Cardiovascular health in pregnant women and their children

The Generation R Study

Romy Gaillard

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The Generation R Study

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Het Generation R Onderzoek

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Chapter 1

General introduction

General introduction

Introduction

Cardiovascular disease is a major public health problem in the general adult population.[1] Cardiovascular disease leads to over 17.3 million deaths per year and is the leading cause of death and disability worldwide.[1] In the Netherlands, cardiovascular disease accounts for approximately 30% of current mortality rates among men and women.[2] Because of the clinical impact that cardiovascular and metabolic diseases have at older ages, research into related risk factors has mostly been focused on adults. However, in the last decades, an accumulating body of evidence suggested that cardiovascular health in younger age groups also has major long-term public health implications.

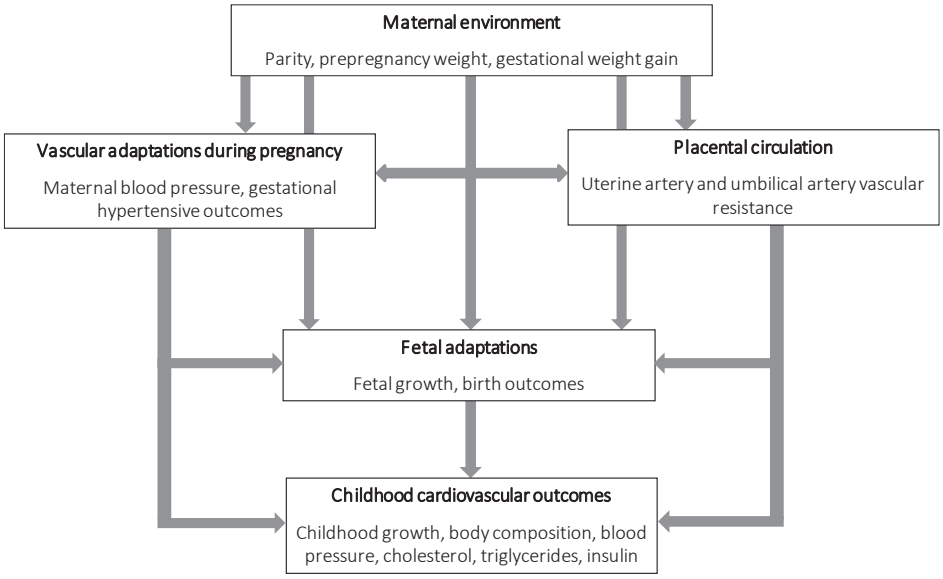
First, cardiovascular health status of women of reproductive age may complicate pregnancy. Obesity and insulin resistance are associated with increased risks of infertility in women.[3] During pregnancy, important adaptions occur in the maternal circulation and metabolism to meet the increased metabolic demands of the mother and fetus. Cardiovascular adaptations include an initial fall in systemic vascular tone, an increase in cardiac output and an expansion of plasma volume, which subsequently leads to gradual lowering of the systolic and diastolic blood pressure until mid-pregnancy and a rise from mid-pregnancy to delivery. Pregnancy also leads to adaptions in maternal glucose homeostasis and higher maternal cholesterol levels. Normally, these adaptations result in a better placental perfusion and nutrient supply to the fetus. However, suboptimal adaptations, which might occur due to adverse maternal cardiovascular health status, may lead to increased risks of pregnancy complications.[4] Suboptimal hemodynamic adaptions may lead to gestational hypertensive disorders, whereas suboptimal glucose metabolism adaptations may lead to gestational diabetes. Maternal obesity, hypertension and diabetes during pregnancy are associated with increased risks of maternal and perinatal mortality and morbidity.[5-7] Also, women who suffered from pregnancy complications have higher risks of cardiovascular disease and type 2 diabetes many decades after their pregnancy.[8-10]

Second, maternal cardiovascular health during pregnancy may have long-term offspring consequences. Maternal gestational hypertensive disorders are associated with increased risks of delivering preterm and small size for gestational age infants[6], whereas gestational diabetes leads to higher risks of delivering large size for gestational age infants and neonatal hypoglycemia.[11] Studies have demonstrated that these associations are not only present in the extremes of maternal disease status, but also across the full range of maternal cardiovascular health status.[12,13] Large-scale epidemiological studies have also shown that children born with a low birth weight have higher risks of cardiovascular disease and type 2 diabetes in adulthood.[14-18] Based on these findings, it has been hypothesized that adverse exposures, acting at different stages of fetal and early postnatal development, lead to permanent adaptations in the structure, physiology and function of various organ systems. This early programming contributes to short-term survival, but increases the susceptibility of cardiovascular and metabolic diseases

in later life.[19] This hypothesis is not only supported by these observational studies showing that both low and high birth weight are associated with the risk of obesity, cardiovascular disease and type 2 diabetes in adulthood, but also by various mechanistic animal studies.[19,20] Thus, previous research suggests that both restricted and excessive nutritional in utero environments may lead to cardiovascular disease in later life.

In summary, cardiovascular health and disease in pregnant women and their children is important for clinically relevant, adverse short-term and long-term health outcomes. Identifying factors influencing cardiovascular health in pregnant women and their children, may help to develop future preventive strategies that improve cardiovascular health throughout the life course and in future generations. Therefore, studies presented in this thesis were designed to identify maternal, placental and fetal factors and critical developmental periods during pregnancy associated with cardiovascular health outcomes in mothers and children (**Figure 1.1**).

Figure 1.1. Overview of the hypotheses for the associations of maternal, placental and fetal factors with cardiovascular health in pregnant women and children studied in this thesis



Maternal factors and critical periods during pregnancy

Various maternal socio-demographic and lifestyle related characteristics have been associated with adverse maternal and fetal pregnancy outcomes. These factors include, among others, maternal educational level, dietary factors and smoking during pregnancy. Not much is known about the role of maternal physical factors at the start of pregnancy. Therefore, the studies presented in this thesis are focused on the associations of maternal parity and weight throughout pregnancy with maternal and childhood cardio-

vascular development. We also studied the role of placental hemodynamic function and fetal growth in relation to pregnancy complications and childhood outcomes.

Maternal parity

Maternal nulliparity is an important risk factor for maternal pregnancy complications, such as pre-eclampsia.[21] Also, it is well-known that children from nulliparous mothers are smaller than children from multiparous mothers.[22] It has been suggested that the difference in birth weight between firstborn and second-born children is approximately 200 grams, which is of similar magnitude as the influence of maternal smoking during pregnancy on birth weight. Among multiparous mothers only, there is a much smaller increase in birth weight with each following pregnancy.[22] The mechanisms underlying these associations are largely unknown, but may involve permanent adaptations in the maternal vasculature following pregnancy, which leads to a more favorable environment for both placental development and fetal nutrition in consecutive pregnancies.[23] The long-term consequences of maternal nulliparity for cardiovascular health of offspring remain unclear. As in Western countries, there is a high percentage of one-child families, maternal nulliparity may be an important risk factor for maternal and fetal pregnancy complications and adverse cardiovascular health outcomes in the offspring.[24]

Maternal prepregnancy body mass index and gestational weight gain

Overweight and obesity, defined as a body mass index (BMI) ≥25 kg/m^2 and BMI ≥30 kg/m^2, respectively, are common in both Western and non-Western countries.[25] Worldwide, the prevalence of overweight and obesity has nearly doubled in the last 20 years.[25] The strong increase in overweight and obesity prevalences is also present among women of reproductive age and children.[26] Maternal prepregnancy obesity is an important risk factor for maternal and fetal pregnancy complications and for childhood obesity.[27,28] It has been suggested that a maternal obesogenic environment during pregnancy leads to higher maternal plasma concentrations of glucose, amino acids and free fatty acids with increased placental transfer of nutrients during fetal development. This might cause permanent changes in appetite, energy metabolism and neuroendocrine function of offspring, predisposing an individual to a greater risk of obesity and cardiovascular disease in later life.[29] In line with this hypothesis, both epidemiological studies and animals studies have shown that maternal gestational diabetes and prepregnancy obesity are associated with higher fetal growth rates and higher birth weight, and increased risks of obesity in the offspring.[29] However, it remains unclear whether these associations are also present across the whole range of maternal prepregnancy body mass index. Next to maternal obesity at the start of pregnancy, weight gain during pregnancy may also affect maternal and childhood outcomes.[30-33] Gestational weight gain is a complex trait, which reflects multiple components including maternal nutritional status, tissue expansion due to fat storage and fluid expansion, and growth of fetus, placenta and uterus.[30] Not much is known about the effects of gestational weight

gain, independent of maternal prepregnancy body mass index, or about critical periods of gestational weight gain on maternal and childhood outcomes. As both maternal prepregnancy body mass index as well as gestational weight gain may be important modifiable factors for improving maternal health and health of offspring, obtaining a better understanding of these associations and their underlying mechanisms is of importance for preventive strategies.

Placental vascular function

The placenta forms the active interface between the maternal and fetal blood circulations and regulates both maternal physiological changes during pregnancy as well fetal nutrient supply and fetal development. To meet the increasing demands of the rapidly developing fetus, changes in the placental vasculature occur during pregnancy. Maternal blood enters the intervillious space in the placenta through the spiral arteries, which descend from the uterine arteries. Normally, during early pregnancy, the spiral arteries are remodeled due to trophoblastic invasion, which changes the spiral arteries from narrow muscular vessels into wide non-muscular arteries. Together with other maternal hemodynamic adaptations, this leads to the development of a high-flow and low-resistance circulation. On the fetal side, blood enters the placenta through the umbilical arteries, which form a capillary network in the terminal villi of the villous tree, which floats freely in the maternal blood in the intervillous space. The fetal villous and capillary surface areas increase during pregnancy to allow sufficient blood flow for the developing fetus. Impaired placentation leading to abnormal placental perfusion and placental damage may be a key factor in the development of pre-eclampsia and intrauterine fetal growth restriction.[6,34] Suboptimal placental growth and function may also persistently influence growth and cardiovascular function in later life.[35] Previous studies among adults suggested associations of both low and high placental weight with adverse cardiovascular outcomes in later life, but results are not consistent.[36] Placental weight is only a crude measure of placental growth and more detailed measures of placental function, assessed during pregnancy, might give further insight in long-term consequences of placental dysfunction.

Fetal and early childhood growth

Low and high birth weight are associated with cardiovascular disease in adulthood.[14-18] Birth weight is unlikely to be a causal factor per se leading to cardiovascular disease in later life. Birth weight is merely an end-point of different fetal exposures and growth patterns, and the starting point of childhood growth. Longitudinal studies showed that the risk of cardiovascular disease is highest among adults who were born with a low birth weight and had a high postnatal weight gain.[37,38] Thus, these studies suggest that there may be critical periods of growth in fetal and early postnatal life that influence the development of cardiovascular disease in later life. From a biological and preventive

perspective, it is of great importance to identify these specific critical periods for fetal and childhood growth.

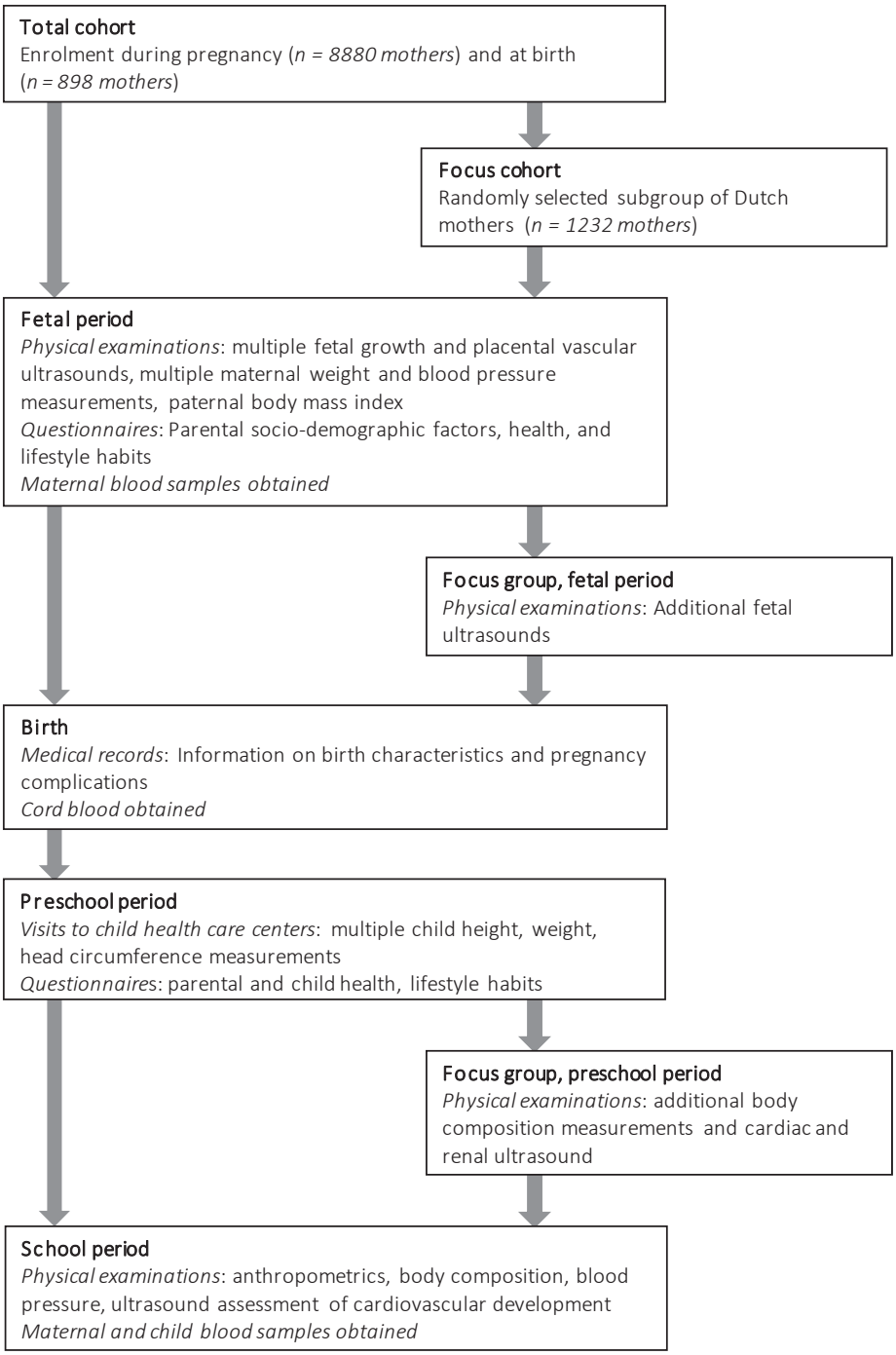
General aim of this thesis

The general aim of this thesis was to identify maternal, placental and fetal factors leading to adverse cardiovascular outcomes in pregnant women and their children.

General design

The studies presented in this thesis were embedded in the Generation R Study, a population based prospective cohort study from fetal life until young adulthood in Rotterdam, The Netherlands.[39] The Generation R Study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life and childhood. All pregnant women living in the study area with a delivery date between April 2002 and January 2006 were eligible for enrolment in this study. Enrolment was aimed at early pregnancy, but was possible until the birth of the child. In total, 9778 mothers were enrolled in the study, of whom 8880 (91%) were included during pregnancy (**Figure 1.2**). Assessments were planned in early pregnancy (<18 weeks of gestation), mid-pregnancy (18 - 25 weeks of gestation) and late pregnancy (≥25 weeks of gestation), and included parental physical examinations, maternal blood and urine collection, fetal ultrasound examinations, and self-administered questionnaires. In the preschool period, from birth to 4 years of age, data collection was performed in all children by questionnaires and visits to the routine child health care centers. All children were invited to a dedicated research center in the Erasmus MC – Sophia Children's Hospital to participate in detailed body composition and cardiovascular follow-up measurements at the age of 6 years. Measurements during this visit included anthropometrics, body composition, cardiovascular development and body fluid specimen collection.

Figure 1.2. Design and data collection in the Generation R Study



Outline of this thesis

The objectives are addressed in several studies presented in this thesis. In Chapter 2, studies on maternal influences on maternal and childhood outcomes are described. In Chapter 2.1, we examined whether maternal blood pressure tracks during pregnancy, and whether this tracking is influenced by maternal characteristics and associated with the risk of gestational hypertensive disorders. The influence of maternal parity on maternal pregnancy-related hemodynamic adaptations, placental vascular function and pregnancy complications, and childhood cardiovascular development is described in Chapter 2.2 and Chapter 2.3, respectively. We studied the associations of maternal prepregnancy body mass index and weight gain during pregnancy with maternal pregnancy-related hemodynamic adaptions and the risk of pregnancy complications, and childhood cardiovascular development in Chapter 2.5, 2.6, 2.7, respectively.

In Chapter 3, we present studies focused on the associations of placental hemodynamic function and fetal growth with maternal and childhood outcomes. The influence of placental hemodynamic function on maternal and fetal pregnancy complications and cardiovascular development in childhood is studied in Chapter 3.1 and Chapter 3.2, respectively. In Chapter 3.3, we examined whether fetal growth characteristics track during pregnancy and are associated with the risk of adverse birth outcomes. Chapter 3.4 describes the association of first trimester fetal growth restriction with cardiovascular development in childhood.

Finally, Chapter 4 provides a general discussion in which the studies described in this thesis are described in broader context, and implications and suggestions for future research are discussed.

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Chapter 2

Maternal influences

Chapter 2.1

Blood pressure tracking and the risks of gestational hypertensive disorders

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Adapted from Eur Heart J. 2011;32(24):3088-97

Abstract

Aims: Blood pressure tracking can be used to examine the predictability of future values by early measurements. In a population-based prospective cohort study, among 8482 pregnant women, we examined whether blood pressure in early pregnancy tracks to third trimester and whether this tracking is influenced by maternal characteristics and is associated with the risk of gestational hypertensive disorders.

Methods and results: Blood pressure was measured in each trimester of pregnancy. Information about doctor-diagnosed pregnancy-induced hypertension and pre-eclampsia was obtained from medical records. Correlation coefficients between first and third trimester for systolic and diastolic blood pressure were 0.47 and 0.46, respectively. The Odds Ratio (OR) for staying in the highest tertile from first to third trimester for systolic blood pressure was 3.09 (95% Confidence Interval (CI): 2.73, 3.50) and for diastolic blood pressure 3.28 (95% CI: 2.90, 3.69). Blood pressure tracking coefficients were lower in younger, shorter, and non-European women and in women with higher gestational weight gain. Systolic and diastolic blood pressure changes from second to third trimester, but not from first to second trimester, were positively associated with the risks of pregnancy-induced hypertension and pre-eclampsia.

Conclusions: Blood pressure tracks moderately during pregnancy and is influenced by maternal characteristics. Second to third trimester increases in systolic and diastolic blood pressure are associated with an increased risk of gestational hypertensive disorders.

Introduction

Gestational hypertensive disorders complicate about 7% of all pregnancies and are associated with increased risks of both maternal and perinatal morbidity and mortality.[1,2] Blood pressure measurement is an important screening test used in obstetric care to detect or predict gestational hypertensive disorders.[2] However, the predictive accuracy of blood pressure measurement in early pregnancy still remains controversial.[3,4] A review among 34 studies showed that in first and second trimester, systolic and diastolic blood pressure predicted pre-eclampsia poorly.[3] This review compiled many studies with major methodological differences. The examined populations varied widely in their a priori risk of pre-eclampsia and blood pressure was measured at very different timepoints in pregnancy. Also, many studies used different definitions of gestational hypertensive disorders.[5] Some studies suggested that blood pressure development differs between pregnancies uncomplicated and complicated by gestational hypertensive disorders and that small differences in blood pressure development may already occur in the first half of pregnancy.[4,6]

Tracking is used to describe the longitudinal development of a variable and focuses on the maintenance of one's relative position in a distribution of values over time.[7,8] Tracking can also be used to examine the predictability of future values by early measurements.[7,8] Examining tracking during pregnancy might give further insight in the predictive value of blood pressure measurement early in pregnancy. However, to the best of our knowledge, not much is known about blood pressure tracking during pregnancy.

Therefore, we examined in a population-based prospective cohort study among 8482 pregnant women, whether blood pressure in early pregnancy tracks to third trimester, and whether this tracking is influenced by maternal characteristics and is associated with the risk of gestational hypertensive disorders.

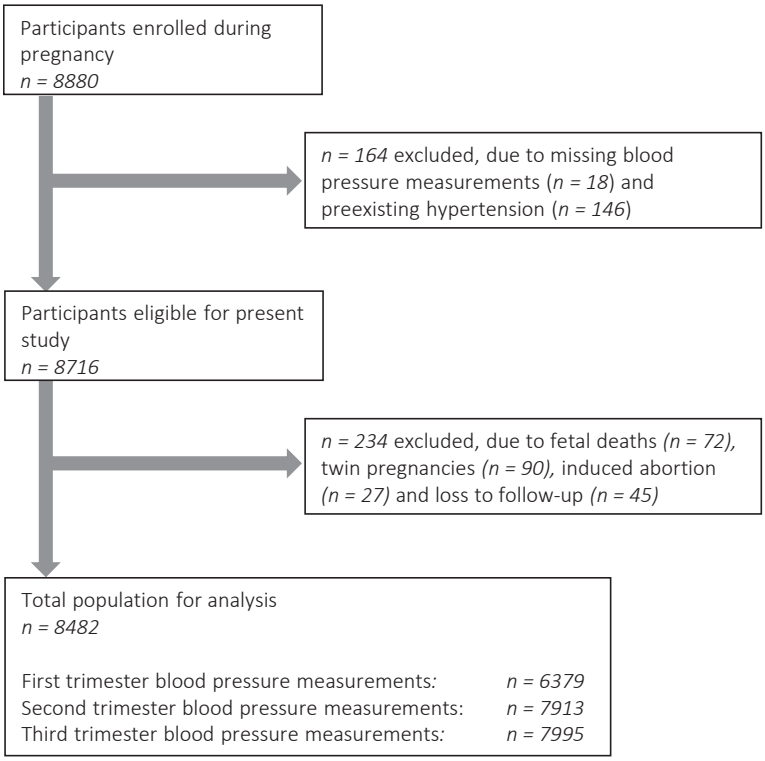
Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards based in Rotterdam, the Netherlands.[9,10] The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center in Rotterdam (MEC 198.782/2001/31). Written consent was obtained from all participating women. Assessments during pregnancy were planned in first, second, and third trimester. The individual timing of these assessments depended on the gestational age at enrolment. In total, 8880 women were enrolled during pregnancy. For the present study, we excluded women without any blood pressure measurement (*n = 18*). Also, we excluded women with pre-existent hypertension (*n = 146*) and pregnancies leading to fetal death (*n = 72*), induced abortion (*n = 27*), loss to follow-up (*n = 45*), and

twin pregnancies (*n = 90*). Thus, the cohort for analysis comprised 8482 pregnant women (**Figure 2.1.1**).

Figure 2.1.1. Flow chart of the participants



Blood pressure

Blood pressure was measured with the validated Omron 907® automated digital oscillometric sphygmanometer (OMRON Healthcare Europe B.V. Hoofddorp, the Netherlands).[11] All participants were seated in upright position with back support, and were asked to relax for 5 minutes. A cuff was placed around the non-dominant upper arm, which was supported at the level of the heart, with the bladder midline over the brachial artery pulsation. In case of an upper arm exceeding 33 cm, a larger cuff (32 – 42 cm) was used. The mean value of 2 blood pressure readings over a 60s interval was documented for each participant. In total, blood pressure was measured in 6379 women in first trimester (median 13.2 weeks of gestation, 95% range 9.8 – 17.6), in 7913 women in second trimester (median 20.4 weeks of gestation, range 18.5 – 23.6), and in 7995 women in third trimester (median 30.2 weeks of gestation, 95% range 28.4 – 32.9). For the analysis, 22.287 blood pressure measurements were available. Three, two, and one

blood pressure measurements were available for 5857, 2091, and 534 women, respectively.

Pregnancy-induced hypertension and pre-eclampsia

Information on pregnancy complications was obtained from medical records. Women suspected of pregnancy complications based on these records were crosschecked with the original hospital charts. Details of these procedures have been described elsewhere.[12]

Briefly, the following criteria were used to identify women with pregnancy-induced hypertension: development of systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24 h urine collection containing at least 300 mg of protein) were used to identify women with pre-eclampsia.[13] Information on pregnancy complications was available for 8236 women.

Covariates

Gestational age was established by fetal ultrasound examination during the first ultrasound visit.[10] Maternal age was assessed at enrolment. During visits in first, second, and third trimester, maternal anthropometrics were measured at one of the research centers. Height (cm) and weight (kg) were measured without shoes and heavy clothing and body mass index (kg/m^2) was calculated for each pregnancy period. We defined gestational weight gain as the difference between weight before pregnancy and weight in third trimester. Information on educational level, ethnicity, and parity was obtained at enrolment. Information about smoking, alcohol consumption, and caffeine intake was assessed by questionnaires in each trimester.[10]

Statistical analysis

First, we analyzed the longitudinal systolic and diastolic blood pressure patterns in women with uncomplicated pregnancies and women with pregnancies complicated by hypertensive disorders using unbalanced repeated measurement regression models. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data.[14] Using fractional polynomials of gestational age, the best-fitting models were constructed. For this analysis, we categorized women in three categories: uncomplicated pregnancy, pregnancy-induced hypertension, and pre-eclampsia. The categories were included in these models as intercept and as an interaction term with gestational age.

To examine whether women maintain their position in the distribution of blood pressure (tracking), we estimated the Pearson's correlation coefficients and categorized systolic blood pressure, diastolic blood pressure, and mean arterial pressure in tertiles

in first and third trimester. We used logistic regression models to calculate the Odds Ratio (OR) to remain in the same blood pressure tertile from first to third trimester. Next, we examined whether maternal characteristics influence blood pressure tracking. We categorized each maternal characteristic and for each category we estimated Pearson's correlation coefficients and blood pressure tracking coefficients using linear regression models. We further examined the associations of blood pressure change during pregnancy with the risks of pregnancy-induced hypertension and pre-eclampsia using multiple logistic regression models.

These models were adjusted for gestational age at intake, gestational age at each pregnancy period, maternal age, educational level, parity, ethnicity, prepregnancy body mass index, gestational weight gain, smoking habits, alcohol consumption, and caffeine intake. Missing data of the covariates were imputed using multiple imputation. The percentages of missing values within the population for analysis were lower than or equal to 15%, except for prepregnancy body mass index (19.4%) and gestational weight gain (23.1%). The repeated measurement analysis was performed using the Statistical Analysis System version 9.2 (SAS, Institute Inc., Cary, NC, USA), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). P-values are two-tailed. All presented Confidence Intervals (CIs) are calculated at the 95% level.

Results

Subject characteristics

Table 2.1.1 shows that, of all women, 306 women developed pregnancy-induced hypertension and 168 women developed pre-eclampsia. Women who developed pregnancy-induced hypertension and pre-eclampsia were more often nulliparous and had a higher prepregnancy body mass index. From first trimester onwards systolic blood pressure, diastolic blood pressure, and mean arterial pressure were higher for women who developed pregnancy-induced hypertension and pre-eclampsia in later pregnancy (Table 2.1.2).

Table 2.1.1. Subject characteristics by pregnancy health (*n = 8236*)[1]

Characteristics	Non-hypertensive complicated pregnancy *n = 7762*	Pregnancy-induced hypertension *n = 306*	Pre-eclampsia *n = 168*	P-value[3]
Age (yrs)	29.7 (5.3)	30.0 (5.1)	28.8 (5.3)	0.086
Height (cm)	167.1 (7.4)	168.6 (7.2)	165.7 (7.3)	0.001
Weight (kg)	65.5 (12.0)	74.9 (18.4)	68.5 (15.0)	0.001
Prepregnancy body mass index (kg/m^2)	23.4 (4.1)	26.3 (6.2)	24.8 (5.3)	0.001
Gestational weight gain (kg)	10.4 (5.0)	11.5 (6.9)	10.6 (6.5)	0.007
Parity (% nulliparous)	53.9	74.5	78.0	0.001
Gestational age at intake (wks)[2]	14.5 (10.4, 28.9)	13.7 (9.5, 24.0)	14.6 (10.3, 24.4)	0.011
Highest completed education (%)				
Primary school	10.6	7.8	12.5	0.016
Secondary school	41.7	48.4	49.4	
Higher education	38.7	39.2	28.0	
Missings	9.1	4.6	10.1	
Ethnicity (%)				
European	52.7	70.3	47.6	0.001
Non-European	39.7	26.8	44.6	
Missings	7.6	2.9	7.7	
Alcohol consumption (%)				
No	42.5	40.5	47.6	0.241
Yes	43.4	48.7	41.1	
Missings	14.1	10.8	11.3	
Smoking habits (%)				
No	63.8	63.7	63.7	0.527
Yes	21.6	25.2	22.6	
Missings	14.5	11.1	13.7	
Caffeine intake (%)				
No	4.3	3.6	4.2	0.797
Yes	87.4	91.2	85.7	
Missings	8.3	5.2	10.1	

[1]Values are means (standard deviation) or percentages. [2]Median (95% range). [3]Differences in subject characteristics between the groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.

