.09)*	0.06 (0.03 to 0.10)*	0.07 (0.03 to 0.12)*	0.02 (-0.03 to 0.06)
Maternal BMI model[c]	0.01 (-0.02 to 0.04)	0.02 (-0.01 to 0.06)	0.02 (-0.03 to 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)*

2.2

Maternal glucose concentrations in early pregnancy and childhood liver fat accumulation

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ABSTRACT

Background and aims: Gestational diabetes seems to be associated with offspring non-alcoholic fatty liver disease. We hypothesized that maternal glucose concentrations across the full range may have persistent effects on offspring liver fat accumulation.

Methods: In a multi-ethnic population-based prospective cohort study among 2,168 women and their offspring, maternal early-pregnancy glucose concentrations were measured at a median of 13.1 weeks' gestation (95% range 9.6 to 17.2 weeks). Liver fat fraction was measured at 10 years by magnetic resonance imaging. Non-alcoholic fatty liver disease was defined as liver fat fraction ≥5.0%. We performed analyses among all mothers with different ethnic backgrounds and those of European ancestry only.

Results: The multi-ethnic group had a median maternal early-pregnancy glucose concentration of 4.3 mmol/l (IQR 3.9 to 4.9) and a 2.8% (n=60) prevalence of non-alcoholic fatty liver disease. The models adjusted for child age and sex only showed that in the multi-ethnic group higher maternal early-pregnancy glucose concentrations were associated with higher liver fat accumulation and higher odds of non-alcoholic fatty liver disease, but these associations attenuated into non-significance after adjustment for potential confounders. Among mothers of European ancestry only, maternal early-pregnancy glucose concentrations were associated with increased odds of non-alcoholic fatty liver disease (OR 1.95 (95% CI: 1.32 to 2.88 after adjustment for confounders) per 1 mmol/l increase in maternal early-pregnancy glucose concentration). These associations were not explained by maternal pre-pregnancy and childhood BMI, visceral fat and metabolic markers.

Conclusions: In this study, maternal early-pregnancy glucose concentrations were only among mothers of European ancestry associated with offspring non-alcoholic fatty liver disease. The associations of higher maternal early-pregnancy glucose concentrations with offspring non-alcoholic fatty liver disease may differ between ethnic groups.

INTRODUCTION

Pre-existing diabetes and gestational diabetes are complicating up to 25% of pregnancies (1-3). Recent studies suggest that gestational diabetes leads to impaired offspring cardiovascular and metabolic health in childhood and adulthood (4-7). The observed associations seem not to be restricted to the clinical diagnosis of gestational diabetes but are also present across the full range of maternal glucose concentrations (8, 9). Previous studies suggest that gestational diabetes is also associated with offspring markers of liver pathology (10-15). Results from animal studies suggest that offspring of maternal pregnancy hyperglycemia are predisposed to develop liver steatosis (12-15). In humans, a case-control study among 25 mothers showed that intrahepatocellular lipid content, as measured by magnetic resonance spectroscopy, was increased in neonates of mothers with both obesity and gestational diabetes compared to neonates of mothers with both normal weight and without gestational diabetes (11). Another study among 1,215 mother-child pairs reported that maternal pregnancy diabetes or glycosuria was associated with an increased risk for ultrasound-diagnosed non-alcoholic fatty liver disease at 17.8 years of age, independent of maternal pre-pregnancy BMI (10). We have previously shown that maternal early-pregnancy glucose metabolism is associated with childhood glucose metabolism, but not with other childhood cardiometabolic outcomes after adjustment for maternal pre-pregnancy BMI (8). Also liver fat accumulation is related to risk factors for cardiometabolic disease, independent of total body fat (16, 17). We hypothesized that higher maternal glucose concentrations across the full range in early pregnancy are associated with liver fat accumulation in the offspring. Such associations may predispose individuals to liver and cardio-metabolic disease in later life.

We assessed the associations of maternal early-pregnancy glucose concentrations with offspring liver fat accumulation and non-alcoholic fatty liver disease with magnetic resonance imaging (MRI) at 10 years of age in a multi-ethnic population-based prospective cohort among 2,168 mothers and their children. Because both glucose concentrations, liver fat and the associations between them may differ between ethnic groups, we performed analyses in the full multi-ethnic group and in the group of European ancestry only.

METHODS

Study population

This study was embedded in the Generation R Study. This is a multi-ethnic population-based prospective cohort from early fetal life onwards, based in Rotterdam, the Netherlands (18). The study has been approved by the Medical Ethical Committee of the Erasmus University Medical Center in Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained for all participants (19). All pregnant women were enrolled between 2001 and 2005. The enrollment procedure has been described in detail previously (20). In total, 8,879 women were enrolled during pregnancy, of whom 6,099 were enrolled in early pregnancy, had measurements of glucose concentrations available and had singleton pregnancies. MRI-based liver fat measurements at 10 years of age were available in a subgroup of 2,168 of their children (**Figure 2.2.1**). None of these children had a history of jaundice, medication use, alcohol use, smoking, or drug use, based on information from questionnaires at 10 years of age. Missing measurements were mainly due to whether or not the child attended the MRI subgroup study at 10 years of age, lost to follow-up, no data on liver fat or MRI artifacts (19).

Maternal early-pregnancy glucose and insulin concentrations

Non-fasting blood samples were collected once in early pregnancy at 13.1 median weeks' gestation (95% range, 9.6 to 17.2), as previously described (8, 21). Briefly, venous blood samples were collected from pregnant women. Although samples were at least 30 minutes post-meal, we had no information on the exact time interval of the post-meal fasting duration and therefore consider all samples random. Glucose concentration (mmol/l) is an enzymatic quantity and was measured with c702 module on the Cobas 8000 analyzer (Roche, Almere, the Netherlands). Insulin concentration (pmol/l) was measured with electrochemiluminescence immunoassay on the Cobas e411 analyzer (Roche). Quality control samples demonstrated intra- and inter-assay CVs of 0.9% and 1.2% for glucose concentrations and of 1.3% and 2.5% for insulin concentrations, respectively. Information on pre-existing diabetes was obtained from self-reported questionnaires and on gestational diabetes from medical records after delivery (19). Gestational diabetes was diagnosed by a community midwife or an obstetrician according to Dutch midwifery and obstetric guidelines (19, 22). The following criteria were used: either a random glucose level >11.0 mmol/l, a fasting glucose ≥7.0 mmol/l, or a fasting glucose between 6.1 and 6.9 mmol/l with a subsequent abnormal glucose tolerance test (22). In clinical practice and for this study sample, an abnormal glucose tolerance test was defined as a glucose level greater than 7.8 mmol/l after glucose intake.

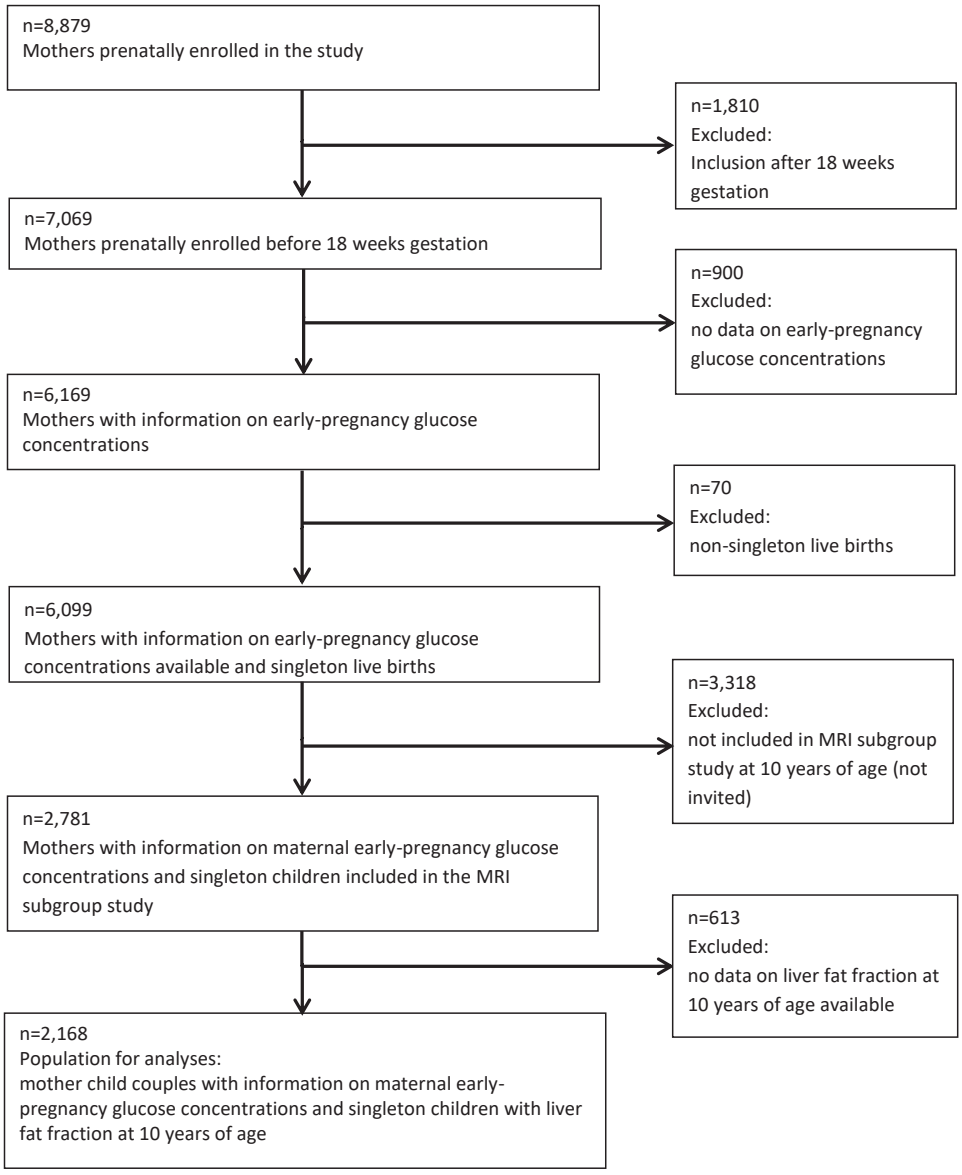


FIGURE 2.2.1. Flow chart of the study participants

Liver fat at 10 years

We measured liver fat using a 3.0 Tesla MRI scanner (Discovery MR750w, GE Healthcare, Milwaukee, Wisconsin, United States) as described previously (19, 23-25). A liver fat scan

was performed using a single-breath-hold, 3D volume and a special 3-point proton density weighted Dixon technique (IDEAL IQ) for generating a precise liver fat fraction image (26). The IDEAL IQ scan is based on a carefully tuned 6-echo echo planar imaging acquisition. The obtained fat fraction maps were analyzed by the Precision Image Analysis (PIA) (Kirkland, Washington, United States) using the sliceOmatic (TomoVision, Magog, Canada) software package. All extraneous structures and any image artifacts were removed manually (27). Liver fat fraction was determined by taking four samples of at least 4cm^2 from the central portion of the hepatic volume. Subsequently, the mean signal intensities were averaged to generate an overall mean liver fat estimation. Liver fat measured with IDEAL IQ using MRI is reproducible, highly precise and validated in adults (28, 29). Non-alcoholic fatty liver disease was defined as liver fat ≥5.0% (23, 29, 30). We studied liver fat fraction across the full range and dichotomized in low, <5.0%, and high, ≥5.0%, based on the clinical cutoff for non-alcoholic fatty liver disease (31). As a sensitivity analysis, we dichotomized liver fat into low, ≤2.0%, and high, >2.0%, based on the median liver fat fraction in our population and on previous work from our group describing that liver fat accumulation above 2.0% is already associated with an increased cardio-metabolic risk profile in children (16).

Covariates

Information was obtained by questionnaires on maternal age, parity, ethnicity, education level, smoking, alcohol consumption, folic acid supplement use, pre-pregnancy weight, and total daily energy intake during pregnancy (18). We categorized ethnicity into European (Dutch n=1,258 (58.8%) and other European n=168 (7.7%)) versus Non-European (Cape Verdean n=98 (4.6%), other African n=21 (1.0%), Dutch Antillean n=42 (2.0%), Surinamese n=172 (8.0%), American n=43 (2.0%), Asian n=48 (2.1%), Indonesian n=75 (3.5%), Turkish n=117 (5.5%), Moroccan n=95 (4.4%), Oceanian n=4 (0.2%)). We measured maternal height without shoes at intake and calculated pre-pregnancy BMI. Non-fasting venous blood samples were obtained in early pregnancy, total cholesterol (mmol/l), triglyceride (mmol/l) and high-density lipoprotein cholesterol (HDL) (mmol/l) concentrations were analyzed. Low-density lipoprotein (LDL) (mmol/l) concentrations were calculated using the Friedewald equation (32). Maternal dyslipidemia was defined as having three or more out of the following four adverse factors: total cholesterol above the seventy-fifth percentile to triglycerides above the seventy-fifth percentile to HDL cholesterol below the twenty-fifth percentile, and LDL cholesterol above the seventy-fifth percentile of our study population. Information on child gestational age at birth, sex, and birthweight was obtained from medical records (19). We obtained information on breastfeeding in infancy by questionnaire (19). Non-fasting blood samples were collected to determine concentrations of insulin, total cholesterol, triglycerides, HDL

cholesterol and LDL cholesterol at 6 years of age (33). At the 10 years of age follow-up visit, we measured childhood height and weight, both without shoes and heavy clothing, and calculated BMI and sex- and age-adjusted childhood BMI standard deviation score (SDS) based on Dutch reference growth charts (Growth Analyzer 4.0, Dutch Growth Research Foundation) (34). Visceral fat mass was obtained by MRI scans, as described previously (19, 35). Physical activity and screen time were assessed with questionnaires at 10 years of age (36). Non-fasting venous blood samples were obtained and we measured glucose and insulin concentrations.

Statistical analysis

We conducted a non-response analysis to compare characteristics of mothers and children with and without liver MRI scan measurements with Student's t-tests, Mann-Whitney tests and Chi-square tests. Second, we used linear and logistic regression models to assess associations of maternal early-pregnancy glucose concentrations across the full range with liver fat accumulation and with the odds of non-alcoholic fatty liver disease. Potential covariates were first selected based on previous literature, their association with both the exposure and the outcome or a change in the effect estimates of >10% in the basic model as shown with the Directed Acyclic Graph (**Supplementary Figure S2.2.1**), subsequently we performed a backward model selection analysis (10, 37). The basic model was adjusted for child sex and age 10 years at follow-up measurements. The main confounder model was additionally adjusted for maternal ethnicity, education, and child physical activity. We further adjusted any significant association in the main model for maternal pre-pregnancy BMI, dyslipidemia, and child metabolic markers at 6 years, BMI at 10 years, visceral fat mass at 10 years, and glucose concentrations at 10 years to explore whether any significant association was explained by these covariates (3, 8, 38).

Because both glucose concentrations, liver fat and the associations between them may differ between ethnic groups, we performed analyses in the full multi-ethnic group and in the European ancestry only groups (**Supplementary Table S2.2.1**) (39). Unfortunately, the other ethnic subgroups were too small to perform ethnic specific analyses. As sensitivity analysis, first, we repeated all analyses using maternal early-pregnancy insulin concentrations as exposure as another marker of maternal glucose metabolism in early pregnancy. Maternal early-pregnancy insulin concentrations were natural log-transformed before the SDS construction due to the skewed distribution. Second, to assess the associations of maternal early-pregnancy glucose concentrations with a potentially clinically relevant liver fat cutoff, we repeated the analyses using liver fat dichotomized in low, ≤2.0%, and high, >2.0%, liver fat. Third, we explored whether

our observed associations were affected by specific subgroups in our study population. We first excluded women with the pre-existing diabetes or gestational diabetes (total n=28) to focus specifically on a non-diabetic population. Second, we excluded women with glucose concentrations sampled at >14 weeks' gestation to assess the associations of first trimester maternal glucose concentrations with liver fat accumulation at school age (n=702); The distribution of liver fat was skewed and natural log-transformed values were used in all linear regression analyses. Missing data in the covariates were multiple-imputed using Markov chain Monte Carlo approach. Five imputed datasets were created and analyzed together. All statistical analyses were performed using the Statistical Product and Service Solutions (SPSS) Statistics version 25.0 for Windows (IBM, Chicago, Illinois, United States).

RESULTS

Subject characteristics

The median maternal early-pregnancy glucose concentration was 4.3 mmol/l (95% range, 3.0 to 6.4, interquartile range(IQR) 3.9 to 4.9). The median liver fat fraction was 2.0% (95% range, 1.2-5.2%, IQR 1.7 to 2.5) and the prevalence of non-alcoholic fatty liver disease was 2.8% (n=60) in children at 10 years of age (**Table 2.2.1**). Mothers of children with non-alcoholic fatty liver disease had a higher BMI, were less often from European ancestry, had slightly higher level of educational attainment, and those children had higher BMI and visceral fat mass compared to children without non-alcoholic fatty liver disease in the full multi-ethnic group (**Table 2.2.1**). In the European ancestry only group, mothers of children with non-alcoholic fatty liver disease had higher glucose concentrations in early pregnancy, and those children were less active compared to children without non-alcoholic fatty liver disease (**Table 2.2.2**). Mothers of the European ancestry only group had similar glucose concentrations and had slightly higher level of educational attainment compared to the full multi-ethnic group (**Supplementary Table S2.2.2**).The correlation coefficient for the correlation between maternal early-pregnancy glucose and maternal pre-pregnancy BMI was 0.15 (**Supplementary Table S2.2.3**). Non-response analyses showed that participants without outcome measurements had mothers with a slightly lower level of educational attainment (**Supplementary Table S2.2.4**).

TABLE 2.2.1. Maternal and child characteristics by offspring non-alcoholic fatty liver disease status - full multi-ethnic group

	Total group n=2,168	NAFLD no n=2,108	NAFLD yes n=60	*p* value
Maternal characteristics				
Age at enrollment, years	30.8 (4.6)	30.9 (4.6)	30.1 (6.0)	0.36
Gestational age at glucose/insulin measurement, weeks	13.1 (9.6 to 17.2)	13.1 (9.6 to 17.2)	13.1 (11.2 to 17.9)	0.20
Pre-pregnancy body mass index, kg/m^2	22.5 (18.1 to 35.2)	22.4 (18.1 to 34.9)	24.9 (18.3 to 42.8)	<0.01
Parity, nulliparous	1,317 (61.0)	1,284 (61.2)	33 (55.0)	0.33
Ethnicity, European	1,426 (66.6)	1,401 (67.3)	25 (42.4)	<0.01
Education, higher	1,115 (53.6)	1,099 (54.2)	16 (29.1)	<0.01
Smoking during pregnancy, continued	334 (18.7)	329 (19.0)	5 (10.4)	0.14
Alcohol consumption, during pregnancy	622 (37.2)	609 (37.5)	13 (28.3)	0.20
Folic acid supplement use, yes	1,024 (71.4)	994 (71.5)	30 (68.2)	0.64
Daily energy intake, kcal/day	2,060 (572)	2,061 (571)	2,053 (610)	0.93
Dyslipidemia	233 (10.7)	226 (10.7)	7 (11.7)	0.82
Glucose, mmol/l	4.4 (0.8)	4.4 (0.8)	4.6 (1.0)	0.12
Insulin, pmol/l	113.1 (19.8 to 669.6)	112.8 (19.7 to 673.2)	171.0 (22.8 to 672.6)	0.09
Pre-existing Diabetes	6 (0.3)	5 (0.3)	1 (1.9)	0.04
Gestational Diabetes	22 (1.1)	22 (1.1)	0 (0)	0.43
Child characteristics				
Sex, female	1,113 (51.3)	1,082 (51.3)	31 (51.7)	0.96
Birthweight, grams	3,447 (548)	3,475 (549)	3,347 (535)	0.15
Gestational age at birth, weeks	40.3 (36.0, 42.4)	40.3 (36.0, 42.4)	39.9 (34.5 to 42.8)	0.08
Ever breastfed, yes	1,761 (93.0)	1,721 (93.1)	40 (87.0)	0.11
Insulin at 6 years, pmol/l	113.5 (18.1 to 409.9)	113.1 (17.7 to 409.8)	130.7 (34.1 to 412.5)	0.42
Total cholesterol at 6 years, mmol/l	4.2 (0.6)	4.2 (0.6)	4.4 (0.7)	0.09
LDL cholesterol at 6 years, mmol/l	2.4 (0.6)	2.4 (0.6)	2.4 (0.6)	0.61
HDL cholesterol at 6 years, mmol/l	1.3 (0.3)	1.3 (0.3)	1.4 (0.3)	0.49
Triglycerides at 6 years, mmol/l	1.0 (0.4 to 2.4)	1.0 (0.4 to 2.4)	1.1 (0.4 to 3.1)	0.10
Age 10 years at outcome follow-up measurements, years	9.8 (0.4)	9.8 (0.3)	9.9 (0.5)	0.34
Playing sports at 10 years, hours/day	1.3 (0.3 to 3.5)	1.3 (0.3 to 3.5)	1.1 (0.1 to 3.5)	0.15
Screen time at 10 years, ≥2 hours/day	852 (51.5)	824 (51.2)	28 (62.2)	0.15
Body mass index at 10 years, kg/m^2	16.9 (14.0 to 24.3)	16.9 (14.0 to 23.9)	21.9 (15.5 to 31.0)	<0.01
Visceral fat mass at 10 years, grams	369.0 (164 to 1,005)	364.1 (163 to 948)	804.4 (242 to 1,849)	<0.01
Glucose at 10 years, mmol/l	5.2 (0.9)	5.3 (0.9)	5.1 (0.7)	0.34
Insulin at 10 years, pmol/l	180.8 (37.1 to 625.7)	180,0 (36.8 to 610,5)	208.8 (41.7 to 830.5)	0.09
Liver fat fraction at 10 years, %	2.0 (1.2 to 5.2)	2.0 (1.2 to 4.0)	6.5 (5.1 to 20.4)	<0.01
Liver fat dichotomized, high ≥2.0%	1,086 (50.1)	1,026 (48.7)	60 (100)	<0.01
Non-alcoholic fatty liver disease	60 (2.8)	-	-	-

Values are observed and represent numbers (valid %), means (SD), or medians (95% range).

TABLE 2.2.2. Maternal and childhood characteristics by offspring non-alcoholic fatty liver disease status - European only group

	Europeans only n=1,426	NAFLD no n=1,401	NAFLD yes n=25	*p* value
Maternal characteristics				
Age at enrollment, years	31.7 (4.0)	31.7 (4.0)	31.1 (5.0)	0.49
Gestational age at glucose/insulin measurement, weeks	12.8 (9.6 to 17.0)	12.9 (9.6 to 16.8)	12.4 (10.9 to 17.0)	0.62
Prepregnancy body mass index, kg/m^2	22.2 (18.1 to 34.3)	22.2 (18.1 to 31.3)	24.3 (18.1 to 34.2)	0.05
Parity, nulliparous	901 (63.3)	513 (36.7)	10 (40.0)	0.73
Education, higher	923 (65.4)	914 (65.9)	9 (36.0)	<0.01
Smoking during pregnancy, continued	217 (18.7)	215 (18.9)	2 (9.5)	0.28
Alcohol consumption, during pregnancy	414 (38.2)	407 (38.3)	7 (33.3)	0.64
Folic acid supplement use, yes	662 (70.6)	648 (70.4)	14 (77.8)	0.50
Daily energy intake, kcal/day	2,053 (587)	2,055 (586)	1,966 (651)	0.51
Dyslipidemia	141 (9.9)	140 (10.0)	1 (4.0)	0.32
Glucose, mmol/l	4.4 to 0.8	4.4 (0.8)	5.0 (1.2)	<0.01
Insulin, pmol/l	102.1 (19.2 to 518.6)	102.1 (19.1 to 440.7)	103.9 (19.8 to 846.0)	0.15
Pre-existing Diabetes	2 (0.2)	2 (0.2)	0 (0)	0.85
Gestational Diabetes	15 (1.1)	15 (1.1)	0 (0)	0.61
Child characteristics				
Sex, female	722 (50.6)	708 (50.5)	14 (56.0)	0.59
Birthweight, grams	3,500 to 540	3,500 (540)	3,447 (521)	0.62
Gestational age at birth, weeks	40.3 (36.0 to 42.4)	40.3 (36.0 to 42.1)	40.0 (37.0 to 42.6)	0.62
Ever breastfed, yes	1,195 (92.1)	1,177 (92.0)	18 (94.7)	0.66
Insulin at 6 years, pmol/l	115.1 (18.5 to 394.3)	114.1 (18.3 to 394.5)	155.4 (60.3 to 398.2)	0.06
Total cholesterol at 6 years, mmol/l	4.2 (0.6)	4.2 (0.6)	4.4 (0.8)	0.15
LDL cholesterol at 6 years, mmol/l	2.3 (0.6)	2.3 (0.6)	2.5 (0.7)	0.45
HDL cholesterol at 6 years, mmol/l	1.3 (0.3)	1.3 (0.3)	1.4 (0.3)	0.34
Triglycerides at 6 years, mmol/l	1.0 (0.4 to 2.3)	1.0 (0.4 to 2.3)	0.9 (0.4 to 2.4)	0.60
Age 10 years at outcome follow-up measurements, years	9.8 (0.3)	9.8 (0.3)	9.8 (0.3)	0.82
Playing sports at 10 years, hours/day	1.4 (0.4 to 3.5)	1.4 (0.4 to 3.5)	1.2 (0.1 to 2.5)	<0.01
Screen time at 10 years, ≥2 hours/day	552 (45.9)	538 (45.6)	14 (63.6)	0.09
Body mass index at 10 years, kg/m^2	16.6 (14.0 to 22.6)	16.6 (14.0 to 22.0)	21.3 (16.2 to 28.6)	<0.01
Visceral fat mass at 10 years, grams	371.7 (168 to 981)	369.9 (168 to 920)	782,1 (301 to 1,360)	<0.01
Glucose at 10 years, mmol/l	5.3 (1.0)	5.3 (1.0)	5.2 (0.8)	0.76
Insulin at 10 years, pmol/l	172.1 (34.9 to 577,9)	171.2 (34.8 to 573.7)	212.8 (40.8 to 826.0)	0.15
Liver fat fraction at 10 years, %	2.0 (1.2 to 4.5)	2.0 (1.2 to 4.0)	6.2 (5.1 to 14.0)	<0.01
Liver fat dichotomized, high ≥2.0%	687 (48.2)	662 (47.3)	25 (100.0)	<0.01
Non-alcoholic fatty liver disease	25 (1.8)	-	-	-

Values are observed and represent numbers (valid %), means (SD), or medians (95% range).

Maternal early-pregnancy glucose concentrations and childhood liver fat

In the full multi-ethnic group, results from the basic models showed that higher maternal early-pregnancy glucose concentrations were associated with higher liver fat accumulation (difference 0.04 (95% Confidence Interval (CI): 0.02 to 0.07) SDS per 1 mmol/l increase in maternal early-pregnancy glucose concentration) and with increased odds of non-alcoholic fatty liver disease (odds ratio (OR) 1.27 (95% CI: 1.10 to 1.46) per 1 mmol/l increase in maternal early-pregnancy glucose concentration) (**Table 2.2.3**). These associations attenuated into non-significance in the main confounder model. In mother-child pairs of European ancestry only, higher maternal early-pregnancy glucose concentrations were associated with increased odds of non-alcoholic fatty liver disease (OR 1.95 (95% CI: 1.32 to 2.88) per 1 mmol/l increase in maternal early-pregnancy glucose concentration in the main confounder model). These associations were not explained by maternal pre-pregnancy BMI, and dyslipidemia. Also, childhood metabolic markers at 6 years, BMI and visceral fat mass or glucose concentrations at 10 years of age, did not explain the observed associations (**Table 2.2.4**). Maternal glucose concentrations were not associated with liver fat accumulation among mother-child pairs of European ancestry only (**Table 2.2.4**).

TABLE 2.2.3. Associations between maternal early-pregnancy glucose concentrations with childhood liver fat fraction and non-alcoholic fatty liver disease in the full multi-ethnic group

	Liver Fat at School Age n=2,168			
Maternal early-pregnancy glucose mmol/l	**Difference liver fat fraction SDS (95% Confidence Interval)**	**p value**	**Odds ratio NAFLD yes/no (95% Confidence Interval)**	**p value**
Basic model	0.04 (0.01 to 0.07)	0.12	1.26 (1.09 to 1.45)	0.11
Main confounder model	0.03 (-0.02 to 0.08)	0.27	1.20 (0.90 to 1.59)	0.21
Maternal body mass index model	0.01 (-0.04 to 0.05)	0.84	1.18 (0.87 to 1.59)	0.30
Maternal dyslipidemia model	0.03 (-0.02 to 0.08)	0.29	1.25 (0.93 to 1.67)	0.14
Child metabolic markers at 6 years model	0.03 (-0.02 to 0.08)	0.27	1.24 (0.93 to 1.66)	0.15
Child body mass index at 10 years model	0.01 (-0.04 to 0.06)	0.68	1.13 (0.84 to 1.53)	0.42
Child visceral fat mass at 10 years model	0.02 (-0.02 to 0.06)	0.47	1.30 (0.95 to 1.79)	0.11
Child glucose concentrations at 10 years model	0.03 (-0.02 to 0.08)	0.30	1.26 (0.94 to 1.69)	0.12

Values are regression coefficients (95% CIs) from linear regression models that reflect differences in liver fat fraction in SDS per maternal early-pregnancy glucose concentrations in mmol/l. Values are ORs (95% CIs) that reflect the risk of NAFLD per

maternal early-pregnancy glucose concentrations in mmol/l. Basic model: adjusted for child sex and age 10 years at outcome follow-up measurements. Main model: basic model additionally adjusted for maternal ethnicity, education, child physical activity. Maternal BMI model: main model additionally adjusted for maternal pre-pregnancy BMI. Maternal dyslipidemia model: main model additionally adjusted for maternal dyslipidemia in early pregnancy. Child metabolic markers at 6 years model: main model additionally adjusted for child insulin, total cholesterol, LDL and HDL-cholesterol and triglycerides concentrations at 6 years of age. Child BMI model: main model additionally adjusted for child BMI at 10 years of age. Child visceral fat mass model: main model additionally adjusted for child MRI-measured visceral fat mass at 10 years of age. Child glucose concentrations model: main model additionally adjusted for child glucose concentrations at 10 years of age. NAFLD was defined as "yes" when liver fat ≥5.0% and as "no" when liver fat <5.0%. Abbreviations: BMI, Body Mass Index; NAFLD, non-alcoholic fatty liver disease; SDS, standard deviation score.

TABLE 2.2.4. Associations between maternal early-pregnancy glucose concentrations with childhood liver fat fraction and non-alcoholic fatty liver disease in the group of European ancestry only

	Liver Fat at School Age n=1,426			
Maternal early-pregnancy glucose mmol/l	**Difference liver fat fraction SDS (95% Confidence Interval)**	**p value**	**Odds ratio NAFLD yes/no (95% Confidence Interval)**	**p value**
Basic model	0.03 (-0.03 to 0.08)	0.38	1.93 (1.31 to 2.84)	<0.01
Main confounder model	0.02 (-0.04 to 0.08)	0.49	1.95 (1.32 to 2.88)	<0.01
Maternal body mass index model	0.00 (-0.06 to 0.06)	0.90	1.86 (1.24 to 2.78)	<0.01
Maternal dyslipidemia model	0.02 (-0.04 to 0.08)	0.49	1.92 (1.30 to 2.86)	<0.01
Child metabolic markers at 6 years model	0.02 (-0.04 to 0.08)	0.50	1.96 (1.31 to 2.95)	<0.01
Child body mass index model	0.01 (-0.05 to 0.06)	0.78	1.66 (1.04 to 2.64)	0.03
Child visceral fat mass at 10 years model	0.00 (-0.05 to 0.06)	0.89	1.82 (1.19 to 2.79)	<0.01
Child glucose concentrations model	0.02 (-0.04 to 0.08)	0.50	1.95 (1.32 to 2.88)	<0.01

Values are regression coefficients (95% CIs) from linear regression models that reflect differences in liver fat fraction in SDS per maternal early-pregnancy glucose concentrations in mmol/l in mother-child pairs of European ancestry only. Values are ORs (95% CIs) that reflect the risk of NAFLD per maternal early-pregnancy glucose concentrations in mmol/l. Basic model: adjusted for child sex and age at outcome follow-up measurements. Main model: basic model additionally adjusted for maternal

education, child physical activity. Maternal BMI model: main model additionally adjusted for maternal pre-pregnancy BMI. Maternal dyslipidemia model: main model additionally adjusted for maternal dyslipidemia in early pregnancy. Child metabolic markers at 6 years model: main model additionally adjusted for child insulin, total cholesterol, LDL and HDL-cholesterol and triglycerides concentrations at 6 years of age. Child BMI model: main model additionally adjusted for child BMI at 10 years of age. Child visceral fat mass model: main model additionally adjusted for child MRI-measured visceral fat mass at 10 years of age. Child glucose concentrations model: main model additionally adjusted for child glucose concentrations at 10 years of age. NAFLD was defined as "yes" when liver fat ≥5.0% and as "no" when liver fat <5.0%. Abbreviations: BMI, Body Mass Index; NAFLD, non-alcoholic fatty liver disease; SDS, standard deviation score.

Sensitivity analyses

When we repeated the main analyses by using insulin concentrations we observed largely the same patterns and tendencies as for glucose concentrations (**Supplementary Table S2.2.5**). When we repeated the analyses with childhood liver fat accumulation categorized into ≤2.0% versus >2% we observed odds in similar direction but smaller as for maternal early-pregnancy glucose concentrations with non-alcoholic fatty liver disease (**Supplementary Table S2.2.6**). No differences in findings were present when mothers with pre-existing diabetes or gestational diabetes or mothers with glucose measurements after 14 weeks gestation were excluded from the analyses in both the full multi-ethnic group and the European ancestry only group. (**Supplementary Table S2.2.7** and **Supplementary Table S2.2.8**).

DISCUSSION

In this prospective cohort study, we observed that maternal early-pregnancy glucose concentrations were only among mothers of European ancestry associated with offspring non-alcoholic fatty liver disease. These associations were not explained by maternal pre-pregnancy BMI, and dyslipidemia. Also, childhood metabolic markers at 6 years, or BMI, visceral fat mass and glucose concentrations at 10 years, did not explain the observed associations. No associations were observed in the full group.

Interpretation of main findings

Non-alcoholic fatty liver disease ranges from liver steatosis, to fibrosis, cirrhosis, and eventually end-stage liver disease (40). In adults, non-alcoholic fatty liver disease is associated with type 2 diabetes, cardiovascular disease, dyslipidemia, and metabolic syndrome (16, 31, 40, 41). We previously reported that elevated liver fat is associated

with an adverse cardiometabolic risk profile in children (16). Gestational diabetes and hyperglycemia diagnosed in second half of pregnancy are associated with an altered offspring body fat composition, cardiovascular and metabolic health (4-6, 38, 42). Studies in women with gestational diabetes showed an association with offspring markers of liver pathology (10, 11). These findings, together with observations from animal studies, suggest that maternal gestational hyperglycemia might be related to offspring liver fat development (12-14). More specifically, early pregnancy might be a critical period for effects of intrauterine maternal glucose exposure on liver health, because the embryonic development of the metabolic systems and of the placenta already occurs in the first weeks after conception (43). Therefore, we hypothesized that higher maternal glucose concentrations across the full range in early pregnancy are associated with liver fat accumulation in offspring.

In this study, in children 10 years of age we did not observe that maternal early-pregnancy glucose concentrations were associated with childhood liver fat accumulation and with risk of non-alcoholic fatty liver disease. Because both glucose concentrations, liver fat and the associations between ethnic subgroups strongly differ, we performed analyses in the full multi-ethnic group and in the group of European ancestry only. In the European ancestry only group, the largest ethnic subgroup, we observed an almost 2-fold increase in odds of non-alcoholic fatty liver disease, independent of maternal pre-pregnancy BMI and dyslipidemia, childhood metabolic markers at 6 years, or BMI, visceral fat mass and of glucose concentrations at 10 years. This may suggest that there is also an intrauterine effect of maternal early-pregnancy glucose concentrations on childhood liver fat accumulation through other pathways than through maternal pre-pregnancy or child BMI, or child glucose concentrations in this subgroup. Due to smaller sample sizes for the other individual ethnic subgroups, we could not test these associations in each ethnic subgroup separately. We did not observe associations of maternal early-pregnancy glucose concentrations with liver fat across the full range in the total study sample and in the largest ethnic subgroup. The lack of association in the total group might be due to a modifying effect of ethnicity with per ethnic subgroup opposite directions of effect estimates. The lack of association in the largest ethnic subgroup could be due to the moderate sample size, together with the relatively small variability in liver fat accumulation in this population of children. Further studies are needed to explore these associations among higher-risk populations and evaluating liver fat accumulation in older offspring.

The underlying pathogenic mechanisms behind the abnormal metabolic risk profile in offspring of mothers with gestational diabetes are largely unknown. Animal studies have suggested that *in utero* exposure to high glucose concentrations may induce

ectopic fat storage (12-14). For instance, mouse models of maternal insulin resistance have shown impairment of gene expression involved in fatty acid oxidative capacity and lipogenesis in offspring liver (14, 15, 44). The accelerated hepatic fat storage in mouse offspring appear to persist into adulthood, suggesting a lasting impact of the maternal intrauterine environment on pathways of hepatic lipid metabolism (15, 44). Another speculation is that the higher insulin resistance in the offspring of mothers with gestational diabetes is associated with higher liver fat accumulation, although the direction of effect is not yet defined (8, 16). In mothers with gestational diabetes a higher risk for non-alcoholic liver fat disease after pregnancy is observed, supporting the hypothesis of a link between insulin resistance and liver fat accumulation (45).

Given the high prevalence of both obesity and impaired glucose metabolism in preconceptional women, these may represent pivotal targets if proven causal for public health in preventing offspring obesity and metabolic disease, like non-alcoholic fatty liver disease (1-3). Our findings emphasize the importance of developing preventive strategies before and in early pregnancy to improve liver and metabolic health outcomes in children. Further studies should characterize the maternal metabolic environment in early pregnancy to provide insights into the causality of early-life determinants of non-alcoholic fatty liver disease taking into account ethnic background.

Methodological considerations

The population-based prospective longitudinal design of this study together with the large sample size with data collection from early pregnancy onwards and the availability of MRI-measured liver fat fraction at 10 years of age are major strengths of this study. The children who underwent MRI measurements at 10 years of age constitute a subgroup of the full Generation R Study population. This may have led to biased effect estimates if associations were different between those included and not included in the analyses, which seems unlikely since the non-response analysis showed hardly any differences. The prevalence of gestational diabetes in our sample was lower than expected (1.1% versus 2-5% in the general Dutch population (46)). likely due to the use of medical records after delivery to obtain information on the diagnosis of gestational diabetes and to lack of universal screening, which may have led to misclassification. The low prevalence of gestational diabetes may also indicate a selection towards a non-diabetic population and might affect the generalizability of our findings. Accurate diagnosis of gestational diabetes is difficult. A fasting glucose greater than 7.0 mmol/l might also represent pre-existing diabetes and a fasting glucose between 6.1 and 6.9 mmol/l might also represent impaired glucose tolerance, instead of gestational diabetes. We verified information about gestational diabetes from medical records. However, glucose testing for diagnosis

of gestational diabetes was not yet routinely performed in our cohort study. Therefore, we may have missed the clinical diagnosis of gestational diabetes among women with relatively higher glucose concentrations. Our findings might be partly explained by women with higher glucose concentrations who were not diagnosed with gestational diabetes. Further studies are needed to replicate our findings among more higher risk populations, including women with impaired glucose tolerance from preconception and early pregnancy onwards and women at higher risk to develop gestational diabetes. The small number of children with non-alcoholic fatty liver disease is likely explained by the fact that we measured liver fat in a relatively healthy study population at a young age, which could have limited our statistical power to detect significant associations and may affect the generalizability of our findings. The main analyses focused on non-alcoholic fatty liver disease were based on only 60 in the full and 25 children in the Europeans ancestry only group with MRI-diagnosed non-alcoholic fatty liver disease. Therefore, these results need to be interpreted carefully and need further replication. We obtained random maternal glucose concentrations once during pregnancy at non-fixed times throughout the day. Due to our study design, we were not able to collect repeated fasting blood samples. As glucose concentrations throughout the day are influenced by multiple factors such as dietary intake and exercise, this may have led to non-differential misclassification, causing an underestimation of our associations. We did not have information on 1 hour and 2 hour postprandial glucose concentrations available. However, previous studies, including studies from our cohort, have shown that random maternal gestational glucose concentrations in pregnancy are related to the risks of gestational diabetes, adverse birth outcomes, childhood obesity, childhood cardiac ventricular structure and function, and altered childhood glucose metabolism (8, 21, 47, 48). These associations were in the same direction as the associations shown for maternal fasting glucose concentrations and postprandial glucose concentrations with these adverse outcomes (37, 49). Further studies are needed using repeated detailed maternal glucose measurements, including fasting glucose concentrations and postprandial glucose measurements to replicate our findings. Ideally, these studies should already measure maternal glucose metabolism prior to pregnancy to reflect maternal glucose metabolism in the preconception period. Information on many covariates was available, yet some residual confounding may have influenced the results.

CONCLUSIONS

Maternal early-pregnancy glucose concentrations were only among mothers of European ancestry associated with offspring non-alcoholic fatty liver disease. These

associations were independent of maternal pre-pregnancy and childhood BMI, visceral fat and metabolic markers. No associations were observed in the full multi-ethnic group. Further studies are needed to explore the causality of the observed associations. Optimizing maternal pre-pregnancy BMI and glucose concentrations could be starting points for prevention strategies to improve liver health among future generations.

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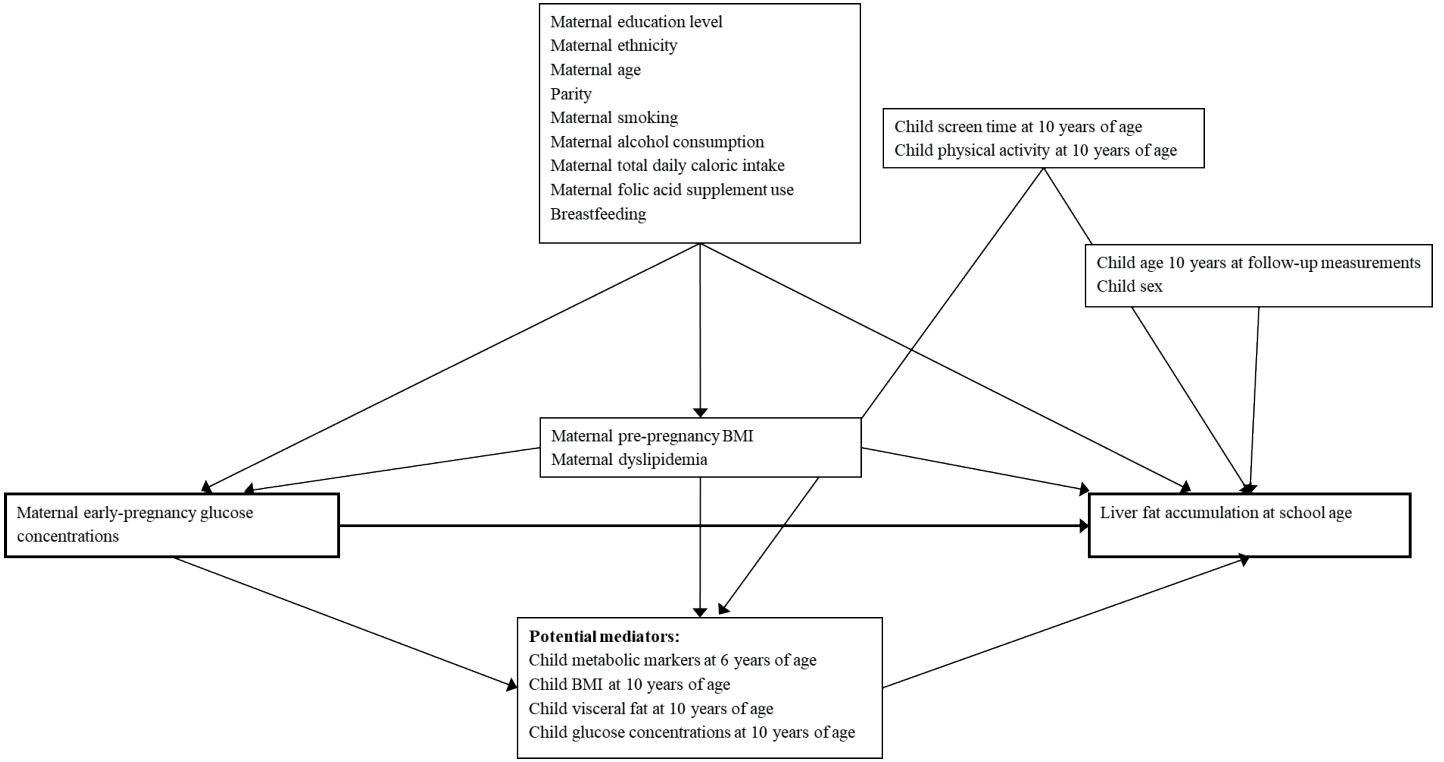
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SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIGURE S2.2.1. Directed Acyclic Graph

Covariate selection was primarily based on the Directed Acyclic Graph and subsequent on backward model selection analysis. The final model included child sex and age 10 years at follow-up measurements, maternal ethnicity, education, and child physical activity. We selected maternal ethnicity, education, smoking, alcohol consumption, folic acid supplement use, and child physical activity and screen time in the model, based on previous literature, their association with both the exposure and the outcome or change in effect estimates of >10% in the basic model(1, 2). Thereafter, we selected variables for the main model using backward selection and stopped when all *p* values <0.20(3). Maternal age, parity, and total daily caloric intake, and breastfeeding were not included in the main model as they did not affect the observed associations. Maternal smoking, alcohol consumption, folic acid supplement and child screen time were removed with backward selection from the main model having a *p* value >0.20(3). To observe the added confounding effect of maternal pre-pregnancy BMI in the observed associations we created an extra model, the maternal BMI model additionally adjusted for maternal pre-pregnancy BMI. Previous studies have suggested that maternal pre-pregnancy BMI largely explains the associations between gestational diabetes and offspring outcomes(4, 5). Next to this, we assessed the possible confounding effect of maternal dyslipidemia in an extra model, the maternal dyslipidemia model: main model additionally adjusted for maternal dyslipidemia. As we showed in our Directed Acyclic Graph, child metabolic markers at 6 years of age, BMI at 10 years, visceral fat mass at 10 years of age or child glucose concentrations at 10 years of age may mediate potential associations between maternal glucose concentrations and liver fat accumulation at school age. To explore the mediating role of child metabolic markers at 6 years of age, BMI at 10 years, visceral fat mass at 10 years As we showed in our Directed Acyclic Graph, child BMI at 10 years of age or child glucose concentrations at 10 years of age may mediate potential associations between maternal glucose concentrations and liver fat accumulation at school age. To explore the mediating role of child BMI at 10 years of age and child glucose concentrations at 10 years of age, we additionally corrected for these characteristics in separate models.

SUPPLEMENTARY TABLE S2.2.1. Differences between groups of various ethnic backgrounds

Maternal ethnicity	Glucose, mmol/l	Liver fat fraction, %	Non-alcoholic fatty liver disease
Total group (n=2,141)	4.44 (0.8)	2.00 (1.2 to 5.2)	60 (2.8)
European			
Dutch (n=1,258)	4.42 (0.8)	1.97 (1.2 to 4.5)	23 (1.8)
Other European (n=168)	4.29 (0.7)	2.07 (1.2 to 4.7)	2 (1.2)
Non-European			
Cape Verdean (n=98)	4.51 (0.9)	2.22 (1.2 to 8.4)	6 (6.1)
Other African (n=21)	4.40 (0.7)	2.11 (1.3 to 4.8)	0 (0.0)
Dutch Antillean (n=42)	4.46 (1.1)	1.81 (1.3 to 10.9)	1 (2.4)
Surinamese (n=172)	4.48 (0.8)	2.00 (1.2 to 5.5)	6 (3.5)
American (n=43)	4.30 (0.7)	2.06 (1.3 to 5.6)	1 (2.3)
Asian (n=48)	4.85 (1.1)	1.87 (1.3 to 4.4)	0 (0.0)
Indonesian (n=75)	4.44 (0.9)	1.99 (1.2 to 7.0)	4 (5.3)
Turkish (n=117)	4.51 (0.8)	2.45 (1.2 to 13.8)	13 (11.1)
Moroccan (n=95)	4.58 (0.9)	2.03 (1.3 to 8.9)	3 (3.2)
Oceanian (n=4)	4.28 (0.8)	1.78 (1.5 to 3.2)	0 (0.0)

Values are observed and represent numbers (valid %), means (SD), or medians (95% range).

SUPPLEMENTARY TABLE S2.2.2. Maternal and childhood characteristics for the full multi-ethnic group and by European ancestry only group versus other ancestry group

	Total group n=2,168	Europeans only n=1,426	Other ancestry n=715	*p* value
Maternal characteristics				
Age at enrollment, years	30.8 (4.6)	31.7 (4.0)	29.2 (5.3)	<0.01
Gestational age at glucose/insulin measurement, weeks	13.1 (9.6 to 17.2)	12.8 (9.6 to 17.0)	13.5 (9.6 to 17.5)	<0.01
Pre-pregnancy body mass index, kg/m^2	22.5 (18.1 to 35.2)	22.2 (18.1 to 34.3)	23.4 (18.0 to 36.6)	<0.01
Parity, nulliparous	1,317 (61.0)	901 (63.3)	404 (57.0)	0.01
Ethnicity, European	1,426 (66.6)	-	-	-
Education, higher	1,115 (53.6)	923 (65.4)	191 (28.5)	<0.01
Smoking during pregnancy, continued	334 (18.7)	217 (18.7)	110 (18.2)	0.80
Alcohol consumption, during pregnancy	854 (51.1)	554 (51.1)	201 (35.6)	0.31
Folic acid supplement use, yes	1,024 (71.4)	662 (70.6)	350 (73.4)	0.27
Daily energy intake, kcal/day	2,060 (572)	2053 (587)	2080 (540)	0.40
Glucose, mmol/l	4.4 (0.8)	4.4 (0.8)	4.5 (0.9)	0.01
Insulin, pmol/l	113.1 (19.8 to 669.6)	102.1 (19.2 to 518.6)	142.8 (22.8 to 964.8)	<0.01
Pre-existing Diabetes	6 (0.3)	2 (0.2)	4 (0.6)	0.08
Gestational Diabetes	22 (1.1)	15 (1.1)	7 (1.0)	0.86
Child characteristics				
Sex, female	1,113 (51.3)	722 (50.6)	375 (52.4)	0.43
Birthweight, grams	3,447 (548)	3,500 (540)	3349 (551)	<0.01
Gestational age at birth, weeks	40.3 (36.0 to 42.4)	40.3 (36.0 to 42.4)	40.1 (35.8 to 42.4)	<0.01
Ever breastfed, yes	1,761 (93.0)	1,195 (92.1)	552 (95.0)	0.02
Age 10 years at outcome follow-up measurements, years	9.8 (0.4)	9.8 (0.3)	9.9 (0.4)	<0.01
Playing sports at 10 years, hours/day	1.3 (0.3 to 3.5)	1.4 (0.4 to 3.5)	1.2 (0.2 to 3.6)	<0.01
Screen time at 10 years, ≥2 hours/day	852 (51.5)	552 (45.9)	292 (65.9)	<0.01
Body mass index at 10 years, kg/m^2	16.9 (14.0 to 24.3)	16.6 (14.0 to 22.6)	17.4 (13.9 to 26.1)	<0.01
Glucose at 10 years, mmol/l	5.2 (0.9)	5.3 (1.0)	5.2 (0.9)	0.31
Insulin at 10 years, pmol/l	180.8 (37.1 to 625.7)	172.1 (34.9 to 577.9)	193.4 (38.2 to 726.6)	<0.01
Liver fat fraction at 10 years, %	2.0 (1.2 to 5.2)	2.0 (1.2 to 4.5)	2.5 (1.3 to 6.5)	<0.01
Liver fat dichotomized, high ≥2.0%	1,086 (50.1)	687 (48.2)	383 (53.6)	0.02
Non-alcoholic fatty liver disease	60 (2.8)	25 (1.8)	35 (4.9)	<0.01

Values are observed and represent numbers (valid %), means (SD), or medians (95% range). Differences were tested using Student t tests and Mann-Whitney tests for normally and non-normally distributed variables, respectively, and $\chi 2$ test was used for dichotomous variables. Number of missings per covariate: maternal ethnicity, n=27 (1.2%); maternal educational level, n=86 (4.0%); smoking during pregnancy, n=384 (17.7%); alcohol consumption, n=497 (22.9%); folic acid supplement use during pregnancy, n=733 (33.8%); pre-existing diabetes n=254 (11.7%); gestational diabetes, n=133 (6.1%); ever breastfed, n=274 (12.6%); screen time, n=514 (23.7%).

SUPPLEMENTARY TABLE S2.2.3. Correlation coefficients between maternal glucose and insulin concentrations, childhood liver fat fraction and non-alcoholic fatty liver disease, and body mass index of mother and child

	Maternal glucose	Maternal insulin	Maternal pre-pregnancy BMI	Child liver fat	Child NAFLD	Child BMI
Maternal glucose	1	0.53*	0.15*	0.01	0.02	0.03
Maternal insulin	0.53*	1	0.23*	0.05**	0.04	0.07*
Maternal pre-pregnancy BMI	0.15*	0.23*	1	0.18*	0.09*	0.35*
Child liver fat	0.01	0.05**	0.18*	1	0.28*	0.37*
Child NAFLD	0.02	0.04	0.09*	0.28*	1	0.19*
Child BMI	0.03	0.07*	0.35*	0.37*	0.19*	1

Values are Spearman correlation coefficients. P value <0.01, ** P value <0.05.*

SUPPLEMENTARY TABLE S2.2.4. Comparison of characteristics between mothers and children with and without outcome measurements

	Participants	Non-participants	
	n=2,168	n=613	*p* value
Maternal characteristics			
Age at enrollment, years	30.8 (4.6)	30.5 (5.0)	0.15
Gestational age at glucose/insulin measurement, weeks	13.1 (9.6 to 17.2)	13.2 (9.8 to 17.6)	0.06
Pre-pregnancy body mass index, kg/m^2	22.5 (18.1 to 35.2)	22.6 (18.1 to 33.6)	0.83
Parity, nulliparous	1,317 (61.0)	367 (60.5)	0.80
Ethnicity, European	1,426 (66.6)	379 (62.6)	0.07
Education, higher	1,115 (53.6)	266 (46.0)	<0.01
Smoking during pregnancy, continued	334 (18.7)	92 (17.8)	0.63
Alcohol consumption, during pregnancy	854 (51.1)	226 (47.0)	0.11
Folic acid supplement use, yes	1,024 (71.4)	300 (70.8)	0.81
Daily energy intake, kcal/day	2,060 (572)	2,015 (550)	0.16
Glucose, mmol/l	4.4 (0.8)	4.4 (0.8)	0.74
Insulin, pmol/l	113.1 (19.8 to 669.6)	116.7 (19.7 to 575.6)	0.75
Pre-existing Diabetes	6 (0.3)	0 (0.0)	0.19
Gestational Diabetes	22 (1.1)	7 (1.2)	0.78
Child characteristics			
Sex, female	1,113 (51.3)	294 (48.0)	0.14
Birthweight, grams	3,447 (548)	3,421 (530)	0.29
Gestational age at birth, weeks	40.3 (36.0 to 42.4)	40.3 (36.3 to 42.3)	0.90
Ever breastfed, yes	1,761 (93.0)	462 (93.0)	0.99

Values are observed and represent numbers (valid %). means (SD), or medians (95% range). Differences were tested using Student t tests and Mann-Whitney tests for normally and non-normally distributed variables, respectively, and $\chi 2$ test was used for dichotomous variables. Number of missings per covariate in participants: maternal ethnicity, n=27 (1.2%); maternal educational level, n=86 (4.0%); smoking during pregnancy, n=384 (17.7%); alcohol consumption, n=497 (22.9%); folic acid supplement use during pregnancy, n=733 (33.8%); pre-existing diabetes n=254 (11.7%); gestational diabetes, n=133 (6.1%); ever breastfed, n=274 (12.6%); screen time, n=514 (23.7%).

SUPPLEMENTARY TABLE S2.2.5. Associations between maternal early-pregnancy glucose and insulin concentrations SDS with childhood liver fat fraction and non-alcoholic fatty liver disease

	Liver Fat at School Age n=2,168			
Maternal early-pregnancy glucose SDS	**Difference liver fat fraction SDS (95% Confidence Interval)**	**p value**	**Odds ratio NAFLD yes/no (95% Confidence Interval)**	**p value**
Basic model	0.03 (0.01 to 0.06)	0.12	1.22 (1.08 to 1.37)	0.11
Main confounder model	0.02 (-0.02 to 0.07)	0.27	1.17 (0.92 to 1.48)	0.21
Maternal body mass index model	0.00 (-0.04 to 0.05)	0.84	1.15 (0.89 to 1.48)	0.30
Maternal dyslipidemia model	0.02 (-0.02 to 0.07)	0.29	1.20 (0.94 to 1.54)	0.14
Child metabolic markers at 6 years model	0.02 (-0.02 to 0.07)	0.27	1.20 (0.94 to 1.54)	0.15
Child body mass index at 10 years model	0.01 (-0.03 to 0.05)	0.68	1.11 (0.86 to 1.44)	0.42
Child visceral fat mass at 10 years model	0.01 (-0.02 to 0.05)	0.47	1.25 (0.95 to 1.64)	0.11
Child glucose concentrations at 10 years model	0.02 (-0.02 to 0.07)	0.30	1.22 (0.95 to 1.56)	0.12
Maternal early-pregnancy insulin SDS				
Basic model	0.06 (0.02 to 0.10)	<0.01	1.23 (0.95 to 1.59)	0.11
Main confounder model	0.03 (-0.01 to 0.08)	0.12	1.09 (0.84 to 1.41)	0.51
Maternal body mass index model	0.00 (-0.04 to 0.05)	0.85	1.01 (0.78 to 1.31)	0.96
Maternal dyslipidemia model	0.02 (-0.02 to 0.07)	0.30	1.06 (0.81 to 1.38)	0.69
Child metabolic markers at 6 years model	0.03 (-0.01 to 0.08)	0.15	1.06 (0.81 to 1.39)	0.65
Child body mass index at 10 years model	0.01 (-0.03 to 0.05)	0.52	0.95 (0.73 to 1.25)	0.73
Child visceral fat mass at 10 years model	0.02 (-0.02 to 0.05)	0.45	1.07 (0.80 to 1.43)	0.64
Child glucose concentrations at 10 years model	0.03 (-0.01 to 0.07)	0.16	1.06 (0.81 to 1.39)	0.66

Values are regression coefficients (95% CIs) from linear regression models that reflect differences in liver fat fraction in SDS per maternal early-pregnancy glucose or insulin concentrations in SDS. Values are ORs (95% CIs) that reflect the risk of NAFLD at 10 years of age per maternal early-pregnancy glucose or insulin concentrations in SDS. Basic model: adjusted for child sex and age 10 years at outcome follow-up measurements. Main model: basic model additionally adjusted for maternal ethnicity, education, child physical activity. Maternal BMI model: main model additionally adjusted for maternal pre-pregnancy BMI. Maternal dyslipidemia model: main model additionally adjusted for maternal dyslipidemia in early pregnancy. Child metabolic markers at 6 years model: main model additionally adjusted for child insulin, total-cholesterol, LDL and HDL-cholesterol and triglycerides concentrations at 6 years of age. Child BMI model: main model additionally adjusted for child BMI at 10 years of age. Child visceral fat mass model: main model additionally adjusted for child MRI-measured visceral fat mass at 10 years of age. Child glucose concentrations model: main model additionally adjusted for child glucose concentrations at 10 years of age. NAFLD was defined as "yes" when liver fat ≥5.0% and as "no" when liver fat <5.0%. Abbreviations: BMI, Body Mass Index; NAFLD, non-alcoholic fatty liver disease; SDS, standard deviation score.

SUPPLEMENTARY TABLE S2.2.6. Associations between maternal early-pregnancy glucose with childhood liver fat fraction below or above 2% liver fat

	Liver Fat at School Age n=2,168	
Maternal early-pregnancy glucose mmol/l	**Odds ratio Liver fat >2% yes/no (95% Confidence Interval)**	**p value**
Basic model	1.05 (1.00 to 1.11)	0.34
Main confounder model	1.04 (0.94 to 1.15)	0.49
Maternal body mass index model	1.00 (0.90 to 1.11)	0.95
Maternal dyslipidemia model	1.04 (0.94 to 1.14)	0.50
Child metabolic markers at 6 years model	1.04 (0.94 to 1.15)	0.48
Child body mass index model	1.01 (0.91 to 1.12)	0.89
Child visceral fat mass at 10 years model	1.02 (0.92 to 1.14)	0.69
Child glucose concentrations model	1.03 (0.92 to 1.15)	0.66

Values are ORs (95% CIs) that reflect the risk of more than 2% liver fat at 10 years of age per maternal early-pregnancy glucose concentrations in mmol/l. Basic model: adjusted for child sex and age 10 years at outcome follow-up measurements. Main model: basic model additionally adjusted for maternal ethnicity, education, child physical activity. Maternal BMI model: main model additionally adjusted for maternal pre-pregnancy BMI. Maternal dyslipidemia model: main model additionally adjusted for maternal dyslipidemia in early pregnancy. Child metabolic markers at 6 years model: main model additionally adjusted for child insulin, total-cholesterol, LDL and HDL-cholesterol and triglycerides concentrations at 6 years of age. Child BMI model: main model additionally adjusted for child BMI at 10 years of age. Child visceral fat mass model: main model additionally adjusted for child MRI-measured visceral fat mass at 10 years of age. Child glucose concentrations model: main model additionally adjusted for child glucose concentrations at 10 years of age. Liver fat >2% was defined as "yes" when liver fat >2.0% and as "no" when liver fat ≤2.0%. Abbreviations: BMI, Body Mass Index; SDS, standard deviation score.

SUPPLEMENTARY TABLE S2.2.7. Sensitivity analyses on the associations between maternal early-pregnancy glucose with childhood liver fat fraction and non-alcoholic fatty liver disease among the full multi-ethnic group

	Liver Fat at School Age			
Maternal early-pregnancy glucose mmol/l	**Difference liver fat fraction SDS (95% Confidence Interval)**	***p* value**	**Odds ratio NAFLD yes/no (95% Confidence Interval)**	***p* value**
Women without pre-existing or gestational diabetes (n=1,771)				
Basic model	0.04 (0.00 to 0.08)	0.15	1.20 (1.02 to 1.41)	0.27
Main confounder model	0.03 (-0.03 to 0.08)	0.38	1.15 (0.83 to 1.60)	0.39
Maternal body mass index model	0.00 (-0.05 to 0.06)	0.91	1.09 (0.77 to 1.53)	0.63
Maternal dyslipidemia model	0.02 (-0.03 to 0.08)	0.40	1.15 (0.83 to 1.60)	0.39
Child metabolic markers at 6 years model	0.03 (-0.03 to 0.08)	0.36	1.16 (0.83 to 1.62)	0.38
Child body mass index model	0.01 (-0.04 to 0.06)	0.68	1.08 (0.77 to 1.50)	0.66
Child visceral fat mass at 10 years model	0.02 (-0.03 to 0.07)	0.37	1.21 (0.85 to 1.74)	0.29
Child glucose concentrations model	0.02 (-0.03 to 0.08)	0.40	1.18 (0.85 to 1.64)	0.34
Women included before 14 weeks gestation (n=1,466)				
Basic model	0.03 (0.00 to 0.06)	0.40	1.20 (1.00 to 1.45)	0.32
Main confounder model	0.02 (-0.04 to 0.08)	0.48	1.23 (0.85 to 1.78)	0.27
Maternal body mass index model	0.00 (-0.06 to 0.06)	0.95	1.15 (0.78 to 1.69)	0.48
Maternal dyslipidemia model	0.02 (-0.04 to 0.08)	0.53	1.22 (0.85 to 1.77)	0.29
Child metabolic markers at 6 years model	0.02 (-0.04 to 0.08)	0.48	1.25 (0.86 to 1.81)	0.25
Child body mass index model	0.01 (-0.05 to 0.06)	0.74	1.12 (0.78 to 1.63)	0.54
Child visceral fat mass at 10 years model	0.01 (-0.05 to 0.06)	0.78	1.22 (0.82 to 1.81)	0.34
Child glucose concentrations model	0.02 (-0.04 to 0.08)	0.50	1.25 (0.86 to 1.81)	0.24

Values are regression coefficients (95% CIs) from linear regression models that reflect differences in liver fat fraction in SDS per maternal early-pregnancy glucose concentrations in mmol/l. Values are ORs (95% CIs) that reflect the risk of NAFLD at 10 years of age per maternal early-pregnancy glucose in mmol/l. Basic model: adjusted for child sex and age 10 years at outcome follow-up measurements. Main model: basic model additionally adjusted for maternal ethnicity, education, child physical activity. Maternal BMI model: main model additionally adjusted for maternal pre-pregnancy BMI. Maternal dyslipidemia model: main model additionally adjusted for maternal dyslipidemia in early pregnancy. Child metabolic markers at 6 years model: main model additionally adjusted for child insulin, total-cholesterol, LDL and HDL-cholesterol and triglycerides concentrations at 6 years of age. Child BMI model: main model additionally adjusted for child BMI at 10 years of age. Child visceral fat mass model: main model additionally adjusted for child MRI-measured visceral fat mass at 10 years of age. Child glucose concentrations model: main model additionally adjusted for child glucose concentrations at 10 years of age. NAFLD was defined as "yes" when liver fat ≥5.0% and as "no" when liver fat <5.0%. Abbreviations: BMI, Body Mass Index; NAFLD, non-alcoholic fatty liver disease; SDS, standard deviation score.

SUPPLEMENTARY TABLE S2.2.8. Sensitivity analyses on the associations between maternal early-pregnancy glucose with childhood liver fat fraction and non-alcoholic fatty liver disease among the European ancestry only group

	Liver Fat at School Age			
Maternal early-pregnancy glucose mmol/l	**Difference liver fat fraction SDS (95% Confidence Interval)**	***p* value**	**Odds ratio NAFLD yes/no (95% Confidence Interval)**	***p* value**
Women without pre-existing or gestational diabetes (n=1,184)				
Basic model	0.03 (-0.03 to 0.10)	0.29	1.95 (1.29 to 2.97)	<0.01
Main confounder model	0.03 (-0.03 to 0.09)	0.31	1.99 (1.30 to 3.05)	<0.01
Maternal body mass index model	0.01 (-0.05 to 0.07)	0.69	1.96 (1.27 to 3.01)	<0.01
Maternal dyslipidemia model	0.03 (-0.03 to 0.09)	0.36	1.98 (1.29 to 3.03)	<0.01
Child metabolic markers at 6 years model	0.03 (-0.03 to 0.09)	0.34	2.02 (1.30 to 3.12)	<0.01
Child body mass index model	0.02 (-0.04 to 0.07)	0.62	1.67 (1.02 to 2.72)	0.04
Child visceral fat mass at 10 years model	0.02 (-0.04 to 0.08)	0.51	1.91 (1.21 to 3.01)	<0.01
Child glucose concentrations model	0.03 (-0.03 to 0.09)	0.36	2.00 (1.30 to 3.07)	<0.01
Women included before 14 weeks gestation (n=1,037)				
Basic model	0.01 (-0.06 to 0.08)	0.76	1.81 (1.18 to 2.77)	<0.01
Main confounder model	0.01 (-0.06 to 0.07)	0.82	1.80 (1.17 to 2.76)	<0.01
Maternal body mass index model	-0.01 (-0.08 to 0.05)	0.69	1.72 (1.10 to 2.69)	0.02
Maternal dyslipidemia model	0.01 (-0.06 to 0.07)	0.85	1.86 (1.20 to 2.89)	<0.01
Child metabolic markers at 6 years model	0.01 (-0.06 to 0.07)	0.86	1.88 (1.20 to 2.95)	<0.01
Child body mass index model	-0.01 (-0.07 to 0.06)	0.87	1.58 (0.95 to 2.62)	0.08
Child visceral fat mass at 10 years model	-0.01 (-0.07 to 0.05)	0.71	1.70 (1.07 to 2.68)	0.03
Child glucose concentrations model	0.01 (-0.06 to 0.07)	0.86	1.82 (1.18 to 2.81)	<0.01

Values are regression coefficients (95% CIs) from linear regression models that reflect differences in liver fat fraction in SDS per maternal early-pregnancy glucose concentrations in mmol/l in mother-child pairs of European ancestry only. Values are ORs (95% CIs) that reflect the risk of NAFLD per maternal early-pregnancy glucose concentrations in mmol/l. Basic model: adjusted for child sex and age at outcome follow-up measurements. Main model: basic model additionally adjusted for maternal education, child physical activity. Maternal BMI model: main model additionally adjusted for maternal pre-pregnancy BMI. Maternal dyslipidemia model: main model additionally adjusted for maternal dyslipidemia in early pregnancy. Child metabolic markers at 6 years model: main model additionally adjusted for child insulin, total-cholesterol, LDL and HDL-cholesterol and triglycerides concentrations at 6 years of age. Child BMI model: main model additionally adjusted for child BMI at 10 years of age. Child visceral fat mass model: main model additionally adjusted for child MRI-measured visceral fat mass at 10 years of age. Child glucose concentrations model: main model additionally adjusted for child glucose concentrations at 10 years of age. NAFLD was defined as "yes" when liver fat ≥5.0% and as "no" when liver fat <5.0%. Abbreviations: BMI, Body Mass Index; NAFLD, non-alcoholic fatty liver disease; SDS, standard deviation score.

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2.3

Maternal early-pregnancy glucose concentrations and alterations in cardiac structure and function in childhood

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ABSTRACT

Backgroud and aims: Gestational diabetes mellitus has been associated with offspring cardiac congenital malformations, ventricular hypertrophy, and diastolic dysfunction in large observational cohort studies and experimental animal models. We assessed the associations of maternal random glucose concentrations across the full range with childhood cardiac ventricular structure and function.

Methods: In a population-based prospective cohort among 1,959 women and their offspring, maternal random glucose concentrations were measured at a median 13.1 weeks' gestation (95% range 10.5-16.8 weeks). We obtained offspring cardiac outcomes, relative to body size, through cardiac MRI at 10 years.

Results: The mean maternal random glucose concentration was 4.4 mmol/L (SD 0.8). The highest quintile of maternal glucose concentrations, compared with the lowest quintile, was associated with a lower childhood left ventricular mass (-0.19 SD score (SDS), 95% CI -0.31 to -0.07) and left ventricular end-diastolic volume (-0.17 SDS, 95% -0.28 to -0.05). Also, higher maternal glucose concentrations across the full range per 1 mmol/L increase were associated with a lower childhood left ventricular mass and left ventricular end-diastolic volume (P values ≤0.05). Adjustment for maternal prepregnancy BMI, gestational age, and weight at birth or childhood BMI and blood pressure did not influence the effect estimates. Maternal glucose concentrations were not significantly associated with childhood right ventricular end-diastolic volume or left and right ventricular ejection fraction.

Conclusions: Higher maternal random glucose concentrations in the first half of pregnancy are associated with a lower childhood left ventricular mass and left ventricular end-diastolic volume, with the strongest associations for childhood left ventricular mass. These associations were not explained by maternal, birth, or childhood characteristics. Further studies are needed to replicate these findings using repeated maternal glucose measurements throughout pregnancy and offspring cardiac outcomes throughout childhood and adulthood.

INTRODUCTION

Diabetes in pregnancy is an important risk factor for congenital heart disease (1). Children exposed to both maternal pregestational and gestational diabetes mellitus have increased risks of defects in cardiogenesis, including septal defects and hypoplastic left heart syndrome (2). Several studies have reported that pregestational and gestational diabetes mellitus are also associated with subclinical cardiac changes in fetal and infant life, including a higher ventricular mass and lower ventricular diastolic function (3-5). Studies among pregnant women without diabetes observed that offspring exposed to higher gestational glucose concentrations, but below diagnostic thresholds of gestational diabetes mellitus, had an increased risk of cardiac structural defects (6, 7). These observations, together with findings from studies among diabetic and nondiabetic animals, suggest that maternal hyperglycemia may have a direct effect on fetal cardiac development (8-10). The human heart is the first functional organ to develop, and development already starts in the early embryonic stage. Already from early pregnancy onward, higher maternal glucose concentrationsmay influence growth and proliferation of fetal cardiomyocytes and subsequently affect myocardial structure and function (5, 10-13). Thus far, it remains unknown whether these cardiac alterations in early life, in response to higher maternal gestational glucose concentrations, also have consequences for offspring cardiac development in later life. We hypothesized that higher maternal glucose concentrations from early pregnancy onward are associated with persistent offspring cardiac adaptations, including altered left and right ventricular dimensions and a lower ventricular function in childhood. We especially expected the right ventricle, as the dominant ventricle in fetal life, to be affected. We examined, in a population-based prospective cohort study among 1,959 mothers and their children, the associations of maternal random glucose concentrations in the first half of pregnancy with childhood left and right ventricular structure and function measured by cardiac MRI at 10 years.

METHODS

Study design and study sample

This study was embedded in the Generation R Study, a population-based prospective cohort study in Rotterdam, the Netherlands (14). Approval was obtained from the local Medical Ethical Committee (Erasmus University Medical Center, Rotterdam, the Netherlands). Written consent was obtained from participants' parents. The study enrolled 7,145 pregnantwomen,18 weeks' gestation, of whom 6,099 had singleton

pregnancies and glucose measurements available, of which 3,811 had offspring with follow-up visits at 10 years (**Supplementary Figure S2.3.1**). As a result of later implementation of MRI scanswithin follow-up visits, a subgroup of 2,294 children was scanned, of which 1,965 had good-quality cardiac MRI measurements. After exclusion of children with cardiac abnormalities in their medical history (n=6), our population for analysis consisted of 1,959 mothers and children.

Maternal random glucose and insulin concentrations

Nonfasting random venous blood samples were collected once at 13.1 weeks' gestation (95% range 10.5 to 16.8) by research nurses and briefly stored at room temperature and subsequent temporarily stored on ice. Blood samples were obtained after at least 30 min of fasting, because of which we considered the samples as random nonfasting samples. Blood was collected in EDTA tubes, and processing was aimed to finish within a maximum of 3 h after sampling. Glucose (mmol/L) was measured with c702 module on the Cobas 8000 analyzer. Insulin (pmol/L) was measured with electrochemiluminescence immunoassay on the Cobas e411 analyzer. We considered maternal glucose concentrations as our main exposure. As a secondary exposure, we used maternal insulin concentrations as another marker of maternal glucose metabolism and for its potential additional effect on offspring cardiac development through other alterations in maternal metabolism as a consequence of insulin insensitivity and altered placental development (15, 16).

Cardiac measurements

At 10 years, we performed childhood cardiac MRI using a wide-bore GE Discovery MR 750 3T scanner (General Electric, Milwaukee, MI) (17). We included left ventricular mass, left and right ventricular end-diastolic volume, and left and right ventricular ejection fraction as outcomes, based on our hypothesis that higher maternal glucose concentrations directly affect embryonic and fetal cardiomyocyte development and proliferation leading to alterations in right and left ventricular structure and function in later life (9). Histograms of outcomes are shown in Supplementary Materials 1. Because cardiac outcomes are strongly dependent on childhood size, we corrected all cardiac outcomes measures for body surface area (BSA), leading to normally distributed BSA-corrected outcomes (18). To further enable comparison of effect sizes for associations of maternal glucose metabolism with childhood left and right ventricular outcomes, we constructed SDscores (SDS) of outcomes (details in **Supplementary Materials S2.3.1**). As a secondary outcome and as a potential mediator, we measured childhood blood pressure at 10 years.

Covariates

Information on maternal age, ethnicity, educational level, parity, prepregnancy weight, folic acid supplement use, alcohol consumption, smoking, total caloric intake, nausea and vomiting, and diagnosis of type 1 or 2 diabetes before pregnancy was obtained through participants' questionnaires (14). Maternal height was measured at intake without shoes. BMI was calculated. Information on diagnosis of gestational diabetes mellitus, gestational hypertensive disorders, and the child's sex, gestational age, and weight at birth was obtained from medical records (14). Gestational diabetes mellitus was diagnosed by a community midwife or an obstetrician according to Dutch midwifery and obstetric guidelines using the following criteria: a random glucose level >11.0 mmol/L, a fasting glucose ≥7.0 mmol/L, or a fasting glucose between 6.1 and 6.9 mmol/L with a subsequent abnormal glucose tolerance test result. In clinical practice and for this study sample, an abnormal glucose tolerance test result was defined as 1-h postprandial glucose concentration .7.8 mmol/L after an oral glucose load of 75 g. Screening for gestational diabetes mellitus was conducted in women with one or more of the following risk factors according to Dutch midwifery and obstetric guidelines: gestational diabetes mellitus in a previous pregnancy, BMI >30 kg/m^2 at the first prenatal visit, macrosomia or large-for-gestational-age newborn in a previous pregnancy, first-degree relative with diabetes, high-risk ethnicities, unexplained intrauterine death in a previous pregnancy, or polycystic ovarian syndrome. We further refer to pregestational and gestational diabetes mellitus as (pre)gestational diabetes mellitus. At 10 years, we measured the child's BMI (14). This measurement preceded the cardiac MRI by a median of 1.1 month (95% range 0.0 to 2.2).

Statistical analyses

First, we performed a nonresponse analysis to compare characteristics of mothers and children with cardiac MRI measurements to those without. Second, we assessed associations of maternal random glucose concentrations with childhood cardiac outcomes. Based on previous studies showing linear associations of higher maternal glucose concentrations with perinatal complications, childhood BMI, and glucose metabolism, we hypothesized a linear tendency for associations of maternal glucose concentrations with offspring cardiac outcomes (19-22). We first categorized maternal glucose concentrations into quintiles, based on the distribution of maternal glucose concentrations, to assess whether associations were restricted to women with relatively high glucose concentrations and to explore linearity. We plotted unadjusted means of childhood cardiac outcomes per maternal glucose concentration quintile and examined the associations of the higher maternal glucose concentrations quintiles with childhood cardiac outcomes, compared with the lowest quintile, using multiple linear regression

models. Next, we assessed the associations of maternal glucose concentrations continuously per 1 mmol/L increase with childhood cardiac outcomes to explore the continuous associations across the full range of maternal glucose concentrations, which is not fully captured by the quintile analyses. We assessed linearity by visualizing the data by categorizing maternal glucose concentrations into deciles and by testing for quadratic terms for maternal glucose concentrations within our models. We also estimated cubic spline models, with additional boundary knots at the 5th and 95th percentile, and assessed whether this significantly improved model fit. These analyses showed the linear model was the best fit for the data. Linear regression model assumptions were fulfilled. We constructed five different models, based on a directed acyclic graph analysis (**Supplementary Materials S2.3.2**): 1. basic model adjusted for gestational age at blood sampling, child's age and sex, and time difference between the BSA and MRI measurement; 2. confounder model, as main model: basic model additionally adjusted for maternal ethnicity, educational level, folic acid supplement use, alcohol consumption, smoking, and gestational hypertensive disorders; 3. maternal BMI model: confounder model additionally adjusted for maternal prepregnancy BMI; 4. birth model: maternal BMI model additionally adjusted for the child's gestational age and weight at birth; and 5. child model: birth model additionally adjusted for childhood concurrent BMI and blood pressure. We tested statistical interaction terms for maternal prepregnancy BMI, fetal sex, and child's concurrent BMI, but none were significant. Additionally, we repeated all analyses using maternal random insulin concentrations as exposure. To enable comparison of effect sizes for the associations of different measures of maternal glucose metabolism with childhood cardiac outcomes, these analyses were performed using maternal random glucose and insulin concentrations in SDS. We log-transformed maternal insulin concentrations before the construction of the SDS given its skewed distribution. To assess robustness of findings, we performed several sensitivity analyses: 1) excluding women with (pre)gestational diabetes mellitus to assess the associations of maternal glucose concentrations within a population without diabetes; 2) excluding women with the diagnosis of gestational hypertensive disorders; and 3) excluding women with glucose concentrations sampled at >14 weeks' gestation to assess the associations of first trimester maternal glucose concentrations with offspring cardiac development. Multiple imputations of covariates (pooled results of five imputed data sets) were performed. All analyses were performed using SPSS 24.0 for Windows software (IBM, Armonk, NY), except for spline analyses, which were conducted in R 3.6.3 software with the splines package.

RESULTS

Subject characteristics

Table 2.3.1 reports that the mean maternal random glucose concentration was 4.4 mmol/L (SD 0.8). The glucose concentration in 0.3% of women was ≥7.8 mmol/L. Population characteristics according to quintiles of maternal glucose concentrations are provided in **Supplementary Table S2.3.1**. Nonresponse analysis showed that mothers of children with cardiac MRI measurements at 10 years, compared with those without, had slightly higher glucose concentrations (**Supplementary Table S2.3.2**).

Maternal glycemia and childhood cardiac function and structure

Figure 2.3.1 shows that the unadjusted means of childhood left ventricular mass and that the left and right ventricular end-diastolic volumes were lowest in the highest maternal glucose concentrations quintile. In the confounder models, compared with children from mothers with glucose concentrations in the lowest quintile, children from mothers with glucose concentrations in the highest quintile had a lower left ventricular mass (20.19 SDS, 95% CI 20.31 to 20.07) and a lower left ventricular end-diastolic volume (20.17 SDS, 95% CI 20.28 to 20.05). A similar nonsignificant tendency was present for childhood right ventricular end-diastolic volume. No consistent associations of maternal glucose concentrations quintiles with left and right ventricular ejection fraction were present. In the basic models, higher maternal glucose concentrations across the full range were associated with a lower childhood left ventricular mass, left ventricular end-diastolic volume, and right ventricular end-diastolic volume (all P values ≤0.05) (**Table 2.3.2**). In the confounder models, higher maternal glucose concentrations were associated with a lower childhood left ventricular mass and lower left ventricular end-diastolic volume only (20.06 SDS (95% CI 20.10 to 20.01) and 20.04 SDS (95% CI 20.09 to 0.00), per 1 mmol/L increase in maternal glucose concentration, respectively). Adjustment for maternal prepregnancy BMI and child's gestational age and weight at birth and childhood BMI and blood pressure did not materially change these effect estimates. There was a nonsignificant tendency for an association of higher maternal glucose concentrations with a lower childhood right ventricular ejection fraction. Higher maternal glucose concentrations were not associated with childhood blood pressure, which we considered as a potential mediator of the associations of maternal glucose concentrations with childhood cardiac outcomes (**Supplementary Table S2.3.3**).

TABLE 2.3.1. Characteristics of the study population

	Total group (n=1959)
Maternal Characteristics	
Age at enrolment, mean (SD), years	30.9 (4.6)
Gestational age at intake, median (95%), weeks	13.1 (10.5 to 16.8)
Prepregnancy body mass index, median (95%), kg/m^2	22.5 (18.7to 31.6)
Gestational weight gain, mean (SD), kg	15.1 (5.8)
Parity, n nulliparous (%)	1190 (60.7)
Ethnicity, n Dutch or European (%)*	1306 (66.7)
Education level, n high (%)	1011 (51.6)
Income, n high (%)	1374 (70.1)
Smoking during pregnancy, n yes (%)	418 (21.3)
Alcohol consumption during pregnancy, n yes (%)	1063 (54.3)
Folic acid supplement use, n yes (%)	1262 (64.4)
Glucose, mean (SD), mmol/l	4.4 (0.8)
First quintile, mean (SD), mmol/l	3.4 (0.4)
Second quintile, mean (SD), mmol/l	4 (0.1)
Third quintile, mean (SD), mmol/l	4.3 (0.1)
Fourth quintile, mean (SD), mmol/l	4.8 (0.1)
Fifth quintile, mean (SD), mmol/l	5.7 (0.6)
Insulin, median (95%), pmol/l	113.0 (24.4 to 502.8)
First quintile, median (95% range), pmol/l	31.8 (16.4 to 44.4)
Second quintile, median (95% range), pmol/l	64.4 (47.8 to 82.9)
Third quintile, median (95% range), pmol/l	113.1 (86.7 to 145.0)
Fourth quintile, median (95% range), pmol/l	195.9 (152.0 to 251.4)
Fifth quintile, median (95% range), pmol/l	373.1 (266.9 to 887.6)
(Pre)Gestational diabetes, n (%)	11 (0.6)
Gestational hypertensive disorders, n (%)	131 (6.7)
Birth characteristics	
Sex, n female (%)	1028 (52.5)
Birthweight, mean (SD), grams	3448 (546)
Gestational age at birth, median (95%), weeks	40.3 (37.1 to 42.1)
Child Characteristics at 10 years	
Age, mean (SD), years	9.9 (9.5 to 11.6)
Height, mean (SD), cm	141.6 (6.7)
Weight, median (95% range), kg	33.8 (26.4 to 48.8)
Body mass index, median (95%), kg/m^2	16.9 (14.4 to 22.9)
Body surface area, median (95%), m^2	1.1 (1.0 to 1.4)

TABLE 2.3.1. Continued

	Total group (n=1959)
Blood pressure	
Systolic, mean (SD), mmHg	103.1 (7.9)
Diastolic, mean (SD), mmHg	58.5 (6.4)
Cardiac MRI measures	
Left ventricular mass, median (95%), gram	47.5 (34.5 to 67.9)
Left ventricular end-diastolic-volume, median (95%), ml	98.7 (73.7 to 132.7)
Left ventricular ejection fraction, mean (SD), %	58.4 (4.6)
Right ventricular end-diastolic-volume, median (95%), ml	98.2 (71.3 to 134.8)
Right ventricular ejection fraction, mean (SD), %	58.2 (4.9)

For normal distributed data, the mean with standard deviation is stated. For non-normally distributed data, the median with 95% range is stated. SD: Standard Deviation

Number of missings per covariate: maternal ethnicity n=24 (1.2%), maternal educational level n=82 (4.2%), folic acid supplement use during pregnancy n=432 (22.1%), alcohol consumption n=216 (11.0%), smoking during pregnancy n=197 (10.1%), gestational hypertensive disorders n=33 (1.7%).

**Maternal ethnicities within our the study population included Dutch n=1148 (58.6%), European n=158 (8.1%), Surinamese n=154 (7.9%), Turkish n=102 (5.2%), Moroccan n=85 (4.3%), Cape Verdian n=84 (4.3%), Indonesian n=70 (3.6%), Asian n=41 (2.1%), Dutch Antilles n=35 (1.8%), American n=24 (1.2%) and other ethnicities (all <1% per ethnicity)*

Additional analyses

Higher maternal insulin concentrations were associated with lower childhood left and right ventricular end-diastolic volume (all P values ≤0.05), but not with left ventricular mass and left and right ventricular ejection fraction (**Supplementary Table S2.3.4**). Strength of these associations was comparable to maternal glucose concentrations. Excluding mothers with (pre)gestational diabetes mellitus or gestational hypertensive disorders had no effect on the associations (**Supplementary Table S2.3.5**). When we repeated the analyses among women with glucose concentrations <14 weeks' gestation available, effect estimates for the association with childhood left ventricular mass were similar but borderline significant, effect estimates for the associations with childhood left ventricular end-diastolic volume were in similar direction but attenuated toward nonsignificant, and the effect estimates for the association with childhood right ventricular ejection fraction was in similar direction and slightly stronger (**Supplementary Table S2.3.5**).

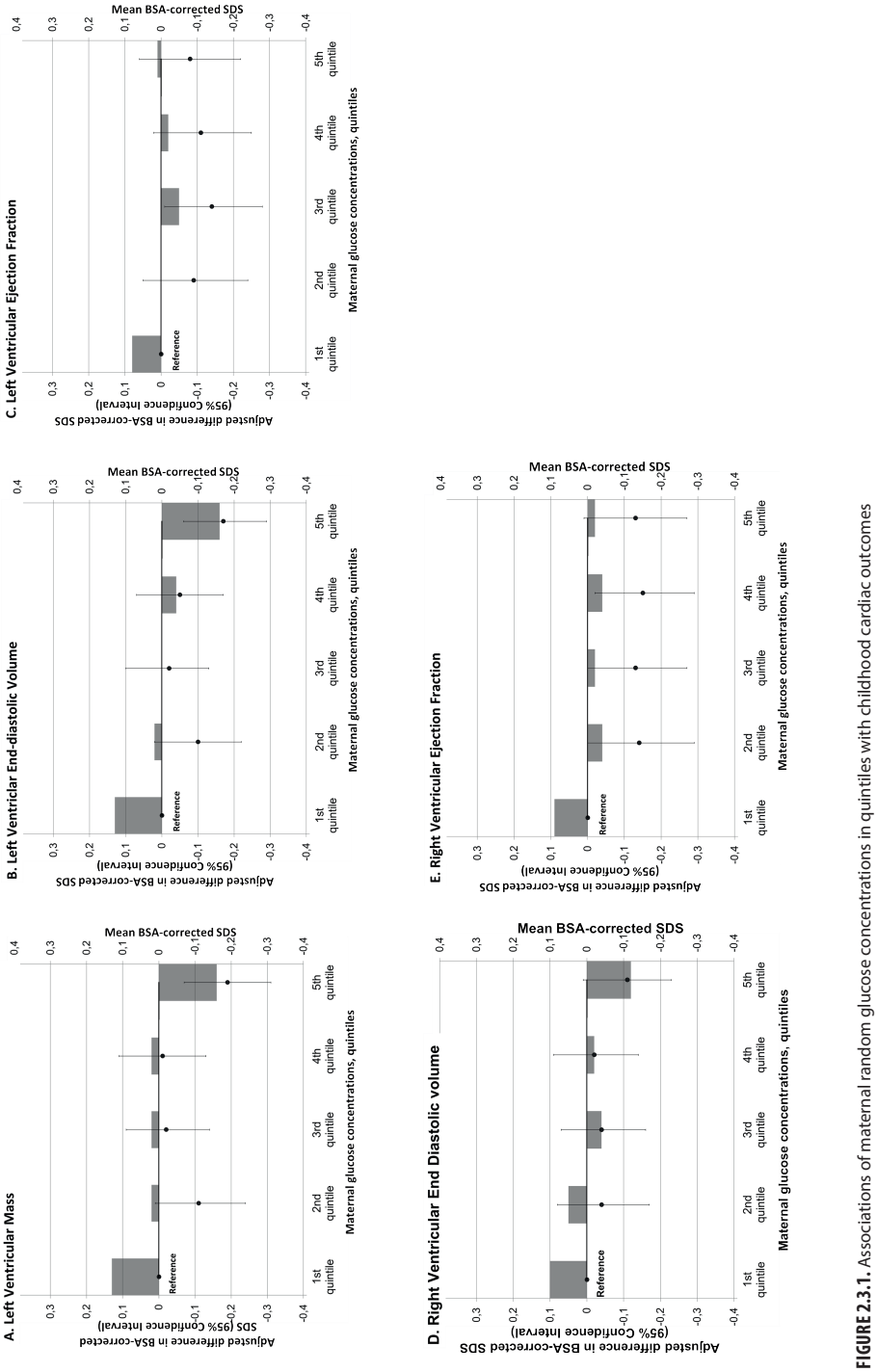


FIGURE 2.3.1. Associations of maternal random glucose concentrations in quintiles with childhood cardiac outcomes

TABLE 2.3.2. Maternal random glucose concentrations across the full range and childhood cardiac outcomes at 10 years of age

Model	Left ventricular Mass (SDS)	p-value	Left ventricular end-diastolic-volume (SDS)	p-value	Left ventricular ejection fraction (SDS)	p-value	Right ventricular end-diastolic-volume (SDS)	p-value	Right ventricular ejection fraction (SDS)	p-value
Maternal glucose (mmol/l)										
Basic model[a]	-0.06 (-0.11 to -0.02)	0.01	-0.06 (-0.10 to -0.01)	0.02	-0.04 (-0.09 to 0.02)	0.16	-0.05 (-0.09 to 0.00)	0.05	-0.05 (-0.10 to 0.01)	0.09
Confounder model[b]	-0.06 (-0.10 to -0.01)	0.02	-0.05 (-0.09 to 0.00)	0.05	-0.05 (-0.10 to 0.01)	0.15	-0.04 (-0.08 to 0.01)	0.13	-0.05 (-0.10 to 0.01)	0.10
Maternal BMI model[c]	-0.05 (-0.10 to -0.01)	0.03	-0.04 (-0.09 to 0.00)	0.07	-0.05 (-0.10 to 0.01)	0.08	-0.03 (-0.08 to 0.02)	0.19	-0.06 (-0.11 to 0.00)	0.04
Birth model[d]	-0.05 (-0.10 to -0.01)	0.02	-0.05 (-0.09 to 0.00)	0.04	-0.05 (-0.10 to 0.01)	0.09	-0.04 (-0.08 to 0.01)	0.12	-0.05 (-0.11 to 0.00)	0.05
Child model[e]	-0.05 (-0.10 to 0.00)	0.04	-0.04 (-0.09 to 0.00)	0.06	-0.04 (-0.10 to 0.01)	0.12	-0.03 (-0.08 to 0.02)	0.19	-0.05 (-0.11 to 0.00)	0.05

Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood cardiac outcomes in SDS per 1 mmol/l increase in maternal random glucose concentrations. SDS: standard deviation score, BMI: Body Mass Index

[a]*Basic model is adjusted for gestational age at enrolment, child's age and sex at follow up measurements and time difference between measurement of child's body surface area and cardiac MRI*

[b]*Confounder model is the basic model additionally adjusted for maternal ethnicity, maternal educational level, folic acid supplement use during pregnancy, alcohol consumption and smoking during pregnancy, gestational hypertensive disorders*

[c]*Maternal BMI model is the confounder model additionally adjusted for maternal prepregnancy body mass index*

[d]*Birth model is the maternal BMI model additionally adjusted for gestational age and weight at birth*

[e]*Child model is the birth model additionally adjusted for child's body mass index and blood pressure at 10 years of age*

Black circles represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood cardiac outcomes in Standard Deviation Score (SDS) for maternal glucose concentrations in quintiles compared to the lowest quintile (left y-axis). Regression models were adjusted for gestational age at enrolment, child's age and sex at follow up measurements and time difference between measurement of child's BSA and cardiac MRI, maternal ethnicity, educational level, folic acid supplement use during pregnancy, alcohol consumption and smoking during pregnancy, gestational hypertensive disorders. Bars represent the unadjusted means of Body Surface Area-corrected Standard Deviation Scores of childhood cardiac outcomes (right y-axis). Mean (SD) per quintile of maternal glucose concentrations in mmol/l are 3.4 (0.4) for quintile 1, 4.0 (0.1) for quintile 2, 4.3 (0.1) for quintile 3, 4.8 (0.1) for quintile 4 and 5.7 (0.6) for quintile 5. Median (95% range) per quintile of maternal glucose concentrations in mmol/l are 3.6 (2.6 to 3.8) for quintile 1, 4.0 (3.9 to 4.1) for quintile 2, 4.3 (4.2 to 4.5) for quintile 3, 4.8 (4.6 to 5.0) for quintile 4 and 5.5 (5.1 to 6.8) for quintile 5.

DISCUSSION

In a population without diabetes, higher maternal random glucose concentrations in the first half of pregnancy, especially maternal glucose concentrations within the highest quintile, were associated with a lower childhood left ventricular mass and left ventricular end-diastolic volume. The strongest association was present for childhood left ventricular mass. These associations were not explained by maternal, birth, and childhood characteristics.

Interpretation of main findings

Maternal hyperglycemia during pregnancy seems associated with an altered fetal cardiac development. (Pre)gestational diabetes mellitus is associated with congenital heart defects and with alterations in fetal cardiac structure and function within the normal range of cardiac development (1-7, 12, 23). Studies among women with diabetes measuring fetal ventricular mass, ventricular filling velocities (early [E]-to-late [A] ratio), and isovolumetric relaxation time, showed a higher left ventricular mass and lower left and right ventricular diastolic function in fetuses of mothers with poor glycemic control compared with mothers with good glycemic control (3, 23). Studies among newborns of mothers with diabetes showed similar results (4, 24). One small study suggested that pathologic ventricular hypertrophy in newborns of mothers with diabetes normalized within the first 6 months of life (25). Associations may not be limited to populations with diabetes only. A study showed that in 277 pregnant women without diabetes, offspring exposed to higher random glucose concentrations in midpregnancy had a higher

risk of tetralogy of Fallot but not of transposition of the great arteries (7). Similarly, a study among 19,171 pregnant women without diabetes showed that higher maternal glucose concentrations and higher postprandial glucose concentrations after an oral glucose tolerance test were associated with increased risks of any offspring congenital heart defect (6).

These findings, together with those from animal studies, suggest that maternal gestational glucose concentrations are an important factor influencing cardiogenesis (11). It is likely that women who develop gestational diabetes mellitus or hyperglycemia later in pregnancy already have a suboptimal glucose metabolism in the first half of pregnancy (26). As embryonic and fetal cardiac development starts in the 1st weeks after conception, this may be a critical period for potential adverse effects of a suboptimal maternal glucose metabolism on embryonic and fetal cardiac development (11). We observed that higher maternal random glucose concentrations, especially those within the highest quintile, were associated with a lower childhood left ventricular mass and left ventricular end-diastolic volume. These findings were already observed in a population of relatively lean women with random glucose concentrations largely within the normal range.

The association of higher maternal glucose concentrations with a lower childhood left ventricular end-diastolic volume is in line with studies among populations with diabetes focused on fetal cardiac adaptations. Ventricular end-diastolic volume defines the ventricular ability to fill during the diastolic phase. Filling during the diastolic phase could be affected by increased stiffness and decreased relaxation of the ventricles caused by structural changes in the myocardium (27, 28). Animal studies have shown a decreased number of cardiomyocytes and transient hypertrophy after exposure to higher maternal glucose concentrations. Ventricular end-diastolic volume is one of the main determinants of stroke volume, and to maintain an adequate stroke volume, a lower ventricular end-diastolic volume may need to be compensated in the systolic phase, leading to an altered systolic function in the long-term (29). Nonsignificant tendencies were found for associations of higher maternal glucose concentrations with lower childhood ventricular ejection fraction, a measure of ventricular systolic function. Associations with ejection fraction may become more apparent at older ages. Thus, our findings suggest that higher maternal random glucose concentrations, already within the normal range, are associated with childhood ventricular diastolic function and filling capacity.

In contrast to findings of studies in fetal and infant life, we observed that higher maternal random glucose concentrations, especially those within the highest quintile, were

associated with a lower childhood left ventricular mass. Effect estimates were consistent among all sensitivity analyses, but became borderline significant among women with first trimester glucose concentrations available. This is most likely due to smaller sample size. These findings are in line with observations in experimental animal models which show that the influence of maternal glycemia on cardiac development may be different in childhood and adulthood than in fetal life and infancy (8, 10). Higher fetal glucose and insulin concentrations, in response to maternal glycemia, may decrease the number of cardiomyocytes but simultaneously accelerate individual cardiomyocyte growth (8-10). This may result in ventricular hypertrophy in fetal and early postnatal life, due to accelerated growth of individual cardiomyocytes, but a decreased ventricular mass in later life resulting from the reduction in cardiomyocyte number (10). When our results are compared with those from previous studies, differences in study design and populations should be considered, because most previous studies were conducted in pregnant women with diabetes and focused on offspring left ventricular mass during fetal and early postnatal life (3, 4). Thus, our study shows for the first time that in a population without diabetes, higher maternal glucose concentrations within the normal range are associated with a lower childhood left ventricular mass.

Contrary to our prior hypothesis that the right ventricle might be more affected by intrauterine exposure to higher maternal glucose concentrations, we mainly observed associations with offspring left cardiac outcomes. We observed a tendency for associations of higher maternal glucose concentrations with a lower childhood right ventricular ejection fraction, which were slightly stronger in the sensitivity analysis with maternal glucose concentrations <14 weeks' gestation available. This could suggest that maternal glucose metabolism impacts embryonic right ventricular development. During fetal transition to extrauterine life, major adaptations in the cardiovascular system occur, and the afterload for the left ventricle increases strongly compared with the right ventricle. As a result of the higher workload of the left ventricle during postnatal life, alterations in the left ventricle in response to a suboptimal maternal glucose metabolism may be more pronounced in childhood. We observed the strongest association with childhood left ventricular mass. Similarly, the associations of higher maternal glucose concentrations might be stronger with offspring right ventricular mass than with right ventricular end-diastolic volume or ejection fraction. At 10 years, right ventricular mass cannot be measured accurately with MRI because the right ventricular wall is too thin and is prone to measurement error (29). Studies among offspring at older ages should evaluate whether maternal glycemia is associated with right ventricular mass and should compare the strength of associations of maternal glycemia with right and left ventricular outcomes. Furthermore, studies using detailed measurements of embryonic

and fetal cardiac development, including advanced ultrasound techniques, can be used to provide insight into critical periods of maternal glucose metabolism on right and left embryonic and fetal cardiac development.

We used maternal insulin concentrations as an additional measure of maternal glucose metabolism. We observed that higher maternal insulin concentrations were also associated with a lower childhood left ventricular end-diastolic volume, with a similar strength as maternal glucose concentrations but not with childhood left ventricular mass. Higher maternal insulin concentrations were also associated with lower childhood right ventricular end-diastolic volume. On the basis of experimental animal models, it seems that primarily maternal glucose concentrations would directly affect embryonic and fetal cardiomyocyte development (3). Maternal insulin does not cross the placenta but does affect maternal metabolism and placental development, which may also indirectly influence offspring cardiac development through, for example, alterations in fetal-placental blood flow patterns (15, 16). Experimental studies need to distinguish whether maternal insulin concentrations have a potential indirect effect, in addition to the effect of maternal glucose concentrations, on embryonic and fetal cardiac development.

Effect estimates for the associations of maternal random glucose concentrations with a lower childhood left ventricular mass and left ventricular end-diastolic volume were small but important from a cardiovascular developmental perspective. Our findings add to evidence suggesting that maternal glucose metabolism during pregnancy may directly affect offspring cardiac development, because associations were not explained by maternal, birth, or childhood characteristics. Few studies have focused on how childhood cardiac development relates to adult cardiac structure and function and cardiovascular morbidity. Our findings are in line with previous studies showing associations of fetal growth restriction and small-size-for-gestational age at birth with lower childhood left ventricular mass and left ventricular end-diastolic volume (17, 30). Fetal growth restriction and small-size-for-gestational-age at birth are risk factors for cardiovascular diseases in adulthood, which may suggest that these childhood cardiac alterations may be related to adverse cardiac health outcomes in later life (31, 32). Left ventricular mass tracks throughout childhood into adulthood, but whether left ventricular end-diastolic volume tracks into adulthood remains unknown (33, 34). Studies among adult populations have shown that increased left ventricular mass is associated with a higher risk of cardiovascular morbidity, but whether a lower left ventricular mass is related to cardiovascular diseases is unclear (35). Among adult populations, both an increased and a reduced left ventricular end-diastolic volume are associated with a higher risk of cardiovascular morbidity, even with preserved ejection

fraction, but these associations have not been assessed from childhood onward (36, 37). Further studies need to replicate our findings and assess the longterm implications for offspring cardiac development. Intervention studies are needed to obtain further insight into the causality of these observed associations and the possibilities to improve offspring cardiovascular health by optimizing maternal glucose status during pregnancy.

Methodological considerations

We had a prospective design with data collection from early pregnancy onward, including detailed measurements of childhood cardiac development using cardiac MRI scans. The number of cases of (pre)gestational diabetes mellitus and offspring cardiac abnormalities was relatively low. Women with pregestational diabetes mellitus may have been reluctant to participate, and children with cardiac abnormalities could be lost to follow-up, which may have led to selection bias. However, because we aimed to assess associations of maternal glycemia with offspring cardiac alterations within the normal range, it is unlikely that this biased results. We obtained information on the diagnosis of gestational diabetes mellitus from medical records. Glucose testing for the diagnosis of gestational diabetes mellitus was not performed in all women for study purposes, which may have led to misclassification. The nonresponse analysis, low number of cases, and relatively lean population suggests a selection to a relatively healthy population, which may affect the generalizability of our findings. Studies among higher-risk populations, such as women with obesity or with a suboptimal glucose metabolism, need to replicate findings. We obtained random maternal glucose concentrations once during pregnancy at nonfixed times throughout the day. Owing to our study design, we were not able to collect repeated fasting blood samples. Glucose concentrations throughout the day are influenced by multiple factors, such as dietary intake and exercise, and although blood samples were stored on ice for a maximum of 3 h, blood glucose concentrations may decline in EDTA tubes. These factors may have led to nondifferential misclassification, causing an underestimation of our associations. However, previous studies, including studies from our cohort, showed that random maternal gestational glucose concentrations are related to the risks of gestational diabetes mellitus, adverse birth outcomes, childhood obesity, and altered glucose metabolism (22, 38-40). These associations were in a similar direction as those for maternal fasting and postprandial glucose concentrations with these adverse outcomes (19, 20). Timing of sampling of maternal glucose concentrations in our study is relatively broad, but <18 weeks' gestation, covering the first half of pregnancy. This classification was aligned with the logistics of the study. Further studies need to replicate our findings using repeated maternal fasting and postprandial glucose measurements. These

studies should already measure glucose before pregnancy, because maternal glycemia in the first half of pregnancy is likely to reflect maternal glucose before conception, and repeatedly throughout pregnancy from the first trimester onward, to identify critical periods of maternal glycemia for offspring cardiac development. Information on multiple maternal and childhood characteristics was available, but residual confounding may have influenced results.

CONCLUSION

Higher maternal random glucose concentrations in the first half of pregnancy, already within the normal range, were associated with a lower childhood left ventricular mass and lower left ventricular end-diastolic volume at 10 years. The strongest association was present for childhood left ventricular mass. Maternal, birth, and childhood characteristics did not explain these associations. Further studies are needed to replicate our findings and to assess the long-term associations of maternal glucose metabolism with offspring cardiac.

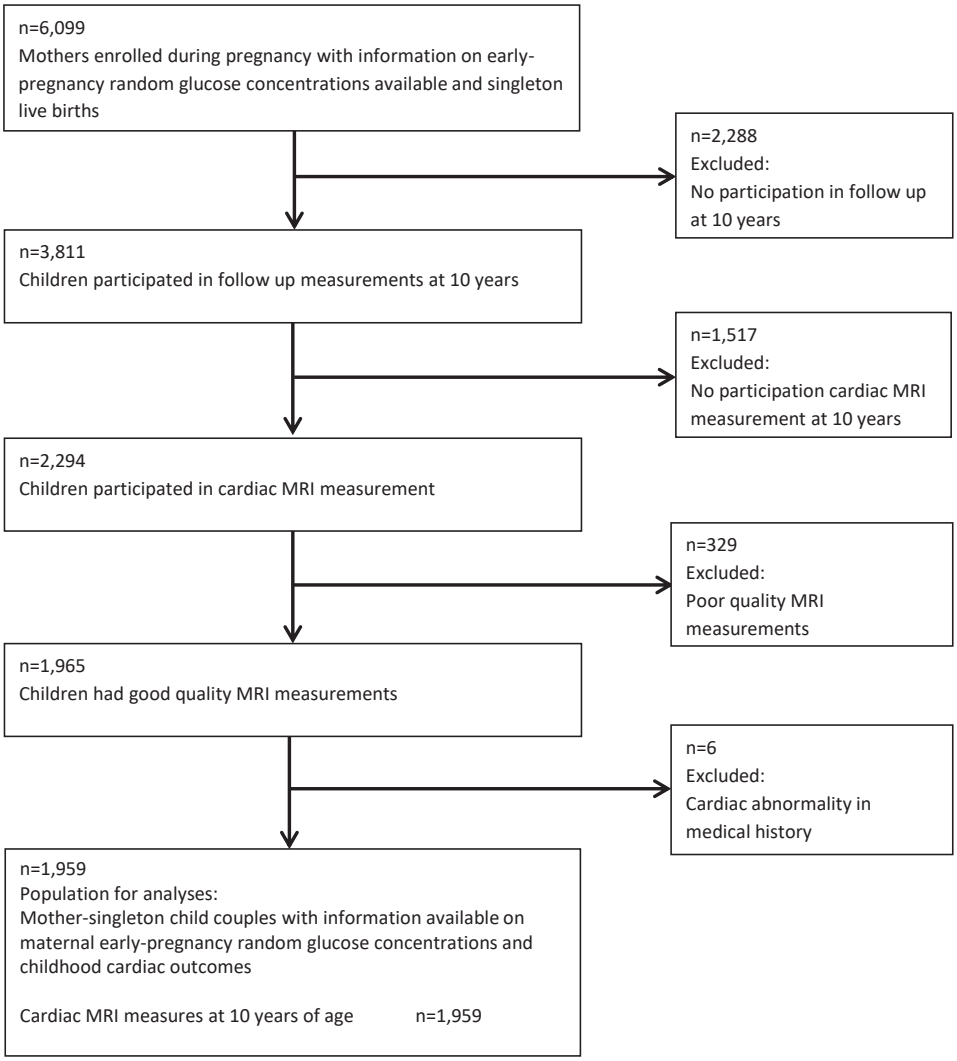
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SUPPLEMENTARY MATERIAL

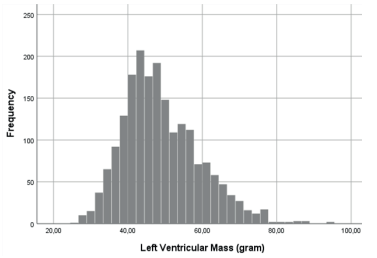


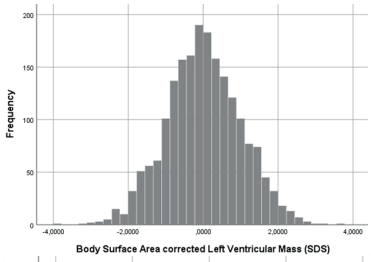
SUPPLEMENTARY FIGURE S2.3.1. Flow chart of the study participants

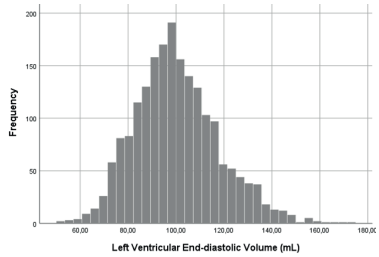
Supplementary Materials S2.3.1. Childhood cardiac MRI measurements and correction for body surface area

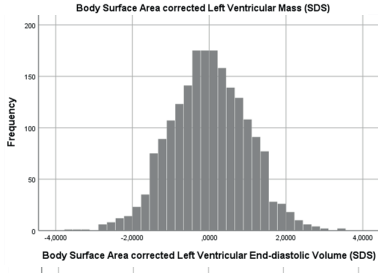
The histograms of the absolute cardiac outcomes are shown below. Childhood left and right ventricular ejection fraction had a normal distribution, and left ventricular mass and left and right end-diastolic volume showed a more skewed distribution, as shown in the histograms below. As childhood cardiac outcomes are strongly dependent on childhood size, we corrected all childhood cardiac outcomes for body surface area (BSA) (1). We used Generalized Additive Models for Location, Size and Shape (GAMLSS) in R, version 3.2.0 (R Core Team, Vienna, Austria) (2). These models enable flexible modelling, taking into account the distribution of the outcome variable(3). Worm plots and Akaike Information Criterion were used in sensitivity analyses to obtain the best model fit. Weight and length were measured at the research center. BSA was computed using the Haycock formula (BSA (m2) = 0.024265 × weight (kg)0.5378 × height (cm)0.396. To enable comparison of our effect sizes for associations of maternal glucose metabolism with childhood left and right ventricular structural and functional measures, we constructed childhood BSA-adjusted cardiac outcomes Standard Deviation Scores (SDS). This led to normally distributed childhood BSA adjusted cardiac outcomes SDS as shown in the histograms.

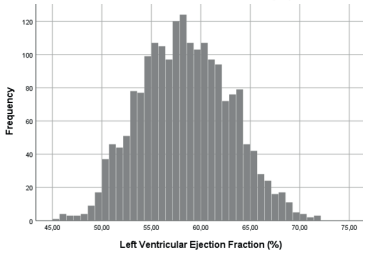
Distribution of uncorrected childhood cardiac outcomes in their original unit and BSA-corrected childhood cardiac outcomes in SDS

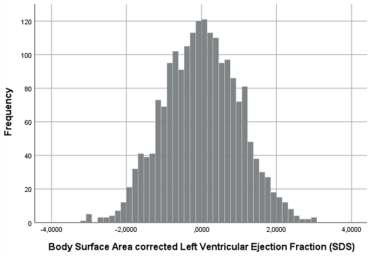


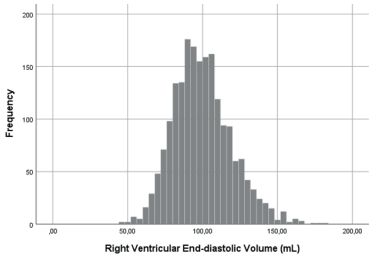


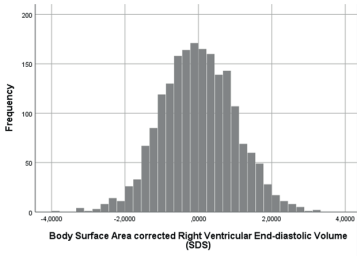


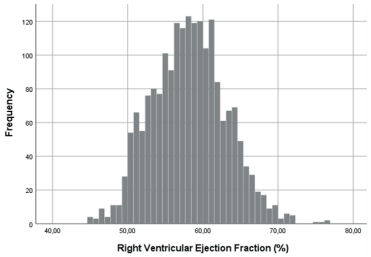


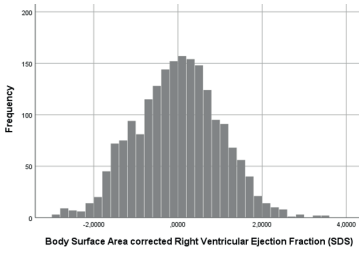






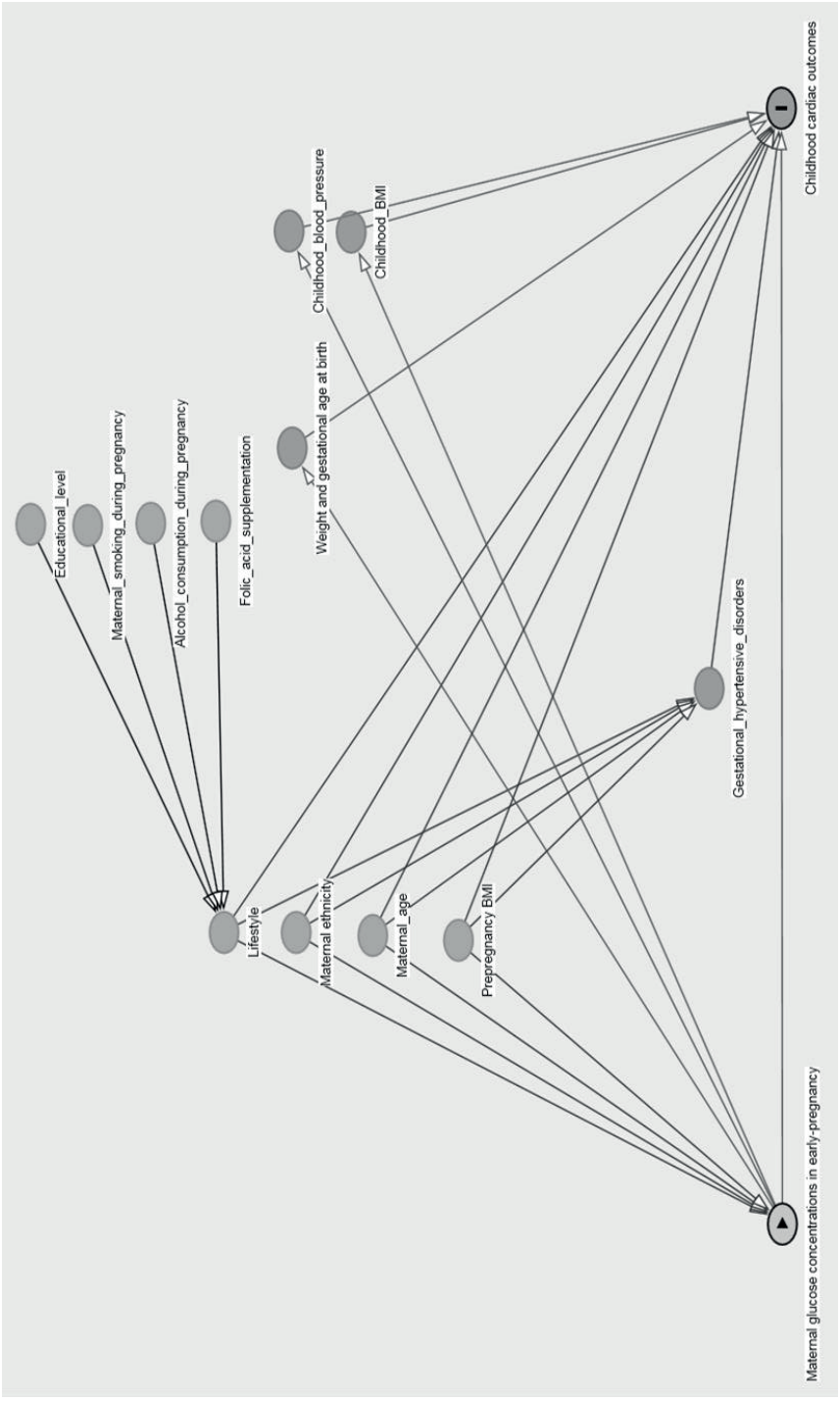






Supplementary Materials S2.3.2. Model selection based on a Directed Acyclic Graph representing the confounding and mediating pathways between maternal glucose concentrations in early-pregnancy and childhood cardiac outcomes

We selected maternal ethnicity, educational level, folic acid supplement use, alcohol consumption, smoking status and gestational hypertensive disorders in the model, based on their association with exposures and outcomes or change in effect estimates of >10% in our study sample (4-7). Maternal nausea and vomiting, and total caloric intake were not included in the confounder model as they did not affect the observed associations. Higher maternal glucose concentrations during pregnancy are an important risk factor for the risks of preterm birth and delivering a high or low birthweight infant (8-10). Multiple studies have shown that higher maternal glucose concentrations during pregnancy are associated with offspring BMI and blood pressure (11-13). In turn, these adverse birth outcomes and childhood characteristics are associated with childhood cardiac function and structure (14-17). As we showed in our DAG, gestational age and weight at birth and childhood BMI and blood pressure may mediate potential associations between maternal glucose concentrations and childhood cardiac outcomes. To explore the mediating role of birth characteristics and child's concurrent BMI and blood pressure, we additionally corrected for these birth and child characteristics in separate models.


Educational_level
Maternal_smoking_during_pregnancy
Alcohol_consumption_during_pregnancy
Folic_acid_supplementation
Childhood_blood_pressure
Childhood_BMI
Weight and gestational age at birth
Childhood cardiac outcomes
Lifestyle
Maternal ethnicity
Maternal_age
Prepregnancy BMI
Gestational_hypertensive_disorders
Maternal glucose concentrations in early-pregnancy

SUPPLEMENTARY TABLE S2.3.1. Baseline characteristics according to quintiles of maternal early-pregnancy random glucose concentrations

	Glucose concentrations Quintile 1 (n=433)	Glucose concentrations Quintile 2 (n=327)
Maternal Characteristics		
Age at enrolment, mean (SD), years	30.4 (4.7)	30.9 (4.7)
Gestational age at intake, median (95%), weeks	13.2 (10.7 to 16.9)	13.4 (10.9 to 16.9)
Prepregnancy body mass index, median (95%), kg/m^2	21.9 (18.5 to 28.6)	22.3 (18.5 to 30.2)
Gestational weight gain, mean (SD), kg	15.5 (5.0)	15.4 (6.0)
Parity, n nulliparous (%)	273 (63.2)	207 (63.5)
Ethnicity, n Dutch or European (%)	298 (69.5)	219 (67.6)
Education level, n high (%)	229 (52.9)	170 (52.0)
Income, n high (%)	258 (68.1)	187 (63.6)
Smoking during pregnancy, n yes (%)	99 (22.9)	66 (20.2)
Alcohol consumption during pregnancy, n yes (%)	254 (65.3)	174 (61.5)
Folic acid supplement use, n yes (%)	280 (84.1)	198 (81.1)
Glucose, mean (SD), mmol/l	3.4 (0.4)	4 (0.1)
Insulin, median (95%), pmol/l	65.3 (20.0 to 264.2)	63.1 (21.9 to 303.5)
Pre(gestational) diabetes, n (%)	2 (0.5)	0 (0.0)
Gestational hypertensive disorders, n (%)	24 (5.7)	16 (5.0)
Birth characteristics		
Sex, n female (%)	227 (52.4)	162 (49.5)
Birthweight, mean (SD), grams	3420 (554)	3417 (552)
Gestational age at birth, median (95%), weeks	40.3 (37.5 to 42.1)	40.3 (36.6 to 42.0)
Child Characteristics at 10 years		
Age, mean (SD), years	10.3 (0.7)	10.2 (0.7)
Body mass index, median (95%), kg/m^2	10.3 (0.7)	10.2 (0.7)
Body surface area, median (95%), m^2	1.1 (1.0 to 1.4)	1.2 (1.0 to 1.4)
Blood pressure		
Systolic, mean (SD), mmHg	102.8 (7.7)	102.7 (7.9)
Diastolic, mean (SD), mmHg	57.9 (6.4)	58.2 (6.4)
Cardiac MRI measures		
Left ventricular mass, median (95%), gram	47.9 (35.0 to 71.1)	48.2 (33.9 to 67.9)
Left ventricular end-diastolic volume, median (95%), ml	100.3 (74.5 to 133.9)	99.1 (72.1 to 134.0)
Left ventricular ejection fraction, mean (SD), %	58.8 (4.6)	58.4 (4.8)
Right ventricular end-diastolic volume, median (95%), ml	100.4 (70.4 to 138.4)	99.0 (70.4 to 138.4)
Right ventricular ejection fraction, mean (SD), %	58.7 (5.0)	58.0 (4.9)

Glucose concentrations Quintile 3 (n=432)	Glucose concentrations Quintile 4 (n=389)	Glucose concentrations Quintile 5 (n=378)
31.0 (4.7)	31.2 (4.4)	31.2 (4.4)
13.0 (10.2 to 16.9)	12.8 (9.9 to 16.4)	13.3 (10.6 to 16.7)
22.4 (18.7 to 31.7)	22.8 (18.7 to 32.1)	23.5 (18.9 to 35.3)
14.8 (5.4)	15.2 (6.2)	14.5 (6.4)
247 (57.8)	235 (60.7)	228 (60.6)
279 (65.6)	273 (70.7)	237 (62.7)
202 (46.8)	209 (53.7)	200 (52.9)
234 (61.9)	223 (67.2)	207 (63.1)
100 (23.10	84 (21.6)	69 (18.3)
230 (60.5)	211 (61.2)	194 (56.1)
267 (82.4)	271 (87.1)	246 (78.1)
4.3 (0.1)	4.8 (0.1)	5.7 (0.6)
86.8 (22.8 to 333.8)	148.4 (38.3 to 441.2)	294.5 (83.6 to 821.3)
0 (0.0)	2 (0.5)	7 (1.9)
32 (7.5)	27 (7.1)	32 (8.6)
234 (54.2)	202 (51.9)	203 (53.7)
3422 (542)	3510 (534)	3471 (545)
40.1 (37.1 to 42.0)	40.3 (37.7 to 42.1)	40.2 (37.3 to 42.1)
10.1 (0.6)	10.1 (0.6)	10.1 (0.6)
10.1 (0.6)	10.1 (0.6)	10.1 (0.6)
1.1 (1.0 to 1.4)	1.1 (1.0 to 1.4)	1.1 (1.0 to 1.5)
103.3 (8.1)	103.4 (8.0)	103.2 (7.5)
59.0 (6.5)	58.5 (6.1)	59.0 (6.4)
47.2 (36.3 to 67.9)	48.1 (34.4 to 66.9)	46.3 (33.3 to 66.9)
98.6 (75.1 to 133.1)	97.9 (74.5 to 130.1)	97.1 (72.6 to 130.9)
58.2 (4.4)	58.3 (4.7)	58.4 (4.5)
97.8 (71.8 to 134.4)	97.2 (72.1 to 134.0)	97.5 (70.5 to 132.8)
58.1 (4.8)	58.0 (5.1)	58.2 (4.7)

SUPPLEMENTARY TABLE S2.3.2. Non-response analysis for children with and without cardiac MRI follow up at 10 years of age

	Follow up at 10 years (n=1959)	No follow up at 10 years (n=4140)	P-value*
Maternal Characteristics			
Age at enrolment, mean (SD), years	30.9 (4.6)	29.2 (5.2)	0.00
Gestational age at intake, median (95%), weeks	13.1 (10.5 to 16.8)	13.4 (10.5 to 17.4)	0.00
Prepregnancy BMI, median (95%), kg/m^2	22.5 (18.7 to 31.6)	22.6 (18.7 to 32.6)	0.72
Gestational weight gain, mean (SD), kg	15.1 (5.8)	15.1 (5.8)	0.95
Parity, No. nulliparous (%)	1191 (61.1)	2278 (55.5)	0.00
Ethnicity, no. Dutch or European (%)	1306 (67.5)	2259 (57.6)	0.00
Education level, n high (%)	1011 (53.8)	1534 (40.5)	0.00
Income, n high (%)	1374 (70.1)	2097 (50.7)	0.00
Smoking during pregnancy, n yes (%)	418 (23.7)	1102 (30.1)	0.00
Alcohol consumption during pregnancy, n yes (%)	1063 (61.0)	1826 (50.6)	0.00
Folic acid supplement use, n yes (%)	1262 (82.6)	2217 (70.8)	0.00
Glucose, mean (SD), mmol/l	4.4 (0.8)	4.4 (0.8)	0.04
Insulin, median (95%), pmol/l	113.0 (24.4 to 502.8)	115.9 (24.9 to 555.3)	0.17
Pregestational diabetes, n (%)	11 (0.6)	74 (1.9)	0.24
Gestational hypertensive disorders	131 (6.8)	296 (7.1)	0.42
Child Characteristics			
Sex, n female (%)	1028 (52.5)	1985 (47.9)	0.00
Birthweight, mean (SD), grams	3448 (546)	3402 (571)	0.00
Gestational age at birth, median (95%), weeks	40.3 (37.1 to 42.1)	40.1 (36.7 to 42.0)	0.00

For normal distributed data, the mean with standard deviation is stated. For non-normally distributed data, the median with 95% range is stated.

**Differences in subject characteristics between the groups were evaluated using unpaired t-tests for the parametric continuous variables, Mann-Whitney U tests for the non-parametric continuous variables and chi square tests for proportions.*

SUPPLEMENTARY TABLE S2.3.3. Maternal early-pregnancy random glucose and insulin concentrations SDS and childhood cardiac outcomes at 10 years of age

	Left ventricle mass (SDS)	p-value	Left ventricle end-diastolic volume (SDS)	p-value	Left ventricle ejection fraction (SDS)	p-value	Right ventricle end-diastolic volume (SDS)	p-value	Right ventricle ejection fraction (SDS)	p-value
Maternal glucose (SDS)										
Basic model[a]	-0.05 (-0.09 to -0.01)	0.01	-0.05 (-0.09 to -0.01)	0.02	-0.03 (-0.08 to 0.01)	0.05	-0.04 (-0.08 to 0.00)	0.05	-0.04 (-0.08 to 0.01)	0.09
Confounder model[b]	-0.05 (-0.09 to -0.01)	0.02	-0.04 (-0.08 to 0.00)	0.05	-0.03 (-0.08 to 0.01)	0.15	-0.03 (-0.07 to 0.01)	0.13	-0.04 (-0.08 to 0.01)	0.10
Maternal BMI model[c]	-0.04 (-0.08 to -0.01)	0.03	-0.04 (-0.07 to 0.00)	0.07	-0.04 (-0.09 to 0.01)	0.08	-0.03 (-0.06 to 0.01)	0.19	-0.05 (-0.09 to 0.00)	0.04
Birth model[d]	-0.05 (-0.08 to -0.01)	0.02	-0.04 (-0.08 to 0.00)	0.04	-0.04 (-0.08 to 0.01)	0.09	-0.03 (-0.07 to 0.01)	0.12	-0.05 (-0.09 to 0.00)	0.05
Child model[e]	-0.04 (-0.08 to 0.00)	0.04	-0.04 (-0.07 to 0.00)	0.06	-0.04 (-0.08 to 0.01)	0.12	-0.03 (-0.06 to 0.01)	0.19	-0.04 (-0.09 to 0.00)	0.05
Maternal insulin (SDS)										
Basic model[a]	-0.01 (-0.05 to 0.03)	0.54	-0.08 (-0.12 to -0.04)	0.00	0.00 (-0.04 to 0.05)	0.97	-0.07 (-0.11 to -0.03)	0.00	-0.01 (-0.06 to 0.03)	0.63
Confounder model[b]	0.00 (-0.04 to 0.04)	0.98	-0.05 (-0.09 to -0.01)	0.01	0.01 (-0.04 to 0.05)	0.83	-0.05 (-0.09 to -0.01)	0.02	-0.01 (-0.05 to 0.04)	0.70
Maternal BMI model[c]	0.00 (-0.04 to 0.04)	0.87	-0.05 (-0.09 to -0.01)	0.01	0.00 (-0.05 to 0.04)	0.87	-0.04 (-0.08 to 0.00)	0.04	-0.02 (-0.07 to 0.03)	0.38
Birth model[d]	0.00 (-0.04 to 0.05)	0.86	-0.05 (-0.09 to -0.01)	0.01	-0.01 (-0.05 to 0.04)	0.85	-0.04 (-0.08 to -0.01)	0.03	-0.02 (-0.07 to 0.03)	0.40
Child model[e]	0.00 (-0.04 to 0.04)	0.85	-0.05 (-0.09 to -0.01)	0.01	0.00 (-0.05 to 0.04)	0.86	-0.04 (-0.08 to -0.01)	0.03	-0.02 (-0.07 to 0.03)	0.41

Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood Body Surface Area corrected cardiac outcomes in SDS per SDS increase in maternal glucose and insulin concentrations. Insulin concentrations were log transformed. SDS: standard deviation score, BMI: Body Mass Index

[a]*Basic model includes gestational age at enrolment, child's age and sex at follow up measurements and time difference between measurement of child's body surface area and cardiac MRI*

[b]*Confounder model is the basic model adjusted for gestational age at enrolment, child's age and sex at follow up measurements and time difference between measurement of child's body surface area and cardiac MRI, maternal ethnicity, maternal educational level, folic acid supplement use during pregnancy, alcohol consumption and smoking during pregnancy, gestational hypertensive disorders*

[c]*Maternal BMI model is the confounder model additionally adjusted for maternal prepregnancy body mass index*

[d]*Birth model is the maternal BMI model additionally adjusted for gestational age and weight at birth*

[e]*Child model includes the birth BMI model additionally adjusted for child's body mass index and blood pressure at 10 years of age*

SUPPLEMENTARY TABLE S2.3.4. Maternal early-pregnancy random glucose concentrations with childhood blood pressure at 10 years of age

	Childhood systolic blood pressure (SDS)	p-value	Childhood diastolic blood pressure (SDS)	p-value
Maternal glucose (SDS)				
Basic model[a]	0.02 (-0.03 to 0.07)	0.44	0.06 (0.00 to 0.11)	0.04
Confounder model[b]	0.01 (-0.04 to 0.06)	0.74	0.05 (-0.01 to 0.10)	0.09
Maternal BMI model[c]	-0.01 (-0.06 to 0.04)	0.70	0.04 (-0.02 to 0.09)	0.16
Birth model[d]	-0.01 (-0.06 to 0.04)	0.70	0.04 (-0.01 to 0.09	0.14
Child model[e]	-0.01 (-0.06 to 0.04)	0.78	0.04 (-0.01 to 0.10)	0.13

Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood blood pressure in SDS per SDS increase in maternal glucose and insulin concentrations. Insulin concentrations were log transformed. SDS: standard deviation score, BMI: Body Mass Index

[a]*Basic model includes gestational age at enrolment, child's age and sex at follow up measurements and time difference between measurement of child's body surface area and cardiac MRI*

[b]*Confounder model is the basic model adjusted for gestational age at enrolment, child's age and sex at follow up measurements and time difference between measurement of child's body surface area and cardiac MRI, maternal ethnicity, maternal educational level, folic acid supplement use during pregnancy, alcohol consumption and smoking during pregnancy, gestational hypertensive disorders*

[c]*Maternal BMI model is the confounder model additionally adjusted for maternal prepregnancy body mass index*

[d]*Birth model is the maternal BMI model additionally adjusted for gestational age and weight at birth*

[e]*Child model includes the birth BMI model additionally adjusted for child's body mass index at 10 years of age*

SUPPLEMENTARY TABLE S2.3.5. Sensitivity analyses for the associations of maternal early-pregnancy random glucose concentrations with childhood cardiac outcomes at 10 years of age

Model	Left ventricular Mass (SDS)	p-value	Left ventricular end-diastolic volume (SDS)	p-value	Left ventricular ejection fraction (SDS)	p-value	Right ventricular end-diastolic volume (SDS)	p-value	Right ventricular ejection fraction (SDS)	p-value
Maternal glucose (mmol/l)										
Women without pregestational and gestational diabetes (n=1948)										
Basic model[a]	-0.06 (-0.11 to -0.01)	0.01	-0.05 (-0.10 to 0.00)	0.03	-0.03 (-0.09 to 0.02)	0.26	-0.04 (-0.09 to 0.01)	0.08	-0.04 (-0.09 to 0.02)	0.16
Confounder model[b]	-0.06 (-0.10 to -0.01)	0.02	-0.04 (-0.09 to 0.01)	0.10	-0.03 (-0.09 to 0.02)	0.26	-0.03 (-0.08 to 0.02)	0.19	-0.04 (-0.09 to 0.02)	0.17
Maternal BMI model[c]	-0.05 (-0.10 to -0.01)	0.03	-0.04 (-0.09 to 0.01)	0.11	-0.04 (-0.10 to 0.02)	0.15	-0.03 (-0.07 to 0.02)	0.26	-0.05 (-0.10 to 0.01)	0.08
Birth model[d]	-0.05 (-0.10 to -0.01)	0.03	-0.04 (-0.09 to 0.00)	0.06	-0.04 (-0.09 to 0.02)	0.18	-0.03 (-0.08 to 0.01)	0.17	-0.05 (-0.10 to 0.01)	0.10
Child model[e]	-0.05 (-0.10 to 0.00)	0.03	-0.04 (-0.09 to 0.01)	0.09	-0.04 (-0.09 to 0.02)	0.20	-0.03 (-0.07 to 0.02)	0.23	-0.05 (-0.10 to 0.01)	0.10
Women without gestational hypertensive disorders (n=1828)										
Basic model[a]	-0.06 (-0.11 to -0.02)	0.01	-0.06 (-0.10 to -0.01)	0.02	-0.04 (-0.09 to 0.02)	0.16	-0.05 (-0.09 to 0.00)	0.05	-0.05 (-0.10 to 0.01)	0.09
Confounder model[b]	-0.06 (-0.10 to -0.01)	0.02	-0.06 (-0.10 to -0.01)	0.02	-0.04 (-0.09 to 0.01)	0.15	-0.04 (-0.08 to 0.01)	0.12	-0.05 (-0.10 to 0.01)	0.09
Maternal BMI model[c]	-0.05 (-0.10 to -0.01)	0.03	-0.05 (-0.09 to 0.00)	0.05	-0.05 (-0.10 to 0.01)	0.08	-0.03 (-0.08 to 0.02)	0.19	-0.06 (-0.11 to 0.00)	0.04
Birth model[d]	-0.05 (-0.10 to -0.01)	0.02	-0.04 (-0.09 to 0.00)	0.06	-0.05 (-0.10 to 0.01)	0.09	-0.04 (-0.08 to 0.01)	0.13	-0.06 (-0.11 to 0.00)	0.04
Child model[e]	-0.05 (-0.10 to 0.00)	0.04	-0.05 (-0.09 to 0.00)	0.04	-0.04 (-0.10 to 0.01)	0.11	-0.03 (-0.08 to 0.02)	0.20	-0.05 (-0.11 to 0.00)	0.05
Women with first trimester glucose concentrations available (n=1336)[f]										
Basic model[a]	-0.07 (-0.12 to -0.01)	0.03	-0.03 (-0.09 to 0.02)	0.25	-0.06 (-0.12 to 0.00)	0.06	-0.02 (-0.08 to 0.03)	0.40	-0.07 (-0.14 to -0.01)	0.02
Confounder model[b]	-0.06 (-0.11 to 0.00)	0.06	-0.02 (-0.07 to 0.04)	0.54	-0.06 (-0.12 to 0.01)	0.08	-0.01 (-0.07 to 0.05)	0.71	-0.07 (-0.13 to -0.01)	0.04
Maternal BMI model[c]	-0.05 (-0.11 to 0.01)	0.09	-0.04 (-0.07 to 0.04)	0.60	-0.06 (-0.13 to 0.00)	0.05	0.00 (-0.06 to 0.05)	0.88	-0.08 (-0.14 to -0.01)	0.02
Birth model[d]	-0.05 (-0.11 to 0.01)	0.09	-0.02 (-0.08 to 0.03)	0.43	-0.06 (-0.12 to 0.01)	0.08	-0.01 (-0.07 to 0.04)	0.66	-0.07 (-0.14 to -0.01)	0.03
Child model[e]	-0.05 (-0.10 to 0.01)	0.07	-0.02 (-0.07 to 0.04)	0.50	-0.05 (-0.12 to 0.01)	0.11	-0.01 (-0.06 to 0.05)	0.75	-0.07 (-0.14 to 0.00)	0.04

Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood Body Surface Area corrected cardiac outcomes in SDS per mmol/l increase in maternal glucose concentrations. SDS: standard deviation score, BMI: Body Mass Index

[a]Basic model is adjusted for gestational age at enrolment, child's age and sex at follow up measurements and time difference between measurement of child's body surface area and cardiac MRI

[b]Confounder model is the basic model additionally adjusted, maternal ethnicity, maternal educational level, folic acid supplement use during pregnancy, alcohol consumption and smoking during pregnancy, gestational hypertensive disorders (the sensitivity analyses among women without gestational hypertensive disorders was not adjusted for gestational hypertensive disorders)

[c]Maternal BMI model is the confounder model additionally adjusted for maternal prepregnancy body mass index

[d]Birth model is the maternal BMI model additionally adjusted for gestational age and weight at birth

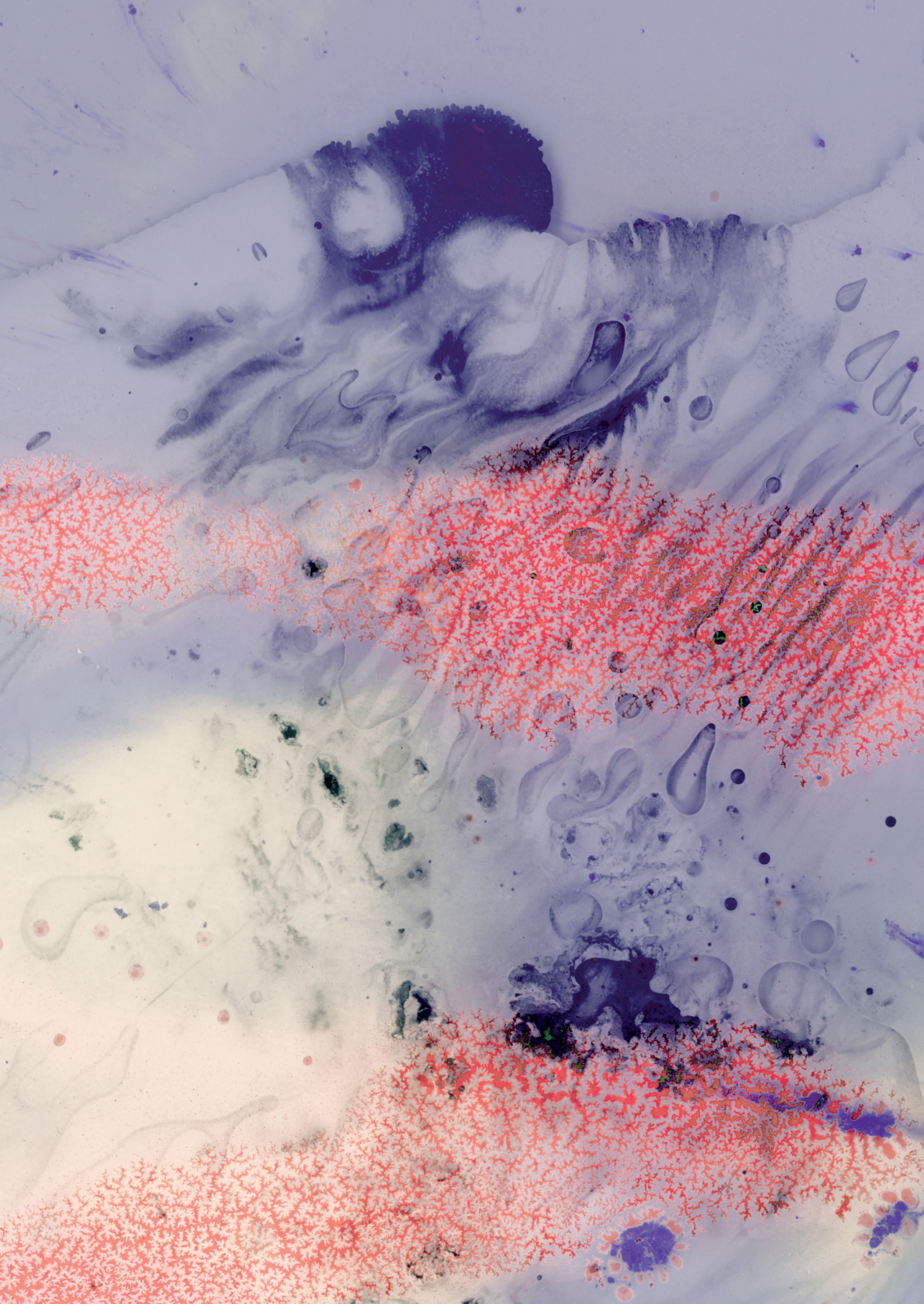
[e]Child model is the birth model additionally adjusted for child's body mass index and blood pressure at 10 years of age

[f]Women with glucose concentrations available <14 weeks gestation had the same mean of glucose concentrations as women with ≥14 weeks gestation glucose concentrations available (mean 4.4 mmol/l, SD 0.8)

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03

Maternal dietary influences during pregnancy

3.1

Maternal dietary glycemic index, fetal growth and birth outcomes

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ABSTRACT

Purpose: Maternal hyperglycemia is associated with adverse birth outcomes. Maternal dietary glycemic index and load influence postprandial glucose concentrations. We examined the associations of maternal early pregnancy dietary glycemic index and load with fetal growth and risks of adverse birth outcomes.

Methods: In a population-based cohort study of 3,471 pregnant Dutch women, we assessed dietary glycemic index and load using a food frequency questionnaire at median 13.4 (95% range 10.6 to 21.2) weeks gestation. We measured fetal growth in mid- and late-pregnancy by ultrasound and obtained birth outcomes from medical records.

Results: Mean maternal early pregnancy dietary glycemic index and load were 57.7 (SD 3.3, 95% range 52.8 to 63.5) and 155 (SD 47, 95% range 87 to 243), respectively. Maternal early pregnancy dietary glycemic index was not associated with fetal growth parameters. A higher maternal early pregnancy dietary glycemic load was associated with a higher fetal abdominal circumference and estimated fetal weight in late-pregnancy (p values < 0.05), but not with mid-pregnancy or birth growth characteristics. A higher maternal early pregnancy dietary glycemic index was associated with a lower risk of a large-for-gestational-age newborn (p value < 0.05). Maternal early pregnancy glycemic index and load were not associated with other adverse birth outcomes.

Conclusion: Among pregnant women without an impaired glucose metabolism, a higher early pregnancy dietary glycemic load was associated with higher late-pregnancy fetal abdominal circumference and estimated fetal weight. No consistent associations of maternal dietary glycemic index and load with growth parameters in mid-pregnancy and at birth were present. A higher glycemic index was associated with a lower risk of a large-for-gestational-age newborn.

INTRODUCTION

Maternal hyperglycemia during pregnancy is a well-known risk factor for adverse birth outcomes, such as macrosomia and neonatal hypoglycemia (1). Accumulating evidence suggests that early pregnancy is a critical period for the adverse effects of high maternal glucose concentrations on embryonic and placental development (2, 3). High maternal glucose concentrations from early pregnancy onwards may cause alterations in embryonic and placental development, and lead to an increased transfer of glucose to the developing fetus, predisposing to increased fetal growth and fat deposition and alterations in fetal metabolism. These fetal adaptations may, subsequently, predispose to increased risks of adverse birth outcomes (1).

During pregnancy, most transfer of glucose across the placenta occurs in the postprandial state. These postprandial glucose concentrations are mainly determined by maternal dietary carbohydrate intake (4). The dietary glycemic index and glycemic load are measures that can be used to qualify and quantify the maternal postprandial glycemic response to the maternal dietary carbohydrate intake. These measures influence postprandial glucose available for maternal energy, storage, and transfer to the fetus (5, 6). Intervention studies suggested that a low-glycemic index diet during the second half of pregnancy may reduce birthweight and infant adiposity in women with gestational diabetes or an impaired glucose metabolism (7, 8). No increased risks of delivering a small-for-gestational-age infant were observed in these intervention studies. However, an observational study among pregnant women not at risk of an impaired glucose metabolism reported that a lower.

Maternal dietary glycemic index in the second half of pregnancy was associated with an increased risk of delivering a small-for-gestational-age infant (9). In pregnant women without an impaired glucose metabolism, not much is known about the effects of maternal dietary glycemic index and load during early pregnancy on directly measured fetal growth throughout pregnancy and the risks of adverse birth outcomes. We hypothesized that a lower maternal dietary glycemic index and load in early pregnancy might reduce the risks of fetal overgrowth and macrosomia, but might also lead to increased risks of fetal undergrowth and low birthweight, especially among a general, healthy population.

Therefore, in a population-based prospective cohort study among 3,471 pregnant women without an impaired glucose metabolism, we examined the associations of maternal early pregnancy dietary glycemic index and load within a low-to-normal range with fetal growth throughout pregnancy and the risks of adverse birth outcomes.

METHODS

Study design and study sample

This study was embedded in the Generation R study, a population-based prospective birth cohort study in Rotterdam, The Netherlands. Details of the study have been described previously (10). Written informed consent was obtained from all women at enrollment between April 2002 and January 2006. The response rate at baseline was 61%, which was calculated by dividing the number of participating live born children by the total number of live born children born in the study area during the inclusion period. The study was approved by the Medical Ethical Committee of Erasmus MC University Medical Center in Rotterdam, The Netherlands (MEC 198.782/2001/31). In total, 4,544 Dutch women were enrolled during pregnancy. During early pregnancy, information on dietary intake was available in 3,558 Dutch women. After exclusion of women with pre-gestational diabetes and non-singleton live births, the final study sample consisted of 3,471 pregnant women and their newborns (**Supplementary Figure S3.1.1**).

Maternal dietary glycemic index and load

We obtained information on maternal dietary intake during early pregnancy at a median of 12.9 weeks gestation (95% range 10.4 to 16.8) by a semi-quantitative 293-item Food Frequency Questionnaire (FFQ) (11). The FFQ was validated against three 24-h dietary recalls in 71 pregnant women with Dutch ethnicity living in Rotterdam. Intra-class correlation coefficients for macronutrient intakes ranged from 0.50 to 0.70 and were 0.54 for carbohydrate intake (12). The average energy intake and carbohydrate intake was calculated using the Dutch Food Composition Table 2006 (13). Next, we calculated the maternal early pregnancy dietary glycemic index and load. The dietary glycemic index provides information on the quality of the glycemic response to a carbohydrate containing food product and is more often used in intervention studies and clinical settings (14, 15). The dietary glycemic load additionally takes the amount of carbohydrate intake into account and, therefore, provides additional information on maternal postprandial glucose concentrations, but this measure may be more prone to measurement errors (5, 6, 16). In line with previous observational studies, we calculated both maternal dietary glycemic index and load for the current study (17-19). To calculate maternal early pregnancy dietary glycemic index and load, glycemic index values were assigned to each individual food item in the FFQ. Glycemic index values were obtained from the glycemic index database on the Dutch diet published by the Medical Research Council Human Nutrition Research (MRC HNR), Cambridge, United Kingdom, using glucose as a reference (glycemic index for glucose equal to 100) (20). Using this database, we obtained direct matches for 84.3% of the food items. For the food items

that could not directly be matched in the database, glycemic index values for similar food items were obtained from proxies (87.8%) or from glycemic index databases of MRC HNR for other countries (9.8%). If no equivalent food item was available for a food item, an arbitrary value of 70 was assigned according to the procedure developed by the MRC HNR (2.4%) (18, 20).

The mean maternal dietary glycemic index per day was calculated by summing the product of the carbohydrate intake of each food item with its glycemic index, which was divided by the total amount of carbohydrates consumed per day. The mean maternal dietary glycemic load was calculated by summing the product of the carbohydrate intake of each food item with the glycemic index of that specific food item. We constructed quartiles and standard deviation scores of maternal dietary glycemic index and glycemic load.

Intervention studies stimulate a low-glycemic index diet by recommending an exchange of high-glycemic index products for low-glycemic index products, which results in a low mean dietary glycemic index (19, 21). In line with these studies, we aimed to explore the effects of a low-glycemic index diet on fetal growth and birth characteristics and the risk of adverse birth outcomes as a secondary analysis. We categorized the mean maternal dietary glycemic index per day into a low, normal, and high-glycemic index diet, using similar cut-offs as used for individual food products (low-glycemic index diet (≤ 55), a normal-glycemic index diet (56 to 69), and a high-glycemic index diet (≥ 70)). We consider this approach in line with intervention studies who recommend a low-glycemic index diet through eating low-glycemic index food products (19, 21).

Fetal growth and adverse birth outcomes

We performed fetal ultrasound examinations to assess fetal growth during mid- and late-pregnancy at a median gestational age of 20.5 (95% range 19.0 to 22.6) and 30.4 (95% range 28.9 to 32.2) weeks, respectively. Gestational age was established during early pregnancy based on crown-rump length. During mid- and late-pregnancy, we measured femur length, abdominal circumference, and head circumference to the nearest millimeter using standardized ultrasound procedures (22). Head circumference, abdominal circumference, and femur length were used to estimate fetal weight by using the Hadlock equation (23). Longitudinal growth curves and gestational-age-adjusted standard deviation scores (SDS) were constructed for all fetal growth measurements (24). These gestational-age-adjusted SDS were based on reference growth curves from the whole study population, and represent the equivalent of *z* scores.

We obtained data on gestational age, weight, length, and head circumference at birth from medical records. Because head circumference and length were not routinely measured at birth, fewer measurements were available (n=1,942 for head circumference and n=2,323 for length at birth). Gestational-age-adjusted SDS for birthweight, length, and head circumference were constructed using North European growth standards (25). Based on international guidelines, we defined small-for-gestational-age and large-for-gestational-age at birth as the lowest and the highest ten percentiles of gestational-age-adjusted birthweight within our study population, respectively (19, 26). Preterm birth was defined as a gestational age at birth<37 weeks (25). Information on caesarian delivery was obtained from medical records.