Table 2.1.2. Blood pressure levels during pregnancy (*n = 8236*)[1]

Pregnancy period	Non-hypertensive complicated pregnancy *n = 7762*	Pregnancy-induced hypertension *n = 306*	Pre-eclampsia *n = 168*	P-value[2]
First trimester				
Systolic blood pressure (mmHg)	114.7 (11.8)	124.1 (12.3)	119.7 (12.4)	0.001
Diastolic blood pressure (mmHg)	67.5 (9.0)	75.7 (10.1)	72.7 (10.2)	0.001
Mean arterial pressure (mmHg)	83.2 (8.9)	91.8 (9.8)	88.3 (9.9)	0.001
Second trimester				
Systolic blood pressure (mmHg)	115.8 (11.6)	126.2 (12.3)	120.9 (12.9)	0.001
Diastolic blood pressure (mmHg)	66.4 (8.9)	75.9 (9.2)	73.4 (9.4)	0.001
Mean arterial pressure (mmHg)	82.9 (8.8)	92.6 (9.1)	89.2 (9.5)	0.001
Third trimester				
Systolic blood pressure (mmHg)	117.4 (11.6)	128.8 (12.9)	124.9 (13.1)	0.001
Diastolic blood pressure (mmHg)	68.2 (8.8)	79.1 (9.7)	76.7 (9.4)	0.001
Mean arterial pressure (mmHg)	84.6 (8.6)	95.7 (9.5)	92.8 (9.4)	0.001

[1]Values are means (standard deviation). [2]Differences in blood pressure levels between the groups were evaluated using one-way ANOVA tests.

Longitudinally measured blood pressure and gestational hypertensive disorders

Figure 2.1.2 shows the systolic and diastolic blood pressure development during pregnancy. Systolic blood pressure was higher from first trimester onward in women who developed pregnancy-induced hypertension and pre-eclampsia. The steepest increase in systolic blood pressure was observed in women who developed pre-eclampsia. Diastolic blood pressure showed a mid-pregnancy dip, with an increase thereafter in pregnant women without hypertensive disorders. In women with pregnancies complicated by pregnancy-induced hypertension and pre-eclampsia, a minor dip was observed in early pregnancy. Diastolic blood pressure was the highest throughout pregnancy for women who developed pregnancy-induced hypertension, but the steepest increase in diastolic blood pressure was observed for women who developed pre-eclampsia. The exact regression coefficients for gestational age-independent (intercept) and gestational age-dependent differences (interaction hypertensive complication and gestational age) are given in the Supplementary Material Table S2.1.1.

Figure 2.1.2. Blood pressure patterns in uncomplicated and complicated pregnancies

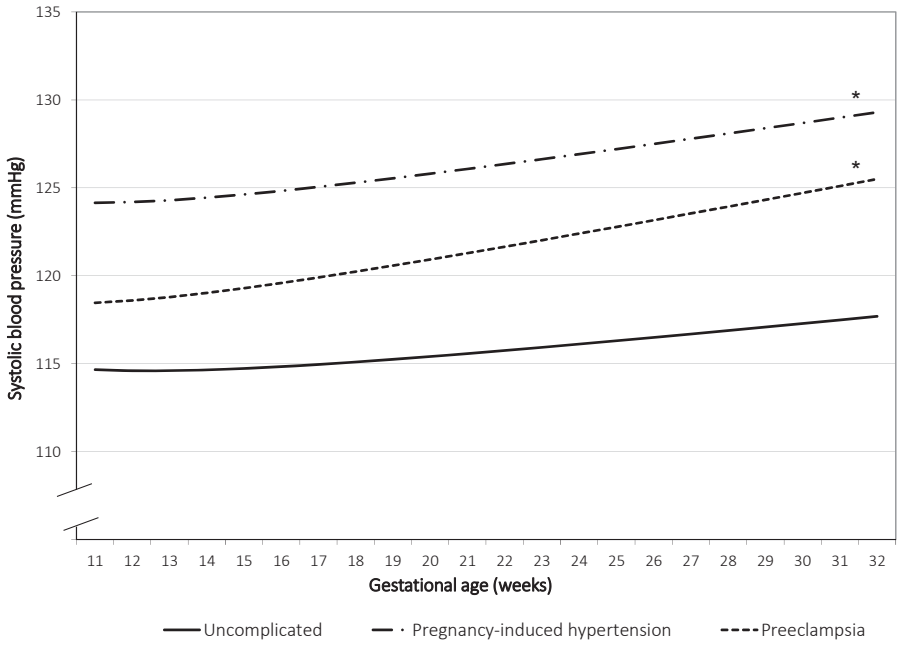


Figure 2.1.2a. Systolic blood pressure

Change in systolic blood pressure in mmHg for women with a pregnancy complicated by pregnancy-induced hypertension and women with a pregnancy complicated by pre-eclampsia compared with women with an uncomplicated pregnancy based on repeated measurement analysis (systolic blood pressure = ß0 + ß1 × hypertensive complication + ß2 × gestational age + ß3 × gestational age^{-2} + ß4 × hypertensive complication × gestational age). P-value reflects the significance level of ß4, which reflects the difference in change in blood pressure per week per pregnancy hypertensive complication, when compared with uncomplicated pregnancies. Estimates are given in Supplementary material Table S2.1.1. *P <0.05.

Figure 2.1.2. Blood pressure patterns in uncomplicated and complicated pregnancies (*continued*)

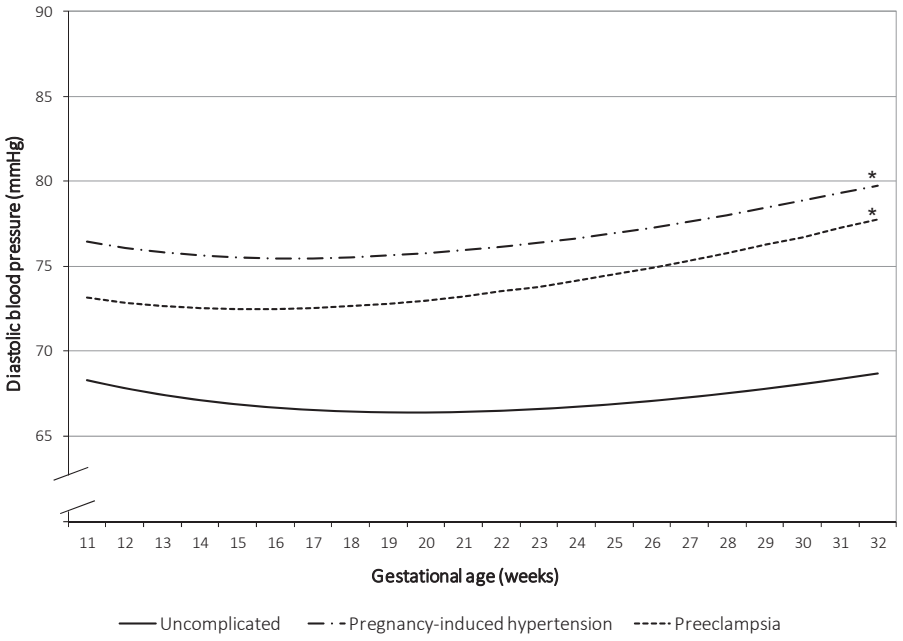


Figure 2.1.2b. Diastolic blood pressure

Change in diastolic blood pressure in mmHg for women with a pregnancy complicated by pregnancy-induced hypertension and women with a pregnancy complicated by pre-eclampsia compared with women with an uncomplicated pregnancy based on repeated measurement analysis (diastolic blood pressure = ß0 + ß1 × hypertensive complication + ß2 × gestational age + ß3 × gestational age$^{0.5}$ + ß4 × hypertensive complication × gestational age). P-value reflects the significance level of ß4, which reflects the difference in change in blood pressure per week per pregnancy hypertensive complication, when compared with uncomplicated pregnancies. Estimates are given in **Supplementary Material Table S2.1.1**. *P <0.05.

Blood pressure tracking during pregnancy

Correlation coefficients between first and third trimester for systolic and diastolic blood pressure and mean arterial pressure were 0.47, 0.46, and 0.49, respectively. The specific scatterplots are given in **Supplementary Figures S2.1.1–S2.1.3.**

Table 2.1.3 shows that for systolic blood pressure, about 55% of the women, who started in the highest tertile in first trimester remained in the highest tertile in third trimester, while approximately 29% and 15% were in the middle and lowest tertiles, respectively. Similar patterns were observed for diastolic blood pressure and mean arterial pressure. The ORs for staying in the upper tertile from first to third trimester for systolic blood pressure and diastolic blood pressure were 3.09 (95% CI: 2.73, 3.50) and 3.28 (95% CI: 2.90, 3.69), respectively. A similar trend was observed for tertiles of mean arterial pressure. Blood pressure tracking coefficients were lower in younger, shorter, and non-European women and in women with higher gestational weight gain (**Table 2.1.4**). Corresponding correlation coefficients are given in **Supplementary Table S2.1.2.**

Table 2.1.5 shows that systolic and diastolic blood pressure change from first to second trimester was not associated with the risk of pregnancy-induced hypertension. Diastolic blood pressure change from first to second trimester was associated with the risk of pre-eclampsia (OR 1.20 (95% CI: 1.01, 1.44) per standard deviation of blood pressure change. Second to third trimester changes in diastolic blood pressure and mean arterial pressure were associated with the risk of pregnancy-induced hypertension (OR 1.20 (95% CI: 1.06, 1.35) and OR 1.18 (95% CI: 1.04, 1.33) per standard deviation of blood pressure change, respectively). Second to third trimester changes in systolic blood pressure, diastolic blood pressure, and mean arterial pressure were associated with the risk of pre-eclampsia (OR 1.22 (95% CI: 1.04, 1.43), OR 1.22 (95% CI: 1.03, 1.43), and OR 1.26 (95% CI: 1.07, 1.48) per standard deviation of blood pressure change, respectively).

Table 2.1.3. Blood pressure tracking from first to third trimester (*n = 6053*)[1,2]

	Tertiles third trimester			
Tertiles first trimester	First	Second	Third	
Systolic blood pressure				*n*
First	2.73 (2.43, 3.07) **	0.90 (0.80, 1.01)	0.33 (0.28, 0.37) **	*2228*
	n = 1202 (53.9%)	*n = 667* (29.9%)	*n = 359* (16.1%)	
Second	0.92 (0.81, 1.03)	1.19 (1.06, 1.34) **	0.94 (0.83, 1.05)	*2028*
	n = 701 (34.6%)	*n = 678* (33.4%)	*n = 649* (32.0%)	
Third	0.29 (0.25, 0.34) **	0.92 (0.81, 1.04)	3.09 (2.73, 3.50) **	*1797*
	n = 284 (15.8%)	*n = 524* (29.2%)	*n = 989* (55.0%)	
n	*2187*	*1869*	*1997*	*6053*
Diastolic blood pressure				*n*
First	3.32 (2.95, 3.72) **	0.80 (0.71, 0.90) **	0.29 (0.25, 0.33) **	*2209*
	n = 1269 (57.4%)	*n = 609* (27.6%)	*n = 331* (15.0%)	
Second	0.76 (0.67, 0.85) **	1.42 (1.26, 1.60) **	0.95 (0.84, 1.07)	*1865*
	n = 626 (33.6%)	*n = 658* (35.3%)	*n = 581* (31.2%)	
Third	0.32 (0.29, 0.37) **	0.86 (0.76, 0.98) *	3.28 (2.90, 3.69) **	*1979*
	n = 371 (18.7%)	*n = 551* (27.8%)	*n = 1057* (53.4%)	
n	*2266*	*1818*	*1969*	*6053*
Mean arterial pressure				*n*
First	3.44 (3.06, 3.87) **	0.73 (0.65, 0.81) **	0.27 (0.23, 0.31) **	*2095*
	n = 1146 (54.7%)	*n = 650* (31.0%)	*n = 299* (14.3%)	
Second	0.67 (0.60, 0.75) **	1.48 (1.33, 1.66) **	1.01 (0.89, 1.14)	*1957*
	n = 587 (30.0%)	*n = 775* (39.6%)	*n = 595* (30.4%)	
Third	0.29 (0.25, 0.34) **	0.89 (0.79, 1.01)	3.40 (2.69, 3.50) **	*2001*
	n = 302 (15.1%)	*n = 595* (29.7%)	*n = 1104* (55.2%)	
n	*2035*	*2020*	*1998*	*6053*

[1]Values are Odds Ratios (95% CI) (number and percentage of women that remain in the same tertile) to remain in the same tertiles of systolic blood pressure, diastolic blood pressure and mean arterial pressure from first to third trimester. Estimates are from multiple imputed data. [2]Model is adjusted for gestational age at intake, gestational age in each pregnancy period, maternal age, educational level, parity, ethnicity, prepregnancy body mass index, gestational weight gain, smoking habits, alcohol consumption and caffeine intake. *P-value <0.05. **P-value <0.01.

Table 2.1.4. Maternal characteristics and blood pressure tracking coefficients[1]

	Systolic blood pressure		Diastolic blood pressure		Mean arterial pressure	
Maternal characteristics	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Age (yrs)						
< 25 years (*n = 1801*)	0.43 (0.38, 0.49)	<0.001	0.37 (0.31, 0.42)	<0.001	0.37 (0.31, 0.42)	<0.001
25-35 years (*n = 5432*)	0.48 (0.45, 0.50)	<0.001	0.47 (0.45, 0.50)	<0.001	0.47 (0.45, 0.50)	<0.001
> 35 years (*n = 1249*)	0.41 (0.34, 0.47)	<0.001	0.47 (0.40, 0.53)	<0.001	0.47 (0.40, 0.53)	<0.001
	Interaction P=0.820		*Interaction P<0.001*		*Interaction P=0.027*	
Height (cm)						
< 165 cm (*n = 3677*)	0.42 (0.39, 0.46)	<0.001	0.42 (0.38, 0.45)	<0.001	0.44 (0.41, 0.48)	<0.001
165-175 cm (*n = 3626*)	0.46 (0.42, 0.49)	<0.001	0.47 (0.44, 0.51)	<0.001	0.50 (0.47, 0.53)	<0.001
> 175 cm (*n = 1149*)	0.44 (0.39, 0.49)	<0.001	0.48 (0.43, 0.53)	<0.001	0.50 (0.45, 0.55)	<0.001
	Interaction P=0.166		*Interaction P<0.001*		*Interaction P=0.001*	
Prepregnancy body mass index (kg/m^2)						
Normal (*n = 4968*)	0.44 (0.41, 0.46)	<0.001	0.43 (0.40, 0.46)	<0.001	0.46 (0.43, 0.49)	<0.001
Overweight (*n = 1298*)	0.45 (0.39, 0.51)	<0.001	0.39 (0.34, 0.45)	<0.001	0.42 (0.37, 0.48)	<0.001
Obesity (*n = 567*)	0.44 (0.35, 0.52)	<0.001	0.48 (0.39, 0.56)	<0.001	0.50 (0.42, 0.58)	<0.001
	Interaction P=0.590		*Interaction P=0.715*		*Interaction P=0.592*	
Gestational weight gain (kg)						
< 7 kg (*n = 1638*)	0.47 (0.42, 0.51)	<0.001	0.48 (0.44, 0.53)	<0.001	0.50 (0.46, 0.54)	<0.001
7-11.9 kg (*n = 2877*)	0.44 (0.41, 0.48)	<0.001	0.46 (0.42, 0.49)	<0.001	0.48 (0.44, 0.51)	<0.001
> 12 kg (*n = 2010*)	0.45 (0.40, 0.49)	<0.001	0.43 (0.39, 0.48)	<0.001	0.47 (0.43, 0.51)	<0.001
	Interaction P=0.014		*Interaction P<0.001*		*Interaction P=0.005*	
Parity						
Nulliparous (*n = 4666*)	0.45 (0.42, 0.48)	<0.001	0.43 (0.40, 0.46)	<0.001	0.46 (0.43, 0.49)	<0.001
Multiparous (*n = 3711*)	0.46 (0.43, 0.50)	<0.001	0.47 (0.43, 0.50)	<0.001	0.50 (0.46, 0.53)	<0.001
	Interaction P=0.574		*Interaction P=0.099*		*Interaction P=0.115*	
Highest completed education						
Primary school (*n = 896*)	0.43 (0.35, 0.51)	<0.001	0.43 (0.35, 0.51)	<0.001	0.47 (0.40, 0.55)	<0.001
Secondary school (*n = 3572*)	0.48 (0.44, 0.51)	<0.001	0.46 (0.43, 0.50)	<0.001	0.50 (0.46, 0.53)	<0.001
Higher education (*n = 3244*)	0.45 (0.43, 0.48)	<0.001	0.45 (0.42, 0.49)	<0.001	0.48 (0.44, 0.51)	<0.001
	Interaction P=0.693		*Interaction P=0.968*		*Interaction P=0.615*	
Ethnicity						
European (*n = 4508*)	0.45 (0.42, 0.48)	<0.001	0.49 (0.46, 0.52)	<0.001	0.51 (0.48, 0.54)	<0.001
Non-European (*n = 3335*)	0.43 (0.39, 0.47)	<0.001	0.39 (0.35, 0.43)	<0.001	0.43 (0.39, 0.47)	<0.001
	Interaction P=0.448		*Interaction P<0.001*		*Interaction P=0.001*	
Alcohol consumption						
No (*n = 3620*)	0.46 (0.43, 0.50)	<0.001	0.46 (0.42, 0.49)	<0.001	0.49 (0.46, 0.52)	<0.001
Yes (*n = 3676*)	0.45 (0.42, 0.49)	<0.001	0.45 (0.42, 0.48)	<0.001	0.48 (0.45, 0.51)	<0.001
	Interaction P=0.433		*Interaction P=0.666*		*Interaction P=0.553*	
Smoking habits						
None (*n = 5045*)	0.47 (0.44, 0.50)	<0.001	0.47 (0.44, 0.49)	<0.001	0.50 (0.47, 0.53)	<0.001
Yes (*n = 1847*)	0.42 (0.37, 0.47)	<0.001	0.42 (0.37, 0.47)	<0.001	0.45 (0.40, 0.49)	<0.001
	Interaction P=0.072		*Interaction P=0.079*		*Interaction P=0.042*	
Caffeine intake						
No (*n = 359*)	0.49 (0.38, 0.60)	<0.001	0.54 (0.45, 0.64)	<0.001	0.55 (0.46, 0.65)	<0.001
Yes (*n = 7404*)	0.46 (0.43, 0.48)	<0.001	0.45 (0.43, 0.47)	<0.001	0.48 (0.46, 0.50)	<0.001
	Interaction P=0.672		*Interaction P=0.550*		*Interaction P=0.504*	

[1]Values are regression coefficients (95% CI) from first to third trimester for systolic blood pressure, diastolic blood pressure and mean arterial pressure.

Table 2.1.5. Blood pressure development and the risks of pregnancy-induced hypertension and pre-eclampsia (*n = 8236*)[1,2]

Pregnancy period	Pregnancy-induced hypertension	Pre-eclampsia
First to second trimester		
Systolic blood pressure	1.06 (0.93, 1.20)	1.00 (0.84, 1.20)
Diastolic blood pressure	1.05 (0.92, 1.20)	1.20 (1.01, 1.44) *
Mean arterial pressure	1.06 (0.93, 1.21)	1.14 (0.95, 1.37)
Second to third trimester		
Systolic blood pressure	1.09 (0.97, 1.23)	1.22 (1.04, 1.43) *
Diastolic blood pressure	1.20 (1.06, 1.35) **	1.22 (1.03, 1.43) *
Mean arterial pressure	1.18 (1.04, 1.33) **	1.26 (1.07, 1.48) **
First to third trimester		
Systolic blood pressure	1.15 (1.01, 1.31) *	1.23 (1.02, 1.47) *
Diastolic blood pressure	1.28 (1.12, 1.46) **	1.42 (1.18, 1.70) **
Mean arterial pressure	1.27 (1.11, 1.45) **	1.40 (1.16, 1.67) **

[1]Values are Odds Ratios (95% CI) that reflect the difference in risks of pregnancy-induced hypertension and pre-eclampsia per standard deviation change in blood pressure level between trimesters. Estimates are from multiple imputed data. [2]Model is adjusted for gestational age at intake, gestational age at each pregnancy period, maternal age, educational level, ethnicity, parity, prepregnancy body mass index, gestational weight gain, smoking habits, alcohol consumption and caffeine intake. *P-value <0.05. **P-value <0.01.

Discussion

Results from this prospective cohort study showed that gestational blood pressure development is different from first trimester onwards between non-hypertensive pregnancies and pregnancies complicated by gestational hypertensive disorders. Systolic and diastolic blood pressure and mean arterial pressure track moderately during pregnancy. This tracking is influenced by maternal characteristics. Systolic and diastolic blood pressure changes from second to third trimester are positively associated with the risk of gestational hypertensive disorders.

Methodological considerations

Some methodological issues need to be considered. One of the strengths of this study was the prospective data collection from early pregnancy onwards. We had a large sample size of 8482 participants with 22.287 blood pressure measurements. The response rate at baseline for participation in the study was 61%. The non-response would lead to biased effect estimates if the associations would be different between those included and not included in the analyses. However, this seems unlikely because biased estimates in large cohort studies mainly arise from loss to follow-up rather than from non-response at baseline.[15] Detailed information about a large number of potential confounding factors was available in this study. However, because of the observational design, residual confounding due to other socio-demographic and lifestyle related determinants might still be an issue. In addition, information on many covariates in this study was self-reported, which may have resulted in underreporting of certain adverse lifestyle-related determinants. Furthermore, blood pressure has a large within subject-variation and is also liable to measurement error. Measurement error might cause an underestimation of the true tracking correlation of blood pressure.[7] However, when

tracking is used to examine the predictive value of early measurements to identify those at risk, measurement error will not bias the results, because measurement error also occurs in real clinical setting.[7] Finally, we had a relative small number of cases of pregnancy-induced hypertension and pre-eclampsia, which might indicate a selection towards a healthy, low-risk population. It might be of interest to perform a similar analysis in a high risk, hospital-based population.

Blood pressure development during pregnancy

Several studies have reported differences in blood pressure development between non-hypertensive-complicated pregnancies and pregnancies complicated by pregnancy-induced hypertension or pre-eclampsia.[5,6] A previous study among 202 primigravid women at high risk for gestational hypertensive disorders observed differences in the circadian variability of systolic and diastolic blood pressure between uncomplicated pregnancies and pregnancies complicated by gestational hypertensive disorders. Pregnancies leading to gestational hypertensive disorders had elevated blood pressure levels in first trimester.[6] In the same study, the known second trimester blood pressure dip was not present in complicated pregnancies, and blood pressure increased strongly in complicated pregnancies, particularly in those complicated by pre-eclampsia. We observed similar differences in the blood pressure patterns using office blood pressure measurements. Although we did not observe an absence of the mid-pregnancy dip in pregnancies complicated by gestational hypertensive disorders, we did observe that the mid-pregnancy dip was smaller and tended to occur earlier in pregnancy. We also observed a larger increase in blood pressure levels from second to third trimester in complicated pregnancies, particularly for pregnancies complicated by pre-eclampsia. Even though these observed differences in blood pressure development are highly statistically significant, it needs to be considered that both systolic blood pressure and diastolic blood pressure were within the physiological range of blood pressure variability. However, these differences might provide clues on how to earlier identify those women at increased risk of gestational hypertensive disorders.

Blood pressure tracking

We have previously shown that obese and overweight women already had a higher blood pressure in first trimester, when compared with normal weight women. These differences remained stable throughout pregnancy.[16] Our current study shows that systolic blood pressure, diastolic blood pressure, and mean arterial pressure track moderately from first to third trimester. Blood pressure tracking in pregnancy might help to early identify those women that are at high risk to develop gestational hypertensive disorders. Several variables have been identified that might influence or predict tracking in studies among children and adults. It has been shown that length of follow-up is inversely associated with the tracking correlation.[17,18] We observed that the tracking correlation for systolic and diastolic blood pressure was stronger between first and second

trimester and second and third trimester compared with the tracking correlation between first and third trimester. Also, some studies have suggested that blood pressure tracking is different in different ethnic populations.[17,19,20] Accordingly, we observed differences in tracking coefficients for diastolic blood pressure and mean arterial pressure in European women and non-European women. Furthermore, age, overweight, and weight change have been suggested to influence tracking.[17,20,21] A study among men and women showed the tracking correlation for different age categories; for women aged 20 – 24, the tracking correlation for systolic blood pressure was 0.43 and the tracking correlation for diastolic blood pressure was 0.59, while for women aged 35 – 39 the tracking correlation was 0.64 and 0.68, respectively.[20] A study among Australian children reported that tracking of blood pressure, especially systolic blood pressure, was influenced by body mass index and change in body mass index.[21] Those individuals in the highest quartile of body mass index and those individuals in the highest quartile of weight gain had higher risks of persistence of high blood pressure levels. Similarly, maternal age, prepregnancy body mass index, and gestational weight gain might influence tracking. We observed that especially tracking of diastolic blood pressure and mean arterial pressure were influenced by maternal characteristics such as in older age and lower gestational weight gain.

Finally, systolic blood pressure, diastolic blood pressure, and mean arterial pressure tracked equally. However, diastolic blood pressure and mean arterial pressure were more strongly associated with the risks of pregnancy-induced hypertension and preeclampsia when compared with systolic blood pressure. This might indicate that diastolic blood pressure and mean arterial pressure have a higher predictive accuracy for gestational hypertensive disorders than systolic blood pressure.

Conclusion

Blood pressure tracks moderately during pregnancy. Second to third trimester increases in systolic and diastolic blood pressure are associated with the risk of gestational hypertensive disorders. Blood pressure tracking is related to maternal characteristics. Further research is needed focused on factors influencing blood pressure tracking and their associations with gestational hypertensive disorders.

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Supplementary Material

Supplementary Table S2.1.1. Longitudinal associations between pregnancy hypertensive complications and systolic and diastolic blood pressure[1]

Hypertensive complication	Intercept (mmHg)	P-value[2]	Slope (mmHg / week of gestation)	P-value[2]
	Difference in systolic blood pressure			
Uncomplicated	110.6	<0.001	*Reference*	
Pregnancy-induced hypertension	118.9	<0.001	0.10	0.020
Pre-eclampsia	112.3	0.273	0.19	0.002
	Difference in diastolic blood pressure			
Uncomplicated	97.2	<0.001	*Reference*	
Pregnancy-induced hypertension	103.8	<0.001	0.14	<0.001
Pre-eclampsia	99.8	0.031	0.20	<0.001

[1]Values are based on repeated non-linear regression models and reflect the change in blood pressure in mmHg per pregnancy hypertensive complication compared to the reference group of women with an uncomplicated pregnancy. [2]P-value reflects the significance level of the estimate.