Covariates

Information on maternal age, educational level (primary education finished, secondary education finished, and higher education finished), parity (nulliparous and multiparous), folic acid supplement use (yes/no), and daily nausea for past three months (yes/no) and daily vomiting for past 3 months (yes/no) was collected by questionnaire at enrollment. We measured maternal height at enrollment and obtained information on maternal pre-pregnancy weight through questionnaire and calculated pre-pregnancy body mass index (BMI) (10). Information on maternal smoking (yes/no) and alcohol consumption (yes/no) was assessed by repeated questionnaires throughout pregnancy (10). Information on gestational diabetes was obtained through medical records.

Statistical analyses

First, we performed a non-response analysis comparing Dutch women with and without information available on early pregnancy dietary glycemic index and load. We further compared population characteristics according to maternal dietary glycemic index quartiles using Chi-square tests for categorical variables and one-way ANOVA for continuous variables. Second, we examined the associations of maternal early pregnancy dietary glycemic index and load with fetal growth patterns from mid-pregnancy onwards using unbalanced repeated measurement regression models. We included maternal early pregnancy dietary glycemic index and load quartiles in these models as intercept and as interaction term with gestational age to estimate fetal growth rates over time. To further assess the associations of maternal dietary glycemic index and load with fetal growth characteristics in each pregnancy period in detail, we examined the associations of maternal early pregnancy dietary glycemic index and load in quartiles and per SDS change with each fetal growth characteristics in each pregnancy period and at birth using linear regression models. In the analyses with maternal early pregnancy dietary glycemic index and load in quartiles, we assessed

whether associations were restricted to women with a relatively low or high dietary glycemic index and load and explored whether there was a linear tendency present. We used quartiles based on variability between the categories of maternal dietary glycemic index and load and to maintain statistical power. Next, we assessed the associations of maternal early pregnancy dietary glycemic index and load continuously per 1-SDS increase with fetal growth characteristics in each pregnancy period and at birth to explore the continuous associations across the low-to-normal range of maternal early pregnancy dietary glycemic index and load, which is not fully captured by the quartile analyses. First, we only adjusted for gestational age at study enrollment. Subsequently, we additionally adjusted these models for maternal age, parity, educational level, pre-pregnancy BMI, early pregnancy total daily energy intake, smoking during pregnancy, alcohol use during pregnancy, daily nausea, and vomiting during early pregnancy and fetal sex, as nutritional exposures are prone to confounding by other maternal socio-demographic and lifestyle characteristics. Variables were selected based on literature and included in the final model when the covariate caused a ≥ 10% change in the effect estimate (27-29). We did not adjust for gestational weight gain, as fetal growth is a major component of gestational weight gain and additional adjustment of gestational weight gain would thus lead to over adjustment. Finally, we assessed the associations of maternal early pregnancy dietary glycemic index and load in quartiles and per SDS change with the risks of adverse birth outcomes using logistic regression models with similar adjustment. To assess whether the effects were different for mothers with a different pre-pregnancy BMI and/or child's sex, we tested for interactions between maternal dietary glycemic index and load and maternal pre-pregnancy BMI and child's sex in the models described above, but none were significant (30, 31).

We performed several sensitivity analyses: (1) as a secondary analysis, we further explored the associations of a low-glycemic index diet as compared to a normal-glycemic index diet, according to our predefined categories, with fetal growth and the risks of adverse birth outcomes; (2) as we were interested in the effects of maternal dietary glycemic index and load among low-risk pregnant women, we repeated the analyses excluding women with gestational diabetes, excluding women with overweight or obesity and excluding women aged>35 years, respectively (15, 32).

To reduce selection bias due to missing data, multiple imputations of covariates (pooled results of five imputed datasets) were be performed (33). The repeated measurement analyses were performed using the Statistical Analysis System version 9.4 (SAS Institute, Cary, NC, USA). All other analyses were performed using the Statistical Package of Social Sciences version 24.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Subject characteristics

Mean maternal dietary glycemic index and load were 57.7 (SD 3.3, 95% range 52.8 to 63.5) and 155 (SD 47, 95% range 87 to 243), respectively (**Table 3.1.1**). 705 (20.3%) women consumed a low-glycemic index diet (mean dietary glycemic index per day ≤ 55) and no women consumed a high-glycemic index diet (mean glycemic index per day ≥ 70). Women within the higher dietary glycemic index quartiles were more likely to be younger, multiparous, lower educated, had a higher pre-pregnancy BMI, higher total energy intake, and smoked more often during pregnancy. Fetal growth characteristics according to maternal dietary glycemic index quartiles are given in **Supplementary Table S3.1.1**. The non-response analysis showed that women with information on dietary intake were more likely to be multiparous and higher educated compared to women without these data (**Supplementary Table S3.1.2**).

Maternal dietary glycemic index and load and fetal growth

Figure 3.1.1 shows fetal head circumference, length, and weight growth patterns from mid-pregnancy onwards for quartiles of maternal dietary glycemic index and load. As compared to the lowest quartile of maternal dietary glycemic index, the highest quartile of maternal dietary glycemic index tended to be associated with lower fetal head circumference, length, and weight growth rates from late-pregnancy onwards, but only for fetal length, the *p* value for interaction of maternal dietary glycemic index quartiles with gestational age was significant (p value < 0.05). No consistent associations of maternal dietary glycemic load quartiles with fetal growth patterns were present (regression coefficients for gestational age-independent and gestational age-dependent effects in **Supplementary Table S3.1.3**).

Maternal dietary glycemic index within a low-to-normal range was not associated with fetal growth characteristics in each pregnancy period or at birth in the basic or adjusted models (**Table 3.1.2**). In contrast, higher maternal dietary glycemic load within a low-to-normal range was associated with a higher fetal abdominal circumference and fetal estimated weight in late-pregnancy, with stronger associations after adjustment for maternal socio-demographic and lifestyle factors (differences in late-pregnancy fetal abdominal circumference and estimated fetal weight SDS 0.08 (95% CI 0.02 to 0.15), 0.07 (95% CI 0.00 to 0.14) per SDS increase in glycemic load, respectively]. However, no associations of maternal dietary glycemic load with fetal growth characteristics in mid-pregnancy or at birth were present. **Supplementary Table S3.1.4** and **S3.1.5**

TABLE 3.1.1. Population characteristics according to maternal dietary glycemic index quartiles

	Total group *(n=3,471)*	Glycemic index quartile 1 *(n=867)*	Glycemic index quartile 2 *(n=868)*	Glycemic index quartile 3 *(n=868)*	Glycemic index quartile 4 *(n=868)*	P-value[a]
Maternal characteristics						
Maternal age at enrolment, mean (SD), years	31.4 (4.4)	32.3 (4.0)	31.7 (4.1)	31.1 (4.4)	30.4 (4.9)	0.00
Gestational age at enrolment, median (95%), weeks	13.4 (10.6 to 21.2)	13.4 (10.9 to 21.2)	13.4 (10.5 to 21.6)	13.4 (10.6 to 21.6)	13.4 (10.4 to 20.8)	0.93
Parity, n nulliparous (%)	2,076 (59.9)	542 (62.7)	538 (62.0)	513 (59.2)	483 (55.9)	0.02
Prepregnancy weight status, overweight or obese, n (%)	685 (22.9)	138 (18.3)	164 (22.2)	194 (25.9)	189 (25.3)	0.01
Gestational weight gain, mean (SD), g/week	10.8 (4.4)	10.7 (4.1)	10.8 (4.5)	10.9 (4.4)	10.8 (4.7)	0.71
Education, n high (%)	2,026 (59.1)	605 (70.4)	536 (62.3)	489 (57.3)	396 (46.4)	0.00
Glycemic index, mean (SD)	57.7 (3.3)	53.8 (1.4)	56.5 (0.6)	58.6 (0.7)	62.1 (1.9)	n.a.
Glycemic load, mean (SD)	155 (47)	132 (33)	147 (39)	160 (43)	179 (56)	0.00
Carbohydrate intake, mean (SD), g/d	267 (75)	246 (60)	261 (68)	272 (74)	288 (88)	0.00
Protein intake, mean (SD), g/d	79 (19)	82 (18)	80 (19)	79 (19)	75 (20)	0.00
Fat intake, mean (SD), g/d	86 (24)	85 (24)	87 (24)	88 (25)	85 (25)	0.02
Fiber intake, mean (SD), g/d	23 (7)	25 (7)	24 (7)	23 (7)	21 (7)	0.00
Total energy intake, mean (SD), kcal/d	2,145 (511)	2,063 (453)	2,132 (495)	2,183 (516)	2,201 (564)	0.00
Folic acid supplement use, n yes (%)	2,532 (72.9)	658 (75.9)	638 (73.5)	648 (74.7)	588 (67.7)	0.00
Alcohol use during pregnancy, n yes (%)	2,117 (66.3)	581 (72.8)	554 (69.6)	506 (63.1)	476 (59.6)	0.00
Smoking during pregnancy, n yes (%)	833 (26.9)	152 (19.0)	181 (22.5)	226 (28.0)	274 (33.9)	0.00
Nausea during early-pregnancy, n (%)	880 (27.7)	181 (22.8)	207 (26.2)	249 (31.2)	243 (30.5)	0.00
Vomiting during early-pregnancy, n (%)	145 (4.6)	20 (2.5)	36 (4.6)	42 (5.3)	47 (5.9)	0.01
Gestational diabetes, n (%)	31 (0.9)	6 (0.7)	6 (0.7)	9 (1.1)	10 (1.2)	0.64
Birth characteristics						
Sex, n male (%)	1,753 (50.5)	404 (46.6)	475 (54.7)	411 (47.4)	463 (53.3)	0.00
Gestational age at birth, median (95% range), weeks	40.3 (37.0, 42.1)	40.3 (36.9, 42.1)	40.3 (37.1, 42.1)	40.1 (37.1, 42.1)	40.3 (36.3, 42.1)	0.59
Birthweight, mean (SD), g	3,489 (554)	3,498 (584)	3,500 (512)	3,505 (553)	3,452 (565)	0.17
Preterm birth, n (%)	162 (4.7)	43 (5.0)	34 (3.9)	30 (3.5)	55 (6.3)	0.02
Small-for-gestational-age, n (%)	345 (10.0)	85 (9.8)	82 (9.5)	87 (10.1)	91 (10.5)	0.91
Large-for-gestational-age, n (%)	345 (10.0)	105 (12.1)	85 (9.8)	89 (10.3)	66 (7.6)	0.02

[a]*P-values were obtained by ANOVA test for continuous variables or by chi-square test for categorical variables.*

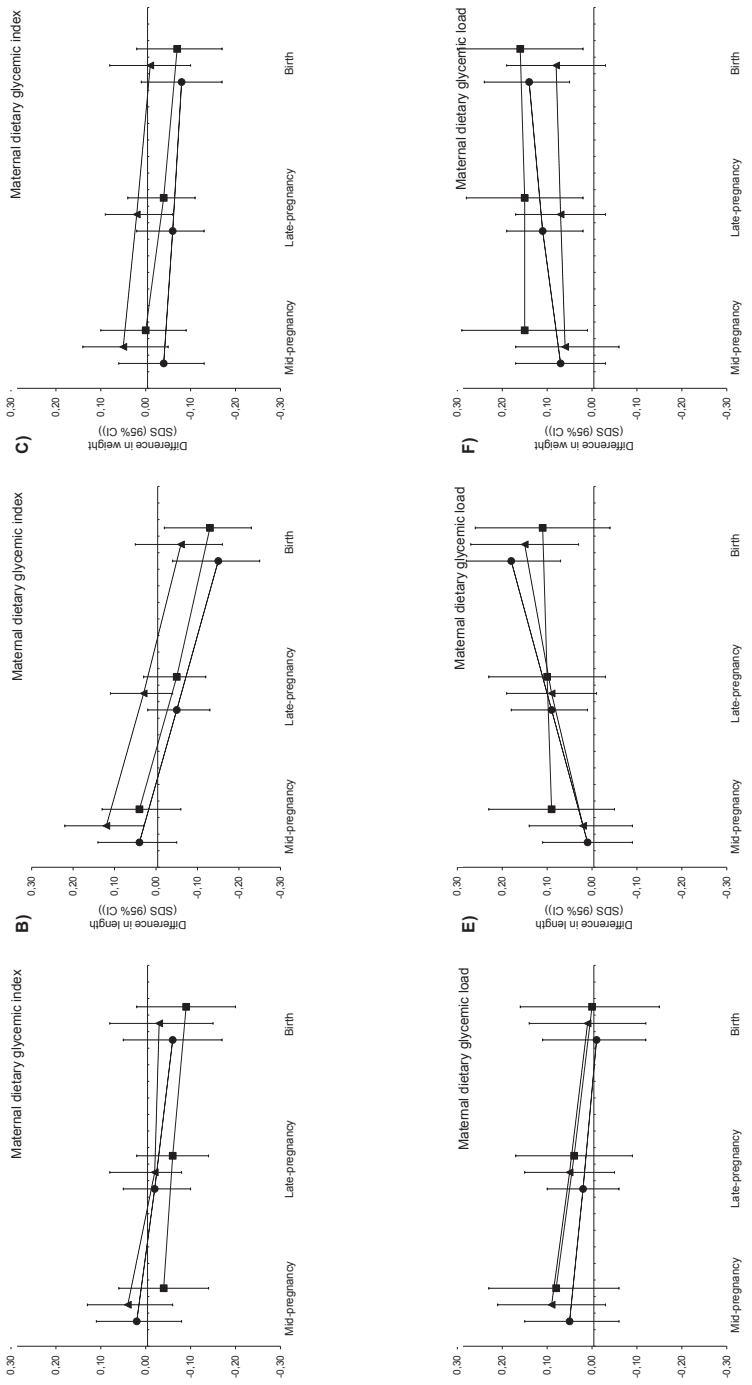


FIGURE 3.1.1. Associations of maternal dietary glycemic index and load with fetal growth patterns from mid-pregnancy onwards

show that when we analyzed associations of maternal dietary glycemic index and load in quartiles with fetal growth characteristics in each pregnancy period, similar findings were present.

Differences in fetal growth rates for the upper three maternal dietary glycemic index quartiles (Figure 3.1.1a, 3.1.1b, 3.1.1c) and the upper three maternal dietary glycemic load quartiles (Figure 3.1.1d, 3.1.1e, 3.1.1f), as compared to the lowest maternal dietary glycemic index and load quartile, respectively. Circles represent the second quartile, triangles the third quartile and squares the fourth quartile of maternal dietary glycemic index and load, respectively. Results are based on repeated measurement regression models and reflect the differences in gestational-age-adjusted SDS scores of fetal head circumference, length and weight growth for the three highest maternal dietary glycemic index and load quartiles compared the lowest maternal dietary glycemic index and load quartile (reference group represented as zero line). The models were adjusted for gestational age at intake, maternal age, parity, pre-pregnancy BMI, maternal education, smoking during pregnancy, alcohol use during pregnancy, nausea during early-pregnancy, vomiting during early-pregnancy, early-pregnancy total daily energy intake, and fetal sex. We only observed a significant interaction for maternal dietary glycemic index quartiles with gestational age for fetal length. Regression coefficients for gestational age-independent and gestational age-dependent effects are given in Supplementary Table S3.1.3.

Maternal dietary glycemic index and load and the risk of adverse birth outcomes

Higher maternal dietary glycemic index within a low-to-normal range was not associated with the risks of preterm birth, delivering a small-for-gestational-age infant or caesarian delivery (**Table 3.1.3**). Higher maternal dietary glycemic index within a low-to-normal range was associated with a lower risk of delivering a large-for-gestational-age newborn in the basic model, which was not explained by adjustment for maternal socio-demographic or lifestyle factors (Odds ratio for the risk of a large-for-gestational-age newborn in the adjusted model: 0.86 (95% CI 0.76 to 0.98) per SDS increase in dietary glycemic index). No associations of maternal dietary glycemic load within a low-to-normal range with adverse birth outcomes were present in the basic or adjusted models. When we analyzed maternal dietary glycemic index and load in quartiles, similar findings were present (**Supplementary Table S3.1.6** and **S3.1.7**).

TABLE 3.1.2. Associations of maternal early-pregnancy dietary glycemic index and load with fetal growth and birth characteristics[a]

	Difference in head circumference SDS (95% CI)	Difference in abdominal circumference SDS (95% CI)	Difference in length SDS (95% CI)	Difference in weight SDS (95% CI)
Maternal early-pregnancy glycemic index (SDS)				
		Mid-pregnancy		
	n=3351	*n=3354*	*n=3352*	*n=3336*
Basic model[b]	-0.02 (-0.06 to 0.02)	-0.01 (-0.04 to 0.02)	0.02 (-0.01 to 0.06)	0.01 (-0.03 to 0.04)
Adjusted model[c]	-0.01 (-0.05 to 0.02)	0.00 (-0.04 to 0.03)	0.02 (-0.02 to 0.05)	0.01 (-0.03 to 0.04)
		Late-pregnancy		
	n=3365	*n=3391*	*n=3400*	*n=3387*
Basic model[b]	-0.03 (-0.06 to 0.01)	0.01 (-0.03 to 0.04)	-0.02 (-0.05 to 0.02)	0.00 (-0.03 to 0.04)
Adjusted model[c]	-0.02 (-0.05 to 0.02)	0.02 (-0.02 to 0.05)	0.00 (-0.04 to 0.03)	0.01 (-0.02 to 0.05)
		Birth		
	n=1942		*n=2323*	*n=3456*
Basic model[b]	-0.03 (-0.08 to 0.02)	n.a.	-0.02 (-0.07 to 0.02)	-0.03 (-0.06 to 0.00)
Adjusted model[c]	-0.02 (-0.07 to 0.03)	n.a.	-0.01 (-0.06 to 0.04)	-0.02 (-0.06 to 0.01)
	Difference in head circumference SDS (95% CI)	Difference in abdominal circumference SDS (95% CI)	Difference in length SDS (95% CI)	Difference in weight SDS (95% CI)
Maternal early-pregnancy glycemic load (SDS)				
		Mid-pregnancy		
	n=3351	*n=3354*	*n=3352*	*n=3336*
Basic model[b]	0.01 (-0.02 to 0.05)	0.01 (-0.02 to 0.05)	0.02 (-0.01 to 0.05)	0.02 (-0.01 to 0.05)
Adjusted model[c]	0.01 (-0.06 to 0.08)	0.03 (-0.04 to 0.10)	0.06 (-0.01 to 0.12)	0.06 (-0.01 to 0.12)
		Late-pregnancy		
	n=3365	*n=3391*	*n=3400*	*n=3387*
Basic model[b]	0.00 (-0.03 to 0.04)	0.03 (0.00 to 0.07)	0.01 (-0.03 to 0.04)	0.03 (0.00 to 0.07)
Adjusted model[c]	-0.02 (-0.09 to 0.05)	0.08 (0.01 to 0.15)*	0.00 (-0.07 to 0.07)	0.07 (0.00 to 0.14)
		Birth		
	n=1942		*n=2323*	*n=3456*
Basic model[b]	0.01 (-0.04 to 0.06)	n.a.	0.02 (-0.03 to 0.06)	0.02 (-0.02 to 0.05)
Adjusted model[c]	0.04 (-0.06 to 0.14)	n.a.	0.00 (-0.09 to 0.09)	-0.01 (-0.07 to 0.06)

*n.a.: not available *P-value<0.05*

[a]Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in standard deviation score of fetal growth and birth characteristics per one increase in standard deviation of maternal dietary glycemic index and load intake during early-pregnancy.

[b]Basic models were adjusted for gestational age at study enrolment

[c]Adjusted models were the basic models additionally adjusted for maternal age, parity, educational level, prepregnancy BMI, early-pregnancy total daily energy intake, smoking during pregnancy, alcohol use during pregnancy, daily nausea and vomiting during early-pregnancy and fetal sex

TABLE 3.1.3. Associations of maternal early-pregnancy dietary glycemic index and load with the risks of adverse birth outcomes[a]

	Preterm birth OR (95% CI) *(Ncases=162)*	Small-for-gestational age at birth OR (95% CI) *(Ncases =345)*	Large-for-gestational age at birth OR (95% CI) *(Ncases =345)*	Caesarian delivery OR (95% CI) *(Ncases =410)*
Maternal early-pregnancy glycemic index (SDS)				
Basic model[b]	1.13 (0.97 to 1.32)	1.04 (0.93 to 1.16)	0.86 (0.77 to 0.96)*	0.93 (0.84 to 1.04)
Adjusted model[c]	1.12 (0.95 to 1.32)	1.01 (0.90 to 1.14)	0.86 (0.76 to 0.97)*	0.98 (0.89 to 1.11)
Maternal early-pregnancy glycemic load (SDS)				
Basic model[b]	1.01 (0.86 to 1.18)	1.03 (0.92 to 1.15)	0.94 (0.84 to 1.05)	0.93 (0.84 to 1.03)
Adjusted model[c]	1.26 (0.92 to 1.71)	0.97 (0.78 to 1.21)	0.80 (0.64 to 1.02)	1.01 (0.82 to 1.25)

**P-value<0.05.*

[a]Values are odds ratios (95% Confidence Interval) obtained from logistic regression analysis reflecting the differences in odds of adverse birth outcomes per standard deviation change of maternal dietary glycemic index and glycemic load intake during early-pregnancy.

[b]Basic models were adjusted for gestational age at study enrolment

[c]Adjusted models were the basic models additionally adjusted for maternal age, parity, educational level, prepregnancy BMI, early-pregnancy total daily energy intake, smoking during pregnancy, alcohol use during pregnancy, daily nausea and vomiting during early-pregnancy and fetal sex

Sensitivity analyses

In a secondary analysis, no associations of a maternal low-glycemic index diet, based on comparison to individual food product classifications, as compared to a normal-glycemic index diet with fetal growth characteristics or the risks of adverse birth outcomes were present (results not shown). When we excluded women with gestational diabetes, we observed similar results (results not shown). When we repeated the analyses among normal weight women or women aged < 35 years, we observed largely similar effect estimates (**Supplementary Tables S3.1.8** and **S3.1.9**).

DISCUSSION

Among pregnant women without an impaired glucose metabolism, we observed that maternal early pregnancy dietary glycemic index across was not associated with fetal growth parameters, whereas a higher maternal early pregnancy dietary glycemic load was associated with a higher fetal abdominal circumference and estimated fetal weight in late-pregnancy only. A higher glycemic index, but not load, was associated with a lower risk of a large-for-gestational-age newborn.

Interpretation of main findings

There is increasing interest in targeting maternal dietary glycemic index and load during pregnancy as a lifestyle intervention to improve pregnancy and birth outcomes. Small interventions studies among pregnant women with gestational diabetes, impaired glucose tolerance or obesity, have already shown that a lower glycemic index diet from the second half of pregnancy onwards improves maternal glucose concentrations and lowers the risk of delivering a large-for-gestational-age newborn (19, 34). With dietary interventions, these studies achieved a median maternal dietary glycemic index around 50 or lower in their intervention groups and compared these effects to a normal or high maternal dietary glycemic index. Far less is known about the effects of maternal dietary glycemic index and load on birth outcomes among populations not at risk for an impaired glucose metabolism.

A few previous studies focused on the associations of maternal dietary glycemic index and load with birth characteristics and the risks of adverse birth outcomes among general, healthy populations, but no studies focused on directly measured fetal growth characteristics (9, 17, 18, 30). These studies differed strongly with regards to the methods used to calculate maternal dietary glycemic index and load, the timing of the dietary assessments, studied populations, and adjustment for maternal socio-demographic and lifestyle characteristics. An observational study among 47,003 Danish pregnant women reported that a higher maternal dietary glycemic load, but not index, in mid-pregnancy was associated with a higher birthweight and an increased risk of delivering a large-for-gestational-age newborn (30). A study among 1,082 multi-ethnic non-diabetic pregnant women from USA showed that the lowest quintile of maternal mid-pregnancy dietary glycemic index, but not load, was associated with a lower birthweight and an increased risk of delivering a small-for-gestational-age infant. Using white bread instead of glucose as a reference, the glycemic index in this study varied < 71 for the lowest quintile to > 85 for the highest quintile. No associations of the highest quintile of maternal mid-pregnancy dietary glycemic index or load with a higher birthweight and increased risk of delivering a large-for gestational-age-infant were observed (9). Contrarily, a study among 842 low-risk Irish pregnant women reported no associations of maternal dietary glycemic index and load in early pregnancy continuously with birthweight or adverse birth outcomes, after adjusting for maternal age, pre-pregnancy BMI, and parity and considering multiple testing (17). Similarly, a study among 906 low-risk pregnant women from the UK showed no associations of maternal early or late-pregnancy dietary glycemic index and load continuously with fat and lean mass at birth (18). The mean and variability of the glycemic index in these two studies were comparable to ours.

In line with these previous studies focused on maternal early pregnancy dietary glycemic index and load, we observed that women within our study consumed diet with a relatively low mean dietary glycemic index. No consistent associations of maternal early pregnancy dietary glycemic index and load across the low-to-normal range with birthweight and the risks of adverse birth outcomes were observed. We did observe that a higher maternal early pregnancy dietary glycemic load, especially within the highest quartile, was associated with a higher late-pregnancy fetal abdominal circumference and estimated fetal weight, but findings were not consistent across pregnancy and may reflect a chance finding. However, fetal fat development mainly occurs in late-pregnancy and abdominal circumference is an important indicator of fetal fat deposition (35). This could suggest that a higher maternal early pregnancy dietary glycemic load may rather affect fetal body composition than growth, which is also suggested by the previous studies conducted in infants (36, 37).

Contrary to our prior hypothesis, we observed that a higher maternal early pregnancy dietary glycemic index within a low-to-normal range was associated with lower fetal length growth rates from late-pregnancy onwards and with a lower risk of delivering a large-for-gestational-age newborn only. These association were not explained by maternal socio-demographic and lifestyle characteristics. It could reflect a chance finding. Our study population is a relatively healthy population not at high risk of an impaired glucose tolerance. We only included Dutch women without pre-gestational diabetes and we observed largely similar results for women with a normal weight, younger than 35 years old, and without gestational diabetes. Possibly, the range of maternal dietary glycemic index within our population reflects a relatively healthy range for women at a low risk of an impaired glucose metabolism in early pregnancy. Maternal dietary glycemic index within this range may be not related to increased risks of fetal undergrowth or overgrowth. The timing of dietary glycemic index assessment in early pregnancy may also be important. Maternal insulin sensitivity is much higher in early pregnancy as compared to mid- and late-pregnancy, which leads to smaller fluctuations in postprandial glycemic responses to carbohydrate containing foods in early pregnancy (5). Potential adverse effects of a higher maternal dietary glycemic index on fetal growth and the risk of macrosomia may be more pronounced in the second half of pregnancy, when pregnant women are physiologically more insulin resistant and the postprandial glycemic response shows larger fluctuations. Finally, postprandial peaks in maternal glucose concentrations and subsequent peak increases in fetal glucose concentrations may rather have an effect on fetal body composition and fetal metabolism than on skeletal growth, by affecting fetal development of adipocytes and the cardio-metabolic system (18, 36, 37). This hypothesis is supported by the associations which we observed of a higher maternal early pregnancy glycemic load with fetal abdominal circumference

and estimated fetal weight in late-pregnancy when fetal fat accumulation occurs. Further studies using multiple assessments of dietary intake throughout pregnancy are needed to examine the detailed associations of maternal dietary glycemic index and load with both fetal and neonatal growth and body composition.

Importantly, in a secondary analysis, we observed no increased risks of preterm birth, small-for-gestational-age at birth, or caesarian delivery, as complication of abnormal fetal growth, among women consuming a low-glycemic index diet, as compared to women consuming a normal-glycemic index diet. The mean dietary glycemic index of women consuming a low-glycemic index diet within our study was largely similar to the mean dietary glycemic index reported in intervention studies stimulating a low-glycemic index diet through advising low-glycemic index food products (19, 21). This suggests that even among pregnant populations without an impaired glucose metabolism, a diet with lower glycemic index products in early pregnancy does not appear to be associated with fetal growth restriction and related adverse birth outcomes. These findings are important from a public health perspective, as there is an increasing interest in stimulating a diet with low-glycemic index products during pregnancy to improve birth and childhood outcomes. Our findings suggest that adhering to a diet with low-glycemic index products may be a safe intervention during pregnancy without adverse effects on fetal growth and birth outcomes in women without an impaired glucose metabolism. The beneficial effects of a lower dietary glycemic index and load within general, healthy populations on fetal growth and birth outcomes remain to be determined.

Strengths and limitations

Strengths of this study were the prospective study design, large sample size, and repeatedly measured fetal growth data from mid-pregnancy onwards available. Limitations of this study should also be taken into account when interpreting results. First, the response rate at baseline for participating in the Generation R study cohort was 61%. The non-response would have led to biased effect estimates if the associations were different between those included and not included in the analyses. However, this seems unlikely because biased estimates in large cohort studies often arise from loss to follow-up rather than from non-response at baseline (38). Second, we did not have information on previous gestational diabetes or polycystic ovarian syndrome, which are also associated with an increased risk of an impaired glucose metabolism. Although we expect the number of cases of previous gestational diabetes and polycystic ovarian syndrome to be low, as we had a relatively healthy population, this may have affected our results. Further studies excluding these women should replicate our findings. The

selection towards a relatively healthy Dutch population may affect the generalizability of our findings and might have led to reduced statistical power. Most women had a dietary glycemic index and load within the normal range and the number of adverse birth outcomes was also relatively low. Further studies are needed among multi-ethnic populations with a more diverse dietary intake to replicate our findings. Third, even though the FFQ is widely used for dietary assessment in observational studies, measurement of food intake by an FFQ may be affected by measurement error, recall bias, and reporting bias. Subsequent calculation of the dietary glycemic index and load from the FFQ may further be affected by uncertainty induced by preparation of foods, mixed dishes, variations of food products of time, or unavailability of specific food products (20). Fourth, we obtained information on maternal dietary intake only once during pregnancy. Further studies from preconception onwards are needed using repeated assessments of maternal dietary intake prior and throughout pregnancy to obtain further insight into critical periods for the influence of maternal dietary glycemic quality and quantity on embryonic and fetal development and adverse birth outcomes. Finally, although were able to adjust for multiple confounding factors, there might still be residual confounding as in any observational study.

CONCLUSION

Among pregnant women without an impaired glucose metabolism, a higher maternal early pregnancy dietary glycemic load was associated with a higher fetal abdominal circumference and estimated fetal weight in late-pregnancy. Maternal dietary glycemic index and load were not consistently associated with fetal growth parameters in mid-pregnancy and at birth. A higher glycemic index was associated with a lower risk of a large-for-gestational-age newborn. Further studies with a larger variability in maternal dietary glycemic index and load among multi-ethnic low-risk populations are needed to assess whether a lower glycemic index diet is a feasible lifestyle intervention to improve fetal growth and birth outcomes.

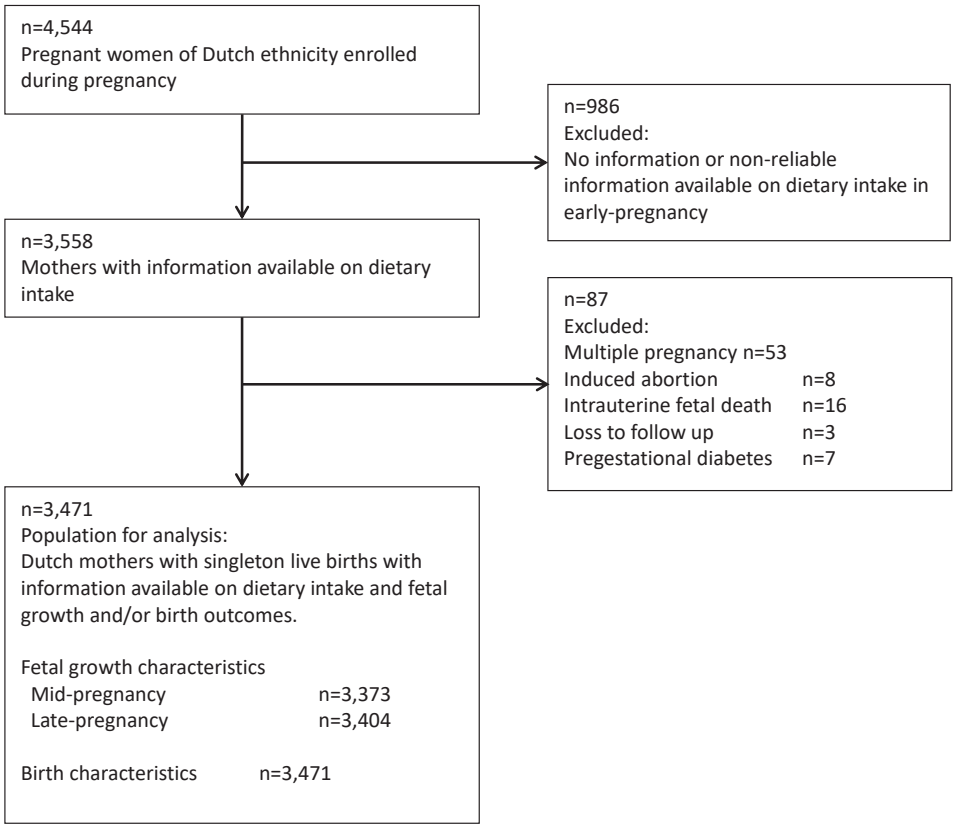
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SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIGURE S3.1.1. Flow chart of the study population

SUPPLEMENTARY TABLE S3.1.1. Fetal growth characteristics according to maternal dietary glycemic index quartiles

	Total group	Glycemic index quartile 1	Glycemic index quartile 2	Glycemic index quartile 3	Glycemic index quartile 4	P-value[a]
Mid-pregnancy						
Gestational age at ultrasound measurement, mean (SD)	20.6 (1.1)	20.6 (1.1)	20.6 (1.1)	20.6 (1.0)	20.7 (1.1)	0.13
Head circumference, mean (SD), mm	179 (14)	180 (14)	179 (14)	179 (13)	180 (13)	0.56
Abdominal circumference, mean (SD), mm	157 (14)	157 (15)	157 (13)	156 (14)	158 (14)	0.22
Femur length, mean (SD), mm	33 (3)	33 (3)	33 (3)	33 (3)_	34 (3)	0.43
Estimated fetal weight, mean (SD), g	379 (87)	381 (93)	376 (84)	378 (84)	383 (86)	0.35
Late-pregnancy						
Gestational age at ultrasound measurement, mean (SD)	30.4 (1.0)	30.5 (1.0)	30.5 (1.0)	30.4 (1.0)	30.4 (1.1)	0.12
Head circumference, mean (SD), mm	286 (12)	287 (12)	286 (12)	285 (13)	285 (12)	0.10
Abdominal circumference, mean (SD), mm	265 (16)	266 (16)	265 (16)	265 (17)	265 (17)	0.29
Femur length, mean (SD), mm	57 (3)	58 (3)	57 (3)	57 (3)	57 (3)	0.27
Estimated fetal weight, mean (SD), g	1633 (259)	1646 (249)	1627 (258)	1630 (269)	1628 (259)	0.39

[a]*P-values were obtained by ANOVA-tests*

SUPPLEMENTARY TABLE S3.1.2. Non-response analysis for women with a singleton live birth and information dietary intake during early-pregnancy, compared to women with a singleton live birth without data on dietary intake during early-pregnancy

	Women with information on dietary glycemic index (n=3,471)	Women without information on dietary glycemic index (n=924)	P-value[a]
Maternal characteristics			
Maternal age at enrolment, mean (SD), years	31.4 (4.4)	31.7 (5.2)	0.09
Parity, n nulliparous (%)	2,076 (59.9)	379 (46.1)	<0.01
Pre-pregnancy BMI group, n (%)			0.08
Underweight	25 (5.7)	25 (5.8)	
Normal weight	306 (70.2)	305 (71.1)	
Overweight	72 (16.5)	70 (16.3)	
Obese	33 (7.6)	29 (6.8)	
Gestational weight gain, mean (SD), g/week	10.8 (4.4)	11.2 (4.8)	0.08
Education, n high (%)	2,026 (59.1)	464 (52.5)	<0.01
Folic acid supplement use, n yes (%)	2,532 (72.9)	342 (82.0)	<0.01
Alcohol use during pregnancy, yes (%)	2,117 (66.3)	275 (58.8)	<0.01
Smoking during pregnancy, n (%)	833 (26.9)	213 (26.1)	0.19
Nausea during early-pregnancy, n (%)	880 (27.7)	141 (30.7)	0.23
Vomiting during early-pregnancy, n (%)	145 (4.6)	37 (8.1)	<0.01
Gestational diabetes, n (%)	31 (0.9)	13 (1.5)	0.12
Child characteristics			
Sex, n (%) male	1,753 (50.5)	493 (51.5)	0.58
Gestational age at birth, median (95%)	40.3 (37.0, 42.1)	40.0 (36.7, 42.0)	<0.01
Birthweight, mean (SD), g	3,489 (554)	3445 (593)	0.04

BMI: body mass index.

[a]P-values were obtained by independent t-test or Mann-Whitney U-test for continuous variables and chi-square tests for categorical variables.

SUPPLEMENTARY TABLE S3.1.3. Regression coefficients of longitudinal associations between maternal dietary glycemic index quartiles with fetal growth patterns[a]

	Intercept Head circumference (SDS)	Slope Head circumference (SDS)	Intercept Length (SDS)	Slope Length (SDS)	Intercept Weight (SDS)	Slope Weight (SDS)
Maternal dietary glycemic index						
Quartile 1	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Quartile 2	0.096 (-0.153 to 0.281)	-0.004 (-0.011 to 0.003)	0.229 (0.029 to 0.429)	-0.009 (-0.012 to -0.005)	0.007 (-0.167 to 0.180)	-0.002 (-0.007 to 0.003)
Quartile 3	0.108 (-0.111 to 0.0.326)	-0.004 (-0.011 to 0.004)	0.300 (0.100 to 0.500)	-0.009 (-0.015 to -0.025)	0.103 (-0.071 to 0.278)	-0.003 (-0.008 to 0.002)
Quartile 4	0.018 (-0.200 to 0.235)	-0.003 (-0.010 to 0.004)	0.200 (-0.001 to 0.400)	-0.008 (-0.015 to -0.002)	0.076 (-0.100 to 0.251)	-0.004 (-0.009 to 0.001)
Maternal dietary glycemic load						
Quartile 1	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Quartile 2	0.103 (-0.117 to 0.323)	-0.003 (-0.010 to 0.004)	-0.149 (-0.352 to 0.054)	0.008 (0.001 to 0.012)	0.004 (-0.173 to 0.182)	0.003 (-0.002 to 0.009)
Quartile 3	0.168 (-0.059 to 0.394)	-0.004 (-0.011 to 0.003)	-0.104 (-0.314 to 0.107)	0.006 (0.000 to 0.013)	0.032 (-0.153 to 0.218)	0.001 (-0.004 to 0.006)
Quartile 4	0.162 (-0.080 to 0.405)	-0.004 (-0.011 to 0.003)	0.078 (-0.149 to 0.304)	0.001 (-0.006 to 0.007)	0.137 (-0.067 to 0.342)	0.001 (-0.005 to 0.006)

[a]*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 per quartile of dietary glycemic index and glycemic load intake during early-pregnancy, compared with the lowest quartile of maternal dietary glycemic index and load as the reference group adjusted for gestational age at study enrolment, maternal age, parity, pre-pregnancy BMI, maternal education, smoking during pregnancy, alcohol use during pregnancy, nausea during early-pregnancy, vomiting during early-pregnancy, early-pregnancy total daily energy intake and fetal sex*

SUPPLEMENTARY TABLE S3.1.4. Associations of maternal dietary glycemic index in quartiles with fetal and birth characteristics[a]

Maternal dietary glycemic index	Head circumference (SDS)		Abdominal circumference (SDS)		Length (SDS)		Weight (SDS)	
	Basic model[b]	Adjusted model[c]	Basic model[b]	Adjusted model[c]	Basic model[b]	Adjusted model[c]	Basic model[b]	Adjusted model[c]
				Mid pregnancy				
Quartile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quartile 2	0.04 (-0.06 to 0.14)	0.03 (-0.07 to 0.12)	-0.06 (-0.16 to 0.04)	-0.06 (-0.16 to 0.04)	0.02 (-0.07 to 0.12)	0.02 (-0.08 to 0.11)	-0.04 (-0.14 to 0.06)	-0.04 (-0.14 to 0.05)
Quartile 3	0.03 (-0.07 to 0.13)	0.05 (-0.05 to 0.15)	-0.04 (-0.14 to 0.06)	-0.03 (-0.12 to 0.07)	0.15 (0.05 to 0.24)	0.12 (0.02 to 0.22)*	0.05 (-0.05 to 0.15)	0.05 (-0.05 to 0.14)
Quartile 4	-0.05 (-0.15 to 0.05)	-0.04 (-0.14 to 0.06)	-0.05 (-0.15 to 0.04)	-0.03 (-0.13 to 0.07)	0.03 (-0.06 to 0.13)	0.02 (-0.08 to 0.11)	-0.02 (-0.11 to 0.08)	-0.01 (-0.11 to 0.09)
				Late pregnancy				
Quartile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quartile 2	-0.04 (-0.14 to 0.06)	-0.06 (-0.15 to 0.04)	-0.09 (-0.19 to 0.01)	-0.09 (-0.19 to 0.01)	-0.03 (-0.13 to 0.06)	-0.02 (-0.12 to 0.07)	-0.08 (-0.18 to 0.02)	-0.08 (-0.17 to 0.02)
Quartile 3	-0.03 (-0.12 to 0.07)	0.00 (-0.10 to 0.09)	-.0.01 (-0.11 to 0.09)	0.01 (-0.10 to 0.09)	0.03 (-0.07 to 0.12)	0.03 (-0.07 to 0.12)	0.01 (-0.09 to 0.11)	0.01 (-0.08 to 0.11)
Quartile 4	-0.09 (-0.19 to 0.00)	-0.07 (-0.17 to 0.03)	-0.04 (-0.13 to 0.06)	-0.02 (-0.11 to 0.08)	-0.07 (-0.17 to 0.02)	-0.05 (-0.15 to 0.04)	-0.06 (-0.15 to 0.04)	-0.03 (-0.13 to 0.07)
				Birth				
Quartile 1	Ref.	Ref.	n.a.	n.a.	Ref.	Ref.	Ref.	Ref.
Quartile 2	-0.03 (-0.17 to 0.12)	-0.02 (-0.16 to 0.12)	n.a.	n.a.	-0.19 (-0.32 to -0.06)*	-0.19 (-0.32 to -0.06)*	-0.07 (-0.16 to 0.03)	-0.07 (-0.16 to 0.03)
Quartile 3	-0.07 (-0.21 to 0.07)	-0.07 (-0.21 to 0.08)	n.a.	n.a.	-0.08 (-0.21 to 0.05)	-0.07 (-0.20 to 0.06)	0.00 (-0.10 to 0.10)	-0.01 (-0.11 to 0.08)
Quartile 4	-0.09 (-0.23 to 0.05)	-0.07 (-0.22 to 0.07)	n.a.	n.a.	-0.13 (-0.26 to 0.00)*	-0.09 (-0.23 to 0.04)	-0.09 (-0.18 to 0.01)	-0.07 (-0.16 to 0.03)

**P<0.05.*

[a]Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in standard deviation change of fetal and birth growth parameters per quartile of maternal dietary glycemic index during early-pregnancy as compared to the lowest quartile

[b]Basic models were adjusted for gestational age at study enrolment

[c]Adjusted models were the basic models additionally adjusted for maternal age, parity, educational level, prepregnancy BMI, early-pregnancy total daily energy intake, smoking during pregnancy, alcohol use during pregnancy, daily nausea and vomiting during early-pregnancy and fetal sex

SUPPLEMENTARY TABLE S3.1.5. Associations of maternal dietary glycemic load in quartiles with fetal and birth characteristicsa

Maternal dietary glycemic load	Head circumference (SDS)		Abdominal circumference (SDS)		Length (SDS)		Weight (SDS)	
	Basic model[b]	Adjusted model[c]	Basic model[b]	Adjusted model[c]	Basic model[b]	Adjusted model[c]	Basic model[b]	Adjusted model[c]
				Mid pregnancy				
Quartile 1	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Quartile 2	0.06 (-0.04 to 0.15)	0.07 (-0.05 to 0.18)	0.13 (0.03 to 0.22)*	0.15 (0.04 to 0.26)*	0.03 (-0.06 to 0.13)	0.06 (-0.05 to 0.16)	0.10 (0.01 to 0.20)*	0.13 (0.03 to 0.24)*
Quartile 3	0.09 (-0.01 to 0.19)	0.09 (-0.01 to 0.25)	0.06 (-0.04 to 0.15)	0.10 (-0.02 to 0.23)	0.02 (-0.08 to 0.11)	0.07 (-0.06 to 0.16)	0.04 (-0.05 to 0.14)	0.11 (-0.02 to 0.23)
Quartile 4	0.06 (-0.04 to 0.16)	0.10 (-0.07 to 0.30)	0.09 (-0.01 to 0.19)	0.19 (0.02 to 0.35)*	0.06 (-0.03 to 0.16)	0.14 (-0.03 to 0.30)	0.10 (0.00 to 0.19)*	0.21 (0.04 to 0.37)*
				Late pregnancy				
Quartile 1	Ref.	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Quartile 2	0.07 (-0.03 to 0.17)	0.04 (-0.07 to 0.14)	0.07 (-0.02 to 0.17)	0.07 (-0.04 to 0.18)	0.09 (-0.01 to 0.18)	0.08 (-0.03 to 0.18)	0.09 (-0.01 to 0.19)	0.09 (-0.02 to 0.20)
Quartile 3	0.07 (-0.03 to 0.17)	0.03 (-0.09 to 0.16)	0.03 (-0.07 to 0.13)	0.05 (-0.07 to 0.18)	0.05 (-0.04 to 0.14)	0.06 (-0.06 to 0.18)	0.04 (-0.05 to 0.14)	0.07 (-0.06 to 0.20)
Quartile 4	0.01 (-0.08 to 0.11)	-0.01 (-0.17 to 0.15)	0.10 (0.00 to 0.19)	0.16 (0.00 to 0.33)*	0.05 (-0.05 to 0.14)	0.07 (-0.09 to 0.23)	0.10 (0.00 to 0.20)	0.17 (0.00 to 0.34)*
				Birth				
Quartile 1	*Ref.*	*Ref.*	n.a.	n.a.	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Quartile 2	-0.02 (-0.16 to 0.12)	-0.05 (-0.20 to 0.11)	n.a.	n.a.	0.19 (0.06 to 0.32)*	0.14 (-0.01 to 0.28)	0.16 (0.06 to 0.25)*	0.11 (0.00 to 0.21)
Quartile 3	0.00 (-0.15 to 0.14)	-0.02 (-0.21 to 0.17)	n.a.	n.a.	0.17 (0.03 to 0.30)*	0.13 (-0.05 to 0.30)	0.06 (-0.04 to 0.15)	0.02 (-0.10 to 0.14)
Quartile 4	-0.02 (-0.16 to 0.12)	-0.03 (-0.27 to 0.22)	n.a.	n.a.	0.08 (-0.05 to 0.21)	0.06 (-0.17 to 0.28)	0.10 (0.00 to 0.20)*	0.07 (-0.09 to 0.23)

**P<0.05.*

[a]Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in standard deviation change of fetal and birth growth parameters per quartile of maternal dietary glycemic load during early-pregnancy as compared to the lowest quartile

[b]Basic models were adjusted for gestational age at study enrolment

[c]Adjusted models were the basic models additionally adjusted for maternal age, parity, educational level, prepregnancy BMI, early-pregnancy total daily energy intake, smoking during pregnancy, alcohol use during pregnancy, daily nausea and vomiting during early-pregnancy and fetal sex

SUPPLEMENTARY TABLE S3.1.6. Associations of maternal dietary glycemic index in quartiles with the risk of adverse birth outcomes[a]

Maternal dietary glycemic index	Preterm birth OR (95% CI) *(Ncases=162)*	Small-for-gestational age at birth OR (95% CI) *(Ncases =345)*	Large-for-gestational age at birth OR (95% CI) *(Ncases =345)*	Caesarian section OR (95% CI) *(Ncases =410)*
Basic model[b]				
Quartile 1	*Ref.*	*Ref.*	*Ref.*	*Ref*
Quartile 2	0.78 (0.49 to 1.23)	0.96 (0.70 to 1.33)	0.79 (0.58 to 1.07)	0.83 (0.62 to 1.11)
Quartile 3	0.68 (0.43 to 1.10)	1.03 (0.75 to 1.41)	0.83 (0.62 to 1.12)	0.78 (0.58 to 1.04)
Quartile 4	1.30 (0.87 to 1.97)	1.07 (0.79 to 1.47)	0.60 (0.43 to 0.83)*	0.84 (0.63 to 1.12)
Adjusted model[c]				
Quartile 1	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Quartile 2	0.76 (0.48 to 1.21)	0.95 (0.69 to 1.32)	0.77 (0.57 to 1.06)	0.86 (0.64 to 1.15)
Quartile 3	0.68 (0.42 to 1.11)	1.03 (0.74 to 1.42)	0.81 (0.60 to 1.11)	0.85 (0.63 to 1.15)
Quartile 4	1.28 (0.83 to 1.98)	1.01 (0.73 to 1.41)	0.60 (0.43 to 0.84)*	1.00 (0.74 to 1.135)

**P<0.05.*

[a]Values are odds ratios (95% Confidence Interval) obtained from logistic regression analysis reflecting the differences in odds of adverse birth outcomes per quartile of maternal dietary glycemic index during early-pregnancy as compared to the lowest quartile

[b]Basic models were adjusted for gestational age at study enrolment

[c]Adjusted models were the basic models additionally adjusted for maternal age, parity, educational level, prepregnancy BMI, early-pregnancy total daily energy intake, smoking during pregnancy, alcohol use during pregnancy, daily nausea and vomiting during early-pregnancy and fetal sex

SUPPLEMENTARY TABLE S3.1.7. Associations of maternal dietary glycemic load in quartiles with the risk of adverse birth outcomes[a]

Maternal dietary glycemic load	Preterm birth OR (95% CI) *(Ncases=162)*	Small-for-gestational age at birth OR (95% CI) *(Ncases =345)*	Large-for-gestational age at birth OR (95% CI) *(Ncases =345)*	Caesarian section OR (95% CI) *(Ncases =410)*
Basic model[b]				
Quartile 1	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Quartile 2	0.71 (0.45 to 1.13)	0.79 (0.58 to 1.09)	1.10 (0.81 to 1.50)	0.85 (0.64 to 1.13)
Quartile 3	0.98 (0.63 to 1.50)	0.89 (0.65 to 1.22)	0.88 (0.63 to 1.21)	0.74 (0.55 to 0.99)
Quartile 4	0.96 (0.62 to 1.48)	1.01 (0.75 to 1.38)	0.99 (0.72 to 1.35)	0.80 (0.60 to 1.07)
Adjusted model[c]				
Quartile 1	*Ref.*	*Ref.*	*Ref.*	*Ref.*
Quartile 2	0.88 (0.52 to 1.48)	0.80 (0.56 to 1.16)	1.00 (0.70 to 1.42)	0.87 (0.62 to 1.20)
Quartile 3	1.34 (0.75 to 2.39)	0.82 (0.54 to 1.25)	0.80 (0.52 to 1.23)	0.76 (0.51 to 1.12)
Quartile 4	1.55 (0.73 to 3.33)	0.85 (0.49 to 1.46)	0.90 (0.52 to 1.57)	0.90 (0.53 to 1.50)

**P<0.05.*

[a]Values are odds ratios (95% Confidence Interval) obtained from logistic regression analysis reflecting the differences in odds of adverse birth outcomes per quartile of maternal dietary glycemic load during early-pregnancy as compared to the lowest quartile

[b]Basic models were adjusted for gestational age at study enrolment

[c]Adjusted models were the basic models additionally adjusted for maternal age, parity, educational level, prepregnancy BMI, early-pregnancy total daily energy intake, smoking during pregnancy, alcohol use during pregnancy, daily nausea and vomiting during early-pregnancy and fetal sex

SUPPLEMENTARY TABLE S3.1.8. Associations of maternal dietary glycemic index and load in women with a BMI <25 kg/m^2 with fetal and birth parameters

	Difference in head circumference SDS (95% CI)	Difference in abdominal circumference SDS (95% CI)	Difference in length SDS (95% CI)	Difference in weight SDS (95% CI)
Maternal early-pregnancy glycemic index (SDS)				
		Mid-pregnancy		
	n=2,533	*n=2,538*	*n=2,539*	*n=2,528*
Basic model[b]	-0.01 (-0.05 to 0.03)	0.00 (-0.04 to 0.04)	0.02 (-0.01 to 0.06)	0.02 (-0.02 to 0.06)
Adjusted model[c]	-0.01 (-0.05 to 0.04)	0.01 (-0.03 to 0.05)	0.02 (-0.02 to 0.05)	0.02 (-0.02 to 0.06)
		Late-pregnancy		
	n=2,535	*n=2,559*	*n=2,563*	*n=2,555*
Basic model[b]	-0.02 (-0.06 to 0.02)	0.00 (-0.04 to 0.04)	-0.01 (-0.05 to 0.03)	0.00 (-0.04 to 0.04)
Adjusted model[c]	-0.01 (-0.05 to 0.03)	0.02 (-0.02 to 0.06)	0.01 (-0.03 to 0.05)	0.02 (-0.02 to 0.07)
		Birth		
	n=1,479		*n=1,757*	*n=2,605*
Basic model[b]	-0.01 (-0.07 to 0.05)	n.a.	0.00 (-0.06 to 0.05)	-0.01 (-0.06 to 0.03)
Adjusted model[c]	-0.00 (-0.06 to 0.07)	n.a.	0.02 (-0.04 to 0.07)	-0.01 (-0.05 to 0.03)
Maternal early-pregnancy glycemic load (SDS)				
		Mid-pregnancy		
	n=2,533	*n=2,538*	*n=2,539*	*n=2,528*
Basic model[b]	0.03 (-0.01 to 0.08)	0.03 (-0.01 to 0.07)	0.03 (-0.01 to 0.07)	0.04 (-0.01 to 0.08)
Adjusted model[c]	0.04 (-0.04 to 0.12)	0.06 (-0.02 to 0.13)	0.09 (0.02 to 0.17)*	0.09 (0.02 to 0.17)*
		Late-pregnancy		
	n=2,535	*n=2,559*	*n=2,563*	*n=2,555*
Basic model[b]	0.02 (-0.03 to 0.06)	0.05 (0.01 to 0.08)*	0.01 (-0.03 to 0.05)	0.04 (0.00 to 0.08)*
Adjusted model[c]	0.01 (-0.07 to 0.09)	0.11 (0.03 to 0.19)*	0.03 (-0.05 to 0.10)	0.10 (0.02 to 0.18)*
		Birth		
	n=1,479		*n=1,757*	*n=2,605*
Basic model[b]	0.03 (-0.03 to 0.09)	n.a.	0.04 (-0.02 to 0.09)	0.02 (-0.02 to 0.06)
Adjusted model[c]	0.09 (-0.03 to 0.21)	n.a.	0.03 (-0.08 to 0.14)	0.03 (-0.05 to 0.11)

n.a.: not available

**P<0.05*

[a]Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in standard deviation score of fetal growth and birth characteristics per one increase in standard deviation of maternal dietary glycemic index and load intake during early-pregnancy

[b]Basic models were adjusted for gestational age at study enrolment

[c]Adjusted models were the basic models additionally adjusted for maternal age, parity, educational level, prepregnancy BMI, early-pregnancy total daily energy intake, smoking during pregnancy, alcohol use during pregnancy, daily nausea and vomiting during early-pregnancy and fetal sex

SUPPLEMENTARY TABLE S3.1.9. Associations of maternal dietary glycemic index and load in women aged <35 years with fetal and birth parameters

	Difference in head circumference SDS (95% CI)	Difference in abdominal circumference SDS (95% CI)	Difference in length SDS (95% CI)	Difference in weight SDS (95% CI)
Maternal early-pregnancy glycemic index (SDS)				
		Mid-pregnancy		
	n=2,751	*n=2,754*	*n=2,750*	*n=2,738*
Basic model[b]	-0.02 (-0.06 to 0.02)	0.01 (-0.05 to 0.02)	0.02 (-0.02 to 0.06)	0.00 (-0.04 to 0.04)
Adjusted model[c]	0.00 (-0.04 to 0.04)	0.00 (-0.04 to 0.04)	0.02 (-0.02 to 0.06)	0.01 (-0.03 to 0.05)
		Late-pregnancy		
	n=2,748	*n=2,771*	*n=2,781*	*n=2,769*
Basic model[b]	-0.03 (-0.06 to 0.01)	0.01 (-0.03 to 0.05)	-0.02 (-0.06 to 0.02)	0.00 (-0.04 to 0.04)
Adjusted model[c]	0.00 (-0.04 to 0.03)	0.03 (-0.01 to 0.07)	-0.01 (-0.04 to 0.03)	0.02 (-0.02 to 0.06)
		Birth		
	n=1,575		*n=1,903*	*n=2,824*
Basic model[b]	-0.02 (-0.07 to 0.04)	n.a.	-0.03 (-0.08 to 0.03)	0.00 (-0.04 to 0.04)
Adjusted model[c]	-0.01 (-0.07 to 0.05)	n.a.	0.00 (-0.06 to 0.05)	0.00 (-0.04 to 0.04)
Maternal early-pregnancy glycemic load (SDS)				
		Mid-pregnancy		
	n=2,751	*n=2,754*	*n=2,750*	*n=2,738*
Basic model[b]	0.01 (-0.03 to 0.05)	0.01 (-0.02 to 0.05)	0.01 (-0.03 to 0.05)	0.02 (-0.02 to 0.05)
Adjusted model[c]	0.02 (-0.06 to 0.10)	0.03 (-0.05 to 0.10)	0.07 (-0.01 to 0.14)	0.06 (-0.01 to 0.14)
		Late-pregnancy		
	n=2,748	*n=2,771*	*n=2,781*	*n=2,769*
Basic model[b]	0.00 (-0.04 to 0.03)	0.03 (0.01 to 0.06)*	0.00 (-0.04 to 0.04)	0.02 (-0.02 to 0.06)
Adjusted model[c]	0.00 (-0.07 to 0.07)	0.10 (0.03 to 0.18)*	0.02 (-0.05 to 0.09)	0.10 (0.02 to 0.17)*
		Birth		
	n=1,575		*n=1,903*	*n=2,824*
Basic model[b]	0.02 (-0.04 to 0.07)	n.a.	0.00 (-0.05 to 0.06)	0.01 (-0.03 to 0.05)
Adjusted model[c]	0.07 (-0.05 to 0.18)	n.a.	-0.01 (-0.11 to 0.10)	0.02 (-0.05 to 0.10)

*n.a.: not available *P<0.05*

[a]Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in standard deviation score of fetal growth and birth characteristics per one increase in standard deviation of maternal dietary glycemic index and load intake during early-pregnancy

[b]Basic models were adjusted for gestational age at study enrolment

[c]Adjusted models were the basic models additionally adjusted for maternal age, parity, educational level, prepregnancy BMI, early-pregnancy total daily energy intake, smoking during pregnancy, alcohol use during pregnancy, daily nausea and vomiting during early-pregnancy and fetal sex

3.2

Maternal dietary glycemic index and childhood general, abdominal and ectopic fat accumulation

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ABSTRACT

Background and aims: Maternal hyperglycemia during pregnancy is an important risk factor for childhood adiposity. Maternal dietary glycemic index during pregnancy directly influences maternal and fetal glucose concentrations. We examined the associations of maternal early-pregnancy dietary glycemic index with offspring general, abdominal and ectopic fat accumulation among normal weight and overweight or obese pregnant women and their offspring.

Methods: In a population-based cohort study among 2488 Dutch pregnant women and their children, we assessed maternal dietary glycemic index by food frequency questionnaire at median 13.4 (95% range 10.7 to 21.1) weeks gestation. Dietary glycemic index was used continuously and categorized into low (≤55), normal (56–69) and high (≥70) glycemic index diet. We measured offspring BMI, total fat mass and android/gynoid fat mass ratio by DXA, and visceral fat mass and liver fat fraction by MRI at 10 years.

Results: No associations of maternal early-pregnancy dietary glycemic index with offspring adiposity were present among women with a normal weight and their children. Among women with overweight or obesity and their children, 1-Standard Deviation Score (SDS) increase in maternal early-pregnancy dietary glycemic index was associated with higher childhood BMI (0.10 SDS, 95% Confidence Interval (CI) 0.01 to 0.19), total fat mass index (0.13 SDS, 95% CI 0.05 to 0.22), visceral fat mass index (0.19 SDS, 95% CI 0.07 to 0.32) and tended to be associated with a higher android/gynoid fat mass ratio (0.09 SDS, 95% CI -0.01 to 0.19) and higher risk of childhood overweight (Odds Ratio (OR) 1.20, 95% CI 0.97 to 1.48). Women with overweight or obesity consuming an early-pregnancy low-glycemic index diet, as compared to an early-pregnancy normal-glycemic index diet, had children with lower BMI, total fat mass index, visceral fat mass index and android/gynoid fat mass ratio at 10 years (p-values<0.05). No women consumed a high-glycemic index diet. No associations were explained by maternal socio-economic, lifestyle and dietary characteristics, birth or childhood characteristics. No associations with liver fat fraction were present.

Conclusions: In women with overweight or obesity and their children, a higher maternal early-pregnancy dietary glycemic index is associated with childhood general, abdominal and visceral fat accumulation, but not with liver fat. Intervention studies among overweight and obese pregnant women may need to target the dietary glycemic index to prevent childhood adiposity.

INTRODUCTION

Childhood overweight and obesity are major public health problems and associated with cardio-metabolic morbidity and premature mortality (1, 2) Childhood BMI is most commonly used as a measure to assess the risk of adverse cardio-metabolic health outcomes. However, ectopic fat accumulation, especially visceral fat and liver fat accumulation, are even more closely related to the development of an impaired glucose metabolism, low-grade systemic inflammation and dyslipidemia and subsequent cardio-metabolic diseases (3, 4).

Accumulating evidence suggests that fetal life is a critical period for establishing the risk of obesity and ectopic fat accumulation in later life (5, 6) Maternal nutrition is an important factor for fetal development as it directly influences fetal nutrient supply (7) The maternal dietary glycemic index and load influence maternal postprandial glucose concentrations, which is a main nutritional determinant of fetal growth, adipocyte development and metabolism (5, 6, 8). A maternal diet with a high glycemic index and load may increase glucose transfer to the fetus, adversely affecting fetal growth and body composition (9, 10). These effects may be even stronger among overweight or obese pregnant women, who are more likely to have an unhealthy diet and a suboptimal glucose metabolism (5). Animal studies have already shown that a maternal low-glycemic index diet during pregnancy reduces offspring weight and visceral and liver fat accumulation, but associations among humans are not known (11, 12). An observational study among 906 pregnant women and their offspring showed that a higher maternal dietary glycemic index during early-pregnancy, but not during late-pregnancy, was associated with a higher childhood total body fat mass (13).

We hypothesized that a higher maternal early-pregnancy dietary glycemic index and load, especially among women with overweight or obesity, may be modifiable risk factors for the development of obesity and ectopic fat accumulation in the offspring. Therefore, in a population-based prospective cohort study among 2488 pregnant women and their children, we examined the associations of maternal early-pregnancy dietary glycemic index and load with childhood BMI, total fat mass, android/gynoid fat mass ratio, visceral fat mass, liver fat and the risk of overweight at 10 years. We examined these associations among women with a normal weight and women with overweight or obesity and their offspring separately.

METHODS

Study design and study sample

This study was embedded in the Generation R study, a population based prospective birth cohort study in Rotterdam, the Netherlands (14). Written informed consent was obtained from all women. The study was approved by the local Medical Ethical Committee (MEC 198.782/2001/31). In total, 4096 Dutch women were enrolled during pregnancy of which 3558 women had information on dietary intake available. We excluded women with pregestational diabetes (n=7) and non-singleton live births (n=80). Of the remaining 3471 women and their offspring, 2488 participated in follow up measurements at 10 years (**Supplementary Figure S3.2.1**). Due to later implementation of MRI scans within follow-up visits, we only had measurements of visceral fat or liver fat fraction available within a subgroup of 1397 children.

Maternal dietary glycemic index and load

We obtained information on maternal dietary intake during early-pregnancy at a median of 13.4 weeks gestation (95% range 9.9 to 22.6) by a semi-quantitative 293-item Food Frequency Questionnaire (FFQ) (14). The FFQ was validated against three 24-h dietary recalls and biomarkers from blood samples in 80 pregnant women with Dutch ethnicity living in Rotterdam, the Netherlands, which is directly comparable to our study population. Energy-adjusted intra-class correlation coefficients for macronutrient intakes ranged from 0.41 to 0.88 and was 0.60 for carbohydrate intake (15). The average energy intake and carbohydrate intake was calculated using the Dutch Food Composition Table 2006 (16). To calculate maternal early-pregnancy dietary glycemic index and load, we assigned glycemic index values to each individual food item in the FFQ. We obtained glycemic index values from the glycemic index database on the Dutch diet published by the Medical Research Council Human Nutrition Research (MRC HNR), Cambridge, United Kingdom, using glucose as reference (glycemic index for glucose equal to 100). We calculated the mean glycemic index and load per day (17). We considered the dietary glycemic index as our main exposure, as the dietary glycemic index provides information on the quality of the glycemic response to a carbohydrate containing food product and is more often used in intervention studies and clinical settings (9, 18). We included dietary glycemic load as a secondary exposure, as this measure takes the amount of carbohydrate intake into account and therefore provides additional information on maternal postprandial glucose concentrations, but this measure may be more prone to measurement error (19-21). We constructed standard deviation scores to analyze maternal early-pregnancy dietary glycemic index and glycemic load across the full range.

In line with previous intervention studies, we also categorized the mean maternal early-pregnancy dietary glycemic index per day into a low-, normal-, and high-glycemic index diet, using similar cut-offs as used for individual food products (low-glycemic index diet (≤55), a normal-glycemic index diet (56–69) and a high-glycemic index diet (≥70)) (22, 23).

Childhood general, abdominal and ectopic fat

At the age of 10 years, we measured height and weight without shoes and heavy clothing and calculated Body Mass Index (BMI) (kg/m^2). Childhood BMI sex-and-age-adjusted standard (SDS) were constructed based on Dutch reference growth charts (Growth Analyzer 4.0, Dutch Growth Research Foundation) (24). We defined childhood overweight or obesity by categorizing childhood weight status according to the International Obesity Task Force cut-offs (25). Overweight and obesity were combined into one category and under- and normal weight were combined into one category. We measured total, android and gynoid body fat mass by Dual-Energy X-ray absorptiometry (DXA) (iDXA; General Electrics–Lunar, 2008, Madison, WI) and calculated android/gynoid fat mass ratio (26). Visceral fat and liver fat fraction were obtained from MRI scans as described previously (27). Childhood body fat mass is strongly influenced by height of the child (28). To enable assessment of the associations of maternal early-pregnancy dietary glycemic index and load with childhood adiposity outcomes independent of childhood size, we estimated the optimal adjustment for childhood height using log–log regressions (details in **Supplementary Methods 3.2.1**) (28, 29). We calculated total fat mass index (total fat mass/height^4) and visceral fat mass index (visceral fat mass/height^3).

Covariates

Information on maternal age, educational level, parity, folic acid supplement use, prepregnancy weight and diagnosis of pre-gestational diabetes were obtained through questionnaire at enrolment. Information on smoking, alcohol consumption, vomiting and maximum weight during pregnancy was obtained through questionnaires throughout pregnancy. Information on maternal dietary energy, fiber, fat and protein intake during pregnancy was obtained with the FFQ. As a proxy measurement of maternal overall diet quality, the Dietary Approaches to Stop Hypertension (DASH) diet score was derived from the FFQ (30-32). The score is composed of 8 food components, based mainly on the Fung method with a scoring system based on quintile rankings and included the intake of total grains, vegetables, fruits, non-full-fat dairy products, and nuts/seeds/legumes and the intake of red and processed meats, sugar-sweetened beverages/sweets/added sugars and sodium. A lower maternal DASH diet score characterizes a

lower overall dietary quality (32). We measured maternal height at enrolment and calculated prepregnancy body mass index (BMI) (14). Information on gestational diabetes, child's gestational age and weight at birth and sex were obtained from medical records. Information on breastfeeding and the timing of introduction to solid foods was assessed by questionnaires during infancy, and the average time watching television was assessed by a questionnaire at 10 years of age (14).

Statistical analyses

First, we performed a non-response analysis to compare characteristics of women with and without offspring follow-up measurements available. Second, we examined the associations of maternal early-pregnancy dietary glycemic index SDS and maternal dietary glycemic index categories with childhood BMI, total fat mass index, android/gynoid fat mass ratio, visceral fat mass index, liver fat fraction and the risk of obesity using linear and logistic regression models. We constructed five different models, based on a Directed Acyclic Graph (DAG) analysis to identify which factors may act as confounders or potential mediators in these associations (**Supplementary Methods S3.2.2**): 1) the basic model, adjusted for gestational age at intake, fetal sex and child's age at follow up visit; 2) the confounder model (main model), the basic model additionally adjusted for potential confounders. We only included maternal age, parity, educational level, prepregnancy BMI, smoking during pregnancy, vomiting during pregnancy, and daily total energy intake during pregnancy to the confounder model based on their association with the exposure and outcome of interest and a >10% change of the effect estimate after adding these covariates to the basic model (33-35). Based on these criteria, alcohol consumption and folic acid supplementation were not selected for inclusion in the confounder model.; 3) the birth model, the confounder model additionally adjusted for gestational-age-and-sex-adjusted birthweight; 4) the child model, the birth model additionally adjusted for infant breastfeeding, solid food introduction and television watching at 10 years; 5) the maternal diet model, the confounder model additionally adjusted for gestational weight gain and maternal fiber, protein and fat intake during pregnancy to assess the effects of other important maternal dietary factors strongly related to the glycemic index and overall diet quality on the observed associations. Based on our hypothesis that effects may be stronger among women with overweight or obesity, we performed all analyses in the total population and stratified for mothers with a prepregnancy BMI<25 kg/m^2 and BMI≥25 kg/m^2. We also formally tested for interactions of maternal prepregnancy BMI continuously with maternal early-pregnancy dietary glycemic index continuously for all childhood outcomes. We added the interaction term of maternal prepregnancy BMI with maternal early-pregnancy dietary glycemic index to linear regression models including maternal prepregnancy

BMI, early-pregnancy dietary glycemic index and gestational age at intake, fetal sex, and child's age at follow up visit. Significant interactions of maternal prepregnancy BMI with maternal early-pregnancy dietary glycemic index were present for childhood total fat mas index (p=0.00) and childhood visceral fat mass index (p=0.02), but not for childhood BMI (p=0.20), childhood android/gynoid fat mass ratio (p=0.17) and childhood liver fat (p=0.86). We performed four sensitivity analyses to assess the robustness of our findings: 1) we repeated the analyses using maternal early-pregnancy dietary glycemic index in quartiles to further explore whether associations were stronger for women consuming a higher dietary glycemic index within our study population; 2) we repeated the analyses using maternal early-pregnancy dietary glycemic load as an exposure to assess the potential additional effect of quantity of maternal dietary carbohydrate intake; 3) we repeated the analyses additionally adjusting for the maternal DASH diet score to assess the potential effect of overall maternal diet quality on the associations; 4) we repeated the analyses excluding mothers with gestational diabetes, as we were interested in the effects of maternal early-pregnancy dietary glycemic index among a non-diabetic population. To reduce selection bias due to missing data, multiple imputations of covariates (pooled results of 5 imputed datasets) were performed (36). The analyses were performed using the Statistical Package of Social Sciences version 24.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Subject characteristics

Table 3.2.1 shows that the overall mean maternal early-pregnancy dietary glycemic index was 57.6 (SD 3.3) and was comparable for women with a normal weight and women with overweight or obesity. Within our population, 20.9% women a low-glycemic index diet, 79.1% women consumed a normal-glycemic index diet and no women consumed a high-glycemic index diet based on comparison to individual food products classifications. Non-response analyses showed that women with and without offspring participating in follow-up measurements had a similar early-pregnancy dietary glycemic index and prepregnancy BMI (**Supplementary Table S3.2.1**).

TABLE 3.2.1. Population characteristics according to maternal prepregnancy BMI

	Total group (n=2,488)	Women with BMI<25 kg/m² (n=1,681)	Women with BMI≥25kg/m² (n=480)
Maternal characteristics			
Maternal age at enrolment, mean (SD), years	31.8 (4.1)	31.9 (4.2)	31.5 (4.0)
Gestational age at enrolment, median (95% range), weeks	13.4 (9.9 to 22.6)	13.4 (9.9 to 22.5)	13.3 (10.1 to 23.2)
Parity, n nulliparous (%)	1,549 (62.3)	1071 (63.8)	288 (60.0)
Pre-pregnancy BMI, median (95% range)	22.3 (18.4 to 33.3)	21.6 (18.1 to 24.7)	27.5 (25.1 to 38.1)
Gestational weight gain, mean (SD), kg/week	0.35 (0.14)	0.36 (0.13)	0.33 (0.18)
Education, n high (%)	1,566 (62.9)	1128 (67.9)	234 (49.0)
Dietary glycemic index, mean (SD)	57.7 (3.5)	57.6 (3.3)	57.9 (3.1)
First quartile, mean (SD)	53.7 (1.4)	53.7 (1.4)	53.8 (1.3)
Second quartile, mean (SD)	56.4 (0.6)	56.4 (0.6)	56.4 (0.6)
Third quartile, mean (SD)	58.5 (0.7)	58.5 (0.7)	58.5 (0.7)
Fourth quartile, mean (SD)	62.0 (1.9)	62.0 (1.8)	61.9 (1.9)
Dietary glycemic load, mean (SD)	154.0 (46.1)	154.7 (45.7)	151.1 (47.6)
Low glycemic index diet, n yes (%)	522 (20.9)	376 (22.4)	80 (16.5)
Carbohydrate intake, mean (SD), g/d	266 (74)	268 (73)	260 (76)
Protein intake, mean (SD), g/d	79 (19)	80 (19)	78 (18)
Fat intake, mean (SD), g/d	87 (24)	87 (24)	84 (23)
Fiber intake, mean (SD), g/d	24 (7)	24 (7)	22 (6)
Total energy intake, mean (SD), kcal/d	2145 (500)	2160 (499)	2090 (498)
Dietary Approaches to Stop Hypertension Diet Score, mean (SD)	25 (4)	25 (5)	24 (4)
Folic acid supplement use, n yes (%)	1,865 (75.0)	1319 (91.7)	372 (90.1)
Alcohol use during pregnancy, n yes (%)	1,577 (63.4)	1141 (71.4)	277 (60.1)
Smoking during pregnancy, n yes (%)	510 (20.5)	366 (22.8)	96 (20.9)

TABLE 3.2.1. Continued

	Total group (n=2,488)	Women with BMI<25 kg/m² (n=1,681)	Women with BMI≥25kg/m² (n=480)
Maternal characteristics			
Vomiting during early-pregnancy, n (%)	93 (3.7)	55 (3.5)	27 (5.9)
Gestational diabetes, n yes (%)	20 (0.8)	9 (0.6)	10 (2.2)
Birth/infant characteristics			
Sex, n female (%)	1,255 (50.4)	838 (49.9)	252 (52.5)
Gestational age at birth, median (95% range), weeks	40.3 (36.0 to 42.4)	40.3 (36.3 to 42.4)	40.3 (35.7 to 42.4)
Birthweight, mean (SD), g	3498 (537)	3490 (528)	3560 (535)
Ever breastfed, n yes (%)	2006 (80.6)	1390 (93.3)	352 (87.8)
Introduction of solid foods before 6 months, yes (%)	1633 (65.6)	1098 (86.7)	302 (90.7)
Child characteristics at 10 years			
Age, median (95% range), years	9.7 (9.4 to 10.2)	9.7 (9.3 to 10.4)	9.7 (9.3 to 10.7)
Average television watching time >2 hours/day n yes (%)	511 (20.5)	308 (18.3)	137 (28.3)
BMI, median (95% range), kg/m²	16.6 (14.3 to 21.3)	16.4 (14.3 to 20.3)	17.5 914.6 to 23.8)
Total fat mass, median (95% range), g	8,038 (4,463 to 18,644)	7,718 (4421 to 17191)	9,710 (4,792 to 22,408)
Android/gynoid fat mass ratio, median (95% range)	0.23 (0.15 to 0.45)	0.23 (0.15 to 0.40)	0.26 (0.16 to 0.50)
Visceral fat mass, median (95% range), g	372 (166 to 928)	353 (157 to 820)	457 (197 to 1,198)
Liver fat fraction, median (95% range), %	2.0 (1.2 to 4.6)	1.9 (1.3 to 3.5)	2.2 (1.4 to 4.8)
Overweight or obese, n yes (%)	285 (11.4)	129 (7.7)	120 (25.0)

Maternal early-pregnancy dietary glycemic index and childhood general, abdominal and ectopic fat accumulation

Table 3.2.2 shows that among the total population and among normal weight women and their children, maternal early-pregnancy dietary glycemic index was not associated with childhood BMI, total body fat or ectopic fat accumulation. Women with overweight or obesity and their children, a higher maternal early-pregnancy dietary glycemic index was associated with a higher childhood BMI, total fat mass index and visceral fat mass index and tended to be associated with a higher childhood android/gynoid fat mass ratio in the confounder model (differences: 0.10 SDS (95% confidence interval (CI) 0.01 to 0.18), 0.13 SDS (95% CI 0.05 to 0.22), 0.19 SDS (95% CI 0.07 to 0.32) and 0.09 SDS (95% CI -0.01 to 0.19) per SDS increase in maternal dietary glycemic index, respectively). Additional adjustment for gestational-age-and-sex-adjusted birthweight, child characteristics and additional maternal dietary characteristics did not explain these associations. No associations with liver fat fraction were present. Women with overweight or obesity, a higher maternal early-pregnancy dietary glycemic index also tended to be associated with a higher risk of childhood overweight, but the association was not significant (Odds Ratio (OR) 1.20, 95% CI 0.97 to 1.48 per SDS increase in maternal dietary glycemic index) (**Table 3.2.3**). Maternal early-pregnancy dietary glycemic index was not associated with the risk of childhood overweight in the total population or among normal weight women.

Figure 3.2.1 shows the associations of maternal dietary glycemic index categorized into low and normal-glycemic index diet according to individual food products classification with childhood general, abdominal and ectopic fat. Among the total population and among normal weighted women and their children, a maternal low-glycemic index diet, as compared to a normal-glycemic index diet, was not associated with childhood BMI, total body fat or ectopic fat. Among women with overweight or obesity and their children, a maternal low-glycemic index diet during pregnancy, as compared to a maternal normal-glycemic index diet, was associated with a lower childhood BMI (–0.35 SDS, 95% CI -0.58 to –0.13), total fat mass index (–0.35 SDS, 95% CI 0.58 to 0.13), android/gynoid fat mass ratio (–0.26 SDS, 95% CI -0.52 to 0.00), visceral fat mass index (–0.50 SDS, 95% CI -0.84 to –0.17) and tended to be associated with a lower risk of childhood overweight (OR 0.57, 95% CI 0.31 to 1,05). No associations with liver fat fraction were present.

TABLE 3.2.2. Associations of maternal early-pregnancy dietary glycemic index with childhood general, abdominal and ectopic fat accumulation

	Effect estimates for childhood outcome per SDS increase in maternal early-pregnancy glycemic index		
	Total group	**Women with BMI<25 kg/m²**	**Women with BMI≥25kg/m²**
		Difference in BMI SDS (95% CI)	
	n=2483	*n=1920*	*n=563*
Basic model[a]	0.05 (0.01 to 0.09)**	0.02 (-0.02 to 0.06)	0.10 (0.01 to 0.19)*
Confounder model[b]	0.02 (-0.01 to 0.06)	0.00 (-0.04 to 0.04)	0.10 (0.01 to 0.18)*
Birth model[c]	0.02 (-0.02 to 0.06)	0.00 (-0.04 to 0.04)	0.10 (0.01 to 0.18)*
Child model[d]	0.02 (-0.02 to 0.05)	-0.01 (-0.05 to 0.03)	0.11 (0.02 to 0.19)*
Maternal diet model[e]	0.01 (-0.03 to 0.05)	-0.03 (-0.07 to 0.02)	0.11 (0.02 to 0.21)*
		Difference in total fat mass index SDS (95% CI)	
	n=2455	*n=1898*	*n=557*
Basic model[a]	0.07 (0.03 to 0.11)**	0.03 (-0.01 to 0.07)	0.15 (0.06 to 0.24)**
Confounder model[b]	0.04 (0.00 to 0.07)	0.01 (-0.03 to 0.05)	0.13 (0.05 to 0.22)**
Birth model[c]	0.04 (0.00 to 0.07)	0.01 (-0.03 to 0.05)	0.13 (0.05 to 0.22)**
Child model[d]	0.03 (-0.01 to 0.07)	0.00 (-0.04 to 0.04)	0.14 (0.05 to 0.22)**
Maternal diet model[e]	0.01 (-0.03 to 0.05)	-0.03 (-0.07 to 0.02)	0.13 (0.03 to 0.23)*
		Difference in android/gynoid ratio SDS (95% CI)	
	n=2458	*n=1901*	*n=557*
Basic model[a]	0.04 (0.00 to 0.08)*	0.01 (-0.03 to 0.05)	0.11 (0.00 to 0.21)
Confounder model[b]	0.01 (-0.03 to 0.05)	-0.01 (-0.06 to 0.03)	0.09 (-0.01 to 0.19)
Birth model[c]	0.01 (-0.03 to 0.05)	-0.01 (-0.06 to 0.03)	0.09 (-0.01 to 0.19)
Child model[d]	0.01 (-0.04 to 0.04)	-0.02 (-0.07 to 0.02)	0.09 (-0.01 to 0.19)
Maternal diet model[e]	-0.02 (-0.07 to 0.02)	-0.06 (-0.11 to -0.01)	0.09 (-0.03 to 0.20)
		Difference visceral fat mass index SDS (95% CI)	
	n=1246	*n=956*	*n=290*
Basic model[a]	0.08 (0.02 to 0.13)**	0.02 (-0.05 to 0.08)	0.23 (0.10 to 0.36)**
Confounder model[b]	0.04 (-0.01 to 0.10)	0.00 (-0.07 to 0.06)	0.19 (0.07 to 0.32)**
Birth model[c]	0.04 (-0.01 to 0.10)	0.00 (-0.07 to 0.06)	0.19 (0.07 to 0.32)**
Child model[d]	0.04 (-0.02 to 0.09)	-0.01 (-0.07 to 0.05)	0.19 (0.07 to 0.32)**
Maternal diet model[e]	0.02 (-0.04 to 0.08)	-0.02 (-0.10 to 0.05)	0.17 (0.03 to 0.31)**
		Difference in liver fat fraction SDS (95% CI)	
	n=1395	*n=1074*	*n=321*
Basic model[a]	0.00 (-0.05 to 0.06)	0.00 (-0.06 to 0.05)	0.00 (-0.14 to 0.13)
Confounder model[b]	-0.03 (-0.08 to 0.03)	-0.01 (-0.07 to 0.05)	-0.05 (-0.18 to 0.08)
Birth model[c]	-0.02 (-0.08 to 0.03)	-0.01 (-0.07 to 0.05)	-0.05 (-0.18 to 0.08)

TABLE 3.2.2. Continued

	Effect estimates for childhood outcome per SDS increase in maternal early-pregnancy glycemic index		
	Total group	**Women with BMI<25 kg/m²**	**Women with BMI≥25kg/m²**
Child model[d]	-0.03 (-0.08 to 0.03)	-0.02 (-0.08 to 0.04)	-0.05 (-0.19 to 0.08)
Maternal diet model[e]	-0.06 (-0.12 to 0.00)	-0.05 (-0.12 to 0.02)	-0.07 (-0.22 to 0.08)

**P<0.05 **P<0.01. SDS: standard deviation scores*

*Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in standard deviation score of childhood adiposity outcomes per SDS increase in maternal early-pregnancy dietary glycemic index. One SDS maternal early-pregnancy dietary glycemic index corresponds to an increase of glycemic index of 3.5. P-values for interaction terms maternal prepregnancy BMI*maternal dietary glycemic index for each individual childhood adiposity outcome were 0.11 for BMI, 0.00 for total fat mass index, 0.08 for android/gynoid fat mass ratio, 0.00 for visceral fat mass index and 0.89 for liver fat fraction.*

[a]Basic models were adjusted for gestational age at intake, fetal sex and child's age at follow up

[b]Confounder models were the basic models additionally adjusted for maternal age, maternal educational level, maternal prepregnancy BMI, smoking during pregnancy, vomiting during early-pregnancy, daily total energy intake

[c]Birth models were the confounder models additionally adjusted for gestational-age-and-sex adjusted birthweight

[d]Child models were the birth models, additionally adjusted for infant breastfeeding, introduction of solid foods and average television watching time

[e]Maternal diet models were the confounder models additionally adjusted for gestational weight gain and maternal fiber, fat and protein intake

TABLE 3.2.3. Associations of maternal early-pregnancy dietary glycemic index with risk of childhood overweight

	Odds Ratio for risk of childhood overweight per SDS increase in maternal early-pregnancy glycemic index		
	Total group	**Women with BMI<25 kg/m²**	**Women with BMI≥25kg/m²**
Basic model[a]	1.12 (0.99 to 1.26)	1.00 (0.85 to 1.19)	1.21 (0.99 to 1.48)
Confounder model[b]	1.05 (0.92 to 1.20)	0.94 (0.79 to 1.12)	1.20 (0.97 to 1.48)
Birth model[c]	1.06 (0.92 to 1.21)	0.94 (0.79 to 1.12)	1.21 (0.98 to 1.49)
Child model[d]	1.04 (0.91 to 1.20)	0.92 (0.76 to 1.10)	1.24 (0.99 to 1.54)
Maternal diet model[e]	0.98 (0.85 to 1.14)	0.84 (0.68 to 1.03)	1.19 (0.94 to 1.49)

SDS: standard deviation scores

*Values represent odds ratios (95% confidence interval) from logistic regression models that reflect differences in standard deviation score of risk of childhood overweight per SDS in maternal early-pregnancy dietary glycemic index. One SDS maternal early-pregnancy dietary glycemic index corresponds to an increase of glycemic index of 3.5. p-value for interaction term maternal prepregnancy weight status*maternal dietary glycemic index was 0.19.*

[a]Basic models were adjusted for gestational age at intake, fetal sex and child's age at follow up

[b]Confounder models were the basic models additionally adjusted for maternal age, maternal educational level, maternal prepregnancy BMI, smoking during pregnancy, vomiting during early-pregnancy, daily total energy intake

[c]Birth models were the confounder models additionally adjusted for gestational-age-and-sex adjusted birthweight

[d]Child models were the birth models, additionally adjusted for infant breastfeeding, introduction of solid foods and average television watching time

[e]Maternal diet models were the confounder models additionally adjusted for gestational weight gain and maternal fiber, fat and protein intake

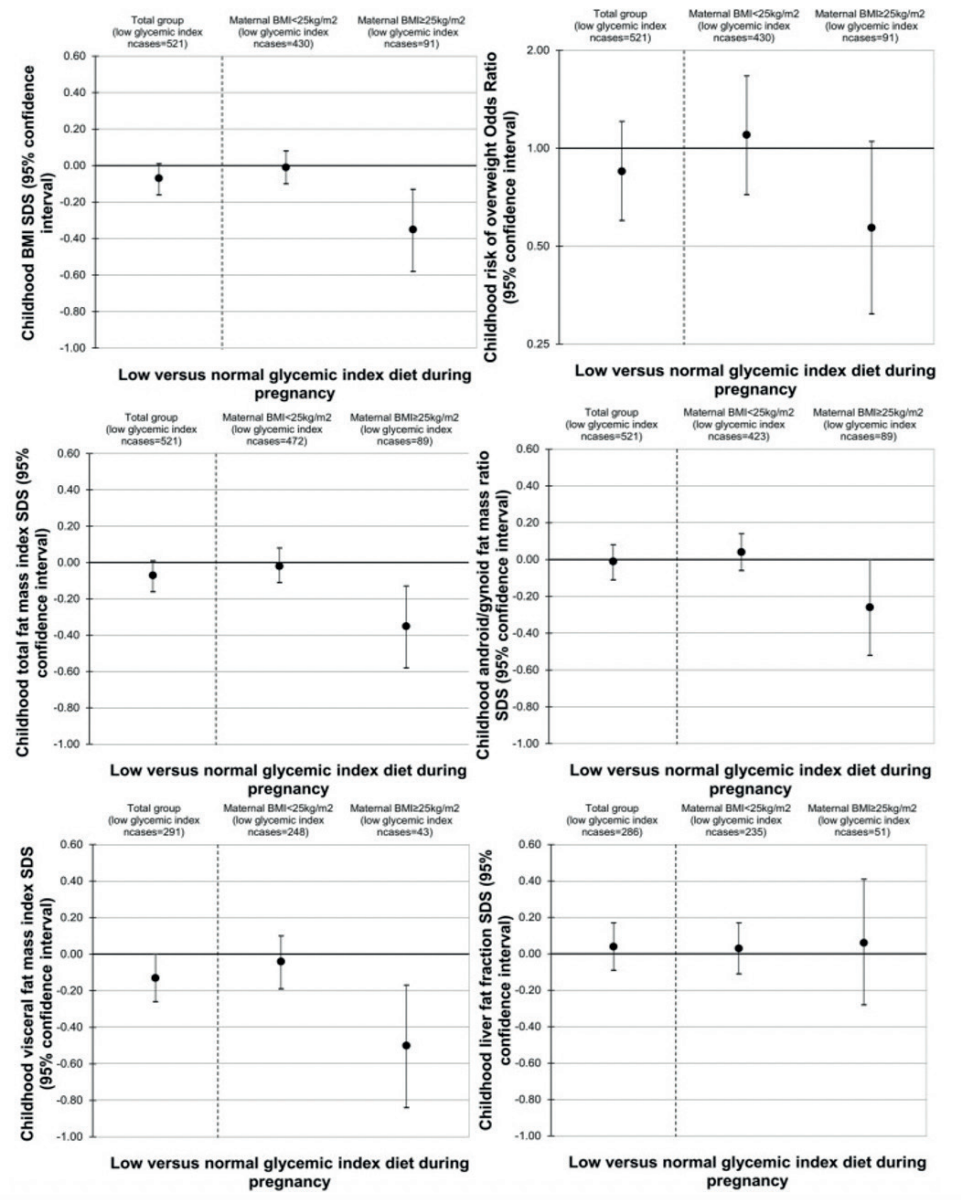


FIGURE 3.2.1. Associations of maternal early-pregnancy low-glycemic index diet as compared to maternal early-pregnancy normal-glycemic index diet with childhood adiposity outcomes

Cut offs for a low-, and normal-glycemic index diet are based on comparison to individual food products classifications (≤55 and 56–69 for a low-, and normal-glycemic index diet, respectively). No women within our population consumed a high-glycemic index diet (≥70). Ncases represents the number of women who consumed a low-glycemic index diet within the specified group. Values represent regression coefficients and odds ratios (ORs) (95% Confidence Intervals) from linear and logistic regression models respectively, that reflect differences in standard deviation scores for childhood adiposity outcomes and differences in risk for childhood overweight for a maternal low-glycemic index diet as compared to a normal-glycemic index diet. Associations were adjusted for maternal age, maternal educational level, maternal prepregnancy BMI, smoking during pregnancy, vomiting during early-pregnancy and daily total energy intake.

Sensitivity analyses

When repeating analyses using the maternal early-pregnancy dietary glycemic index in quartiles, associations of the highest quartile with childhood general, abdominal and ectopic fat accumulation as compared to the lowest quartile, were similar as for the analyses using the maternal early-pregnancy dietary glycemic continuously (**Supplementary Table S3.2.2**). Among the total population, a higher maternal early-pregnancy glycemic load was associated with a higher child total fat mass, whereas among normal weight women and their children no associations were present. Among women with overweight or obesity, a higher maternal early-pregnancy dietary glycemic load was associated with a higher childhood BMI, total fat mass index, visceral fat mass index, and higher risk of childhood overweight in the confounder models (**Supplementary Tables S3.2.3** and **S3.2.4**). After adjustment for the maternal DASH diet score, effects estimates were similar to those observed after adjustment for maternal dietary characteristics (**Supplementary Table S3.2.5**). Excluding women with gestational diabetes from the analyses did not change our findings (**Supplementary Table S3.2.6**).

DISCUSSION

Among women with overweight or obesity and their children, a higher maternal early-pregnancy dietary glycemic index was associated with higher childhood BMI, total body fat, abdominal fat and visceral fat accumulation, but not with liver fat accumulation. These associations were not explained by maternal socio-demographic, lifestyle and other dietary characteristics, birth or child characteristics. In normal weight women, no associations of maternal early-pregnancy dietary glycemic index with childhood general, abdominal or ectopic fat accumulation were present.

Interpretation of main findings

The maternal dietary glycemic index during pregnancy is receiving increasing interest as a potential modifiable target to improve birth outcomes and reduce the risk of offspring obesity (37). The dietary glycemic index during pregnancy directly influences maternal postprandial glucose concentrations. Postprandial peaks in maternal glucose concentrations and subsequent peak increases in fetal glucose and insulin concentrations could irreversibly affect offspring adiposity development by altering fetal growth, development of adipocytes and metabolism (6, 8). Intervention studies have already shown that stimulating a low-glycemic index diet during pregnancy in women at increased risk of an impaired glucose metabolism may reduce the risk of macrosomia and adiposity in infant offspring (38, 39). The long-term effects of a higher maternal dietary glycemic index during pregnancy on offspring adiposity and ectopic fat accumulation are not well-known. A study among 842 Irish mother–child pairs observed no association of maternal dietary glycemic index assessed between 12 and 16 weeks gestation with offspring BMI or waist circumference at 5 years after adjustment for maternal prepregnancy BMI (40). The mean dietary glycemic index was 58.9 within this study population. Contrarily, a study among 906 mother–child pairs in the United Kingdom reported that a higher maternal dietary glycemic index and load in early-pregnancy, but not late-pregnancy, were associated with a higher offspring total body fat measured by DXA at 4 and 6 years of age after adjustment for maternal prepregnancy BMI. This study reported a mean dietary glycemic index of 59.6 in early-pregnancy and 58.9 in late-pregnancy. No differences in associations among women with a normal weight and women with overweight or obesity and their offspring were present (13). Animal studies showed that a maternal high-glycemic index diet during pregnancy was associated with higher offspring visceral fat mass and a transient higher liver fat, which normalized in adolescence (11, 41).

Partly in line with these previous studies, we observed that among women with overweight or obesity and their children, a higher maternal early-pregnancy dietary glycemic index across the full range was associated with a higher childhood BMI and total body fat. In addition, we showed that a higher maternal early-pregnancy dietary glycemic index was associated with higher childhood abdominal and visceral fat accumulation. In line with previous human studies, we observed that women within our study consumed a diet with a relatively low mean dietary glycemic index of 57.7. Associations for maternal dietary glycemic load were in similar direction, which suggests that the observed associations of maternal early-pregnancy dietary glycemic index with childhood adiposity are not fully explained by the amount of carbohydrate intake. The associations for maternal dietary glycemic load were slightly weaker, possibly due to

more measurement error and confounding in assessment of the dietary glycemic load than dietary glycemic index or a small effect of the amount of carbohydrate intake (42). As compared to a maternal normal-glycemic index diet, a maternal low-glycemic index diet was associated with lower childhood BMI, total fat mass, android/gynoid fat mass and visceral fat accumulation. We observed no associations with childhood liver fat accumulation, what could be explained by our relatively healthy population, but also, as suggested by an animal study, the effects on liver fat fraction may be more pronounced in infancy (11). Associations were only present among women with prepregnancy overweight or obesity and their children. Additional adjustment for prepregnancy BMI did not affect the observed associations within this group of women and their offspring. This is in line with intervention studies mainly reporting beneficial effects of a low-glycemic index diet during pregnancy on pregnancy outcomes among women at risk of an impaired glucose metabolism (22). Overall, our findings suggest that among women with prepregnancy overweight or obesity and their children, a higher maternal early-pregnancy dietary glycemic index is associated with higher childhood higher general, abdominal and visceral fat accumulation at 10 years, but not with liver fat accumulation. No associations are present among normal weight women and their offspring.

The mechanisms underlying the observed associations are not well known. Our findings were not explained by maternal socio-economic, lifestyle, birth or childhood characteristics. Additional adjustment for other maternal macronutrients did not explain the associations, nor did additional adjustment for overall dietary quality by additionally adjusting for the maternal DASH diet score (43). This suggests that observed associations are less likely to only reflect effects of an overall unhealthy lifestyle and diet among women with overweight or obesity, but that a higher maternal early-pregnancy dietary glycemic index may have a direct effect on offspring adipose tissue development. A maternal diet during pregnancy with a higher glycemic index and subsequent peaks in postprandial glucose concentrations increase glucose transfer to the developing embryo or fetus. Women with overweight or obesity have a more pronounced insulin resistance during pregnancy, causing larger fluctuations in postprandial glucose concentrations and higher glucose transfer to the developing embryo or fetus (5, 6, 44). Higher embryonic and fetal glucose concentrations accelerate embryonic and fetal growth and may alter development of adipocytes, pancreatic endocrine and hepatic metabolic function (6, 8, 45). These alterations may not only directly increase fetal adipose tissue accumulation, but could also cause fat accumulation in postnatal life due to irreversible changes in fatty acid oxidation, lipogenesis, and lipoprotein export (46, 47). Experimental studies need to identify mechanisms underlying the associations of maternal early-pregnancy dietary glycemic index on offspring adiposity development.

The observed associations of maternal early-pregnancy dietary glycemic index with childhood adiposity outcomes among women with overweight or obesity and their children were relatively small, but important from a public health perspective. It is well-known that childhood adiposity tracks into adulthood (48-52). We observed the strongest effect of the maternal early-pregnancy dietary glycemic index with childhood visceral fat accumulation. Visceral fat accumulation is known to cause systemic inflammation and is strongly linked to an impaired glucose metabolism and an adverse lipid profile (48). In adulthood, visceral fat accumulation strongly increases the risk of type 2 diabetes, cardiovascular disease and premature mortality, even irrespective of general fat mass (3, 52). Maternal prepregnancy overweight and obesity are associated with increased offspring risks of obesity and ectopic fat accumulation (3, 4, 6). Especially in this high risk group, insight into modifiable lifestyle factors from preconception onwards is needed to develop interventions to improve offspring adiposity outcomes and related cardio-metabolic health. Intervention studies among women with overweight or obesity should reveal whether stimulating a low-glycemic index diet already from preconception or early-pregnancy reduces the risk of childhood obesity and increased general, abdominal and visceral fat in the offspring.

Methodological considerations

Strengths of this study are the prospective design, large sample size, and the use of detailed measures of childhood adiposity obtained with DXA and MRI. 72% of children from Dutch mothers with early-pregnancy dietary glycemic index available participated in follow-up measurements. As we observed no differences in early-pregnancy dietary glycemic index and prepregnancy BMI between mothers with and without offspring participating in follow up measurements, we consider bias due to loss the follow-up unlikely. The selection towards a relatively healthy Dutch population with a relatively low mean dietary glycemic index may affect the generalizability of our findings. Further studies are needed to replicate our findings among multi-ethnic populations with a more diverse dietary intake. Even though the FFQ is a validated questionnaire widely used for dietary assessment in observational studies, measurement of food intake by a FFQ may be affected by over- or underreporting of dietary intake during pregnancy. Although the mean dietary glycemic index is in line with previous studies, this limitation of the FFQ may explain the relatively low mean dietary glycemic index within our study population and lead to an underestimation of results (13, 40). However, a study performed within a study population directly comparable to our study population validate the FFQ using three 24-h dietary recalls and biomarkers from blood samples and showed only a slight underestimation of carbohydrate intake. Calculation of the dietary glycemic index from the FFQ may further be affected by uncertainty induced by preparation of foods,

mixed dishes, variations of food products over time or unavailability of specific food products (17). Further studies using different methods to assess the dietary glycemic index in low and high-risk pregnant populations are needed to replicate our results. We adjusted our analyses for multiple confounding factors, but residual confounding may still be present.

CONCLUSIONS

Among women with overweight or obesity and their children, a higher maternal early-pregnancy dietary glycemic index was associated with a higher childhood BMI, general and abdominal fat accumulation and visceral fat accumulation, but not with liver fat accumulation. Intervention studies among overweight and obese pregnant women may need to target the dietary glycemic index to prevent childhood adiposity.

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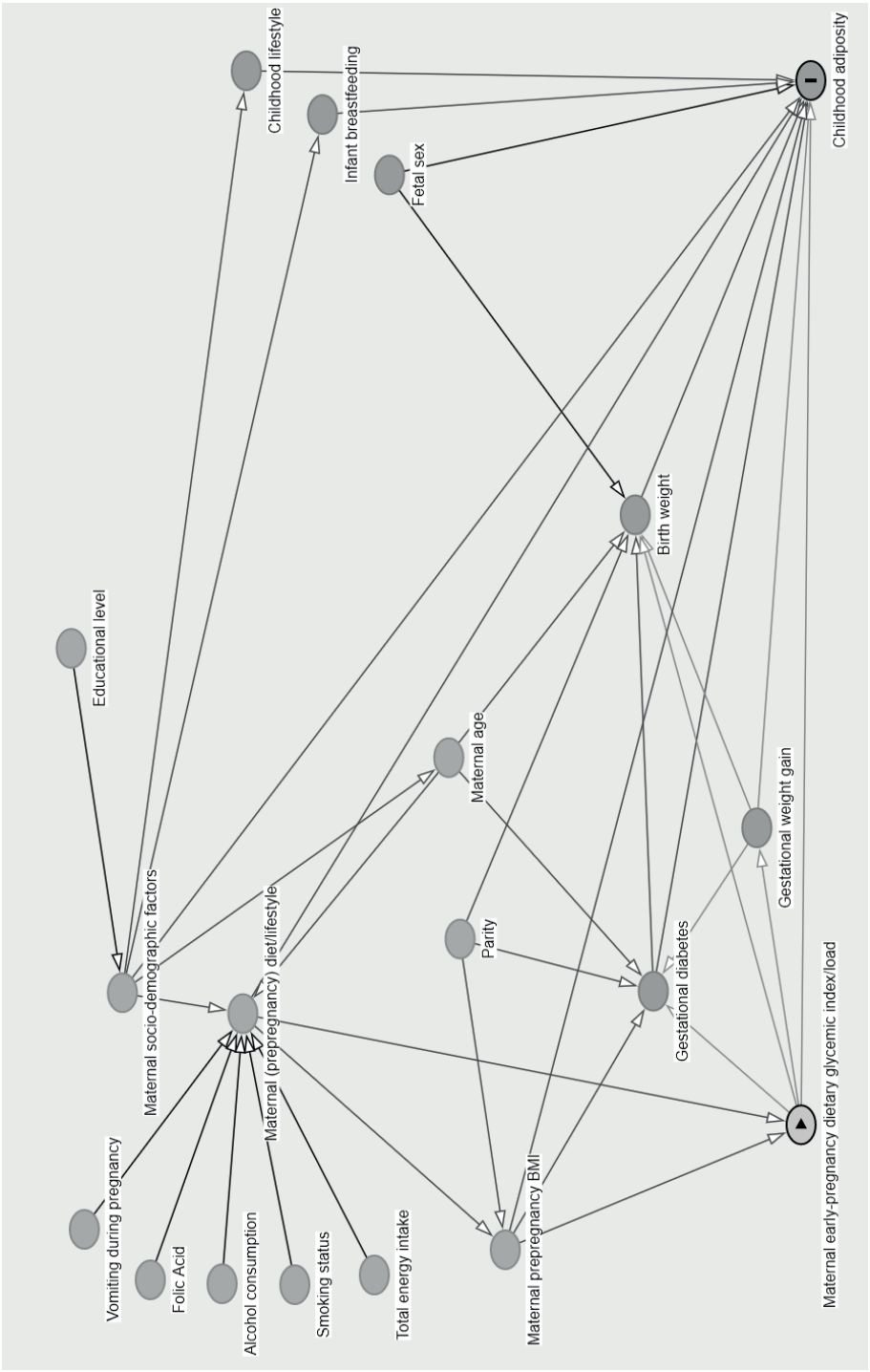
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SUPPLEMENTARY MATERIAL

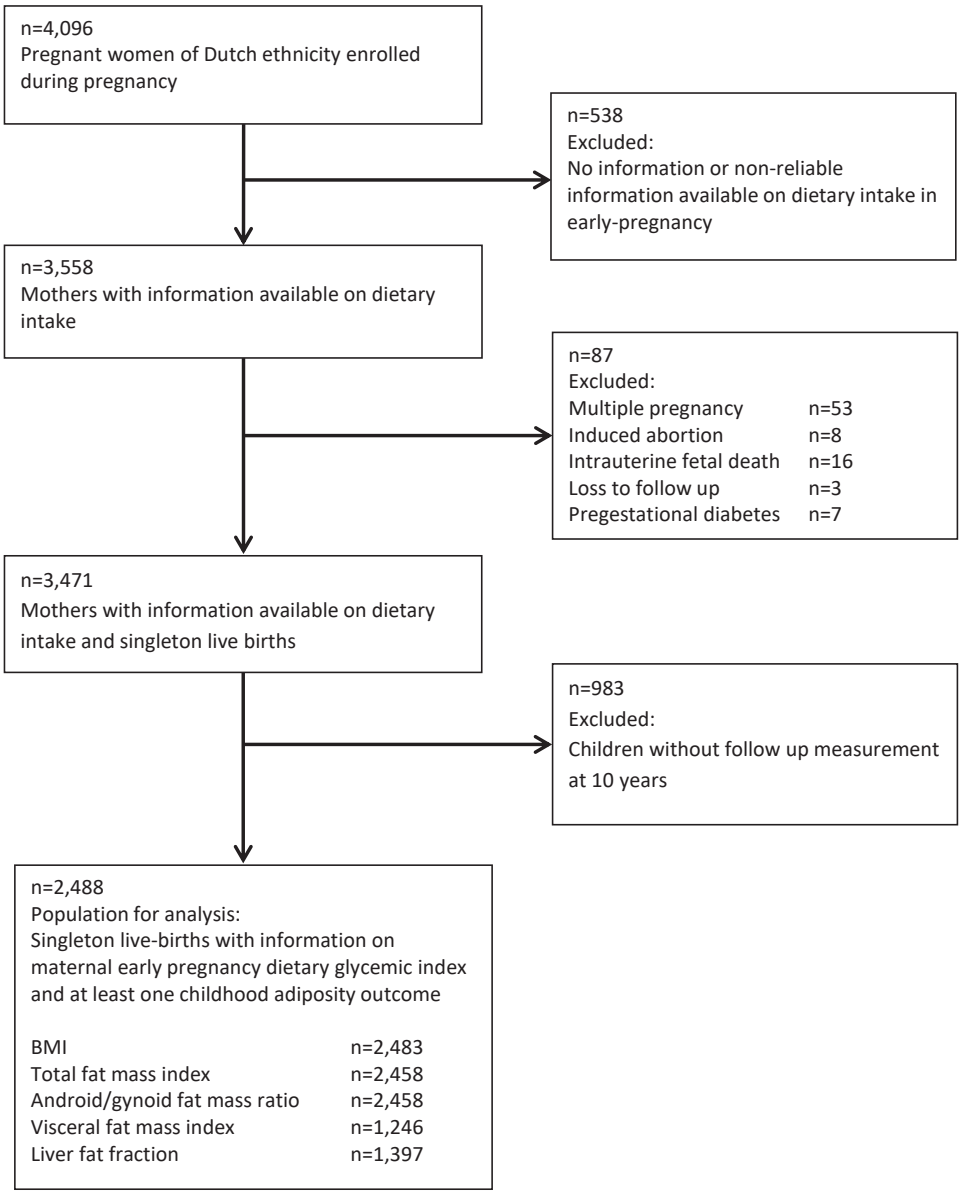
Supplementary Methods S3.2.1. Log-log regression analyses

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, 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 and visceral fat mass divided by height^3.

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SUPPLEMENTARY METHODS S3.2.2. Directed Acyclic Graph analysis



SUPPLEMENTARY FIGURE S3.2.1. Flow chart of the study participants

SUPPLEMENTARY TABLE S3.2.1. Non-response analysis for Dutch women with information on dietary glycemic index available and singleton livebirths and their offspring with and without participation in follow up measurements at 10 years

	Participation in offspring follow up measurements at 10 years (n=2,488)	No participation in offspring follow up measurements at 10 years (n=983)	p-value
Maternal characteristics			
Maternal age at enrolment, mean (SD), years	31.8 (4.1)	30.3 (4.8)	0.00
Gestational age at enrolment, median (95% range), weeks	13.4 (9.9 to 22.6)	13.4 (9.7 to 24.0)	0.85
Parity, n nulliparous (%)	1,549 (62.3)	532 (54.0)	0.00
Pre-pregnancy BMI, median (95% range)	22.3 (18.4 to 33.3)	22.2 (18.0 to 34.1)	0.72
Gestational weight gain, mean (SD), kg/week	0.35 (0.14)	0.36 (0.15)	0.04
Education, n high (%)	1,566 (62.9)	461 (47.2)	0.00
Glycemic index, mean (SD)	57.7 (3.5)	58.0 (3.2)	0.40
Glycemic load, mean (SD)	154.0 (46.1)	156.1 (48.4)	0.24
Low glycemic index, n (%)	522 (20.9)	183 (26.0)	0.10
Carbohydrate intake, mean (SD), g/d	266 (74)	269 (78)	0.20
Protein intake, mean (SD), g/d	79 (19)	78 (20)	0.10
Fat intake, mean (SD), g/d	87 (24)	86 (25)	0.05
Fiber intake, mean (SD), g/d	24 (7)	23 (7)	0.23
Total energy intake, mean (SD), kcal/d	2145 (500)	2,145 (538)	0.05
Folic acid supplement use, n yes (%)	1,865 (75.0)	672 (83.1)	0.00
Alcohol use during pregnancy, n yes (%)	1,577 (63.4)	540 (25.5)	0.00
Smoking during pregnancy, n yes (%)	510 (20.5)	324 (34.9)	0.00
Vomiting during early-pregnancy, n yes (%)	93 (3.7)	52 (5.7)	0.05
Gestational diabetes, n (%)	20 (0.8)	12 (1.3)	0.24
Birth/infant characteristics			
Sex, n female (%)	1,255 (50.4)	468 (47.3)	0.09
Gestational age at birth, median (95% range), weeks	40.3 (36.0 to 42.4)	40.1 (35.3 to 42.3)	0.00
Birthweight, mean (SD), g	3498 (537)	3,465 (596)	0.00
Ever breastfed, yes (%)	2006 (80.6)	587 (85.7)	0.00
Introduction of solid foods before 6 months, yes (%)	1633 (65.6)	444 (87.2)	0.75

BMI: body mass index. P-values were obtained by independent t-test or Mann-Whitney U-test for continuous variables and chi-square tests for categorical variables.

SUPPLEMENTARY TABLE S3.2.2. Associations of maternal early-pregnancy dietary glycemic index in quartiles with childhood general, abdominal and ectopic fat accumulation

	Effect estimates for childhood outcome for maternal early-pregnancy glycemic index in quartiles		
	Total group	**Women with BMI<25 kg/m²**	**Women with BMI≥25kg/m²**
		Difference in BMI SDS (95% CI)	
	2,483	*1,920*	*563*
First quartile (n=621)	*Reference*	*Reference*	*Reference*
Second quartile (n=621)	0.08 (-0.02 to 0.18)	-0.01 (-0.12 to 0.10)	0.41 (0.16 to 0.66)*
Third quartile (n=619)	0.08 (-0.03 to 0.18)	0.05 (-0.06 to 0.16)	0.19 (-0.07 to 0.44)
Fourth quartile (n=622)	0.06 (-0.05 to 0.16)	-0.02 (-0.13 to 0.10)	0.31 (0.06 to 0.57)*
		Difference in total fat mass index SDS (95% CI)	
	2,455	*1,898*	*557*
First quartile (n=611)	*Reference*	*Reference*	*Reference*
Second quartile (n=618)	0.13 (0.03 to 0.24)*	0.07 (-0.05 to 0.18)	0.42 (0.17 to 0.67)*
Third quartile (n=611)	0.07 (-0.04 to 0.17)	0.04 (-0.08 to 0.15)	0.22 (-0.04 to 0.47)
Fourth quartile (n=615)	0.10 (-0.01 to 0.21)	0.02 (-0.10 to 0.14)	0.40 (0.15 to 0.66)*
		Difference in android/gynoid ratio SDS (95% CI)	
	2,458	*1,901*	*557*
First quartile (n=611)	*Reference*	*Reference*	*Reference*
Second quartile (n=619)	0.07 (-0.04 to 0.19)	0.02 (-0.10 to 0.14)	0.30 (0.02 to 0.58)*
Third quartile (n=613)	0.00 (-0.11 to 0.12)	-0.04 (-0.16 to 0.08)	0.17 (-0.12 to 0.45)
Fourth quartile (n=615)	0.01 (-0.11 to 0.12)	-0.05 (-0.17 to 0.08)	0.22 (-0.07 to 0.52)
		Difference visceral fat mass index SDS (95% CI)	
	1,246	*956*	*290*
First quartile (n=307)	*Reference*	*Reference*	*Reference*
Second quartile (n=313)	0.18 (0.02 to 0.33)*	0.09 (-0.09 to 0.26)	0.56 (0.19 to 0.92)*
Third quartile (n=321)	0.09 (-0.7 to 0.25)	0.05 (-0.12 to 0.22)	0.32 (-0.04 to 0.69)
Fourth quartile (n=305)	0.10 (-0.07 to 0.26)	-0.06 (-0.24 to 0.12)	0.65 (0.28 to 1.02)*
		Difference in liver fat fraction SDS (95% CI)	
	1,395	*1,074*	*321*
First quartile (n=351)	*Reference*	*Reference*	*Reference*
Second quartile (n=347)	-0.02 (-0.17 to 0.13)	-0.01 (-0.17 to 0.16)	-0.05 (-0.43 to 0.34)
Third quartile (n=355)	-0.09 (-0.24 to 0.07)	-0.06 (-0.23 to 0.10)	-0.15 (-0.54 to 0.23)
Fourth quartile (n=344)	-0.05 (-0.21 to 0.11)	0.01 (-0.16 to 0.18)	-0.23 (-0.62 to 0.16)

**P<0.05 SDS: standard deviation scores. Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in standard deviation score of childhood adiposity outcomes for the upper three maternal dietary glycemic index quartiles as compared to the lowest quartile.*

[a]Basic models were adjusted for gestational age at intake, fetal sex and child's age at follow up

[b]Confounder models were the basic models additionally adjusted for maternal age, maternal educational level, maternal prepregnancy BMI, smoking during pregnancy, vomiting during early-pregnancy, daily total energy intake

[c]Birth models were the confounder models additionally adjusted for gestational-age-and-sex adjusted birthweight

[d]Child models were the birth models, additionally adjusted for infant breastfeeding, introduction of solid foods and average television watching time

[e]Maternal diet models were the confounder models additionally adjusted for gestational weight gain and maternal fiber, fat and protein intake

SUPPLEMENTARY TABLE S3.2.3. Associations of maternal early-pregnancy dietary glycemic load with childhood general, abdominal and ectopic fat accumulation

	Effect estimates for childhood outcome per SDS increase in maternal early-pregnancy glycemic load		
	Total group	**Women with BMI<25 kg/m²**	**Women with BMI≥25kg/m²**
		Difference in BMI SDS (95% CI)	
	2,483	*1,920*	*563*
Basic model[a]	-0.01 (-0.04 to 0.03)	0.00 (-0.04 to 0.04)	0.02 (-0.07 to 0.10)
Confounder model[b]	0.09 (0.02 to 0.15)	0.07 (0.00 to 0.15)	0.12 (-0.04 to 0.28)
Birth model[c]	0.08 (0.02 to 0.15)	0.07 (0.00 to 0.14)	0.13 (-0.03 to 0.28)
Child model[d]	0.08 (0.01 to 0.15)	0.06 (-0.01 to 0.13)	0.15 (-0.01 to 0.30)
Maternal diet model[e]	0.05 (-0.14 to 0.24)	-0.09 (-0.30 to 0.12)	0.51 (0.03 to 0.98)
		Difference in total fat mass index SDS (95% CI)	
	2,455	*1,898*	*557*
Basic model[a]	-0.01 (-0.05 to 0.03)	-0.01 (-0.05 to 0.03)	0.04 (-0.04 to 0.13)
Confounder model[b]	0.08 (0.01 to 0.15)*	0.06 (-0.02 to 0.13)	0.16 (0.00 to 0.32)
Birth model[c]	0.08 (0.01 to 0.15)*	0.06 (-0.02 to 0.13)	0.15 (0.00 to 0.33)
Child model[d]	0.07 (0.01 to 0.14)*	0.05 (-0.03 to 0.12)	0.17 (0.01 to 0.34)*
Maternal diet model[e]	0.07 (-0.12 to 0.26)*	-0.08 (-0.30 to 0.13)	0.61 (0.13 to 1.08)*
		Difference in android/gynoid ratio SDS (95% CI)	
	2,458	*1,901*	*557*
Basic model[a]	0.00 (-0.04 to 0.04)	-0.01 (-0.05 to 0.04)	0.03 (-0.07 to 0.12)
Confounder model[b]	0.04 (-0.04 to 0.11)	0.02 (-0.06 to 0.10)	0.09 (-0.09 to 0.28)
Birth model[c]	0.03 (-0.04 to 0.11)	0.02 (-0.06 to 0.11)	0.09 (-0.09 to 0.28)
Child model[d]	0.03 (-0.04 to 0.11)	0.01 (-0.07 to 0.10)	0.10 (-0.09 to 0.28)
Maternal diet model[e]	-0.09 (-0.30 to 0.11)	-0.22 (-0.46 to 0.01)	0.35 (0.19 to 0.90)
		Difference visceral fat mass index SDS (95% CI)	
	1,246	*956*	*290*
Basic model[a]	-0.03 (-0.09 to 0.02)	-0.03 (-0.09 to 0.03)	-0.01 (-0.13 to 0.12)
Confounder model[b]	0.10 (-0.01 to 0.20)	0.04 (-0.07 to 0.16)	0.30 (0.06 to 0.54)*
Birth model[c]	0.09 (-0.01 to 0.20)	0.04 (-0.07 to 0.16)	0.30 (0.05 to 0.54)*
Child model[d]	0.08 (-0.03 to 0.18)	0.02 (-0.09 to 0.14)	0.30 (0.05 to 0.55)*
Maternal diet model[e]	0.01 (-0.27 to 0.30)	-0.15 (-0.47 to 0.17)	0.67 (-0.01 to 1.34)
		Difference in liver fat fraction SDS (95% CI)	
	1,395	*1,074*	*321*
Basic model[a]	0.00 (-0.06 to 0.05)	-0.01 (-0.07 to 0.05)	0.07 (-0.04 to 0.19)
Confounder model[b]	0.04 (-0.06 to 0.14)	0.05 (-0.06 to 0.16)	0.02 (-0.24 to 0.27)
Birth model[c]	0.03 (-0.07 to 0.14)	0.05 (-0.06 to 0.16)	0.01 (-0.25 to 0.26)

SUPPLEMENTARY TABLE S3.2.3. Continued

	Effect estimates for childhood outcome per SDS increase in maternal early-pregnancy glycemic load		
	Total group	**Women with BMI<25 kg/m²**	**Women with BMI≥25kg/m²**
Child model[d]	0.03 (-0.08 to 0.13)	0.04 (-0.07 to 0.15)	-0.01 (-0.26 to 0.25)
Maternal diet model[e]	0.32 (-0.59 to -0.04)	-0.22 (-0.52 to 0.08)	-0.67 (-1.37 to 0.03)

**P<0.05 SDS: standard deviation scores. Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in standard deviation score of childhood adiposity outcomes per SDS increase in maternal early-pregnancy dietary glycemic load.*

[a]Basic models were adjusted for gestational age at intake, fetal sex and child's age at follow up

[b]Confounder models were the basic models additionally adjusted for maternal age, maternal educational level, maternal prepregnancy BMI, smoking during pregnancy, vomiting during early-pregnancy, daily total energy intake

[c]Birth models were the confounder models additionally adjusted for gestational-age-and-sex adjusted birthweight

[d]Child models were the birth models, additionally adjusted for infant breastfeeding, introduction of solid foods and average television watching time

[e]Maternal diet models were the confounder models additionally adjusted for gestational weight gain and maternal fiber, fat and protein intake

SUPPLEMENTARY TABLE S3.2.4. Associations of maternal early-pregnancy dietary glycemic load with risk of childhood overweight

	Effect estimates for risk of childhood overweight per SDS increase in maternal early-pregnancy glycemic load		
	Total group	**Women with BMI<25 kg/m²**	**Women with BMI≥25kg/m²**
Basic model[a]	1.03 (0.91 to 1.17)	0.98 (0.82 to 1.17)	1.17 (0.97 to 1.41)
Confounder model[b]	1.25 (0.98 to 1.60)	1.09 (0.78 to 1.52)	1.47 (1.00 to 2.17)
Birth model[c]	1.25 (0.98 to 1.60)	1.08 (0.77 to 1.51)	1.49 (1.01 to 2.20)*
Child model[d]	1.24 (1.10 to 1.86)*	1.05 (0.75 to 1.47)	1.56 (1.05 to 2.32)*
Maternal diet model[e]	1.05 (0.53 to 2.10)	0.60 (0.23 to 1.54)	2.01 (0.67 to 6.05)

**P<0.05 SDS: standard deviation scores. Values represent odds ratios (95% confidence interval) from logistic regression models that reflect differences in standard deviation score of childhood risk of overweight per SDS in maternal early-pregnancy dietary glycemic load.*

[a]Basic models were adjusted for gestational age at intake, fetal sex and child's age at follow up

[b]Confounder models were the basic models additionally adjusted for maternal age, maternal educational level, maternal prepregnancy BMI, smoking during pregnancy, vomiting during early-pregnancy, daily total energy intake

[c]Birth models were the confounder models additionally adjusted for gestational-age-and-sex adjusted birthweight

[d]Child models were the birth models, additionally adjusted for infant breastfeeding, introduction of solid foods and average television watching time

[e]Maternal diet models were the confounder models additionally adjusted for gestational weight gain and maternal fiber, fat and protein intake

SUPPLEMENTARY TABLE S3.2.5. Associations of maternal early-pregnancy dietary glycemic index with childhood general, abdominal and ectopic fat accumulation after adjustment for maternal DASH diet score

	Effect estimates for childhood outcome per SDS increase in maternal early-pregnancy glycemic load		
	Total group	**Women with BMI<25 kg/m²**	**Women with BMI≥25kg/m²**
		Difference in BMI SDS (95% CI)	
	2483	*1920*	*563*
Confounder model additionally adjusted for maternal DASH diet score[a]	0.04 (0.00 to 0.08)	0.01 (-0.04 to 0.05)	0.12 (0.02 to 0.21)*
		Difference in total fat mass index SDS (95% CI)	
	2455	*1898*	*557*
Confounder model additionally adjusted for maternal DASH diet score[a]	0.03 (-0.01 to 0.01)	-0.01 (-0.05 to 0.04)	0.13 (0.03 to 0.23)*
		Difference in android/gynoid ratio SDS (95% CI)	
	2458	*1901*	*557*
Confounder model additionally adjusted for maternal DASH diet score[a]	-0.01 (-0.05 to 0.04)	-0.04 (-0.09 to 0.01)	0.08 (-0.03 to 0.19)
		Difference visceral fat mass index SDS (95% CI)	
	1246	*956*	*290*
Confounder model additionally adjusted for maternal DASH diet score[a]	0.04 (-0.02 to 0.10)	0.00 (-0.08 to 0.07)	0.17 (0.03 to 0.31)*
		Difference in liver fat fraction SDS (95% CI)	
	1395	*1074*	*321*
Confounder model additionally adjusted for maternal DASH diet score[a]	-0.03 (-0.09 to 0.03)	-0.02 (-0.09 to 0.05)	-0.05 (-0.19 to 0.09)

**P<0.05 SDS: standard deviation scores. CI is confidence interval. DASH: Dietary Approaches to Stop Hypertension*

Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in standard deviation score of childhood adiposity outcomes per SDS increase in maternal early-pregnancy dietary glycemic index adjusted according to confounder models with additional adjustment for maternal DASH diet score

[a]Confounder models were adjusted for gestational age at intake, fetal sex and child's age at follow up, maternal age, maternal educational level, maternal prepregnancy BMI, smoking during pregnancy, vomiting during early-pregnancy, daily total energy intake

SUPPLEMENTARY TABLE S3.2.6. Associations of maternal early-pregnancy dietary glycemic index with childhood general, abdominal and ectopic fat accumulation among women without gestational diabetes

	Effect estimates for childhood outcome per SDS increase in maternal early-pregnancy glycemic index excluding women with gestational diabetes		
	Total group	**Women with BMI<25 kg/m²**	**Women with BMI≥25kg/m²**
		Difference in BMI SDS (95% CI)	
	n=2463	*n=1911*	*n=552*
Confounder model[a]	0.02 (-0.02 to 0.06)	0.00 (-0.04 to 0.05)	0.08 (-0.02 to 0.17)
		Difference in total fat mass index SDS (95% CI)	
	n=2435	*n=1889*	*n=546*
Confounder model[a]	0.03 (-0.01 to 0.07)	0.00 (-0.04 to 0.05)	0.13 (0.04 to 0.23)**
		Difference in android/gynoid ratio SDS (95% CI)	
	n=2438	*n=1892*	*n=546*
Confounder model[a]	0.01 (-0.03 to 0.05)	-0.01 (-0.06 to 0.03)	0.08 (-0.03 to 0.18)
		Difference visceral fat mass index SDS (95% CI)	
	n=1241	*n=954*	*n=287*
Confounder model[a]	0.06 (0.00 to 0.12)	0.01 (-0.06 to 0.08)	0.22 (0.09 to 0.35)**
		Difference in liver fat fraction SDS (95% CI)	
	n=1392	*n=1074*	*n=318*
Confounder model[a]	-0.03 (-0.08 to 0.03)	0.00 (-0.07 to 0.07)	-0.03 (-0.18 to 0.11)

**P<0.05 **P<0.01 SDS: standard deviation scores. CI is confidence interval*

*[a]Confounder models were the basic models additionally adjusted for maternal age, maternal educational level, maternal prepregnancy BMI, smoking during pregnancy, vomiting during early-pregnancy, daily total energy intake*P<0.05 **P<0.01 SDS: standard deviation scores. CI is confidence interval*

[a]Confounder models were the basic models additionally adjusted for maternal age, maternal educational level, maternal prepregnancy BMI, smoking during pregnancy, vomiting during early-pregnancy, daily total energy intake

3.3

Maternal plasma polyunsaturated fatty acids and childhood liver fat accumulation

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ABSTRACT

Background: Maternal polyunsaturated fatty acids (PUFA) concentrations in pregnancy are essential for fetal lipid metabolism and adipocyte differentiation. Whether suboptimal maternal gestational PUFA concentrations adversely affect offspring liver fat development is unknown.

Objective: We examined the associations of maternal n-3 and n-6 PUFA concentrations in pregnancy with childhood liver fat accumulation.

Design: In a population-based prospective cohort study among 2,424 mother-child pairs, we measured maternal total and individual n-3 and n-6 PUFA plasma concentrations at mean gestational age (SD) of 20.6 (1.1) weeks. Childhood liver fat fraction was obtained by MRI at 10 years. Non-alcoholic fatty liver disease was a liver fat fraction ≥5.0%.

Results: We observed that 1-Standard deviation (SD) higher maternal n-3 PUFA concentrations, especially DHA, was associated with a lower childhood liver fat fraction (-0.07 SD-score (95% CI -0.11 to -0.02) for both total n-3 PUFA and DHA concentrations). Of n-6 PUFAs, 1-SD higher maternal DGLA concentrations was associated with a higher childhood liver fat fraction (0.06 SD-score (95% CI 0.02 to 0.10)). Associations were not explained by maternal or childhood socio-demographic and lifestyle characteristics. Associations were stronger among boys and less consistent among girls. Among boys, higher maternal total n-3 PUFA concentrations were associated with a lower risk of childhood non-alcohol fatty liver disease (Odds Ratio 0.42 (95% CI 0.25 to 0.70)).

Conclusions: Maternal lower n-3 PUFA and higher n-6 PUFA concentrations in pregnancy are associated with offspring liver fat accumulation in childhood. Optimizing maternal PUFA concentrations during pregnancy may be a target for preventing liver fat accumulation in their offspring.

INTRODUCTION

Liver steatosis is the most common cause of chronic liver disease in childhood (1). Children with liver steatosis have a strongly increased risk of cardio-metabolic disease in later life, such as type 2 diabetes and end-stage liver disease in adulthood (1, 2). A higher amount of liver fat is even related to an adverse cardio-metabolic risk profile already in childhood (3, 4). It has been suggested that fetal life is a critical period for developing liver steatosis later in life (1, 5, 6). In the same cohort as the current study, we have previously shown that children with fetal growth restriction followed by infant catch up growth have an increased liver fat fraction (7). More specifically, the maternal diet during pregnancy and subsequent fetal nutrient availability could contribute to fetal liver adipose tissue deposits (1, 8, 9). Maternal dietary intake of poly-unsaturated fatty acids (PUFA) is essential for fetal growth and adipose tissue development, as the fetus fully depends on maternal PUFA concentrations (10, 11). Animal studies have shown that higher maternal omega-3 (n-3) PUFA concentrations may inhibit offspring liver fat storage, while higher maternal omega-6 (n-6) PUFA concentrations could stimulate offspring hepatic adipocyte differentiation (12-16). Human studies have suggested that suboptimal maternal PUFA concentrations during pregnancy may be related to development of childhood adiposity (17-19). However, the effects of maternal PUFA concentrations during pregnancy on childhood liver fat development remain unknown. As childhood liver steatosis is an emerging public health problem, identification of modifiable early-life risk factors is important for strategies which aim to prevent liver fat accumulation and associated cardio-metabolic risk from childhood onwards.

We hypothesized that lower maternal n-3 PUFA concentrations and higher n-6 PUFA concentrations during pregnancy lead to increased childhood liver fat accumulation, predisposing offspring to risks of adverse cardio-metabolic outcomes later in life. Therefore, in a population-based prospective cohort among 2,424 mothers and their children we examined the associations of maternal n-3 and n-6 PUFA concentrations during pregnancy with childhood liver fat accumulation and the risk of non-alcohol fatty liver disease (NAFLD) at the age of 10 years.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands (20). Written informed consent was obtained from all women. The study has been approved by the

Medical Ethical Committee of the Erasmus MC, University Medical Center in Rotterdam (MEC 198.782/2001/31). In total, 8,879 women were enrolled during pregnancy, of whom 6,997 had information on PUFA concentrations in pregnancy available. We excluded twin pregnancies (n=73). Of these women, 4,140 children participated in follow up measurements at ten years of age (**Supplementary Figure S3.3.1**). Due to later implementation of MRI scans within follow-up visits, we only had measurements in a subgroup of our population for liver fat fraction (n=2,424). Missing measurements were mainly due to whether the child attended the MRI subgroup, loss to follow up, no data on liver fat or MRI artifacts (20).

Maternal PUFA concentrations

Maternal venous samples were drawn at a mean gestational age (SD) of 20.6 (1.1) weeks). To analyze PUFA concentrations, EDTA plasma samples were selected and transported to the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich Medical Center. After being thawed, the analysis of plasma glycerophospholipid PUFAs composition was performed by a sensitive and precise high-throughput method, suitable in large epidemiological studies (21). PUFA concentrations were expressed as a proportion of total fatty acids present in the chromatogram (weight percentage, wt%) (22, 23). Based on findings from previous studies, we selected maternal PUFAs for our analyses, which previously have been shown to be associated with an increased risk of cardiovascular and metabolic outcomes in adults and maternal and fetal pregnancy outcomes (24-28). Total n-3 PUFA concentrations included α-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3), docosahexaenoic acid (DHA, C22:6n-3). Total n-6 PUFA concentrations included linoleic acid (LA, C18:2n-6), γ-linolenic acid (GLA, C18:3n-6), eicosadienoic acid (EDA, C20:2n-6), dihomo-gamma-linolenic acid (DGLA, C20:3n6), arachidonic acid (AA, C20:4n-6), docosatetraenoic acid (DTA, C22:4n-6). The n-6/n-3 ratio was calculated as the sum of all n-6 PUFA divided by the sum of all n-3 PUFA.

Childhood liver fat

At 10 years of age, we measured child's liver fat fraction obtained from MRI scans as described in detail previously (29). All children underwent imaging using a 3.0-T MRI scanner (Discovery MR750w; GE Healthcare). The liver fat scan was performed using a single-breath-hold, 3D volume and a special 3-point proton density weighted Dixon technique (IDEAL IQ) for generating a precise liver fat fraction image (30). The IDEAL IQ scan is based on a carefully tuned 6-echo echo planar imaging acquisition. The obtained fat-fraction maps were analyzed by the Precision Image Analysis (PIA, Kirkland,

Washington, United States) using the sliceOmatic (TomoVision, Magog, Canada) software package. Liver fat fraction was determined by taking four samples of at least 4 cm^2 from the central portion of the hepatic volume. Subsequently, the mean signal intensities were averaged to generate an overall mean liver fat estimation. NAFLD was defined as liver fat fraction ≥5.0% (31, 32).

Covariates

Information on maternal age, educational level and ethnicity was obtained at the enrolment (20). We measured maternal height and obtained information about maternal pre-pregnancy weight by questionnaire to calculate prepregnancy body mass index (BMI). We measured systolic blood pressure at study enrolment (20). Information on maternal smoking and folic acid supplement use was assessed by questionnaires during pregnancy. We used a food frequency questionnaire to assess maternal caloric intake during pregnancy (33, 34). Weight gain up to 30 weeks of gestation was calculated as the difference between maternal weight measured at a median (95% range) gestational age of 30.2 (28.4 to 32.6) and self-reported weight before pregnancy. Information about pregnancy complications, sex, gestational age and weight at birth was obtained from medical records (20). Information about breastfeeding, timing of introduction of solid foods and average TV watching time was obtained by questionnaires in infancy and childhood (20).

Statistical analysis

First, we performed a non-response analysis to compare characteristics of mothers and children with and without childhood liver fat measurements available using Student's t-tests, Mann-Whitney tests and Chi-square tests. Second, we examined the associations of maternal total and individual n-3 and n-6 PUFA concentrations during pregnancy with childhood liver fat fraction and the risk of NAFLD using linear and logistic regression models, respectively. Because of its skewed distribution, liver fat fraction was log-transformed. To enable comparison of effect estimates, we constructed standard deviation scores (SDS) for maternal PUFA concentrations and childhood liver fat fraction. We assessed associations in different models, constructed based on a Directed Acyclic Graph (DAG) analysis to identify which factors may act as confounders or potential mediators (**Supplementary Methods S3.3.1**): 1) a basic model including gestational age at maternal blood sampling and child's sex age at liver fat measurement; 2) a maternal model, which is the basic model additionally adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking, folic acid supplement use and daily caloric intake during pregnancy, systolic blood pressure at study enrolment, gestational weight gain up to 30 weeks of gestation, gestational

hypertensive disorders and gestational diabetes, based on their associations with the exposures and outcomes in literature. 3) a child model, which is the maternal model additionally adjusted for child characteristics, including the gestational-age-and-sex-adjusted birthweight, breastfeeding, timing of introduction of solid foods and average TV watching time (24, 35). Because of the statistically significant interaction of child's sex with maternal n-3 PUFA concentrations and the n-6/n-3 PUFA ratio for the associations with childhood liver fat fraction, we performed analyses for the total group and stratified by offspring sex. Finally, to examine whether potential associations of maternal PUFA status during pregnancy with childhood liver fat accumulation were present independent of the child's BMI, we used conditional regression analyses. We regressed childhood liver fat fraction on BMI to create standardized residuals for liver fat independent of child's BMI and examined the associations of maternal PUFA concentrations with these standardized residuals as outcomes using linear regression models (36). These analyses enable further exploration whether potential associations with childhood liver fat reflect an excess liver fat accumulation or rather represent an overall higher BMI in general. To reduce selection bias due to missing data, multiple imputations of covariates (pooled results of 5 imputed datasets) were performed (37). All analyses are performed using the Statistical Package of Social Sciences version 24.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Subject characteristics

Table 3.3.1 shows maternal and childhood characteristics. Of all participating children, 63 (3%) had NAFLD. The mean (SD) maternal n-3 and n-6 PUFA concentrations in pregnancy were 109.1 (27.4) mg/L and 607.2 (88.8) mg/L, respectively (**Table 3.3.2**). Characteristics were largely similar among boys and girls (**Supplementary Table S3.3.1**). Non-response analyses showed that compared to mothers without offspring liver fat measurements available, those with offspring liver fat measurements available had slightly higher total n-3 PUFA, but lower total n-6 PUFA concentrations and had offspring with a higher birthweight (**Supplementary Tables S3.3.2 and S3.3.3**).

TABLE 3.3.1. Characteristics for mothers and their children

	Total group (n=2,424)
Maternal Characteristics	
Age, mean (SD), years	30.9 (4.7)
Gestational age at enrolment, median (95% range), weeks	13.5 (9.8 to 22.1)
Gestational age at blood sampling, mean (SD), weeks	20.6 (1.1)
Ethnicity, n European (%)	1,571 (66)
Education, n higher education (%)	1,206 (52)
Parity, n nulliparous (%)	1,442 (60)
Pre-pregnancy BMI, median (95% range), kg/m^2	22.5 (18.0, 35.1)
Gestational weight gain, mean (SD) kg	0.34 (0.16)
Smoking during pregnancy, n no (%)	1,659 (77)
Folic acid supplement use, n yes (%)	1,507 (80)
Maternal dietary energy intake, mean (SD), kcal/day	2,075 (540)
Early-pregnancy systolic blood pressure, mean (SD), mmHg	116 (12)
Pregnancy complications,	
Gestational diabetes, n. yes (%)	18 (1)
Gestational hypertensive disorder, n yes (%)	143 (6)
Birth and infant characteristics	
Sex, n girls (%)	1,217 (50)
Birthweight, mean (SD), g	3,454 (540)
Gestational age at birth, median (95% range), weeks	40.1 (35.9, 42.4)
Ever breastfed, n yes (%)	1,943 (94)
Introduction of solid food after 6 months, n yes (%)	199 (11)
Child Characteristics at 10 years	
Age, mean (SD), years	9.8 (0.3)
Average TV watching time, n < 2 hours/day (%)	1,371 (71)
Body mass index, median (95% range), kg/m^2	17.5 (14.0 to 24.3)
Total fat mass, median (95% range), g	8,438 (4,474 to 21,535)
Liver fat fraction, median (95% range), (%)	2.0 (1.2 to 5.2)
Non-alcohol fatty liver disease, n yes (%)	63 (3)

Values represent mean (SD), median (95% range) or number of participants (valid %).

TABLE 3.3.2. Maternal poly-unsaturated fatty acid concentrations in pregnancy

	Absolute concentrations (mg/L)	Percentage by weight of total sum of fatty acids (%)
Total PUFA concentrations	716.4 (98.4)	43.7 (1.9)
Total n-3 PUFA concentrations	109.1 (27.4)	6.7 (1.5)
ALA	5.3 (1.8)	0.3 (0.1)
EPA	9.3 (5.6)	0.6 (0.3)
DPA	12.5 (4.2)	0.8 (0.2)
DHA	80.5 (20.4)	4.9 (1.1)
Total n-6 PUFA concentrations	607.2 (88.8)	37.1 (2.4)
LA	362.4 (63.1)	22.2 (2.8)
GLA	1.5 (0.7)	0.1 (0.0)
EDA	8.5 (1.9)	0.5 (0.1)
DGLA	61.7 (16.3)	3.7 (0.7)
AA	158.3 (32.4)	9.7 (1.5)
DTA	7.0 (2.1)	0.4 (0.1)
Total n-6/n-3 ratio	5.9 (1.6)	NA

PUFA: poly-unsaturated fatty acids, ALA: α-linolenic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid, LA: linoleic acid, GLA: γ-linolenic acid, EDA: eicosadienoic acid, DGLA: dihomo-gamma-linolenic acid, AA: arachidonic acid, DTA: docosatetraenoic acid. Values represent mean (SD)

Maternal n-3 PUFA concentrations and childhood liver fat

Table 3.3.3 shows that after adjustment for maternal factors, 1-Standard deviation (SD) higher maternal total n-3 PUFA concentrations and especially docosahexaenoic acid (DHA) concentrations were associated with a -0.07 SDS (95% CI -0.11 to -0.02) lower childhood liver fat fraction. Additional adjustment for child characteristics did not affect the effect estimates. Results from the conditional analyses suggested that the associations of maternal total n-3 PUFA and DHA concentrations with childhood liver fat fraction were independent of childhood BMI (**Supplementary Table S3.3.4**). Higher maternal n-3 PUFA concentrations were not associated with the risk of childhood NAFLD (**Figure 3.3.1**).

Analyses stratified by sex showed that the observed associations were only present in boys, but not among girls (**Supplementary Table S3.3.5**). Among boys, also higher maternal EPA concentrations were associated with a lower childhood liver fat fraction ($p<0.05$). One SD higher maternal total n-3 PUFA concentrations was associated with a lower risk of NAFLD among boys only (Odds Ratio 0.42 (95% CI 0.25 to 0.70)) (**Supplementary Figure S3.3.2**).

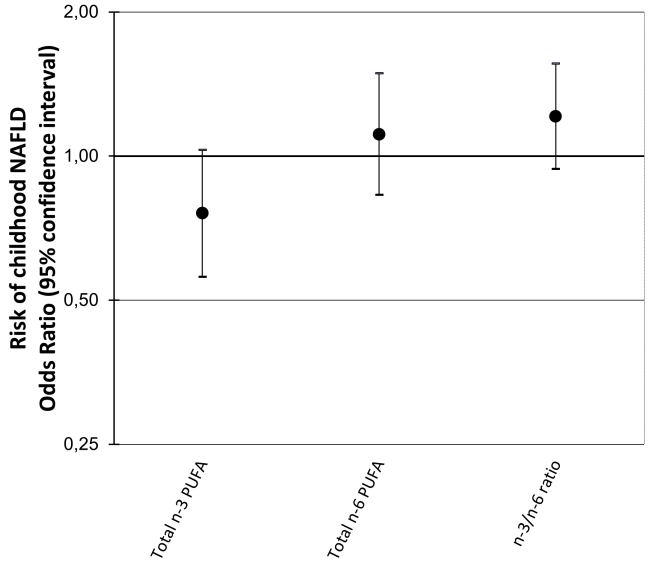


FIGURE 3.3.1. Associations of maternal n-3 and n-6 poly-unsaturated fatty acid concentrations with the risk of childhood non-alcohol fatty liver disease

PUFA: poly-unsaturated fatty acids SDS: standard deviation score. Values represent odds ratios (95% confidence interval) from logistic regression models that reflect the risks of childhood non-alcohol fatty live disease per SD increase in maternal PUFA concentrations adjusted for adjusted for gestational age at maternal blood sampling, child's age and sex and maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, blood pressure at enrolment, smoking, folic acid supplement use and total caloric intake during pregnancy, gestational weight gain up to 30 weeks of gestation, and pregnancy complications. Estimates are based on multiple imputed data.

Maternal n-6 PUFA concentrations and childhood liver fat

Table 3.3.3 shows that of all n-6 PUFAs, only 1-SD higher maternal n-6 PUFA dihomo-gamma-linolenic acid (DGLA) concentrations were associated with a 0.06 SDS (95% CI 0.02 to 0.10) higher liver fat fraction in the maternal model. Additional adjustment for child characteristics did not affect the effect estimate. The associations of maternal DGLA concentrations with childhood liver fat fraction were independent of childhood BMI (**Supplementary Table S3.3.4**). Higher maternal total n-6 PUFA concentrations were not associated with the risk of childhood NAFLD (**Figure 3.3.1**). Sex stratified analyses showed that the observed associations were similar for boys and girls (**Supplementary Table S3.3.5, Figure S3.3.2**).

TABLE 3.3.3. Associations of maternal n-3 poly-unsaturated fatty acid concentrations with childhood liver fat accumulation at ten years

Total n-3 PUFA concentrations (SDS)	Differences in childhood liver fat fraction (95% confidence interval) in SDS (n=2,424)	Total n-6 PUFA concentrations (SDS)	Differences in childhood liver fat fraction (95% confidence interval) in SDS (n=2,424)
Basic model[1]	-0.11 (-0.15 to -0.07)**	Basic model[1]	0.07 (0.04 to 0.10)**
Maternal model[2]	-0.07 (-0.11 to -0.02)**	Maternal model[2]	0.01 (-0.04 to 0.05)
Child model[4]	-0.06 (-0.10 to -0.02)**	Child model[3]	NA
ALA		**LA**	
Basic model[1]	-0.04 (-0.06 to 0.00)	Basic model[1]	0.02 (0.00 to 0.05)
Maternal model[2]	0.01 (-0.03 to 0.05)	Maternal model[2]	0.01 (-0.03 to 0.05)
Child model[4]	NA	Child model[3]	NA
EPA		**GLA**	
Basic model[1]	-0.07 (-0.10 to -0.04)**	Basic model[1]	0.04 (0.02 to 0.06)**
Maternal model[2]	-0.04 (-0.08 to 0.00)	Maternal model[2]	0.03 (-0.01 to 0.07)
Child model[3]	-0.04 (-0.07 to 0.00)	Child model[3]	NA
DPA		**EDA**	
Basic model[1]	-0.07 (-0.11 to -0.03)**	Basic model[1]	0.01 (0.00 to 0.03)
Maternal model[2]	-0.03 (-0.07 to 0.01)	Maternal model[2]	0.01 (-0.03 to 0.05)
Child model[3]	NA	Child model[3]	NA
DHA		**DGLA**	
Basic model[1]	-0.10 (-0.12 to -0.08)**	Basic model[1]	0.08 (0.06 to 0.09)**
Maternal model[2]	-0.07 (-0.11 to -0.02)**	Maternal model[2]	0.06 (0.02 to 0.10)**
Child model[3]	-0.06 (-0.11 to -0.02)**	Child model[3]	0.06 (0.02 to 0.10)**
		AA	
		Basic model[1]	0.02 (0.01 to 0.04)**
		Maternal model[2]	-0.04 (-0.08 to 0.01)

TABLE 3.3.3. Continued

Total n-3 PUFA concentrations (SDS)	Differences in childhood liver fat fraction (95% confidence interval) in SDS (n=2,424)	Total n-6 PUFA concentrations (SDS)	Differences in childhood liver fat fraction (95% confidence interval) in SDS (n=2,424)
		Child model[3]	NA
		DTA	
		Basic model[1]	0.07 (0.05 to 0.08)**
		Maternal model[2]	0.03 (-0.01 to 0.07)
		Child model[3]	NA

SDS: standard deviation score, PUFA: poly-unsaturated fatty acids, ALA: α-linolenic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid, LA: linoleic acid, GLA: γ-linolenic acid, EDA: eicosadienoic acid, DGLA: dihomo-gamma-linolenic acid, AA: arachidonic acid, DTA: docosatetraenoic acid, NA: not applicable

*Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal PUFA concentrations. Estimates are based on multiple imputed data. SDS is standard deviation score, NA is not applicable *p<0.05 **p<0.01*

[1]Basic model includes gestational age at maternal blood sampling, child's age and sex.

[2]Maternal model includes the basic model adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, blood pressure at enrolment, smoking, folic acid supplement use and total caloric intake during pregnancy, gestational weight gain up to 30 weeks of gestation, and pregnancy complications.

[3]Child model is the maternal model additionally adjusted for gestational-age-and-sex-adjusted birthweight, breastfeeding, timing of introduction of solid foods and average TV watching time.

Maternal n-6/n-3 PUFA ratio and childhood liver fat

One SD higher maternal n-6/n-3 PUFA ratio was associated with 0.06 SDS (95% CI 0.02 to 0.11) higher childhood liver fat fraction, but not with the risk of childhood NAFLD (**Figure 3.3.1**). Additional adjustment for child characteristics did not affect the effect estimate (**Supplementary Table S3.3.6**). The associations of maternal n-6/n-3 PUFA ratio with childhood liver fat fraction were also independent of childhood BMI (**Supplementary Table S3.3.4**).

Sex stratified analyses showed that the observed associations were only present in boys and not among girls (**Supplementary Table S3.3.6**).

DISCUSSION

Lower maternal n-3 PUFA and higher maternal n-6 PUFA concentrations during pregnancy were associated with increased childhood liver fat accumulation, especially among boys. The strongest effects were present for DHA and DGLA. Higher maternal total n-3 PUFA concentrations during pregnancy were associated with a lower risk of childhood NAFLD among boys, but not among girls.

Interpretation of main findings

Maternal n-3 PUFA concentrations are important for fetal growth and development and may reduce the risk of preterm birth and low birthweight (38, 39). Besides improving birth outcomes, optimizing maternal PUFA status during pregnancy, characterized by increasing maternal n-3 PUFA and lowering maternal n-6 PUFA concentrations, may also have a beneficial effect on offspring adiposity development (40). In a previous study from our cohort, we observed that higher maternal n-3 PUFA concentrations and lower maternal n-6 PUFA concentrations were associated with a lower childhood total body fat mass and android/gynoid fat mass ratio at six years of age (19). Rodent studies have suggested that higher maternal n-3 PUFA concentrations during pregnancy may reduce hepatic fat storage in offspring, through the effects on lipid metabolism and lipid gene expression in the liver (14, 15, 41-43). On the other hand, higher n-6 PUFA concentrations could stimulate hepatic adipocyte differentiation, leading to liver fat accumulation (14, 16). Previous meta-analyses in non-pregnant populations suggested that higher n-3 PUFA concentrations could reduce liver fat in both children and adults (44, 45). Based on these studies, we hypothesized for the current study that lower maternal n-3 PUFA concentrations and higher n-6 PUFA concentrations during pregnancy increases offspring liver fat development.

We observed that lower maternal n-3 PUFA and higher n-6 PUFA concentrations during pregnancy were associated with increased childhood liver fat accumulation. The strongest effects were present for the individual n-3 PUFA DHA and the individual n-6 PUFA DGLA. This is largely in line with findings from previous studies that showed that mainly maternal DHA and DGLA concentrations are associated with offspring general and abdominal fat accumulation (19, 46-48). When stratifying on sex, the associations of maternal n-3 PUFA concentrations in pregnancy with a childhood liver fat accumulation and the risk of NAFLD were only present among boys, while we observed no sex-dependent differences for associations of maternal n-6 PUFAs. Differences of associations between boys and girls could be due to the differences in n-3 PUFA metabolism between boys and girls or to differences in effects on gene expression between boys and girls (49, 50). Mechanistic studies, including animal studies, should identify the pathways underlying the sex-specific differences of the associations of maternal PUFA concentrations with offspring liver fat accumulation.

Maternal and childhood socio-demographic and lifestyle characteristics did not explain our observed associations, substantiating the hypothesis that maternal PUFA concentrations during pregnancy may have a direct effect on offspring liver fat development. Associations were also independent of childhood BMI. These findings suggest that associations are likely to not just reflect a higher overall fat, but that these children exposed to suboptimal maternal PUFA concentrations during pregnancy have excess liver fat development. This is important, as liver steatosis is highly indicative for an adverse cardio-metabolic health, even in children with a normal BMI (3).