Supplementary Table S2.1.2. Maternal characteristics and blood pressure correlation coefficients[1]

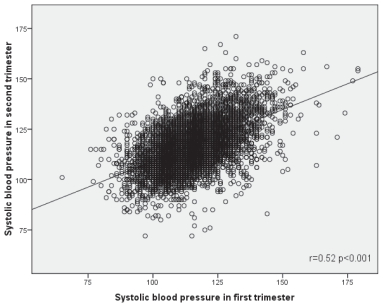
	Systolic blood pressure		Diastolic blood pressure		Mean arterial pressure	
Maternal characteristics	Correlation Coefficient	P-value	Correlation Coefficient	P-value	Correlation Coefficient	P-value
Age (yrs)						
< 25 years (*n = 1801*)	0.41	<0.001	0.36	<0.001	0.40	<0.001
25 - 35 years (*n = 5432*)	0.49	<0.001	0.49	<0.001	0.53	<0.001
> 35 years (*n = 1249*)	0.41	<0.001	0.45	<0.001	0.46	<0.001
	Interaction P=0.820		*Interaction P<0.001*		*Interaction P=0.027*	
Height (cm)						
< 165 cm *(n = 3677*)	0.43	<0.001	0.41	<0.001	0.45	<0.001
165 -175 cm (*n = 3626*)	0.46	<0.001	0.48	<0.001	0.50	<0.001
> 175 cm (*n = 1149*)	0.49	<0.001	0.51	<0.001	0.55	<0.001
	Interaction P=0.166		*Interaction P<0.001*		*Interaction P=0.001*	
Prepregnancy body mass index (kg/m^2)						
Normal (*n = 4968*)	0.44	<0.001	0.42	<0.001	0.45	<0.001
Overweight (*n = 1298*)	0.45	<0.001	0.40	<0.001	0.43	<0.001
Obesity (*n = 567*)	0.46	<0.001	0.50	<0.001	0.53	<0.001
	Interaction P=0.590		*Interaction P=0.715*		*Interaction P=0.592*	
Gestational weight gain (kg)						
< 7 kg (*n = 1638*)	0.51	<0.001	0.51	<0.001	0.54	<0.001
7-11.9 kg (*n = 2877*)	0.45	<0.001	0.46	<0.001	0.49	<0.001
> 12 kg (*n = 2010*)	0.45	<0.001	0.42	<0.001	0.47	<0.001
	Interaction P=0.014		*Interaction P<0.001*		*Interaction P=0.005*	
Parity						
Nulliparous (*n = 4666*)	0.46	<0.001	0.44	<0.001	0.48	<0.001
Multiparous (*n = 3711*)	0.46	<0.001	0.47	<0.001	0.50	<0.001
	Interaction P=0.574		*Interaction P=0.099*		*Interaction P=0.115*	
Highest completed education						
Primary school (*n = 896*)	0.42	<0.001	0.42	<0.001	0.46	<0.001
Secondary school (*n = 3572*)	0.47	<0.001	0.47	<0.001	0.49	<0.001
Higher education (*n = 3244*)	0.48	<0.001	0.46	<0.001	0.50	<0.001
	Interaction P=0.693		*Interaction P=0.968*		*Interaction P=0.615*	

Supplementary Table S2.1.2. Maternal characteristics and blood pressure correlation coefficients[1] (*continued*)

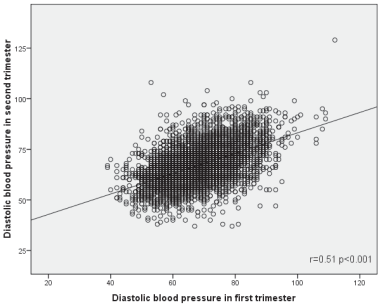
Maternal characteristics	Systolic blood pressure		Diastolic blood pressure		Mean arterial pressure	
	Correlation Coefficient	P-value	Correlation Coefficient	P-value	Correlation Coefficient	P-value
Ethnicity						
European (*n = 4508*)	0.47	<0.001	0.50	<0.001	0.52	<0.001
Non-European (*n = 3335*)	0.42	<0.001	0.39	<0.001	0.43	<0.001
	Interaction P=0.448		*Interaction P<0.001*		*Interaction P=0.001*	
Alcohol consumption						
No (*n = 3620*)	0.47	<0.001	0.46	<0.001	0.50	<0.001
Yes (*n = 3676*)	0.46	<0.001	0.45	<0.001	0.49	<0.001
	Interaction P=0.433		*Interaction P=0.666*		*Interaction P=0.553*	
Smoking habits						
None (*n = 5045*)	0.49	<0.001	0.47	<0.001	0.51	<0.001
Yes (*n = 1847*)	0.41	<0.001	0.42	<0.001	0.44	<0.001
	Interaction P=0.072		*Interaction P=0.079*		*Interaction P=0.042*	
Caffeine intake						
No (*n = 359*)	0.47	<0.001	0.58	<0.001	0.56	<0.001
Yes (*n = 7404*)	0.46	<0.001	0.45	<0.001	0.49	<0.001
	Interaction P=0.672		*Interaction P=0.550*		*Interaction P=0.504*	

[1]Values are correlation coefficients from first to third trimester for systolic blood pressure, diastolic blood pressure and mean arterial pressure.

Supplementary Figure S2.1.1. Correlation of blood pressure between first and second trimester



2.1.1a. Systolic blood pressure



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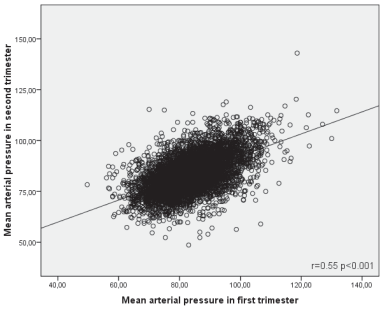
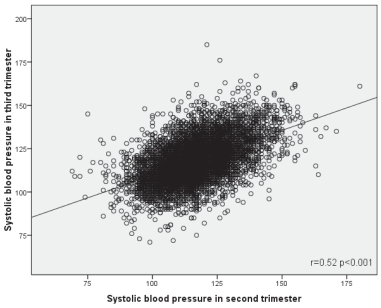
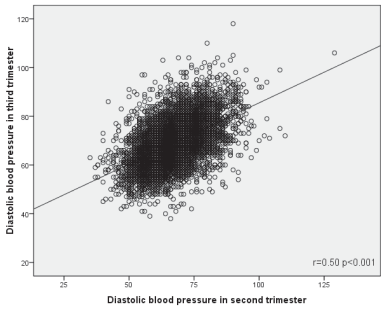


2.1.1c. Mean arterial pressure

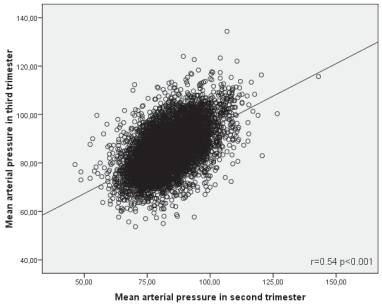
Supplementary Figure S2.1.2. Correlation of blood pressure between second and third trimester



2.1.2a. Systolic blood pressure

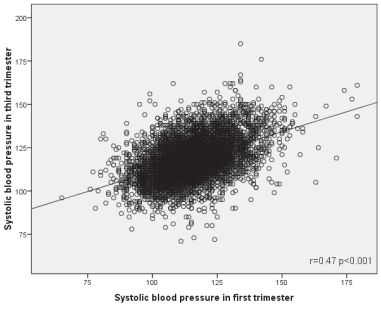


2.1.2b. Diastolic blood pressure

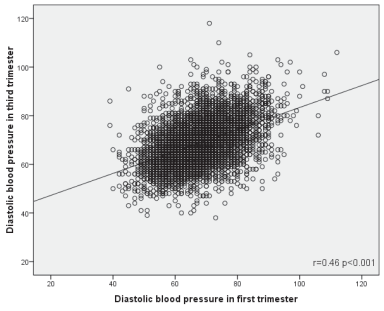


2.1.2c. Mean arterial pressure

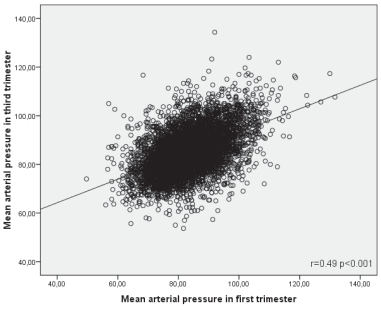
Supplementary Figure S2.1.3. Correlation of blood pressure between first and third trimester



2.1.3a. Systolic blood pressure



2.1.3b. Diastolic blood pressure



2.1.3c. Mean arterial pressure

Chapter 2.2

Maternal parity and hemodynamic adaptations during pregnancy

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Abstract

Background: It has been suggested that maternal vascular adaptations during pregnancy differ between nulliparous and multiparous women. Therefore, we examined the associations of parity with blood pressure, hemodynamic placental function during pregnancy and the risks of gestational hypertensive disorders.

Methods: The study was embedded in a population-based prospective cohort study among 8377 pregnant women. Information about parity and gravidity was obtained at enrollment. Blood pressure was repeatedly measured in each trimester and mean pulsatility and resistance indices of uterine artery were measured in second and third trimesters. Information on gestational hypertension and pre-eclampsia was available from medical records.

Results: As compared with nulliparous women, multiparous women had a lower systolic and diastolic blood pressure in each trimester of pregnancy and slightly higher second and third trimester uterine artery resistance and pulsatility indices (all P-values <0.05), but a lower risk of third trimester uterine artery notching (Odds Ratio (OR) 0.67 (95% Confidence Interval (CI): 0.53, 0.84)). The risks of gestational hypertension and pre-eclampsia were lower among multiparous women as compared with nulliparous women (OR 0.32 (95% CI: 0.24, 0.43) and OR 0.24 (95% CI: 0.16, 0.37), respectively). Among multiparous women only, we did not observe associations of parity with hemodynamic parameters.

Conclusions: Nulliparous pregnant women have higher blood pressure levels throughout pregnancy and higher risks of notching and gestational hypertensive disorders. The first pregnancy might be a major risk factor for maternal hemodynamic maladaptations and vascular complications. Further studies are needed to explore the underlying mechanisms and consequences for fetal growth and development.

Introduction

Gestational hypertension and pre-eclampsia are common pregnancy complications and are considered to have at least part of their origin in cardiovascular maladaptation in early pregnancy because of suboptimal placentation.[1–3] Previously, we have shown that maternal age, smoking, folic acid supplement use, and maternal caffeine intake influence maternal hemodynamic adaptations during pregnancy.[4–6] During normal pregnancy, physiological cardiovascular adaptations occur to meet demands of the rapidly developing fetus. These cardiovascular adaptations include an initial fall in systemic vascular tone in order to increase the cardiac output and an expanding plasma volume,[2,7,8] which subsequently leads to gradual lowering of the systolic and diastolic blood pressure until mid-pregnancy and rise from mid-pregnancy to delivery.[4,5] Cardiovascular maladaptation during pregnancy may lead to gestational hypertension and pre-eclampsia in extreme cases, but also to differences in blood pressure and hemodynamic placental function within a normal population.[9] Parity might influence these cardiovascular adaptations during pregnancy.[7,10,11] Blood pressure levels have been found to be higher in nulliparous women than in multiparous women.[7,12] Also, nulliparous women seem to have a higher risk of pre-eclampsia and gestational hypertension as compared with multiparous women,[13–15] but results are not consistent.[16–18] Not much is known about the effect of parity on longitudinal blood pressure development and hemodynamic placental function during pregnancy. It is also not known whether there is an optimum number for previous pregnancies with regard to cardiovascular adaptations during pregnancy.

Therefore, we examined in a population-based prospective cohort study among 8377 pregnant women, the associations of parity with blood pressure and hemodynamic placental function in different trimesters and the risks of gestational hypertension and pre-eclampsia.

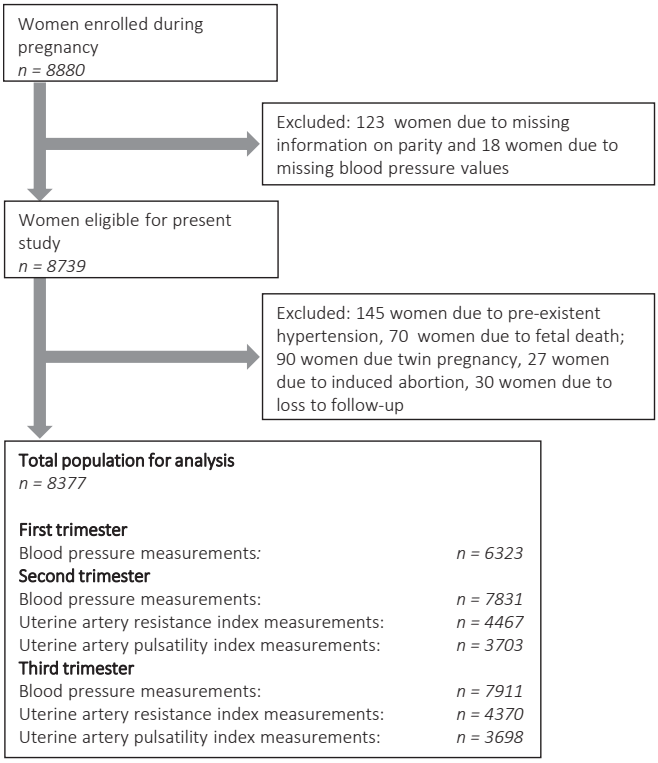
Methods

Design

This study was embedded in the Generation R study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, The Netherlands.[19,20] The study has been approved by the medical Ethical Committee of Erasmus Medical Center in Rotterdam. Written consent was obtained from all participating women.[21] All pregnant women were enrolled between 2001 and 2005. Response rate at birth was 61%. Enrollment was aimed in first trimester, but allowed until delivery. In total, 8880 women were enrolled during pregnancy. For the present study, we excluded women without information about parity (*n = 123*). Next, we excluded women without any blood pressure measurement (*n = 18*). Also, we excluded women with pre-existing hypertension

(*n = 145*). Since we restricted our analysis to low risk pregnancies, we excluded pregnancies leading to fetal death (*n = 70*), induced abortion (*n = 27*), loss to follow-up (*n = 30*) and twin pregnancies (*n = 90*). Similar results were found after including fetal deaths in the analyses. Thus, the cohort for analysis comprised 8377 women (**Figure 2.2.1**).

Figure 2.2.1. Flow chart of participants



Parity and gravidity assessment

Information about parity and gravidity was obtained by questionnaire at enrollment. First, parity was categorized into six categories (0, 1, 2, 3, 4, and ≥5). Because of smaller number of cases, the highest three parity categories were combined for assessing the associations of parity with risks of notching, gestational hypertension and pre-eclampsia (0, 1, 2, and ≥3). Parity and gravidity were highly correlated (r = 0.82; P <0.001). Primary analyses were performed for parity and sensitivity analyses were performed using gravidity instead of parity to examine whether the associations differed between parity and gravidity.

Blood pressure measurement

Blood pressure was measured in each trimester with an Omron® 907 automated digital oscillometric sphygmomanometer, which was validated in adults (OMRON Healthcare Europe B.V., Hoofddorp, The Netherlands).[22] All participants were seated in upright position with back support and were asked to relax for 5 minutes. A cuff was placed around a nondominant upper arm which was supported at the level of the heart, with bladder midline over the brachial artery pulsation. In case of an upper arm exceeding 33 cm, a larger cuff (32 – 42) was used. The mean value of two blood pressure readings over a 60s interval was documented for each participant. For the analysis, 22.065 blood pressure measurements were available. Three, two, and one blood pressure measurements were available for 5816, 2056, and 505 women, respectively.

Hemodynamic placental function and placental weight measurement

Ultrasound examinations to assess uteroplacental vascular resistance were performed in second trimester (gestational age 20 weeks) and third trimester (gestational age 30 weeks). Uteroplacental vascular resistance was assessed by uterine artery resistance index, uterine artery pulsatility index and presence of third trimester notching in uterine arteries. The right and left uterine arteries were identified at the apparent crossover with external iliac arteries on color Doppler and pulsed wave Doppler was used to obtain the waveforms. The high-pass filter was set at 100 Hz and the transducer was placed in the lower lateral quadrant of the abdomen angled medially. The insonation angle was kept as close to 0° as possible and always below 20°. Only wave-forms with clear outline were accepted. For each measurement, three consecutive uniform waveforms were recorded by pulsed Doppler ultrasound during fetal apnea and without fetal movements. The mean of three measurements was used for further analyses. Raised uterine artery resistance indices indicate increased placental resistance.[23] A notch, which reflects an abnormal waveform resulting from increased blood flow resistance in uterine artery, was considered to be present when there was a clearly defined upturn of the flow velocity waveform at the beginning of diastole, which was present in all three waveforms, on both occasions when each uterine artery was sampled.[24] Medical records completed by midwives and obstetricians were used to obtain information about placental weight (g). Placental function was measured in one of the two research centers in *n = 4467* and *n = 4370* women for uterine artery resistance index and in *n = 3703* and *n = 3698* women for uterine artery pulsatility index in second and third trimester, respectively.

Gestational hypertension and pre-eclampsia

Information on pregnancy complications was obtained from medical records. Women suspected of pregnancy complications based on these records were crosschecked with the original hospital charts. Details of these procedures have been described

elsewhere.[25] Briefly, the following criteria were used to identify women with gestational hypertension: development of systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as two or more dipstick reading of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24-h urine collection containing at least 300 mg of protein) were used to identify women with pre-eclampsia.

Covariates

Gestational age was established by fetal ultrasound examination during first ultrasound visit.[20] Maternal age was assessed at intake. Weight and height were repeatedly measured and body mass index was calculated for each trimester.[26] Information about educational level, ethnicity, and folic acid supplementation use was obtained at enrollment. Information about smoking and alcohol consumption was assessed by questionnaires in each trimester.

Statistical analysis

First, the associations of parity with repeatedly measured systolic and diastolic blood pressures were analyzed using unbalanced repeated measurement regression models. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data.[27] They are described in detail in the Supplementary material. In short, using fractional polynomials of gestational age, the best fitting models were constructed. Parity categories were included in these models as intercept and as an interaction term with gestational age. Next, we examined the associations of parity with systolic and diastolic blood pressure in each trimester and with placental vascular resistance (uterine artery resistance index and uterine artery pulsatility index) in second and third trimester using linear regression models. For these models, we examined whether the residuals were normally distributed using normal probability plots, whether the variance of the residuals was homoscedastic and whether the regression models were linear.[28] The associations of parity with the risks of third trimester notching, gestational hypertension and pre-eclampsia were assessed using multiple logistic regression models. All models were adjusted for maternal age, gestational age at enrollment, gestational age at time of measurement, educational level, ethnicity, smoking habits, alcohol consumption and folic acid supplement use. Maternal age and body mass index were entered into linear and logistic regression models as continuous variables. We used Markov chain Monte Carlo approach for multiple imputation of missing values in the covariates. Five imputed datasets were created and analyzed together. The percentages of missing values within population for analysis were lower than 15% except for folic acid supplement use (25.2%).The repeated measurement analyses were performed using SAS version 9.2 (SAS, Cary, NC), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were

performed using Statistical Package of Social Sciences version 17.0 for Windows (SPSS, Chicago, IL, USA).

Results

Subject characteristics

Table 2.2.1 shows subject characteristics according to parity. Multiparous women were more frequently less higher educated, of non-European origin and were less likely to consume alcohol during pregnancy. From first trimester onwards, systolic and diastolic blood pressure levels were lower among multiparous women (Table 2.2.2).

Table 2.2.1. Subjects' characteristics by parity (*n = 8377*)[1]

Parity	0 *n = 4666*	1 *n = 2499*	2 *n = 858*	3 *n = 254*	4 *n = 68*	≥5 *n = 32*	P-value[3]
Maternal characteristics							
Age, years	28.3 (5.3)	30.5 (4.8)	32.1 (4.3)	33.1 (4.1)	35.3 (3.6)	36.4 (3.9)	<0.001
Body mass index, kg/m^2	24.2 (4.1)	24.9 (4.4)	26.3 (4.8)	27.6 (5.2)	28.3 (4.4)	27.8 (3.8)	<0.001
Gestational age at intake, weeks[2]	14.2 (10.3, 24.9)	14.3 (10.1, 24.9)	15.4 (10.5, 30.1)	15.9 (11.1, 31.2)	17.5 (11.6, 33.1)	20.6 (9.1, 33.6)	<0.001
Education, %							
Primary school	7.9	11.8	22.5	33.6	47.3	69.8	<0.001
Secondary school	47.4	45.3	43.6	44.8	49.1	21.8	
Higher education	44.7	42.9	33.9	21.6	3.6	8.4	
Ethnicity, %							
European	60.6	59.8	44.6	29.8	18.3	18.3	<0.001
Non-European	39.4	40.2	55.4	70.2	81.7	81.7	
Alcohol consumption, %							
No	63.2	59.8	65.9	77.0	86.5	86.4	<0.001
Yes	36.8	40.2	34.1	23.0	13.5	13.6	
Smoking habits, %							
No	83.0	82.8	82.4	83.4	78.8	81.0	<0.001
Yes	17.0	17.2	17.6	16.4	21.2	19.0	
Folic acid supplements, %							
None	23.0	30.2	48.7	65.0	82.2	95.2	<0.001
1st 10 weeks	34.4	28.8	26.1	16.0	15.6	-	
Periconception use	42.6	41.0	25.2	19.0	2.2	4.8	
Pregnancy complications, %							
Pre-eclampsia	3.0	1.2	0.2	2.4	-	-	<0.001
Gestational hypertension	5.2	2.4	1.5	2.4	1.5	3.2	<0.001

[1]Values are means (standard deviation) or percentages. [2]Median (95% range). [3]Differences in subject characteristics between groups were evaluated using one-way-ANOVA-tests for continuous variables and Chi-square tests for proportions.

Table 2.2.2. Blood pressure and placental flow characteristics by parity (*n = 8377*)[1]

Parity	0	1	2	3	4	≥5	
	n = 4666	*n = 2499*	*n = 858*	*n = 254*	*n = 68*	*n = 32*	P-value[2]
First trimester							
Systolic blood pressure (mmHg)	115.9 (12.2)	114.2 (11.7)	114.4 (11.5)	114.3 (11.2)	113.2 (12.7)	112.0 (11.9)	<0.001
Diastolic blood pressure (mmHg)	68.4 (9.3)	67.1 (9.3)	67.6 (8.9)	68.3 (8.6)	67.6 (11.7)	68.2 (10.2)	<0.001
Second trimester							
Systolic blood pressure (mmHg)	117.5 (11.8)	115.1 (11.7)	114.7 (11.7)	115.2 (11.1)	115.9 (12.3)	112.8 (13.6)	<0.001
Diastolic blood pressure (mmHg)	67.6 (9.0)	65.9 (9.2)	66.1 (9.1)	66.7 (9.0)	68.2 (10.1)	64.2 (7.7)	<0.001
Uterine artery resistance index	0.53 (0.09)	0.54 (0.08)	0.55 (0.09)	0.56 (0.08)	0.56 (0.08)	0.60 (0.09)	<0.001
Uterine artery pulsatility index	0.89 (0.27)	0.90 (0.25)	0.91 (0.26)	0.94 (0.25)	0.96 (0.30)	1.11 (0.42)	0.01
Third trimester							
Systolic blood pressure (mmHg)	119.2 (11.8)	116.6 (11.7)	116.2 (12.1)	116.0 (10.9)	117.0 (12.1)	115.6 (13.7)	<0.001
Diastolic blood pressure (mmHg)	69.9 (9.0)	67.5 (9.0)	67.1 (9.4)	67.5 (8.6)	68.0 (10.0)	70.3 (10.6)	<0.001
Uterine artery resistance index	0.47 (0.08)	0.48 (0.07)	0.49 (0.07)	0.51 (0.07)	0.51 (0.07)	0.53 (0.06)	<0.001
Uterine artery pulsatility index	0.72 (0.19)	0.74 (0.18)	0.75 (0.18)	0.82 (0.20)	0.77 (0.17)	0.83 (0.15)	<0.001

[1]Values are means (standard deviation). [2]Differences in subject characteristics between groups were evaluated using one-way-ANOVA-tests for continuous variables.

Parity and blood pressure in different trimesters

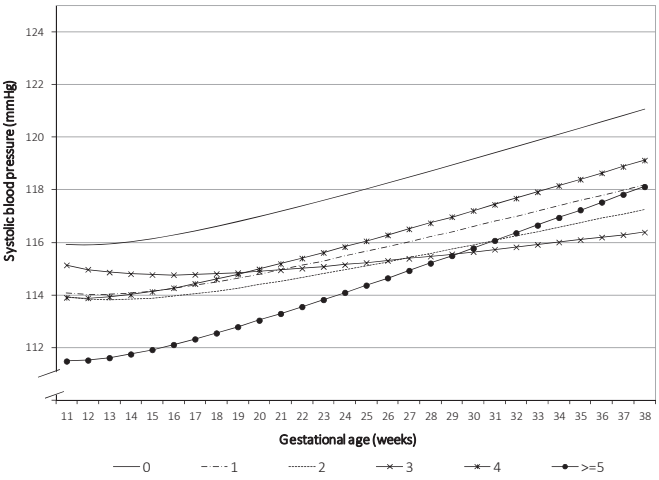
Figure 2.2.2 shows that longitudinal measured systolic blood pressure throughout pregnancy was highest among nulliparous women. Also, the greatest increase in systolic blood pressure occurred among nulliparous women. Diastolic blood pressure showed a mid-pregnancy dip for all parity categories. The steepest increase in diastolic blood pressure was observed in nulliparous women and in women with five or more children. The exact regression coefficients are given in Supplementary Table S2.2.1. Cross-sectional analyses showed that, as compared with nulliparous women, multiparous women had lower first, second, and third trimester systolic blood pressure (differences for multiparous vs. nulliparous women: −2.17 mmHg (95% Confidence Intervals (CI): −2.77, −1.58), −2.74 mmHg (95% CI: −3.27, −2.22), and −2.83 mmHg (95% CI: −3.36, −2.29), respectively) and diastolic blood pressure (differences: −1.78 mmHg (95% CI: −2.25, −1.31), −2.29 mmHg (95% CI: −2.71, −1.88), and −3.05 mmHg (95% CI: −3.47, −2.65), respectively). Similar differences for systolic and diastolic blood pressure were found when we used gravidity instead of parity (Supplementary Tables S2.2.2 and S2.2.3).

Parity, hemodynamic placental function and placental weight

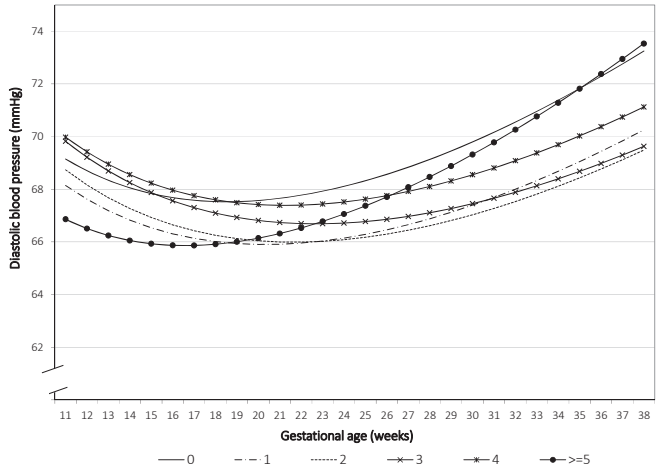
Table 2.2.3 shows that as compared with nulliparous women, multiparous women had slightly higher uterine artery resistance index (differences in uterine artery resistance indices in second and third trimester: 0.007 (95% CI: 0.001, 0.013), 0.007 (95% CI: 0.001, 0.013), respectively). We observed similar tendencies for second and third trimester uterine artery pulsatility indices. As compared with nulliparous women, multiparous women had a higher placental weight (difference in placental weight: 23.15 g (95%

CI: 15.11, 31.19).When we used gravidity instead of parity, we observed similar results (**Supplementary Table S2.2.4**).

Figure 2.2.2. Blood pressure patterns during pregnancy in different categories of parity



2.2.2a. Systolic blood pressure



2.2.2b. Diastolic blood pressure

Change patterns in systolic and diastolic blood pressure in mmHg during pregnancy for women who had given birth once, women who had given birth twice, women who had given birth thrice, women who had given birth four times and women who had given birth five times or more as compared with women who had not given birth before based on repeated measurement analysis. Systolic blood pressure (SBP) = $\beta_0 + \beta_1 \times$ parity $+ \beta_2 \times$ gestational age $+ \beta_3 \times$ gestational age$^{-2} + \beta_4 \times$ parity $\times$ gestational age. Diastolic blood pressure (DBP) = $\beta_0 + \beta_1 \times$ parity $+ \beta_2 \times$ gestational age $+ \beta_3 \times$ gestational age$^{0.5} + \beta_4 \times$ parity $\times$ gestational age. In these models '$\beta_0 + \beta_1 \times$ parity' reflects the intercept and '$\beta_2 \times$ gestational age $+ \beta_3 \times$ gestational age^{-2}' reflects the slope of change in blood pressure per week for systolic blood pressure, and '$\beta_2 \times$ gestational age $+ \beta_3 \times$ gestational age$^{0.5}$,' reflects the slope of change in blood pressure per week for diastolic blood pressure. Our term of interest was '$\beta_4 \times$ parity $\times$ gestational age' which reflects the difference in change in blood pressure per week per parity category for either systolic or diastolic blood pressure. Estimates and P-values are given in **Supplementary Table S2.2.1.**

Parity and risk of notching and gestational hypertensive disorders

The risk of notching was decreased among multiparous women as compared with nulliparous women (Odds Ratio (OR) 0.67 (95% CI: 0.53, 0.84). As compared with nulliparous women, the risks of gestational hypertension and pre-eclampsia were lower among multiparous women (OR 0.32 (95% CI: 0.24, 0.43), OR 0.24 (95% CI: 0.16, 0.37) for gestational hypertension and pre-eclampsia, respectively) (**Table 2.2.4**). Among multiparous women only, we did not observe consistent associations of parity with the risks of gestational hypertensive disorders. Effect estimates in unadjusted analyses showed a similar direction and strength as compared with the full model. Using gravidity instead of parity, we observed similar results (**Supplementary Table S2.2.5**).