The mechanisms underlying the associations of suboptimal maternal PUFA concentrations with increased childhood liver fat accumulation remain largely unclear. Higher maternal n-6 PUFA and subsequent higher fetal n-6 PUFA concentrations can promote fetal adipocyte differentiation through peroxisome proliferator-activated receptor activation, stimulating triglyceride storage and inflammatory processes (16, 51, 52). This is crucial for liver fat development, as hepatic fat content mainly consists of triglyceride storage (53). On the other hand, increase in maternal n-3 PUFA concentrations inhibits fetal adipocyte differentiation and lipid storage, decreasing adipose tissue deposition (51, 52, 54). We observed the strongest associations for maternal DHA with childhood liver fat accumulation. DHA can reduce triglyceride concentrations and lipoprotein lipase activity, which are highly involved in liver fat storage (15, 55). Besides, misbalances in maternal PUFA concentrations could cause DNA methylation patterns that determine expression of lipogenic genes in the liver (41, 56).

The observed associations of maternal PUFA concentrations with childhood liver fat accumulation were relatively small, but are important from a public health perspective. Adequate maternal PUFA intake, mainly focused on increasing DHA, during pregnancy has already been a target of interest due to its beneficial effects on birth outcomes (38, 39). Our study underlines the importance of optimization of maternal PUFA concentrations during pregnancy, not only for birth outcomes, but also for long-term offspring liver outcomes. As DHA can easily be supplemented in the maternal diet, our findings may offer an important target during pregnancy for the early prevention of childhood liver steatosis (43, 57). Further studies need to be conducted to assess whether adequate maternal PUFA intake during pregnancy could reduce offspring liver accumulation, risk of NALFD and subsequent offspring cardio-metabolic risk.

Methodological considerations

Strengths of this study are the prospective design, large sample size, and the use of detailed measures of childhood liver fat obtained with MRI. The children who underwent MRI measurements at 10 years consisted of a random subgroup of the Generation R Study. We observed that mothers of children that were included had higher total n-3 and lower n-6 PUFA concentrations in comparison to those not participating in follow up measurements, which may have led to selection bias and an underestimation of results. Maternal PUFA concentrations were measured only once in mid-pregnancy. It is known that PUFA concentrations measured in plasma may reflect a period of dietary intake of approximately two weeks (58). Further studies using repeatedly measured maternal PUFA concentrations throughout pregnancy should evaluate critical periods for maternal PUFA concentrations to affect fetal fat development. Although we were able to adjust our analyses for multiple confounding factors, residual confounding due to for example childhood nutritional intake may be present.

CONCLUSIONS

Lower maternal n-3 PUFA concentrations and higher n-6 PUFA concentrations in pregnancy are associated with higher childhood liver fat accumulation, especially among boys. Optimization of maternal PUFA concentrations during pregnancy could be a potential target to reduce the risk of childhood liver steatosis.

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SUPPLEMENTARY MATERIAL

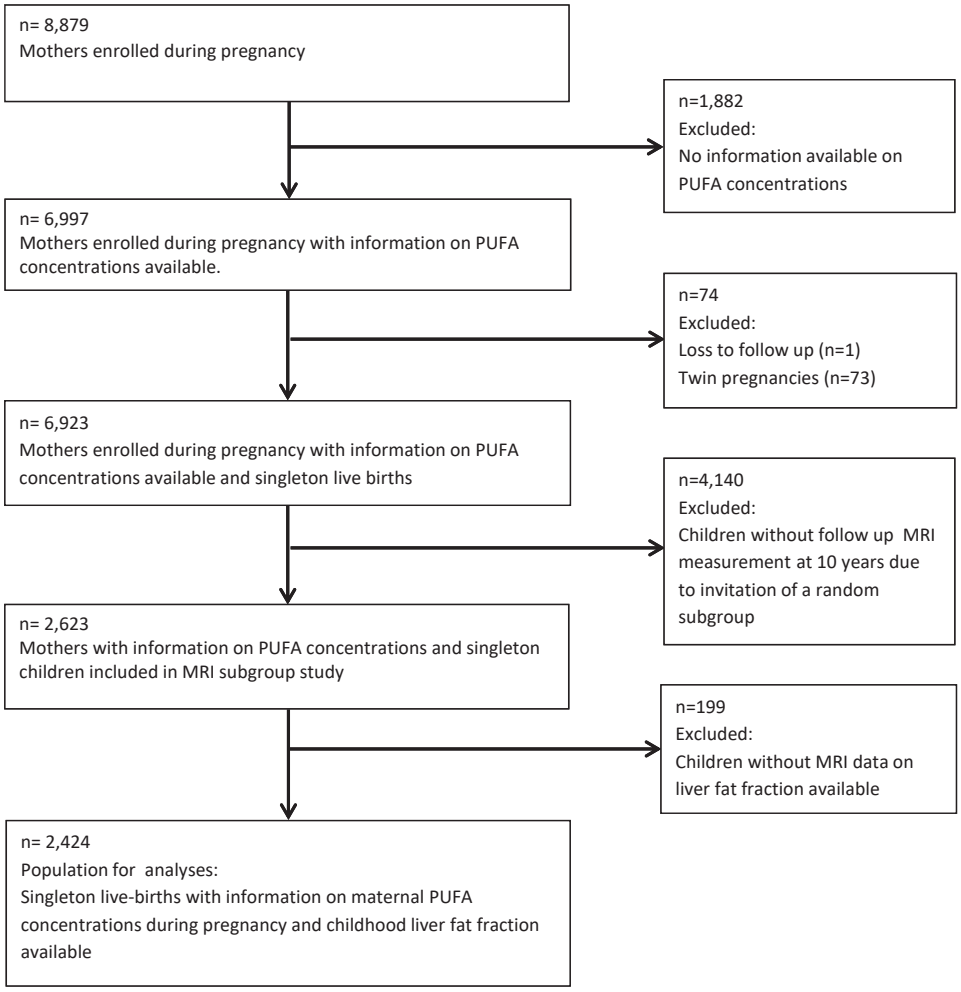
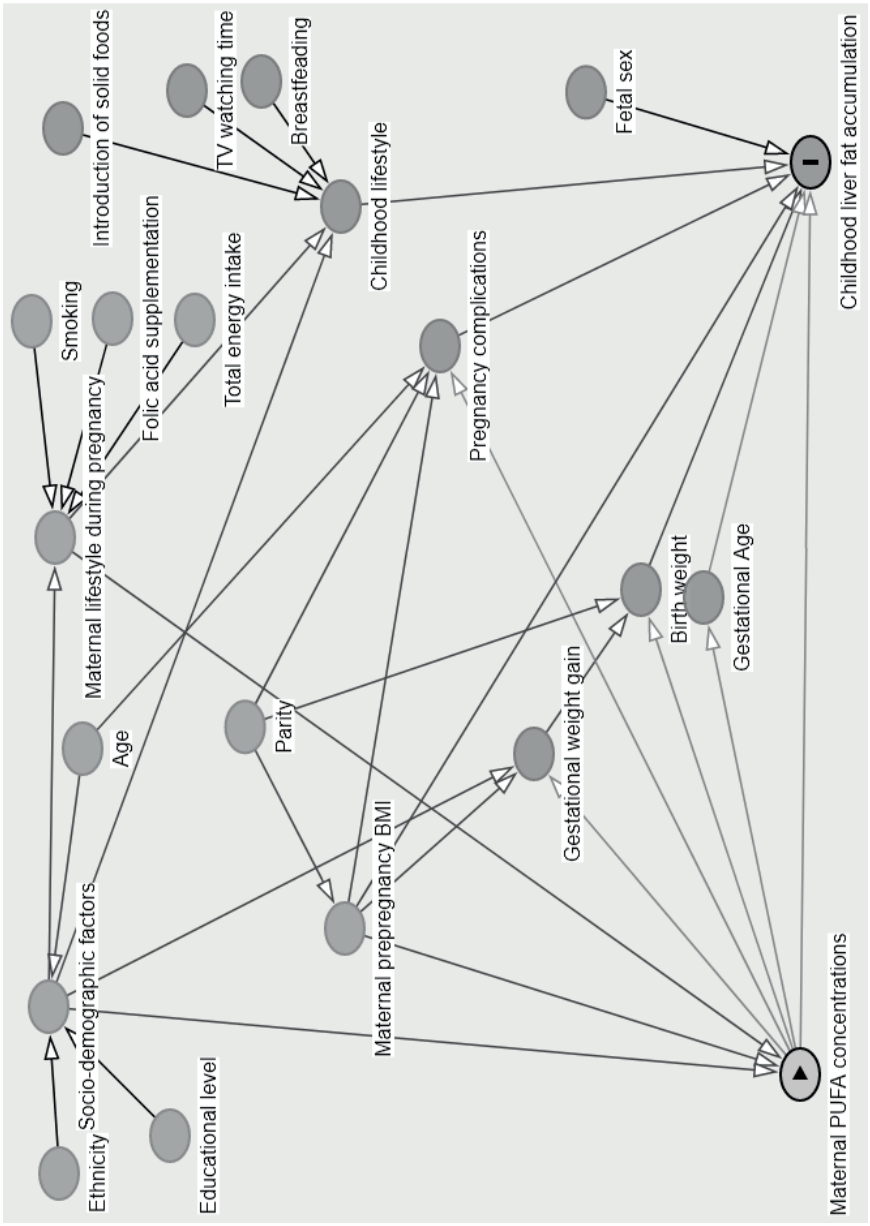


FIGURE S3.3.1. Flow chart of the study participants



SUPPLEMENTARY METHODS S3.3.1. Directed Acyclic Graph analysis

SUPPLEMENTARY TABLE S3.3.1. Population characteristics for boys and girls separately

	Boys (n=1,207)	Girls (n=1,217)
Maternal Characteristics		
Age, mean (SD), years	31.0 (4.8)	30.8 (4.7)
Gestational age at enrolment, median (95% range), weeks	14.4 (9.7 to 22.1)	13.5 (10.0 to 22.1)
Gestational age at blood sampling, mean (SD), weeks	20.7 (1.1)	20.6 (1.1)
Ethnicity, n European (%)	779 (65)	792 (66)
Education, n higher education (%)	607 (53)	599 (51)
Parity, n nulliparous (%)	692 (58)	750 (62)
Pre-pregnancy BMI, median (95% range), kg/m^2	22.5 (18.0 to 35.5)	22.6 (18.2 to 35.0)
Gestational weight gain, mean (SD) kg	0.34 (0.15)	0.34 (0.16)
Smoking during pregnancy, n no (%)	822 (77)	835 (25)
Folic acid supplement use, n yes (%)	735 (80)	772 (81)
Maternal dietary energy intake, mean (SD), kcal/day	2079 (525)	2071 (54)
Early-pregnancy systolic blood pressure, mean (SD), mmHg	116 (12)	116 (12)
Pregnancy complications,		
Gestational diabetes, n. yes (%)	8 (1)	10 (1)
Gestational hypertensive disorder, n yes (%)	71 (6)	72 (6)
Birth and infant characteristics		
Birthweight, mean (SD), g	3536 (551)	3373 (545)
Gestational age at birth, median (95% range), weeks	40.3 (36.0 to 42.4)	40.1 (35.7 to 42.1)
Ever breastfed, n yes (%)	944 (94)	999 (94)
Introduction of solid food after 6 months, n yes (%)	98 (11)	101 (11)
Child Characteristics at 10 years		
Age, mean (SD), years	9.8 (0.4)	9.8 (0.3)
Average TV watching time, n < 2 hours/day (%)	672 (69)	699 (72)
Body mass index, median (95% range), kg/m^2	16.8 (14.0 to 23.9)	17.1 (14.0 to 24.5)
Liver fat fraction, median (95% range), (%)	2.0 (1.20 to 5.19)	2.01 (1.27 to 5.20)
Non-alcohol fatty liver disease, n yes (%)	31 (3)	32 (3)

Values represent mean (Standard Deviation), median (95% range) or number of participants (valid %).

SUPPLEMENTARY TABLE S3.3.2. Comparison of characteristics mothers and children with and without childhood liver fat outcomes at ten years

Characteristics	Participation in follow-up MRI measurements (n=2,424)	No participation in follow-up MRI measurements (n=4,499)	P-value
Maternal Characteristics			
Age, mean (SD), years	30.9 (4.7)	29.1 (5.4)	0.00
Gestational age at enrolment, median (95% range), weeks	13.5 (9.8 to 22.1)	14.2 (9.8 to 23.2)	0.00
Ethnicity, n European (%)	1,571 (66)	2,242 (50)	0.00
Education, n higher education (%)	1,206 (52)	1,495 (37)	0.00
Parity, n nulliparous (%)	1,442 (60)	2,402 (54)	0.00
Pre-pregnancy BMI, median (95% range), kg/m^2	22.5 (18.0, 35.1)	22.7 (17.9 to 35.1)	0.20
Gestational weight gain, mean (SD) kg	0.34 (0.16)	0.34 (0.16)	0.67
Smoking during pregnancy, n no (%)	1,659 (77)	2,767 (70)	0.00
Folic acid supplement use, n yes (%)	1,507 (80)	2,198 (67)	0.00
Maternal dietary energy intake, mean (SD), kcal/day	2,075 (540)	2,020 (583)	0.00
Early-pregnancy systolic blood pressure, mean (SD), mmHg	116 (12)	115 (12)	0.02
Pregnancy complications,			
Gestational diabetes, n. yes (%)	18 (1)	52 (1)	0.09
Gestational hypertensive disorder, n yes	143 (6)	262 (6)	0.99
Birth and infant characteristics			
Sex, n girl (%)	1,217 (50)	2,210 (49)	0.39
Birthweight, mean (SD), g	3,454 (540)	3,340 (564)	0.00
Gestational age at birth, median (95% range), weeks	40.1 (35.9, 42.4)	40.1 (35.4 to 42.3)	0.00
Ever breastfed, n yes (%)	1,943 (94)	2,621 (91)	0.00
Introduction of solid food after 6 months, n yes (%)	199 (11)	194 (10)	0.27

Values represent mean (Standard Deviation), median (95% range) or number of subjects valid (%).

SUPPLEMENTARY TABLE S3.3.3. Comparison of maternal poly-unsaturated fatty acid concentrations in mothers with and without childhood liver fat outcomes at ten years

Maternal PUFAs concentrations	Participation in follow-up MRI measurements (n=2,424)	No participation in follow-up MRI measurements (n=4,499)	P-value
Total PUFA concentrations			
Absolute concentrations, mg/L	716.4 (98.4)	702.0 (98.7)	0.00
Percentage by weight of total sum of fatty acids	43.7 (1.9)	44.9 (2.0)	0.01
Total n-3 PUFA			
Absolute concentrations, mg/L	109.1 (27.4)	100.3 (26.8)	0.00
Percentage by weight of total sum of fatty acids	6.7 (1.5)	6.3 (1.4)	0.00
ALA			
Absolute concentrations, mg/L	5.3 (1.8)	4.9 (1.9)	0.00
Percentage by weight of total sum of fatty acids	0.3 (0.1)	0.30 (0.1)	0.00
EPA			
Absolute concentrations, mg/L	9.3 (5.6)	8.0 (5.1)	0.00
Percentage by weight of total sum of fatty acids	0.6 (0.3)	0.5 (0.3)	0.00
DPA			
Absolute concentrations, mg/L	12.5 (4.2)	11.6 (4.3)	0.00
Percentage by weight of total sum of fatty acids	0.8 (0.2)	0.7 (0.2)	0.00
DHA			
Absolute concentrations, mg/L	80.5 (20.3)	74.3 (19.9)	0.00
Percentage by weight of total sum of fatty acids	4.9 (1.1)	4.6 (1.1)	0.00
Total n-6 PUFA			
Absolute concentrations, mg/L	607.2 (88.8)	601.7 (89.5)	0.01
Percentage by weight of total sum of fatty acids	37.1 (2.4)	37.6 (2.6)	0.00
LA			
Absolute concentrations, mg/L	362.4 (63.1)	361.0 (62.7)	0.39
Percentage by weight of total sum of fatty acids	22.1 (2.8)	22.6 (2.9)	0.00
GLA			
Absolute concentrations, mg/L	1.5 (0.7)	1.5 (0.7)	0.51
Percentage by weight of total sum of fatty acids	0.1 (0.0)	0.1 (0.0)	0.00
EDA			
Absolute concentrations, mg/L	8.5 (1.9)	8.5 (1.9)	0.48
Percentage by weight of total sum of fatty acids	0.5 (0.1)	0.53 (0.1)	0.00
DGLA			
Absolute concentrations, mg/L	61.7 (16.3)	58.8 (17.0)	0.00
Percentage by weight of total sum of fatty acids	3.7 (0.7)	3.7 (0.8)	0.04
AA			
Absolute concentrations, mg/L	158.3 (32.4)	155.8 (33.0)	0.00

SUPPLEMENTARY TABLE S3.3.3. Continued

Maternal PUFAs concentrations	Participation in follow-up MRI measurements (n=2,424)	No participation in follow-up MRI measurements (n=4,499)	P-value
Percentage by weight of total sum of fatty acids	9.7 (1.5)	9.7 (1.6)	0.07
DTA			
Absolute concentrations, mg/L	7.0 (2.1)	7.0 (2.3)	0.38
Percentage by weight of total sum of fatty acids	0.42 (0.11)	0.44 (0.12)	0.00
Total n-6/n-3 PUFAs ratio			
Absolute concentrations, mg/L	5.9 (1.6)	6.4 (1.8)	0.00
Percentage by weight of total sum of fatty acids	-	-	-

PUFA: poly-unsaturated fatty acids, ALA: α-linolenic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid, LA: linoleic acid, GLA: γ-linolenic acid, EDA: eicosadienoic acid, DGLA: dihomo-gamma-linolenic acid, AA: arachidonic acid, DTA: docosatetraenoic acid.
Values represent mean (Standard Deviation).

SUPPLEMENTARY TABLE S3.3.4. Associations of maternal n-3 and n-6 poly-unsaturated fatty acid concentrations with childhood liver fat accumulation at ten years conditional for childhood BMI

	Differences in childhood liver fat fraction (95% confidence interval) in SDS (n=2,424)
Total n-3 PUFA concentrations (SDS)	-0.07 (-0.11 to -0.02)**
ALA	0.01 (-0.04 to 0.05)
EPA	-0.03 (-0.07 to 0.01)
DPA	-0.04 (-0.08 to 0.00)
DHA	-0.07 (-0.11 to -0.03)**
Total n-6 PUFA concentrations (SDS)	-0.01 (-0.05 to 0.04)
LA	-0.01 (-0.05 to 0.04)
GLA	0.03 (-0.01 to 0.07)
EDA	0.02 (-0.02 to 0.06)
DGLA	0.06 (0.02 to 0.11)**
AA	-0.04 (-0.08 to 0.01)
DTA	0.02 (-0.02 to 0.07)
n-3/n-6 ratio	0.06 (0.02 to 0.11)**

PUFA: poly-unsaturated fatty acids, ALA: α-linolenic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid, LA: linoleic acid, GLA: γ-linolenic acid, EDA: eicosadienoic acid, DGLA: dihomo-gamma-linolenic acid, AA: arachidonic acid, DTA: docosatetraenoic acid.
*Values represent regression coefficients (95% confidence interval) from conditional linear regression models that reflect the difference standard residual change of childhood outcomes conditional on BMI per SDS change in maternal PUFA concentrations adjusted for gestational age at maternal blood sampling, child's age and sex, maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, blood pressure at enrolment, smoking, folic acid supplement use and total caloric intake during pregnancy, gestational weight gain up to 30 weeks of gestation, and pregnancy complications. Estimates are based on multiple imputed data. **p<0.01.*

SUPPLEMENTARY TABLE S3.3.5. Associations of maternal n-3 poly-unsaturated fatty acid concentrations with childhood liver fat accumulation at ten years among boys and girls separately

	Differences in childhood fat outcomes (95% confidence interval) in SDS	
Maternal n-3 PUFA concentrations (SDS)	**Boys (n=1,207)**	**Girls (n=1,217)**
Total n-3 PUFAs		
Basic model[1]	-0.15 (-0.21 to -0.09)**	-0.06 (-0.12 to -0.01)*
Maternal model[2]	-0.11 (-0.18 to -0.05)**	-0.03 (-0.08 to 0.03)
Child model[3]	-0.11 (-0.17 to -0.05)**	-0.03 (-0.08 to 0.03)
ALA		
Basic model[1]	-0.03 (-0.09 to 0.03)	-0.05 (-0.10 to 0.01)
Maternal model[2]	0.00 (-0.06 to 0.06)	0.01 (-0.05 to 0.06)
Child model[3]	NA	NA
EPA		
Basic model[1]	-0.10 (-0.16 to -0.04)**	-0.04 (-0.09 to 0.01)
Maternal model[2]	-0.06 (-0.12 to 0.00)*	-0.01 (-0.07 to 0.04)
Child model[3]	-0.06 (-0.12 to 0.00)*	-0.02 (-0.07 to 0.04)
DPA		
Basic model[1]	-0.08 (-0.14 to -0.02)**	-0.05 (-0.11 to 0.01)
Maternal model[2]	-0.04 (-0.10 to 0.02)	-0.02 (-0.08 to 0.04)
Child model[3]	NA	NA
DHA		
Basic model[1]	-0.15 (-0.20 to -0.09)**	-0.06 (-0.11 to 0.00)*
Maternal model[2]	-0.11 (-0.17 to -0.05)**	-0.03 (-0.08 to 0.03)
Child model[3]	-0.11 (-0.17 to -0.05)**	-0.03 (-0.08 to 0.03)

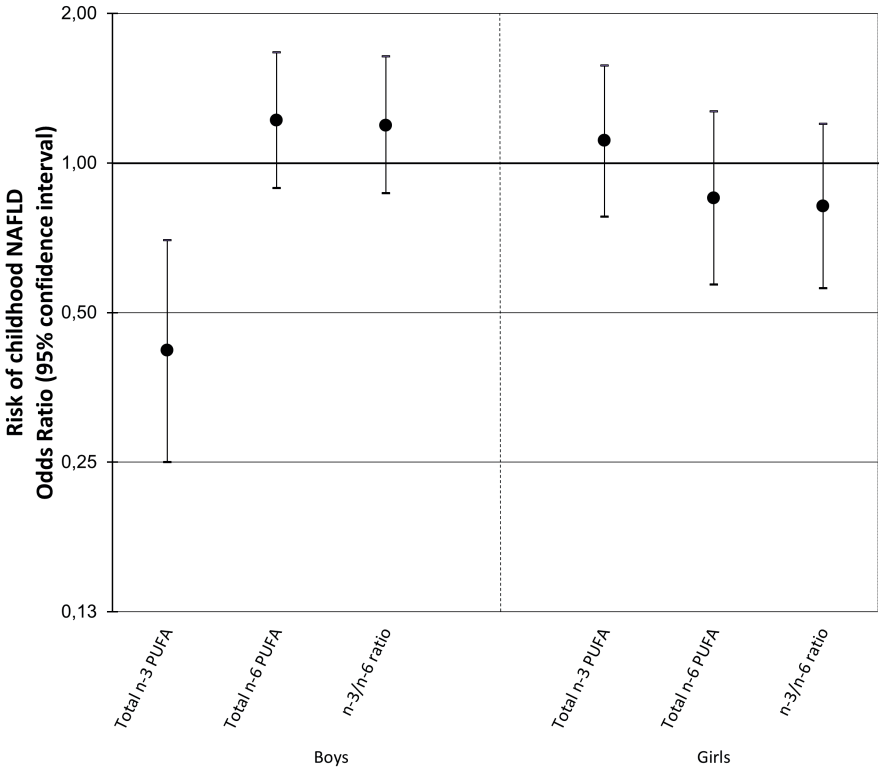
PUFA: poly-unsaturated fatty acids, ALA: α-linolenic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid

*Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal PUFA concentrations. Estimates are based on multiple imputed data. *p<0.05 **p<0.01*

[1]Basic model includes gestational age at maternal blood sampling and child's age.

[2] Maternal model includes the basic model adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, blood pressure at enrolment, smoking, folic acid supplement use and total caloric intake during pregnancy, gestational weight gain up to 30 weeks of gestation, and pregnancy complications.

[3]Child model is the maternal model additionally adjusted for gestational-age-and-sex-adjusted birthweight, breastfeeding, timing of introduction of solid foods and average TV watching time.



SUPPLEMENTARY FIGURE S3.3.2. Associations of maternal n-3 and n-6 poly-unsaturated fatty acid concentrations with the risk of childhood non-alcohol fatty liver disease among boys and girls separately

PUFA: poly-unsaturated fatty acids Values represent odds ratios (95% confidence interval) from logistic regression models that reflect the risks of childhood non-alcohol fatty live disease per SD increase in maternal PUFA concentrations adjusted for adjusted for gestational age at maternal blood sampling, child's age and sex and maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, blood pressure at enrolment, smoking, folic acid supplement use and total caloric intake during pregnancy, gestational weight gain up to 30 weeks of gestation, and pregnancy complications. Estimates are based on multiple imputed data. SDS is standard deviation score.

SUPPLEMENTARY TABLE S3.3.5. Associations of maternal n-6 poly-unsaturated fatty acid concentrations with childhood liver fat accumulation at ten years among boys and girls separately

	Differences in childhood fat outcomes (95% confidence interval) in SDS	
Total n-6 PUFA concentrations (SDS)	**Boys (n=1,207)**	**Girls (n=1,217)**
Basic model[1]	0.08 (0.03 to 0.14)	0.05 (-0.01 to 0.11)
Maternal model[2]	0.03 (-0.03 to 0.10)	-0.01 (-0.08 to 0.05)
Child model[3]	NA	NA
LA		
Basic model[1]	0.04 (-0.02 to 0.09)	0.00 (-0.06 to 0.05)
Maternal model[2]	0.03 (-0.04 to 0.09)	-0.01 (-0.07 to 0.05)
Child model[3]	NA	NA
GLA		
Basic model[1]	0.04 (0.01 to 0.07)	0.04 (-0.02 to 0.09)
Maternal model[2]	0.03 (-0.03 to 0.09)	0.03 (-0.03 to 0.09)
Child model[3]	NA	NA
EDA		
Basic model[1]	0.03 (-0.03 to 0.09)	0.00 (-0.06 to 0.06)
Maternal model[2]	0.02 (-0.04 to 0.08)	0.00 (-0.05 to 0.06)
Child model[3]	NA	NA
DGLA		
Basic model[1]	0.08 (0.02 to 0.14)**	0.08 (0.02 to 0.13)**
Maternal model[2]	0.06 (0.00 to 0.12)*	0.05 (-0.01 to 0.11)
Child model[3]	NA	NA
AA		
Basic model[1]	0.02 (-0.04 to 0.07)	0.03 (-0.02 to 0.09)
Maternal model[2]	-0.04 (-0.10 to 0.02)	-0.03 (-0.09 to 0.03)
Child model[3]	NA	NA
DTA		
Basic model[1]	0.08 (0.02 to 0.14)*	0.06 (0.00 to 0.11)
Maternal model[2]	0.04 (-0.02 to 0.10)	0.02 (-0.04 to 0.08)
Child model[3]	NA	NA

PUFA: poly-unsaturated fatty acids, ALA: α-linolenic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid

*Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal PUFA concentrations. Estimates are based on multiple imputed data. *p<0.05 **p<0.01*

[1]Basic model includes gestational age at maternal blood sampling and child's age.

[2] Maternal model includes the basic model adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, blood pressure at enrolment, smoking, folic acid supplement use and total caloric intake during pregnancy, gestational weight gain up to 30 weeks of gestation, and pregnancy complications.

[3]Child model is the maternal model additionally adjusted for gestational-age-and-sex-adjusted birthweight, breastfeeding, timing of introduction of solid foods and average TV watching time.

SUPPLEMENTARY TABLE S3.3.6. Associations of maternal n-6/n-3 poly-unsaturated fatty acid ratio with childhood liver fat accumulation at ten years

	Differences in childhood liver fat fraction (95% confidence interval) in SDS		
Maternal n-6/n-3 PUFA (SDS)	Total group (n=2,424)	Boys (n=1,207)	Girls (n=1,217)
Basic model[1]	0.11 (0.07 to 0.15)**	0.15 (0.09 to 0.21)**	0.07 (0.01 to 0.13)*
Maternal model[2]	0.06 (0.02 to 0.11)**	0.12 (0.05 to 0.18)**	0.02 (-0.04 to 0.08)
Child model[3]	0.06 (0.02 to 0.11)**	0.11 (0.05 to 0.18)**	0.02 (-0.04 to 0.08)

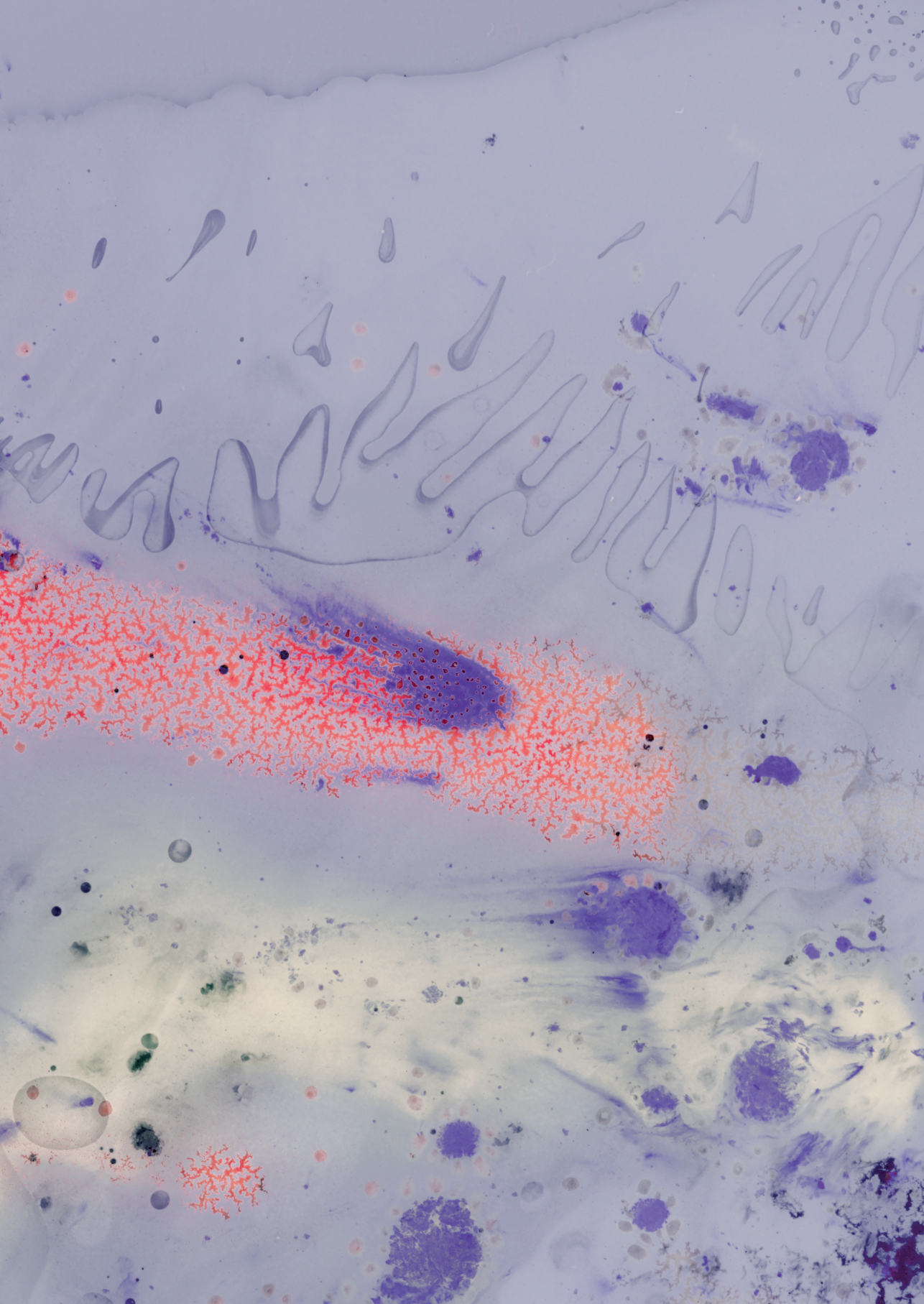
PUFA: poly-unsaturated fatty acids

*Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal PUFA concentrations. Estimates are based on multiple imputed data. SDS is standard deviation score. *p<0.05 **p<0.01*

[1] Basic model includes gestational age at maternal blood sampling, child's age and sex.

[2] Maternal model includes the basic model adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, blood pressure at enrolment, smoking, folic acid supplement use and total caloric intake during pregnancy, gestational weight gain up to 30 weeks of gestation, and pregnancy complications.

[3]Child model is the maternal model additionally adjusted for gestational-age-and-sex-adjusted birthweight, breastfeeding, timing of introduction of solid foods and average TV watching time.



04

Risk prediction of offspring health outcomes

4.1

Maternal prepregnancy body mass index, metabolic profiling and the prediction of birthweight

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ABSTRACT

Context: Maternal prepregnancy BMI has a strong influence on gestational metabolism, but detailed metabolic alterations are unknown.

Objective: First, to examine the associations of maternal prepregnancy BMI with maternal early-pregnancy metabolite alterations. Second, to identify an early-pregnancy metabolite profile associated with birthweight in women with a higher prepregnancy BMI that improved prediction of birthweight compared to glucose and lipid concentrations.

Design, setting and participants: Prepregnancy BMI was obtained in a subgroup of 682 Dutch pregnant women from the Generation R prospective cohort study.

Main outcome measures: Maternal non-fasting targeted amino acid, non-esterified fatty acid, phospholipid and carnitine concentrations measured in blood serum at mean gestational age of 12.8 weeks. Birthweight, obtained from medical records.

Results: A higher prepregnancy BMI was associated with 72 altered amino acid, non-esterified fatty acid, phospholipid and carnitine concentrations and 6 metabolite ratios reflecting Krebs cycle, inflammatory, oxidative stress and lipid metabolic processes (p-values<0.05). Using penalized regression models, a metabolite profile was selected including 15 metabolites and 4 metabolite ratios, based on its association with birthweight in addition to prepregnancy BMI. The adjusted R^2 of birthweight was 6.1% for prepregnancy BMI alone, 6.2% after addition of glucose and lipid concentrations and 12.9% after addition of the metabolite profile.

Conclusions: A higher maternal prepregnancy BMI was associated with altered maternal early-pregnancy amino acids, non-esterified fatty acids, phospholipids and carnitines. Using these metabolites, we identified a maternal metabolite profile which improved prediction of birthweight in women with a higher prepregnancy BMI compared to glucose and lipid concentrations.

INTRODUCTION

Overweight or obesity in women prior and during pregnancy is a major risk factor for birth complications, including delivering a large-for-gestational-age newborn (1, 2). These associations of a higher maternal prepregnancy BMI with a higher birthweight are not confined to the extremes, but are already present across the full ranges of maternal prepregnancy BMI and birthweight (3). Although the association of a higher maternal prepregnancy BMI with a higher birthweight is well known, the underlying mechanisms are not understood.

Maternal prepregnancy BMI has a strong influence on maternal metabolism during pregnancy, leading to an increased and suboptimal composition of fetal nutrient supply (1, 2). It has been proposed that these alterations in fetal nutrient supply cause increased fetal growth and stimulate adiposity development, leading to a higher birthweight (1, 2). Higher maternal glucose and lipid concentrations during pregnancy have already been shown to be important factors leading to increased fetal growth, but only partly explain the associations of a higher maternal prepregnancy BMI with a higher birthweight (4). Metabolomics techniques offer the opportunity to obtain a detailed characterization of maternal metabolism during pregnancy and may enable identification of novel metabolic pathways in the associations of a higher maternal prepregnancy BMI with macrosomia (5). Recent studies already reported that a higher maternal BMI during pregnancy was associated with altered maternal metabolite concentrations throughout pregnancy, in particular with alterations in branched chain amino acids (BCAA) and non-esterified fatty acid (NEFA) serum concentrations (6-9). Other studies observed associations of altered maternal amino acids (AA), phospholipids (PL) and carnitines (Carn) serum concentrations in the second half of pregnancy with higher birthweight (10, 11). A previous study among 400 pregnant women showed that altered maternal acylcarnitine, lipid, carbohydrate and organic acid related metabolites associated with a higher maternal BMI in second half of pregnancy could improve prediction of birthweight and fat mass in the newborn (12). Identifying detailed maternal early-pregnancy metabolic profiles involved in the association of a higher maternal prepregnancy BMI with higher offspring birthweight may provide more insight into the mechanisms underlying this well-known association and offer novel biomarkers for early identification of pregnant women at increased risk of delivering a large-for-gestational-age newborn.

Therefore, in a subgroup of 682 Dutch women participating in a population-based prospective cohort study from early-pregnancy onwards, we first examined the associations of maternal prepregnancy BMI across the full range with maternal non-

fasting early-pregnancy serum concentrations of amino acids (AA), non-esterified fatty acids (NEFA), phospholipids (PL) and carnitines (Carn). Second, we explored the predictive value of identified maternal early-pregnancy metabolites alterations on offspring birthweight in addition to maternal glucose and lipid concentrations.

METHODS

Study Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood in Rotterdam, the Netherlands (13). Study approval was obtained 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 women participating in the study. In total 8,879 women were enrolled during pregnancy in the Generation R Study. Metabolomics data were available in a preselected subsample of 1041 Dutch mother–child pairs, of whom 814 had early-pregnancy metabolomics data available (13). This subsample is a random group of mothers and their children of Dutch ethnicity selected for additional measurements within the Generation R study, already at the start of our cohort study (14). Dutch ethnicity of participants in this samples was defined as having both parents born in the Netherlands, according the classification of Statistics Netherlands (15). Prepregnancy BMI was available in 690 of these women. After exclusion fetal deaths (n=7) and women without data on offspring birthweight available (n=1), our population for analyses consisted of 682 women (**Supplementary Figure S4.1.1**).

Maternal prepregnancy BMI

Information on maternal prepregnancy weight was obtained through questionnaires at enrolment (17). At enrolment, height was obtained at research center without shoes, and prepregnancy BMI was calculated (correlation coefficient with BMI based on measured weight at enrolment 0.96). For analyses, we used prepregnancy BMI continuously and categorized into four categories: underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (>25 kg/m^2), and obesity (≥30 kg/m^2). To increase statistical power, we combined underweight (n=18) with normal weight (n=482) into one category and overweight (n=132) with obesity (n=50) into one category.

Maternal serum metabolite measurement

Maternal early-pregnancy non-fasting random venous blood samples were collected at study enrolment at a mean (standard deviation (SD)) gestational age of 12.8 (1.7)

weeks by research nurses at one of the dedicated research centers (18). A targeted metabolomics analysis was performed at LMU Munich to determine the serum concentrations (mmol/L) of AA, NEFA, PL, including diacyl-phosphatidylcholines (PC. aa), acyl-alkyl-phosphatidylcholines (PC.ae), acyl-lysophosphatidylcholines (Lyso. PC.a), alkyl-lysophosphatidylcholines (Lyso.PC.e) and sphingomyelines (SM), and Carn including free carnitine (Free Carn) and acyl-carnitines (Carn.a), as described previously (14). IUPAC-IUB Nomenclature was used for notation of AA (15). The following notation was used 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 of an ester bond ('acyl-') and the 'e' represents an ether bond ('alkyl-'). 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, for each metabolite we excluded batches with an intra-batch CV higher than 25% (6-8, 19). 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 (7). Metabolites and participants with more than 50% of missing values were excluded. Missing metabolite values of the remaining metabolites and participants were imputed using the Random Forest algorithm (R package *missForest) (*7, 20, 21).

Individual metabolites were clustered in 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) (22). As we expected fetal growth to by mainly affected by Krebs cycle, inflammation, oxidative stress and glucose and lipid metabolic processes due to a higher maternal prepregnancy BMI, we computed the following ratios: AA ratios Asn/Asp and Gln/Glu as indicators for anaplerosis or replenishing of Krebs cycle metabolites; NEFA.18:1/NEFA.18:0 and NEFA.16:1/NEFA/16:0 ratios as markers of stearoyl-CoA desaturase-1 activity which is associated with increased fat accumulation and reduced fatty acid oxidation; ΣPC.aa/ΣPC. ae, reflecting oxidative stress ΣLyso.PC.a/ΣPC.aa, as a lipid biomarker of inflammation; lyso.PC.a.C16:0 + lyso.PC.a.C18:0)/ΣPC.aa as a proinflammatory biomarker; (lyso. PC.a.C18:1 + lyso.PC.a.C18:2)/ΣPC.aa as an anti-inflammatory biomarker, Carn.a ratios (Carn.a .C:16:0/free carnitine (Carn) and Carn.a.C2:0/Carn.a.C16:0) as markers of carnitine palmitoyl transferase-1 activity (CPT1) and fatty acid β-oxidation, respectively and Val/ PC.ae.C:32.2 as a marker of insulin resistance (13, 23-28). To correct for right skewedness,

individual metabolite concentrations and metabolite ratios were square root transformed or log transformed. To enable comparison of the effect estimates, standard deviation scores (SDS) were calculated for individual metabolite concentrations.

Birthweight

Information about offspring sex, gestational age and weight at birth was obtained from medical records (13). Gestational-age-and-sex-adjusted SDS for weight at birth were constructed using North European growth standards as the reference growth curve and represent the equivalent of z-scores (29). A large-for-gestational-age newborn (LGA) was defined as the highest ten percentiles of gestational age- and sex-adjusted birthweight in the study cohort.

Covariates

Information on maternal age, educational level, parity, folic acid supplementation and smoking and alcohol consumption during pregnancy were obtained through questionnaires (13). Information on maternal daily dietary energy intake during pregnancy was obtained with a Food Frequency Questionnaire (30). Systolic blood pressure was measured at the research center prior to venous blood sampling at study enrolment (31). Triglycerides, High Density Lipoprotein-cholesterol (HDL-cholesterol) and glucose concentrations were analyzed in the same venous blood samples as used for metabolomics analyses (18). To enable comparison of effect estimates, standard deviation scores (SDS) were calculated for triglycerides, HDL-cholesterol and glucose, of which triglycerides was first log-transformed because of non-normality.

Statistical analysis

First, we performed a non-response analysis comparing characteristics of women with metabolomics data and information on prepregnancy BMI available to women with metabolomics data available but without information on prepregnancy BMI. Second, we assessed population characteristics according to maternal prepregnancy weight status. Third, we assessed the associations of maternal prepregnancy BMI across the full range and in clinical categories with maternal early-pregnancy individual metabolite concentrations, metabolite ratios and metabolite groups. Analyses were adjusted for gestational age at blood sampling, maternal age, educational level, parity, smoking, alcohol use, folic acid supplementation, total dietary energy intake, early-pregnancy systolic blood pressure and fetal sex. Confounder selection was based on a Directed Acyclic Graph (DAG) and association with exposure and outcomes in existing literature (DAG shown in **Supplementary Figure 4.1.2**). Fourth, we used a penalized regression method (lasso regression) to select, from the identified altered maternal early-pregnancy

metabolites, a combination of metabolites that were jointly associated with birthweight in addition to maternal prepregnancy BMI. Lasso regression is a highly useful method for developing a model with a high number of predictors which are highly correlated, such as our metabolite data (32). We used maternal prepregnancy BMI and birthweight as continuous exposure and outcome respectively, because of the continuous associations reported in previous studies and to maintain statistical power (7, 10-12). A 10-fold cross-validation was performed and the penalty parameter value yielding the smallest prediction error was used. Selection of maternal early-pregnancy metabolites was done in regression models including maternal prepregnancy BMI across the full range and all selected confounders, which could not be penalized. We compared the predictive performance for offspring birthweight of three linear regression models: 1) including only maternal prepregnancy BMI and confounders (BMI model); 2) including maternal prepregnancy BMI, confounders, maternal triglycerides, HDL-cholesterol and glucose concentrations (conventional biomarker model) and 3) including maternal prepregnancy BMI, confounders, and the set of selected maternal early-pregnancy metabolites associated with birthweight (metabolite model). Predictive performance per model was assessed by explained variance expressed as the adjusted R^2, obtained from the three linear regression models. We also assessed the explained variance of the model including maternal prepregnancy BMI, conventional biomarkers and selected maternal early-pregnancy metabolites. As an additional analysis, we assessed whether the maternal early-pregnancy metabolite profile selected on birthweight continuously could aid in the prediction of the risk of LGA at birth. We obtained the Area Under the Receiving Operator Curve (AUC)) with predicted probabilities obtained from logistic regression models for the risk of LGA at birth, for the maternal BMI model, conventional maternal biomarker model and the maternal early-pregnancy metabolite model. Nominal and Benjamini-Hochberg false discovery rate (FDR) corrected p-values were obtained from regression models (33). Because of the explorative purpose of the study, we maintained a p-value<0.05 in the analyses of maternal prepregnancy BMI with maternal early-pregnancy metabolites as a threshold for metabolites to be included in the penalized regression model. Missing values of covariates were imputed using Multiple Imputation, and we used pooled results from five imputed datasets. 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

Subject characteristics

Table 4.1.1 shows that the median (95% range) maternal prepregnancy BMI was 22.6 (18.5 to 33.3) kg/m^2. Mean (SD) birthweight was 3538 (511) grams. Birthweight was slightly lower among normal weight pregnant women as compared to overweight or obese pregnant women. Non-response analyses showed that women with metabolomics data and information on preprepregnancy BMI available had a similar offspring birthweight as compared those without information on prepregnancy BMI available (**Supplementary Table S4.1.1**).

TABLE 4.1.1. Population characteristics

	Total group (n=682)	Prepregnancy underweight or normal weight (n=500)	Prepregnancy overweight or obesity (n=182)
Maternal characteristics			
Maternal age at enrolment, mean (SD), years	31.4 (4.2)	31.4 (4.2)	31.4 (4.0)
Gestational age at enrolment, mean (SD), weeks	13.1 (1.7)	13.3 (1.7)	12.8 (1.7)
Pre-pregnancy BMI, median (95% range)	22.6 (18.4 to 33.3)	21.6 (18.3 to 24.7)	27.7 (25.1 to 37.4)
Parity, n nulliparous (%)	418 (61)	317 (63)	101 (55)
Education, n high (%)	436 (64)	348 (70)	88 (49)
Folic acid supplement use, n yes (%)	546 (91)	402 (92)	144 (90)
Alcohol use during pregnancy, n yes (%)	442 (69)	336 (72)	106 (61)
Smoking during pregnancy, n yes (%)	154 (24)	111 (24)	43 (25)
Total energy intake, mean (SD), kcal/d	2125 (490)	2147 (489)	2046 (491)
Systolic blood pressure, median (95% range), mmHg	118 (97 to 146)	116 (95 to 139)	124 (104 to 156)
Glucose concentrations, mean (SD), mmol/L	4.4 (0.8)	4.3 (0.8)	4.6 (0.9)
Triglycerides median (95% range), mmol/L	1.2 (0.7 to 2.6)	1.3 (0.6 to 2.4)	1.4 (0.8 to 2.6)
HDL-cholesterol, mean (SD), mmol/L	1.8 (0.3)	1.8 (0.3)	1.7 (0.3)
Birth characteristics			
Sex, n female (%)	315 (46)	226 (45)	89 (49)
Gestational age at birth, median (95% range), weeks	40.3 (36.6 to 42.4)	40.3 (36.6 to 42.4)	40.4 (36.7 to 42.4)
Birthweight, mean (SD), g	3538 (511)	3523 (491)	3578 (560)
Large-for-gestational-age newborn, n (%)	69 (10)	45 (9)	23 (13)

Values represent mean (SD), median (95% range) or number of participants (valid %).

Maternal prepregnancy BMI and early-pregnancy metabolite concentrations

Of the overall metabolite groups, a higher maternal prepregnancy BMI was associated with higher NEFA and SM concentrations only, which remained after multiple testing correction (16). A higher maternal prepregnancy BMI was associated with alterations of 72 individual early-pregnancy AA, NEFA, PC.aa, PC.ae, lyso.PC.a, SM and Carn.a metabolite concentrations (all p-values<0.05), but not with alterations in lyso.PC.e and free carnitine metabolite concentrations (**Figure 4.1.1a**). After multiple testing correction, 43 associations of maternal prepregnancy BMI with individual metabolites from the AA, NEFA, PC.aa, lyso.PC.a, SM and Carn.a groups remained significant (FDR-corrected p-values<0.05). The strongest associations were present for SM.a.C34:2, SM.a.C36:2, and SM.a.C36:3 (difference in maternal metabolite concentrations for SM.a.C34:2 0.08, 95% CI 0.06 to 0.10 SDS, for SM.a.C36:2 0.09, 95% CI 0.06 to 0.11 and for SM.a.C36:3 0.08, 95% CI 0.06 to 0.10 SDS per kg/m^2 increase in maternal prepregnancy BMI). A higher maternal prepregnancy BMI was associated with higher NEFA 16:1/16:0, NEFA 18:1/18:0 and ΣPC.aa/ΣPC.ae ratios, and lower Asn/Asp, Gln/Glu and lyso.PC.a.C:18.1+C:18.2/ΣPC.aa ratios (p-values<0.05), all remaining significant after multiple testing correction (FDR-corrected p-values<0.05) (**Table 4.1.2**).

Partly in line with the associations of maternal prepregnancy BMI across the full range, 46 maternal early-pregnancy AA, NEFA, PC.aa, PC.ae, lyso.PC.a, SM and Carn.a metabolite concentrations were higher in overweight and obese pregnant women, as compared to normal weight pregnant women (p-values<0.05) (**Figure 4.1.1b**). After multiple testing correction, the associations of maternal prepregnancy BMI with 20 individual AA, PC.aa, lyso.PC.a and SM metabolite concentrations remained significant (FDR-corrected p-values<0.05). women with overweight or obesity had higher NEFA 16:1/16:0, NEFA 18:1/18:0 and ΣPC.aa/ΣPC.ae ratios, but lower Asn/Asp, lyso.PC.a.C:18/PC.aa ratios as compared to normal weight women, remaining significant after multiple testing correction (FDR-corrected p-values<0.05) (**Table 4.1.2**). Effect estimates for associations of maternal prepregnancy BMI continuously and in categories with all individual maternal early-pregnancy metabolite concentrations are shown in **Supplementary Figure S4.1.3 and Table S4.1.3**.

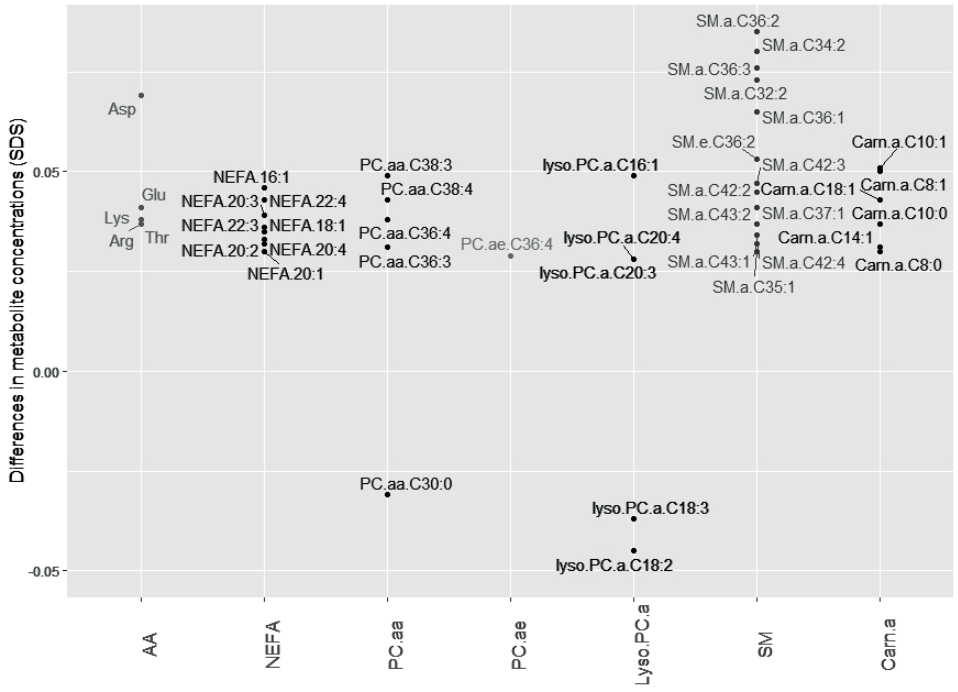


FIGURE 4.1.1A. Associations of maternal prepregnancy BMI with maternal early-pregnancy metabolites

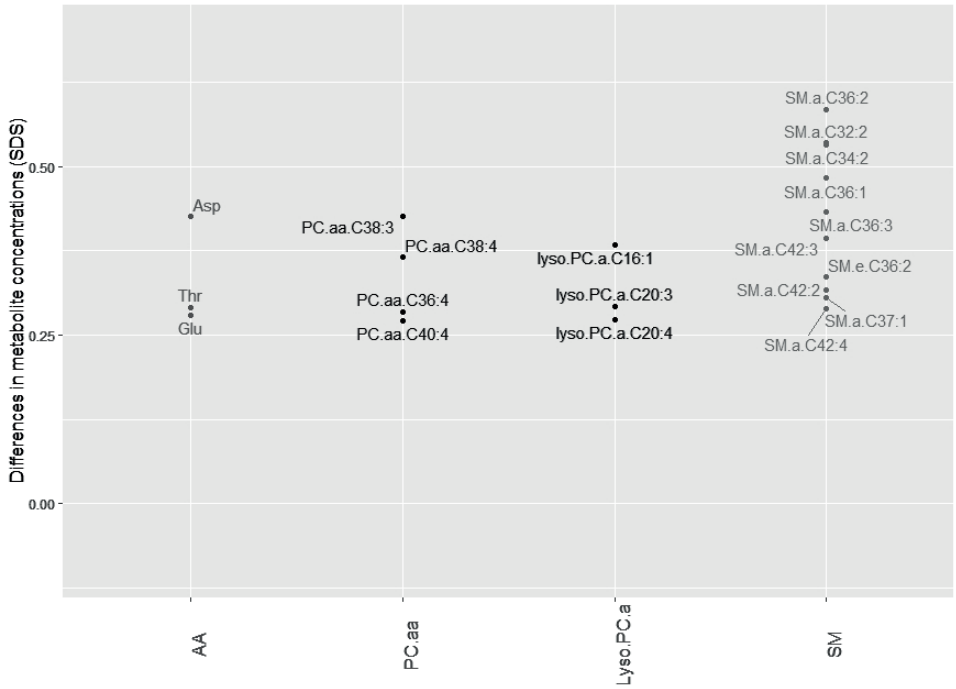


FIGURE 4.1.1B. Significant associations of maternal prepregnancy overweight or obesity with maternal early-pregnancy metabolites.

Abbreviations: AA amino acid, NEFA non-esterified fatty acid, PC.aa diacyl-phosphatidylcholines, PC.ae acyl-alkyl-phosphatidylcholines, Lyso.PC.a acyl-lysophosphatidylcholines, SM sphingomyelines Carn.a acylcarnitines. Regression coefficients were obtained from linear regression models that reflect the difference in maternal early-pregnancy metabolite concentrations in SDS per kg/m² increase in maternal prepregnancy BMI (Figure 4.1.1a) and difference in maternal early-pregnancy metabolite concentrations in SDS for women with overweight or obesity as compared to normal weight women (Figure 4.1.1b) of associations with false discovery rate corrected p-values<0.05. Models were adjusted for gestational age at blood sampling, age, educational level, parity, smoking, alcohol consumption, folic acid supplementation, daily total energy intake, systolic blood pressure and fetal sex.

TABLE 4.1.2. Associations of maternal prepregnancy BMI with maternal early-pregnancy metabolite ratios

	Maternal prepregnancy BMI (kg/m²)				Maternal prepregnancy overweight or obesity		
Early-pregnancy maternal metabolite ratios	**Difference in metabolite concentration (SDS) (95% CI) per kg/m² increase in BMI)**	**p-value**	**FDR corrected p-value**	**Maternal normal prepregnancy weight**	**Difference in metabolite concentration (SDS) (95% CI))**	**p-value**	**FDR corrected p-value**
Asn/Asp	-0.08 (-0.10 to -0.06)	0.000	0.000	*Reference*	-0.51 (-0.69 to -0.33)	0.000	0.000
Gln/Glu	-0.04 (-0.06 to 0.01)	0.002	0.004	*Reference*	-0.16 (-0.35 to 0.03)	0.090	0.165
NEFA.18:1/18:0	0.04 (0.02 to 0.06)	0.000	0.001	*Reference*	0.23 (0.04 to 0.42)	0.016	0.035
NEFA.16:1/16:0	0.05 (0.03 to 0.07)	0.000	0.000	*Reference*	0.25 (0.06 to 0.43)	0.010	0.035
Carn.a.16.0/free carnitine	0.01 (-0.01 to 0.03)	0.413	0.504	*Reference*	0.06 (-0.12 to 0.25)	0.505	0.694
Carn.a.C:2/C:16	-0.03 (-0.03 to 0.02)	0.784	0.863	*Reference*	-0.02 (-0.21 to 0.17)	0.822	0.849
ΣLyso.PC.a/ΣPC.aa	-0.02 (-0.04 to 0.00)	0.109	0.171	*Reference*	-0.13 (-0.31 to 0.05)	0.165	0.260
(Lyso.PC.a.C:18.1+C:18.2)/ΣPC.aa	-0.06 (-0.08 to -0.04)	0.000	0.000	*Reference*	-0.32 (-0.50 to -0.14)	0.001	0.003
(Lyso.PC.a.C:16:0+C18:0)/ΣPC.aa	0.00 (-0.02 to 0.02)	0.929	0.929	*Reference*	-0.05 (-0.23 to 0.13)	0.570	0.697
Val/PC.ae.C:32.2	0.01 (-0.01 to 0.04)	0.237	0.326	*Reference*	0.02 (-0.17 to 0.20)	0.849	0.849
ΣPC.aa/ΣPC.ae	0.03 (0.01 to 0.05)	0.016	0.029	*Reference*	0.23 (0.05 to 0.40)	0.014	0.035

Values represent regression coefficients (95% confidence interval) and corresponding p-values and false discovery rate corrected p-values from linear regression models that reflect the difference in maternal early-pregnancy metabolite ratios in SDS per kg/m² increase in BMI and for women with overweight or obesity as compared to normal weight women. Models were adjusted for gestational age at blood sampling, age, educational level, parity, smoking, alcohol consumption, folic acid supplementation, daily total energy intake, systolic blood pressure and fetal sex.

Maternal prepregnancy BMI, early-pregnancy metabolites and birthweight

A higher maternal prepregnancy BMI was significantly associated with a higher birthweight (0.03, 95% CI 0.01 to 0.05 SDS per kg/m^2 increase in maternal prepregnancy BMI) (**Table 4.1.3**). Based on their joint association with birthweight, a combination of 15 individual maternal early-pregnancy metabolites and 4 metabolite ratios were selected using lasso regression models retaining maternal prepregnancy BMI in the model (**Table 4.1.3**). Adding these selected individual maternal early-pregnancy metabolites and metabolite ratios to the model resulted in a higher effect estimate for the association of maternal prepregnancy BMI with birthweight (0.05, 95% CI 0.03 to 0.07 SDS per kg/m^2 increase in maternal prepregnancy BMI). The explained variance of the model for offspring birthweight including maternal prepregnancy BMI and the individual maternal early-pregnancy metabolites and metabolites ratios was 12.9% (SD of the residuals 0.90), which was higher than the explained variance of the model only including maternal prepregnancy BMI (6.1%, SD of the residuals 0.93) and the model including maternal prepregnancy BMI and conventional maternal biomarkers (6.2%, SD of the residuals 0.93). For presentation purposes, the predicted values versus observed values for all models are shown in **Supplementary Figure S4.1.3** and show more accurate predictions of the model including maternal early-pregnancy metabolites and metabolite ratios as compared to the model only including maternal prepregnancy BMI and the model including maternal prepregnancy BMI and conventional maternal biomarkers. A model including maternal preprepregnancy BMI, conventional biomarkers and the maternal early-pregnancy metabolites and metabolite ratios had an explained variance of 13.0%.

Predictive performance for the risk of LGA at birth improved after addition of the maternal early-pregnancy metabolite profile (AUC: 0.76, 95% CI 0.70 to 0.82), when compared to the performance of models only including maternal prepregnancy BMI (AUC: 0.67, 95% CI 0.61 to 0.73) and the model including maternal prepregnancy BMI and conventional maternal biomarkers (AUC: 0.69, 95% CI 0.62 to 0.76) (**Supplementary Table S4.1.4**).

TABLE 4.1.3. Selected models for the prediction of birthweight

Models	Included in the model	Difference in birthweight (SDS) (95% CI)	Adjusted R^2	Standard deviation of the residuals
BMI model[a]	Prepregnancy BMI	0.03 (0.00 to 0.05)	6.1%	0.93
Biomarker model[b]	Prepregnancy BMI	0.02 (0.00 to 0.04)	6.2%	0.93
	Glucose	0.06 (-0.01 to 0.14)		
	Triglycerides	0.01 (-0.07 to 0.05)		
	HDL-cholesterol	-0.03 (-0.11 to 0.05)		
Metabolite model[c]	Prepregnancy BMI	0.05 (0.03 to 0.07)	12.9%	0.90
	Gln	0.03 (-0.05 to 0.13)		
	Lys	-0.04 (-0.12 to 0.04)		
	NEFA.18:2	0.08 (-0.04 to 0.19)		
	NEFA.20:3	0.06 (-0.06 to 0.18)		
	NEFA.22:3	-0.15 (-0.26 to -0.05)		
	PC.aa.C30:0	0.05 (-0.04 to 0.15)		
	PC.aa.C38:3	0.14 (0.00 to 0.27)		
	PC.ae.C34:4	-0.14 (-0.24 to -0.05)		
	lyso.PC.a.C16:1	-0.23 (-0.33 to -0.12)		
	lyso.PC.a.C20:4	0.04 (-0.07 to 0.14)		
	SM.a.C36:1	-0.05 (-0.22 to 0.12)		
	SM.a.C36:2	-0.15 (-0.34 to 0.04)		
	SM.a.C40:2	0.13 (0.01 to 0.25)		
	SM.a.C42:4	0.12 (-0.01 to 0.26)		
	Carn.a.C10:1	-0.09 (-0.18 to 0.00)		
	Asn/Asp ratio	0.03 (-0.05 to 0.11)		
	Gln/Glu ratio	0.03 (-0.06 to 0.12)		
	NEFA.16:1/16:0 ratio	-0.06 (-0.13 to 0.01)		
	ΣPC.aa/ΣPC.ae ratio	-0.01 (-0.10 to 0.07)		

Values represent regression coefficients (95% confidence interval) and adjusted R^2 obtained from linear regression models

[a]BMI model includes maternal prepregnancy BMI, gestational age at blood sampling, age, educational level, parity, smoking, alcohol consumption, folic acid supplementation, daily total energy intake, systolic blood pressure and fetal sex

[b]Biomarker model includes the BMI model with additional adjustment for maternal glucose, triglycideride and HDL-cholesterol concentrations

[c]Metabolite model includes the BMI model with additional adjustment for selected maternal early-pregnancy metabolites

DISCUSSION

A higher maternal prepregnancy BMI was associated with alterations in individual maternal early-pregnancy metabolite concentrations from the AA, NEFA, PC.aa, PC.ae, lyso.PC.a, SM and Carn.a groups and alterations in metabolite ratios marking processes of the Krebs cycle, inflammation, oxidative stress and lipid metabolism. Using these altered maternal early-pregnancy metabolites and metabolite ratios, we identified an early-pregnancy maternal metabolite profile consisting of 15 metabolites and 4 metabolite ratios, which was associated with a higher birthweight in addition to maternal prepregnancy BMI. Use of this identified maternal metabolite profile together with maternal prepregnancy BMI resulted in a better prediction of birthweight than a model with maternal prepregnancy BMI alone or maternal prepregnancy BMI and maternal early-pregnancy glucose and lipid concentrations.

Interpretation of main findings

Overweight and obesity are well-known to be associated with major alterations in metabolism, especially glucose and lipid metabolism. However, detailed underlying metabolic processes in these associations remain to be elucidated (9). Previous studies among non-pregnant populations suggested mainly associations of a higher BMI with higher NEFA and BCAA concentrations, but also reported less consistent associations with alterations in other AA and PL (9). Among pregnant populations, only a few studies have assessed the associations of maternal BMI with detailed metabolite profiles in pregnancy and these studies showed inconsistent results (6, 7, 12). A study from the United States among 167 pregnant women using a targeted metabolomics approach observed that a higher maternal prepregnancy BMI was associated with alterations in second trimester maternal AA, first and second trimester NEFAs, first and third trimester PL, but not with Carn.a throughout pregnancy (6). A multinational study among 400 pregnant women observed a cross-sectional association of a higher maternal BMI at 28 weeks gestation with altered AA, Carn.a, carbohydrates and fatty acids concentrations using a targeted and non-targeted metabolomics approach (12). A Spanish study among 200 pregnant women reported associations of a higher prepregnancy BMI with mainly higher maternal BCAA concentrations at delivery and less strong associations with alterations in maternal NEFA.22:4, PC.aa.C38:4, SM.C32:2, SM.C34.2 and Carn.a.C4:0 concentrations (7).

Largely in line with previous studies, we observed that a higher maternal prepregnancy BMI was associated with alterations in maternal early-pregnancy AA, NEFA, PL, including PC.aa, PC.ae, lyso.PC.a, lyso.PC.e and SM, and Carn.a concentrations. We observed the

strongest associations for higher SM.a.36:2, SM.a.C34:2, SM.a.C36:3, and SM.a.C32:2 concentrations, which may be involved in the development of insulin resistance (34). In contrast to these previous studies among non-pregnant and pregnant populations, we did not find associations of a higher maternal prepregnancy BMI with higher concentrations of the BCAA, Valin, Leucin and Isoleucin. These metabolites are markers of insulin resistance (34). The lack of associations in the current study with BCAA could be due to our relatively healthy population, with low mean glucose concentrations. It could also be due to our non-fasting samples and the timing in early-pregnancy, as insulin resistance may be more pronounced in the fasting state and later in pregnancy (6, 35). In line with previous studies, we focused on metabolite ratios that are markers for processes in the Krebs cycle, inflammation, oxidative stress, lipid metabolism and insulin resistance (7, 13, 23-28). We observed associations of a higher maternal prepregnancy BMI with metabolite ratios reflecting reduced anaplerosis or replenishing of the Krebs cycle metabolites, stearoyl-CoA desaturase-1 activity, anti-inflammatory biomarkers and oxidative stress. Thus, our results suggest that a maternal higher prepregnancy BMI is associated with alterations in maternal early-pregnancy AA, NEFA, PC.aa, PC.ae, lyso.PC.a, SM and Carn.a metabolite concentrations, with the strongest effect on SM metabolites, and with metabolite ratios marking processes in the Krebs cycle, inflammation, oxidative stress and lipid metabolism.

Hyperglycemia and dyslipidemia during pregnancy in response to a higher prepregnancy BMI only partly explain the associations of a higher maternal prepregnancy BMI with a higher birthweight (1, 2, 4). The role of altered metabolites during early-pregnancy in the associations of a higher maternal prepregnancy BMI with a higher birthweight is largely unknown. Recently, a study among 400 pregnant women used targeted and non-targeted metabolomics approaches to obtain fasting and 1 hour metabolites (12). This study showed associations of a higher maternal BMI in second half of pregnancy with alterations in AA, NEFA, carnitines and sugars/alcohols metabolite concentrations. These metabolites improved the prediction of birthweight by increasing the explained variance for birthweight from 4.4 to 6.5% after addition of maternal fasting metabolites to a model with maternal prepregnancy BMI and from 7.1 to 9.2% after addition of 1 hour metabolites. A study among 8,212 women obtained nuclear magnetic resonance-derived metabolite concentrations in mid-pregnancy (36). They observed that addition of 66 metabolites, including AA, fatty acids, phospholipids, apolipoproteins, cholesterol and very low density lipoprotein, to a risk factor model with maternal age, pregnancy BMI, ethnicity and parity, improved prediction of large-for-gestational-age newborns. The AUC was 0.71 (95% CI 0.66 to 0.75) for the risk factor model alone and increased to 0.75 (95% CI 0.70 to 0.79) after addition of the 66 metabolites. Largely in line with the metabolites identified in these previous studies, we observed that a

maternal early-pregnancy metabolite profile including altered AA, NEFA and Carn.a and PL concentrations and metabolite ratios marking processes in the Krebs cycle, inflammation and lipid metabolism was associated with a higher birthweight in addition to maternal prepregnancy BMI. The explained variation improved from 6.1% for the model only including maternal prepregnancy BMI and confounders to 12.9% for the model additionally including the maternal early-pregnancy metabolite profile.

Maternal metabolic disturbances, due to a higher maternal prepregnancy BMI, can affect fetal growth and development directly through altering fetal exposure to an adverse maternal metabolite profile, but also by affecting placental development and leading to an altered regulation of maternal nutrient transfer to the fetus (1, 37). In the identified maternal metabolite profile, we observed an effect of AA, Glutamin and Lysin, which are important for protein synthesis, essential for fetal growth (19, 38, 39). The highest number of metabolites selected in the maternal early-pregnancy metabolite profile were from the NEFA and PL groups, which underlines the importance of lipid metabolism in affected fetal growth in women with a higher prepregnancy BMI. Animal studies and studies among women with gestational diabetes have suggested that increased NEFA are transported to the fetus leading to increased fetal growth and adiposity development (40, 41). Higher maternal NEFA metabolite concentrations may also have an effect on placental development, increasing transfer of triglycerides to the fetus, altering fetal lipid metabolism and adipose tissue development (42). Increase of maternal PL, especially SM, is a normal physiological process during pregnancy to preserve fetal nutrient supply, but the fetus does not depend on maternal PL (19). While SM inhibits cholesterol absorption, excess increase in maternal SM can cause increased insulin resistance leading to higher maternal and fetal glucose concentrations and subsequent accelerated fetal growth (34). Metabolite ratios selected in the maternal early-pregnancy metabolite profile were markers of reduced anaplerosis and replenishing processes of metabolites in the Krebs cycle, and altered lipid metabolism and oxidative stress. These processes are well known to be influenced by a higher BMI and also affect placental development and fetal growth (43). Thus, our findings suggest that an altered maternal early-pregnancy metabolite profile seems to be involved in pathways underlying the associations of a higher maternal prepregnancy BMI with a higher birthweight. Further studies among larger multi-ethnic populations with higher variability in metabolite concentrations and birthweight are needed to replicate our findings.

The identification of a maternal early-pregnancy metabolite profile that relates to the associations of a higher maternal prepregnancy BMI with a higher birthweight is important from an etiological and public health perspective. Our findings may

contribute to the understanding of mechanisms underlying the associations of a higher maternal prepregnancy BMI with a higher birthweight. Associations for maternal prepregnancy overweight or obesity with maternal early-pregnancy metabolites and metabolite ratios were largely similar as for maternal prepregnancy BMI across the full range. These findings suggest that effects of a higher maternal prepregnancy BMI on early-pregnancy metabolites are not limited to thresholds of disease, but are already present within a healthy range. Importantly, the identified maternal early-pregnancy metabolite profile improved prediction of birthweight in addition to maternal prepregnancy BMI and had a better predictive performance than conventional maternal biomarkers including glucose and lipid concentrations. Although the variance explained of birthweight strongly improved after addition of the maternal metabolite profile to the model, the overall variance explained remained relatively low and this prediction model cannot be directly translated into clinical practice. However, we consider this identified maternal early-pregnancy metabolite profile important as it provides novel insight into potential novel markers for more accurate prediction of birthweight after replication and validation. Further studies are needed focused on the development of more advanced prediction models and identification of novel markers before and during pregnancy to further improve the prediction of birthweight. After replication of our findings and incorporation in more advanced prediction models, determination of the maternal metabolite profile in early-pregnancy may enable more accurate identification of women with a higher prepregnancy BMI at increased risk of delivering a large-for-gestational age newborn and provide novel targets for interventions.

Methodological considerations

We obtained metabolomics data in a subgroup of our multi-ethnic cohort, which consists of Dutch participants only (13). Ethnicity is likely to have a major influence on the metabolome, via both genetic and environmental factors (44). By performing our study within an ethnic homogenous population, we reduced the risks of potential residual confounding or effect modification by ethnicity. This is especially important since little is known about the influence of maternal prepregnancy BMI on early-pregnancy metabolite adaptations. However, our selected study population may affect the generalizability of our findings. Further studies are needed to replicate our findings among multi-ethnic populations and to explore whether ethnic-specific effects are present. As we used self-reported prepregnancy weight, misclassification bias may be an issue. However, we observed a high correlation between self-reported prepregnancy weight and early-pregnancy weight measured at enrollment at the research center. We used non-fasting blood samples for metabolomics analyses and as a consequence dietary intake may have influenced the metabolomics data. Although metabolomics

research is generally performed using fasting samples, a large cohort study among 6,671 adults observed a better biological reproducibility of non-fasting samples compared to fasting samples and suggested that non-fasting samples may be more useful for the prediction of subsequent disease, as the human physical state is non-fasting the majority of the day (45). We used a targeted metabolomics approach, allowing us to optimize the quantification of the metabolites of interest, but relevant biological pathways might be missed. Further studies using both untargeted and targeted metabolomics in fasting and non-fasting serum samples are needed to replicate our findings and to identify further novel pathways. Finally, we adjusted our analyses for many potential confounders. However, due to the observational nature of the study, residual confounding cannot be excluded.

CONCLUSIONS

A higher maternal prepregnancy BMI is associated with alterations in maternal early-pregnancy AA, NEFA, PC.aa, PC.ae, lyso.PC.a, SM and Carn.a metabolite concentrations. Using these altered metabolites, we identified a maternal early-pregnancy metabolite profile, which improves the prediction of birthweight in addition to maternal prepregnancy BMI and has a better performance for the prediction of birthweight than conventional maternal biomarkers. These findings are important from an etiological perspective and may enable early identification of pregnant women at increased risk of delivering a large-for-gestational-age newborn after further replication.

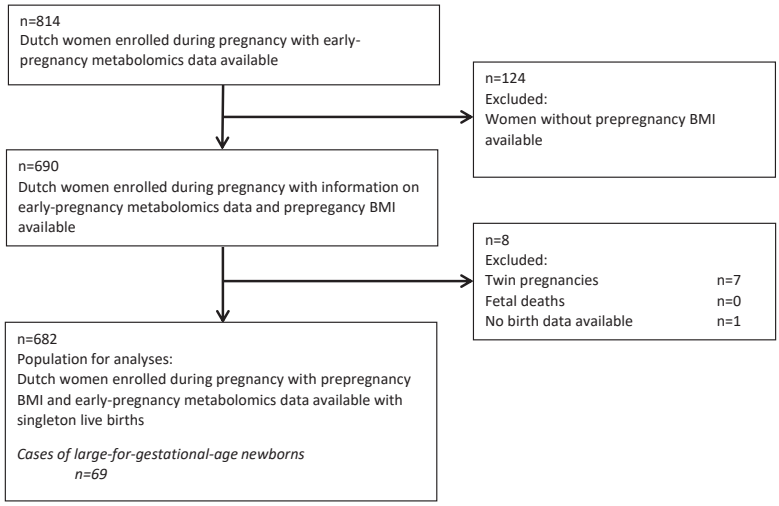
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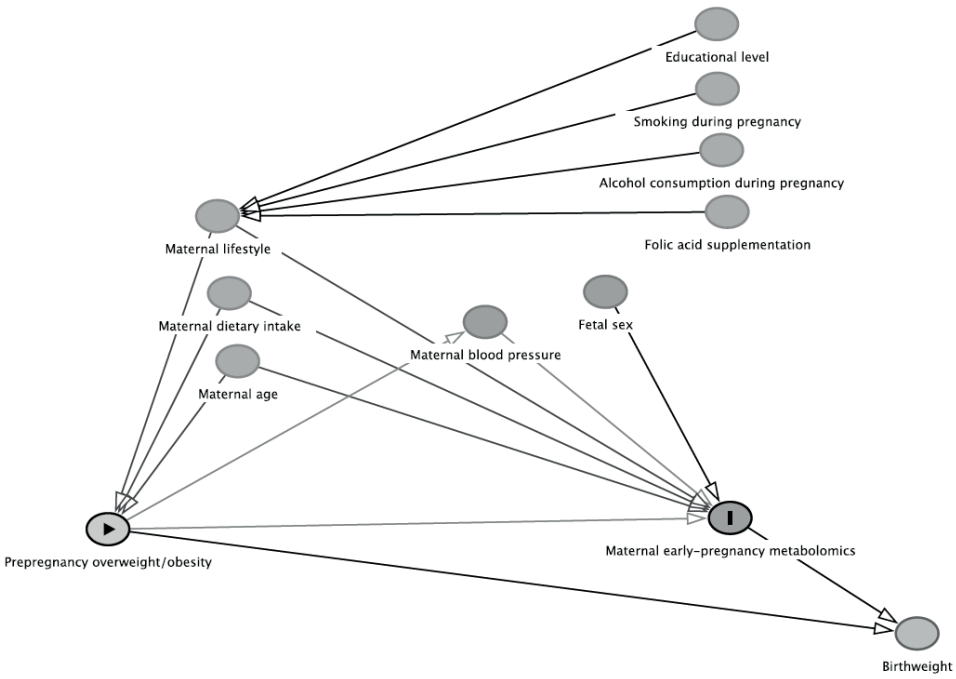
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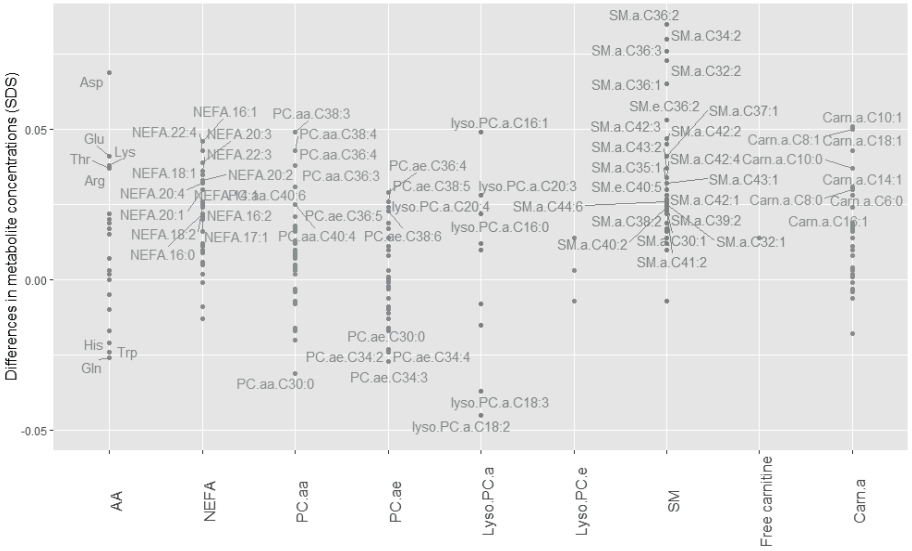
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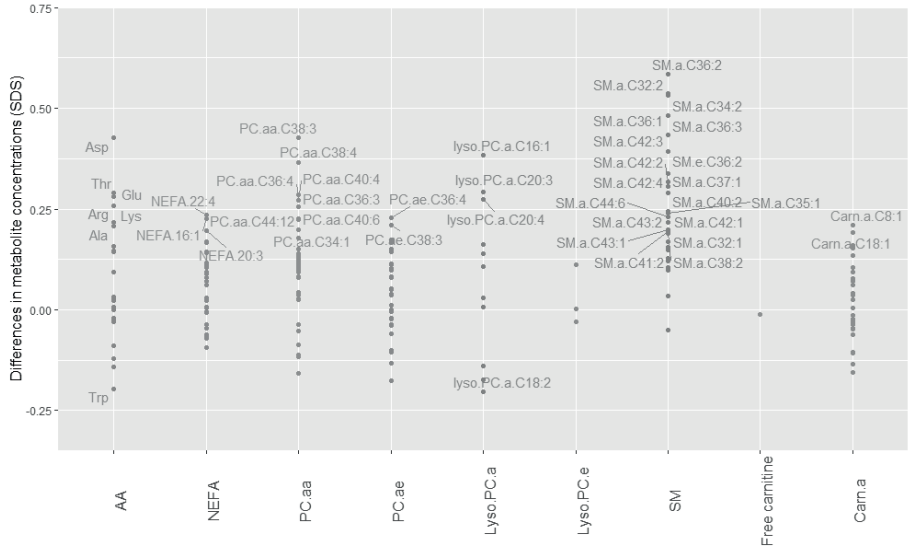
SUPPLEMENTARY FIGURE S4.1.1. Flow chart of study participants



SUPPLEMENTARY FIGURE S4.1.2. Directed Acyclic Graph



SUPPLEMENTARY FIGURE S4.1.3A. Associations of maternal prepregnancy BMI with maternal early-pregnancy metabolites



SUPPLEMENTARY FIGURE S4.1.3B. Associations of maternal prepregnancy overweight or obesity with maternal early-pregnancy metabolites

Abbreviations: AA amino acid, NEFA non-esterified fatty acid, PC.aa diacyl-phosphatidylcholines, PC.ae acyl-alkyl-phosphatidylcholines, Lyso.PC.a acyl-lysophosphatidylcholines, Lyso.PC.e alkyl–lysophosphatidylcholines, SM sphingomyelines Carn.a acylcarnitines. Regression coefficients were obtained from linear regression models that reflect the difference in maternal early-pregnancy metabolite concentrations in SDS per kg/m² increase in maternal prepregnancy BMI (Figure 4.1.1a) and difference in maternal early-pregnancy metabolite concentrations in SDS for women with overweight or obesity as compared to normal weight women (Figure 4.1.1b). Metabolite concentrations significantly associated with prepregnancy BMI and overweight or obesity (False discovery rate-adjusted p<0.05) are labeled by name of the metabolite. Models were adjusted for gestational age at blood sampling, age, educational level, parity, smoking, alcohol consumption, folic acid supplementation, daily total energy intake, systolic blood pressure and fetal sex.

SUPPLEMENTARY TABLE S4.1.1. Non-response analyses for women with metabolomics data available with and without information on prepregnancy BMI

	Women with metabolomics data and information on prepregnancy BMI available (n=690)	Women with metabolomics data available without information on prepregnancy BMI (n=124)	p-value*
Maternal characteristics			
Maternal age at enrolment, mean (SD), years	31.4 (4.2)	31.4 (3.5)	0.99
Gestational age at blood sampling, mean (SD), weeks	13.2 (1.7)	13.0 (1.7)	0.01
Parity, n nulliparous (%)	423 (61)	72 (58)	0.50
Education, n high (%)	440 (64)	69 (14)	0.15
Folic acid supplement use, n yes (%)	552 (91)	54 (92)	0.84
Alcohol use during pregnancy, n yes (%)	449 (69)	58 (68)	0.92
Smoking during pregnancy, n yes (%)	157 (24)	25 (29)	0.34
Total energy intake, mean (SD), kcal/d	2129 (490)	2138 (508)	0.86
Systolic blood pressure, median (95% range), mmHg	118 (97 to 146)	117 (101 to 151)	0.37
Birth characteristics			
Sex, n female (%)	318 (46)	55 (44)	0.72
Gestational age at birth, median (95% range), weeks	40.3 (36.0 to 42.4)	40.2 (33.6 to 42.5)	0.79
Birthweight, mean (SD), g	3524 (528)	3545 (529)	0.68

**p-values were obtained using t-test or Mann-Whitney U tests for continuous variables and chi-square tests for categorical variables*

SUPPLEMENTARY TABLE S4.1.2. Associations of maternal prepregnancy BMI with maternal early-pregnancy metabolite groups

	Prepregnancy BMI (kg/m²)			Prepregnancy normal weight	Prepregnancy overweight or obesity		
Early-pregnancy metabolite group	**Difference in metabolite group (SDS) (95% CI)**	**P-value**	**FDR-adjusted p-value**	**Difference in metabolite group (SDS) (95% CI)**	**Difference in metabolite group (SDS) (95% CI)**	**P-value**	**FDR adjusted p-value**
Amino acids (AA)	0.01 (-0.012;0.032)	0.388	0.61	*Reference*	0.135 (-0.051;0.322)	0.155	0.447
Branched-chain AA	0.003 (-0.019;0.026)	0.761	0.897	*Reference*	0.001 (-0.184;0.186)	0.992	0.992
Aromatic AA	0 (-0.022;0.022)	0.990	0.99	*Reference*	0.006 (-0.178;0.19)	0.949	0.985
Essential AA	0.012 (-0.011;0.034)	0.305	0.506	*Reference*	0.076 (-0.11;0.262)	0.422	0.633
Non-essential AA	0.008 (-0.015;0.03)	0.504	0.652	*Reference*	0.162 (-0.025;0.348)	0.090	0.33
Non-esterified fatty acids (NEFA)	0.029 (0.007;0.052)	0.009	0.045	*Reference*	0.137 (-0.05;0.323)	0.151	0.447
Saturated NEFA	0.02 (-0.003;0.042)	0.084	0.214	*Reference*	0.1 (-0.087;0.287)	0.294	0.537
Mono-unsaturated NEFA	0.038 (0.016;0.06)	0.001	0.007	*Reference*	0.185 (-0.001;0.371)	0.052	0.245
Poly-unsaturated NEFA	0.027 (0.005;0.05)	0.016	0.068	*Reference*	0.094 (-0.093;0.281)	0.325	0.537
Diacyl-phosphatidylcholines (PC.aa)	0.022 (0;0.044)	0.050	0.151	*Reference*	0.211 (0.028;0.394)	0.024	0.158
Saturated PC.aa	-0.002 (-0.024;0.02)	0.830	0.908	*Reference*	0.039 (-0.145;0.223)	0.675	0.845
Mono-unsaturated PC.aa	0.016 (-0.006;0.038)	0.154	0.318	*Reference*	0.19 (0.007;0.373)	0.042	0.23
Poly-unsaturated PC.aa	0.023 (0.001;0.045)	0.041	0.136	*Reference*	0.211 (0.028;0.394)	0.024	0.158
Acyl-alkyl-phosphatidylcholines (PC.ae)	0.008 (-0.015;0.03)	0.499	0.652	*Reference*	0.095 (-0.091;0.282)	0.317	0.537
Saturated PC.ae	0.008 (-0.014;0.03)	0.454	0.651	*Reference*	0.089 (-0.096;0.273)	0.346	0.544
Mono-unsaturated PC.ae	-0.003 (-0.025;0.019)	0.791	0.9	*Reference*	0.061 (-0.125;0.246)	0.522	0.749
Poly-unsaturated PC.ae	0.009 (-0.014;0.031)	0.446	0.651	*Reference*	0.096 (-0.091;0.284)	0.312	0.537
Acyl-lysophosphatidylcholines (Lyso.PC.a)	0.007 (-0.014;0.029)	0.513	0.652	*Reference*	0.105 (-0.077;0.286)	0.258	0.537
Saturated Lyso.PC.a	0.021 (-0.001;0.043)	0.059	0.162	*Reference*	0.163 (-0.018;0.345)	0.078	0.324
Mono-unsaturated Lyso.PC.a	-0.005 (-0.027;0.016)	0.630	0.77	*Reference*	0.094 (-0.088;0.277)	0.311	0.537
Poly-unsaturated Lyso.PC.a	-0.029 (-0.051;-0.008)	0.008	0.045	*Reference*	-0.096 (-0.279;0.088)	0.307	0.537

SUPPLEMENTARY TABLE S4.1.2. Continued

	Prepregnancy BMI (kg/m²)			Prepregnancy normal weight	Prepregnancy overweight or obesity		
Early-pregnancy metabolite group	**Difference in metabolite group (SDS) (95% CI)**	**P-value**	**FDR-adjusted p-value**	**Difference in metabolite group (SDS) (95% CI)**	**Difference in metabolite group (SDS) (95% CI)**	**P-value**	**FDR adjusted p-value**
Alkyl-lysophosphatidylcholines (Lyso.PC.e)	-0.001 (-0.023;0.022)	0.952	0.981	*Reference*	0.016 (-0.17;0.202)	0.866	0.985
Saturated Lyso.PC.e	-0.002 (-0.024;0.02)	0.853	0.908	*Reference*	0.005 (-0.181;0.191)	0.955	0.985
Mono-unsaturated Lyso.PC.e	0.012 (-0.011;0.034)	0.307	0.506	*Reference*	0.101 (-0.083;0.286)	0.283	0.537
Sphingomyelines (SM)	0.042 (0.02;0.064)	0.000	0.002	*Reference*	0.313 (0.13;0.497)	0.001	0.014
Saturated SM	0.015 (-0.007;0.037)	0.190	0.368	*Reference*	0.047 (-0.139;0.234)	0.620	0.845
Mono-unsaturated SM	0.032 (0.01;0.054)	0.005	0.03	*Reference*	0.238 (0.053;0.423)	0.012	0.13
Poly-unsaturated SM	0.048 (0.027;0.07)	0.000	0.000	*Reference*	0.363 (0.181;0.545)	0.000	0.003
Free carnitine	0.014 (-0.008;0.036)	0.210	0.386	*Reference*	-0.012 (-0.195;0.171)	0.899	0.985
Acyl-carnitines (Carn.a)	0.024 (0.002;0.046)	0.036	0.131	*Reference*	0.038 (-0.148;0.224)	0.691	0.845
Small-chain Carn.a	0.017 (-0.005;0.039)	0.137	0.301	*Reference*	0.01 (-0.175;0.196)	0.914	0.985
Medium-chain Carn.a	0.042 (0.02;0.064)	0.000	0.002	*Reference*	0.133 (-0.054;0.32)	0.163	0.447
Large-chain Carn.a	0.018 (-0.004;0.041)	0.106	0.249	*Reference*	0.04 (-0.148;0.227)	0.679	0.845

Abbreviations: AA amino acid, NEFA non-esterified fatty acid, PC.aa diacyl-phosphatidylcholines, PC.ae acyl-alkyl-phosphatidylcholines, Lyso.PC.a acyl-lysophosphatidylcholines, Lyso.PC.e alkyl–lysophosphatidylcholines, SM sphingomyelines Carn.a acylcarnitines. Values represent regression coefficients (95% confidence interval) and corresponding p-values and false discovery rate corrected p-values from linear regression models that reflect the difference in maternal early-pregnancy metabolite group concentrations in SDS per kg/m2 increase in BMI and for women with overweight or obesity as compared to normal weight women. Models were adjusted for gestational age at blood sampling, age, educational level, parity, smoking, alcohol consumption, folic acid supplementation, daily total energy intake, systolic blood pressure and fetal sex.

SUPPLEMENTARY TABLE S4.1.3. Effect estimates for associations of maternal prepregnancy BMI with maternal early-pregnancy metabolites

	Prepregnancy BMI (kg/m²)			Prepregnancy normal weight	Prepregnancy overweight or obesity		
Metabolite	Difference in metabolite concentration (SDS) (95% CI) (95% ci)	FDR adjusted p-value	FDR adjusted p-value	Difference in maternal metabolite concentration (SDS) (95% CI)	Difference in maternal metabolite concentration (SDS) (95% CI)	p-value	FDR adjusted p-value
Ala	0.024 (0.001 to 0.046)	0.043	0.043	*Reference*	0.242 (0.055 to 0.428)	0.011	0.112
Arg	0.038 (0.016 to 0.061)	0.001	0.001	*Reference*	0.268 (0.082 to 0.454)	0.005	0.067
Asn	-0.016 (-0.039 to 0.007)	0.177	0.177	*Reference*	-0.094 (-0.283 to 0.094)	0.328	0.711
Asp	0.065 (0.043 to 0.087)	0.000	0.000	*Reference*	0.368 (0.186 to 0.549)	0.000	0.002
Cit	-0.006 (-0.028 to 0.017)	0.634	0.634	*Reference*	-0.018 (-0.205 to 0.169)	0.853	0.935
Gln	-0.023 (-0.047 to 0)	0.054	0.054	*Reference*	0.011 (-0.183 to 0.205)	0.910	0.958
Glu	0.035 (0.013 to 0.058)	0.002	0.002	*Reference*	0.223 (0.034 to 0.412)	0.021	0.17
Gly	0.018 (-0.005 to 0.041)	0.123	0.123	*Reference*	0.173 (-0.017 to 0.362)	0.074	0.381
His	-0.019 (-0.041 to 0.004)	0.107	0.107	*Reference*	-0.038 (-0.224 to 0.148)	0.691	0.881
Ile	0.008 (-0.015 to 0.031)	0.511	0.511	*Reference*	0.063 (-0.127 to 0.253)	0.516	0.838
Leu	0.008 (-0.015 to 0.031)	0.500	0.500	*Reference*	0.014 (-0.175 to 0.203)	0.882	0.95
Lys	0.043 (0.02 to 0.065)	0.000	0.000	*Reference*	0.239 (0.050 to 0.427)	0.013	0.119
Met	-0.013 (-0.036 to 0.010)	0.275	0.275	*Reference*	-0.079 (-0.270 to 0.112)	0.419	0.778
Orn	0.026 (0.003 to 0.048)	0.026	0.026	*Reference*	0.134 (-0.051 to 0.320)	0.157	0.555
Phe	0.021 (-0.003 to 0.044)	0.081	0.081	*Reference*	0.173 (-0.015 to 0.361)	0.073	0.381
Pro	0.013 (-0.01 to 0.036)	0.280	0.280	*Reference*	0.208 (0.016 to 0.400)	0.034	0.264
Trp	-0.018 (-0.041 to 0.005)	0.117	0.117	*Reference*	-0.137 (-0.322 to 0.049)	0.150	0.555
Ser	-0.01 (-0.033 to 0.014)	0.414	0.414	*Reference*	0.023 (-0.168 to 0.214)	0.812	0.919
Thr	0.038 (0.016 to 0.06)	0.001	0.001	*Reference*	0.297 (0.114 to 0.480)	0.002	0.028
Tyr	0.009 (-0.014 to 0.032)	0.450	0.450	*Reference*	0.053 (-0.135 to 0.242)	0.580	0.867
Val	0.012 (-0.011 to 0.035)	0.317	0.317	*Reference*	0.068 (-0.120 to 0.255)	0.479	0.807

SUPPLEMENTARY TABLE S4.1.3. Continued

	Prepregnancy BMI (kg/m²)			Prepregnancy normal weight	Prepregnancy overweight or obesity		
Metabolite	**Difference in metabolite concentration (SDS) (95% CI) (95% ci)**	**FDR adjusted p-value**	**FDR adjusted p-value**	**Difference in maternal metabolite concentration (SDS) (95% CI)**	**Difference in maternal metabolite concentration (SDS) (95% CI)**	**p-value**	**FDR adjusted p-value**
Cys	0.029 (0.007 to 0.052)	0.011	0.011	*Reference*	0.068 (-0.121 to 0.258)	0.482	0.807
NEFA.14.0	-0.006 (-0.029 to 0.016)	0.583	0.583	*Reference*	-0.114 (-0.304 to 0.077)	0.242	0.648
NEFA.14.1	0.015 (-0.008 to 0.038)	0.204	0.204	*Reference*	-0.010 (-0.200 to 0.181)	0.921	0.958
NEFA.15.0	-0.014 (-0.037 to 0.009)	0.218	0.218	*Reference*	-0.134 (-0.326 to 0.057)	0.170	0.565
NEFA.16.0	0.014 (-0.009 to 0.037)	0.226	0.226	*Reference*	0.031 (-0.159 to 0.222)	0.746	0.900
NEFA.16.1	0.035 (0.012 to 0.057)	0.002	0.002	*Reference*	0.124 (-0.062 to 0.311)	0.192	0.597
NEFA.16.2	0.017 (-0.006 to 0.040)	0.145	0.145	*Reference*	-0.052 (-0.243 to 0.138)	0.593	0.867
NEFA.17.0	-0.002 (-0.025 to 0.021)	0.841	0.841	*Reference*	-0.065 (-0.258 to 0.128)	0.511	0.838
NEFA.17.1	0.015 (-0.008 to 0.038)	0.208	0.208	*Reference*	0.004 (-0.188 to 0.197)	0.964	0.974
NEFA.17.2	0.005 (-0.018 to 0.028)	0.661	0.661	*Reference*	-0.089 (-0.280 to 0.101)	0.359	0.729
NEFA.18.0	-0.011 (-0.033 to 0.012)	0.363	0.363	*Reference*	-0.068 (-0.258 to 0.123)	0.486	0.807
NEFA.18.1	0.025 (0.003 to 0.048)	0.027	0.027	*Reference*	0.082 (-0.106 to 0.270)	0.394	0.758
NEFA.18.2	0.017 (-0.006 to 0.039)	0.147	0.147	*Reference*	0.018 (-0.171 to 0.207)	0.854	0.935
NEFA.18.3	0.005 (-0.017 to 0.028)	0.652	0.652	*Reference*	-0.025 (-0.213 to 0.163)	0.795	0.919
NEFA.19.1	0.005 (-0.018 to 0.028)	0.691	0.691	*Reference*	-0.034 (-0.226 to 0.159)	0.734	0.900
NEFA.20.1	0.021 (-0.002 to 0.043)	0.074	0.074	*Reference*	0.031 (-0.158 to 0.220)	0.748	0.900
NEFA.20.2	0.023 (0.000 to 0.045)	0.051	0.051	*Reference*	0.048 (-0.141 to 0.237)	0.622	0.867
NEFA.20.3	0.031 (0.008 to 0.054)	0.008	0.008	*Reference*	0.130 (-0.062 to 0.321)	0.184	0.597
NEFA.20.4	0.024 (0.001 to 0.046)	0.038	0.038	*Reference*	0.087 (-0.098 to 0.273)	0.356	0.729
NEFA.20.5	0.000 (-0.022 to 0.022)	0.988	0.988	*Reference*	-0.091 (-0.276 to 0.093)	0.332	0.711
NEFA.22.3	0.026 (0.004 to 0.049)	0.023	0.023	*Reference*	0.049 (-0.140 to 0.237)	0.613	0.867

SUPPLEMENTARY TABLE S4.1.3. Continued

	Prepregnancy BMI (kg/m²)			Prepregnancy normal weight	Prepregnancy overweight or obesity		
Metabolite	Difference in metabolite concentration (SDS) (95% CI) (95% ci)	FDR adjusted p-value	FDR adjusted p-value	Difference in maternal metabolite concentration (SDS) (95% CI)	Difference in maternal metabolite concentration (SDS) (95% CI)	p-value	FDR adjusted p-value
NEFA.22.4	0.032 (0.010 to 0.054)	0.005	0.005	*Reference*	0.130 (-0.056 to 0.315)	0.170	0.565
NEFA.22.5	0.010 (-0.013 to 0.033)	0.386	0.386	*Reference*	-0.017 (-0.208 to 0.175)	0.865	0.942
NEFA.22.6	0.001 (-0.021 to 0.023)	0.943	0.943	*Reference*	-0.093 (-0.279 to 0.093)	0.328	0.711
NEFA.24.0	-0.011 (-0.033 to 0.012)	0.363	0.363	*Reference*	-0.057 (-0.245 to 0.131)	0.553	0.856
NEFA.24.1	-0.002 (-0.025 to 0.022)	0.895	0.895	*Reference*	-0.023 (-0.220 to 0.173)	0.816	0.919
NEFA.24.2	0.009 (-0.014 to 0.032)	0.432	0.432	*Reference*	0.043 (-0.149 to 0.234)	0.662	0.881
NEFA.24.4	0.012 (-0.010 to 0.035)	0.272	0.272	*Reference*	0.011 (-0.173 to 0.195)	0.908	0.958
NEFA.24.5	0 (-0.023 to 0.023)	0.974	0.974	*Reference*	-0.072 (-0.263 to 0.119)	0.460	0.807
NEFA.26.0	0.009 (-0.014 to 0.031)	0.446	0.446	*Reference*	0.056 (-0.130 to 0.241)	0.557	0.856
NEFA.26.1	0.006 (-0.017 to 0.028)	0.611	0.611	*Reference*	-0.029 (-0.217 to 0.158)	0.758	0.902
NEFA.26.2	0.002 (-0.02 to 0.024)	0.851	0.851	*Reference*	-0.079 (-0.263 to 0.104)	0.397	0.758
lyso.PC.a.C14.0	0.017 (-0.005 to 0.039)	0.135	0.135	*Reference*	0.170 (-0.015 to 0.355)	0.072	0.381
lyso.PC.a.C16.0	0.024 (0.001 to 0.046)	0.037	0.037	*Reference*	0.172 (-0.017 to 0.361)	0.075	0.381
lyso.PC.a.C16.1	0.051 (0.030 to 0.073)	0.000	0.000	*Reference*	0.389 (0.207 to 0.571)	0.000	0.001
lyso.PC.a.C18.0	0.01 (-0.013 to 0.032)	0.408	0.408	*Reference*	0.093 (-0.095 to 0.281)	0.331	0.711
lyso.PC.a.C18.1	-0.012 (-0.034 to 0.011)	0.315	0.315	*Reference*	0.050 (-0.139 to 0.239)	0.606	0.867
lyso.PC.a.C18.2	-0.037 (-0.059 to -0.015)	0.001	0.001	*Reference*	-0.143 (-0.328 to 0.042)	0.130	0.524
lyso.PC.a.C18.3	-0.031 (-0.053 to -0.008)	0.008	0.008	*Reference*	-0.119 (-0.309 to 0.072)	0.223	0.639
lyso.PC.a.C20.3	0.029 (0.007 to 0.052)	0.011	0.011	*Reference*	0.286 (0.099 to 0.472)	0.003	0.045
lyso.PC.a.C20.4	0.025 (0.003 to 0.047)	0.024	0.024	*Reference*	0.247 (0.066 to 0.429)	0.008	0.093
lyso.PC.a.C20.5	-0.006 (-0.029 to 0.016)	0.575	0.575	*Reference*	-0.135 (-0.321 to 0.052)	0.158	0.555

SUPPLEMENTARY TABLE S4.1.3. Continued

	Prepregnancy BMI (kg/m²)			Prepregnancy normal weight	Prepregnancy overweight or obesity		
Metabolite	Difference in metabolite concentration (SDS) (95% CI) (95% ci)	FDR adjusted p-value	FDR adjusted p-value	Difference in maternal metabolite concentration (SDS) (95% CI)	Difference in maternal metabolite concentration (SDS) (95% CI)	p-value	FDR adjusted p-value
lyso.PC.a.C22.6	-0.01 (-0.032 to 0.013)	0.403	0.403	*Reference*	-0.012 (-0.199 to 0.174)	0.896	0.955
lyso.PC.e.C16.0	0.005 (-0.018 to 0.029)	0.650	0.650	*Reference*	0.006 (-0.189 to 0.201)	0.951	0.971
lyso.PC.e.C18.0	-0.009 (-0.032 to 0.014)	0.455	0.455	*Reference*	-0.057 (-0.248 to 0.135)	0.562	0.856
lyso.PC.e.C18.1	0.014 (-0.009 to 0.037)	0.223	0.223	*Reference*	0.102 (-0.088 to 0.291)	0.292	0.703
PC.aa.C30.0	-0.031 (-0.053 to -0.009)	0.007	0.007	*Reference*	-0.104 (-0.291 to 0.082)	0.274	0.681
PC.aa.C30.3	0.011 (-0.012 to 0.034)	0.343	0.343	*Reference*	0.061 (-0.130 to 0.253)	0.530	0.847
PC.aa.C32.0	0.001 (-0.021 to 0.024)	0.917	0.917	*Reference*	0.050 (-0.139 to 0.239)	0.604	0.867
PC.aa.C32.1	0.017 (-0.005 to 0.039)	0.138	0.138	*Reference*	0.166 (-0.021 to 0.353)	0.082	0.381
PC.aa.C32.2	-0.013 (-0.036 to 0.009)	0.244	0.244	*Reference*	-0.068 (-0.255 to 0.119)	0.479	0.807
PC.aa.C32.3	0.012 (-0.010 to 0.035)	0.285	0.285	*Reference*	0.110 (-0.080 to 0.300)	0.258	0.657
PC.aa.C34.1	0.015 (-0.008 to 0.037)	0.200	0.200	*Reference*	0.168 (-0.019 to 0.356)	0.078	0.381
PC.aa.C34.2	0.009 (-0.013 to 0.031)	0.425	0.425	*Reference*	0.086 (-0.096 to 0.269)	0.354	0.729
PC.aa.C34.3	0.004 (-0.018 to 0.026)	0.738	0.738	*Reference*	0.035 (-0.152 to 0.222)	0.711	0.888
PC.aa.C34.4	0.016 (-0.006 to 0.038)	0.155	0.155	*Reference*	0.122 (-0.063 to 0.306)	0.196	0.597
PC.aa.C34.5	-0.019 (-0.041 to 0.003)	0.086	0.086	*Reference*	-0.111 (-0.293 to 0.070)	0.230	0.64
PC.aa.C36.0	0.009 (-0.014 to 0.032)	0.437	0.437	*Reference*	0.050 (-0.143 to 0.243)	0.612	0.867
PC.aa.C36.1	-0.006 (-0.028 to 0.017)	0.613	0.613	*Reference*	0.069 (-0.118 to 0.256)	0.472	0.807
PC.aa.C36.2	-0.008 (-0.03 to 0.015)	0.501	0.501	*Reference*	0.008 (-0.177 to 0.194)	0.930	0.958
PC.aa.C36.3	0.031 (0.009 to 0.053)	0.006	0.006	*Reference*	0.245 (0.060 to 0.430)	0.010	0.099
PC.aa.C36.4	0.034 (0.012 to 0.056)	0.003	0.003	*Reference*	0.246 (0.062 to 0.430)	0.009	0.098
PC.aa.C36.5	0.004 (-0.018 to 0.026)	0.707	0.707	*Reference*	0.031 (-0.154 to 0.215)	0.745	0.900

SUPPLEMENTARY TABLE S4.1.3. Continued

	Prepregnancy BMI (kg/m²)			Prepregnancy normal weight	Prepregnancy overweight or obesity		
Metabolite	Difference in metabolite concentration (SDS) (95% CI) (95% ci)	FDR adjusted p-value	FDR adjusted p-value	Difference in maternal metabolite concentration (SDS) (95% CI)	Difference in maternal metabolite concentration (SDS) (95% CI)	p-value	FDR adjusted p-value
PC.aa.C36.6	-0.005 (-0.027 to 0.017)	0.681	0.681	*Reference*	-0.013 (-0.198 to 0.171)	0.888	0.951
PC.aa.C38.0	0.016 (-0.007 to 0.038)	0.175	0.175	*Reference*	0.088 (-0.101 to 0.278)	0.363	0.729
PC.aa.C38.2	0.018 (-0.005 to 0.04)	0.130	0.130	*Reference*	0.126 (-0.063 to 0.315)	0.190	0.597
PC.aa.C38.3	0.048 (0.025 to 0.07)	0.000	0.000	*Reference*	0.407 (0.222 to 0.592)	0.000	0.001
PC.aa.C38.4	0.036 (0.014 to 0.058)	0.002	0.002	*Reference*	0.306 (0.123 to 0.489)	0.001	0.021
PC.aa.C38.5	0.005 (-0.018 to 0.027)	0.690	0.690	*Reference*	0.081 (-0.109 to 0.271)	0.402	0.760
PC.aa.C38.6	0.008 (-0.014 to 0.03)	0.480	0.480	*Reference*	0.068 (-0.118 to 0.253)	0.476	0.807
PC.aa.C40.0	0.005 (-0.019 to 0.028)	0.702	0.702	*Reference*	0.008 (-0.184 to 0.200)	0.934	0.958
PC.aa.C40.1	-0.003 (-0.026 to 0.02)	0.809	0.809	*Reference*	-0.010 (-0.204 to 0.184)	0.918	0.958
PC.aa.C40.2	-0.021 (-0.045 to 0.002)	0.072	0.072	*Reference*	-0.188 (-0.379 to 0.002)	0.053	0.371
PC.aa.C40.3	-0.002 (-0.025 to 0.021)	0.860	0.860	*Reference*	-0.003 (-0.194 to 0.188)	0.976	0.981
PC.aa.C40.4	0.018 (-0.004 to 0.041)	0.109	0.109	*Reference*	0.220 (0.035 to 0.405)	0.020	0.170
PC.aa.C40.5	-0.01 (-0.032 to 0.013)	0.411	0.411	*Reference*	0.044 (-0.146 to 0.234)	0.651	0.881
PC.aa.C40.6	0.02 (-0.002 to 0.043)	0.079	0.079	*Reference*	0.186 (-0.002 to 0.373)	0.052	0.371
PC.aa.C42.0	0.014 (-0.009 to 0.037)	0.243	0.243	*Reference*	0.104 (-0.086 to 0.294)	0.283	0.689
PC.aa.C42.5	-0.022 (-0.045 to 0.000)	0.055	0.055	*Reference*	-0.084 (-0.273 to 0.105)	0.386	0.753
PC.aa.C43.6	0.003 (-0.02 to 0.025)	0.819	0.819	*Reference*	0.059 (-0.127 to 0.245)	0.534	0.847
PC.aa.C44.12	0.016 (-0.006 to 0.039)	0.162	0.162	*Reference*	0.179 (-0.008 to 0.366)	0.062	0.379
PC.ae.C30.0	-0.023 (-0.045 to -0.001)	0.042	0.042	*Reference*	-0.108 (-0.294 to 0.078)	0.254	0.657
PC.ae.C32.0	-0.005 (-0.028 to 0.018)	0.657	0.657	*Reference*	0.028 (-0.163 to 0.220)	0.772	0.912
PC.ae.C32.1	-0.008 (-0.031 to 0.014)	0.473	0.473	*Reference*	0.020 (-0.170 to 0.210)	0.838	0.934

SUPPLEMENTARY TABLE S4.1.3. Continued

	Prepregnancy BMI (kg/m²)			Prepregnancy normal weight	Prepregnancy overweight or obesity		
Metabolite	Difference in metabolite concentration (SDS) (95% CI) (95% ci)	FDR adjusted p-value	FDR adjusted p-value	Difference in maternal metabolite concentration (SDS) (95% CI)	Difference in maternal metabolite concentration (SDS) (95% CI)	p-value	FDR adjusted p-value
PC.ae.C32.2	-0.013 (-0.035 to 0.009)	0.261	0.261	*Reference*	-0.005 (-0.190 to 0.180)	0.959	0.974
PC.ae.C34.0	-0.019 (-0.042 to 0.004)	0.104	0.104	*Reference*	-0.085 (-0.275 to 0.105)	0.382	0.753
PC.ae.C34.1	-0.003 (-0.026 to 0.019)	0.779	0.779	*Reference*	0.020 (-0.166 to 0.206)	0.832	0.932
PC.ae.C34.2	-0.026 (-0.048 to -0.003)	0.024	0.024	*Reference*	-0.107 (-0.292 to 0.079)	0.260	0.657
PC.ae.C34.3	-0.028 (-0.05 to -0.006)	0.014	0.014	*Reference*	-0.178 (-0.363 to 0.008)	0.061	0.379
PC.ae.C34.4	-0.022 (-0.044 to 0.000)	0.056	0.056	*Reference*	-0.124 (-0.312 to 0.063)	0.194	0.597
PC.ae.C36.0	-0.007 (-0.03 to 0.016)	0.553	0.553	*Reference*	0.009 (-0.180 to 0.198)	0.926	0.958
PC.ae.C36.1	-0.005 (-0.028 to 0.018)	0.663	0.663	*Reference*	0.052 (-0.134 to 0.239)	0.582	0.867
PC.ae.C36.2	-0.016 (-0.038 to 0.005)	0.141	0.141	*Reference*	-0.049 (-0.229 to 0.132)	0.598	0.867
PC.ae.C36.3	-0.004 (-0.026 to 0.019)	0.733	0.733	*Reference*	0.037 (-0.150 to 0.225)	0.697	0.882
PC.ae.C36.4	0.023 (0.001 to 0.045)	0.045	0.045	*Reference*	0.169 (-0.016 to 0.354)	0.073	0.381
PC.ae.C36.5	0.019 (-0.004 to 0.041)	0.105	0.105	*Reference*	0.100 (-0.087 to 0.287)	0.296	0.703
PC.ae.C38.0	0.003 (-0.019 to 0.025)	0.797	0.797	*Reference*	0.084 (-0.100 to 0.268)	0.370	0.736
PC.ae.C38.2	-0.009 (-0.032 to 0.013)	0.428	0.428	*Reference*	-0.044 (-0.231 to 0.144)	0.649	0.881
PC.ae.C38.3	0.016 (-0.007 to 0.039)	0.166	0.166	*Reference*	0.170 (-0.018 to 0.357)	0.077	0.381
PC.ae.C38.4	0.012 (-0.011 to 0.035)	0.303	0.303	*Reference*	0.117 (-0.071 to 0.304)	0.222	0.639
PC.ae.C38.5	0.021 (-0.002 to 0.043)	0.071	0.071	*Reference*	0.120 (-0.068 to 0.308)	0.211	0.632
PC.ae.C38.6	0.018 (-0.005 to 0.04)	0.124	0.124	*Reference*	0.070 (-0.117 to 0.257)	0.464	0.807
PC.ae.C40.0	0.01 (-0.012 to 0.033)	0.367	0.367	*Reference*	0.070 (-0.118 to 0.258)	0.468	0.807
PC.ae.C40.1	-0.009 (-0.031 to 0.014)	0.452	0.452	*Reference*	-0.026 (-0.212 to 0.161)	0.788	0.919
PC.ae.C40.2	-0.002 (-0.024 to 0.021)	0.889	0.889	*Reference*	-0.032 (-0.218 to 0.154)	0.740	0.900

SUPPLEMENTARY TABLE S4.1.3. Continued

	Prepregnancy BMI (kg/m²)			Prepregnancy normal weight	Prepregnancy overweight or obesity		
Metabolite	Difference in metabolite concentration (SDS) (95% CI) (95% ci)	FDR adjusted p-value	FDR adjusted p-value	Difference in maternal metabolite concentration (SDS) (95% CI)	Difference in maternal metabolite concentration (SDS) (95% CI)	p-value	FDR adjusted p-value
PC.ae.C40.3	-0.003 (-0.026 to 0.020)	0.807	0.807	*Reference*	0.040 (-0.152 to 0.232)	0.682	0.881
PC.ae.C40.4	0.005 (-0.018 to 0.028)	0.657	0.657	*Reference*	0.115 (-0.076 to 0.307)	0.239	0.647
PC.ae.C40.5	0.009 (-0.014 to 0.032)	0.443	0.443	*Reference*	0.057 (-0.131 to 0.246)	0.552	0.856
PC.ae.C40.6	-0.002 (-0.025 to 0.020)	0.850	0.850	*Reference*	-0.023 (-0.210 to 0.164)	0.808	0.919
PC.ae.C42.1	0.006 (-0.017 to 0.028)	0.624	0.624	*Reference*	0.118 (-0.070 to 0.306)	0.218	0.639
PC.ae.C42.3	-0.014 (-0.036 to 0.009)	0.233	0.233	*Reference*	-0.015 (-0.202 to 0.172)	0.877	0.95
PC.ae.C42.4	0.000 (-0.024 to 0.023)	0.966	0.966	*Reference*	0.073 (-0.118 to 0.265)	0.453	0.807
PC.ae.C42.5	0.011 (-0.012 to 0.033)	0.365	0.365	*Reference*	0.111 (-0.078 to 0.301)	0.250	0.657
PC.ae.C42.6	0.008 (-0.015 to 0.031)	0.490	0.490	*Reference*	0.096 (-0.093 to 0.284)	0.321	0.711
SM.a.C30.1	0.021 (-0.001 to 0.044)	0.064	0.064	*Reference*	0.096 (-0.092 to 0.283)	0.319	0.711
SM.a.C32.1	0.022 (0.000 to 0.044)	0.050	0.050	*Reference*	0.151 (-0.034 to 0.336)	0.110	0.465
SM.a.C32.2	0.070 (0.048 to 0.091)	0.000	0.000	*Reference*	0.486 (0.304 to 0.668)	0.000	0.000
SM.a.C33.1	0.008 (-0.014 to 0.030)	0.465	0.465	*Reference*	0.055 (-0.130 to 0.240)	0.558	0.856
SM.a.C34.1	0.015 (-0.007 to 0.037)	0.188	0.188	*Reference*	0.112 (-0.074 to 0.298)	0.239	0.647
SM.a.C34.2	0.072 (0.051 to 0.094)	0.000	0.000	*Reference*	0.455 (0.275 to 0.635)	0.000	0.000
SM.a.C35.0	0.010 (-0.014 to 0.033)	0.414	0.414	*Reference*	-0.002 (-0.196 to 0.192)	0.982	0.982
SM.a.C35.1	0.024 (0.002 to 0.046)	0.034	0.034	*Reference*	0.179 (-0.006 to 0.364)	0.059	0.379
SM.a.C36.1	0.059 (0.037 to 0.081)	0.000	0.000	*Reference*	0.421 (0.236 to 0.605)	0.000	0.000
SM.a.C36.2	0.077 (0.056 to 0.099)	0.000	0.000	*Reference*	0.500 (0.318 to 0.682)	0.000	0.000
SM.a.C36.3	0.070 (0.048 to 0.091)	0.000	0.000	*Reference*	0.365 (0.184 to 0.547)	0.000	0.002
SM.a.C37.1	0.042 (0.019 to 0.064)	0.000	0.000	*Reference*	0.284 (0.096 to 0.471)	0.003	0.047

SUPPLEMENTARY TABLE S4.1.3. Continued

	Prepregnancy BMI (kg/m²)			Prepregnancy normal weight	Prepregnancy overweight or obesity		
Metabolite	Difference in metabolite concentration (SDS) (95% CI) (95% ci)	FDR adjusted p-value	FDR adjusted p-value	Difference in maternal metabolite concentration (SDS) (95% CI)	Difference in maternal metabolite concentration (SDS) (95% CI)	p-value	FDR adjusted p-value
SM.a.C38.2	0.022 (0.000 to 0.044)	0.054	0.054	*Reference*	0.163 (-0.022 to 0.348)	0.085	0.383
SM.a.C38.3	0.018 (-0.005 to 0.040)	0.123	0.123	*Reference*	0.105 (-0.084 to 0.294)	0.276	0.681
SM.a.C39.1	0.006 (-0.017 to 0.029)	0.602	0.602	*Reference*	0.087 (-0.100 to 0.274)	0.363	0.729
SM.a.C39.2	0.022 (0.000 to 0.044)	0.055	0.055	*Reference*	0.115 (-0.072 to 0.303)	0.228	0.640
SM.a.C40.2	0.019 (-0.003 to 0.041)	0.095	0.095	*Reference*	0.192 (0.008 to 0.377)	0.041	0.311
SM.a.C40.5	0.016 (-0.006 to 0.039)	0.150	0.150	*Reference*	0.133 (-0.052 to 0.319)	0.159	0.555
SM.a.C41.1	0.016 (-0.006 to 0.038)	0.149	0.149	*Reference*	0.076 (-0.108 to 0.260)	0.417	0.778
SM.a.C41.2	0.018 (-0.004 to 0.040)	0.113	0.113	*Reference*	0.156 (-0.030 to 0.341)	0.100	0.443
SM.a.C42.1	0.021 (-0.001 to 0.044)	0.062	0.062	*Reference*	0.171 (-0.015 to 0.357)	0.072	0.381
SM.a.C42.2	0.037 (0.015 to 0.059)	0.001	0.001	*Reference*	0.245 (0.062 to 0.428)	0.009	0.098
SM.a.C42.3	0.039 (0.017 to 0.061)	0.001	0.001	*Reference*	0.323 (0.138 to 0.507)	0.001	0.014
SM.a.C42.4	0.028 (0.005 to 0.050)	0.016	0.016	*Reference*	0.232 (0.049 to 0.416)	0.013	0.119
SM.a.C42.6	0.012 (-0.011 to 0.034)	0.305	0.305	*Reference*	0.089 (-0.097 to 0.275)	0.348	0.729
SM.a.C43.1	0.025 (0.003 to 0.047)	0.027	0.027	*Reference*	0.134 (-0.049 to 0.317)	0.151	0.555
SM.a.C43.2	0.030 (0.008 to 0.052)	0.008	0.008	*Reference*	0.151 (-0.031 to 0.334)	0.105	0.456
SM.a.C44.6	0.020 (-0.003 to 0.043)	0.085	0.085	*Reference*	0.179 (-0.009 to 0.367)	0.062	0.379
SM.e.C36.2	0.045 (0.023 to 0.067)	0.000	0.000	*Reference*	0.258 (0.073 to 0.443)	0.006	0.083
SM.e.C38.3	-0.008 (-0.03 to 0.015)	0.496	0.496	*Reference*	-0.067 (-0.254 to 0.120)	0.481	0.807
SM.e.C40.5	0.023 (0.000 to 0.046)	0.046	0.046	*Reference*	0.133 (-0.057 to 0.324)	0.171	0.565
Carn	0.012 (-0.011 to 0.034)	0.318	0.318	*Reference*	-0.039 (-0.225 to 0.146)	0.677	0.881
Carn.a.C10.0	0.027 (0.004 to 0.049)	0.020	0.020	*Reference*	0.041 (-0.148 to 0.23)	0.669	0.881

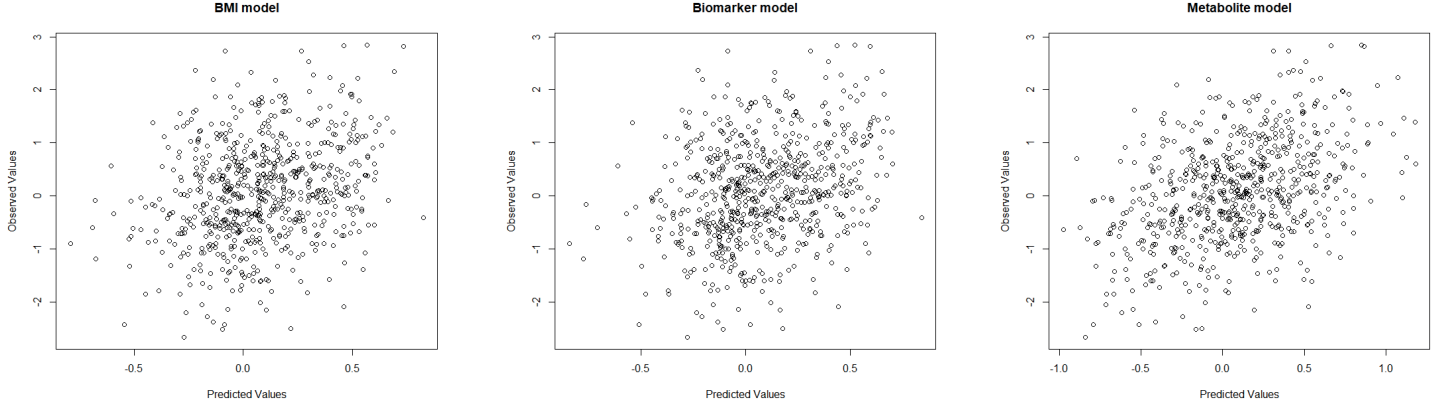
SUPPLEMENTARY TABLE S4.1.3. Continued

	Prepregnancy BMI (kg/m²)			Prepregnancy normal weight	Prepregnancy overweight or obesity		
Metabolite	Difference in metabolite concentration (SDS) (95% CI) (95% ci)	FDR adjusted p-value	FDR adjusted p-value	Difference in maternal metabolite concentration (SDS) (95% CI)	Difference in maternal metabolite concentration (SDS) (95% CI)	p-value	FDR adjusted p-value
Carn.a.C10.1	0.044 (0.022 to 0.067)	0.000	0.000	*Reference*	0.094 (-0.095 to 0.283)	0.329	0.711
Carn.a.C12.0	0.012 (-0.010 to 0.035)	0.292	0.292	*Reference*	0.025 (-0.163 to 0.213)	0.796	0.919
Carn.a.C14.1	0.026 (0.003 to 0.048)	0.028	0.028	*Reference*	0.042 (-0.147 to 0.231)	0.663	0.881
Carn.a.C14.2	0.010 (-0.013 to 0.033)	0.397	0.397	*Reference*	-0.024 (-0.214 to 0.165)	0.801	0.919
Carn.a.C15.0	0.001 (-0.022 to 0.024)	0.938	0.938	*Reference*	-0.048 (-0.238 to 0.142)	0.623	0.867
Carn.a.C16.0	0.017 (-0.006 to 0.039)	0.154	0.154	*Reference*	0.041 (-0.149 to 0.230)	0.675	0.881
Carn.a.C16.0.Oxo	-0.007 (-0.030 to 0.016)	0.536	0.536	*Reference*	-0.039 (-0.229 to 0.151)	0.688	0.881
Carn.a.C16.1	0.021 (-0.001 to 0.044)	0.068	0.068	*Reference*	0.074 (-0.114 to 0.263)	0.442	0.807
Carn.a.C16.2	0.010 (-0.012 to 0.033)	0.365	0.365	*Reference*	0.036 (-0.150 to 0.223)	0.703	0.884
Carn.a.C18.0	-0.001 (-0.024 to 0.021)	0.905	0.905	*Reference*	-0.097 (-0.285 to 0.091)	0.312	0.711
Carn.a.C18.1	0.038 (0.015 to 0.060)	0.001	0.001	*Reference*	0.141 (-0.046 to 0.328)	0.139	0.542
Carn.a.C18.2	0.014 (-0.009 to 0.037)	0.240	0.240	*Reference*	-0.039 (-0.230 to 0.151)	0.686	0.881
Carn.a.C18.2.OH	0.003 (-0.019 to 0.026)	0.767	0.767	*Reference*	-0.062 (-0.250 to 0.127)	0.522	0.841
Carn.a.C2.0	0.012 (-0.011 to 0.034)	0.308	0.308	*Reference*	-0.026 (-0.211 to 0.159)	0.783	0.919
Carn.a.C20.0	0.007 (-0.016 to 0.030)	0.551	0.551	*Reference*	-0.041 (-0.233 to 0.150)	0.673	0.881
Carn.a.C20.1	0.003 (-0.020 to 0.026)	0.810	0.810	*Reference*	-0.051 (-0.242 to 0.140)	0.598	0.867
Carn.a.C20.3	-0.001 (-0.024 to 0.023)	0.953	0.953	*Reference*	-0.030 (-0.223 to 0.162)	0.759	0.902
Carn.a.C20.4	-0.005 (-0.028 to 0.018)	0.676	0.676	*Reference*	-0.035 (-0.226 to 0.156)	0.720	0.894
Carn.a.C3.0	-0.008 (-0.031 to 0.015)	0.503	0.503	*Reference*	-0.139 (-0.326 to 0.048)	0.145	0.555
Carn.a.C3.0.DC	0.016 (-0.007 to 0.038)	0.177	0.177	*Reference*	-0.065 (-0.249 to 0.119)	0.489	0.807
Carn.a.C4.0	-0.019 (-0.041 to 0.003)	0.097	0.097	*Reference*	-0.143 (-0.328 to 0.043)	0.132	0.524

SUPPLEMENTARY TABLE S4.1.3. Continued

	Prepregnancy BMI (kg/m²)			Prepregnancy normal weight	Prepregnancy overweight or obesity		
Metabolite	**Difference in metabolite concentration (SDS) (95% CI) (95% ci)**	**FDR adjusted p-value**	**FDR adjusted p-value**	**Difference in maternal metabolite concentration (SDS) (95% CI)**	**Difference in maternal metabolite concentration (SDS) (95% CI)**	**p-value**	**FDR adjusted p-value**
Carn.a.C5.0	0.009 (-0.014 to 0.032)	0.432	0.432	*Reference*	0.039 (-0.150 to 0.228)	0.686	0.881
Carn.a.C6.0	0.021 (-0.002 to 0.043)	0.077	0.077	*Reference*	0.100 (-0.088 to 0.287)	0.299	0.703
Carn.a.C6.0.OH	0.011 (-0.011 to 0.034)	0.314	0.314	*Reference*	0.052 (-0.131 to 0.236)	0.576	0.867
Carn.a.C8.0	0.021 (-0.001 to 0.044)	0.065	0.065	*Reference*	0.018 (-0.171 to 0.206)	0.853	0.935
Carn.a.C8.1	0.043 (0.021 to 0.065)	0.000	0.000	*Reference*	0.164 (-0.02 to 0.347)	0.080	0.381
Carn.a.C8.2	-0.001 (-0.024 to 0.022)	0.956	0.956	*Reference*	-0.150 (-0.34 to 0.040)	0.123	0.511

Abbreviations: AA amino acid, NEFA non-esterified fatty acid, PC.aa diacyl-phosphatidylcholines, PC.ae acyl-alkyl-phosphatidylcholines, Lyso.PC.a acyl-lysophosphatidylcholines, Lyso.PC.e alkyl–lysophosphatidylcholines, SM sphingomyelines Carn.a acylcarnitines. Values represent regression coefficients (95% confidence interval) and corresponding p-values and false discovery rate corrected p-values from linear regression models that reflect the difference in maternal early-pregnancy metabolite concentration in SDS per kg/m² increase in BMI and for women with overweight or obesity as compared to normal weight women. Models were adjusted for gestational age at blood sampling, age, educational level, parity, smoking, alcohol consumption, folic acid supplementation, daily total energy intake, systolic blood pressure and fetal sex.



SUPPLEMENTARY FIGURE S4.1.4. Observed versus prediction values for linear regression models for birthweight

Scatterplots represent observed and predicted values for birthweight in SDS. Predicted values for birthweight were obtained from linear regression models.
[a]BMI model includes prepregnancy BMI, gestational age at blood sampling, age, educational level, parity, smoking, alcohol consumption, folic acid supplementation, daily total energy intake, systolic blood pressure and fetal sex
[b]Biomarker model includes the BMI model with additional adjustment for glucose, triglyciderides and HDL-cholesterol
[c]Metabolite model includes the BMI model with additional adjustment for prepregnancy BMI, Gln, Lys, NEFA.18:2, NEFA.20:3, NEFA.22:3, PC.aa.C30:0, PC.aa.C38:3, PC.ae.C34:4, lyso.PC.a.C16:1, lyso.PC.a.C20:4, SM.a.C36:1, SM.a.C36:2, SM.a.C40:2, SM.a.C42:4, Carn.a.C10:1, Asn/Asp ratio, Gln/Glu ratio, NEFA.16:1/16:0 ratio, ΣPC.aa/ΣPC.ae ratio

SUPPLEMENTARY TABLE S4.1.4. Predictive performance of models for the risk prediction of large-for-gestational-age newborns

Models	AUC (95% CI)
BMI model[a]	0.67 (0.61 to 0.73)
Biomarker model[b]	0.69 (0.62 to 0.76)
Metabolite model[c]	0.76 (0.70 to 0.82)

[a]*BMI model includes prepregnancy BMI, gestational age at blood sampling, age, educational level, parity, smoking, alcohol consumption, folic acid supplementation, daily total energy intake, systolic blood pressure and fetal sex*

[b]*Biomarker model includes the BMI model with additional adjustment for glucose, triglyciderides and HDL-cholesterol*

[c]*Metabolite model includes the BMI model with additional adjustment for prepregnancy BMI, Gln, Lys, NEFA.18:2, NEFA.20:3, NEFA.22:3, PC.aa.C30:0, PC.aa.C38:3, PC.ae.C34:4, lyso.PC.a.C16:1, lyso.PC.a.C20:4, SM.a.C36:1, SM.a.C36:2, SM.a.C40:2, SM.a.C42:4, Carn.a.C10:1, Asn/Asp ratio, Gln/Glu ratio, NEFA.16:1/16:0 ratio, ΣPC.aa/ΣPC.ae ratio*

4.2

Prediction of healthy pregnancies in women with overweight or obesity

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Submitted

ABSTRACT

Background/Objective: Women with overweight and obesity receive an uniform approach of intensified antenatal care due to their increased risk of pregnancy complications, even though not all of these high-risk women develop complications. We aimed to develop a prediction model based on maternal characteristics for the prediction of healthy pregnancy outcomes pregnant women with overweight or obesity. Second, we assessed whether paternal characteristics and maternal early-pregnancy metabolites can improve prediction of a healthy pregnancy outcome.

Subjects/Methods: In a population-based cohort study among a subsample of 1,180 Dutch women with overweight or obesity, we developed a prediction model using 32 maternal early- and mid-pregnancy socio-demographic, lifestyle, physical, and pregnancy-related characteristics. We assessed the additional predictive performance of paternal characteristics and maternal early-pregnancy metabolites. A healthy pregnancy outcome was defined as a pregnancy without fetal death, gestational hypertension, preeclampsia, gestational diabetes, caesarian section, preterm birth, large-for-gestational-age at birth, macrosomia, postpartum weight retention and offspring obesity at 5 years.

Results: In total, 25% of pregnant women with overweight or obesity had a healthy pregnancy outcome. Maternal early-pregnancy age, marital status, parity, BMI, and mid-pregnancy gestational weight gain, systolic blood pressure and estimated fetal weight were selected into the model using backward selection. This model had a moderate predictive performance with an Area Under the Receiver Operating Characteristic Curve of 0.65 (95% confidence interval 0.61 to 0.68) and sensitivity of 23% at 90% specificity. Paternal characteristics and maternal early-pregnancy metabolites did not improve the predictive performance.

Conclusions: In pregnant women with overweight or obesity, maternal characteristics can moderately predict a healthy pregnancy outcome. Paternal characteristics and maternal early-pregnancy metabolites have no incremental value for prediction of a healthy pregnancy outcome. Further studies need to identify novel markers enabling more accurate prediction of a healthy pregnancy outcome in women with overweight or obesity.

INTRODUCTION

Overweight or obesity among women is currently the most common medical disorder in pregnancy (1-3). Women with overweight or obesity during pregnancy not only have strongly increased risks of maternal and neonatal morbidity and mortality, but also of long-term adverse maternal and offspring health outcomes, including postpartum weight retention and offspring obesity (4, 5). For prevention and management of these risks, guidelines recommend intensified antenatal monitoring and care for pregnant women with obesity (6-8). Nevertheless, a substantial proportion of women with overweight or obesity will have an uncomplicated pregnancy and may receive redundant intensified antenatal care (9). A personalized risk assessment, using well-known risk factors associated with adverse pregnancy and long-term health outcomes, could enable tailored antenatal care in these high-risk women. Such approach is essential to avoid unnecessary medicalization of pregnancy, to improve pregnancy outcomes and to reduce health care burden and costs (10-12).

In a subsample of 1,180 Dutch pregnant women with overweight or obesity and their offspring from a population-based cohort study, we aimed to develop a prediction model using maternal socio-demographic, lifestyle, physical and pregnancy-related characteristics in the first half of pregnancy to predict a healthy pregnancy outcome. Second, we used paternal characteristics and maternal early-pregnancy metabolites to explore whether innovative measurements could improve prediction of a healthy pregnancy outcome in addition to well-known clinical risk factors.

METHODS

Subjects

This study was embedded in a subgroup of the Generation R Study, a population-based prospective cohort study from fetal life until adulthood in Rotterdam, the Netherlands (13). Study approval was obtained by the Medical Ethical Committee of the Erasmus Medical Center, University Medical Center, Rotterdam (MEC 198.782/2001/31). In total, 8,879 women were enrolled during pregnancy. Written informed consent was obtained from all participant. For the current study, we only included women with a Dutch ethnicity. Of Dutch women, 1,200 had prepregnancy or early-pregnancy overweight or obesity. After exclusion of non-singleton pregnancies, induced abortions and women without information on birth characteristics, population for analyses consisted of 1,180 women (**Supplementary Figure S4.2.1**). For secondary analyses, paternal characteristics were available in 1,023 of the women and early-pregnancy metabolites

were available in a preselected subgroup of 273 of the women (14). We focused on an ethnic homogeneous sample to increase accuracy of predictor selection by eliminating statistical noise induced by ethnic variation in predictors and outcomes (15). When predicting healthy pregnancies within high risk women who may be eliminated from intensified care, only strong predictors should be selected to achieve a highly accurate prediction model. We assessed generalizability of the developed prediction model in non-Dutch women with overweight or obesity in the Generation R Study (n=1,584).

Maternal clinical candidate predictors

For development of prediction models, we included characteristics well-known to be associated with the risk of adverse pregnancy outcomes and clustered them based on their assessment in early- or mid-pregnancy (2-4).

Early-pregnancy cluster: Information on age, ethnicity, educational level, income, marital status, parity, history of obstetric complications, folic acid supplementation and smoking during pregnancy was obtained from questionnaires (13). History of obstetric complications included stillbirth, miscarriage, pre-eclampsia, gestational hypertensive disorders, gestational diabetes, caesarian section, low birth weight, macrosomia. Dietary intake was assessed by a Food Frequency Questionnaire (16). At a median gestational age of 12.9 (95% range 9.6 to 17.3) weeks, we measured height, weight and blood pressure at the research center (13). BMI (kg/m^2) was calculated (17). Non-fasting venous blood samples were sampled to analyze glucose (mmol/l), triglyceride (mmol/l), High Density Lipoprotein-cholesterol (HDL-cholesterol) (mmol/l) and C-reactive protein (CRP) (mg/l), Soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) concentrations. Detailed procedures for biomarker analyses are described elsewhere (13, 18-22). CRP concentrations >10 mg/l were excluded to eliminate high CRP concentrations due to acute infections.

Mid-pregnancy cluster: At a median gestational age of 20.6 (95% range 18.7 to 23.3) weeks, weight was measured and mid-pregnancy weight gain in kilograms/week was calculated as mid-pregnancy weight-early-pregnancy/difference in gestational age. We measured mid-pregnancy systolic and diastolic blood pressure and obtained vitamin D, sFlt-1 and PlGF concentrations from sampled venous blood samples (18). Using ultrasound examinations we obtained estimated fetal weight, umbilical artery pulsatility index and uterine artery resistance index as described previously (23-25). Gestational-age adjusted standard deviation-scores for estimated fetal weight ware based on reference growth charts from the whole study population and represent the equivalent of z-scores.

Paternal characteristics and maternal metabolites

Paternal characteristics: Of participating partners, 934 (91%) were known to be the biological father. We obtained information on paternal age, ethnicity, educational level, BMI and smoking by questionnaire (13).

Metabolomics: Metabolomics analysis was done in the same venous blood samples as used for clinical biomarker analyses, as in detailed described previously (26). Shortly, a targeted metabolomics analysis was performed at LMU Munich to determine serum concentrations (mmol/L) of 195 amino acids (AA), non-esterified fatty acids (NEFA), phospholidipds, including diacyl-phosphatidylcholines, acyl-alkyl-phosphatidylcholines, acyl-lysophosphatidylcholines, alkyl-lysophosphatidylcholines and sphingomyelines, and carnitines including free carnitine and acyl-carnitines, as in detail described previously (26). IUPAC-IUB Nomenclature was used for notation of AA (27). For notation of NEFA X:Y, X denotes the length of the carbon chain, Y the number of double bonds.

Healthy pregnancy outcome

A healthy pregnancy outcome was defined as the absence of the following outcomes: intrauterine fetal death, gestational hypertension, pre-eclampsia, gestational diabetes, caesarian section, preterm birth, large-for-gestational-age (LGA) at birth, macrosomia, maternal postpartum weight retention and offspring overweight or obesity.

Information on maternal pregnancy complications was obtained from medical records (13). Preterm birth was defined as gestational age at birth <37 weeks. LGA was the highest ten percentiles of gestational age- and sex-adjusted birthweight (28). Macrosomia was birthweight >4000 grams. At a visit at the research center at child's age of 6 years, we measured maternal weight at 6 years postpartum. Maternal postpartum weight gain was defined as having a postpartum weight higher than the prepregnancy weight, calculated as maternal weight 6 years postpartum – maternal prepregnancy weight. Child's height and weight were measured and BMI was calculated (n=891). If child's BMI at 6 years was missing, we used the last growth measured at the Community Child Health Centers (median age 3.8, 95% range 2.0 to 4.0) (n=144) (29). We categorized childhood weight status in underweight/normal weight and overweight/obesity. If information on an outcome was missing, it was considered as a non-adverse outcome.

Statistical analysis

Nominal and non-linear candidate predictors were categorized into clinical categories or quintiles and one missing category to allow for missing values when using the final

model in clinical setting. Linear candidate predictors were used continuously and imputed with mean or median. In case of missing values in clinical practice, the mean or median can be inserted. For model selection, we used two multivariable logistic regression models to evaluate whether accurate prediction could already be performed in early-pregnancy or required mid-pregnancy characteristics. We started only selecting characteristics from the early-pregnancy cluster using backward selection and stopped when all p-values<0.20. After selection from the early-pregnancy cluster, we assessed model performance by assessing the area under the receiver operating curve (AUC), sensitivity and positive likelihood ratio at 70, 80, and 90% specificity. We extended this model by including the cluster of mid-pregnancy characteristics. Based on the log-likelihood ratio, we evaluated whether the cluster of mid-pregnancy characteristics improved the model and further selected variables from this cluster using a similar approach. After model selection, we again assessed model performance of the full maternal model and compared AUCs of the early-pregnancy and the full model using DeLong test (30). To explore generalizability to multi-ethnic populations, we assessed model performance of the final model in women with a non-Dutch ethnicity.

As secondary analyses, we assessed the incremental predictive value of paternal characteristics and maternal metabolites. Because of their less established associations with a healthy pregnancy outcome, we used forward selection to select these candidate predictors (threshold p-value<0.20). Clusters of selected paternal characteristics and maternal metabolites were separately added to the full maternal model and we compared the model performance.

We performed several sensitivity analyses: 1) only including women with measured early-pregnancy overweight or obesity 2) only including women with full information on all adverse outcomes available, 3) excluding fetal deaths from the composite healthy pregnancy outcome and 4) excluding postpartum weight retention and childhood overweight or obesity from the composite healthy pregnancy outcome. The statistical analyses were performed using the Statistical Package of Social Sciences version 24.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Subject characteristics

Figure 4.2.1 shows that 293 (25%) of overweight and obese pregnant women had a healthy pregnancy outcome. Of women with an adverse pregnancy outcome, 447 (50%) had more than one adverse pregnancy outcome. **Table 4.2.1** shows characteristics of women with overweight or obesity according to a healthy or adverse pregnancy outcome. Women with an adverse pregnancy outcome were more often obese.

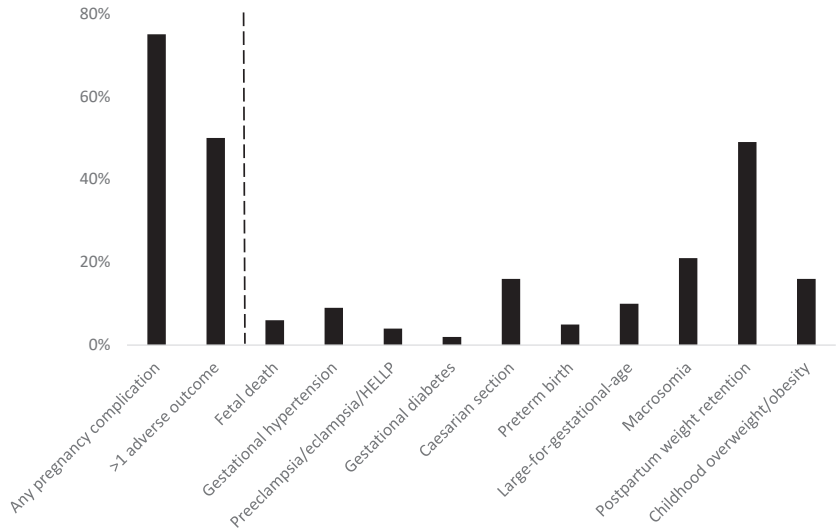


FIGURE 4.2.1. Prevalence of adverse outcomes

TABLE 4.2.1. Population characteristics

	Total group (n=1,180)	Healthy pregnancy outcome (n=293)	Any adverse pregnancy outcome (n=887)
Early-pregnancy characteristics			
Gestational age at measurement, median (95% range), weeks	12.9 (9.6 to 17.3)	12.9 (8.2 to 17.2)	12.9 (9.8 to 17.4)
Age, mean (SD), years	31.1 (4.4)	30.2 (4.5)	31.3 (4.4)
Prepregnancy Body Mass Index, median (95% range), kg/m^2	26.6 (23.0 to 38.1)	26.5 (23.4 to 36.7)	26.7 (23.0 to 38.5)
Prepregnancy obesity, n yes (%)	228 (23)	42 (19)	186 (24)
Early-pregnancy Body Mass Index, median (95% range), kg/m^2	27.6 (25.0 to 38.6)	27.7 (25.1 to 38.9)	27.7 (25.1 to 38.9)
Early-pregnancy obesity, n yes (%)	297 (28)	63 (24)	234 (30)
Parity, n multiparous (%)	518 (44)	140 (48)	378 (43)
Education, n higher education (%)	522 (45)	119 (41)	403 (46)
Income, n >2200 euro (%)	714 (71)	167 (68)	547 (71)
Marital status, n married or living together (%)	1070 (94)	254 (90)	816 (95)
History of obstetric complications, n no (%)	392 (97)	97 (97)	295 (97)
Smoking, n no (%)	779 (72)	185 (70)	594 (72)
Folic acid supplementation, n yes (%)	837 (86)	190 (83)	647 (88)
Fruit consumption, n ≥200grams/day, n yes (%)	638 (54)	164 (64)	474 (61)
Vegetable consumption, n ≥250grams/day, n yes (%)	67 (6)	18 (7)	49 (6)
Energy intake, mean (SD), kcal/day	2090 (508)	2062 (517)	2101 (505)
Carbohydrate intake, mean (SD), g/day	256 (75)	252 (78)	257 (74)
Fat intake, mean (SD), g/day	84 (24)	83 (23)	84 (24)
Protein intake, mean (SD), g/day	77 (19)	76 (20)	78 (19)
Systolic blood pressure, mean (SD), mmHg	123 (13)	122 (13)	122 (13)
Diastolic blood pressure, mean (SD), mmHg	73.1 (9.9)	72 (10)	73 (10)
Glucose, mean (SD), mmol/L	4.5 (0.9)	4.4 (0.7)	4.5 (0.9)
HDL-concentrations, mean (SD), mmol/L	1.7 (0.3)	1.7 (0.3)	1.7 (0.3)
Triglycerides concentrations, median (95% range), mmol/L	1.4 (0.7 to 2.8)	1.4 (0.7 to 2.7)	1.4 (0.7 to 2.8)
CRP concentrations, median (95% range), mg/L	4.9 (0.9 to 9.6)	5.2 (0.8 to 9.7)	4.8 (0.9 to 9.6)
Placental growth factor, median (95% range), mom	0.99 (0.42 to 4.21)	1.05 (0.39 to 3.86)	0.99 (0.39 to 4.31)
sFlt-1, median, (95% range), mom	1.00 (0.41 to 2.60)	1.02 (0.42 to 2.59)	0.99 (0.39 to 2.62)
Mid-pregnancy characteristics			
Gestational age at measurement, median (95% range), weeks	20.6 (18.7 to 23.3)	20.4 (18.7 to 23.3)	20.5 (18.8 to 23.5)
Mid-pregnancy weight, median (95% range), kg/m^2	84.0 (69.0 to 116.0)	82.0 (67.5 to 112.2)	84.8 (70.0 to 117.0)
Gestational weight gain, median (95% range), kg/week	0.29 (-0.19 to 0.71)	0.24 (-0.24 to 0.67)	0.30 (-0.15 to 0.72)
Systolic blood pressure, mean (SD), mmHg	123 (12)	122 (11)	125 (13)
Diastolic blood pressure, mean (SD), mmHg	72 (10)	71 (9)	72 (10)
Vitamin D concentrations, median (95% range), nmol/L	60.1 (16.3 to 121.9)	59.9 (13.5 to 114.2)	60.3 (16.6 to 122.7)
Placental growth factor, median (95% range), mom	1.00 (0.39 to 3.15)	0.97 (0.37 to 3.39)	1.01 (0.40 to 2.93)
sFlt-1, median, (95% range), mom	1.00 (0.33 to 3.15)	0.99 (0.31 to 2.99)	1.00 (0.33 to 3.48)

TABLE 4.2.1. Continued

	Total group (n=1,180)	Healthy pregnancy outcome (n=293)	Any adverse pregnancy outcome (n=887)
Estimated fetal weight, mean (SD), SDS	0.01 (1.00)	-0.14 (0.97)	0.05 (1.00)
Uterine artery resistance index, mean (SD), SDS	0.00 (1.00)	0.03 (0.97)	-0.01 (1.01)
Umbilical artery pulsatility index, mean (SD), SDS	0.00 (1.00)	0.08 (1.03)	-0.02 (0.99)
Birth characteristics			
Sex, n female (%)	594 (51)	146 (50)	448 (51)
Gestational age at birth, median (95%), weeks	40.3 (35.5 to 42.3)	40.3 (37.1 to 42.3)	40.3 (34.4 to 42.3)
Birthweight, mean (SD), grams	3534 (591)	3370 (389)	3590 (635)

Percentage are valid percentages

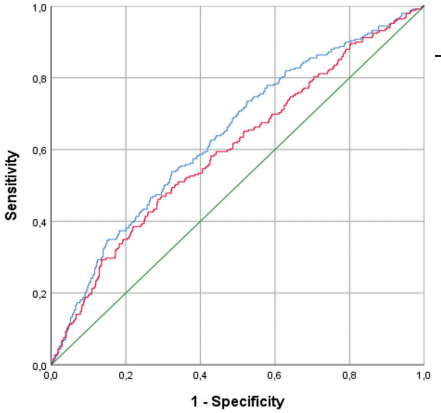
Model selection

From the early-pregnancy maternal candidate predictors, maternal age, marital status, parity, BMI, systolic blood pressure and CRP concentrations were selected in the model using backward selection (**Table 4.2.2**). This early-pregnancy model had an AUC of 0.61 (95% CI 0.58 to 0.65) with a sensitivity of 23% and positive likelihood ratio of 2.3 at 90% specificity. From the maternal mid-pregnancy candidate predictors, gestational weight gain, systolic blood pressure and estimated fetal weight in mid-pregnancy were selected in the model. Addition of these mid-pregnancy characteristics resulted in maternal early-pregnancy systolic blood pressure being removed from the model. The full model, including early-, and mid-pregnancy characteristics, had an AUC of 0.65 (95% CI 0.61 to 0.68) with a sensitivity of 23% and positive likelihood ratio of 2.3 at 90% specificity (p=0.016 in comparison to the early-pregnancy model). Effect estimates for the selected risk factors are shown in **Table 4.2.3**. A pregnant woman with overweight or obesity and with a healthy risk profile has a 56% chance of a healthy pregnancy outcome, whereas a women with an unhealthy risk profile has a 15% chance of a healthy pregnancy outcome (**Figure 4.2.2**).

Among women with a non-Dutch ethnicity, the largest ethnic groups were Turkish (n=354), Surinamese (n=288) and Moroccan (n=293). In non-Dutch women, the full maternal model had an AUC of 0.62 (95% CI 0.59 to 0.65).