Table 2.2.3. Associations of parity with uterine artery vascular resistance and placental weight (*n = 8377*)[1]

Parity	Second trimester uterine artery resistance index[4] *n = 4467*	Third trimester uterine artery resistance index[5] *n = 4370*	Second trimester uterine artery pulsatility index[6] *n = 3703*	Third trimester uterine artery pulsatility index[7] *n = 3698*	Differences in placental Weight[2,8] *n = 6049*
0	*Reference* *n = 2558*	*Reference* *n = 2487*	*Reference* *n = 2131*	*Reference* *n = 2115*	*Reference* *n = 3466*
≥1	0.007 (0.001, 0.013) *n = 1909*	0.007 (0.001, 0.013) *n = 1883*	0.009 (−0.010, 0.038) *n = 1572*	0.009 (−0.004, 0.023) *n = 1583*	23.15 (15.11, 31.19)* *n = 2583*
1	0.006 (0, 0.012) *n = 1328*	0.005 (−0.001, 0.11) *n = 1311*	0.006 (−0.014, 0.03) *n = 1091*	0.006 (−0.008, 0.02) *n = 1098*	23.37 (14.81, 31.93)* *n = 1729*
2	0.009 (−0.001, 0.019) *n = 412*	0.008 (0, 0.017) *n = 420*	0.013 (−0.019, 0.05) *n = 344*	0.008 (−0.015, 0.03) *n = 353*	22.79 (9.52, 36.05)* *n = 602*
3	0.021 (0.004, 0.04) *n = 124*	0.026 (0.011, 0.04)* *n = 119*	0.035 (−0.022, 0.09) *n = 101*	0.068 (0,029, 0.11)* *n = 102*	39.46 (16.92, 62.00)* *n = 179*
4	0.021 (−0.009, 0.05) *n = 35*	0.012 (−0.017, 0.04) *n = 25*	0.046 (−0.060, 0.15) *n = 27*	0.006 (−0.076, 0.09) *n = 22*	79.99 (39.46, 120.56)* *n = 52*
≥5	0.055 (−0.001, 0.11) *n = 10*	0.029 (−0.024, 0.08) *n = 8*	0.193 (0.015, 0.37) *n = 9*	0.066 (−0.068, 0.19) *n = 8*	−6.84 (−69.57, 55.89) *n = 21*
Trend[3]	*0.006 (0.003, 0.01)**	*0.005 (0.002, 0.01)**	*0.012 (-0.001, 0.02)*	*0.010 (0.002, 0.02)**	*13.34 (8.69, 17.99)**

[1]Values are regression coefficients (95% Confidence Interval) and reflect differences in uterine artery resistance and pulsatility indices and placental weight for different parity categories as compared to nulliparous women. All values were adjusted for gestational age at time of measurement, maternal age, body mass index, ethnicity, education, folic acid supplementation, smoking and alcohol consumption. [2]Differences in placental weight (g) for different parity categories as compared to nulliparous women. [3]Tests for trend were based on multiple linear regression models with parity as a continuous variable. [4]R^2 = 0.2. [5]R^2 = 0.2.[6]R^2 = 0.2.[7]R^2 = 0.2.[8]R^2 = 0.5. *P-value <0.05.

Discussion

Results from this prospective population-based cohort study showed that as compared with multiparous women, nulliparous women have a higher systolic and diastolic blood pressure in each trimester of pregnancy and lower uterine artery resistance indices. Nulliparous women had higher risks of third trimester uterine artery notching, and higher risks of gestational hypertension and pre-eclampsia. We did not observe significant associations of parity with placental hemodynamics among multiparous women.

Table 2.2.4. Associations of parity with notching and gestational hypertensive disorders (*n = 8377*) [1]

Parity	Notching OR (95% CI)	Gestational hypertension OR (95% CI)	Pre-eclampsia OR (95% CI)
0	*Reference*	*Reference*	*Reference*
n = 4666	n_{cases} *= 314*	n_{cases} *= 228*	n_{cases} *= 131*
≥1	0.67 (0.53, 0.84)*	0.32 (0.24, 0.43)*	0.24 (0.16, 0.37)*
n = 3711	n_{cases} *= 156*	n_{cases} *= 78*	n_{cases} *= 36*
1	0.67 (0.52, 0.85)*	0.37 (0.27, 0.50)*	0.31 (0.20, 0.48)*
n = 2499	n_{cases} *= 108*	n_{cases} *= 57*	n_{cases} *= 28*
2	0.59 (0.39, 0.90)*	0.19 (0.10, 0.35)*	0.04 (0.01, 0.19)*
n = 858	n_{cases} *= 32*	n_{cases} *= 13*	n_{cases} *= 2*
≥3	0.86 (0.48, 1.54)	0.28 (0.12, 0.61)*	0.25 (0.10, 0.63)*
n = 354	n_{cases} *= 16*	n_{cases} *= 8*	n_{cases} *= 6*
Trend[2]	*0.81 (0.71, 0.94)**	*0.49 (0.40, 0.60)**	*0.39 (0.29, 0.52)**

[1]Values are Odds Ratio and 95% Confidence Interval that indicate the differences in risks of developing a notch, gestational hypertension and pre-eclampsia in different categories of parity compared to reference group of nulliparous women. Values were adjusted for maternal age, body mass index, educational level, ethnicity, folic acid supplements, smoking and alcohol consumption. [2]Tests for trend were based on logistic regression models with parity as a continuous variable. *P-value <0.05.

Methodological considerations

One of the strengths of this study was the prospective data collection from early pregnancy onwards. We had a large sample size of 8377 participants with 22.065 blood pressure measurements. The response rate of the study was 61%. The percentages of women from ethnic minority groups and lower socio-economic status were slightly lower than expected from population figures in Rotterdam. This might indicate a selection toward a relatively healthy population, and might affect the generalizability of our results. However, it is unlikely that non-response has led to biased estimates, because biased estimates in large cohort studies mainly arise from loss to follow-up rather than from nonresponse at baseline.[29] Furthermore, not all women were already enrolled in the study in first trimester. Therefore, we did not have first trimester blood pressure measurements in ~25% of the participating women.[20] It seems unlikely that late enrollment has biased our results. We observed small differences in the associations of parity with the risk of gestational hypertensive disorders between women who were enrolled during first trimester or later in pregnancy. Detailed information about a large number of potential confounding factors was available in this study. However, because of the observational design, residual confounding because of other socio-demographic and lifestyle related determinants might still be an issue. In addition, information on many covariates in this study was self-reported, which may have resulted in underreporting of certain adverse lifestyle related determinants. Blood pressure and uteroplacental vascular resistance indices measurements provided only a fraction of 24h profile and may have been conducted under circumstances that influenced the measurements, which might have led to random misclassification and an underestimation of the observed differences. Finally, we had relatively small numbers of women with notching (*n = 470*), gestational hypertension cases (*n = 306*) and pre-eclampsia cases (*n = 167*). This might also reflect a selection toward a relatively healthy, low risk population.

Parity, blood pressure, and hemodynamic placental function

Higher parity and gravidity have been hypothesized as possible protective factors for gestational hypertensive disorders. We have shown that parity influences systolic and diastolic blood pressure levels during pregnancy from first trimester onwards. For all parity categories, parous women had lower blood pressure levels as compared with nulliparous women in each trimester. This is in accordance with observations in smaller previous studies.[7,12] A study among 6662 women observed higher mean blood pressure levels in nulliparous gravidas compared with blood pressure levels in parous gravidas.[8] Another study among 600 pregnant women showed a greater increase of blood pressure in nulliparous than in multiparous women during pregnancy.[7] However, not all previous studies showed associations of parity with blood pressure.[16,30] A study among a small cohort of 205 normotensive white pregnant women did not observe differences in blood pressure in relation to parity.[16] In another prospective cohort study among 366 pregnant women, there was no difference in arterial blood pressure during pregnancy between nulliparous and multiparous women who remained normotensive during pregnancy.[30] These differences in results might be explained by the smaller study samples and selection of study participants. In our study, additional exclusion of women with gestational hypertension and pre-eclampsia from our analysis did not change our results (data not shown).

Results in our study indicate that nulliparous women had a lower uterine artery resistance but a higher risk of notching. These results are in line with a previous study among 4132 pregnancies uncomplicated by pre-eclampsia, which observed slightly higher uterine artery resistance index values but less prevalent notching in parous women when compared with nulliparous women.[31] These findings might be explained by effects of parity on spiral arteries. During early placentation, trophoblastic cells infiltrate thick-walled spiral arteries and transform them into thin-walled vessels that can dilate and accommodate increased uteroplacental blood flow. It is possible that some permanent modifications persist in maternal vessels as an effect of this process, altering their compliance in future pregnancies. These changes may explain the lower prevalence of notches we observed in multiparous women.

Parity and risk of gestational hypertension and pre-eclampsia

Nulliparous women had a higher risk of developing both gestational hypertension and pre-eclampsia. Multiple previous studies have examined the associations of parity with gestational hypertensive complications.[1,14] A systemic review of 52 studies reported an increased risk of pre-eclampsia in nulliparous women.[14] The mechanisms by which nulliparous women might have higher blood pressure levels throughout pregnancy and higher risks of gestational hypertensive disorders are not fully understood. It has been suggested that immunological and cardiovascular adaptations occur during the first pregnancy that might trigger altered cardiovascular responses in subsequent pregnancies.[7,32] Another possible mechanism is that initial maternal rejection of placental

cytotrophoblasts lead to inadequately remodeled spiral arteries during first pregnancy.[3,31,32] This might lead to shallow implantation and consequently to downstream hypoxia and appearance of maternal symptoms. In line with this hypothesis, we observed that placental weight was lower among nulliparous women. It is possible that some modifications that lead to a more positive immune response in future pregnancies persist in maternal vessels as an effect of this process.[31] Furthermore, the hormonal milieu of pregnancy has been shown to influence vessel structure, basal tone and reactivity via receptors for chorionic gonadotropin, estradiol, and progesterone located in vascular endothelium and smooth muscles.[11,32] Studies have suggested that these changes occur in early pregnancy and that they might persist.[11,33] This might partly explain the underlying pathway of the observed associations between parity and blood pressure and the risk of gestational hypertensive disorders.

Conclusion

Parity has been suggested as a risk factor for gestational hypertensive disorders. Our study showed that as compared with multiparous women, nulliparous women have higher systolic and diastolic blood pressure in each trimester of pregnancy, and higher risks of third trimester uterine artery notching, and gestational hypertension and pre-eclampsia. We did not observe significant associations of parity with placenta hemodynamics among multiparous women. The first pregnancy might be a major risk factor for hemodynamic maladaptations and vascular complications. Future studies focused on mechanisms underlying the observed associations, particularly focused on early gestation are needed.

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Supplementary Material

Supplementary Methods S2.2.1. Unbalanced repeated measurements regression models

The associations of parity with repeatedly measured systolic and diastolic blood pressure were analysed using unbalanced repeated measurement regression models. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data. Using fractional polynomials of gestational age, the best fitting models were constructed (1). For this analysis, parity was categorized into 6 groups (0; 1; 2; 3; 4; ≥5) and included in these models as intercept and as an interaction term with gestational age. These models can be written as:

Systolic blood pressure = $ß_0 + ß_1 \times \text{parity} + ß_2 \times \text{gestational age} + ß_3 \times \text{gestational age}^{-2} + ß_4 \times \text{parity} \times \text{gestational age}$

Diastolic blood pressure = $ß_0 + ß_1 \times \text{parity} + ß_2 \times \text{gestational age} + ß_3 \times \text{gestational age}^{0.5} + ß_4 \times \text{parity} \times \text{gestational age}$

In these models, '$ß_0 + ß_1 \times$ parity' reflects the intercept. The intercept reflects the mean systolic and diastolic blood pressure value for these parity categories. '$ß_2 \times$ gestational age + $ß_3 \times$ gestational age^{-2}' reflects the slope of change in blood pressure per week for systolic blood pressure, and '$ß_2 \times$ gestational age + $ß_3 \times$ gestational age$^{0.5}$' reflects the slope of change in blood pressure per week for diastolic blood pressure. Main interest was in the term '$ß_4 \times$ parity × gestational age', which reflects the difference in change in blood pressure per week between the different parity categories for systolic and diastolic blood pressure. The exact regression coefficients for gestational age independent (intercept) and gestational age dependent differences (interaction parity and gestational age) are given in the **Supplementary Table S2.2.1** below.

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Supplementary Table S2.2.1. Longitudinal associations between parity and systolic and diastolic blood pressure[1]

Parity	Intercept (mmHg)	P-value[2]	Interaction (mmHg) (95% CI)	P-value[2]
Differences in systolic blood pressure				
0	111.51	<0.0001	*Reference*	
1	110.10	0.003	-0.038 (-0.07, -0.001)	0.04
2	110.28	0.113	-0.068 (-0.12, -0.01)	0.02
3	112.32	0.55	-0.144 (-0.24, -0.03)	0.006
4	109.47	0.48	0.002 (-0.21, 0.21)	0.98
≥5	106.48	0.30	0.054 (-0.31, 0.41)	0.76
Differences in diastolic blood pressure				
0	98.25	<0.0001	*Reference*	
1	98.05	0.59	-0.073 (-0.10, -0.04)	<0.0001
2	99.20	0.11	-0.12 (-0.16, -0.07)	<0.0001
3	100.67	0.02	-0.15 (-0.24, -0.07)	<0.0001
4	100.28	0.36	-0.11 (-0.27, 0.05)	0.20
≥5	94.91	0.37	0.09 (-0.18, 0.37)	0.50

[1]Values are based on repeated non-linear regression models and reflect the change in blood pressure in mmHg per parity category compared to the reference group of nulliparous women. [2]P-value reflects the significance level of the estimate.

Supplementary Table S2.2.2. Cross-sectional associations of parity with blood pressure (*n = 8377*)[1,2]

Parity	First trimester *n = 6323*	Second trimester *n = 7831*	Third trimester *n = 7911*
	Differences in systolic blood pressure[A]		
0	*Reference* *n = 3627*	*Reference* *n = 4410*	*Reference* *n = 4448*
≥1	-2.17 (-2.77, -1.58)* *n = 2696*	-2.74 (-3.27, -2.22)* *n = 3421*	-2.83 (-3.36, -2.29)* *n = 3463*
1	-2.17 (-2.80, -1.53)* *n = 1912*	-2.60 (-3.16, -2.03)* *n = 2333*	-2.70 (-3.29, -2.13)* *n = 2354*
2	-2.15 (-3.20, -1.11)* *n = 569*	-3.22 (-4.11, -2.33)* *n = 774*	-3.20 (-4.10, -2.30)* *n = 791*
3	-2.38 (-4.18, -0.57)* *n = 167*	-3.10 (-4.63, -158)* *n = 229*	-3.39 (-4.93, -1.84)* *n = 231*
4	-2.40 (-6.09, 1.28) *n = 37*	-2.28 (-5.10, 0.53) *n = 62*	-1.78 (-4.69, 1.11) *n = 60*
≥5	-4.16 (-10.85, 2.51) *n = 11*	-5.01 (-9.54, -0.48)* *n = 23*	-2.77 (-7.03, 1.48) *n = 27*
Trend[3]	*-1.14 (-1.51, -0.76)**	*-1.41 (-1.73, -1.09)**	*-1.37 (-1.69, -1.05)**
	Differences in diastolic blood pressure[B]		
0	*Reference* *n = 3627*	*Reference* *n = 4410*	*Reference* *n = 4448*
≥1	-1.78 (-2.25, -1.31)* *n = 2696*	-2.29 (-2.71, -1.88)* *n = 3421*	-3.05 (-3.47, -2.65)* *n = 3463*
1	-1.77 (-2.26, -1.27)* *n = 1912*	-2.16 (-2.60, -1.72)* *n = 2333*	-2.82 (-3.26, -2.37)* *n = 2354*
2	-1.85 (-2.66, -1.03)* *n = 569*	-2.68 (-3.38, -1.98)* *n = 774*	-3.75 (-4.45, -3.05)* *n = 791*
3	-1.76 (-3.17, -0.35)* *n = 167*	-2.89 (-4.08, -1.70)* *n = 229*	-3.96 (-5.16, -2.76)* *n = 231*
4	-1.73 (-4.60, 1.14) *n = 37*	-2.01 (-4.22, 0.18) *n = 62*	-3.62 (-5.88, -1.36)* *n = 60*
≥5	-2.38 (-7.57, 2.81) *n = 11*	-5.92 (-9.47, -2.38)* *n = 23*	-1.02 (-4.35, 2.30) *n = 27*
Trend[3]	*-0.91 (-1.20, -0.61)**	*-1.22 (-1.47, -0.98)**	*-1.54 (-1.79, -1.29)**

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in blood pressure in mmHg between parity categories as compared to the reference group of nulliparous women. Estimates are from multiple imputed data. [2]Models were adjusted for gestational age at visit, maternal age, body mass index, educational level, ethnicity, folic acid supplement use, smoking habits and alcohol consumption. [3]Tests for trend were based on multiple linear regression models with parity as a continuous variable. [A]R^2 for systolic blood pressure in first, second and third trimester = 0.16, 0.16, 0.15, respectively. [B]R^2 for diastolic blood pressure in first, second and third trimester = 0.16, 0.16, 0.14, respectively. *P-value <0.05.

Supplementary Table S2.2.3. Cross-sectional associations of gravidity with blood pressure (*n = 8377*)[1,2]

Gravidity	First trimester *n = 6323*	Second trimester *n = 7831*	Third trimester *n = 7911*
	Differences in systolic blood pressure		
1	*Reference* *n = 2895*	*Reference* *n = 3471*	*Reference* *n = 3504*
2	-1.90 (-2.55, -1.25)* *n = 1942*	-1.77 (-2.34, -1.18)* *n = 2374*	-1.62 (-2.21, -1.03)* *n = 2397*
3	-1.84 (-2.71, -0.96)* *n = 879*	-2.49 (-3.25, -1.72)* *n = 1137*	-1.98 (-2.75, -2.21)* *n = 1158*
4	-3.01 (-4.28, -1.73)* *n = 355*	-2.94 (-4.02, -1.87)* *n = 499*	-3.54 (-4.63, -2.45)* *n = 501*
5	-2.04 (-3.94, 0.13) *n = 147*	-2.01 (-3.57, -0.43)* *n = 212*	-0.65 (-2.25, 0.95) *n = 209*
≥6	-1.71 (-3.95, 0.54) *n = 105*	-3.55 (-5.49, -1.62)* *n = 138*	-3.42 (-5.37, 1.48)* *n = 142*
Trend[3]	*-0.68 (-0.94, -0.42)**	*-0.78 (-1.01, -0.56)**	*-0.66 (-0.88, -0.43)**
	Differences in diastolic blood pressure		
1	*Reference* *n = 2895*	*Reference* *n = 3471*	*Reference* *n = 3504*
2	-1.27 (-1.77, -0.76)* *n = 1942*	-1.62 (-2.07, -1.16)* *n = 2374*	-1.98 (-2.44, -1.53)* *n = 2397*
3	-1.10 (-1.78, -0.42)* *n = 879*	-1.76 (-2.36, -1.16)* *n = 1137*	-2.64 (-3.24, -2.03)* *n = 1158*
4	-2.11 (-3.11, -1.11)* *n = 355*	-2.42 (-3.26, -1.58)* *n = 499*	-3.49 (-4.34, -2.65)* *n = 501*
5	-1.27 (-2.76, 0.22) *n = 147*	-2.07 (-3.31, 0.84)* *n = 212*	-2.48 (-3.74, -1.23)* *n = 209*
≥6	-0.63 (-2.39, 1.13) *n = 105*	-2.12 (-3.63, -0.60)* *n = 138*	-2.43 (-3.95, 0.91)* *n = 142*
Trend[3]	*-0.42 (-0.63, -0.22)**	*-0.58 (-0.75, -0.41)**	*-0.77 (-0.94, -0.59)**

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in blood pressure in mmHg between gravidity categories as compared to the reference group of women who were pregnant for the first time. Estimates are from multiple imputed data. [2]Models were adjusted for gestational age at visit, maternal age, body mass index, educational level, ethnicity, folic acid supplement use, smoking habits and alcohol consumption. [3]Tests for trend were based on multiple linear regression models with gravidity as a continuous variable. *P-value <0.05.

Supplementary Table S2.2.4. Associations of gravidity with uterine artery vascular resistance and placental weight (*n = 8377*)[1]

Gravidity	Second trimester uterine artery resistance index *n = 4467*	Third trimester uterine artery resistance index *n = 4370*	Second trimester uterine artery pulsatility index *n = 3703*	Third trimester uterine artery pulsatility index *n = 3698*	Differences in placental weight[2] *n = 6049*
1	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
	n = 2014	*n = 1978*	*n = 1686*	*n = 1690*	*n = 2755*
2	0 (-0.006, 0.006)	0.002 (-0.003, 0.08)	-0.012 (-0.033, 0.009)	0.003 (-0.012, 0.017)	15.29 (6.49, 24.09)*
	n = 1370	*n = 1338*	*n = 1116*	*n = 1116*	*n = 1744*
3	0.004 (-0.004, 0.013)	0.009 (0.002, 0.016)	-0.006 (-0.033, 0.022)	0.015 (-0.004, 0.035)	26.40 (14.92, 37.88)*
	n = 619	*n = 630*	*n = 516*	*n = 531*	*n = 877*
4	0.021 (0.01, 0.033)*	0.017 (0.007, 0.027)*	0.027 (-0.013, 0.066)	0.03 (0.002, 0.05)*	31.69 (15.69, 47.68)*
	n = 273	*n = 254*	*n = 224*	*n = 212*	*n = 385*
5	0.016 (-0.001, 0.033)	0.022 (0.007, 0.038)*	0.053 (-0.003, 0.11)	0.007 (-0.035, 0.049)	23.9 (0.5, 47.68)
	n = 118	*n = 102*	*n = 99*	*n = 88*	*n = 167*
≥6	0.018 (-0.003, 0.04)	0.018 (-0.001, 0.037)	0.063 (-0.008, 0.135)	0.048 (-0.002, 0.098)	5.5 (-21.91, 33.09)
	n = 73	*n = 68*	*n = 62*	*n = 61*	*n = 121*
Trend[3]	*0.004 (0.002, 0.007)**	*0.005 (0.002, 0.007)**	*0.008 (0, 0.017)*	*0.007 (0.001, 0.013)*	*5.85 (2.62, 9.07)**

[1]Values are regression coefficients (95% Confidence Interval) and reflect differences in uterine artery resistance and pulsatility indices and placental weight for different categories of gravidity compared to women who were pregnant for the first time. All values were adjusted for gestational age at time of measurement, maternal age, body mass index, ethnicity, education, folic acid supplementation, smoking and alcohol consumption. [2]Differences in placental weight (g) for different categories of gravidity as compared to women who were pregnant for the first time. [3]Tests for trend were based on multiple linear regression models with gravidity as a continuous variable. *P-value <0.05.

Supplementary Table S2.2.5. Associations of gravidity with risks of notching and gestational hypertensive disorders[1]

Gravidity		Notching OR (95% CI)	Gestational hypertension OR (95% CI)	Pre-eclampsia OR (95% CI)
1	*n = 3684*	*Reference*	*Reference*	*Reference*
		n_{cases} *= 239*	n_{cases} *= 191*	n_{cases} *= 111*
2	*n = 2518*	0.82 (0.65, 1.04)	0.48 (0.36, 0.64)*	0.39 (0.26, 0.58)*
		n_{cases} *= 129*	n_{cases} *= 74*	n_{cases} *= 35*
3	*n = 1232*	0.78 (0.57, 1.08)	0.29 (0.18, 0.45)*	0.18 (0.09, 0.36)*
		n_{cases} *= 58*	n_{cases} *= 26*	n_{cases} *= 10*
≥4	*n = 943*	0.98 (0.67, 1.42)	0.21 (0.11, 0.37)*	0.21 (0.10, 0.42)*
		n_{cases} *= 44*	n_{cases} *= 15*	n_{cases} *= 11*
Trend[2]		*0.97 (0.88, 1.07)*	*0.63 (0.54, 0.73)**	*0.53 (0.43, 0.65)**

[1]Values are Odds Ratio and 95% Confidence Interval that indicate the differences in risks of notching, gestational hypertension and pre-eclampsia in different categories of gravidity compared to reference group of women were pregnant for the first time. Values were adjusted for maternal age, body mass index, educational level, ethnicity, folic acid supplementation, smoking and alcohol consumption. [2]Tests for trend were based on logistic regression models with gravidity as a continuous variable. *P-value <0.05.

Chapter 2.3

Maternal parity, early growth and childhood cardiovascular risk factors

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Abstract

Background: We examined the associations of maternal parity with fetal and childhood growth characteristics, and childhood cardio-metabolic risk factors in a population-based prospective cohort study among 9031 mothers and their children.

Methods: Fetal and childhood growth were repeatedly measured. We measured childhood anthropometrics, body fat distribution, left ventricular mass, blood pressure, blood lipids and insulin levels at the age of 6 years.

Results: As compared to nulliparous mothers, multiparous mothers had children with higher third trimester fetal head circumference, length and weight growth and lower risks of preterm birth and small size for gestational age at birth, but a higher risk of large size for gestational age at birth (P-values <0.05). Children from multiparous mothers had lower rates of accelerated infant growth, and lower levels of childhood body mass index, total fat mass percentage and total- and LDL-cholesterol than children of nulliparous mothers (P-values <0.05). They also had a lower risk of childhood overweight (Odds Ratio (OR) 0.75 (95% Confidence Interval (CI): 0.63, 0.88)) and tended to have a lower risk of childhood clustering of cardio-metabolic risk factors (OR 0.82 (95% CI: 0.64, 1.05)). Among children from multiparous mothers, we observed consistent tendencies towards lower risks of childhood overweight and lower cholesterol levels with increasing parity (P-value <0.05).

Conclusions: Offspring from nulliparous mothers have lower fetal but higher infant growth rates and higher risks of childhood overweight and adverse metabolic profile. Maternal nulliparity may have persistent cardio-metabolic consequences for the offspring.

Introduction

The first pregnancy is associated with maternal hemodynamic maladaptations and higher risks of vascular complications during pregnancy.[1-3] Maternal and placental hemodynamic maladaptations may adversely affect fetal nutrient supply and fetal growth. Previous studies showed that nulliparous mothers have higher risks of delivering small size for gestational age children.[4,5] Fetal growth restriction and small size for gestational age at birth are associated with increased risks of neonatal morbidity and mortality, and with higher risks of obesity, higher blood pressure levels and insulin resistance in childhood and adulthood.[6-10] Most previous studies used birth weight as proxy for early growth but did not examine the associations of maternal parity with longitudinally measured fetal and childhood growth characteristics. Also, whether maternal nulliparity has persistent cardio-metabolic consequences for the offspring remains unclear.

Therefore, in a population-based prospective cohort study of 9031 mothers and their children, we examined the associations of maternal parity with longitudinally measured fetal and childhood growth characteristics. We also examined the associations of maternal parity with adverse birth outcomes, infant catch-up growth and childhood cardiometabolic risk factors.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards in Rotterdam, the Netherlands.[11] The study has been approved by the Medical Ethical Committee of Erasmus Medical Center, Rotterdam. Written consent was obtained from all participating mothers.[12] Response rate at birth was 61%. In total, 9778 mothers were enrolled in the study. 9147 mothers had information on parity available and gave birth to singleton live-born children. We excluded mothers and children without any fetal or childhood follow-up measurement available. Our cohort for analysis comprised 9031 mothers and their children (**Supplementary Figure S2.3.1**).

Parity assessment

Information about parity (defined as the number of times that a woman had given birth to a fetus with a gestational age of 24 weeks or more, regardless of whether the child was born alive or was stillborn) was obtained by questionnaire at enrollment. Parity was categorized into 4 categories (0; 1; 2; ≥3).

Fetal and early childhood growth measurements

Fetal ultrasound examinations were performed in 2 dedicated research centers in first (median: 13.5 wks of gestation, 95% range: 10.6, 17.5), second (median: 20.6 wks of gestation, 95% range: 18.6, 23.4) and third trimester (median: 30.4 wks of gestation, 95% range: 28.4, 33.0). We established gestational age by using data from the first ultrasound examination.[13] In the second and third trimesters, we measured fetal head circumference, abdominal circumference and femur length to the nearest millimeter using standardized ultrasound procedures.[14] Estimated fetal weight was calculated using the formula of Hadlock et al.[15] Gestational-age-adjusted standard deviation scores (SDS) were constructed for all fetal growth measurements.[13] Information about gender, gestational age, weight, length, and head circumference at birth was obtained from medical records. Gestational-age-adjusted SDS for birth weight, length and head circumference were constructed using North-European growth standards.[16]

Well-trained staff in the Community Health Centers obtained postnatal growth characteristics according to standard schedule and procedures at the ages of 3 months (median: 3.3, 95% range: 3.0, 3.9), 6 months (median: 6.2, 95% range: 5.2, 8.3), 12 months (median: 11.0, 95% range: 10.1, 12.6), 24 months (median: 24.8, 95% range: 23.4, 28.2), 36 months (median: 36.7, 95% range: 35.4, 40.8) and 48 months (median: 45.8, 95% range: 44.5, 48.6). SDS for postnatal growth characteristics were obtained with Dutch growth reference charts (Growth Analyzer 3.0; Dutch Growth Research Foundation, Rotterdam, Netherlands).

Birth outcomes

Preterm birth was defined as a gestational age of <37 weeks at delivery. Low birth weight was defined as birth weight <2500 g. Small size for gestational age at birth and large size for gestational age at birth were defined as a sex and gestational age adjusted birth weight below the 5th percentile (<-1.77 SDS) and above 95th percentile in the study cohort (>1.59 SDS), respectively.

Childhood cardio-metabolic outcomes

At the age of 6 years (median: 72.6 months, 95% range: 68.4, 96.2) childhood height and weight were measured without shoes and heavy clothing in a dedicated research facility in the Erasmus Medical Center, Sophia Children's Hospital. Body mass index was calculated. Overweight and obesity were defined according to the definition of Cole et al.[17] Body fat was measured by Dual-Energy X-ray absorptiometry (DXA) (iDXA, General Electrics – Lunar, 2008, Madison, WI, USA).[18] Total body fat mass percentage was calculated as percentage of total body weight. Android/gynoid fat mass ratio was calculated. Two-dimensional M-mode echocardiographic measurements were performed using methods recommended by the American Society of Echocardiography, and used to calculate the left ventricular mass using the formula derived by Devereux et al.[19,20]

Systolic and diastolic blood pressure were measured at the right brachial artery, four times with one minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus TM (Paramus, NJ, USA). A cuff was selected with a cuff width approximately 40% of the arm circumference and long enough to cover 90% of the arm circumference. Thirty minutes fasting venous blood samples were obtained and cholesterol, Low-Density-Lipoprotein (LDL)-cholesterol, High-Density-Lipoprotein (HDL)-cholesterol, triglycerides and insulin levels were measured. In line with previous definitions used among paediatric populations to define childhood metabolic-syndrome-like-phenotype[21], we defined clustering of cardio-metabolic risk factors as having any of the 3 or more following components: android fat mass percentage ≥75th percentile, systolic blood pressure or diastolic blood pressure ≥75th percentile, HDL-cholesterol ≤25th percentile or triglycerides ≥75th percentile and insulin level ≥75th percentile. We used android fat mass percentage as a proxy for waist circumference as waist circumference is not available in our study.

Covariates

Maternal age, weight and height were assessed at intake.[11] Maternal prepregnancy body mass index was calculated. Information on maternal education level, ethnicity and folic acid supplementation use was obtained at enrolment. Information on smoking and alcohol consumption was assessed by questionnaires during pregnancy. Maternal first trimester nutritional information was obtained by a food frequency questionnaire.[11] Maternal weight gain until a gestational age of 30 weeks (median: 30.2, 95% range: 28.5, 32.9) was measured. We used records from midwives and obstetricians to collect information on pregnancy complications.[22] Information about breastfeeding, timing of introduction of solid foods and average television watching time was obtained by questionnaires.[11]

Statistical analysis

First, we explored the associations of maternal parity with repeatedly measured fetal and childhood growth characteristics (head circumference, (femur) length, and (estimated fetal) weight) using unbalanced repeated measurement regression models. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data.[23] For presentation purposes of the longitudinal analyses, we combined the upper maternal parity categories, and used 3 categories (0, 1, ≥2). We also performed regular multivariate linear regression analyses to analyse the associations of maternal parity with fetal and childhood growth characteristics in absolute values.

Second, we used multivariate logistic regression models to analyze the associations of maternal parity with the risks of adverse birth outcomes. We used multivariate linear regression models to assess the associations of maternal parity with infant growth in different intervals. Finally, we used similar models to analyze the associations of

maternal parity with childhood cardio-metabolic outcomes and the risks of childhood overweight and childhood clustering of cardio-metabolic risk factors. Tests for trend were performed by analysing parity as per original number. Sensitivity analyses were performed among European mothers only. To take into account the potential effect of miscarriages, we performed a sensitivity analysis using maternal gravidity instead of parity for the analyses focused on birth and childhood outcomes.

All models were adjusted for gestational age at enrolment, maternal age, ethnicity, educational level, prepregnancy body mass index, gestational weight gain, smoking and alcohol consumption during pregnancy, folic acid supplementation use, total calorie intake during pregnancy, pregnancy complications, and fetal sex. The models focused on childhood growth outcomes were additionally adjusted for child's age at visit, gestational age at birth, infant breastfeeding, timing of introduction of solid foods and average duration of television watching, whereas the models focused on childhood body fat outcomes and cardio-metabolic outcomes were additionally adjusted for birth weight, child's age at measurement and child's height (body fat outcomes) or child's body mass index (cardio-metabolic outcomes). We tested for potential interactions between maternal parity and birth weight for the analyses focused on postnatal growth and cardio-metabolic outcomes, but no significant interactions were present. Missing data of covariates were imputed using multiple imputations. Analyses were performed using Statistical Package of Social Sciences version 17.0 for windows (SPSS Inc, Chicago, IL, USA) and Statistical Analysis System version 9.2 (SAS, Institute Inc. Cary NC, USA).

Results

Subject characteristics

Characteristics of the participants according to maternal parity are shown in **Table 2.3.1**. **Supplementary Table S2.3.1** shows that mothers with children with follow-up at the age of 6 years were more often higher educated and from European-descent.

Maternal parity and longitudinally measured fetal and childhood growth

Figure 2.3.1 gives the results of the longitudinal analyses, and shows that as compared to nulliparous mothers, multiparous mothers had children with higher fetal head circumference, length and weight growth from third trimester onwards, resulting in a higher head circumference, length and weight at birth (all P-values <0.05). From the postnatal age of 6 months onwards, differences in children's head circumference, height and weight between parity categories became smaller. At the age of 6 years, children of multiparous mothers had a lower stature and a lower weight (P-values <0.05), as compared to children of nulliparous mothers, but no differences in childhood head circumference were present. The associations of maternal parity with fetal and

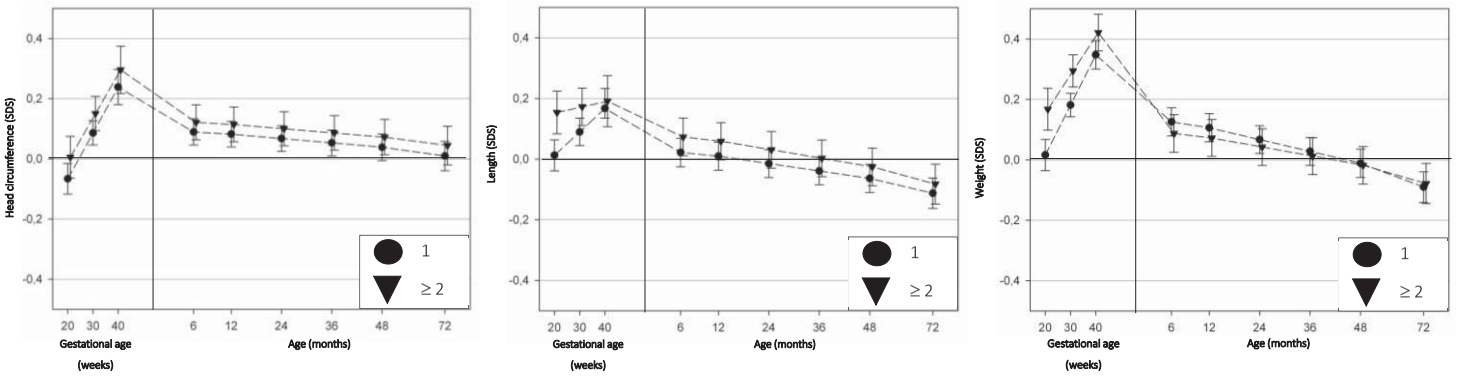
childhood growth characteristics from regular linear regression models in absolute values are given in **Supplementary Tables S2.3.2** and **S2.3.3**.

Table 2.3.1. Characteristics of study population by maternal parity (*n = 9031*)[1]

	Parity				
	0	1	2	≥3	
Characteristics	*n = 4994*	*n = 2721*	*n = 939*	*n = 377*	P-value
Maternal characteristics					
Age, years	28.5 (5.3)	30.8 (4.9)	32.4 (4.5)	34.0 (4.3)	<0.01
Height, cm	167.6 (7.4)	167.2 (7.4)	165.9 (7.2)	164.3 (7.3)	<0.01
Prepregnancy weight, kg	65.2 (12.6)	66.9 (12.5)	69.0 (14.3)	70.2 (12.7)	<0.01
Prepregnancy body mass index, kg/m^2	23.2 (4.2)	23.8 (4.3)	24.9 (4.9)	26.0 (4.8)	<0.01
Gestational weight gain, kg	10.9 (5.0)	9.9 (4.6)	9.8 (5.6)	9.4 (5.8)	<0.01
Gestational age at intake, weeks	13.8 (9.9, 24.2)	13.9 (9.8, 24.6)	14.8 (9.9, 29.8)	16.1 (11.1, 31.4)	<0.01
Education (higher education), %	44.9	43.4	35.9	17.0	<0.01
Ethnicity (European),%	60.9	59.9	45.2	26.3	<0.01
Alcohol consumption during pregnancy (Yes), %	53.0	50.9	42.7	25.1	<0.01
Smoking habits during pregnancy (Yes),%	18.1	18.8	19.8	20.7	0.02
Folic acid supplements (%)					
None	23.0	30.3	48.0	71.8	<0.01
1st 10 weeks	34.4	28.7	26.1	13.7	
Periconception use	42.6	41.0	25.9	14.5	
Total calorie intake (kcal)	2032 (561)	2046 (560)	2056 (616)	2003 (634)	0.58
Pregnancy complications (%)					
Pre-eclampsia	3.1	1.2	0.2	1.8	<0.01
Gestational hypertension	5.3	2.4	1.5	2.4	<0.01
Gestational diabetes	0.7	1.4	1.4	3.3	<0.01
Birth and infant characteristics					
Gestational age at birth, weeks	40.1 (34.7, 42.4)	40.1 (36.1, 42.1)	40.0 (35.7, 42.4)	40.0 (35.8,42.4)	0.52
Male sex, %	50.2	51.2	51.7	51.2	0.77
Birth weight, g	3325 (567)	3502 (532)	3529 (569)	3546 (562)	<0.01
Breastfeeding (Yes), %	93.2	89.4	92.0	94.2	<0.01
Timing of introduction of solid foods (<6 months),%	90.0	88.5	91.2	88.8	0.05
Average duration of television watching (> 2 hours/day) (%)	16.7	20.3	27.7	36.0	<0.01
Childhood characteristics					
Age at follow up, months	72.5 (67.9, 95.1)	72.7 (67.8, 95.9)	72.9 (68.0, 95.2)	73.7 (69.2, 98.0)	<0.01
Height, cm	119.5 (5.9)	119.3 (6.2)	119.7 (6.0)	119.2 (6.4)	0.41
Weight, kg	23.2 (4.1)	23.3 (4.3)	23.8 (5.1)	23.3 (3.9)	0.02
Body mass index, kg/m^2	16.2 (1.8)	16.3 (1.9)	16.5 (2.3)	16.3 (1.8)	<0.01
Total fat mass,%	25.0 (5.6)	24.7 (5.6)	25.2 (6.3)	25.4 (5.7)	0.09
Android/gynoid fat mass ratio, %	25.2 (6.3)	25.0 (6.2)	25.5 (7.2)	25.1 (5.9)	0.43
Left ventricular mass, g	53.4 (11.6)	53.7 (11.7)	54.0 (12.1)	53.5 (12.4)	0.64
Systolic blood pressure, mmHg	102.9 (8.3)	102.4 (8.0)	102.9 (8.3)	104.1 (7.9)	0.02
Diastolic blood pressure, mmHg	60.9 (6.8)	60.4 (6.9)	60.6 (6.7)	61.4 (7.1)	0.05
Cholesterol, mmol/L	4.2 (0.6)	4.2 (0.6)	4.2 (0.6)	4.1 (0.6)	0.20
Low Density Lipoprotein Cholesterol, mmol/L	2.4 (0.6)	2.4 (0.6)	2.3 (0.5)	2.3 (0.5)	0.04
High Density Lipoprotein Cholesterol, mmol/L	1.3 (0.3)	1.4 (0.3)	1.4 (0.3)	1.4 (0.3)	0.02
Triglycerides, mmol/L	1.0 (0.4,2.4)	0.9 (0.4,2.2)	0.9 (0.4,2.7)	0.9 (0.3,2.6)	0.26
Insulin, pmol/L	112.3 (17.5, 398.0)	117.4 (15.8, 395.8)	105.3 (11.4, 393.2)	91.6 (19.7, 487.9)	0.30

[1]Values are means (standard deviation) or median (95% range) or percentages.

Figure 2.3.1. Associations of maternal parity with fetal and childhood growth characteristics[1]



2.3.1a. Head circumference **2.3.1b**. Length **2.3.1c.** Weight

[1]Fetal and childhood head circumference, length and weight growth among different maternal parity categories (1, ≥2) as compared to nulliparity (reference group, shown as zero-line in the graphs). Results are based on repeated measurement regression models. Effect estimates (95% CI represented by error bars) reflect the differences in (gestational)-age-adjusted SDS scores of head circumference, length and weight at 20, 30 and 40 weeks of gestation and at 6, 12, 24, 36, 48 months and 72 months postnatally among different maternal parity categories. As the reference category, nulliparity, is represented by the zero-line in the graphs, non-crossing of the zero-line by the effect estimates and Confidence Intervals for parity=1 and parity≥2, indicates that the effect estimates for these categories are statistically significant. Estimates are from multiple imputed data. Models were adjusted for gestational age at enrolment, maternal age, ethnicity, educational level, prepregnancy body mass index, gestational weight gain, smoking and alcohol consumption during pregnancy, folic acid supplementation use, total calorie intake during pregnancy, pregnancy complications, and fetal sex.

Table 2.3.2. Associations of maternal parity with birth outcomes (*n = 9027*)[1]

Parity	Preterm birth OR (95% CI)	Low birth weight OR (95% CI)	Small size for gestational age OR (95% CI)	Large size for gestational age OR (95% CI)
0	*Reference*	*Reference*	*Reference*	*Reference*
n = 4992	n_{cases} *= 317*	n_{cases} *= 317*	n_{cases} *= 321*	n_{cases} *= 160*
≥1	0.62 (0.50, 0.77)**	0.42 (0.33, 0.53)**	0.44 (0.34, 0.55)**	2.47 (1.97, 3.10)**
n = 4035	n_{cases} *= 168*	n_{cases} *= 121*	n_{cases} *= 124*	n_{cases} *= 286*
1	0.64 (0.51, 0.80)**	0.42 (0.32, 0.55)**	0.45 (0.35, 0.58)**	2.35 (1.85, 2.97)**
n = 2721	n_{cases} *= 114*	n_{cases} *= 79*	n_{cases} *= 85*	n_{cases} *= 182*
2	0.59 (0.41, 0.86)**	0.42 (0.28, 0.63)**	0.43 (0.28, 0.65)**	2.88 (2.08, 4.01)**
n = 937	n_{cases} *= 40*	n_{cases} *= 30*	n_{cases} *= 30*	n_{cases} *= 75*
≥3	0.52 (0.30, 0.90)*	0.36 (0.20, 0.67)**	0.32 (0.17, 0.62)**	3.26 (2.02, 5.27)**
n = 377	n_{cases} *= 17*	n_{cases} *= 13*	n_{cases} *= 11*	n_{cases} *= 30*
Trend[2]	*0.77 (0.67, 0.88)***	*0.60 (0.52, 0.72)***	*0.61 (0.52, 072)***	*1.48 (1.32, 1.65)***
Trend among multiparous mothers only[2]	*0.87 (0.69, 1.09)*	*0.86 (0.66, 1.12)*	*0.86 (0.65, 1.14)*	*1.19 (1.01, 1.40)**

[1]Values are Odds Ratios (95% Confidence Interval) that indicate the differences in risks of preterm birth (gestational age <37 weeks at delivery), low birth weight (birth weight <2500 g), small size for gestational age at birth (sex and gestational-age-adjusted birth weight below 5th percentile) and large size for gestational age at birth (sex and gestational-age-adjusted birth weight above 95th percentile) for different categories of parity compared nulliparous mothers. Estimates are based on multiple imputed data. Models were adjusted for gestational age at enrolment, maternal age, ethnicity, educational level, prepregnancy body mass index, gestational weight gain, smoking and alcohol consumption during pregnancy, folic acid supplementation use, total calorie intake during pregnancy, pregnancy complications, and fetal sex. [2]Tests for trend were based on logistic regression models with parity as a continuous variable. *P-value <0.05.**P-value <0.01.

Maternal parity, birth outcomes and infant growth patterns

Table 2.3.2 shows that as compared to nulliparous mothers, multiparous mothers had lower risks of preterm delivery (Odds Ratio (OR) 0.62 (95% CI: 0.50, 0.77)) and small size for gestational age children (OR 0.44 (95% CI: 0.34, 0.55)) but a higher risk of large size for gestational age children (OR 2.47 (95% CI: 1.97, 3.10)). Trend analyses showed that among all mothers a higher parity was associated with lower risks of preterm birth and small size for gestational age at birth, and with a higher risk of large size for gestational age at birth (all P-values <0.05). These trends, although in similar direction, were not significant among multiparous mothers only. Table 2.3.3 shows that compared to infants of nulliparous mothers, infants of multiparous mothers had a lower weight gain between ages of 0 and 3 months, 3 and 6 months and 6 and 12 months (P-values <0.05). Similar significant but weaker associations were present for height in the first year of life.

Maternal parity and childhood cardio-metabolic risk factors

Table 2.3.4 shows that, as compared to children of nulliparous mothers, children of multiparous mothers had a lower body mass index (difference: -0.12 kg/m^2 (95% CI: -0.22, -0.02)) and total fat mass percentage (difference: -0.42 % (95% CI: -0.69, -0.14)), but not android/gynoid fat mass ratio, at the age of 6 years. Among all mothers and multiparous mothers only, consistent trends in similar direction were present (P for trends <0.05). No significant associations were present of maternal parity with

childhood cardiac outcomes. Trend analyses showed that higher maternal parity was associated with lower total-cholesterol and LDL-cholesterol levels in children (P for trend among all mothers and among multiparous mothers only <0.05), but no associations were present for triglycerides and insulin levels. Children of multiparous mothers had a lower risk of childhood overweight (OR 0.75 (95% CI: 0.63, 0.88)) and tended to have a lower risk of childhood clustering of cardio-metabolic risk factors (OR 0.82 (95% CI: 0.64, 1.05)) as compared to children of nulliparous mothers (**Figure 2.3.2**). We observed significant trends towards a lower risk of childhood overweight and childhood clustering of cardio-metabolic risk factors with increasing parity (P for trend among all mothers <0.05). Among multiparous mothers only, trend analysis showed similar tendencies. We observed similar results when we restricted our analyses to mothers of European origin (**Supplementary Table S2.3.4**). We did not adjust our analyses for previous miscarriages. However, when we repeated our analyses using maternal gravidity instead of maternal parity, effect estimates for the associations with birth and childhood outcomes were in similar direction (results not shown).

Discussion

In this prospective cohort study, we observed that as compared to maternal nulliparity, multiparity was associated with higher fetal growth rates from third trimester onwards and with lower risks of delivering preterm and small size for gestational age infants, but a higher risk of delivering large size for gestational age infants. Children of multiparous mothers had lower rates of accelerated infant growth and a better cardio-metabolic profile at 6 years. Among multiparous mothers only, a higher parity was associated with a lower risk of childhood overweight and a better cholesterol profile.

Methodological considerations

We had a prospective data collection from early fetal life onwards, and a large sample size of 9031 pregnant women and their children. Detailed, repeatedly measured, fetal and childhood growth characteristics were available. A potential limitation might be the response rate of 61%. Pregnant women who participated were more highly educated, healthier and more frequently of Dutch origin than were those who did not participate.[11] Follow-up data at 6 years were available in 69% of our study population. The non-response would lead to biased effect estimates if the associations would be different between those included and not included in the analyses. This seems unlikely.[24] The non-response at baseline and at follow-up might have led to a selection of a more healthy population, and might affect the generalizability of our results. Differences in maternal health and lifestyle-related determinants between families with one child and multiple children might be an important confounding aspect within our study. We had detailed information about a large number of potential confounding socio-demographic and lifestyle-related factors available in this study.

Table 2.3.3. Associations of maternal parity with growth rates in different intervals during infancy[1]

	Length (change in SDS)			Weight (change in SDS)		
Parity	0 – 3 months	3 – 6 months	6 – 12 months	0 – 3 months	3 – 6 months	6 – 12 months
0	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
n	*1488*	*2048*	*2645*	*2755*	*2614*	*2957*
≥1	-0.11 (-0.20, -0.02)*	-0.05 (-0.10, -0.01)*	-0.08 (-0.12, -0.04)**	-0.23 (-0.29, -0.17)**	-0.09 (-0.12, -0.06)**	-0.06 (-0.10, -0.03)**
n	*1203*	*1550*	*1999*	*2098*	*1960*	*2239*
1	-0.10 (-0.20, -0.01)*	-0.04 (-0.09, 0.01)	-0.09 (-0.13, -0.05)**	-0.20 (-0.26, -0.13)**	-0.09 (-0.12, -0.05)**	-0.08 (-0.11, -0.04)**
n	*844*	*1082*	*1414*	*1462*	*1379*	*1581*
2	-0.15 (-0.31, 0.00)*	-0.10 (-0.18, -0.02)*	-0.06 (-0.12, 0.01)	-0.33 (-0.43, -0.23)**	-0.11 (-0.17, -0.06)**	-0.01 (-0.06, 0.05)
n	*270*	*348*	*437*	*473*	*432*	*492*
≥3	0.01 (-0.25, 0.26)	-0.08 (-0.21, 0.04)	-0.03 (-0.13, 0.08)	-0.35 (-0.52, -0.19)**	-0.03 (-0.12, 0.06)	-0.06 (-0.15, 0.03)
n	*89*	*120*	*148*	*163*	*149*	*166*
Trend[2]	*-0.05 (-0.10, 0.01)*	*-0.04 (-0.07, -0.01)***	*-0.03 (-0.05, -0.01)**	*-0.14 (-0.18, -0.10)***	*-0.04 (-0.06, -0.02)***	*-0.02 (-0.05, -0.00)**
Trend among multiparous mothers only[2]	*-0.01 (-0.11, 0.10)*	*-0.04 (-0.09, 0.01)*	*0.04 (-0.01, 0.08)*	*-0.07 (-0.14, -0.01)**	*0.01 (-0.03, 0.04)*	*0.01 (-0.02, 0.05)*

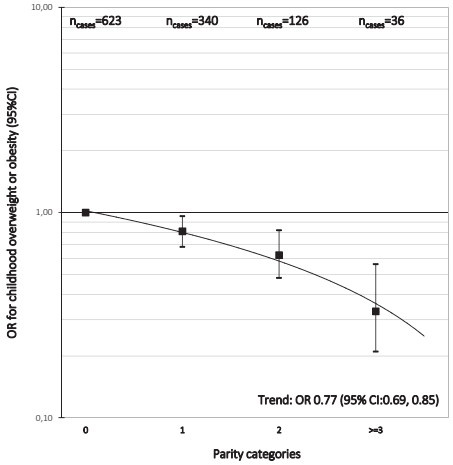
[1]Values are regression coefficients (95% Confidence Interval) from linear regression models and reflect differences in growth rates during the first year of life for different parity categories as compared to nulliparous mothers. Values are based on multiple imputed data. Models were adjusted for gestational age at enrolment, maternal age, ethnicity, educational level, prepregnancy body mass index, gestational weight gain, smoking and alcohol consumption during pregnancy, folic acid supplementation use, total calorie intake during pregnancy, pregnancy complications, and fetal sex, gestational age at birth, infant breastfeeding, timing of introduction of solid foods, average duration of tv watching, and age at visit. [2]Tests for trend were based on multiple linear regression models with parity as a continuous variable. *P-value <0.05. **P-value <0.01.

Table 2.3.4. Associations of maternal parity with childhood cardio-metabolic risk factors (*n = 6295*)[1,2]

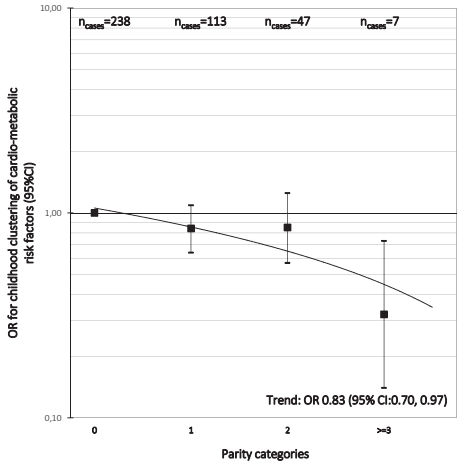
		Difference (95% CI) in childhood cardio-metabolic risk factors for different parity categories							
	n	0	≥1	0	1	2	≥3	*Trend*	*Trend among multiparous mothers only*
Adiposity outcomes									
Body mass index (kg/m^2)	*6295*	*Ref*	-0.12 (-0.22, -0.02)**	*Ref*	-0.09 (-0.19, 0.02)	-0.17 (-0.34, -0.01)*	-0.64 (-0.91, -0.37)**	*-0.13 (-0.19, -0.07)***	*-0.17 (-0.28, -0.06)***
Total fat mass (%)	*6131*	*Ref*	-0.42 (-0.69, -0.14)**	*Ref*	-0.34 (-0.63, -0.05)*	-0.60 (-1.06, -0.15)**	-1.20 (-1.96, -0.44)**	*-0.32 (-0.49, -0.14)***	*-0.30 (-0.60, -0.00)**
Android/gynoid fat mass ratio (%)	*6126*	*Ref*	-0.10 (-0.45, 0.26)	*Ref*	-0.05 (-0.52, 0.33)	-0.12 (-0.70, 0.47)	-1.13 (-2.09, -0.16)*	*-0.19 (-0.41, 0.04)*	*-0.36 (-0.73, 0.02)*
Cardiovascular outcomes									
Left ventricular mass (g)	*5934*	*Ref*	-0.33 (-0.93, 0.27)	*Ref*	-0.38 (-1.01, 0.25)	-0.17 (-1.17, 0.82)	0.07 (-1.53, 1.68)	*-0.05 (-0.43, 0.33)*	*0.27 (-0.37, 0.91)*
Systolic blood pressure (mmHg)	*6055*	*Ref*	-0.36 (-0.83, 0.10)	*Ref*	-0.42 (-0.91, 0.06)	-0.32 (-1.08, 0.45)	0.77 (-0.45, 2.00)	*0.01 (-0.28, 0.30)*	*0.28 (-0.20, 0.75)*
Diastolic blood pressure (mmHg)	*6055*	*Ref*	-0.32 (-0.71, 0.07)	*Ref*	-0.32 (-0.73, 0.08)	-0.38 (-1.03, 0.26)	0.10 (-0.94, 1.14)	*-0.11 (-0.35, 0.14)*	*-0.03 (-0.45, 0.39)*
Metabolic outcomes									
Cholesterol (mmol/L)	*4177*	*Ref*	-0.04 (-0.08, 0.01)	*Ref*	-0.02 (-0.07, 0.03)	-0.09 (-0.17, -0.02)*	-0.13 (-0.24, -0.01)*	*-0.04 (-0.06, -0.01)**	*-0.05 (-0.10, -0.01)**
HDL (mmol/L)	*4181*	*Ref*	0.02 (-0.01, 0.04)	*Ref*	0.02 (-0.00, 0.04)	0.00 (-0.03, 0.04)	0.02 (-0.04, 0.08)	*0.01 (-0.01, 0.02)*	*0.00 (-0.03, 0.02)*
LDL (mmol/L)	*4180*	*Ref*	-0.04 (-0.08, 0.00)*	*Ref*	-0.03 (-0.07, 0.02)	-0.09 (-0.16, -0.03)**	-0.12 (-0.23, -0.02)*	*-0.04 (-0.06, -0.01)***	*-0.06 (-0.10, -0.01)**
Triglyceride (mmol/L)[3]	*4166*	*Ref*	-0.02 (-0.06, 0.02)	*Ref*	-0.03 (-0.06, 0.01)	-0.01 (-0.07, 0.04)	-0.02 (-0.10, 0.07)	*-0.01 (-0.03, 0.01)*	*0.00 (-0.03, 0.04)*
Insulin (pmol/L)[3]	*4138*	*Ref*	0.24 (-0.05, 0.53)	*Ref*	0.30 (0.00, 0.60)	-0.03 (-0.50, 0.44)	0.16 (-0.61, 0.92)	*0.05 (-0.13, 0.23)*	*-0.12 (-0.41, 0.18)*

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in childhood outcomes for different parity categories as compared to nulliparous mothers. Values are based on multiple imputed data. Models were adjusted for gestational age at enrolment, maternal age, ethnicity, educational level, prepregnancy body mass index, gestational weight gain, smoking and alcohol consumption during pregnancy, folic acid supplementation use, total calorie intake during pregnancy, pregnancy complications, and fetal sex, gestational age and weight at birth, infant breastfeeding, timing of introduction of solid foods, average duration of tv watching, and age at measurement. Models for fat mass outcomes were additionally adjusted for current childhood height. Models for cardio-metabolic outcomes were additionally adjusted for current childhood body mass index. [2]Tests for trend were based on multiple linear regression models with parity as a continuous variable. [3]Triglycerides and insulin had a skewed distribution and were logtransformed and square root transformed, respectively.*P-value <0.05. **P-value <0.01.