TABLE 4.2.2. Model selection for no adverse outcome of pregnancy

Model selection based on clusters of maternal clinical candidate predictors



Models	Variables included per model	AUC (95% CI)	Sensitivity at specificity (%)			Positive likelihood ratio at specificity			p-value*
			70%	80%	90%	70%	80%	90%	
Early-pregnancy (red line)	Age + marital status + parity + BMI + early-pregnancy systolic blood pressure + early-pregnancy CRP concentrations	0.61 (0.58 to 0.65)	48	37	23	1.6	1.9	2.3	
Full maternal (blue line)	Age + marital status + parity + BMI + mid-pregnancy gestational weight gain + mid-pregnancy systolic blood pressure + mid-pregnancy estimated fetal weight	0.65 (0.61 to 0.68)	50	41	23	1.7	2.1	2.3	0.016

Models were adjusted for gestational age at early-pregnancy measurement to enable interpretation of effect estimates of biomarkers not standardized

**p-value is obtained using DeLong's test for comparison of the AUC of the full model with the AUC of early-pregnancy model*

TABLE 4.2.3. Effect estimates of characteristics associated with no adverse outcomes

Early-pregnancy characteristics	**Multivariable, early-pregnancy model OR (95% CI)***	**Multivariable, mid-pregnancy model OR (95% CI)***
Intercept	36.80	102.26
Age (per 1 year increase)	0.94 (0.91 to 0.97)	0.94 (0.91 to 0.97)
Marital status		
No partner	*Reference*	*Reference*
Married or in a relationship	0.60 (0.36 to 1.00)	0.58 (0.35 to 0.98)
Missing	0.98 (0.41 to 2.31)	0.88 (0.36 to 2.11)
Parity		
Nulliparous	*Reference*	*Reference*
Multiparous	1.41 (1.06 to 1.87)	1.37 (1.03 to 1.82)
BMI (per 1 kg/m^2 increase)	0.96 (0.92 to 1.00)	0.96 (0.92 to 1.00)
Systolic blood pressure (per 10 mmHg increase)	0.91 (0.81 to 1.03)	
CRP concentrations	1.05 (0.98 to 1.13)	
Mid-pregnancy characteristics		
Gestational weight gain (per 1 kg/week increase)		0.44 (0.22 to 0.89)
Systolic blood pressure (per 10 mmHg)		0.89 (0.79 to 1.00)
Estimated fetal weight		
First quintile		1.10 (0.71 to 1.72)
Second quintile		0.82 (0.51 to 1.29)
Third quintile		*Reference*
Fourth quintile		0.79 (0.50 to 1.26)
Fifth quintile		0.49 (0.30 to 0.82)
Missing		1.18 (0.70 to 1.98)

**All effect estimates were adjusted for gestational age at measurement in early-pregnancy*

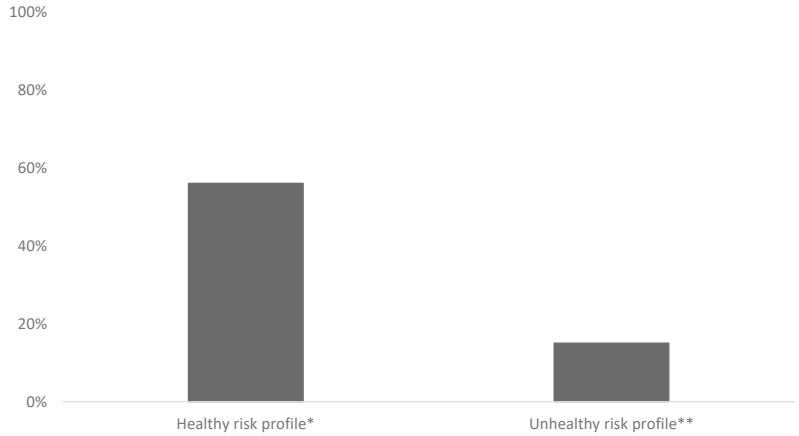


FIGURE 4.2.2. Chances of a healthy pregnancy outcome for women with different combinations of risk factors.

**A health risk profile represents a 26 years old women who is married, multiparous, a BMI of 26 kg/m², 0.3 kg gestational weight gain per week, a mid-pregnancy systolic blood pressure of 110 mmHg, and a mid-pregnancy estimated fetal weight of 0.3 SDS*
***An unhealthy risk profile risk profile represents a 36 years old women without a partner, nulliparous, a BMI of 40 kg/m², 1 kg gestational weight gain per week, a mid-pregnancy blood pressure of 140 mmHg, and a mid-pregnancy estimated fetal weight of 1.5 SDS*

Paternal characteristics and maternal metabolites

Women in the subpopulation with paternal characteristics or maternal early-pregnancy metabolite concentrations available had largely similar characteristics as women in the total population (**Supplementary Table S4.2.1**).

From paternal characteristics, ethnicity and smoking were selected (**Table 4.2.4**). Addition of these paternal characteristics to the full maternal model did not improve model performance (AUC: 0.66 (95% CI 0.63 to 0.70)). From the maternal early-pregnancy metabolites, Arginine, NEFA.14.0, NEFA.14.1, NEFA.16.0, NEFA.17.1 and NEFA.20.3 were selected. Addition of these metabolites to full maternal model did not improve the model performance (AUC: 0.70 (95% CI 0.63 to 0.78), when compared to the performance of the full maternal model when assessed in the subgroup of women with metabolite concentrations available (AUC of the full maternal model in this subgroup 0.69 (95% CI 0.61 to 0.76)).

TABLE 4.2.4. Selection and performance of paternal characteristics and maternal metabolites

No adverse outcome of pregnancy									
			Sensitivity at specificity (%)			**Positive likelihood ratio**			**p-value***
Models	**Variables included per model**	**AUC (95% CI)**	**70%**	**80%**	**90%**	**70%**	**80%**	**90%**	
Paternal characteristics *(n=1,023)*	Full model + paternal ethnicity + paternal smoking	0.66 (0.63 to 0.70)	53	41	26	1.8	2.1	2.6	0.502
Metabolomics (*n=273)*	Full model + Arg + NEFA.14.0 + NEFA.14.1 + NEFA.16.0 + NEFA.17.1 + NEFA.20.3	0.70 (0.63 to 0.78)	56	47	37	1.9	2.4	3.7	0.240

For selection of experimental characteristics, a forward selection procedure was used, with a p-value threshold of <0.20
**p-values are obtained using DeLong's test for comparison of the AUC of the full model with the AUC of the mid-pregnancy model. AUCs of the full model in these selected populations was 0.65 (95% CI 0.61 to 0.69) in the subgroup with paternal characteristics available, 0.69 (95% CI 0.61 to 0.76) in the subgroup with metabolomics available.*

Sensitivity analyses

Model selection and performance was similar to the full maternal model when only including women with early-pregnancy overweight or obesity and not those only with prepregnancy BMI available (n population for analysis=1,027) or when excluding women with fetal deaths (n population for analysis=1,1173) (**Supplementary Table S4.2.2**). Among women with full information on the composite healthy pregnancy outcome available (n population for analysis=948), model selection for a healthy pregnancy outcome was similar, but model performance was slightly lower than in main analyses (AUC 0.60 (95% CI 0.54 to 0.67)) (**Supplementary Table S4.2.2**). When not considering long-term complications as an adverse pregnancy outcome, 644 (55%) of women had a healthy pregnancy outcome (n population for analysis=1,180). Model selection for prediction of a healthy pregnancy outcome excluding postpartum weight gain and offspring overweight or obesity model selection was slightly different, but model performance was largely similar to main analyses (AUC: 0.67 (95% CI 0.64 to 0.70)) (**Supplementary Table S4.2.2**).

DISCUSSION

In this population-based cohort study, one in four women with overweight or obesity during pregnancy had a healthy pregnancy outcome. Common maternal socio-demographic, lifestyle, physical and pregnancy-related characteristics in the first half of pregnancy could moderately predict a healthy pregnancy outcome. Paternal characteristics and maternal early-pregnancy metabolites had no incremental value in the prediction of a healthy pregnancy outcome.

Interpretation of main findings

Women with overweight or obesity during pregnancy are offered uniform intensified antenatal care, due to their increased risk of pregnancy complications and long-term adverse maternal and offspring health outcomes. National and international guidelines on overweight and obesity during pregnancy do not provide sufficient targeted recommendations for tailored antenatal care in these high-risk pregnant women (8, 31, 32). Accurate identification of women with overweight or obesity who will have a healthy pregnancy outcomes is needed to enable a personalized approach of antenatal care (10-12).

Few studies have developed prediction models for a healthy pregnancy outcome in women with obesity during pregnancy (9, 33). A study from the United Kingdom among 1,409 obese pregnant women developed a model including maternal age, parity, systolic blood pressure, HbA1c and plasma adiponectin for the prediction of a healthy pregnancy outcome (AUC of 0.72) (33). In this study, 36% of obese pregnant women had a healthy pregnancy outcome, defined as delivery of a term live-born newborn without antenatal or labour complications. In a Canadian study among 38,055 obese pregnant women, 58% had a healthy pregnancy outcome, defined as delivery of a term live-born newborn without antenatal or labour complications (9). Their model included maternal parity, age, income, ethnicity, weight, placenta-associated plasma protein-A and spontaneously conceived pregnancies and had an AUC of 0.58 for prediction of a healthy pregnancy outcome.

In the current study, we observed that 25% of women with overweight and obesity had a healthy pregnancy outcome, which not only compromised short-term maternal and neonatal outcomes, but also included long-term health outcomes. Maternal gestational overweight and obesity are major risk factors for maternal postpartum weight gain and offspring overweight or obesity, which are strongly related to maternal and offspring cardio-metabolic diseases in later life (4, 34). These outcomes are crucial to take into

account in the risk assessment of pregnancy outcomes among overweight and obese pregnant women. We identified maternal early-pregnancy age, marital status, parity and BMI and mid-pregnancy gestational weight gain, systolic blood pressure and estimated fetal weight as predictors of a healthy pregnancy outcome in women with overweight or obesity during pregnancy. Mid-pregnancy characteristics significantly improved model performance. In line with previous studies, our developed model could moderately predict a healthy pregnancy outcome. In addition to previous studies focusing on women with obesity, we also included women with overweight during pregnancy. Risks of adverse outcomes seem to be already present among pregnant women with a BMI below threshold of obesity, which was confirmed by the additional predictive value of maternal BMI continuously in our model. Thus, our findings suggest that in pregnant women with overweight or obesity, moderate prediction of a health pregnancy outcome can be achieved, when using common maternal characteristics in early-, and mid-pregnancy.

It remains a major challenge to develop models that accurately predict healthy pregnancy outcomes in women with overweight or obesity. There is a need for identification of novel markers to discriminate women with overweight or obesity who will develop adverse pregnancy outcomes from those not. Paternal characteristics may be linked to pregnancy outcomes by affecting semen quality, but also as an additional proxy of family-based lifestyle (35). In the current study, we did not observe an improvement of the prediction model by addition of paternal characteristics, which suggests that the predictive value of family-based lifestyle is mostly captured by maternal factors. Maternal metabolite concentrations during pregnancy have become a relatively new focus for assessing maternal metabolism during pregnancy. In a previous study maternal mid-pregnancy metabolite concentrations improved prediction pregnancy complications on top of common maternal risk factors in normal weight women, but not in women with obesity (36). In the current study, we did not observe an incremental value of maternal metabolites for the prediction of a healthy pregnancy outcome in addition to well-known clinical predictors. As it seems likely that all women with overweight and obesity show subclinical metabolic disruptions during pregnancy, the discriminative power of metabolites for a healthy pregnancy outcome in this high risk population may be limited (4, 5). Thus, paternal characteristics and maternal early-pregnancy metabolite concentrations do not seem to have an incremental value in the prediction of healthy pregnancy outcomes in women with overweight or obesity.

Our findings, together with findings from previous studies, indicate that a substantial proportion of women with overweight or obesity during pregnancy, do not experience complications. This underlines that stratifying women as high risk only based on having

overweight or obesity may be too simplistic. Our developed model was not sufficient to accurately predict a healthy pregnancy outcome. Especially when a prediction model should identify women who can be excluded from intensified antenatal care, a high sensitivity to eliminate possibilities of false-negatives is important. Results from our study, together with results from previous studies, show that highly accurate risk stratification cannot be achieved using common characteristics and less established paternal and maternal metabolomics characteristics. Studies identifying novel markers that improve prediction of healthy pregnancy outcomes in women with overweight and obesity are urgently needed.

Methodological considerations

The major strength of this study is the prospective data collection providing a large amount of high quality data on socio-demographic, lifestyle, physical and pregnancy-related characteristics. Multiple factors were repeatedly available in early- and mid-pregnancy to enable identification of most valuable periods for prediction of a healthy pregnancy outcome. We selected an ethnic homogeneous population, only including Dutch women, to eliminate potential statistical noise or effect modification in predictor selection by ethnicity. This selected population may have led to reduced statistical power and could have affected generalizability. Although model performance was largely similar in women with a non-Dutch ethnicity within our cohort, future models should be developed and validated to meet generalizability to multi-ethnic populations. Although we used validated questionnaires to assess socio-demographic and lifestyle characteristics, questionnaires may still induce information bias.

CONCLUSIONS

Common maternal socio-demographic, lifestyle, physical and pregnancy-related characteristics in the first half of pregnancy can moderately predict a healthy pregnancy outcome in women with overweight or obesity. Paternal characteristics and maternal early-pregnancy metabolites do not improve prediction of a healthy pregnancy outcome. Future studies need to identify novel markers to achieve accurate prediction of healthy pregnancies in overweight and obese pregnant women to enable tailored antenatal care within this high-risk population.

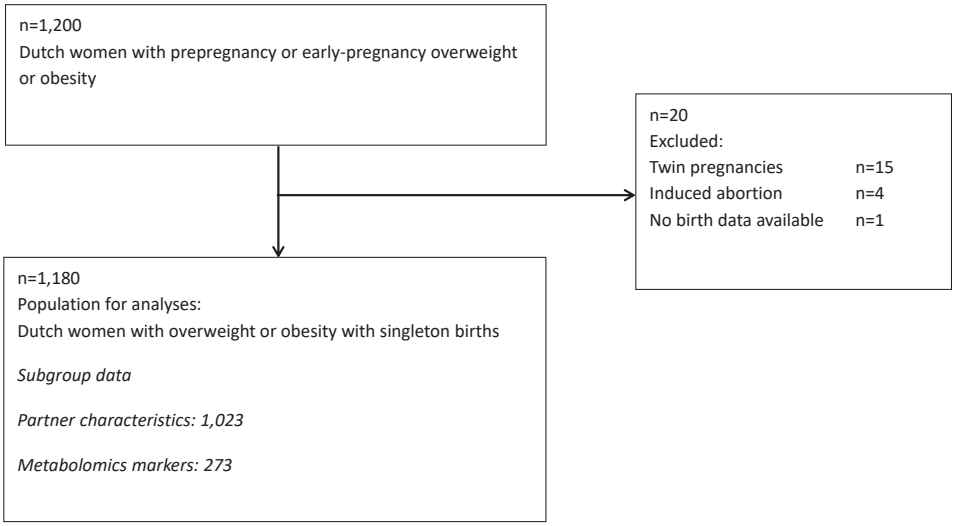
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SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIGURE S4.2.1. Flow chart of study participants

SUPPLEMENTARY TABLE S4.2.1. Characteristics for subgroups for the populations

	Total group (n=1,180)	Paternal characteristics (n=1023)	Metabolomics (n=273)
Early-pregnancy characteristics			
Gestational age at measurement, median (95% range), weeks	12.9 (9.6 to 17.3)	12.9 (9.6 to 17.2)	12.5 (9.0 to 16.8)
Age, mean (SD), years	31.1 (4.4)	31.5 (21.8 to 38.7)	31.4 (4.0)
Prepregnancy Body Mass Index, median (95% range), kg/m^2	26.6 (23.0 to 38.1)	26.6 (23.0 to 38.4)	26.8 (23.2 to 37.4)
Prepregnancy obesity, n yes (%)	228 (23)	194 (22)	50 (23)
Early-pregnancy Body Mass Index, median (95% range), kg/m^2	27.6 (25.0 to 38.6)	27.5 (25.0 to 39.0)	28.8 (25.0 to 37.2)
Early-pregnancy obesity, n yes (%)	297 (28)	257 (28)	78 (29)
Parity, n multiparous (%)	518 (44)	428 (42)	119 (44)
Education, n higher education (%)	522 (45)	471 (47)	133 (49)
Income, n >2200 euro (%)	714 (71)	662 (74)	195 (77)
Marital status, n married or living together (%)	1070 (94)	959 (97)	253 (97)
History of obstetric complications, n no (%)	392 (97)	326 (98)	86 (97)
Smoking, n no (%)	779 (72)	686 (73)	181 (75)
Folic acid supplementation, n yes (%)	837 (86)	744 (88)	199 (90)
Fruit consumption, n ≥200grams/day, n yes (%)	638 (54)	559 (62)	149 (63)
Vegetable consumption, n ≥250grams/day, n yes (%)	67 (6)	58 (6)	12 (5)
Energy intake, mean (SD), kcal/day	2090 (508)	2083 (503)	2071 (480)
Carbohydrate intake, mean (SD), g/day	256 (75)	255 (74)	250 (65)
Fat intake, mean (SD), g/day	84 (24)	83 (24)	84 (24)
Protein intake, mean (SD), g/day	77 (19)	77 (19)	78 (19)
Systolic blood pressure, mean (SD), mmHg	123 (13)	123 (13)	125 (14)
Diastolic blood pressure, mean (SD), mmHg	73.1 (9.9)	73 (10)	75 (11)
Glucose, mean (SD), mmol/L	4.5 (0.9)	4.5 (0.8)	4.5 (0.9)
HDL-concentrations, mean (SD), mmol/L	1.7 (0.3)	1.7 (0.3)	1.7 (0.3)
Triglycerides concentrations, median (95% range), mmol/L	1.4 (0.7 to 2.8)	1.4 (0.7 to 2.7)	1.3 (0.8 to 2.6)
CRP concentrations, median (95% range), mg/L	4.9 (0.9 to 9.6)	4.9 (0.9 to 9.6)	5.3 (0.9 to 9.7)
Placental growth factor, median (95% range), mom	0.99 (0.42 to 4.21)	0.97 (0.42 to 4.22)	0.85 (0.38 to 3.18)
sFlt-1, median, (95% range), mom	1.00 (0.41 to 2.60)	0.99 (0.40 to 2.50)	1.02 (0.41 to 2.67)
Mid-pregnancy characteristics			
Gestational age at measurement, median (95% range), weeks	20.6 (18.7 to 23.3)	20.5 (18.7 to 23.3)	20.4 (18.9 to 22.8)
Mid-pregnancy weight, median (95% range), kg/m^2	84.0 (69.0 to 116.0)	84.0 (69.0 to 116.0)	84.6 (69.0 to 114.6)
Gestational weight gain, median (95% range), kg/week	0.29 (-0.19 to 0.71)	0.29 (-0.19 to 0.70)	0.29 (-0.15 to 0.70)
Systolic blood pressure, mean (SD), mmHg	123 (12)	124 (12)	124 (13)
Diastolic blood pressure, mean (SD), mmHg	72 (10)	72 (10)	73 (11)
Vitamin D concentrations, median (95% range), nmol/L	60.1 (16.3 to 121.9)	61.3 (16.3 to 122.5)	61.6 (18.9 to 117.7)
Placental growth factor, median (95% range), mom	1.00 (0.39 to 3.15)	1.00 (0.38 to 3.22)	1.00 (0.46 to 3.55)
sFlt-1, median, (95% range), mom	1.00 (0.33 to 3.15)	0.98 (0.33 to 3.17)	1.01 (0.32 to 3.53)
Estimated fetal weight, mean (SD), SDS	0.01 (1.00)	0.01 (1.00)	-0.02 (0.92)

SUPPLEMENTARY TABLE S4.2.1. Continued

	Total group (n=1,180)	Paternal characteristics (n=1023)	Metabolomics (n=273)
Uterine artery resistance index, mean (SD), SDS	0.00 (1.00)	-0.01 (0.99)	0.02 (0.94)
Umbilical artery pulsatility index, mean (SD), SDS	0.00 (1.00)	-0.02 (1.00)	-0.05 (0.91)
Birth characteristics			
Sex, n female (%)	594 (51)	511 (50)	125 (46)
Gestational age at birth, median (95%), weeks	40.3 (35.5 to 42.3)	40.3 (35.6 to 42.3)	40.4 (36.1 to 42.4)
Birthweight, mean (SD), grams	3534 (591)	3545 (583)	3588 (576)
Healthy pregnancy outcome, n yes (%)	293 (25)	244 (24)	70 (26)

SUPPLEMENTARY TABLE S4.2.2. Sensitivity analyses for selected outcomes

	No adverse outcome of pregnancy								
			Sensitivity at specificity (%)			Positive likelihood ratio		
Models	**Variables selected**	**AUC (95% CI)**	**70%**	**80%**	**90%**	**70%**	**80%**	**90%**
Only including women with early-pregnancy overweight or obesity (n=1,027)	Similar to full maternal model*	0.64 (0.60 to 0.68)	49	37	22	1.6	1.9	2.2
Complete cases for pregnancy outcomes (n=948)	Similar to full maternal model	0.60 (0.54 to 0.67)	38	31	12	1.3	1.6	1.2
Excluding fetal deaths (n=1173)	Similar to full maternal model	0.65 (0.61 to 0.68)	48	37	23	1.6	1.9	2.3
Excluding long-term outcomes of pregnancy (n=1180)	Age + early-pregnancy BMI + vegetable consumption + protein consumption + early-pregnancy systolic blood pressure + glucose concentrations + early-pregnancy PlGF + gestational weight gain + mid-pregnancy diastolic blood pressure + mid-pregnancy PlGF concentrations + mid-pregnancy sFlt-1 concentrations + estimated fetal weight	0.67 (0.64 to 0.70)	52	39	23	1.7	2.0	2.3

Models were adjusted for gestational age at early-pregnancy measurement to enable interpretation of effect estimates of biomarkers not standardized.
**The full maternal model included maternal age, marital status, parity, BMI, mid-pregnancy gestational weight gain, mid-pregnancy systolic blood pressure and mid-pregnancy estimated fetal weight. The full maternal model had an AUC of 0.65 (95% confidence interval 0.61 to 0.68).*

4.3

Prediction of common birth complications in preconception and early-pregnancy within the general population

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ABSTRACT

Background: Suboptimal maternal health already from preconception onwards is strongly related to an increased risk of birth complications. Screening tools for accurate identification of women at risk of birth complications from preconception onwards are urgently needed.

Objective: To develop a prediction model for birth complications using maternal preconception socio-demographic, lifestyle, medical history and early-pregnancy clinical characteristics in a general multi-ethnic population.

Study design: In a population-based prospective cohort study among 8,340 women, we obtained information on 33 maternal characteristics in preconception and early-pregnancy (<21 weeks gestation). Preterm birth was <37 weeks gestation. Small-for-gestational-age (SGA) and large-for-gestational-age (LGA) at birth were gestational-age-adjusted birthweight in the lowest or highest decile, respectively. Because of their co-occurrence, preterm birth and SGA were a composite outcome.

Results: The basic preconception model consisted of age, ethnicity, parity, body mass index and smoking. This basic preconception model had an area under the receiver operating characteristics curve (AUC) of 0.63 (95% CI 0.61 to 0.65) and 0.64 (95% CI 0.62 to 0.66) for preterm birth/SGA and LGA, respectively. Addition of maternal socio-demographic, lifestyle, medical history and early-pregnancy clinical characteristics significantly improved models. The full model for prediction of preterm birth/SGA had an AUC of 0.66 (95% CI 0.64 to 0.67) with a sensitivity of 22% at 90% specificity. The full model for prediction of LGA had an AUC of 0.67 (95% CI 0.65 to 0.69) with sensitivity of 28% at 90% specificity. Models had a reasonable level of calibration within different socio-economic subsets of our population. Predictive performance was good for various secondary maternal, delivery and neonatal complications.

Conclusion: Maternal preconception and early-pregnancy characteristics, which can easily be obtained in clinical practice, aid in prediction of birth complications within the general population. After external validation, the developed models may serve as a screening tool for early-identification of women at risk of birth complications.

INTRODUCTION

Preterm birth, small-for-gestational-age at birth (SGA) and large-for gestational-age at birth (LGA) are among the most common birth complications, affecting up to 25% of pregnancies (1-3). These birth complications are not only strongly related to maternal and neonatal morbidity and mortality, but also increase the risk of adverse health outcomes in later life (4, 5).

Maternal health and lifestyle during pregnancy are important determinants for fetal development and birth outcomes (6, 7). Preconception and early-pregnancy are crucial periods for the negative effects of adverse maternal lifestyle and physical characteristics on fetal development and birth outcomes (5, 7, 8). Based on these observations, interventions are being developed to optimize maternal health and lifestyle from preconception onwards, but identification women who are likely to benefit most from interventions remains a major challenge (6, 9). Current lifestyle intervention trials often performed risk selection during pregnancy when its often too late for intervention or trials performed selection based on a single risk factor, such as maternal obesity or smoking (10, 11). To increase the impact of integrated lifestyle interventions on birth outcomes, screening tools are needed to enable risk stratification of women already from preconception onwards on a population level.

Therefore, in a population-based prospective cohort study, we developed prediction models for common birth complications using maternal preconception socio-demographic, lifestyle, medical history characteristics and early-pregnancy clinical characteristics and translated these models into a clinical prediction tool. Additionally, we examined whether paternal characteristics improved model performance and examined performance of developed prediction models on maternal, delivery and neonatal complications.

METHODS

Study population

We used data from the Generation R Study, a population-based prospective cohort study in Rotterdam, the Netherlands (12). The study has been approved by the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from all parents. For this study, women were included if they were enrolled in the study in first half of pregnancy and had information available on ≥1 candidate predictor (**Supplementary Figure S4.3.1**). Women with non-

singleton pregnancies (n=94), non-live births (n=103) or missing information on birth outcomes were excluded (n=84), resulting in a population for analyses of 8,340 women. Information of 6,062 fathers was available.

Candidate predictor variables

We selected maternal preconception and early-pregnancy candidate predictors based on a literature search (**Supplementary Methods S4.3.1**). These variables were clustered according to clinical availability and timing of assessment.

Basic preconception characteristics: We obtained information on age, ethnicity, parity, weight just before pregnancy and smoking through questionnaires. Height was measured at study enrolment and pre-pregnancy body mass index (BMI**)** was calculated (12).

Socio-demographic characteristics: We obtained information on educational level, household income, marital status, occupational status and planning of pregnancy through questionnaires (12).

Lifestyle characteristics: Information on alcohol and caffeine consumption and multivitamin and folic acid supplementation before pregnancy was obtained through questionnaires (12). Information about fruit, vegetable and carbohydrate consumption per day and fatty fish consumption per week was from a semi-quantitative food-frequency questionnaire covering the previous three months (13). We assessed dietary carbohydrate quality by calculating mean dietary glycemic index (14). Information on psychological distress was a score >0.71 for the overall psychological symptoms scale on the Brief Symptom Inventory (15).

Medical history characteristics: Information on presence of preexisting chronic diseases (diabetes, cardiovascular, neurological, intestinal or autoimmune disease), history of obstetric complications (stillbirth, miscarriage, pre-eclampsia, gestational hypertensive disorders, gestational diabetes, cesarean section, low birthweight, macrosomia), and consanguinity with the planned biological father was obtained through questionnaires (12). Information on intrauterine fertilization or intracytoplasmic sperm injection to conceive conception was obtained from medical records (12).

Early-pregnancy clinical characteristics: We measured systolic and diastolic blood pressure at a median gestational age of 14.4 (95% range 10.9 to 22.1) weeks) (16). At a median gestational age of 13.2 (95% range 10.5 to 17.2) weeks, we measured hemoglobin (Hb), glucose, total cholesterol, HDL-cholesterol, triglyceride and ferritin concentrations in

venous blood samples (17). At a median gestational age of 20.4 (95% range 18.8 to 22.9) weeks, we measured 25-hydroxyvitamin D (vitamin D) concentrations and omega-3 fatty acids concentrations in venous blood samples (17, 18). Biomarkers were divided into a basic clinical characteristics cluster and a biomarkers cluster.

Paternal characteristics

We obtained information on paternal age, ethnicity, educational level, smoking and alcohol consumption by questionnaire. We measured height, weight, systolic and diastolic blood pressure and calculated BMI (12).

Outcomes

Primary outcomes: Information about sex, gestational age and weight at birth was obtained from medical records (12). Preterm birth was gestational age at birth <37 weeks. Gestational-age-and-sex-adjusted SDSs for weight at birth were constructed using North European growth standards as the reference growth curve (19). SGA and LGA at birth were the lowest and highest ten percentiles of gestational-age-and-sex-adjusted birthweight in the study cohort (19). We additionally tested predictive performance of the developed prediction model on the lowest and highest five percentiles of gestational-age-and-sex-adjusted birthweight. Appropriate size for gestational age at birth was the reference. Using the whole study population as a reference showed similar results (results not shown). Because of their co-occurrence, we combined preterm birth and SGA into a composite outcome to increase statistical power (20). As a sensitivity analysis, we assessed model performance for preterm birth and SGA separately.

Secondary outcomes: 1) maternal complications, including gestational hypertension and preeclampsia; 2) delivery complications, including fetal distress and caesarian section; 3) neonatal complications, including 5 minute Apgar score <7, low birthweight (<2500 grams) and macrosomia (>4000 grams). Information was obtained from medical records.

Statistical analyses

Linear variables were used continuously, non-linear variables as quintiles and categorical variables as common clinical categories (**Supplementary Table S4.3.1)**. Categorized predictors included a missing category to allow for missing values in clinical setting. Missing values in linear candidate predictors were imputed using multiple imputations (pooled results of 5 imputed datasets). Single imputation with the mean or median showed similar results. Within in clinical settings, the mean or median can be inserted in case of missing values. .

Model selection was done for preterm birth/SGA and LGA seperatly. We selected predictors for the models in stages using six multivariable logistic regression models, to evaluate whether accurate prediction of birth complications could already be achieved with a basic model or required a more complex model (**Supplementary Table S4.3.1).** The basic preconception characterstics did not require statistical testing prior to inclusion in the model, because of their well-known associations with birth complications and availability in clincial practice. We extended the basic preconception model by adding clusters of maternal candidate predictors ordered by socio-demographic, lifestyle, medical history, early-pregnancy basic clinical and biomarker characteristics. Based on the log-likelihood ratio, we assessed whether a cluster of variables improved the model and selected variables from this cluster using backward selection with stopping rule $p<0.20$ (21). We included variables of each cluster using an additive approach and tested clusters in order of above apparence. After variable selection, we obtained predicted values from regression models in one imputed dataset and assessed discriminative ability by calculating the Area Under the Receiver Operating Characteristic Curve (AUC), along with sensitivity at different specificities. Results of other imputed datasets were similar (results not shown). We compared AUCs of the basic preconception model with the final model, including selected maternal characteristics using DeLong tests (22). We adjusted effect estimates of the final model for overfitting with an uniform shrinkage factor, calculated using a heuristic formula, and fitted an intercept to linear predictors of shrinked effect estimates (23). We assessed calibration of final models for preterm birth/SGA and LGA unadjusted for overfitting by dividing our study population in three subsets varying in socio-economic status (**Supplementary Table S4.3.2**). We quantified miscalibration using calibration-in-the-large (24). For final models, we tested whether paternal candidate predictors improved model performances. This was a separate analysis, as we only had paternal characteristics available in a subgroup and in clinical practice circumstances may not allow for obtaining paternal characteristics.

We assessed clinical applicability of developed prediction models by: 1) constructing a risk calculator using shrinked effect estimates and calculating risks of a preterm or SGA newborn and LGA newborn for three women with specific combinations of risk factors; 2) calculating the number of women with a predicted probability of ≥20% for preterm birth/SGA or ≥14% for LGA or higher, who would have actually had a preterm birth/SGA and LGA newborn. Cut offs were the highest quartiles of predicted probabilities; and 3) examining performance of final prediction models on secondary maternal, delivery and neonatal complications.

The statistical analyses were performed using the Statistical Package of Social Sciences version 24.0 for Windows (SPSS Inc., Chicago, IL, USA), and R version 3.3.4 (R Foundation for Statistical Computing) (R Core Team 2015).

RESULTS

Study population

Table 4.3.1 shows the maternal and fetal characteristics according to birth outcomes. Of the 8,340 women included, 425 (5.0%) had a newborn born preterm and the mean (SD) birthweight was 3458 (353) grams.

Model selection and performance for preterm birth/SGA and LGA

For preterm birth/SGA, predictive performance of the basic preconception model was moderate (AUC 0.63 (95% Confidence Interval (CI) 0.61 to 0.65) with a sensitivity of 20% at 90% specificity) (**Table 4.3.2**). Maternal characteristics from each cluster improved prediction of the model. Household income, planning of pregnancy, occupational status, weekly alcohol consumption, weekly fatty fish consumption, daily caffeine consumption, presence of a chronic disease and early-pregnancy diastolic blood pressure, hemoglobin and vitamin D concentrations were additionally selected. Performance of the full prediction model for preterm birth/SGA was moderate (AUC of 0.66 (95% CI 0.64 to 0.67) with a sensitivity of 22% at a 90% specificity) and significantly improved in comparison to the basic preconception model. Model performance for preterm birth and SGA separately was slightly lower for preterm birth and slightly higher for SGA than for the composite outcome preterm birth/SGA (results not shown). Performance of the basic preconception model for birthweight within the lowest 5 percentile was moderate (AUC of 0.67 (95% CI 0.64 to 0.70) with a sensitivity of 34% at a 90% specificity), whereas performance of the full model was good (AUC of 0.70 (95% CI 0.68 to 0.73), with a sensitivity of 28% at a 90% specificity). The heuristic shrinkage factor was 0.77 for the final model for preterm birth/SGA (**Table 4.3.3**). The model had a reasonable level of calibration for preterm birth/SGA, with an indication that at higher probabilities the model may slightly overestimate cases of preterm birth/SGA in all three subsets of our population (**Figure 4.3.1**).

TABLE 4.3.1. Population characteristics according to birth outcomes (n total population=8340)

	No adverse birth outcome* (n=6333)	Preterm birth*(n=425)	Small-for-gestational-age* (n=834)	Large-for-gestational-age* (n=834)
Maternal preconception characteristics				
Age, mean (SD), years	29.7 (5.2)	29.3 (5.5)	28.7 (5.7)	30.9 (4.7)
Ethnicity				
Dutch or European, n (%)	3475 (54.9)	204 (48.4)	362 (43.4)	536 (64.3)
Surinamese, n (%)	509 (8.0)	53 (12.5)	137 (16.4)	36 (4.3)
Turkish, n (%)	571 (9.0)	39 (9.2)	66 (7.9)	60 (7.2)
Moroccan, n (%)	441 (7.0)	19 (4.5)	33 (4.0)	53 (6.4)
Cape Verdean or Dutch Antilles, n (%)	433 (6.8)	41 (9.6)	104 (12.5)	38 (4.6)
Other, n (%)	599 (9.5)	44 (10.3)	77 (9.2)	76 (9.1)
Body Mass Index, median (95%), kg/m^2	22.6 (18.0 to 34.9)	22.8 (17.7 to 36.8)	21.9 (17.3 to 34.9)	23.8 (19.1 to 38.8)
Parity, n nulliparous (%)	3474 (54.9)	280 (65.9)	569 (68.2)	228 (40.5)
Education, n higher education (%)	2458 (38.8)	138 (32.5)	255 (30.6)	404 (48.4)
Smoking, n yes (%)	1477 (23.3)	118 (27.8)	516 (33.1)	143 (17.2)
Income, n high (%)	2694 (42.6)	142 (33.4)	248 (29.7)	431 (51.7)
Marital status, n no partner/ stable relation (%)	789 (12.5)	84 (19.8)	176 (21.1)	65 (7.8)
Occupational status, memployed (%)	3405 (53.8)	188 (44.2)	378 (45.3)	496 (59.5)
Planning of pregnancy, n no (%)	1574 (24.9)	118 (27.8)	277 (33.2)	162 (19.4)
Presence of any chronic disease, n no (%)	1922 (30.3)	157 (36.9)	251 (30.1)	236 (28.3)
History of any obstetric complication, n yes (%)†	1907 (30.1)	117 (27.5)	265 (31.8)	251 (30.1)
IVF/ICSI pregnancy, n yes (%)	45 (0.7)	4 (0.9)	5 (0.6)	6 (0.7)
Consanguinity with biological father, n yes (%)	225 (3.6)	13 (3.1)	21 (2.5)	30 (3.6)
Alcohol consumption, n >1x/ week (%)	480 (7.5)	27 (6.3)	42 (5.1)	87 (10.5)
Vegetable consumption, n <250grams/day (%)	4185 (66.1)	269 (63.3)	532 (63.8)	566 (67.9)
Fatty fish consumption, n <1x/ week, (%)	91 (1.4)	8 (1.9)	8 (1.0)	10 (1.2)
Fruit consumption, n <200grams/day (%)	2970 (46.9)	192 (45.2)	419 (50.2)	390 (46.8)

TABLE 4.3.1. Continued

	No adverse birth outcome* (n=6333)	Preterm birth*(n=425)	Small-for-gestational-age* (n=834)	Large-for-gestational-age* (n=834)
Daily dietary glycemic index, mean (SD)	58.2 (3.4)	58.6 (3.8)	58.5 (3.5)	57.9 (3.2)
Caffeine consumption, median (95%), units/day‡	1.5 (0.0 to 6.0)	1.0 (0.0 to 6.1)	1.5 (0.0 to 6.0)	1.5 (0.0 to 6.0)
Multivitamin supplementation, n no (%)	5237 (82.7)	243 (57.2)	171 (20.5)	152 (18.2)
Folic acid supplementation, n no (%)	1343 (21.2)	105 (24.7)	220 (26.4)	140 (16.8)
Psychological distress, n yes (%)	510 (8.1)	43 (10.1)	83 (10.0)	57 (6.8)
Mean systolic blood pressure, mean (SD), mmHg	115 (12)	117 (14)	115 (12)	117 (13)
Mean diastolic blood pressure, mean (SD), mmHg	68 (9)	70 (11)	68 (10)	68 (10)
Hb concentrations, mean (SD), mmol/L	7.5 (0.6)	7.5 (0.7)	7.5 (0.6)	7.6 (0.6)
Glucose, mean (SD), mmol/L	4.4 (0.8)	4.4 (0.9)	4.3 (0.8)	4.6 (0.9)
Total cholesterol concentrations, mean (SD), mmol/L	4.81 (0.85)	4.87 (0.85)	4.76 (0.90)	4.82 (0.89)
HDL-concentrations, mean (SD), mmol/L	1.77 (0.35)	1.77 (0.35)	1.77 (0.34)	1.73 (0.35)
Triglycerides concentrations, mean (SD), mmol/L	1.36 (0.52)	1.42 (0.53)	1.33 (0.51)	1.45 (0.56)
Ferritin concentrations, median (95%), ug/L	52.9 (9.8 to 210.1)	60.5 (10.4 to 257.4)	49.2 (9.7 to 198.5)	48.9 (11.2 to 191.5)
Omega-3 fatty acids concentrations, mean (SD), mg/L	104.1 (27.7)	102.0 (27.8)	99.4 (26.1)	106.2 (26.1)
Vitamin D concentrations, median (95%), nmol/L	47.5 (7.0 to 122.2)	38.2 (7.4 to 120.4)	37.5 (6.3 to 111.4)	31.7 (7.9 to 121.7)
Birth outcomes				
Sex, n female (%)	3168 (50.0)	209 (49.2)	396 (47.5)	410 (49.2)
Gestational age at birth, median (95%), weeks	40.1 (37.4 to 42.4)	35.7 (27.4 to 36.9)	39.9 (34.8 to 42.0)	40.3 (35.8 to 42.4)
Birthweight, mean (SD), grams	3458 (353)	2349 (625)	2720 (379)	4270 (382)

Abbreviations: Hb: Hemoglobin

**newborns with two birth complications (both preterm and small-, or large-for-gestational age) were included in both categories of adverse outcomes*

†History of obstetric complications includes obstetric complications in previous pregnancies (stillbirth, miscarriage, pre-eclampsia, gestational hypertensive disorders, gestational diabetes, caesarian section, low birthweight, macrosomia)

‡1 unit of caffeine consumption represents the equivalent of 1 cup of coffee (90 mg caffeine)

For prediction of LGA, performance of the basic preconception model was moderate (AUC 0.64 (95% CI 0.62 to 0.66) with a sensitivity of 22% at a 90% specificity). Maternal characteristics from each cluster improved prediction of the model. Educational level, household income, daily vegetable consumption, multivitamin supplementation, presence of a chronic disease and early-pregnancy systolic and diastolic blood pressure, hemoglobin, random glucose, triglycerides and ferritin concentrations were additionally selected. Performance of the full model for LGA was moderate (AUC of 0.67 (95% CI 0.66 to 0.69), with a sensitivity of 26% at 90% specificity) and significantly improved in comparison to the basic preconception model (**Table 4.3.2**). Model performance of the basic preconception model for birthweight within the highest 5 percentile was moderate (AUC of 0.67 (95% CI 0.64 to 0.70), with a sensitivity of 34% at a 90% specificity), whereas performance of the full model was good (AUC of 0.70 (95% CI 0.67 to 0.73) with a sensitivity of 31% at a 90% specificity). The heuristic shrinkage factor was 0.76 for LGA (**Table 4.3.3**)**.** The model may underestimate cases of LGA at higher probabilities in women of non-Dutch or European ethnicity and may overestimate cases of LGA at higher probabilities in a population consisting of lower educated women (**Figure 4.3.1**).

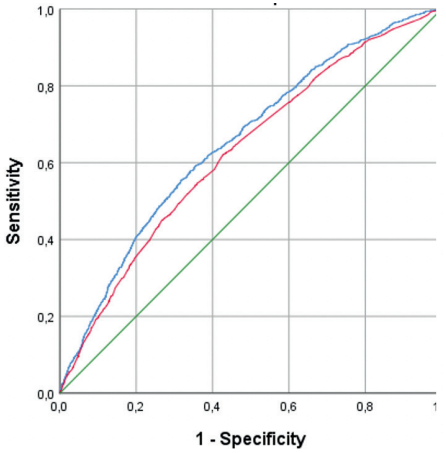
For preterm/SGA was in subset 1 the area under the curve (AUC) 0.65, the intercept -0.04 and the slope 0.98 (**Figure 4.3.1a**), in subset 2 was the AUC 0.66, the intercept 0.03 and the slope 1.04 (**Figure 4.3.1b**) and in subset 3 was the AUC 0.64, the intercept -0.03 and the slope 0.90 (**Figure 4.3.1c**). For LGA was in subset 1 the AUC 0.68, the intercept 0.22 and the slope 1.11 (**Figure 4.3.1d**), in subset 2 was the AUC 0.65, the intercept -0.18 and the slope 0.89 (**Figure 4.3.1e**) and in subset 3 was the AUC 0.68, the intercept -0.16 and the slope 0.94 (**Figure 4.3.1f**).

Paternal analyses

Paternal baseline characteristics are shown in **Supplementary Table S4.3.3**. Paternal characteristics additionally selected for preterm birth/SGA included ethnicity, educational level and BMI, but characteristics did not improve model performance (AUC of 0.66 (95% CI 0.64 to 0.67)) (**Table 4.3.2**). Paternal characteristics additionally selected for LGA included ethnicity, alcohol consumption and diastolic blood pressure and slightly improved model performance (AUC of 0.69 (95% CI 0.67 to 0.70)).

TABLE 4.3.2. Screening performance for preterm birth/small-for-gestational-age at birth and large-for-gestational-age at birth based on maternal characteristics

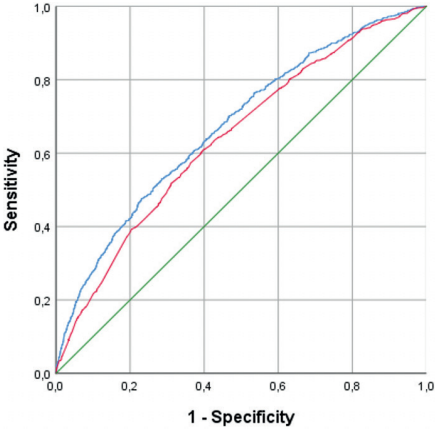
2a. Models for Preterm birth/Small-for-gestational-age



Models	Variables included per model	AUC (95% CI)	Sensitivity at specificity (%)			p-value*
			70%	80%	90%	
Basic preconception (red line)	Age + ethnicity + parity + BMI + smoking	0.63 (0.61 to 0.65)	48	40	20	
Socio-demographic	+ household income + planning of pregnancy + occupational status	0.64 (0.62 to 0.66)	50	37	22	
Lifestyle	+ alcohol consumption + fatty fish consumption + caffeine consumption	0.64 (0.63 to 0.66)	50	38	22	
Medical history	+ presence of chronic disease	0.64 (0.63 to 0.66)	51	38	22	
Early-pregnancy basic clinical	+ diastolic blood pressure + haemoglobin concentrations	0.65 (0.63 to 0.66)	53	38	22	
Final, preterm birth/ SGA-model (blue line)	+ vitamin D concentrations	0.66 (0.64 to 0.67)	54	40	22	<0.01
Paternal model	*+ paternal ethnicity + paternal educational level + paternal BMI*	*0.66 (0.64 to 0.67)*	*53*	*41*	23	

TABLE 4.3.2. Continued

2b. Models for Large-for-Gestational age



Models	Variables included per model	AUC (95% CI)	Sensitivity at specificity (%)			p-value*
			70%	80%	90%	
Basic preconception (red line)	Age + ethnicity + parity + BMI + smoking	0.64 (0.62 to 0.66)	50	39	22	
Socio-demographic	+ educational level + household income	0.65 (0.63 to 0.67)	53	38	25	
Lifestyle	+ vegetable consumption + multivitamin supplementation	0.65 (0.63 to 0.67)	53	40	24	
Medical history	+ presence of chronic disease	0.65 (0.63 to 0.67)	51	39	24	
Early-pregnancy basic clinical	+ systolic blood pressure + diastolic blood pressure + hemoglobin concentrations + glucose concentrations	0.66 (0.64 to 0.68)	54	40	24	
Final, LGA-model (blue line)	+ triglycerides concentrations + ferritin concentrations	0.67 (0.66 to 0.69)	54	41	26	<0.01
Paternal model	*+ paternal ethnicity + paternal alcohol consumption + paternal diastolic blood pressure*	*0.69 (0.67 to 0.70)*	57	44	28	

Abbreviations: AUC: area under the Receiver Operating Curve, CI: confidence interval, SGA: small-for-gestational-age at birth, BMI: Body Mass Index

**p-values are obtained using DeLong's test for comparison of the AUC of the full model with the AUC of the basic preconception model*

TABLE 4.3.3. Effect estimates in the final models for preterm birth/small-for-gestational-age at birth and for large-for-gestational-age at birth

		Preterm birth/SGA		LGA	
Variable	**Categories**	**Odds Ratio (original)**	**Odds Ratio (shrunk)**	**Odds Ratio (original)**	**Odds Ratio (shrunk)**
Intercept		0.04	0.07	0.10	0.11
Age	<25	0.93	0.95	0.79	0.84
	25-35	*Reference*	*Reference*	*Reference*	*Reference*
	>35	1.16	1.12	0.83	0.88
Ethnicity	Dutch or European	*Reference*	*Reference*	*Reference*	*Reference*
	Surinamese	1.65	1.44	0.46	0.57
	Turkish	0.86	0.89	0.65	0.73
	Moroccan	0.56	0.65	0.64	0.73
	Cape Verdian or Dutch Antilles	1.51	1.35	0.62	0.71
	Other	0.95	0.96	0.77	0.83
	Missing	1.03	1.02	0.63	0.72
Prepregnancy BMI	<25 kg/m2	*Reference*	*Reference*	*Reference*	*Reference*
	25.0-30.0 kg/m^2	0.83	0.87	1.61	1.41
	30.1-35.0 kg/m^2	0.76	0.81	2.09	1.71
	>35.0 kg/m^2	0.77	0.83	3.48	2.46
	Missing	1.05	1.04	1.44	1.30
Parity	Nulliparity	1.89	1.59	0.57	0.67
	Multiparous	*Reference*	*Reference*	*Reference*	*Reference*
	Missing	1.50	1.35	1.15	1.11
Smoking status	No	*Reference*	*Reference*	*Reference*	*Reference*
	Yes	1.32	1.23	0.76	0.82
	Missing	0.78	0.83	0.97	0.98
Educational level	Low			1.30	1.21
	High			Reference	Reference
	Missing			0.77	0.82
Household income	Low	1.27	1.19	0.81	0.86
	High	*Reference*	*Reference*	*Reference*	*Reference*
	Missing	1.37	1.26	0.91	0.93
Occupational status	Currently employed	*Reference*	*Reference*		
	Applying for a job	0.81	0.86		
	Unemployed and not applying for a job	1.16	1.12		
	Missing	1.28	1.20		

TABLE 4.3.3. Continued

		Preterm birth/SGA		LGA	
Variable	**Categories**	**Odds Ratio (original)**	**Odds Ratio (shrunk)**	**Odds Ratio (original)**	**Odds Ratio (shrunk)**
Pregnancy planning	Planned pregnancy	*Reference*	Reference		
	Unplanned pregnancy	1.11	1.08		
	Missing	1.06	1.05		
Alcohol consumption	Never or < 1 drink/week	*Reference*	*Reference*		
	>1 drinks/week	0.87	0.90		
	Missing	1.07	1.05		
Vegetable intake	<250 grams/day			0.69	0.77
	≥250 grams/day			*Reference*	*Reference*
	Missing			0.65	0.73
Weekly fatty fish consumption	<1x/week	1.15	1.11		
	1-2x/week	*Reference*	*Reference*		
	>2x/week	2.59	2.01		
	Missing	1.05	1.04		
Daily caffeine consumption	*<2/day*	Reference	Reference		
	≥2/day	1.14	1.10		
	Missing	0.86	0.90		
Multivitamin supplementation	Yes			*Reference*	*Reference*
	No			1.26	1.18
	Missing			1.27	1.19
History of a chronic disease	No	*Reference*	*Reference*	*Reference*	*Reference*
	Yes	1.13	1.10	0.85	0.89
	Missing	1.37	1.26	0.86	0.90
Systolic blood pressure	Per 10 mmHg increase			1.08	1.06
Diastolic blood pressure	Per 10 mmHg increase	1.10	1.07	0.92	0.94
Hb concentrations	First quintile (3.9 to 7.0 mmol/l)	1.17	1.12	1.15	1.11
	Second quintile (7.1 to 7.4 mmol/l)	1.10	1.07	0.97	0.98
	Third quintile (7.5 to 7.6 mmol/l)	*Reference*	*Reference*	*Reference*	*Reference*
	Fourth quintile (7.7 to 8.0 mmol/l)	1.15	1.11	1.25	1.18
	Fifth quintile (8.10 to 11.30 mmol/l)	1.33	1.23	0.92	0.94
	Missing	1.25	1.17	1.09	1.06
Random glucose concentrations	Per mmol/L increase			1.16	1.12

TABLE 4.3.3. Continued

		Preterm birth/SGA		LGA	
Variable	**Categories**	**Odds Ratio (original)**	**Odds Ratio (shrunk)**	**Odds Ratio (original)**	**Odds Ratio (shrunk)**
Triglyceride concentrations	Per mmol/L increase			1.18	1.13
Ferritine concentrations	First quintile (1.5 to 26.4 ug/l)			1.18	1.13
	Second quintile (26.4 to 42.5 ug/l)			0.87	0.91
	Third quintile (42.5 to 62.8 ug/l)			Reference	Reference
	Fourth quintile (62.8 to 95.8 ug/l)			0.69	0.77
	Fifth quintile (95.9 to 390.4 ug/l)			0.86	0.89
	Missing			0.87	0.91
Vitamin D concentrations	Per 10 nmol/l increase	0.94	0.96		

Clinical applicability of maternal prediction models

Risks of preterm birth/SGA or LGA for three women with different risk profiles are shown in **Figure 4.3.2** and were calculated using the risk calculator as screening tool (**Supplementary Material, Excel sheet 1**). For a woman with a healthy risk profile the risk of delivering a preterm/SGA and LGA newborn was 5% and 14%, respectively. For a woman with an unhealthy risk profile the risk was 56% and 17% for preterm/SGA and LGA newborn, respectively. **Figure 4.3.3** shows that of women with a risk >20% for having a preterm birth/SGA newborn, 27% would have actually had a preterm birth/SGA newborn. Of women with a risk of >14% for having a LGA newborn, 20% would have actually had a LGA newborn.

Secondary model performance of the final model for preterm birth/SGA was good for gestational hypertension, preeclampsia, fetal distress, low Apgar score, low birthweight and macrosomia with AUCs ranging from 0.68 (95% CI 0.66 to 0.70) to 0.80 (95% CI 0.78 to 0.83) and slightly lower for caesarean delivering was slightly lower (**Table 4.3.4**). Secondary model performance of the full model for LGA was largely similar (**Table 4.3.4**). Model performance of the basic preconception model for the prediction of secondary outcomes was poorer in comparison to the full maternal prediction models for all secondary outcomes (**Supplementary Table S4.3.4**).

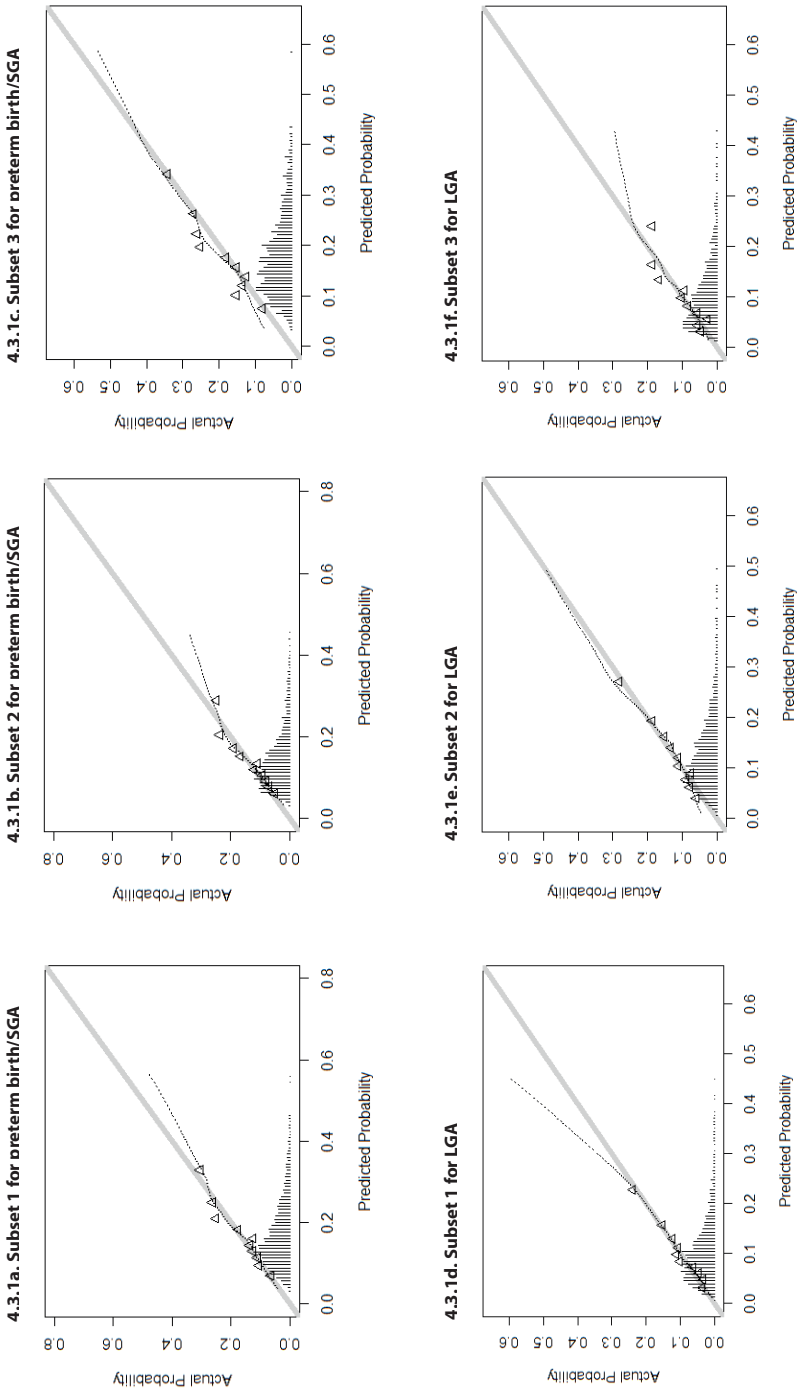


FIGURE 4.3.1. Actual probabilities of preterm birth/SGA and LGA compared with predicted probabilities of preterm birth/SGA and LGA within subsets of the study population based on the final model

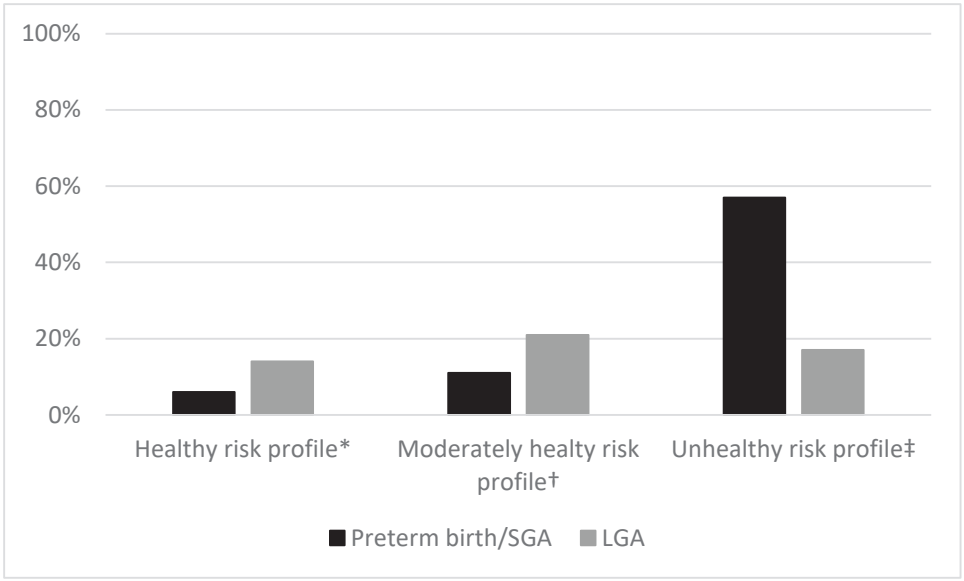


FIGURE 4.3.2. Predicted risks for women at low-, normal- and high risk of an adverse birth outcome obtained from the risk calculator.

*Values are percentages for the risk of having an adverse birth outcome based on the risk calculator developed from the final prediction models for preterm birth/SGA and LGA. *Healthy risk profile represents a women with age 27, Turkish, BMI 20 kg/m², multiparous, non-smoker, high educated, high household income, employed, planned pregnancy, alcohol consumption none, vegetable intake 300 g/day, fatty fish consumption 1-2x/week, caffeine consumption 1 cup/day, multivitamin supplementation yes, chronic disease no, systolic blood pressure 110 mmHg, diastolic blood pressure 70 mmHg, Hb 7.5 mmol/L, glucose concentrations 3.0 mmol/L, triglyceride concentrations 2.0 mmol/L, ferritin concentrations 50 ug/L and vitamin D concentrations 100 nmol/L.*

†Moderately healthy risk profile represents a women with age 26, Dutch, BMI 27 kg/m², nulliparous, smoking no, high educated, high household income, employed, planned pregnancy, alcohol consumption 3 glasses/week, vegetable consumption 300 g/day, fatty fish consumption 1x/week, caffeine consumption none, multivitamin supplementation no, history of chronic disease yes, systolic blood pressure 120 mmHg, diastolic blood pressure 80 mmHg, Hb 7.2 mmol/L, glucose concentrations 4.0 mmol/L, triglyceride concentrations 3.0 mmol/L, ferritin concentrations 50 ug/L and vitamin D concentrations 60 nmol/L.

‡Unhealthy risk profile represents a women age 36, Surinamese, BMI 38 kg/m², nulliparous, smoking yes, low educated, low household income, unemployed and not applying for a job, unplanned pregnancy, alcohol consumption less than 1 drink/day, vegetable intake 100 g/day, fatty fish consumption >2x/week fatty fish, caffeine consumption 5 cups/day, multivitamin supplementation no, history of chronic disease yes, systolic blood pressure 140 mmHg, diastolic blood pressure 95 mmHg, Hb 10.0 mmol/L, glucose concentrations 7.0 mmol/L, triglyceride concentrations 4 mmol/L, ferritin concentrations 100 ug/L and vitamin D concentrations 10 nmol/L.

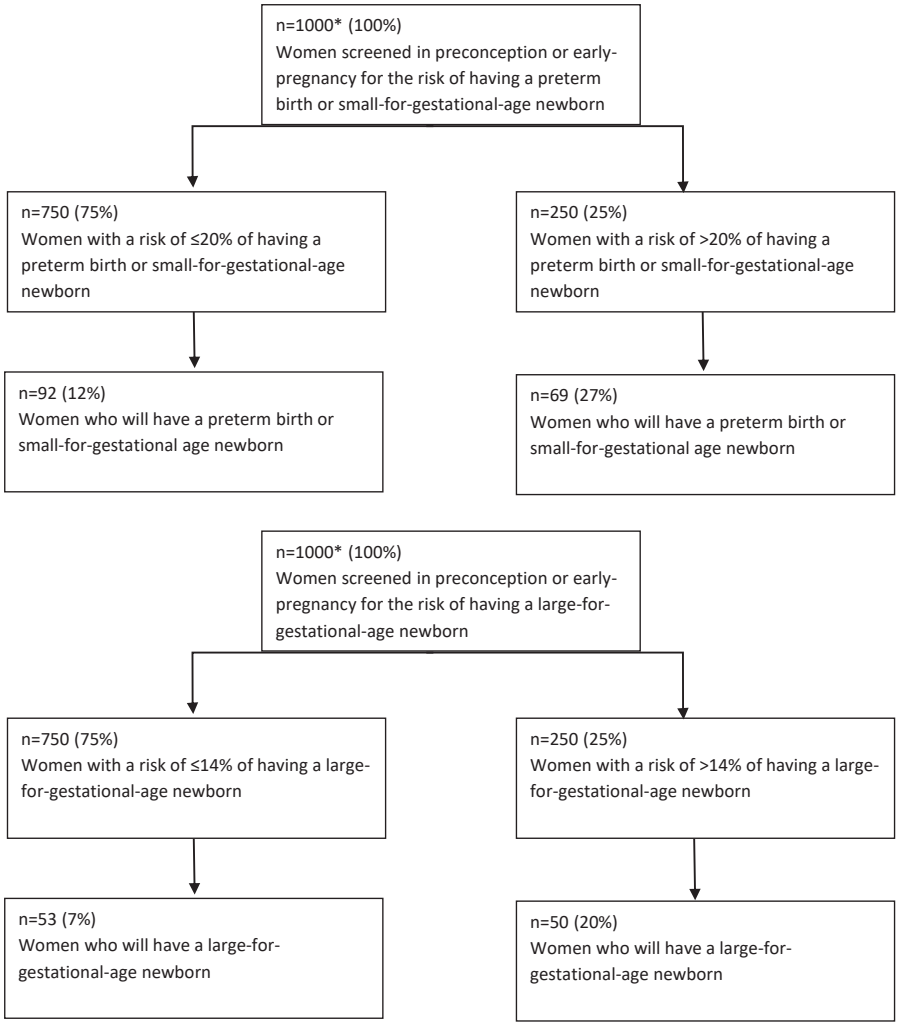


FIGURE 4.3.3. Frameworks of women with an increased risk of preterm birth/small-for-gestational age and Large-for-gestational-age newborns. *For interpretation of this framework, we choose a random number of 1000 women to illustrate how proportions of this framework translate to numbers within an actual population

TABLE 4.3.4. model performance for maternal, delivery and neonatal complications

Maternal pregnancy complications				
3a. Gestational hypertension				
Models	AUC (95% CI)	Sensitivity at specificity (%)		
		70%	80%	90%
Preterm birth/SGA-model*	0.80 (0.78 to 0.83)	75	63	45
LGA-model†	0.81 (0.79 to 0.83)	77	63	44

TABLE 4.3.4. Continued

Maternal pregnancy complications				
3b. Pre-eclampsia				
Models	AUC (95% CI)	Sensitivity at specificity (%)		
		70%	80%	90%
Preterm birth/SGA-model*	0.77 (0.74 to 0.80)	70	58	33
LGA-model†	0.77 (0.74 to 0.81)	72	59	40
Delivery complications				
3c. Fetal distress				
Models	AUC (95% CI)	Sensitivity at specificity (%)		
		70%	80%	90%
Preterm birth/SGA-model*	0.68 (0.66 to 0.70)	53	40	25
LGA-model†	0.68 (0.66 to 0.70)	55	41	23
3d. Caesarian section				
Models	AUC (95% CI)	Sensitivity at specificity (%)		
		70%	80%	90%
Preterm birth/SGA-model*	0.65 (0.64 to 0.67)	50	37	23
LGA-model†	0.66 (0.64 to 0.67)	51	39	24
Neonatal complications				
3e. Low Apgar				
Models	AUC (95% CI)	Sensitivity at specificity (%)		
		70%	80%	90%
Preterm birth/SGA-model*	0.70 (0.65 to 0.76)	60	50	36
LGA-model†	0.72 (0.66 to 0.77)	65	48	35
3e. Low birthweight				
Models	AUC (95% CI)	Sensitivity at specificity (%)		
		70%	80%	90%
Preterm birth/SGA-model*	0.70 (0.67 to 0.73)	59	45	29
LGA-model†	0.70 (0.67 to 0.72)	59	44	27
4e. Macrosomia				
Models	AUC (95% CI)	Sensitivity at specificity (%)		
		70%	80%	90%
Preterm birth/SGA-model*	0.71 (0.67 to 0.74)	61	50	34
LGA-model†	0.71 (0.67 to 0.75)	62	54	35

**Final model with variables selected on preterm birth/SGA include maternal age, ethnicity, parity, Body Mass Index, smoking, household income, planning of pregnancy, occupational status, weekly alcohol consumption, weekly fatty fish consumption, daily caffeine consumption, presence of chronic disease and maternal early-pregnancy diastolic blood pressure, hemoglobin concentrations and vitamin D concentrations.*

†Final model with variables selected on LGA include maternal age, ethnicity, parity, Body Mass Index, smoking, educational level, household income, daily vegetable consumption, presence of chronic disease, multivitamin supplementation and maternal early-pregnancy systolic blood pressure, diastolic blood pressure, hemoglobin concentrations, glucose concentrations, triglyceride concentrations and ferritin concentrations

DISCUSSION

In this population-based prospective cohort study, we observed that basic maternal characteristics in the preconception period can already predict preterm birth or SGA and LGA moderately. Addition of detailed maternal preconception and early-pregnancy characteristics led to a significant improvement of the developed prediction models. Developed prediction models based on preterm birth, SGA and LGA had a good performance for the prediction of secondary maternal, delivery and neonatal complications. Paternal characteristics did not strongly improve prediction of common birth complications.

Strengths and limitations

The major strength of this study is the prospective data collection providing a large amount of maternal high quality data. We considered clinical applicability in development of the prediction models by using a stepwise model estimation. The baseline response rate at birth was 61%, which may have led to a selected population, affecting generalizability. Although we corrected for overfitting to maintain adequate prediction in new populations and models had good calibration in subsets of our population with large in-between differences in socio-demographic characteristics, external validation of the prediction models is needed to assess generalizability to other populations. Although we used validated questionnaires to assess socio-demographic and lifestyle characteristics, questionnaires may still induce measurement error, recall and reporting bias. We only had maternal early-pregnancy non-fasting blood samples. Early-pregnancy biomarkers at least partly reflect maternal biomarkers in the preconception period, but whether these maternal characteristics can be applied in the preconception period for the prediction of birth complications remains to be assessed (25, 26).

Interpretation of main findings

For improvement of birth outcomes, increasing efforts are made to develop strategies for optimization of maternal health and lifestyle from preconception onwards (7, 27). To identify women who will most likely benefit from strategies, tools are urgently needed to enable screening for birth complications from preconception onwards (7).

A few previous studies developed models for the prediction of birth complications (10, 11, 28). These studies focused on prediction during pregnancy, but not in preconception and developed models in selected populations, such as nulliparous women or women with a low socio-economic status. A study among 5,606 nulliparous pregnant women

developed a prediction model for SGA using maternal birthweight, gestational weight gain, biomarkers and ultrasound characteristics at 20 weeks gestation and observed an AUC of 0.69 (10). In the same cohort, predictive performance for LGA using the same characteristics was similar (11). In a study among 263 deprived pregnant women, a model using socio-demographic characteristics for the prediction of the composite outcome preterm birth, low birthweight, intrauterine fetal demise, or neonatal death showed an AUC of 0.79 in training set, but an AUC of 0.63 in validation set (28).

We developed prediction models for preterm birth/SGA and LGA to provide a maternal risk profile already from preconception onwards. While previous studies mainly focused on risk prediction of adverse outcomes in women during pregnancy, identification of women at risk of birth complications during preconception is crucial to increase the impact of intervention strategies focused on optimizing birth outcomes. By translating these two prediction models into a risk calculator, our models can be used as a screening tool to calculate risks for the most common birth complications in women on a population level, already from preconception onwards. As preterm birth, SGA and LGA are major risk factors of maternal and neonatal mortality and morbidity, it is necessary to integrate multiple birth complications in maternal risk stratification. Importantly, we observed a good model performance of the developed prediction models for the detection of secondary maternal, delivery and neonatal complications and of newborns within the more extremes range of abnormal birthweight. This suggests that our developed prediction models are also sufficient for identification of women at increased risks of related pregnancy complications and not only for identification of women at risk of delivering newborns with a constitutionally smaller or higher birthweight. We developed models in a population-based cohort study using an extensive scope of maternal characteristics. We already observed a moderate screening performance for only a small group of maternal preconception characteristics, which can easily and routinely be obtained in clinical practice. Although significant, improvement of the prediction models was relatively small after the addition of more detailed maternal characteristics. This may demonstrate the importance of maternal age, ethnicity, prepregnancy BMI, parity and smoking in clinical risk prediction. However, as our study population was a relatively healthy population, the other more detailed maternal characteristics in our sample may also reflect a relatively healthy range. Possibly, the additional predictive value of maternal socio-demographic, lifestyle, medical history characteristics and early-pregnancy clinical characteristics is stronger at more extreme levels. Paternal characteristics did not strongly improve risk prediction in addition to maternal characteristics. Thus, our findings suggest that prediction of preterm birth, SGA and LGA, but also secondary maternal delivery and neonatal complications, can be performed using maternal characteristics which in clinical practice can already

be obtained in preconception and early-pregnancy. External validation of the model among diverse populations with a higher variability in maternal characteristics is needed to assess whether screening of common birth complications with detailed maternal characteristics, obtained already in the preconception period, leads to clinically relevant effects on screening performance.

When externally validated, our proposed screening tool can provide risk stratification on a population level to aid in the early-identification of women at risk of birth complications. These women could represent a population who may benefit most from lifestyle support for primary prevention and from intensified antenatal care for secondary prevention of birth complications. Accurate identification women at risk of common birth complications already from preconception onwards may enable tailored interventions to prevent neonatal morbidity and mortality and to improve long-term maternal and offspring health. Despite clear benefits of early-identification and subsequent intervention programs for women at increased risk of birth complications, screening also induces false positive cases and medicalization of pregnancies. Future randomized controlled trials need to assess benefits and harms of screening for birth complications. Decision curve analyses can be used to determine appropriate risk cut offs for referral and to assess utility of the prediction model in clinical settings.

CONCLUSIONS

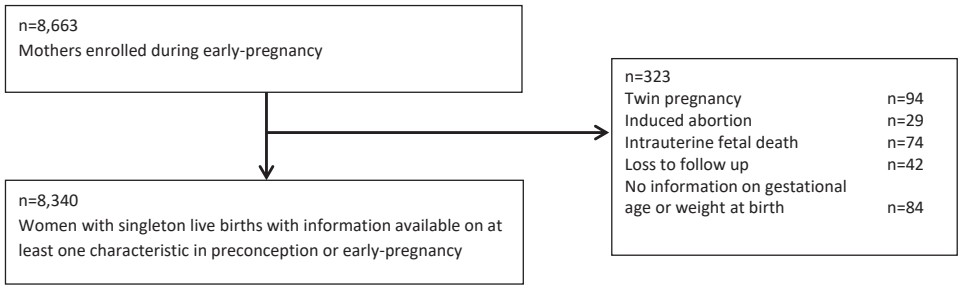
Maternal characteristics, which can easily be obtained in clinical practice already from preconception onwards, aid in the early prediction of common birth complications within the general population. After external model validation, the developed prediction models may serve as a screening tool for the early-identification on a population level of women at risk of birth complications in future intervention studies or clinical practice.