Figure 2.3.2. Associations of maternal parity with childhood overweight and obesity and childhood clustering of cardio-metabolic risk factors[1,2,3,4]



2.3.2a. Overweight and obesity in the offspring



2.3.2b. Clustering of cardio-metabolic risk factors in the offspring

[1]Values are Odds Ratios (95% Confidence Interval) that reflect the difference in risks of childhood overweight and obesity and childhood clustering of cardio-metabolic risk factors for different parity categories (1, 2, ≥3), as compared to nulliparous mothers (0). Estimates are from multiple imputed data. [2]Models were adjusted for gestational age at enrolment, maternal age, ethnicity, educational level, prepregnancy body mass index, gestational weight gain, smoking and alcohol consumption during pregnancy, folic acid supplementation use, total calorie intake during pregnancy, pregnancy complications, and fetal sex, gestational age and weight at birth, infant breastfeeding, timing of introduction of solid foods, average duration of tv watching, and age at measurement. [3]OR for children born from multiparous mothers, as compared to children born from nulliparous mothers for risk of childhood overweight and obesity OR 0.75 (95% CI: 0.63, 0.88) and for childhood clustering of cardio-metabolic risk factors OR 0.82 (95% CI: 0.64, 1.05). [4]Trend among multiparous mothers only for childhood overweight or obesity: OR 0.76 (95% CI: 0.64, 0.90). Trend among multiparous mothers only for childhood clustering of cardio-metabolic risk factors: OR 0.77 (95% CI: 0.58, 1.02).

Extensive adjustment for these factors in our analyses did not explain the associations of maternal parity with birth and childhood outcomes. However, residual confounding due to other lifestyle-related variables, such as maternal and childhood nutritional factors and physical activity, might still be an issue. Also, ethnic background was strongly related to maternal parity in our study, and the influence of ethnic background on childhood outcomes might affect our findings. However, all analyses were adjusted for maternal ethnic background and analyses among European mothers only showed similar results. Finally, we had a relatively small number of cases of adverse birth and childhood outcomes among multiparous mothers within our study, which might explain non-significant findings for these outcomes among multiparous mothers only.

Maternal parity and fetal and childhood outcomes

We observed that nulliparous mothers had children with lower fetal growth rates and higher risks of delivering preterm and small size for gestational age children. Among multiparous women only, trend analysis showed a tendency towards decreasing risks of

adverse birth outcomes with increasing parity. Our findings are in line with a previous study among 25.614 singleton births, which showed that the rates of intra-uterine growth restriction and preterm delivery were higher among nulliparous women as compared to multiparous women.[5] As compared to infants of nulliparous mothers, infants of multiparous mothers had lower rates of accelerated infant growth, lower childhood body mass index, fat mass percentage and cholesterol levels. Among multiparous women only, consistent trends with increasing parity in similar direction were present. Although the observed effect estimates were small and they are mainly of interest from a cardiovascular developmental perspective, previous studies have shown that these childhood cardio-metabolic risk factors tend to track into adulthood and are related to development of cardiovascular disease in later life.[25,26]

In line with our findings, a previous prospective cohort study among 1335 infants showed that infants of nulliparous mothers had dramatic catch-up growth and from 12 months onwards these infants were heavier and taller as compared to infants of multiparous mothers.[27] A study, which examined the combined effect of maternal and child risk factors in generating risk profiles for overweight and obesity among preschool children observed that parity played an important role.[28] Parity has also been identified as an independent determinant of neonatal body composition.[29] Furthermore, a study among 276 men and women reported that adiposity in early adulthood is influenced by maternal parity independent of birth weight and current lifestyle-related factors.[30] In our study, the associations of maternal parity with cardio-metabolic risk factors in childhood were also independent of birth weight and not explained by socio-demographic and lifestyle-related factors. A recent study among 1.065.710 Swedish men also reported that birth order was negatively associated with body mass index.[31]

We observed no associations of maternal parity with childhood left ventricular mass and blood pressure. Studies examining associations of parity with offspring blood pressure have reported conflicting results.[31-36] A study among 3360 children reported that maternal parity was inversely associated with offspring blood pressure.[32] However, this study suggested that as associations of both older and younger siblings with childhood blood pressure were equally strong, the association is likely a postnatal effect instead of a prenatal maternal effect.[32] A study among 453 Brazilian adolescents also showed that significant associations of parity with systolic and diastolic blood pressure disappeared in fully adjusted models, which further supports a postnatal effect.[36]

To our knowledge, no previous studies have examined the associations of maternal parity with childhood metabolic outcomes. The concept of metabolic syndrome in childhood is controversial, as there may be variability in its manifestation with age, gender and ethnicity and there is lack of understanding of underlying pathophysiological mechanisms.[21] However, defining children with clustering of cardio-metabolic risk factors for research purposes might identify children at high risk of cardiovascular and metabolic diseases in later life.[21] We observed that children of nulliparous mothers had higher cholesterol levels and a higher risk of clustering of cardio-metabolic risk factors. A study among young Brazilian adults showed that firstborns had higher cholesterol and triglycerides levels, and a higher metabolic risk as compared to later-borns.[33] The

association of maternal parity with metabolic risk was not explained by birth weight, but was largely explained by rapid postnatal growth.[33] In our study, the associations between maternal parity and childhood overweight and childhood clustering of cardio-metabolic risk factors were not explained by birth weight, but they were also only partly explained by infant growth.

Biological mechanisms

The mechanisms by which nulliparity might be associated with impaired fetal growth, accelerated infant growth and an adverse childhood metabolic profile are not clear. Shared family-based, lifestyle-related, and parenting behaviour factors, which differ between families from nulliparous and multiparous mothers, may explain part of the observed associations. However, since our findings were not explained by a large number of socio-demographic and lifestyle-related characteristics, and we already observed differences in birth and early childhood outcomes, biological mechanisms may also play a role.

Maternal constraint, which involves non-genetic influences by which the mother limits fetal growth, may be greater among nulliparous mothers. Maternal constraint may involve suboptimal adaptations in the utero-placental vasculature.[37] During the first pregnancy the spiral arteries, which provide maternal blood to the placenta, are remodeled.[3] Multiparous women may offer, through remodelling of maternal vascular structures in their previous pregnancies, a more favourable environment for placental development, placental function and fetal nutrition in the next pregnancies.[38-41] Furthermore, differences in maternal metabolic and hormonal environment between nulliparous and multiparous women may influence foeto-placental development.[42,43] Impaired fetal growth, followed by infant catch-up growth may influence risks of adiposity and adverse cardio-metabolic outcomes in later life.[6,44] Animal studies also suggested an increase in fat mass and alterations in endocrine sensitivity in adipose tissue in firstborn offspring, which may also be important risk factors for obesity and related disorders in later life.[40]

Conclusion

We observed that children of nulliparous mothers have slower fetal growth rates and accelerated infant growth rates. Maternal nulliparity is associated with increased risks of adverse birth outcomes, and childhood adiposity and adverse metabolic profile in offspring. Among multiparous mothers only, increasing parity tends to be associated with a decreasing risk of adverse health outcomes in offspring. Maternal nulliparity may have persistent cardio-metabolic consequences for the offspring. Further studies are needed to explore underlying mechanisms.

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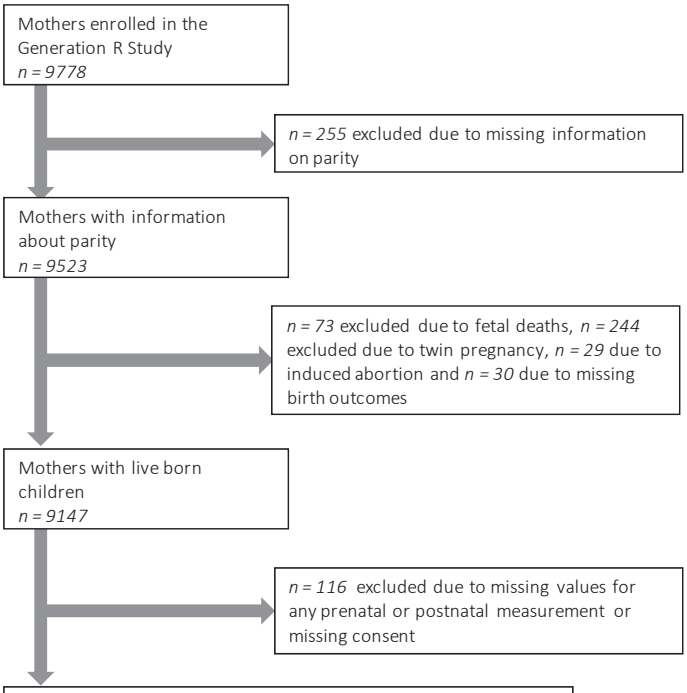
Supplementary Material

Supplementary Figure S2.3.1. Flowchart of the participants



Mothers with information about growth in the offspring
n = 9031

Fetal growth	
Estimated fetal weight in second trimester	*n = 7946*
Estimated fetal weight in third trimester	*n = 8129*
Fetal weight at birth	*n = 8976*
Childhood growth	
6 Months	*n = 6132*
12 Months	*n = 5605*
24 Months	*n = 5062*
36 Months	*n = 4767*
48 Months	*n = 4176*
72 Months	*n = 6304*
Childhood cardio-metabolic outcomes	
Adiposity outcomes	
Body mass index:	*n = 6295*
Body fat distribution:	*n = 6131*
Cardiovascular outcomes	
Left ventricular mass:	*n = 5934*
Systolic blood pressure:	*n = 6055*
Diastolic blood pressure:	*n = 6055*
Metabolic outcomes	
Cholesterol:	*n = 4177*
Triglycerides:	*n = 4166*
Insulin:	*n = 4138*

Supplementary Table S2.3.1. Non-response analysis (*n = 9031*)[1]

Characteristics	Complete population for analysis *n = 9031*	Follow-up at 6 years: Yes *n = 6304*	Follow-up at 6 years: No *n = 2727*
Maternal characteristics			
Age (years)	29.8 (5.3)	30.5 (5.2)	28.4 (5.5)
Height (cm)	167.1 (7.4)	167.5 (7.4)	166.3 (7.4)
Prepregnancy weight (kg)	66.2 (12.8)	66.5 (12.6)	65.7 (13.4)
Prepregnancy body mass index (kg/m^2)	23.6 (4.4)	23.6 (4.2)	23.7 (4.7)
Gestational age at intake (weeks)[2]	14.1 (9.9, 24.8)	13.9 (9.9, 24.4)	14.5 (9.9, 27.5)
Parity (No., %)			
0	4994 (55.3)	3560 (56.5)	1434 (52.6)
1	2721 (30.1)	1911 (30.3)	810 (29.7)
2	939 (10.4)	623 (9.9)	316 (11.6)
≥3	377 (4.2)	210 (3.3)	167 (6.1)
Education (No., %)			
Primary school	926 (11.4)	562 (9.7)	364 (15.6)
Secondary school	3756 (46.1)	2555 (44.0)	1201 (51.3)
Higher education	3470 (42.6)	2696 (46.4)	774 (33.1)
Ethnicity (No., %)			
European	4944 (57.7)	3771 (61.2)	1173 (48.7)
Non- European	3625 (42.3)	2388 (38.8)	1237 (51.3)
Alcohol consumption (No., %)			
No	3674 (49.7)	2384 (46.0)	1290 (58.1)
Yes	3725 (50.3)	2795 (54.0)	930 (41.9)
Smoking habits (No., %)			
No	5460 (72.7)	3876 (74.0)	1584 (70.3)
Yes	2034 (27.1)	1365 (26.0)	669 (29.7)
Folic acid supplements (No., %)			
None	1870 (29.4)	1131 (25.2)	739 (39.5)
1st 10 weeks	1979 (31.1)	1421 (31.7)	558 (29.8)
Periconception use	2511 (39.5)	1937 (43.1)	574 (30.7)
Pregnancy complications (No., %)			
Gestational diabetes	96 (1.1)	62 (1.0)	34 (1.3)
Pre-eclampsia	170 (2.2)	106 (1.9)	64 (2.7)
Gestational hypertension	310 (3.9)	235 (4.2)	75 (3.1)
Birth characteristics			
Gestational age (weeks)[2]	40.1 (35.4, 42.3)	40.1 (35.7, 42.3)	40.0 (34.7, 42.4)
Male sex (No., %)	4578 (50.7)	3156 (50.1)	1423 (52.2)
Birth weight (g)	3409 (565)	3422 (556)	3378 (583)

[1]Values are means (standard deviation) or numbers (percentages). [2]Median (95% range).

Supplementary Table S2.3.2. Associations of maternal parity with fetal growth characteristics during pregnancy and at birth[1,2]

Trimester of measurement and parity category	Head circumference (mm)	Femur length (mm)	Estimated fetal weight (g)
Second trimester			
0	*Reference*	*Reference*	*Reference*
≥1	-0.1 (-0.4, 0.2)	0.1 (0, 0.2)	3.6 (1.4, 5.8)**
1	-0.2 (-0.5, 0.2)	0 (-0.1, 0.1)	2.3 (0, 4.7)*
2	0.1 (-0.4, 0.6)	0.2 (0.1, 0.4)**	7.3 (3.6, 11.0)**
≥3	0 (-0.7, 0.8)	0.3 (0, 0.5)*	9.4 (3.8, 15.0)**
Trend[3]	*0 (-0.2, 0.2)*	*0.1 (0, 0.1)***	*3.0 (1.7, 4.3)***
Third trimester			
0	*Reference*	*Reference*	*Reference*
≥1	0.2 (-0.2, 0.7)	0.2 (0.1, 0.3)**	26.3 (17.3, 35.3)**
1	0.1 (-0.3, 0.6)	0.2 (0.1, 0.3)**	23.9 (14.4, 33.3)**
2	0.6 (-0.1. 1.3)	0.3 (0.1, 0.5)**	33.2 (18.4, 48.0)**
≥3	0.5 (-0.7, 1.6)	0.4 (0.1,0.7)**	38.1 (14.8, 61.5)**
Trend[3]	*0.2 (-0.1, 0.5)*	*0.2 (0.1, 0.2)***	*15.2 (9.8, 20.6)***
At birth	**Head circumference** (cm)	**Birth length** (cm)	**Birth weight** (g)
0	*Reference*	*Reference*	*Reference*
≥1	0.5 (0.4, 0.6)**	0.4 (0.3, 0.6)**	188.9 (169.2, 208.5)**
1	0.5 (0.4, 0.6)**	0.4 (0.3, 0.5)**	174.3 (153.5, 195.2)**
2	0.6 (0.4, 0.8)**	0.5 (0.3, 0.7)**	222.3 (190.0, 254.6)**
≥3	0.9 (0.6, 1.2)**	0.7 (0.3, 1.0)**	284.9 (235.3, 334.5)**
Trend[3]	*0.3 (0.3, 0.4)***	*0.3 (0.2, 0.3)***	*103.4 (91.8, 114.9)***

[1]Results are from linear regression analyses. Values are regression coefficients (95% Confidence Interval) that reflect the differences in each growth characteristic measured in second trimester, third trimester and at birth, for different parity categories as compared to nulliparity. Estimates are based on multiple imputed data. [2]Models were adjusted for gestational age at enrolment, maternal age, ethnicity, educational level, prepregnancy body mass index, gestational weight gain, smoking and alcohol consumption during pregnancy, folic acid supplementation use, total calorie intake during pregnancy, pregnancy complications, and fetal sex. [3]Tests for trend were based on multiple linear regression models with parity as a continuous variable. *P-value <0.05. **P-value <0.01.

Supplementary Table S2.3.3. Associations of maternal parity with childhood growth characteristics during the first 6 years of childhood[1,2]

	Weight				
Parity	12 months (g)	24 months (g)	36 months (g)	48 months (g)	72 months (kg)
0	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
≥1	59.5 (-0.3, 119.3)	-45.2 (-135.7, 45.3)	-77.0 (-193.0, 38.9)	-33.8 (-180.1, 112.4)	-0.3 (-0.6, -0.1)*
1	55.2 (-7.8, 118.2)	-58.4 (-153.9, 37.2)	-88.6 (-210.3, 33.0)	-38.0 (-192.0, 116.1)	-0.3 (-0.5, 0.0)
2	56.7 (-43.8, 157.3)	-18.8 (-172.9, 135.3)	-58.9 (-258.7, 141.0)	-23.5 (-269.6, 222.7)	-0.4 (-0.8, 0.1)
≥3	154.0 (-12.5, 320.5)	90.4 (-161.2, 342.1)	62.7 (-259.3, 384.6)	-3.4 (-403.6, 396.9)	-1.0 (-1.7, -0.3)*
Trend	*42.8 (5.4, 80.1)**	*3.6 (-53.1, 60.3)*	*-13.2 (-86.1, 59.6)*	*14.0 (-77.1, 105.1)*	*-0.2 (-0.4, -0.1)***
	Length				
Parity	12 months (cm)	24 months (cm)	36 months (cm)	48 months (cm)	72 months (cm)
0	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
≥1	-0.0 (-0.2, 0.1)	-0.4 (-0.6, -0.2)**	-0.3 (-0.5, -0.0)*	-0.27 (-0.5, 0.0)	-0.4 (-0.69, -0.13)**
1	-0.1 (-0.2, 0.1)	-0.4 (-0.6, -0.2)**	-0.4 (-0.6, -0.1)**	-0.32 (-0.6, -0.0)*	-0.4 (-0.72, -0.12)**
2	0.1 (-0.2, 0.3)	-0.2 (-0.5, 0.2)	0.1 (-0.3, 0.5)	-0.06 (-0.5, 0.4)	-0.3 (-0.75, 0.19)
≥3	0.3 (-0.1, 0.7)	0.0 (-0.5, 0.6)	0.1 (-0.6, 0.7)	-0.24 (-1.0, 0.5)	-0.9 (-1.68, -0.16)*
Trend	*0.1 (-0.0, 0.1)*	*-0.1 (-0.2, 0.03)*	*-0.03 (-0.2, 0.1)*	*-0.07 (-0.2, 0.1)*	*-0.2 (-0.40, -0.05)***

[1]Results are from linear regression analyses. Values are regression coefficients (95% Confidence Interval) and reflect the differences in growth for each characteristic for different parity categories as compared to nulliparity. Models were adjusted for gestational age at enrolment, maternal age, ethnicity, educational level, prepregnancy body mass index, gestational weight gain, smoking and alcohol consumption during pregnancy, folic acid supplementation use, total calorie intake during pregnancy, pregnancy complications, and fetal sex, gestational age at birth, infant breastfeeding, timing of introduction of solid foods, average duration of tv watching, and age at measurement.[2]Tests for trend were based on multiple linear regression models with parity as a continuous variable. *P-value <0.05. ** P-value <0.01.

Supplementary Table S2.3.4. Associations of maternal parity with childhood cardio-metabolic risk factors among Dutch and European mothers only (*n = 4944*)[1,2]

		Difference (95%CI) in cardio-metabolic outcomes for different parity categories							
	n	0	≥1	0	1	2	≥3	*Trend*	*Trend among multiparous women only*
Adiposity outcomes									
Body mass index (kg/m^2)	*3765*	*Ref*	-0.08 (-0.18, 0.03)	*Ref*	-0.06 (-0.17, 0.06)	-0.14 (-0.33, 0.05)	-0.36 (-0.76, 0.04)	*-0.08 (-0.15, -0.00)***	*-0.14 (-0.29, 0.01)*
Total fat mass (%)	*3668*	*Ref*	-0.33 (-0.64, -0.02)*	*Ref*	-0.30 (-0.62, 0.03)	-0.50 (-1.06, 0.06)	-0.41 (-1.58, 0.76)	*-0.24 (-0.45, -0.02)**	*-0.24 (-0.66, 0.18)*
Android/Gynoid fat mass ratio (%)	*3667*	*Ref*	0.03 (-0.38, 0.44)	*Ref*	0.09 (-0.34, 0.52)	-0.25 (-1.00, 0.49)	0.07 (-1.49, 1.62)	*-0.03 (-0.32, 0.25)*	*-0.29 (-0.85, 0.26)*
Cardiovascular outcomes									
Left ventricular mass (g)	*3544*	*Ref*	-0.35 (-1.12, 0.42)	*Ref*	-0.28 (-1.08, 0.53)	-0.84 (-2.25, 0.57)	0.45 (-2.50, 3.40)	*-0.23 (-0.77, 0.31)*	*0.02 (-1.06, 1.10)*
Systolic blood pressure (mmHg)	*3626*	*Ref*	-0.49 (-1.07, 0.10)	*Ref*	-0.62 (-1.23, -0.01)*	-0.21 (-1.26, 0.84)	2.08 (-0.12, 4.28)	*-0.07 (-0.48, 0.35)*	*0.60 (-0.19, 1.39)*
Diastolic blood pressure (mmHg)	*3626*	*Ref*	-0.46 (-0.95, 0.03)	*Ref*	-0.51 (-1.02, 0.00)	-0.47 (-1.36, 0.42)	1.03 (-0.84, 2.89)	*-0.18 (-0.52, 0.17)*	*0.28 (-0.41, 0.96)*
Metabolic outcomes									
Cholesterol (mmol/L)	*2506*	*Ref*	-0.04 (-0.10, 0.01)	*Ref*	-0.03 (-0.09, 0.03)	-0.08 (-0.18, 0.03)	-0.25 (-0.44, -0.04)*	*-0.05 (-0.08, -0.01)**	*-0.08 (-0.16, -0.01)**
HDL (mmol/L)	*2506*	*Ref*	0.03 (0.00, 0.05)*	*Ref*	0.03 (-0.00, 0.05)	0.06 (0.01, 0.11)*	-0.02 (-0.12, 0.09)	*0.02 (-0.00, 0.04)*	*-0.00 (-0.04, 0.04)*
LDL (mmol/L)	*2506*	*Ref*	-0.06 (-0.11, -0.01)*	*Ref*	-0.04 (-0.10, 0.01)	-0.11 (-0.20, -0.02)*	-0.20 (-0.38, -0.01)*	*-0.05 (-0.09, -0.02)***	*-0.07 (-0.14, -0.01)**
Triglyceride (mmol/L)[3]	*2502*	*Ref*	-0.03 (-0.08, 0.01)	*Ref*	-0.03 (-0.08, 0.01)	-0.05 (-0.12, 0.03)	0.02 (-0.13, 0.17)	*-0.02 (-0.05, 0.01)*	*0.01 (-0.05, 0.06)*
Insulin (pmol/L)[3]	*2486*	*Ref*	0.34 (-0.02, 0.70)	*Ref*	0.44 (0.07, 0.81)*	-0.14 (-0.79, 0.51)	0.06 (-1.26, 1.38)	*0.11 (-0.14, 0.36)*	*-0.37 (-0.85, 0.11)*

[1]Values are linear regression coefficients (95% Confidence Interval) and reflect the differences in childhood outcomes between different categories of parity as compared to nulliparity. Values are based on multiple imputed data. Models were adjusted for gestational age at enrolment, maternal age, educational level, prepregnancy body mass index, gestational weight gain, smoking and alcohol consumption during pregnancy, folic acid supplementation use, total calorie intake during pregnancy, pregnancy complications, and fetal sex, gestational age and weight at birth, infant breastfeeding, timing of introduction of solid foods, average duration of tv watching, and age at measurement. Models for fat mass outcomes were additionally adjusted for current childhood height. Models for cardio-metabolic outcomes were additionally adjusted for current childhood body mass index. [2]Tests for trend were based on multiple linear regression models with parity as a continuous variable. [3]Triglycerides and insulin had a skewed distribution and were logtransformed and square root transformed respectively. *P-value <0.05. **P-value <0.01.

Chapter 2.4

Maternal obesity, excessive gestational weight gain and the risks of pregnancy complications

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Abstract

Objective: The prevalence of overweight and obesity among women of reproductive age is increasing. We aimed to determine risk factors and maternal, fetal and childhood consequences of maternal obesity and excessive gestational weight gain.

Design and Methods: The study was embedded in a population-based prospective cohort study among 6959 mothers and their children. The study was based in Rotterdam, The Netherlands (2001 – 2005).

Results: Maternal lower educational level, lower household income, multiparity, and FTO risk allel were associated with an increased risk of maternal obesity, whereas maternal European ethnicity, nulliparity, higher total energy intake, and smoking during pregnancy were associated with an increased risk of excessive gestational weight gain (all P-values <0.05). As compared to normal weight, maternal obesity was associated with increased risks of gestational hypertension (Odds Ratio (OR) 6.31 (95% Confidence Interval (CI): 4.30, 9.26)), pre-eclampsia (3.61 (95% CI: 2.04, 6.39)), gestational diabetes (OR 6.28 (95% CI: 3.01, 13.06)), caesarean delivery (OR 1.91 (95% CI: 1.46, 2.50)), delivering large size for gestational age infants (OR 2.97 (95% CI: 2.16, 4.08)), and childhood obesity (OR 5.02 (95% CI: 2.97, 8.45)). Weaker associations of excessive gestational weight gain with maternal, fetal and childhood outcomes were observed, with the strongest effects for first trimester weight gain.

Conclusions: Our study shows that maternal obesity and excessive weight gain during pregnancy are associated with socio-demographic, lifestyle, and genetic factors and with increased risks of adverse maternal, fetal and childhood outcomes. As compared to prepregnancy overweight and obesity, excessive gestational weight gain has a limited influence on adverse pregnancy outcomes.

Introduction

Maternal obesity seems to be associated with short-term adverse maternal and fetal outcomes.[1-5] It has also been suggested that maternal obesity is associated with long-term maternal and offspring consequences, such as postpartum weight retention, metabolic syndrome, and obesity in the offspring.[1,3,6] Excessive gestational weight gain might also influence the risk of adverse maternal and fetal outcomes.[4,5,7] The mechanisms of these associations remain unclear, as gestational weight gain reflects both maternal nutritional status, as well as tissue expansion during pregnancy, because of fat storage and fluids.[4] Not much is known about the specific risk factors for maternal obesity and excessive weight gain during pregnancy. Identification of these risk factors and critical periods of gestational weight gain might be useful for the development of preventive strategies.

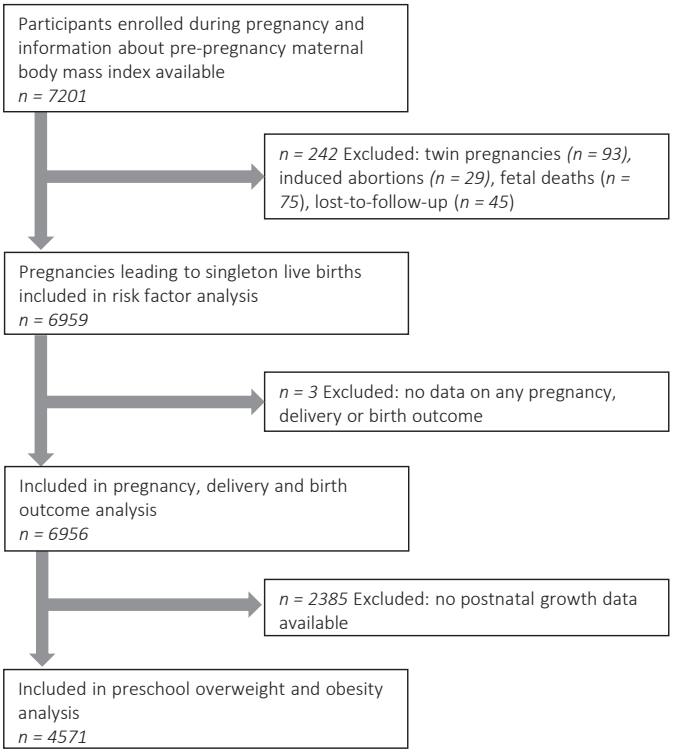
In a population-based prospective cohort study among 6959 mothers and their children, we examined the associations of several socio-demographic, lifestyle, and genetic factors with the risks of maternal obesity and excessive gestational weight gain. Next, we examined the associations of maternal obesity, excessive gestational weight gain, and trimester-specific weight gain with the risks of adverse maternal, fetal, and childhood outcomes.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onward in Rotterdam, the Netherlands.[8] Pregnant women were enrolled between 2001 and 2005. Of all the eligible children in the study area, 61% participated at birth in the study. The Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, approved the study (MEC 198.782/2001/31). Written informed consent was obtained from all mothers.[8] In total, 8880 mothers were enrolled during pregnancy, of whom information about prepregnancy body mass index was available in 7201 subjects. We excluded pregnancies not leading to singleton live births (*n = 242*). The population for analysis was 6959 mothers and their children (**Figure 2.4.1**).

Figure 2.4.1. Selection of study participants



Maternal anthropometrics, obesity, and weight gain during pregnancy

Maternal anthropometrics were measured in the first, second, and third trimester of pregnancy. Height (cm) and weight (kg) were measured without shoes and heavy clothing, and body mass index (kg/m^2) was calculated. Information about maternal weight just before pregnancy was obtained by questionnaire. In our population for analysis, 46.2% of all women were enrolled before a gestational age of 14 weeks. Correlation of prepregnancy weight, obtained by questionnaire, and weight measured at enrolment was 0.95 (P-value <0.001) (regression coefficient for this correlation: 0.93 (95% Confidence Interval (CI): 0.93, 0.94)). Prepregnancy body mass index was categorized into four categories: underweight (<20 kg/m^2), normal weight (20 - 24.9 kg/m^2), overweight (25 - 29.9 kg/m^2), and obesity (≥30 kg/m^2). Weight gain until a gestational age of 30 weeks was measured and available for 6623 mothers. Information about maximum weight during pregnancy was available in a subgroup of 3314 mothers and was assessed by questionnaire 2 months after delivery. Maximum weight from questionnaire and weight measured at 30 weeks were strongly correlated (r = 0.87 (P-value <0.001)). According to Institute of Medicine guidelines, we defined excessive gestational weight gain in relation to maternal prepregnancy body mass index (for underweight and

normal weight mothers: total weight gain >16 kg; for overweight mothers: total weight gain >11.5 kg; for obese mothers: total weight gain >9 kg.[9] Weight gain was further analyzed in each trimester of pregnancy.

Risk factors

Socio-demographic exposures
Maternal age was assessed at intake. The highest completed maternal educational level (primary school; secondary school; higher education) and maternal ethnicity (European; Surinamese; Turkish; Moroccan; Cape-Verdian and Dutch Antilles) were available from questionnaire.[8]

Diet- and lifestyle-related exposures
First trimester nutritional information (total energy intake [kcal], carbohydrates [energy %], fat [energy %], protein [energy %]) was obtained by a food frequency questionnaire at enrolment.[10] Mothers who were enrolled after the first trimester of pregnancy did not receive this food frequency questionnaire. Information about folic acid supplementation use was obtained at enrolment. Information about smoking and alcohol consumption was assessed by questionnaire in each trimester.[8] Maternal smoking and alcohol consumption were categorized in smoking during pregnancy (yes/no) and alcohol consumption during pregnancy (yes/no).

Maternal FTO polymorphism
Maternal genotyping of the FTO polymorphism (rs8050136) was performed using Taqman allelic discrimination assay (Applied Biosystems, Foster City, CA) and Abgene QPCR ROX mix (Abgene, Hamburg, Germany). The genotyping reaction was amplified using the GeneAmpVR PCR system 9600 (95 C [15 min], then 40 cycles of 94 C [15 sec], and 60 C [1 min]). The fluorescence was detected on the 7900HT Fast Real-Time PCR System (Applied Biosystems) and individual genotypes were determined using SDS software (version 2.3, Applied Biosystems).

Paternal exposures
Information on paternal age was obtained at enrolment in the study.[8] At enrolment, paternal height (cm) and weight (kg) were measured and body mass index (kg/m^2) was calculated.[8]

Gestational hypertension, pre-eclampsia, and gestational diabetes

Information on pregnancy complications was obtained from medical records. Details of these procedures have been described elsewhere.[11] Briefly, the following criteria were used to identify women with gestational hypertension: ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as two or more dipstick

reading of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24-h urine collection containing at least 300 mg of protein) were used to identify women with pre-eclampsia.[11] Information about gestational diabetes was obtained from medical records. Gestational diabetes was diagnosed by a community midwife or an obstetrician according to Dutch midwifery and obstetric guidelines using the following criteria: 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.[12] 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.

Delivery and birth complications

Information about assisted delivery, including prelabor rupture of membranes (PROM), Caesarian delivery, ventouse extraction, and postpartum hemorrhage, was obtained from midwife registries and hospital registries at birth. Gestational age was established by fetal ultrasound examination during the first ultrasound visit. Dating of the pregnancy was performed using the first ultrasound measurement of crown-rump length or biparietal diameter, using dating curves derived from this cohort.[13] Gestational age at birth, birth weight, and sex were obtained from midwife and hospital registries at birth.[8] Preterm birth was defined as a gestational age of <37 weeks at birth. Small size for gestational age at birth and large size for gestational age at birth were defined as a gestational age-adjusted birth weight below the 10th percentile and above the 90th percentile in the study cohort.

Childhood overweight and obesity

In children aged 4 years, growth was measured at the Community Child Health Centers.[8] Height and weight were measured in standing position and body mass index (kg/m^2) was calculated. Childhood overweight and obesity were defined by the International Obesity Task Force cutoffs.[14]

Statistical analysis

We examined the associations of risk factors with maternal underweight, overweight, obesity, and excessive gestational weight gain using multivariate logistic regression models. Using similar models, we explored the associations of maternal underweight, overweight, obesity, and excessive gestational weight gain with the risks of pregnancy complications in mothers and children. These models were adjusted for maternal age, educational level, ethnicity, parity, folic acid supplementation use, smoking habits, and alcohol consumption. The models in which we examined maternal overweight and obesity as exposure were also adjusted for maximum gestational weight gain. We tested potential interactions between maternal body mass index and gestational weight gain for these models, but after adjustment for multiple testing, we found no significant

interactions. Furthermore, we used stepwise regression analyses to compare the strength of the associations of prepregnancy overweight and obesity and excessive gestational weight gain with the risks of adverse pregnancy outcomes (data not shown). We performed a sensitivity analysis to examine whether the associations of prepregnancy body mass index with the risk of adverse pregnancy outcomes differed between women enrolled in the first trimester (before 14 weeks of gestation) and women enrolled later in pregnancy. Sensitivity analyses using weight gain until third trimester instead of maximum weight gain were performed for the analyses focused on excessive gestational weight gain and the risk of adverse outcomes. Finally, we examined the associations of trimester-specific weight gain with pregnancy, delivery, fetal, and childhood outcomes using multivariate logistic regression models. Missing data of the covariates were imputed using multiple imputation. The percentages of missing values within the population for analysis were lower than 10%, except for information on maternal nutrition (23.7%) and folic acid supplementation use (17.3%). All analyses were performed using the Statistical Package of Social 17.0 for Windows (SPSS Inc., Chicago, IL).

Results

Subject characteristics

Characteristics of the included mothers, fathers, and children are given in **Table 2.4.1**. Of all mothers, 16.2%, 55.8%, 19.2%, and 8.8% were underweight, normal weight, overweight, and obese, respectively, and 44.5% had excessive weight gain. Subject characteristics according to maternal body mass index category are given in **Supplementary Table S2.4.1**.

Risk factors of maternal overweight and obesity and excessive gestational weight gain

In the multivariate analyses, maternal low educational level, multiparity, no alcohol consumption during pregnancy, FTO risk allele and higher paternal body mass index were all associated with the risk of maternal overweight and obesity (all P-values <0.05) (**Table 2.4.2**). Maternal European ethnicity, nulliparity, higher total energy, carbohydrate, protein and fat intake, no alcohol consumption during pregnancy, smoking during pregnancy, and higher paternal body mass index were associated with a higher risk of excessive gestational weight gain (all P-values <0.05).

Table 2.4.1. Characteristics of mothers, fathers and their children (*n = 6959*)

Characteristics	Value
Maternal characteristics	
Age, median (90% range), years	30.3 (20.4 – 37.9)
Height, mean (SD), cm	167.4 (7.4)
Weight, mean (SD), kg	69.3 (13.1)
Body Mass Index, mean (SD), kg/m^2	23.6 (4.4)
Maximum weight gain, mean (SD), kg	13.6 (8.0)
First trimester weight gain, mean (SD), kg	2.3 (3.6)
Second trimester weight gain, mean (SD), kg	3.3 (2.4)
Third trimester weight gain, mean (SD), kg	5.0 (2.7)
Education, No. (%)	
Primary or secondary	3879 (57.6)
Higher	2852 (42.4)
Household income per month, No. (%)	
< € 1600	1606 (29.3)
> € 1600 - € 2200	834 (15.3)
> € 2200	3035 (55.4)
Race / Ethnicity, No. (%)	
Dutch or European	3958 (57.8)
Surinamese	618 (9.0)
Turkish	640 (9.3)
Moroccan	444 (6.5)
Cape Verdian or Dutch Antilles	496 (7.2)
Others	689 (10.1)
Parity, No. nulliparous (%)	3959 (56.9)
Folic acid supplement use, No. (%)	4085 (71.0)
Diet	
Total energy intake, mean (SD), Kcal	2044 (563)
Carbohydrates, mean (SD), Energy%	48.7 (5.9)
Proteins, mean (SD), Energy%	14.8 (2.5)
Fat, mean (SD), Energy%	36.3 (5.2)
Smoking, No. (%)	1713 (25.9)
Alcohol consumption, No. (%)	3353 (50.5)
FTO rs8050136, No. (%)	
CC	2235 (38.3)
AC	2737 (46.8)
AA	869 (14.9)
Maternal pregnancy complications	
Gestational hypertension, No. (%)	264 (4.0)
Pre-eclampsia, No. (%)	133 (2.1)
Gestational diabetes, No. (%)	70 (1.0)
Prelabour rupture of membranes, No, (%)	260 (3.9)
Postpartum hemorrhage, No. (%)	342 (5.1)
Paternal characteristics	
Age, median (90% range), years	33.1 (22.0 – 44.9)
Height, mean (SD), cm	181.2 (7.7)
Weight, mean (SD), kg	83.5 (11.6)
Body Mass Index, mean (SD), kg/m^2	25.4 (3.2)
Delivery and child characteristics	
Caesarian section, No. (%)	778 (12.3)
Ventouse extraction, No. (%)	858 (13.6)
Males, No. (%)	3518 (51)
Gestational age, median (90% range), weeks	40.1 (36.9 – 42.0)
Preterm birth, No. (%)	354 (5.1)
Birth weight, mean (SD), g	3419 (557)
Small for gestational age[1] (<10th birth centile), No. (%)	680 (9.9)
Large for gestational age[1] (>90th birth centile), No. (%)	692 (10.0)
Preschool overweight and obesity, No. (%)	708 (15.5)

[1]SGA is defined as <10th percentile of age-and sex-adjusted birth weight; LGA is defined as >90th percentile of age-and sex-adjusted birth weight; preterm birth is defined as <37 weeks of gestation.

Table 2.4.2. Risk factors of maternal overweight, obesity and excessive weight gain during pregnancy using multivariate analyses (*n = 6959*)[1]

	Maternal underweight OR (95% CI) *n = 1123*	**Maternal overweight** OR (95% CI) *n = 1334*	**Maternal obesity** OR (95% CI) *n = 611*	**Excessive weight gain** OR (95% CI) *n = 1474*
Maternal risk factors				
Age (1 SD = 5.3y)	0.83 (0.75, 0.93)**	1.03 (0.94, 1.14)	1.04 (0.90, 1.19)	0.97 (0.85, 1.07)
Education				
Primary	0.91 (0.68, 1.22)	1.64 (1.26, 2.12)**	2.48 (1.71, 3.59)**	0.92 (0.62, 1.34)
Secondary	1.00 (0.84, 1.19)	1.39 (1.18, 1.65)**	2.75 (2.12, 3.56)**	1.13 (0.96, 1.36)
Higher	*Reference*	*Reference*	*Reference*	*Reference*
Household income per month				
< €1600	1.10 (0.86, 1.41)	1.05 (0.84, 1.31)	1.36 (1.03, 1.79)*	0.91 (0.69, 1.14)
> €1600 – 2200	1.03 (0.79, 1.34)	1.09 (0.89, 1.35)	1.20 (0.84, 1.72)	0.90 (0.72, 1.12)
> €2200	*Reference*	*Reference*	*Reference*	*Reference*
Ethnicity				
Dutch or European	*Reference*	*Reference*	*Reference*	*Reference*
Non-European	0.94 (0.79, 1.12)	1.23 (1.03, 1.44)*	1.06 (0.87, 1.36)	0.78 (0.65, 0.94)**
Parity				
Nulliparous	*Reference*	*Reference*	*Reference*	*Reference*
Multiparous	0.98 (0.84, 1.15)	1.51 (1.31, 1.75)**	1.68 (1.37, 2.06)**	0.71 (0.61, 0.83)**
Folic acid supplement use				
No	*Reference*	*Reference*	*Reference*	*Reference*
Yes	1.07 (0.86, 1.31)	0.94 (0.78, 1.15)	0.81 (0.61, 1.07)	1.25 (1.00, 1.56)
Total Energy intake (1 SD = 563 kcal)	1.01 (0.93, 1.10)	0.95 (0.88, 1.04)	0.88 (0.79, 0.98)*	1.13 (1.03, 1.23)**
Carbohydrates (1 SD = 6.5% Energy)	1.21 (0.62, 2.36)	1.21 (0.54, 2.70)	5.38 (1.42, 20.21)*	4.49 (1.61, 12.46) **
Proteins (1 SD = 2.6% Energy)	0.94 (0.71, 1.24)	1.18 (0.87, 1.59)	2.23 (1.32, 3.75)**	1.91 (1.26, 2.88)**
Fat (1 SD = 5.6% Energy)	1.19 (0.66, 2.13)	1.12 (0.55, 2.27)	4.51 (1.40, 14.39)*	4.00 (1.62, 9.83)**
Smoking				
No	*Reference*	*Reference*	*Reference*	*Reference*
Yes	1.09 (0.93, 1.29)	0.96 (0.82, 1.13)	1.01 (0.81, 1.25)	2.08 (1.74, 2.48)**
Alcohol				
No	*Reference*	*Reference*	*Reference*	*Reference*
Yes	1.08 (0.92, 1.26)	0.76 (0.65, 0.89)**	0.73 (0.59, 0.90)**	0.83 (0.71, 0.98)*
FTO rs8050136				
CC	*Reference*	*Reference*	*Reference*	*Reference*
AC	1.00 (0.94, 1.16)	1.11 (0.96, 1.28)	1.25 (0.99, 1.58)	1.10 (0.86, 1.41)
AA	0.99 (0.80, 1.25)	1.30 (1.06, 1.58)*	1.64 (1.21, 2.23)**	1.14 (0.95, 1.36)
Paternal risk factors				
Age at intake (1 SD = 5.8 y)	1.07 (0.97, 1.17)	1.02 (0.93, 1.11)	1.05 (0.91, 1.18)	0.98 (0.88, 1.08)
Body mass index (1 SD = 3.5 units)	0.80 (0.73, 0.87)**	1.32 (1.21, 1.44)**	1.53 (1.35, 1.73)**	1.12 (1.02, 1.22)*

Abbreviations: OR; Odds Ratio, CI; Confidence Interval; SD, Standard Deviation

[1]Values are multivariate logistic regression coefficients (95% Confidence Interval). For continuous variables, estimates reflect the risk of maternal underweight, overweight and obesity and excessive gestational weight gain per standard deviation change of the risk factor. For categorical variables or dichotomous variables, the effect estimates represent the risk of maternal underweight, overweight and obesity and excessive gestational weight gain, compared to reference group. Estimates are based on multiple imputed data. *P-value <0.05. **P-value <0.01.

Table 2.4.3. Associations of maternal underweight, overweight, obesity and excessive gestational weight gain with maternal, delivery, birth and childhood complications

	Underweight[1,2] OR (95% CI)	Overweight[1,2] OR (95% CI)	Obesity[1,2] OR (95% CI)	Excessive gestational weight gain[1,3] OR (95% CI)
Maternal complications				
n = 6956	*n = 1123*	*n = 1334*	*n = 611*	*n = 1474*
Gestational hypertension	0.65 (0.41, 1.02)	2.15 (1.55, 2.97)**	6.31 (4.30, 9.26)**	2.07 (1.43, 2.99)**
Pre-eclampsia	1.25 (0.76, 2.06)	1.91 (1.21, 3.00)**	3.61 (2.04, 6.39)**	1.12 (0.67, 1.89)
Gestational diabetes	0.61 (0.18, 2.06)	4.25 (2.32, 7.76)**	6.28 (3.01, 13.06)**	1.54 (0.66, 3.56)
Delivery complications				
n = 6956	*n = 1123*	*n = 1334*	*n = 611*	*n = 1474*
PROM	1.61 (1.17, 2.22)**	0.95 (0.65, 1.37)	1.66 (1.08, 2.55)*	0.69 (0.47, 1.03)
Ventouse extraction	0.98 (0.79, 1.20)	1.00 (0.81, 1.23)	1.12 (0.82, 1.52)	1.21 (0.98, 1.48)
Caesarean section	0.94 (0.74, 1.18)	1.52 (1.24, 1.85)**	1.91 (1.46, 2.50)**	1.26 (1.00, 1.57)*
Postpartum hemorrhage	0.92 (0.66, 1.27)	1.34 (1.01, 1.78)*	1.44 (0.96, 2.16)	1.04 (0.76, 1.42)
Birth complications				
n = 6956	*n = 1123*	*n = 1334*	*n = 611*	*n = 1474*
Preterm birth[4]	1.29 (0.96, 1.72)	1.04 (0.77, 1.42)	1.53 (1.05, 2.20)*	0.67 (0.46, 0.98)*
Large size for gestational age	0.42 (0.30, 0.57)**	1.69 (1.35, 2.12)**	2.97 (2.16, 4.08)**	2.17 (1.72, 2.74)**
Small size for gestational age	1.66 (1.36, 2.07)**	0.81 (0.64, 1.03)	0.54 (0.38, 0.78)**	0.34 (0.26, 0.46)**
5 minute APGAR <7	0.65 (0.30, 1.39)	1.56 (0.90, 2.71)	2.05 (1.04, 4.01)*	1.09 (0.50, 2.39)
Childhood complications				
n = 4571	*n = 736*	*n = 844*	*n = 372*	*n = 1263*
Overweight[5]	0.62 (0.44, 0.87)**	1.48 (1.15, 1.91)**	2.41 (1.75, 3.33)**	1.51 (1.16, 1.97)**
Obesity[5]	0.61 (0.29, 1.28)	1.61 (0.94, 2.74)	5.02 (2.97, 8.45)**	0.93 (0.51, 1.68)

Abbreviations: OR; Odds Ratio, CI; Confidence Interval; PROM, prelabour rupture of membranes

[1]Values are Odds Ratios (95% Confidence Interval) that reflect the difference in risks of complications for underweight, overweight and obese women as compared to women with a normal body mass index, 20 - 24.9 kg/m^2, and for women with excessive gestational weight gain as compared to women with a recommended or less than recommended gestational weight gain. Estimates are from multiple imputed data. [2]Models for underweight, overweight and obesity are adjusted for maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption and gestational weight gain. [3]Models for excessive gestational weight gain are adjusted for maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption. [4]Models are adjusted for gender as well. [5]Models are also adjusted for breastfeeding (yes/no). * P <0.05. **P <0.01.

Maternal body mass index, excessive gestational weight gain and risks of pregnancy, delivery, birth, and childhood outcomes

As compared to normal weight, maternal underweight was associated with a higher risk of PROM (Odds Ratio (OR) 1.61 (95% CI: 1.17, 2.22)) and a higher risk of delivering a small size for gestational age infant (OR 1.66 (95% CI: 1.36, 2.07)), but with a lower risk of delivering a large size for gestational age infant (OR 0.42 (95% CI: 0.30, 0.57)) and a lower risk of childhood overweight of the offspring (OR 0.62 (95% CI: 0.44, 0.87)). As compared to normal weight mothers, mothers with overweight had increased risks of gestational hypertension (OR 2.15 (95% CI: 1.55, 2.97)), pre-eclampsia (OR 1.91 (95% CI: 1.21, 3.00)), gestational diabetes (OR 4.25 (95% CI: 2.32, 7.76)), Caesarean delivery (OR 1.52 (95% CI: 1.24, 1.85)), postpartum hemorrhage (OR 1.34 (95% CI: 1.01, 1.78)), large size for gestational age infants (OR 1.69 (95% CI: 1.35, 2.12)), and childhood overweight (OR 1.48 (95% CI: 1.15, 1.91)) (Table 2.4.3). We observed stronger effect estimates for the associations of maternal obesity with these outcomes. Repeating these analyses

among women who were enrolled during first trimester and among women enrolled later in pregnancy showed that effect estimates differed only slightly between first trimester enrolled and later enrolled women (Supplementary Table S2.4.2).

As compared to low or recommended weight gain, excessive gestational weight gain was associated with a higher risk of gestational hypertension (OR 2.07 (95% CI: 1.43, 2.99)), Caesarean delivery (OR 1.26 (95% CI: 1.00, 1.57)), and large size for gestational age infants (OR 2.17 (95% CI: 1.72, 2.74)), and a lower risk of preterm delivery (OR 0.67 (95% CI: 0.46, 0.98)), and small size for gestational age infants (OR 0.34 (95% CI: 0.26, 0.46)). Excessive gestational weight gain was associated with the risk of childhood overweight (OR 1.51 (95% CI: 1.16, 1.97)). Associations of excessive gestational weight gain with these adverse pregnancy outcomes attenuated when prepregnancy overweight and obesity were included in the model (data not shown). Similar results for the associations with excessive gestational weight gain were found when we used weight in third trimester instead of maximum weight (Supplementary Table S2.4.3).

Trimester specific weight gain and risks of pregnancy, delivery, birth, and childhood outcomes

Table 2.4.4 shows that first trimester weight gain was associated with the risk of gestational hypertension, gestational diabetes, and Caesarean delivery (OR 1.24 (95% CI: 1.12, 1.39), OR 1.29 (95% CI: 1.10, 1.51), and OR 1.19 (95% CI: 1.10, 1.29) per standard deviation of change in gestational weight gain per week, respectively). First trimester weight gain was also associated with the risk of childhood overweight and obesity (OR 1.20 (95% CI: 1.08, 1.34) and OR 1.44 (95% CI: 1.21, 1.70) per standard deviation of change in gestational weight gain per week, respectively). Weight gain in third trimester was associated with the risk of gestational hypertension and pre-eclampsia (OR 1.27 (95% CI: 1.06, 1.51) and OR 1.35 (95% CI: 1.08, 1.69) per standard deviation of change in gestational weight gain per week, respectively). The risks of delivering a large size for gestational age infant and a small size for gestational age infant were influenced by first-, second-, and third-trimester weight gain.

Table 2.4.4. Associations of trimester specific weight gain with maternal, delivery, birth and childhood complications

Complication	First trimester[1,2] OR (95% CI) per sd change in gestational weight gain per week	Second trimester[1,2] OR (95% CI) per sd change in gestational weight gain per week	Third trimester[1,2] OR (95% CI) per sd change in gestational weight gain per week
Maternal complications			
n = 6956	*n = 5695*	*n = 5469*	*n = 3313*
Gestational hypertension	1.24 (1.12, 1.39)**	1.18 (1.03, 1.34)*	1.27 (1.06, 1.51)**
Pre-eclampsia	1.10 (0.92, 1.32)	1.14 (0.96, 1.37)	1.35 (1.08, 1.69)**
Gestational diabetes	1.29 (1.10, 1.51)**	1.31 (1.04, 1.64)*	1.03 (0.72, 1.46)
Delivery complications			
n = 6956	*n = 5695*	*n = 5469*	*n = 3313*
Caesarean delivery	1.19 (1.10, 1.29)**	1.05 (0.96, 1.15)	1.00 (0.90, 1.20)
Birth complications			
n = 6956	*n = 5695*	*n = 5469*	*n = 3313*
Preterm delivery[3]	1.04 (0.93, 1.17)	1.00 (0.88, 1.14)	0.96 (0.80, 1.14)
Large size for gestational age	1.24 (1.14, 1.34)**	1.41 (1.29, 1.53)**	1.42 (1.26, 1.60)**
Small size for gestational age	0.91 (0.82, 0.99)*	0.72 (0.66, 0.80)**	0.74 (0.66, 0.84)**
Childhood complications			
n = 4571	*n = 3812*	*n = 3712*	*n = 2777*
Overweight[4]	1.20 (1.08, 1.34)**	1.17 (1.04, 1.30)**	0.94 (0.83, 1.07)
Obesity[4]	1.44 (1.21, 1.70)**	0.93 (0.75, 1.16)	0.94 (0.73, 1.20)

Abbreviations: OR; Odds Ratio, CI; Confidence Interval; sd; standard deviation

[1]Values are Odds Ratios (95% CI) for the risks of complications per standard deviation change in gestational weight gain per week. Estimates based on multiple imputed data. [2]Models are adjusted for maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption and maternal prepregnancy body mass index. [3]Models are adjusted for gender as well. [4]Models are also adjusted for breastfeeding (yes/no). *P-value <0.05. **P-value <0.01.

Discussion

Results from this prospective cohort study showed that the risks of maternal overweight and obesity were higher among lower educated, non-European origin, and multiparous mothers and mothers with an obese partner. The risk of excessive gestational weight gain was increased by maternal European ethnicity, nulliparity, higher dietary intake, smoking during pregnancy, and having an obese partner. Maternal overweight and obesity were strongly associated with increased risks of gestational hypertensive disorders, gestational diabetes, Caesarean delivery, large size for gestational age infants, and overweight and obesity in the offspring. Excessive gestational weight gain was associated with increased risks of gestational hypertension, Caesarean delivery, large size for gestational age infants and overweight in the offspring. However, the risk of delivering a small size for gestational age infant and the risk of delivering preterm were decreased among women who gained excessively. As compared to prepregnancy overweight and obesity, excessive gestational weight gain tended to have a limited influence on adverse pregnancy outcomes. Prepregnancy overweight and obesity were associated with more adverse pregnancy outcomes compared with excessive gestational weight gain. Furthermore, stepwise regression analysis showed that the effect estimates for the associations of excessive gestational weight gain with pregnancy

complications attenuated when prepregnancy overweight and obesity were taken into account.

Some methodological issues need to be considered. One of the strengths of this study was the prospective data collection from early pregnancy onward. We had a large sample size of 6959 participants. The response rate at baseline for participation in the Generation R Study cohort was 61%. The nonresponse would lead 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 mainly arise from loss to follow-up rather than from nonresponse at baseline.[15] Furthermore, not all women were already enrolled in the study in first trimester. Therefore, we did not have first trimester weight measurements in approximately 53% of the participating women. It seemed unlikely that late enrollment has biased our results. We observed small differences in the effect estimates for the associations of prepregnancy body mass index with the risk of adverse pregnancy outcomes between women who were enrolled during first trimester or later in pregnancy. For all associations, effect estimates were in similar direction in women enrolled during first trimester or later in pregnancy. Detailed information about a large number of potential risk factors and confounding factors was available in this study. However, because of the observational design, residual confounding because of other socio-demographic and lifestyle-related determinants might still be an issue. In addition, information on many covariates in this study was self-reported, which may have resulted in underreporting of certain adverse lifestyle-related determinants. Some data of these covariates were missing. It is unlikely that these data were missing completely at random, so a complete case analysis might lead to biased results. To avoid bias and to maintain statistical power, we used multiple imputations for missing information of the covariates. As compared to the complete case analysis, effect estimates only changed marginally after using multiple imputations to deal with the missing values. Information on maternal prepregnancy weight was self-reported. Self-reported weight tends to be underestimated, so some misclassification might have occurred. Also, maximum weight during pregnancy was self-reported 2 months after delivery. Weight assessment by questionnaire might have led to an underestimation of maximum pregnancy weight. This might have led to an underestimation of the observed effects. However, self-reported prepregnancy weight and weight measured at intake, and self-reported maximum weight and weight measured at 30 weeks of gestation, were highly correlated in our study. Furthermore, for the analyses focused on the associations between trimester-specific weight gain and the risk of adverse pregnancy outcomes, we performed a sensitivity analyses among normal weight women only, as overweight and obese women are more likely to underestimate self-reported weight (results not shown). The effect estimates changed slightly, when overweight and obese women were excluded from the analyses, but were in similar direction. The observed smaller effect sizes might be explained by smaller numbers of subjects and less power to detect differences because of the exclusion of extremes.