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SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIGURE S4.3.1. Flow chart of the study participants

Supplementary Methods S4.3.1. Literature search

((“pregnancy” [mesh]) or pregnancy or (preconception) or (prenatal))
AND (“risk factors” [Mesh] OR “Life Style” [mesh] or “health behavior” [mesh] and (risk factor*) or (life style) or (diet*))
AND (“premature birth” [Mesh] or “Infant, Low Birth Weight” [mesh] or “Pregnancy Complications” [mesh] or (pregnancy outcome) or (birth) or (growth restriction) OR (low birth weight) or macrosom*)
AND ((meta-analysis [Publication Type] OR review [Publication Type] OR systematic review [Publication Type]) AND (english[Language]) AND (("2010"[Date - Publication] : "3000"[Date - Publication])))

SUPPLEMENTARY TABLE S4.3.1. Categories of candidate predictors

	Variable	Categories
Basic preconception characteristics	Age	<25
		25-35
		>35
	Ethnicity	Dutch or European
		Surinamese
		Turkish
		Moroccan
		Cape Verdian or Dutch Antilles
		Other
		Missing
	Parity	Nulliparity
		Multiparous
		Missing
	Prepregnancy BMI	<25.0 kg/m^2
		25.0 to 29.9.0 kg/m^2
		30.0 to 35.0 kg/m^2
		>35.0 kg/m^2
		Missing
	Smoking	No
		Yes
		Missing
Socio-demographic characteristics	Education	Low
		High
		Missing
	Income	Low
		High
		Missing
	Marital status	Married/living together
		No partner/unstable relationship
		Missing
	Occupation	Currently employed
		Applying for a job
		Unemployed and not applying for a job
		Missing
	Pregnancy planning	Planned pregnancy
		Unplanned pregnancy
		Missing

SUPPLEMENTARY TABLE S4.3.1. Continued

	Variable	Categories
Lifestyle characteristics	Alcohol consumption	Never or ≤ 1 drink/week
		>1 drink/week
		Missing
	Multivitamin supplementation	Yes
		No
		Missing
	Folic acid supplementation	Yes
		No
		Missing
	Caffeine consumption	<2/day
		≥2/day
		Missing
	Fruit consumption	≥200 grams/day
		<200 grams/day
		Missing
	Vegetable consumption	≥250 grams/day
		<250 grams/day
		Missing
	Fatty fish consumption	<1x/week
		1-2x/week
		>2x/week
		Missing
	Dietary glycemic index	per 1 increase
	Stress	BSI score ≤0.71
		BSI score >0.71
		Missing

SUPPLEMENTARY TABLE S4.3.1. Continued

	Variable	Categories
Medical history characteristics	History of a chronic disease	No
		Yes
		Missing
	Obstetric complications in history	No
		Yes
		Missing
	IVF or ICSI pregnancy	No
		Yes
		Missing
	Consanguinity with planned biological father	No
		Yes
		Missing
Early-pregnancy basic clinical characteristics	Systolic blood pressure	per 10 mmHg increase
	Diastolic blood pressure	per 10 mmHg increase
	Hb	First quintile (3.9 to 7.0 mmol/l)
		Second quintile (7.1 to 7.4 mmol/l)
		Third quintile (7.5 to 7.6 mmol/l)
		Fourth quintile (7.7 to 8.0 mmol/l)
		Fifth quintile (8.10 to 11.30 mmol/l)
		Missing
	Random Glucose	per 1 mmol/L increase
Early-pregnancy biomarker characteristics	Total cholesterol	Per 1 mmol/L increase
	HDL	per 1 mmol/L increase
	Triglycerides	per 1 mmol/L increase
	Ferritine	First quintile (1.5 to 26.4 ug/l)
		Second quintile (26.4 to 42.5 ug/l)
		Third quintile (42.5 to 62.8 ug/l)
		Fourth quintile (62.8 to 95.8 ug/l)
		Fifth quintile (95.9 to 390.4 ug/l)
		Missing
	Vitamin D	per 10 nmol/l increase
	Omega-3 fatty acids	per 10 mg/l increase

SUPPLEMENTARY TABLE S4.3.1. Continued

	Variable	Categories
Paternal characteristics	Age	<25
		25-35
		>35
	Ethnicity	Dutch or European
		Surinamese
		Turkish
		Moroccan
		Cape Verdian or Dutch Antilles
		Other
		Missing
	Prepregnancy BMI	<25.0 kg/m^2
		25.0 to 29.9.0 kg/m^2
		>30 kg/m^2
		Missing
	Smoking	No
		Yes
		Missing
	Alcohol consumption in past two months	No
		Yes
		Missing
	Systolic blood pressure	per 10 mmHg increase
	Diastolic blood pressure	per 10 mmHg increase

SUPPLEMENTARY TABLE S4.3.2. Socio-demographic characteristics of subsets for calibration

	Subset 1 (n=4,091)	Subset 2 (n=3,035)	Subset 3 (n=1,214)
Maternal socio-demographic characteristics			
Ethnicity			
Dutch or European, n (%)	1,772 (43.3)	2,215 (73.0)	553 (45.6)
Surinamese, n (%)	403 (9.9)	196 (6.5)	123 (10.1)
Turkish, n (%)	506 (12.4)	110 (3.6)	116 (9.6)
Moroccan, n (%)	369 (9.0)	102 (3.4)	69 (5.7)
Cape Verdean or Dutch Antilles, n (%)	364 (8.9)	98 (3.2)	145 (11.9)
Other, n (%)	702 (17.2)	495 (16.3)	217 (17.9)
Education, n higher education (%)	1,939 (47.4)	1,644 (54.2)	346 (28.5)
Income, n high (%)	1,633 (39.9)	720 (23.7)	561 (46.2)
Occupational status, employed n (%)	1,924 (47.0)	2,016 (66.4)	492 (40.5)
Marital status, n no partner/stable relation (%)	468 (11.4)	150 (4.9)	111 (9.1)
Planned pregnancy, n no (%)	1,162 (28.4)	562 (18.5)	388 (32.0)
Birth outcomes			
Preterm birth, n (%)	217 (5.3)	128 (4.2)	80 (6.6)
Small-for-gestational-age, n (%)	436 (10.7)	254 (8.4)	144 (11.9)
Large-for-gestational-age, n (%)	370 (9.0)	356 (11.7)	108 (8.9)

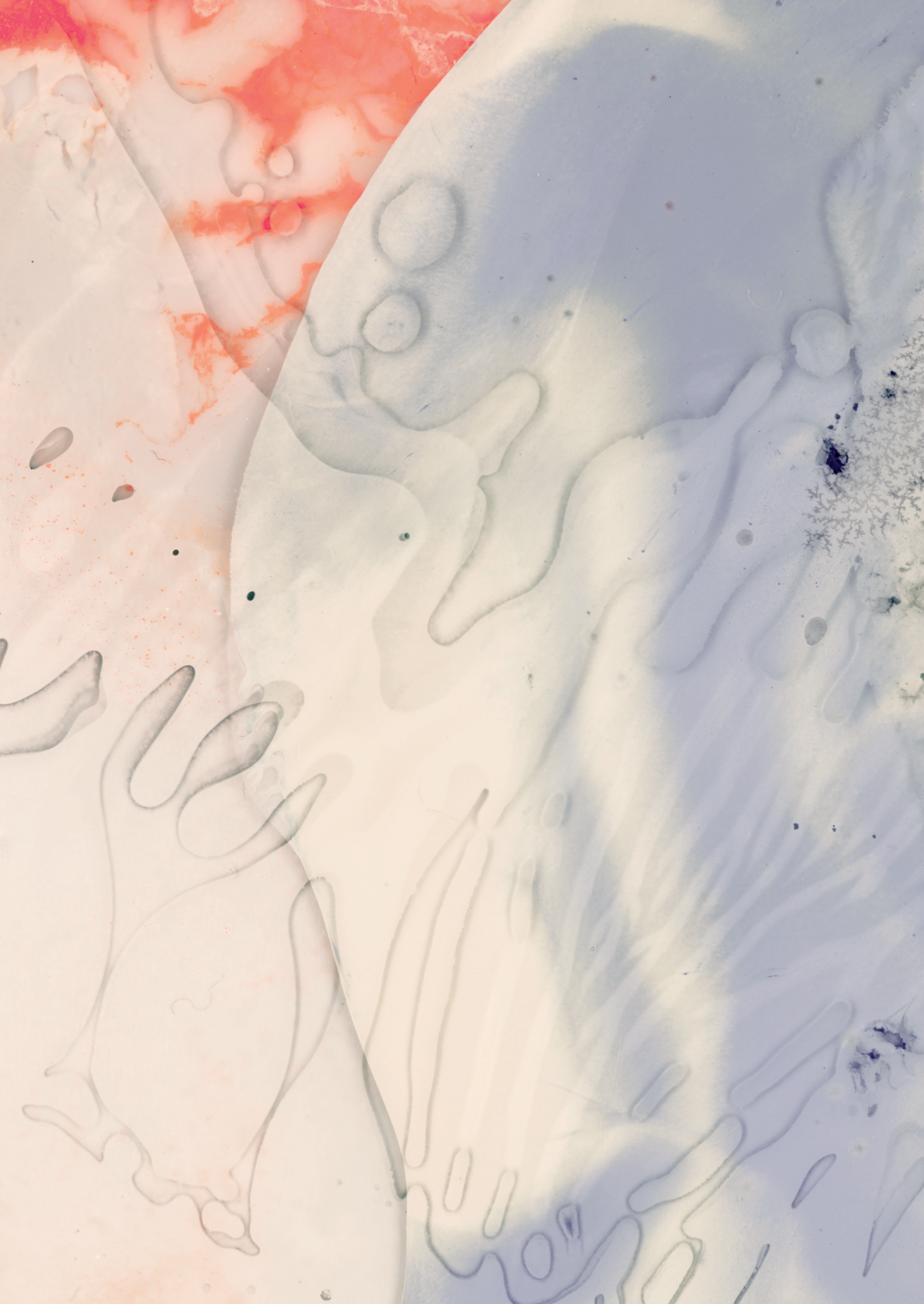
SUPPLEMENTARY TABLE S4.3.3. Paternal characteristics *(n=6,062)*

	No adverse birth outcome* (n=4,631)	Preterm birth*(n=285)	Small-for-gestational-age* (n=545)	Large-for-gestational-age* (n=601)
Paternal characteristics				
Age, mean (SD), years	32.8 (5.7)	32.2 (6.1)	31.8 (6.0)	33.4 (5.1)
Ethnicity				
Dutch or European, n (%)	2,711 (58.5)	155 (54.4)	248 (45.5)	431 (71.7)
Surinamese, n (%)	277 (6.0)	23 (8.1)	73 (13.4)	12 (2.0)
Turkish, n (%)	311 (6.7)	21 (7.4)	37 (6.8)	27 (4.5)
Moroccan, n (%)	185 (4.0)	9 (3.2)	26 (4.8)	19 (3.2)
Cape Verdean or Dutch Antilles, n (%)	235 (5.3)	16 (5.6)	45 (8.2)	17 (2.8)
Other, n (%)	395 (9.0)	24 (9.0)	46 (9.0)	44 (7.6)
Body Mass Index, mean (SD), kg/m^2	25.3 (3.5)	25.3 (3.5)	24.9 (3.5)	25.6 (3.5)
Education, n. higher education (%)	1,985 (42.9)	105 (36.8)	168 (30.8)	316 (52.6)
Smoking, n yes (%)	1,653 (35.7)	106 (37.2)	216 (39.6)	202 (33.6)
Alcohol consumption in past 2 months, n yes (%)	3,173 (68.5)	176 (61.8)	353 (64.8)	451 (75.0)
Mean systolic blood pressure, mean (SD), mmHg	130 (14)	130 (13)	129 (13)	130 (13)
Mean diastolic blood pressure, mean (SD), mmHg	73 (11)	73 (12)	72 (11)	73 (10)

SUPPLEMENTARY TABLE S4.3.4. Model performance for the core model for secondary outcomes

Maternal pregnancy complications and adverse birth outcomes				
4a. Model performance for gestational hypertension and preeclampsia (n total population=8,136, ncases=495)				
Models	AUC (95% CI)	Sensitivity at specificity (%)		
		70%	80%	90%
Core[a]	0.70 (0.67 to 0.72)	60	43	28
4b. Fetal distress (n total population=8,030, ncases=598)				
Models	AUC (95% CI)	Sensitivity at specificity (%)		
		70%	80%	90%
Core[a]	0.70 (0.67 to 0.72)	60	43	28
4c. Model performance for caesarean section (n total population=7,587, ncases=917)				
Models	AUC (95% CI)	Sensitivity at specificity (%)		
		70%	80%	90%
Core[a]	0.64 (0.63 to 0.66)	48	38	22
4d. Model performance for low birthweight (n total population=8,340, ncases=403)				
Models	AUC (95% CI)	Sensitivity at specificity (%)		
		70%	80%	90%
Core[a]	0.66 (0.63 to 0.69)	52	38	22
4e. Model performance for macrosomia (n total population=8,340, ncases=189)				
Models	AUC (95% CI)	Sensitivity at specificity (%)		
		70%	80%	90%
Core[a]	0.68 (0.64 to 0.72)	56	44	33

[a]Core model includes age, ethnicity, prepregnancy Body Mass Index, parity and smoking



05

General discussion

INTRODUCTION

Pregnancy is a critical period in life as many diseases in early and later life may find their origins in the prenatal period (1). Maternal metabolism during preconception and early-pregnancy is crucial for a successful pregnancy and long-term offspring health outcomes. To provide optimal circumstances for embryonic and fetal development, maternal metabolism goes through major adaptations during pregnancy (2, 3). Even small disruptions in maternal metabolism could irreversibly influence fetal development and future offspring health (2). Optimizing maternal metabolism, before and during pregnancy, may be an important target for interventions aiming at early prevention of offspring disease. In order to develop and implement intervention strategies for optimizing maternal metabolism in pregnancy, we first need a better understanding of maternal metabolism in pregnancy and the effects of disruption on fetal development and subsequent offspring health. Further insight into these effects enables identification of factors that may serve as targets for interventions and offer markers for early identification of women and their offspring at risk for complications during pregnancy and childhood.

Therefore, we first aimed to explore the impact of maternal metabolism during pregnancy on offspring cardio-metabolic outcomes. Second, we identified maternal characteristics that can influence maternal metabolism and subsequent offspring health outcomes. Third, we assessed whether these maternal characteristics can be used to develop screening tools for risk prediction of adverse offspring health outcomes (**Figure 5.1)**. This chapter provides the findings of this thesis in the broader context of the literature, an overview of several methodological considerations and provides recommendations for future research and clinical practice.

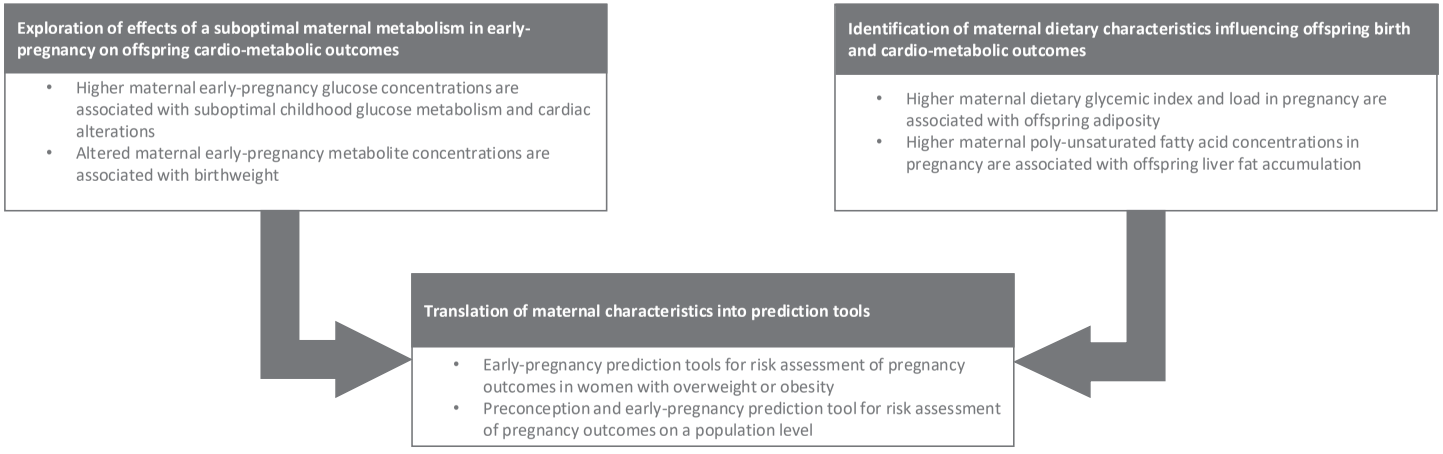


FIGURE 5.1. Outline of aim of this thesis including corresponding main findings

INTERPRETATION OF MAIN FINDINGS

Maternal early-pregnancy glucose metabolism

It is well known that a disrupted maternal metabolism during pregnancy, due to disorders such as obesity, affects fetal development and may have persistent consequences on long-term offspring health (4). More recently, focus has shifted towards a suboptimal maternal metabolism among women who are considered as healthy. In these women, suboptimal adaptations of metabolism to pregnancy may already lead to irreversible consequences in fetal development. Among this population major strides can be made to improve offspring health on a population level.

A disrupted maternal glucose metabolism is gaining interest as crucial factor in the development of offspring pathology. Gestational diabetes is a common metabolic disorder, affecting up to 8% of pregnancies with an ongoing rise in prevalence (5, 6). Definition of gestational diabetes has been a subject of controversy. Until recently, guidelines for the diagnosis of gestational diabetes were based on criteria from 1964 (7). These criteria were designed to recognize and treat hyperglycemia in pregnant women, and to prevent maternal development of long-term type 2 diabetes. Recently, increasing evidence has shown that risk of gestational diabetes is already present in milder forms of hyperglycemia in pregnancy and consequences of gestational diabetes are not limited to maternal risks of type 2 diabetes (8, 9). Milder forms of hyperglycemia in pregnancy also have consequences for the offspring including an increased risk of offspring obesity and glucose tolerance (10, 11). To accurately identify pregnancies at risk of birth complications, new thresholds for diagnosis of gestational diabetes have been proposed in 2010 (12). These guidelines are based on studies focused on maternal glucose concentrations in second half of pregnancy. However, 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 cardio-metabolic development (3, 13, 14).

In this thesis, we observed that higher maternal glucose concentrations already in early-pregnancy were associated with a higher risk of childhood obesity, lower childhood high-density lipoprotein (HDL)-concentrations and higher glucose concentrations at the age of ten years. All associations, except those with childhood glucose concentrations, were explained by maternal prepregnancy BMI. Higher maternal glucose concentrations were not associated with childhood blood pressure and visceral fat accumulation. Our findings are largely in line with previous studies assessing the associations of higher maternal glucose concentrations during the second half of pregnancy with offspring

cardio-metabolic risk factors. A study among 970 Chinese mother-child pairs reported that third-trimester maternal fasting glucose concentrations were associated with a higher risk of offspring obesity, a higher systolic blood pressure and an abnormal glucose tolerance at the age of 7 years, independent of maternal pre-pregnancy BMI (15). A cohort study in the United Kingdom including 2,563 women and their offspring showed that, independent of maternal prepregnancy BMI, glycosuria in mid-pregnancy was associated with a higher offspring BMI and fasting insulin concentrations, but not with blood pressure and lipid concentrations. In addition to these studies, our findings suggest that early-pregnancy may already be a crucial period in which higher maternal glucose concentrations can be used for the identification of children with an increased risk of a suboptimal glucose metabolism. Moreover, our findings underline the importance of maternal weight status prior to pregnancy for childhood cardio-metabolic development. Although mechanisms underlying our observed associations are not fully understood, it could be speculated that higher maternal glucose concentrations cross the placenta and increase fetal glucose and insulin concentrations (3, 16, 17). Fetal hyperglycemia and insulinemia could lead to accelerated fetal growth and disruption of development of organ, endocrine and metabolic systems (3). Besides the direct effects of maternal glucose concentrations on fetal development, maternal higher insulin concentrations could influence placentation, leading to altered fetal nutrient supply and subsequent altered growth and development (16). These alterations in embryonic and fetal growth and development due to higher maternal glucose concentrations already below diagnostic thresholds of gestational diabetes may irreversibly affect offspring cardio-metabolic health in childhood.

Besides focusing on the associations of maternal glucose metabolism during pregnancy on childhood adiposity, blood pressure and lipid and glucose metabolism, we also explored the less studied associations of maternal early-pregnancy glucose concentrations with childhood liver fat accumulation and cardiac development. Results from animal studies suggest that fetal liver fat storage and cardiac development are strongly influenced by maternal and fetal glucose concentrations (18-20). Maternal insulin resistance may cause alterations in gene expression involved in fatty acid oxidative capacity and lipogenesis in offspring liver (19, 20). In contrast to these studies in animals, we did not observe associations of higher maternal early-pregnancy glucose concentrations with childhood liver fat accumulation or the risk of non-alcoholic fatty liver disease among the total multi-ethnic population. The lack of associations could be due to the relatively small variability of liver fat in this population of children and may be more pronounced at older ages, when non-alcoholic fatty liver disease more often arises (21). Glucose metabolism and liver fat development are both influenced by ethnicity. We therefore also focused on the associations of maternal early-pregnancy glucose

concentrations with childhood liver fat among women of European ancestry only, as this is the largest ethnic group in our study population. Within this population, we did observe associations of higher maternal early-pregnancy glucose concentrations with childhood non-alcoholic fatty liver disease. Further studies need to address potential ethnic differences in the associations of maternal glucose concentrations with childhood non-alcoholic fatty liver disease. When focusing on offspring cardiac development, we observed associations of higher maternal early-pregnancy glucose concentrations with alterations in childhood structural development of the left cardiac ventricle. There were no associations with alterations of childhood right ventricle development. As the human heart is the first organ to develop, early-pregnancy may especially be a critical period for higher maternal early-pregnancy glucose concentrations to irreversibly affect embryonic and fetal cardiac development (18, 22-25). Our findings add to existing studies that showed that gestational diabetes is associated with subclinical cardiac changes in fetal and infant life (22, 26, 27). Childhood cardiac development tracks into adulthood, and alterations in left ventricular mass and end-diastolic volume in adulthood are known risk factors of cardiovascular morbidity (28-31). The subclinical alterations we observed in childhood cardiac development may relate to adult cardiovascular morbidity.

In conclusion, our findings support that higher maternal glucose concentrations already below diagnostic thresholds of gestational diabetes, are associated with childhood suboptimal glucose metabolism. Children exposed to higher maternal glucose concentrations may also have alterations in structure and function of the left cardiac ventricle. Results from studies in this thesis suggest that early-pregnancy may already be a critical period for higher maternal glucose concentrations to affect childhood cardio-metabolic development.

Summary

- Children exposed in early-pregnancy to higher maternal glucose concentrations, already below diagnostic thresholds of gestational diabetes, may have a suboptimal glucose metabolism and alterations in left cardiac structure.
- Maternal prepregnancy BMI seems to have a key role in the associations of higher maternal early-pregnancy glucose concentrations with the development of common childhood cardio-metabolic risk factors, including a higher blood pressure, suboptimal lipid metabolism and adiposity development.
- Maternal early-pregnancy glucose concentrations are not associated with childhood liver fat accumulation.

Maternal dietary influences during pregnancy

The maternal diet during pregnancy is essential for fetal development. Maternal nutrient intake not only directly determines fetal nutrient availability, but can also modify fetal development through effects on glucose metabolism (4, 32). As higher glucose concentrations even in women without gestational diabetes seem to adversely affect fetal development and subsequent offspring health, there is an urgent need for optimization of maternal glucose concentrations during pregnancy.

Maternal glucose concentrations during pregnancy are mainly determined by maternal dietary carbohydrate intake. In 1981, the dietary glycemic index and load have been introduced in 1981 as a dietary target in diabetic patients to optimize glucose control (32). The dietary glycemic index and load are measures to qualify and quantify postprandial glycemic response to dietary carbohydrate intake. Consumption of a food product with a higher dietary glycemic index or load, causes higher postprandial glucose concentrations, whereas consumption of food product with a lower glycemic index or load leads to more stable low postprandial glucose concentrations. The dietary glycemic index provides information on the quality of the glycemic response to a carbohydrate containing food product and is more often used in intervention studies and clinical settings (33, 34). The dietary glycemic load additionally takes the amount of carbohydrate intake into account and therefore provides additional information on maternal postprandial glucose concentrations, but the glycemic load may be more prone to measurement errors (32, 35, 36). Among pregnant women at high risk of an impaired glucose metabolism, such as women with obesity or diabetes during pregnancy, a lower dietary glycemic index has already shown to be associated with a lower risk of large-for-gestational-age newborns and lower neonatal skinfold thickness (37, 38). Long-term offspring outcomes have been less studied.

In our studies, we did not limit our population to women with an increased risk of an impaired glucose metabolism. In this non-selected population, we observed no consistent association of a higher maternal early-pregnancy dietary glycemic index and load with fetal growth parameters in mid-pregnancy and at birth. We did observe associations with higher fetal growth parameters in the third trimester. These observations indicate that a higher maternal dietary glycemic index and load may have an effect on fetal adipose tissue development, as the third trimester is the period when fetal fat development is most pronounced. When focusing on long-term childhood adiposity outcomes, we observed that a higher maternal glycemic index and load in early-pregnancy was indeed associated with a higher childhood adiposity, in particular abdominal fat accumulation. A maternal diet during pregnancy with a mean low glycemic index (<55) was associated with an improved fat distribution in these children. These associations were only present in women with prepregnancy overweight or obesity, and not in women with a normal prepregnancy weight. As our findings were not explained by other maternal dietary factors and overall diet quality, our observed associations are less likely to only reflect effects of an overall unhealthy lifestyle and diet among women with overweight or obesity, but support that higher maternal early-pregnancy dietary glycemic index also has a direct effect on offspring adiposity development. For interpretation of these results, it is important to note that due to our use of questionnaires only validated in Dutch women, we limited our studies concerning the dietary glycemic index and load to Dutch women. Regarding birth outcomes, our results are not in line with previous studies (37, 38). A meta-analysis among 11 trials including 1,985 women at risk of an impaired glucose metabolism and their newborns, observed that intervention stimulating a lower glycemic index diet during pregnancy significantly reduced the risk of large-for-gestational-age newborns (37). The contrast of our results to this meta-analysis, is most probably due to our focus on women not selected on an increased risk of an impaired glucose metabolism. The associations of a higher maternal dietary glycemic index and load during pregnancy with childhood adiposity are less studied. Largely in line with our findings, a study among 906 mother-child pairs in the United Kingdom reported that a higher maternal dietary glycemic index and load in early-pregnancy, but not late-pregnancy, were associated with a higher offspring total body fat at 4 and 6 years of age after adjustment for maternal prepregnancy BMI. No differences in associations among normal weight women and women with overweight or obesity and their offspring were present in this study (39). Thus, our findings support previous studies, to suggest that recommending a diet with a low-glycemic index in pregnant women with overweight or obesity may be an useful intervention for the prevention offspring adiposity, in particular visceral fat accumulation. Although we found no support for this recommendation for women

not at increased risk of an impaired glucose metabolism, our findings do suggest that recommending a diet with a low-glycemic index in a non-selected population is a safe intervention, as we also not observed any adverse effects on birth or offspring outcomes among this population.

Besides the maternal dietary glycemic index, maternal polyunsaturated fatty acids (PUFAs) supplementation during pregnancy is receiving an increasing interest for reducing the risk of gestational diabetes and improving offspring health outcomes (40, 41). Maternal PUFA concentrations in blood highly interact with maternal glucose metabolism and a suboptimal PUFA composition in blood seems to be related to insulin resistance (40, 42, 43). An inadequate PUFA intake, characterized as lower n-3 PUFA and higher n-6 PUFA intake, may therefore be a risk factor for the development of insulin resistance in pregnancy. In addition to its effects on maternal glucose metabolism, maternal PUFA plasma concentrations are also essential for fetal growth and adipocyte development, as the fetus fully depends on maternal PUFA concentrations and subsequent placental transfer (44-46). Although a meta-analysis among eleven randomized controlled trials did not observe an effect of maternal n-3 long chain PUFA supplementation on the risk of offspring obesity, both human and animal studies have suggested that an adverse maternal PUFA profile during pregnancy may rather affect offspring's hepatic fat deposition than BMI (46-48). Suboptimal maternal PUFA concentrations could have irreversible direct effects on fetal liver fat development by influencing fetal lipid metabolism, oxidative stress and DNA methylation (49-51). We indeed observed that maternal higher n-3 and lower n-6 PUFA plasma concentrations in pregnancy were associated a lower offspring liver fat. We observed stronger associations for n-3 PUFA concentrations in boys than in girls. In boys, higher maternal n-3 PUFA concentrations were also associated with a lower risk of non-alcoholic fatty liver disease. The associations were independent of child's BMI, which suggests that associations are likely to not just reflect a higher overall fat, but that these children exposed to suboptimal maternal PUFA concentrations during pregnancy have excess liver fat development. This could be due to the reducing effect of maternal n-3 PUFAS on triglyceride concentrations and lipoprotein lipase activity, which are highly involved in liver fat deposition (52, 53). Our findings are largely in line with findings from a previous study from our cohort, observing that higher maternal n-3 PUFA concentrations and lower maternal n-6 PUFA concentrations were associated with a lower childhood total body fat mass and abdominal fat mass at 6 years (47). Optimization of maternal PUFA concentrations in pregnancy may be a target for intervention studies aiming the prevention of offspring non-alcoholic fatty liver disease.

Summary

- In pregnant women, consumption of a higher dietary glycemic load, but not glycemic index, was associated with increased third trimester fetal growth.
- The dietary glycemic index and load during pregnancy were not consistently associated with fetal growth in mid-pregnancy or birth outcomes.
- In women with overweight and obesity during pregnancy, a higher dietary glycemic index was associated with a higher childhood general and abdominal fat, in particular visceral fat accumulation, but not with liver fat.
- Higher maternal omega-3 poly-unsaturated fatty acids and lower maternal omega -6 poly-unsaturated fatty acids concentrations during pregnancy are associated with a lower liver fat accumulation.
- Improvement of maternal dietary glycemic index and poly-unsaturated fatty acids intake during pregnancy may be a useful target for future interventions focusing on reducing childhood adiposity.

Risk prediction of offspring health outcomes

In the early 2000's, perinatal morbidity and mortality was relatively high in the Netherlands (54). New health policies and improved antenatal and neonatal care have led to a fast decline in perinatal morbidity and mortality. Since 2015 that decline has stagnated and the prevalence of perinatal morbidity, including the most common birth complications such as preterm birth and small-for-gestational-age at birth, increased again (55). This may have major adverse consequences for health outcomes in future generations.

The recent increase of perinatal morbidity has been thought to be due to the increase of socio-demographic inequalities and the increase of maternal risk factors, especially the increasing prevalence of maternal overweight and obesity at the start of pregnancy (54-56). This multifactorial etiology of the increase of perinatal morbidity and subsequent adverse offspring outcomes, has led to an urgent call from the Ministry of Health, Welfare and Sport for an integral approach to improve maternal health and lifestyle already prior to pregnancy (57). Multiple studies have aimed to develop interventions to improve maternal lifestyle and health before and during pregnancy (58-61). However, the benefits of these interventions on birth outcomes and offspring health outcomes are very limited. One of the major challenges of improving maternal health and lifestyle through preventive intervention strategies, is the timely and accurate identification of women who may benefit most from lifestyle interventions and intensified antenatal care. In current clinical practice and lifestyle intervention studies, women are often considered at increased risk based on a single risk factor (59, 62, 63). This approach may

be too simplistic, as perinatal morbidity has a multifactorial etiology and risk factors tend to cluster in women. A more detailed, integrated approach to identify the different risk factors within women from preconception onwards may help to improve outcomes. This may facilitate more personalized interventions aiming at the right care for the right women at the right time. In this thesis, we developed models for personalized risk prediction in high-risk women and in the general population.

Risk prediction in high risk populations

The increase in maternal overweight and obesity has a major contribution in the recent increase of perinatal morbidity in the Netherlands (54). Women with overweight and obesity not only have an increased risk of perinatal morbidity but also their offspring is at increased risk of adverse cardio-metabolic long-term outcomes on the long-term. For the prevention and management of these risks, guidelines recommend an uniform approach for all women with overweight or obesity consisting of intensified antenatal monitoring and care (64). A substantial proportion of women with overweight or obesity will have an uncomplicated pregnancy and may receive redundant intensified antenatal care (65). Increasing knowledge on the effects of maternal health and metabolism on fetal development enables us to refine the approach for guidance and improvement of care and outcomes. Differentiation within pregnant women based on multiple characteristics may identify which women need no interventions and those who need tailored interventions based on personal risk factors. A personalized approach of antenatal care in these women is essential for care providers to avoid unnecessary medicalization of pregnancy and to improve pregnancy outcomes, and for health care on a population level to reduce burden and costs (54, 66, 67). We aimed to identify novel and established markers that enable prediction of birth complications in women with a higher prepregnancy BMI.

New markers of maternal metabolism during pregnancy

Metabolomic techniques enable characterization of the maternal metabolic profile in detail. Metabolomics may therefore provide novel insight into the pathway of a higher maternal prepregnancy BMI, maternal metabolism during pregnancy and the risk of large-for-gestational-age newborns (68). Previous studies have already shown an altered maternal metabolome during pregnancy in women with prepregnancy overweight or obesity (69-72). Few studies measuring metabolites in second half of pregnancy, suggested that maternal metabolite concentrations in the second half of pregnancy may improve the prediction of an increased birthweight (73, 74). In this thesis, we observed that a higher maternal prepregnancy BMI was associated with alterations in maternal early-pregnancy amino acid, non-esterified fatty acid,

phospholipid, in particular sphingomyelin, and carnitine concentrations. As fetal growth and development are largely influenced by these maternal metabolites, we hypothesized that these maternal early-pregnancy metabolites may serve as novel markers of metabolism for the improvement of prediction of a large-for-gestational-age newborn in women with a higher prepregnancy BMI. Based on these findings, we proposed a maternal early-pregnancy metabolite profile including amino acids, non-esterified fatty acids, phospholipids and carnitines that can be used in women with an increased prepregnancy BMI, to predict an increased birthweight already from early-pregnancy onwards. Our model was better at predicting a large-for-gestational-age newborn in women with a higher prepregnancy BMI than maternal early-pregnancy glucose concentrations and lipid concentrations. Our identified a maternal early-pregnancy metabolite profile may thus be used for early identification of women at risk of a large-for-gestational-age newborn.

Prediction of healthy pregnancies in women with overweight or obesity

After identification of new markers for prediction of high birthweight in women with a higher BMI, we developed a model that provides personalized prediction of healthy pregnancy outcomes, including absence of long-term maternal and offspring complications, in women with overweight or obesity. For the definition of a healthy pregnancy outcome, we also took into account long-term complications, including maternal and childhood weight status, next to the common short-term pregnancy complications. These long-term complications of maternal overweight and obesity during pregnancy are strongly related to maternal and offspring cardio-metabolic diseases in later life, are therefore crucial to take into account in the risk assessment of pregnancy outcomes among overweight and obese pregnant women (4, 75, 76). In our developed model, common maternal, placental and fetal risk factors or markers of pregnancy complications, could moderately predict an uncomplicated pregnancy. To improve our model, we also explored the incremental value of potential novel markers of pregnancy complications, including maternal early-pregnancy metabolites and paternal characteristics. In contrast to our previously mentioned study focused on prediction of an increased birthweight, maternal metabolites did not significantly improve the prediction of a healthy pregnancy outcome in this model. Also, the effect of paternal characteristics was limited for the prediction of healthy pregnancy outcomes in women with overweight or obesity. Models that have been developed in previous studies also could only moderately identify pregnant women with overweight or obesity who will have a healthy pregnancy outcome (65, 77). Especially when a prediction model should identify women who can be excluded from intensified antenatal care, a model needs to have a high sensitivity to eliminate possibilities of false-negatives. Results from our

study, together with results from previous studies, show that highly accurate prediction of healthy prediction outcomes cannot be achieved using common socio-demographic, lifestyle, physical, pregnancy-related characteristics, nor using maternal metabolites or paternal characteristics. There is a need for novel markers to improve prediction of pregnancy outcomes in these women.

Risk prediction of adverse offspring health outcomes in general populations

Besides focusing on high risk populations, also among the general population increasing efforts are made to prevent from adverse pregnancy outcomes. However, up till now, beneficial effects of interventions are limited (78, 79). One of the main challenges remains targeting women who may benefit most from lifestyle interventions. Ideally, women should already be identified when planning their pregnancy, to allow for optimization of circumstances for pregnancy (80). In the current thesis, we proposed prediction models including maternal characteristics easily obtainable in clinical practice already from preconception onwards that could aid in the prediction of pregnancy complications. Though these models were developed on preterm birth and small-, or-large-for-gestational-age newborns, they were also good at predicting other pregnancy complications, such as preeclampsia and a low Apgar score. Our models were developed in a population-based cohort study using an extensive scope of maternal characteristics. We observed the strongest predictive performance for only a small group of maternal characteristics, including maternal age, ethnicity, prepregnancy BMI, parity and smoking. These characteristics are already known in preconception and can easily, routinely be obtained in clinical practice. Though significant, the improvement of the prediction models was relatively small after the addition of more detailed maternal socio-demographic, lifestyle, medical history and early-pregnancy clinical characteristics. By translating our developed prediction models into one risk calculator, our models can be used after validation as a screening tool to calculate risks for the most common birth complications in women on a population level, already from preconception onwards.

Summary

- A higher maternal prepregnancy BMI is associated with an altered metabolite profile in early-pregnancy. Among women with a higher prepregnancy BMI, maternal early-pregnancy metabolites may serve as novel biomarkers to improve the early identification of women at risk of a large-for-gestational-age newborn.

- Among women with overweight or obesity, maternal sociodemographic, lifestyle, physical, placental and ultrasound characteristics can moderately predict a healthy

pregnancy. The incremental value of maternal early-pregnancy metabolites and paternal characteristics for the prediction of an overall healthy pregnancy outcome in these women is limited.

- On a population level, maternal characteristics easy obtainable from preconception onwards can aid in the prediction of preterm birth, small-for-gestational-age and large-for-gestational-age newborns.

METHODOLOGICAL CONSIDERATIONS

External validity

External validity compromises to what extent results from the studies presented in this thesis are generalizable to other populations. The studies in this thesis were conducted in the Generation R Study. This is a population-based cohort study with a large variability in socio-demographic characteristics between participants. However, we still observed that it compromised a relatively healthy population. This observation is mainly based on the relatively low number of women with gestational diabetes, when compared to the Dutch, and also to the European and North America prevalence. Also, for studies using data on the maternal dietary glycemic index and load and maternal early-pregnancy metabolite concentrations, we were limited to a Dutch population. These selected populations may have affected the generalizability of our findings. Future studies should confirm our findings in multi-ethnic, high-risk populations.

In prediction modelling, generalizability of models could be affected by overfitting of models to the population in which models were developed. Before implementation of the prediction models as a screening tool for risk assessment in clinical settings, more advanced internal validation and external validation of models is needed. External validation of prediction models should be done in other multi-ethnic, low and high risk populations to assess whether models are generalizable to other diverse populations.

INTERNAL VALIDITY

Selection bias

In association studies, selection bias is the distortion of results due to procedures used to select participants in the study. This occurs when women who did not want to participate in the study or were lost to follow up are different from those who did participate in the study or follow up. As previously described, of all children that were

eligible to participate at birth in the inclusion period, 61% participated in the Generation R Study. Compared to what can be expected from the general population of Rotterdam, the percentages of women from lower socio-economic status and from certain ethnic groups were lower. As the Generation R Study is a prospective study, selection on the outcome at baseline cannot be an issue. Previous studies have shown that it is unlikely that results in prospective cohort studies are influenced due to selection by non-response at baseline, but more likely by selective loss-to-follow up. Loss to follow up can lead to selection bias if associations differed between the participants in the study and the participants that left the study. To assess whether this was a problem in the studies presented in this thesis, we performed non-response analyses, in which we assessed whether baseline characteristics differed from women included in the analyses as compared to those who were loss to follow up. We observed that women participating in follow up were most often from European descent and higher educated than those who did not. It is difficult to speculate whether this selection to a more healthy, higher educated study population has led to selection bias. However, we did not observe differences in the exposures between women participating and those loss to follow up in most studies, and therefore selection bias seems to be unlikely.

For the development of prediction models, selection bias may occur by the selection of predictors instead of the selection of participants. This bias, called testimation bias, is induced by stepwise selection of predictors and could lead to an overestimation of the effect of a predictor. Although we used stepwise selection for our prediction models, we do not expect this to have induced testimation bias as we had a strong hypothesis for included candidate predictors and did not observe extreme effects of predictors selected in our final models. Selection bias may also be induced if participants had to leave the study or already had events before tests were performed. This could have occurred in the development of the prediction model for a healthy pregnancy in women with overweight or obesity as we used maternal characteristics throughout pregnancy to develop this model. If women had a fetal death before mid-pregnancy characteristics were obtained, these cases could have influenced model selection or performance. Although we tried to assess whether this occurred through various sensitivity analyses excluding specific outcomes or subpopulations, this still may have affected our final developed model for this study.

Information bias

Information bias, or misclassification, occurs when there are errors in the measurement of the exposure or outcome. Misclassification can either be differential or non-differential. When the measurement error of the outcome depends on the exposure, it involves

differential misclassification. If the measurement error is independent of the exposure, it involves non-differential misclassification. Differential misclassification can distort the association between exposure and outcome. We obtained random maternal glucose concentrations once during pregnancy at non-fixed times throughout the day. Due to our study design, we were not able to collect repeated fasting blood samples. Glucose concentrations throughout the day are influenced by multiple factors such as dietary intake and exercise. These factors may have led to non-differential misclassification, causing an underestimation of our associations. However, previous studies, including studies from our cohort, showed that random maternal gestational glucose concentrations are related to the risks of gestational diabetes, adverse birth outcomes, childhood obesity and altered glucose metabolism (81-84). These associations were in similar direction as those for maternal fasting and postprandial glucose concentrations with these adverse outcomes (8, 85). Timing of sampling of maternal glucose concentrations in our study is relatively broad, but before 18 weeks gestation, covering the first half of pregnancy. This classification was aligned with the logistics of the study. Further studies need to replicate our findings using repeated maternal fasting and postprandial glucose measurements. These studies should already measure glucose prior to pregnancy, as maternal glucose concentrations in the first half of pregnancy are likely to reflect maternal glucose prior to conception, and repeatedly throughout pregnancy from first trimester onwards, to identify critical periods of maternal glucose concentrations for offspring glucose metabolism and cardiac development. Information on maternal dietary glycemic index and load was assessed using a Food Frequency Questionnaire (FFQ). Even though the FFQ is widely used for dietary assessment in observational studies, measurement of food intake by a FFQ may be affected by measurement error, recall bias and reporting bias. Subsequent calculation of the dietary glycemic index from the FFQ may further be affected by uncertainty induced by preparation of foods, mixed dishes, variations of food products of time or unavailability of specific food products (86).

Confounding bias

Confounding is one of the main issues in epidemiological research. Confounding is to misinterpret the effect of the exposure on the outcome, due to an unmeasured factor that is associated with both the exposure and the outcome, but not in the causal pathway between the exposure and the outcome. In the studies included in this thesis aiming to assess associations, confounding was eliminated as much as possible by correction of confounding factors on which data was available. Confounder selection can be based on information on confounding factors from previous studies, literature on the subject, the hypothesis, or statistical assessment. Based on the aim of the study and prior knowledge available, we chose the most optimal method available

for confounder selection for each individual conducted study. Due to the extensive amount of information of mother and child available, we were well able to adjust for confounding. However, residual confounding, which may be caused by unavailable or unconsidered factors, may still result in overestimation of the observed effects. Residual confounding always remains an issue in observational studies, and needs to be considered when interpreting effects.

RECOMMENDATIONS FOR FUTURE RESEARCH

In the present thesis, we showed that in addition to overt gestational diabetes, a suboptimal maternal glucose metabolism and maternal dietary intake in early-pregnancy were associated with adverse childhood cardio-metabolic health outcomes. These associations were already present in women without overt metabolic disorders of pregnancy. In addition we developed risk prediction models for pregnancy outcomes in low- and high-risk populations using established and novel markers. However, several issues remain to be addressed in future studies (**Table 5.1**).

First, studies in this thesis showed that higher maternal early-pregnancy glucose concentrations were associated with multiple childhood cardio-metabolic risk factors. Although these findings suggest that a suboptimal maternal early-pregnancy glucose metabolism and dietary intake affects childhood adiposity development, glucose metabolism and cardiac development, causality of our findings remains to be established. Randomized controlled trials are considered as the gold standard to obtain further insight into the causality. Future randomized controlled trials aiming at optimization of maternal glucose concentrations during early-pregnancy should assess whether the associations of maternal early-pregnancy glucose concentrations with childhood cardio-metabolic outcomes reflect direct effects of maternal glucose concentrations on early intrauterine development. These trials could include different interventions such as lowering the glycemic index or enhancing n-3 PUFA intake. Randomized controlled trials should ideally be conducted among general populations and women at risk of an impaired glucose metabolism, to assess whether recommendation of a diet with a low glycemic index is preferred on a population level, or solely for women at increased risk of an impaired glucose metabolism. Besides randomized controlled trials, observational designs such as Mendelian randomization studies could be helpful in assessing causality, as they are less biased by confounding by using genetic variants associated with the exposure of interest. Sibling studies could also be used to disentangle genetic, lifestyle and intrauterine mechanisms.

Second, the underlying mechanisms of observed associations of maternal glucose metabolism and maternal dietary glycemic index, glycemic load and PUFA concentrations during pregnancy with childhood cardio-metabolic outcomes should be studied in more detail. We took birth and childhood characteristics into account in all of our analyses which did not explain observed associations. These findings suggest an intrauterine effect of suboptimal maternal glucose metabolism, PUFA concentrations and dietary glycemic index on fetal metabolic, adiposity and cardiac development. Future studies should include more detailed assessments of exposures and outcomes, including multiple measurements of glucose intolerance, dietary intake and metabolites already from preconception onwards throughout pregnancy to identify critical periods. With regard to outcome measurements, novel techniques such as 3-dimensional ultrasound, can be used to obtain more detailed information in embryonic and fetal organ and metabolic development. At birth, detailed information on neonatal abdominal and ectopic fat deposition should be included. Besides, observational studies with long-term follow up of offspring should assess the implications of our findings for long-term offspring risk of cardio-metabolic disease, such as type 2 diabetes and myocardial infarction.

Third, we proposed multiple prediction models within this thesis, which require external validation before implementation in clinical settings. External validation of prediction models should be done in other multi-ethnic, lower and higher risk populations to assess whether models are applicable on diverse populations. These studies should also assess whether accurate identification of women at risk of adverse pregnancy outcomes can already be achieved already from preconception onwards. Also, future studies need to evaluate the role of novel markers for the improvement of prediction models and optimal timing of risk assessment. Studies replicating our findings among other low and high-risk populations can establish the role of maternal metabolites, before and during different periods in pregnancy in risk assessment of pregnancy complications and long-term maternal and offspring complications. We were already able to develop models based on maternal socio-demographic, lifestyle, physical, metabolic, placental, ultrasound and paternal characteristics to establish a maternal risk profile. We identified a novel maternal metabolite profile to improve prediction of offspring complications. Studies using untargeted metabolomics approaches or genomics or proteomics analyses can explore other novel markers for the prediction of pregnancy outcomes. We already provide a relatively extensive risk profile, but ideally an integrated maternal risk profile is generated additionally including genomics, proteomics, environmental exposures and social determinants. Novel data driven methods could be used to use all these maternal characteristics together to develop a complete risk profile for optimized identification of those who benefit most from tailored lifestyle interventions

and antenatal care for the improvement of maternal and offspring health outcomes. Finally, after our proposed prediction models are further improved, optimal timing of risk prediction is assessed and models are externally validated, strategies for implementation of prediction models can be explored. For implementation of prediction models, the criteria for screening need to be fulfilled (87). Therefore, future randomized controlled trials implementing proposed prediction models in health care should assess whether the use of our proposed prediction tools indeed enable early-identification of women at risk of adverse pregnancy outcomes. These studies should evaluate whether benefits outweigh the harms of screening for adverse pregnancy outcomes, such as the potential harm of medicalization of pregnancies. Decision curve analyses can be used to determine appropriate risk cut-offs for referral and to assess utility of prediction models in clinical settings.

TABLE 5.1. Recommendations for further research

Major further research focus	Recommendations
Causality	• Randomized controlled trials focused on optimizing maternal early-pregnancy glucose concentrations to improve offspring health outcomes • Randomized controlled trials focused on stimulating a low-glycemic index diet and adequate poly-unsaturated fatty acids intake in early pregnancy to improve maternal glucose metabolism and offspring health outcomes
Underlying mechanisms and long-term implications	• Repeated measurements of maternal glucose metabolism, poly-unsaturated fatty acids concentrations and dietary intake from preconception onwards to identify critical periods • Novel techniques for detailed outcomes measurements such as three-dimensional ultrasound techniques and neonatal fat deposition measurements • Long-term offspring follow up to assess implications for future cardio-metabolic health
Improvement of prediction tools	• Identification of novel markers in the field of genomics, metabolomics and proteomics to improve the prediction of pregnancy outcomes in low-, and high-risk multi-ethnic populations • Evaluation of efficacy of pregnancy models already in the preconception period • Use of data-driven methods to provide integrated risk profiles • External model validation of prediction models in diverse populations
Translation into clinical practice	• Randomized controlled trials assessing the feasibility and optimal cut offs of a low-glycemic index diet and adequate poly-unsaturated fatty acids intake during pregnancy • Randomized controlled trials assessing whether our proposed prediction tools can be useful in the early-identification of women at risk of adverse pregnancy outcomes

IMPLICATIONS FOR CLINICAL PRACTICE AND HEALTH POLICIES

Even though there are key areas for future research that need to be conducted, from the work discussed in this thesis, we can already highlight several implications for health care prior and during pregnancy. We identified that maternal glucose concentrations, even below diagnostic thresholds and in early-pregnancy, may have persistent effects on offspring cardio-metabolic health. These findings suggest that a critical appraisal of current guidelines for screening of glucose intolerance in clinical practice, including screening for glucose intolerance already from early-pregnancy onwards. For optimization of maternal glucose metabolism, we suggest that lowering the dietary glycemic index and load and optimization of PUFA intake would offer an opportunity for the improvement of offspring health. The maternal diet is a key environmental factor during pregnancy. The dietary glycemic index and load can easily be improved by for example recommending whole meal bread instead of white bread. PUFA intake can easily be optimized by recommending eating fatty fish two times a week or use fish oil supplementation. Stimulation of diet with a lower dietary glycemic index and adequate PUFA intake in pregnant women on a population level seems like a safe intervention avoiding medicalization of pregnancy. Future public health strategies should assess the feasibility of stimulating a diet with a low glycemic index and adequate PUFA before and during pregnancy. Second, we propose that risk assessment of pregnancy complications and adverse offspring outcomes requires a more personalized approach. Simple risk assessment based on a single risk factor, neglects that to achieve improvement of pregnancy outcomes, a woman should receive an integral approach. Such approach requires consideration of her full condition and environment. Taking advanced socio-demographic, lifestyle, physical and novel biomarker characteristics into account in risk assessment, enables a more tailored approach of preventive intervention strategies and antenatal care. Third, identification of women who may benefit most of intervention strategies should also be done on a population level. All studies in this thesis were conducted in a population-based cohort study were most participants are considered as metabolic healthy. Our results suggested that even in this population adverse effects of a suboptimal maternal metabolic in this overall healthy population on offspring health outcomes. In these subclinical populations, small shifts towards a healthier population will have an important contribution in the improvement of offspring health outcomes on a population level.

TABLE 5.2. Overview of implications for clinical practice and health policies

Focus of clinical practice and health care policies	Recommendations
Glucose screening	• Critical appraisal of current guidelines in clinical practice regarding timing and cut offs for screening of glucose during pregnancy
Maternal diet	• Focus on lowering the dietary glycemic index and adequate poly-unsaturated fatty acids intake during pregnancy in public health strategies
Personalized risk assessment	• An integral approach in risk assessment taking maternal socio-demographic, lifestyle and physical characteristics before and during pregnancy into account to accurate identify women who are likely to benefit from interventions to improve pregnancy and offspring outcomes
Focus on health during pregnancy on a population level	• Public health strategies should focus on maternal metabolic health before and during pregnancy on a population level

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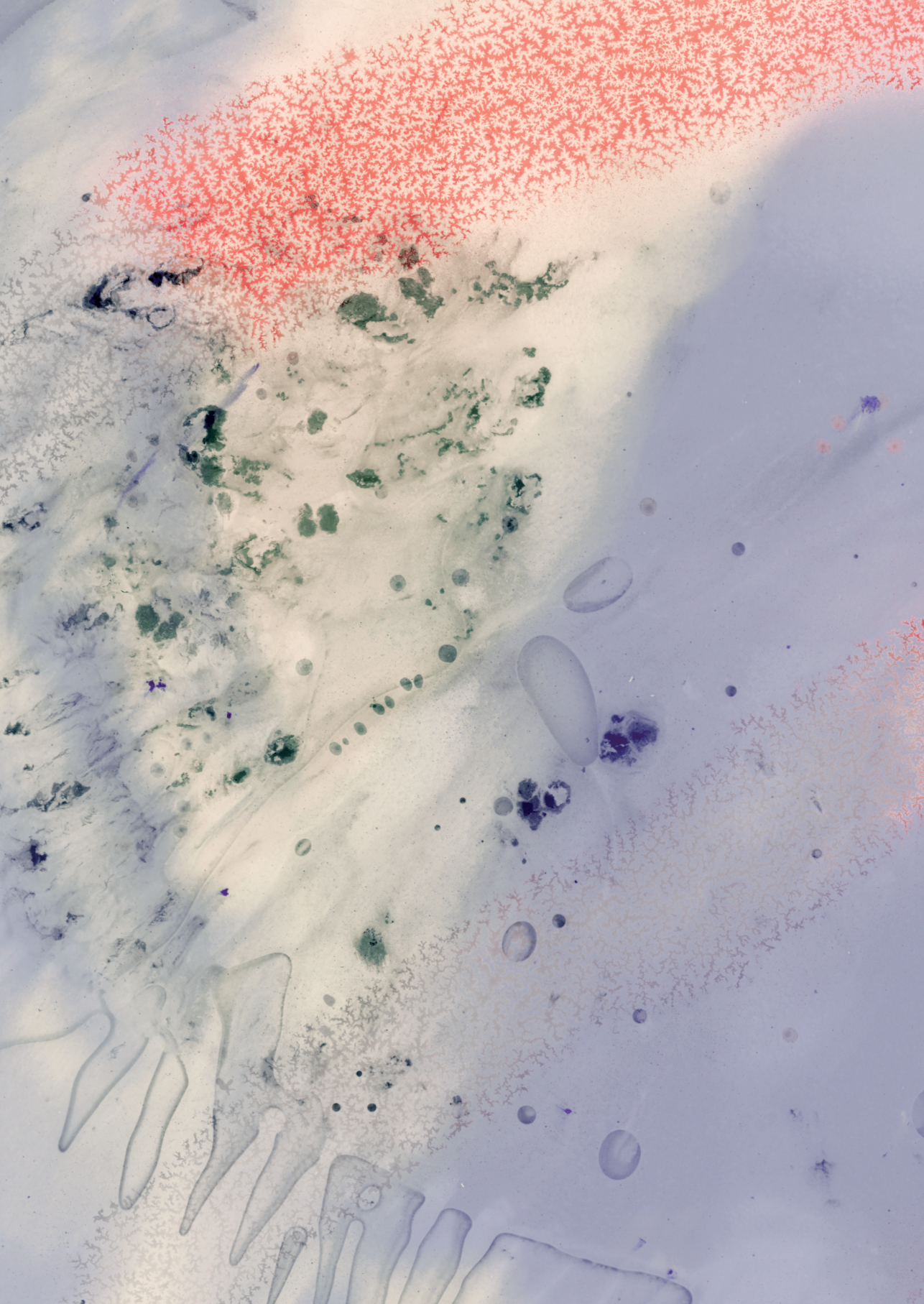
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06

Summary
Samenvatting

SUMMARY

Maternal glucose metabolism goes through major adaptations during pregnancy to provide optimal circumstances for fetal growth and development. Small disruptions of maternal glucose metabolism during pregnancy could already lead to adaptations in fetal development, which may have irreversible consequences for offspring cardio-metabolic health. To prevent adverse cardio-metabolic health outcomes, we need to obtain more insight into the effects of higher maternal glucose concentrations in pregnancy on offspring cardio-metabolic development. Next to maternal glucose concentrations, the maternal diet is an important factor to take into consideration, as it is highly related to glucose metabolism and can be modified during pregnancy. Early identification of women and their children at risk of complications is essential to provide optimal care and deliver timely interventions that may help to improve health of future generations.

The general aims of this thesis were first, to explore the impact of maternal metabolism during pregnancy on offspring birth and cardio-metabolic outcomes; second, to identify maternal characteristics that can influence maternal metabolism and subsequent offspring health outcomes; and third, to assess whether these maternal characteristics can be used to develop screening tools for risk prediction of adverse offspring health outcomes. The studies presented in this thesis were embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, The Netherlands. The Generation R Study is designed to identify early environmental, metabolic and genetic determinants of growth, development and health in fetal life and childhood.

In **Chapter 1,** we provided a background to the studies presented in this thesis, leading to the aims of this thesis.

Based on the studies in **Chapter 2** we concluded that higher maternal glucose concentrations in early-pregnancy are associated with offspring glucose metabolism and cardiac development in childhood, but not other cardio-metabolic risk factors. In **Chapter 2.1,** we observed associations of higher maternal early-pregnancy already below the diagnostic thresholds of diabetes with offspring suboptimal glucose metabolism. The associations of a suboptimal glucose metabolism with childhood blood pressure, lipids and obesity were all explained by maternal prepregnancy BMI. We observed no associations of maternal early-pregnancy glucose concentrations with childhood visceral fat, an important risk factor of cardio-metabolic disease. In **Chapter 2.2** our results showed no associations of maternal glucose concentrations

in early-pregnancy with childhood liver fat accumulation among the total multi-ethnic population. When focusing only on women of European ancestry, higher maternal early-pregnancy glucose concentrations were associated with a higher risk of offspring non-alcoholic fatty liver disease. In **Chapter 2.3,** we observed associations of higher maternal early-pregnancy glucose concentrations with alterations in childhood cardiac development. Especially the left ventricle structure of children exposed to higher maternal glucose concentrations seemed to be affected.

Based on studies in **Chapter 3** we concluded that the maternal dietary glycemic index, glycemic load and maternal poly-unsaturated fatty acids (PUFA) plasma concentrations during pregnancy may influence childhood adiposity development. The dietary glycemic index and load are measures that describe the response of maternal glucose concentrations to carbohydrate consumption, which is an important determinant of glucose concentrations. PUFAs are essential nutrients that can be obtained through fish consumption or through supplements. Results in **Chapter 3.1** showed that the maternal dietary glycemic index and load were not consistently associated with birth outcomes. In **Chapter 3.2** we observed associations of a higher maternal dietary glycemic index and load during pregnancy with childhood adiposity, mainly with a higher visceral fat. The associations of the dietary glycemic index and load with offspring adiposity were only present among women with prepregnancy overweight or obesity. In **Chapter 3.3** we observed that maternal lower omega-3 and higher omega-6 concentrations in plasma were associated with an increased childhood liver fat accumulation. These associations were stronger among boys than girls. In boys, also higher maternal omega-3 PUFA concentrations were associated with a decreased risk of offspring non-alcoholic fatty liver disease.

In **Chapter 4** we presented clinical prediction models that provide risk estimates for several birth outcomes in women already prior and during early pregnancy. In **Chapter 4.1**, we identified a maternal metabolite profile that improves the prediction of a large-for-gestational-age newborn among women with a higher prepregnancy Body Mass Index (BMI). In **Chapter 4.2**, we developed a model for the prediction of a healthy pregnancy in women with overweight or obesity. For this study a healthy pregnancy compromised the absence of short term complications such as gestational diabetes and large-for-gestational-age at birth, and on long-term complications, such as offspring overweight or obesity in childhood. The developed model included maternal age, marital status, BMI, gestational weight gain till second trimester, blood pressure and estimated fetal weight in mid-pregnancy as predictors of a healthy pregnancy. The ability of the model to predict a healthy pregnancy outcome was moderate. In contrast to our study focused on the prediction of an increased birthweight, we observed in this

model that the maternal early-pregnancy metabolite profile had no incremental value for the prediction of an overall healthy pregnancy in overweight or obese women. Also paternal characteristics did not improve the predictive performance of this model. In **Chapter 4.3,** we developed a model for the prediction of birth complications within the general population, without prior selection on maternal weight during pregnancy. This model was designed to predict the most common birth complications, including preterm birth, small-for-gestational-age at birth and large-for-gestational-age at birth, which together affect up to 25% of pregnancies. The model was also good in predicting other birth complications, such as cesarean section and a low Apgar score. Maternal age, ethnicity, parity, BMI and smoking already prior to conception were the strongest predictors for birth complications in this model.

Finally, in **Chapter 5** we provided a general discussion in which we describe the results of our studies in a broader context of existing literature. We elaborated on methodological considerations and provide the clinical implications of our studies and recommendations for future research. The findings in this thesis contribute to early identification of women that may benefit most from timely preventive strategies during pregnancy aiming to improve health in future generations.

SAMENVATTING

Het maternale glucosemetabolisme ondergaat grote veranderingen in de zwangerschap om optimale omstandigheden te creëren voor de groei en ontwikkeling van de foetus. Kleine verstoringen van het maternale glucosemetabolisme kunnen mogelijk al leiden tot suboptimale aanpassingen in de foetale ontwikkeling, welke mogelijk grote gevolgen hebben voor de cardio-metabole gezondheid van het kind. Om nadelige cardio-metabole uitkomsten bij kinderen te voorkomen, moeten we meer inzicht krijgen in het effect van verstoring van het glucosemetabolisme van de moeder in de zwangerschap op de cardio-metabole gezondheid van het kind. Het maternale dieet is hierin ook een belangrijke factor, gezien de nauwe relatie met de glucosehuishouding en de mogelijkheid om het dieet aan te passen tijdens de zwangerschap. Vroege identificatie van vrouwen en hun kinderen met een verhoogd risico op complicaties is essentieel om goede zorg en tijdige interventies al rondom de conceptie te leveren die kunnen bijdragen aan een betere gezondheid van toekomstige generaties.

De doelen van dit proefschrift waren ten eerste, om de effecten van het maternaal glucosemetabolisme in de vroege zwangerschap op de cardio-metabole ontwikkeling van kinderen te onderzoeken; ten tweede om te identificeren of bepaalde maternale dieetfactoren tijdens de zwangerschap invloed hebben op geboorte-, en cardio-metabole uitkomsten van kinderen; en ten derde om deze maternale karakteristieken te vertalen naar predictiemodellen om vrouwen met een verhoogd risico op nadelige kind uitkomsten te kunnen identificeren al in de vroege zwangerschap. De studies in dit proefschrift zijn uitgevoerd binnen de Generation R Studie, een populatie cohortstudie in Rotterdam, Nederland. Binnen de Generation R Studie worden bijna 10,000 moeders en hun kinderen gevolgd vanaf de vroege zwangerschap tot in de adolescentie, met als doel om te onderzoeken welke factoren in het vroege leven invloed hebben op de groei, ontwikkeling en gezondheid van het kind. Hieronder wordt een kort overzicht geven van de individuele studies en resultaten van dit proefschrift.

In **Hoofdstuk 1** werd de achtergrond bij de studies van dit proefschrift beschreven, leidend tot de formulering van de studiedoelen van dit proefschrift.

Uit de studies in **Hoofstuk 2** concludeerden wij dat hogere glucosewaardes bij moeder in de vroege zwangerschap geassocieerd zijn met een suboptimaal glucosemetabolisme en cardiale ontwikkeling, maar niet met andere cardio-metabole risicofactoren bij hun kinderen. Onze resultaten in **Hoofdstuk 2.1** toonden aan dat hogere maternale glucosewaardes in de vroege zwangerschap, zelfs al onder de drempelwaarde voor de diagnose van zwangerschapsdiabetes, geassocieerd waren met een suboptimaal

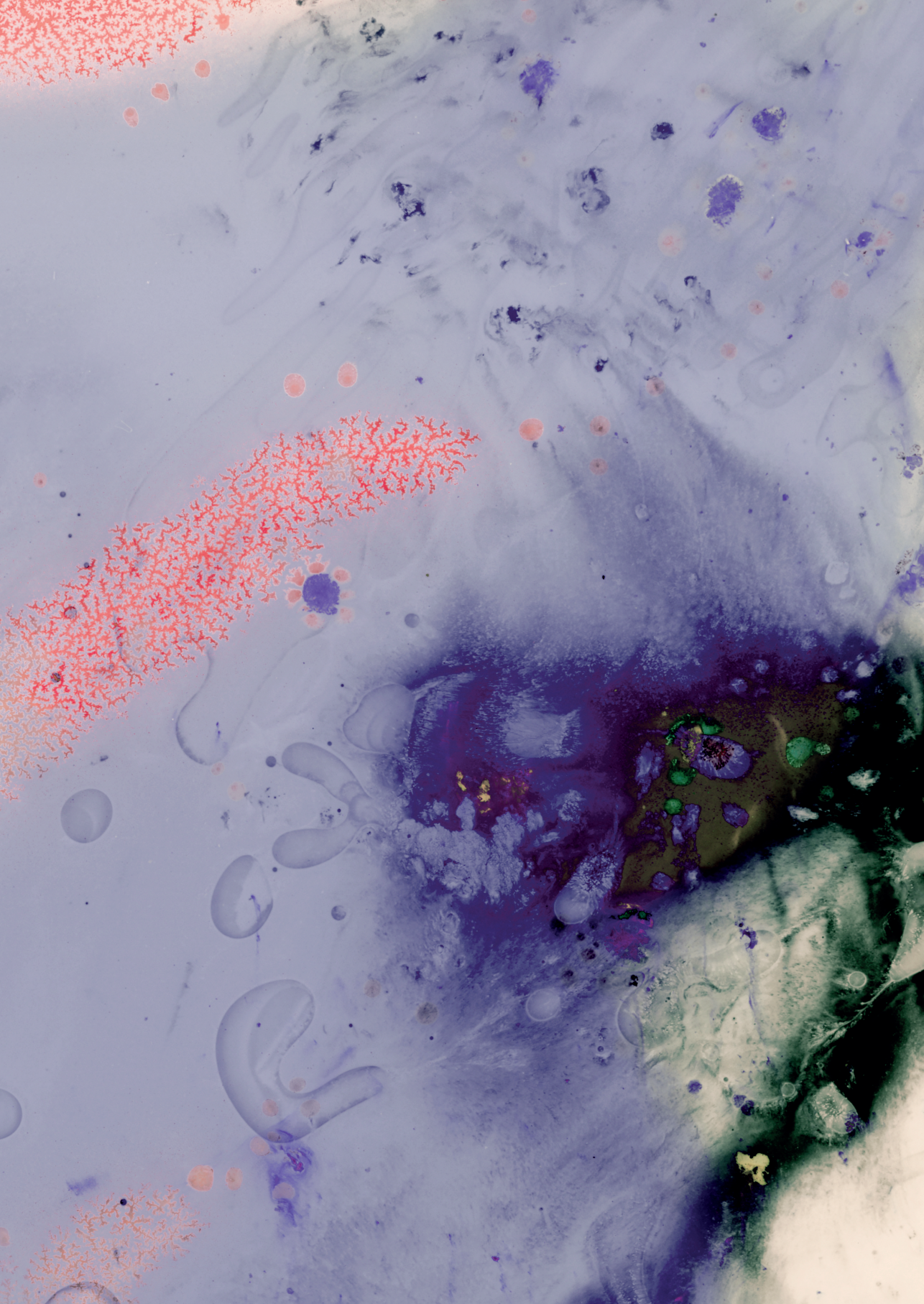
glucosemetabolisme op de kinderleeftijd. De associaties van maternale glucosewaardes in de vroege zwangerschap met de bloeddruk, vetmetabolisme en overgewicht van het kind werden verklaard door het gewicht van de moeder bij aanvang van de zwangerschap. De kinderen van moeders met hogere glucosewaardes tijdens de vroege zwangerschap hadden geen verhoogd visceraal vet, een belangrijke risicofactor voor het krijgen van hart-, en vaatziekten. In **Hoofdstuk 2.2** zagen wij geen associaties tussen glucosewaardes van de moeder tijdens de vroege zwangerschap met de leververvetting van het kind wanneer wij keken naar de totale multi-ethnische populatie. Wanneer wij ons alleen richtten op vrouwen met een Europese achtergrond, zagen wij wel associaties tussen hogere maternale glucose waardes in de vroege zwangerschap en een verhoogd risico op non-alcoholische leververvetting bij hun kinderen. In **Hoofdstuk 2.3** toonden wij aan dat hogere maternale glucosewaardes in de vroege zwangerschap zijn geassocieerd met de cardiale ontwikkeling bij hun kinderen. Met name de linker ventrikels van kinderen blootgesteld aan hogere glucosewaardes in de zwangerschap vertoonden structurele veranderingen.

Op basis van de studies in **Hoofdstuk 3** concludeerden wij dat het maternale dieet, gericht op de glycemische index en glycemische load inname en meervoudig onverzadigde vetzuur (PUFA) concentraties, mogelijk invloed heeft op de vetontwikkeling van het kind. De glycemische index en -load zijn maten die aangeven hoe de glucosewaardes in het bloed reageren op het eten van koolhydraten, en daarmee belangrijke determinanten van glucosewaardes in het bloed. PUFAs zijn essentiële voedingsstoffen die binnenkomen via vis en voedingssupplementen. De resultaten van de studies in **Hoofdstuk 3.1** lieten zien dat de glycemische index en -load in het dieet van de moeder tijdens de zwangerschap niet consistent geassocieerd waren met geboorte uitkomsten. In **Hoofdstuk 3.2** zagen wij dat een maternaal dieet met een hogere glycemische index en -load tijdens de zwangerschap wel geassocieerd was met de vetontwikkeling van het kind, met name met een toename van buikvet. Deze associaties waren alleen aanwezig onder vrouwen met overgewicht of obesitas bij aanvang van de zwangerschap. In **Hoofdstuk 3.3** observeerden wij dat maternaal lagere omega-3 en hogere omega-6 plasma concentraties tijdens de zwangerschap geassocieerd waren met een toename van levervet bij het kind. De associaties waren sterker bij jongens dan bij meisjes. Bij jongens waren hogere maternale omega-3 concentraties ook geassocieerd met een lager risico op non-alcoholische leververvetting op de kinderleeftijd.

In **Hoofdstuk 4** presenteerden wij klinische predictiemodellen om een risicoschatting kunnen geven voor verschillende geboorte uitkomsten onder vrouwen vooraf en in de vroege zwangerschap. In **Hoofdstuk 4.1** identificeerden wij een maternaal metaboliet profiel dat de predictie verbetert van een te hoog geboortegewicht

onder vrouwen met een hogere Body Mass Index (BMI) tijdens de zwangerschap. In **Hoofdstuk 4.2** ontwikkelden wij een predictiemodel om een gezonde zwangerschap te voorspellen onder vrouwen met overgewicht of obesitas tijdens de zwangerschap. Een gezonde zwangerschap omvatte in deze studie zowel de afwezigheid van korte termijn complicaties zoals zwangerschapsdiabetes of een te hoog geboortegewicht, als lange termijn complicaties zoals overgewicht bij het kind. Dit model includeerde maternale leeftijd, burgerlijke status, BMI, gewichtstoename tot het tweede trimester, bloeddruk en foetaal gewicht halverwege de zwangerschap als voorspellers voor een gezonde zwangerschap. Het vermogen van het ontwikkelde predictiemodel om een gezonde uitkomst te voorspellen was redelijk nauwkeurig. In tegenstelling tot onze eerdergenoemde studie gefocust op een te hoog geboortegewicht, zagen we bij dit predictiemodel geen verbetering van de voorspelling van een gezonde zwangerschap wanneer wij het maternaal metaboliet profiel toevoegen. Ook paternale karakteristieken verbeterden het voorspellend vermogen van dit model niet. In **Hoofdstuk 4.3** ontwikkelden wij een predictiemodel voor de voorspelling van geboortecomplicaties onder vrouwen op populatieniveau, zonder een vooraf selectie op hun gewicht tijdens de zwangerschap. Dit model werd ontwikkeld voor de voorspelling van de meest voorkomende geboortecomplicaties, namelijk prematuriteit, te laag en te hoog geboortegewicht, samen verantwoordelijk voor complicaties in bijna een kwart van alle zwangerschappen. Ook andere complicaties rondom de geboorte werden met dit model goed voorspeld, zoals een keizersnede bevalling of een lage Apgar score. In dit model waren maternale leeftijd, etniciteit, pariteit, BMI en het roken bij aanvang van de zwangerschap de sterkste voorspellers voor geboortecomplicaties.

Tenslotte plaatsten wij in **Hoofdstuk 5** de resultaten van de studies in dit proefschrift in een bredere context van de bestaande literatuur en de huidige gezondheidszorg. We bespraken hier de methodologische overwegingen van de uitgevoerde studies, en gingen in op de klinische implicaties en geven aanbevelingen voor toekomstig onderzoek. Deze bevindingen in dit proefschrift dragen bij aan de vroege identificatie van vrouwen die baat hebben bij tijdige preventieve strategieën in de zwangerschap om de cardio-metabole gezondheid van kinderen in toekomstige generaties te bevorderen.



07

Appendices

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LIST OF PUBLICATIONS

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Wahab RJ, Beth SA, Derks IPM, Jansen PW, Moll HA, Kiefte-de Jong JC. Celiac disease autoimmunity and emotional and behavioral problems in childhood. Pediatrics 2019 Oct;144(4):e20183933.

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Wahab RJ, Jaddoe VWV, Van Klaveren D, Vermeulen MJ, Reiss IKM, Steegers EAP, Gaillard R. Risk prediction of common birth complications in preconception and early pregnancy: development of a prediction tool within a population-based prospective cohort. Submitted.

Wahab RJ, Jaddoe VWV, Mezzoiuso AG, Gaillard R. Maternal polyunsaturated fatty acid concentrations during pregnancy and childhood liver fat accumulation. Submitted.

Co-author

Al-Hassany L, **Wahab RJ**, Steegers EAP, Jaddoe VWV, Gaillard R. Smoking cessation in early-pregnancy, gestational weight gain and subsequent risks of pregnancy complications. Eur J Obstet Gynecol Reprod Biol. 2020 Jul 25;253:7-14.

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Geurtsen ML, **Wahab RJ,** Felix JF, Gaillard R, Jaddoe VWV. Early life prediction of child non-alcoholic fatty liver disease. In preparation.

PHD PORTFOLIO

Summary PhD training and teaching activities

Name PhD student:	Rama Wahab
Erasmus MC Department:	Pediatrics
Research School:	Netherlands Institute for Health Sciences
PhD period:	June 2018 – June 2021
Promotor:	Prof. Dr. Jaddoe
Supervisor:	Dr. Gaillard

1. PhD Training	Year	Workload (ECTS)
General courses		
Master's degree Health sciences, specialization Clinical Epidemiology, NIHES, Erasmus University Rotterdam, the Netherlands	2018-2020	70
General academic skills		
EndNote	2018	0.3
Systematic Literature Retrieval	2018	0.3
Good clinical practice	2019	0.3
Basic Course on R	2019	0.3
Scientific integrity course	2020	0.3
Seminars and workshops		
Generation R research meetings, Erasmus MC, The Netherlands	2018-2021	1.0
Generation R mother and child meetings, Erasmus MC, The Netherlands	2018-2021	1.0
ACE Pregnancy & Childhood monthly meetings	2018-2020	1.0
2nd Annual Conference Lifestyle as Medicine, Oegstgeest, The Netherlands	2018	0.2
National and international conferences		
Nederlands Vereniging voor Kindergeneeskunde congres 2019 – '*The influence of maternal glucose concentrations in early-pregnancy on cardio-metabolic risk factors in childhood*' – Oral presentation	2019	0.7
European and international congress on obesity 2020 '*The influence of maternal glucose concentrations in early-pregnancy on cardio-metabolic risk factors in childhood*' – Oral presentation	2020	1.4

1. PhD Training	Year	Workload (ECTS)
European and international congress on obesity 2020 '*Maternal early-pregnancy dietary glycemic index and load, fetal growth and the risk of adverse birth outcomes'* – Oral presentation	2020	0.7
Diabetes in pregnancy study group meeting 2020 *'Maternal dietary glycemic index during pregnancy and childhood adiposity'* - Oral presentation	2020	1.4
Diabetes in pregnancy study group meeting 2020 '*Maternal Early-pregnancy Glycemia and alterations of Cardiac Structure and Function in Childhood*'- Oral presentation	2020	0.7
Diabetes in pregnancy study group meeting 2020 *'Maternal early-pregnancy dietary glycemic index and load and fetal growth and birth characteristics'* - Oral presentation	2020	0.7
Precision Diabetes Medicine conference 2021 '*Maternal Body Mass Index, early-pregnancy metabolite profile and birth weight*' – Poster presentation	2021	0.7
Sophia Research Day 2021 '*Risk prediction for birth complications in preconception and early pregnancy: development of a prediction model within a population-based*' – SLAM presentation	2021	0.7
Pediatric Academic Societies meeting 2021 '*Risk prediction for birth complications in preconception and early pregnancy: development of a prediction model within a population-based*' – Highlighted poster presentation and participation in discussion session	2021	1.4
Other		
Reviewed articles for BJOG, prenatal diagnosis, PLOS ONE, American Journal of Clinical Nutrition, Clinical Nutrition, IJME, International Journal of Obesity, European Journal of Epidemiology	2019-2021	1.0
Collaboration in project with pediatric endocrinology department '*Age of onset of obesity and childhood BMI trajectories in rare genetic obesity disorders*'	2020-2021	1.0

2. Teaching	Year	Workload (ECTS)
Supervising Master's theses		
Lina Al-Hassany, Clinical Epidemiology, NIHES. Project title: *Smoking cessation in early-pregnancy, gestational weight gain and subsequent risks of pregnancy complications*	2019-2020	4.0
Judith Scholing, Vrije Universiteit. Project title: *Maternal early pregnancy dietary glycemic index and load, fetal growth, and the risk of adverse birth outcomes.*	2019	2.0
Angelo Mezzoiuso. Project title: *Associations of maternal plasma PUFA levels during pregnancy with childhood fat accumulation*	2020	2.0

3. Other	Year	Workload (ECTS)
Promeras Board Member – *Representative for PhD students in the Erasmus MC*	2019-2021	4.0

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About the author

Rama Wahab was born on March 17th 1993 in Nijmegen, the Netherlands. In 2011, she started medical school at Leiden University. In the second year of her studies, she started as a research assistant at the department of pediatrics under the supervision of Prof. dr. M.L. Mearin. In 2015, she completed her research internship on coeliac disease in the same department. Having become enthusiastic about doing research, she decided to do a second research internship at the department of pediatrics in the Sophia Children's Hospital, within the Generation R study and under the supervision of Prof. dr. Kiefte-de Jong. Besides research, she followed extracurricular courses in philosophy, art history and literature sciences at Leiden University and on an Erasmus Exchange at Trinity College Dublin, Ireland. After obtaining her medical degree in 2018, she started as a PhD student at the Generation R study under the supervision of Dr. R. Gaillard and Prof. dr. V.W.V. Jaddoe. The focus of her research was on the effects of maternal glucose metabolism in early pregnancy on offspring health outcomes in offspring. The results of this research are presented in this thesis. During her PhD, she acquired a master degree in Clinical Epidemiology at the Erasmus University. She is looking forward to start her clinical experiences in pediatrics early next year.