The risk of maternal obesity and excessive gestational weight gain varied among different ethnic groups and socioeconomic groups, which is in line with previous studies.[16-18] We observed that multiparous women were more frequently obese and had a lower risk of excessive gestational weight gain, as compared to nulliparous women. Accordingly, a study among 57.700 Danish women showed that women with low gestational weight gain were more often multiparous.[17] The risk of overweight and obesity was higher among women who carry the risk variants of the FTO gene. Many studies have already shown an association of the FTO polymorphism with the risk of obesity in children and adults.[19,20] Among pregnant women, the FTO gene has been suggested to influence prepregnancy weight as well.[21] We also showed an association of the FTO gene with the risk of prepregnancy overweight and obesity in pregnant women. However, we did not replicate our findings. Therefore, our results should be considered as hypothesis generating and need replication in further studies. Furthermore, we observed that excessive gestational weight gain was more likely among women who smoked during pregnancy and among women who did not consume alcohol during pregnancy, which is in agreement with the study among Danish women.[17] Higher total energy intake was also associated with an increased risk of excessive gestational weight gain, which has been reported by a previous study.[22]

Previous studies suggested associations between maternal overweight and obesity and the risks of gestational hypertensive disorders and gestational diabetes.[16,23-26] A large review among 13 cohort studies showed that there was a strong positive association between prepregnancy body mass index and pre-eclampsia.[24] Another review suggested that the risk of developing gestational diabetes was two times higher for overweight women and four times higher for obese women compared with normal weight women.[26] We observed similar results as maternal overweight and obesity were strongly associated with the risk of gestational hypertensive disorders and gestational diabetes. For the associations with gestational diabetes, it needs to be noted that accurate diagnosis of gestational diabetes is difficult. A fasting glucose greater than 7.0 mmol/L might also represent preexisting diabetes, and a fasting glucose between 6.1 and 6.9 mmol/L might also represent impaired glucose tolerance, instead of gestational diabetes. Unfortunately, in our study, no data were available on glucose tolerance before pregnancy. Excessive gestational weight gain was associated with the risk of gestational hypertension, but not associated with the risk of gestational diabetes and pre-eclampsia. This might be because of the small number of cases of gestational diabetes and pre-eclampsia in our study population. Overweight and obese mothers, and mothers with excessive weight gain, were at increased risk of Caesarean delivery. This is in line with observations in other studies that examined the association of maternal obesity and antenatal complications.[16-18,27] These associations might be influenced by the effect of obesity and excessive gestational weight gain on birth weight. However, after additional adjustment for birth weight, the associations only changed slightly and remained highly significant (results not shown). The association between maternal obesity and the risk of instrumental delivery remains more controversial. A study among 18.643 women reported that maternal obesity was not associated with the risk of instrumental

delivery.[28] Accordingly, we observed no association of maternal body mass index and excessive gestational weight gain with ventouse extraction. We observed a positive association between prepregnancy obesity and the risk of preterm delivery, which might partly be explained by the association of prepregnancy obesity with the risk of PROM. In our study, we do not have further data available about the specific causes of preterm birth. Further research to assess whether maternal obesity is associated with the risk of idiopathic or indicated preterm birth is necessary. We also observed that the risk of preterm delivery was lower among women who gained excessive weight. Thus far, published studies focused on the associations of maternal anthropometrics with the risk of preterm delivery seem to be inconsistent. Some studies found no association between maternal obesity and preterm delivery, whereas other studies suggested that the risk of preterm birth is higher among obese women.[16,28-30] A study among 76.682 adolescent women reported that the risk of preterm delivery was lower among women who gained excessively, independently of prepregnancy body mass index.[31] It has also been suggested that the association between gestational weight gain and preterm delivery is a modest U-shape.[32] A study among 33.872 women reported that compared with a gestational weight gain of 10 - 14 kg, women who gained less than 10 kg and women who gained more than 20 kg were at increased risk of preterm delivery.[32] In our study population, approximately 65% of the women who gained excessive weight gained below 20 kg. Modest excessive weight gain might have a protective effect for preterm delivery. We observed that maternal obesity and excessive gestational weight gain were associated with an increased risk of large size for gestational age infants and a lower risk of small size for gestational age infants. Similar findings have been reported by other studies.[4,17,18] Previously, we have shown that maternal prepregnancy body mass index is positively associated with birth weight of the offspring.[4] The associations between maternal obesity and excessive gestational weight gain with the risk of delivering a small size for gestational age infant or large size for gestational age infant attenuated after adjustment for gestational hypertensive disorders and gestational diabetes, but remained highly significant (results not shown). Furthermore, multiple studies have suggested that prepregnancy overweight and obesity are associated with an increased risk of longer length of hospital stay, an increased risk of having a neonate with a low Apgar score, and a higher risk of referral to neonatal intensive care unit.[33-35] We also observed that maternal obesity was associated with a higher risk of having a neonate with a low APGAR score. Other information about the neonate's health is not available within our study.

The fetal overnutrition hypothesis suggests that higher maternal plasma concentrations of glucose and free fatty acids because of maternal obesity during pregnancy might increase placental transfer of nutrients during fetal development. This might cause permanent changes in appetite, energy metabolism, and neuro-endocrine function of offspring, predisposing an individual to a greater risk of obesity in later life.[6] In line with this suggested pathway, we observed that maternal overweight and obesity are associated with overweight and obesity in the offspring.

Maternal underweight has also been suggested to be associated with adverse pregnancy outcomes. A large review among 78 studies showed that underweight women had a higher risk of both spontaneous and induced preterm birth and a higher risk of delivering a low birth weight infant.[36] In line with these findings, we observed that maternal underweight was associated with an increased risk of PROM, and an increased risk of delivering a small size for gestational age infant. We did not observe a significant effect on overall preterm birth.

Weight gain during pregnancy may vary greatly, and the effect of gestational weight in first, second, and third trimester on maternal and fetal outcomes might be different. We observed that maternal weight gain was low in first trimester and increased in second and third trimester. Few studies have examined the influence of trimester-specific weight gain on adverse outcomes.[37-39] We observed that weight gain in first trimester was associated with the risk of gestational diabetes and gestational hypertension and weight gain in third trimester was associated with the risk of pre-eclampsia and gestational hypertension. When examining the associations between third-trimester gestational weight gain and the risk of these disorders, it is difficult to differentiate between cause and consequence. The occurring edema might partly explain the excessive gestational weight gain. Further research is necessary to explore reversed causation and to examine underlying mechanisms of these associations. Studies examining the effect of gestational weight gain per trimester have mainly focused on the association of low weight gain and the risk of low birth weight infants.[37-39] A study among 10.696 women showed that low weight gain in second and third trimester, but not in first trimester, was associated with the risk of intrauterine growth retardation.[39] Accordingly, we observed that higher maternal weight gain in second and third trimester was more strongly associated with a lower risk of delivering a small size for gestational age infant, as compared to first-trimester weight gain. Furthermore, higher weight gain in each trimester was associated with a higher risk of delivering a large size for gestational age infant, but the strongest effects of weight gain were during second and third trimester. After additional adjustment for total weight gain and weight gain in the other trimesters, results only changed marginally (results not shown). These associations suggest that the effect of weight gain in early pregnancy might be different from the effect of weight gain later in pregnancy.

Current preventive strategies have mainly focused on restricting gestational weight gain during pregnancy. A meta-analysis of randomized controlled trials, focusing on diet and physical activity during pregnancy as intervention, showed that interventions may be effective to control weight gain during pregnancy.[40] However, as maternal overweight and obesity are strongly associated with short-term and long-term adverse consequences, future preventive strategies should also focus on prepregnancy overweight and obesity.

Conclusion

Maternal socio-demographic characteristics and lifestyle habits are associated with increased risks of maternal obesity and excessive weight gain during pregnancy. Both maternal underweight, overweight, obesity, and excessive gestational weight gain are associated with increased risks of adverse maternal, fetal, and childhood health outcomes. Future preventive strategies, focused on especially prepregnancy body mass index, are needed to improve maternal pregnancy outcomes and health of offspring.

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Supplementary Material

Supplementary Table S2.4.1. Characteristics by maternal body mass index (*n = 6959*)[1,2]

Maternal characteristics	Underweight (<20 kg/m^2) *n = 1123*	Normal (<20-24.9 kg/m^2) *n = 3888*	Overweight (25-29.9 kg/m^2) *n = 1334*	Obesity (≥30 kg/m^2) *n = 611*	P-value[3]
Height (cm), mean (SD)	168.4 (7.1)	167.8 (7.3)	166.3 (7.5)	165.7 (7.5)	<0.01
Prepregnancy weight (kg), mean (SD)	53.6 (5.3)	62.7 (6.4)	74.7 (7.5)	93.4 (13.2)	<0.01
Body mass index (kg/m^2), mean (SD)	18.9 (0.9)	22.6 (1.4)	27.0 (1.4)	34.0 (3.7)	<0.01
Maximum weight gain (kg), mean (SD)	15.0 (5.3)	15.4 (5.4)	14.0 (6.6)	11.3 (8.6)	<0.01
Age (yrs), mean (SD)	29.1 (5.4)	30.0 (5.3)	29.8 (5.2)	29.3 (5.1)	<0.01
Parity, nulliparous (%)	63.4	60.4	47.3	43.5	<0.01
Gestational age at intake (wks), median (95% range)	14.5 (10.2, 28.9)	14.2 (10.4, 25.5)	14.4 (10.2, 24.9)	14.6 (10.5, 28.2)	0.13
Highest completed education (%)					
Primary school	9.1	9.0	15.8	17.5	<0.01
Secondary school	45.7	42.9	50.2	64.3	
Higher education	45.1	46.1	34.0	18.1	
Ethnicity (%)					
European	61.3	62.2	48.9	42.5	<0.01
Non-European	38.7	37.8	51.1	57.5	
Alcohol consumption (%)					
None	45.0	45.2	58.8	66.3	<0.01
Yes	55.0	54.8	41.2	33.7	
Smoking habits (%)					
None	70.8	73.7	75.8	73.9	0.04
Yes	29.2	26.3	24.2	26.1	
Folic acid supplement use (%)					
None	26.5	25.5	34.8	43.7	0.01
Yes	73.5	74.5	65.2	56.3	

[1]Values are means (standard deviation) or percentages. [2]Median (95% range). [3]Differences in subject characteristics between the groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.

Supplementary Table S2.4.2. Associations of prepregnancy overweight and obesity with the risk of adverse pregnancy outcomes according to gestational age at enrollment[1,2]

	Overweight		Obesity	
	First trimester enrolled	Second or third trimester enrolled	First trimester enrolled	Second or third trimester enrolled
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
	n = 601	*n = 733*	*n = 254*	*n = 357*
Maternal complications				
Gestational hypertension	1.78 (1.13, 2.80)*	2.66 (1.66, 4.25)**	6.01 (3.59, 10.01)*	7.18 (4.02, 12.78)**
Pre-eclampsia	1.69 (0.83, 3.44)	2.07 (1.15, 3.73)*	4.06 (1.71, 9.57)**	3.38 (1.57, 7.28)**
Gestational diabetes	3.94 (163, 9.49)**	4.42 (1.91, 10.20)**	7.96 (3.13, 20.19)**	4.26 (1.14, 15.19)*
Delivery complications				
PROM	0.84 (0.45, 1.56)	1.03 (0.65, 1.64)	1.99 (1.01, 3.93)*	1.47 (0.84, 2.58)
Ventouse extraction	1.05 (0.78, 1.41)	0.93 (0.69, 1.26)	1.53 (1.00, 2.32)	0.82 (0.52, 1.29)
Caesarean section	1.62 (1.21, 2.15)**	1.46 (1.09, 1.91)*	1.59 (1.05, 2.40)*	2.24 (1.56, 3.21)**
Postpartum haemorrhage	1.09 (0.71, 1.65)	1.64 (1.11, 2.43)*	1.15 (0.62, 2.12)	1.73 (1.00, 2.99)
Birth complications				
Preterm birth[4]	1.08 (0.67, 1.74)	1.02 (0.68, 1.51)	1.60 (0.86, 2.97)	1.50 (0.92, 2.44)
Large size for gestational age	1.42 (1.02, 1.96)*	2.01 (1.49, 2.70)**	2.53 (1.59, 4.01)**	3.33 (2.27, 4.88)**
Small size for gestational age	0.88 (0.62, 1.25)	0.77 (0.55, 1.05)	0.53 (0.30, 0.93)*	0.55 (0.35, 0.89)*
Childhood complications				
Overweight[5]	1.69 (1.18, 2.43)	1.32 (0.92, 1.87)	2.85 (1.75, 4.63)**	2.15 (1.38, 3.33)**
Obesity[5]	1.72 (0.67, 4.41)	1.56 (0.80, 3.02)	6.37 (2.65, 15.22)**	4.07 (2.10, 7.89)**

Abbreviations: OR; Odds Ratio, CI; Confidence Interval; PROM, prelabour rupture of membranes

[1]Values are Odds Ratios (95% Confidence Interval) that reflect the difference in risks of complications for overweight and obese women as compared to women with a normal body mass index, 20 - 24.9 kg/m^2. Estimates are from multiple imputed data. [2]Models for overweight and obesity are adjusted for maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption and gestational weight gain. [4]Models are adjusted for gender as well. [5]Models are also adjusted for breastfeeding (yes/no). *P-value <0.05. **P-value <0.01.

Supplementary Table S2.4.3. Associations of excessive gestational weight gain with maternal, delivery, birth and childhood complications using weight gain until third trimester (*n = 6956*)

	Excessive gestational weight gain[1,2]
	OR (95% CI)
Maternal complications	*n = 2996*
Gestational hypertension	1.57 (1.21, 2.04)**
Pre-eclampsia	1.20 (0.83, 1.72)
Gestational diabetes	1.90 (1.16, 3.08)*
Delivery complications	*n = 2996*
Caesarean section	1.34 (1.14, 1.58)**
Birth complications	*n = 2996*
Preterm birth[3]	1.06 (0.84, 1.35)
Large size for gestational age	2.08 (1.76, 2.45)**
Small size for gestational age	0.56 (0.47, 0.66)**
Childhood complications	*n = 1949*
Childhood overweight[4]	1.44 (1.21, 1.72)**
Childhood obesity[4]	1.23 (0.82, 1.85)

Abbreviations: OR; Odds Ratio, CI; Confidence Interval

[1]Values are Odds Ratios (95% Confidence Interval) that reflect the difference in risks of complications for women with excessive gestational weight gain as compared to women with a recommended or less than recommended gestational weight gain. Estimates are from multiple imputed data. [2]Models for excessive gestational weight gain are adjusted for maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption. [3]Models are adjusted for gender as well. [4]Models are also adjusted for breastfeeding (yes/no).*P-value <0.05. **P-value <0.01.

Chapter 2.5

Maternal obesity and gestational hypertensive disorders

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Abstract

Objective: We examined the associations of maternal prepregnancy body mass index (BMI) and gestational weight gain with systolic and diastolic blood pressure in different trimesters of pregnancy and the risks of pregnancy-induced hypertension and pre-eclampsia in a population-based prospective cohort study among 6902 mothers.

Methods: Information about maternal weight just before pregnancy was obtained by questionnaires. Maternal anthropometrics and blood pressure were measured in each trimester. Information about gestational hypertensive disorders was available from medical records.

Results: As compared to mothers with a normal weight, maternal obesity (BMI = 30 – 34.9 kg/m^2) and morbid obesity (BMI ≥35 kg/m^2) were associated with higher first trimester systolic blood pressure (differences for obese women and morbidly obese women: 10.80 mmHg (95% Confidence Interval (CI): 9.44, 12.17) and 13.07 mmHg (95% CI: 10.91, 15.23), respectively) and diastolic blood pressure (differences for obese women and morbidly obese women: 8.69 mmHg (95% CI: 7.63, 9.74) and 13.12 mmHg (95% CI: 11.44, 14.79), respectively). Similar differences were observed during second and third trimester. The risks of pregnancy-induced hypertension and pre-eclampsia were increased among obese mothers (Odds Ratio (OR) 4.67 (95% CI: 3.07, 7.09) and OR 2.49 (95% CI: 1.29, 4.78), respectively) and morbidly obese mothers (OR 11.34 (95% CI: 6.80, 18.86) and OR 3.40 (95% CI: 1.39, 8.28), respectively). Maternal weight gain was associated with the risk of pregnancy-induced hypertension.

Conclusions: Maternal obesity and morbid obesity are strongly associated with blood pressure in each trimester, and increased risks of gestational hypertensive disorders.

Introduction

The prevalence of overweight and obesity among women of reproductive age is increasing.[1–3] It is well known that obesity in pregnancy increases the risk for both adverse maternal and neonatal outcomes.[1–4] Obesity has been associated with emergency caesarean section, large size for gestational age at birth, neonatal hypoglycaemia and childhood obesity.[5–8] Maternal obesity seems also to be associated with increased risks of hypertensive disorders. A study among 24.241 nulliparous women observed an increased risk of gestational hypertension and pre-eclampsia for the morbidly obese category, defined as a prepregnancy body mass index of more than 35 kg/m^2, as compared to normal weight women.[6] Furthermore, it has been suggested that maternal weight gain might be associated with the risks of gestational hypertension and pre-eclampsia.[1,2,9] It is not known whether similar associations with gestational hypertensive disorders are present in the lower ranges of body mass index. Also, few studies have examined the effects of maternal overweight and obesity on blood pressure levels during different periods of pregnancy.[4,10,11] The influence of higher body mass index on blood pressure levels might partly explain the observed associations between obesity and the risk of hypertensive disorders during pregnancy.

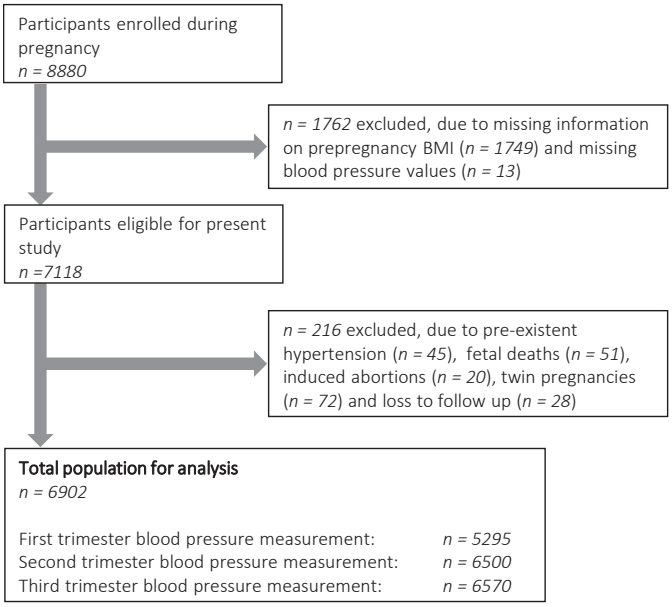
Therefore, we examined in a population-based prospective cohort study among 6902 pregnant women the associations of maternal body mass index and gestational weight gain with systolic and diastolic blood pressure in different trimesters of pregnancy and the risks of pregnancy-induced hypertension and pre-eclampsia.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[12,13] The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center in Rotterdam (MEC 198.782/2001/31). Written consent was obtained from all participating women.[14] All pregnant women were enrolled between 2001 and 2005. Response rate at birth was 61%. Assessments during pregnancy were planned in first, second and third trimester. The individual timing of these assessments depended on the gestational age at enrolment. In total, 8880 women were enrolled during pregnancy. For the present study, we excluded women without information about prepregnancy body mass index (*n = 1749*). Next, we excluded women without blood pressure measurements (*n = 13*). Also, we excluded women with pre-existent hypertension (*n = 45*). As we restricted our analyses to low-risk pregnancies, we excluded pregnancies leading to fetal death (*n = 51*), induced abortions (*n = 20*), loss to follow up (*n = 28*) and twin pregnancies (*n = 72*). Thus, the cohort for analysis consisted of 6902 pregnant women (**Figure 2.5.1**).

Figure 2.5.1. Flowchart of the participants



Maternal anthropometrics and obesity categories

During visits in first, second and third trimester, maternal anthropometrics were measured at one of the research centers. Height (cm) and weight (kg) were measured without shoes and heavy clothing and body mass index (kg/m^2) was calculated for each pregnancy period. Information about maternal weight just before pregnancy was obtained by questionnaires. As enrolment in our study was in pregnancy, we were not able to measure maternal weight before pregnancy. However, in our population for analysis, 46.2% of all women were enrolled before a gestational age of 14 weeks. Correlation of prepregnancy weight obtained by questionnaire and weight measured at enrolment was 0.95 (P-value <0.001). No differences in results were found when we used weight measured at enrolment instead of prepregnancy weight obtained by questionnaire. Prepregnancy body mass index was categorized into five categories as follows: lean (<20 kg/m^2), normal (20 – 24.9 kg/m^2), overweight (25 – 29.9 kg/m^2), obese (30 – 34.9 kg/m^2) and morbidly obese (≥35 kg/m^2). Information about maximum weight during pregnancy was assessed by questionnaire 2 months after delivery and available for only 3609 women. Because of the number of missing values of maximum weight, we defined weight gain as the difference between weight before pregnancy and weight in late pregnancy, which is a measure of weight gain during the first two trimesters.[5] This information was available for 6575 mothers. Maximum weight and weight in late pregnancy were strongly correlated (r = 0.86 (P-value <0.001)). Gestational weight gain was categorized into three categories: less than 7 kg, 7 – 11.9 kg and at least 12 kg.

Blood pressure

Blood pressure was measured with the Omron 907 automated digital oscillometric sphygmomanometer, which was validated in adults (OMRON Healthcare Europe B.V., Hoofddorp, the Netherlands).[15] All participants were seated in upright position with back support and were asked to relax for 5 minutes. A cuff was placed around the non-dominant upper arm, which was supported at the level of the heart, with the bladder midline over the brachial artery pulsation. In case of an upper arm exceeding 33 cm, a larger cuff (32 – 42 cm) was used. The mean value of two blood pressure readings over a 60-s interval was documented for each participant. In total, blood pressure was measured in 5295 women in first trimester (median 13.2 weeks of gestation, 95% range 9.8 – 17.5), in 6500 women in second trimester (median 20.4 weeks of gestation, 95% range 18.5 – 23.6) and in 6570 women in third trimester (median 30.2 weeks of gestation, 95% range 28.5 – 32.9). For the analyses, 18.365 blood pressure measurements were available. Three, two and one blood pressure measurements were available for 4894, 1675 and 333 women, respectively.

Pregnancy-induced hypertension and pre-eclampsia

Information on pregnancy complications was obtained from medical records. Women suspected of pregnancy complications based on these records were crosschecked with the original hospital charts. Details of these procedures have been described elsewhere.[16]

Briefly, the following criteria were used to identify women with pregnancy-induced hypertension: development of systolic blood pressure of at least 140 mmHg and/or diastolic blood pressure of at least 90 mmHg after 20 weeks of gestation in previously normotensive women. These criteria and the presence of proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater or a 24-h urine collection containing at least 300 mg of protein) were used to identify women with pre-eclampsia.

Covariates

Gestational age was established by fetal ultrasound examination during the first ultrasound visit.[13] Maternal age was assessed at intake. Information on educational level, ethnicity, parity and folic acid supplementation use was obtained at enrolment. Information about smoking, alcohol consumption and caffeine intake was assessed by questionnaires in each trimester. Maternal distress was measured by questionnaire at 20 weeks of gestation using the Brief Symptom Inventory,[17] which gives a Global Severity Index. A higher index reflects more stress pregnant women experience.

Statistical analysis

First, the associations of maternal prepregnancy body mass index with repeatedly measured systolic and diastolic blood pressure were analyzed using unbalanced repeated measurement regression models. These models take the correlation between repeated measurements of the same participant into account and allow for incomplete outcome data.[18] Using fractional polynomials of gestational age, the best fitting models were constructed. The prepregnancy body mass index categories were included in these models as intercept and as an interaction term with gestational age. We also examined the associations of prepregnancy body mass index with blood pressure in first, second and third trimester using linear regression models. Next, the associations of prepregnancy body mass index categories with the risks of pregnancy-induced hypertension and pre-eclampsia were assessed using multivariate logistic regression models. Finally, we explored the effect of gestational weight gain on the risks of pregnancy-induced hypertension and pre-eclampsia using multivariate logistic regression models. Tests for trend were performed by treating body mass index as a continuous variable and entering it in the linear or logistic regression models. All models were adjusted for gestational age at visit, maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake and maternal stress. The models in which we examined the effects of prepregnancy body mass index were adjusted for gestational weight gain and the models in which we examined the effects of gestational weight gain were adjusted for prepregnancy body mass index. Missing data of the covariates were imputed using multiple imputation. The percentages of missing values within the population for analysis were lower than or equal to 5%, except for folic acid supplementation use (17.3%) and maternal stress (20.6%). These higher percentages were due to the large number of women who only partially filled out the questionnaire or were not enrolled in first trimester. The repeated measurement analysis was performed using the Statistical Analysis System version 9.2 (SAS Institute Inc., Cary, North Carolina, USA), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Participant characteristics

Characteristics of the included women according to their prepregnancy body mass index are shown in **Table 2.5.1**. Women in the highest body mass index category were more frequently lower educated, had higher maternal stress levels and were less likely to consume alcohol during pregnancy. In total, there were 264 cases of pregnancy-induced hypertension and 131 cases of pre-eclampsia.

Table 2.5.1. Subject characteristics by prepregnancy body mass index (*n = 6902*)[1]

Body Mass Index	Lean (<20 kg/m^2) *n = 1122*	Normal (20-24.9 kg/m^2) *n = 3873*	Overweight (25-29.9 kg/m^2) *n = 1319*	Obese (30-34.9 kg/m^2) *n = 425*	Morbidly obese (≥35 kg/m^2) *n = 163*	P-value[3]
Height (cm)	168.4 (7.1)	167.8 (7.3)	166.3 (7.5)	165.8 (7.3)	165.1 (7.8)	<0.001
Prepregnancy weight (kg)	53.6 (5.3)	62.7 (6.4)	74.7 (7.5)	88.2 (8.8)	105.6 (13.4)	<0.001
Weight gain (kg)	11.2 (5.1)	11.0 (4.5)	9.7 (5.4)	7.7 (6.9)	5.4 (7.1)	<0.001
Age (yrs)	29.1 (5.4)	30.0 (5.3)	29.8 (5.2)	29.5 (5.2)	28.9 (5.1)	<0.001
Parity (%)						
Nulliparous	63.4	60.5	47.3	40.0	50.3	<0.001
Multiparous	36.6	39.5	52.7	60.0	49.7	
Gestational age at intake (wks)[2]	14.5 (10.2, 28.9)	14.2 (10.4, 24.9)	14.4 (10.2, 24.8)	14.8 (10.5, 28.8)	14.5 (10.1, 23.6)	0.07
Highest completed education (%)						
Primary school	9.2	9.0	15.8	19.5	13.8	0.001
Secondary school	45.8	43.0	50.2	61.7	69.7	
Higher education	45.0	48.0	34.0	18.8	16.4	
Ethnicity (%)						
European	61.3	62.2	48.7	42.4	40.8	0.001
Non-European	38.7	37.8	51.3	57.6	59.2	
Maternal stress index[2]	0.17(0.00, 1.51)	0.15(0.00, 1.37)	0.19(0.00, 1.56)	0.17(0.00, 1.46)	0.25(0.00, 1.60)	0.001
Alcohol consumption (%)						
None	45.1	45.2	59.0	65.0	69.7	0.001
First trimester only	14.2	14.2	10.5	13.0	5.8	
Continued	40.7	40.6	30.5	22.0	24.5	
Smoking habits (%)						
None	71.1	74.9	76.4	75.9	70.1	0.03
First trimester only	8.7	8.9	7.2	6.5	8.4	
Continued	20.2	16.3	16.4	17.6	21.5	
Folic acid supplement use (%)						
Preconceptional use	38.3	41.6	37.2	29.4	24.3	0.001
First 10 weeks use	35.2	32.9	28.0	28.2	28.7	
No use	26.5	25.5	34.7	42.4	47.1	
Caffeine intake (%)						
None	4.3	4.1	5.0	5.0	7.7	0.06
<2 units per day	56.9	55.5	53.9	62.5	59.4	
2-5.9 units per day	37.7	39.0	39.5	30.5	32.3	
≥6 units per day	1.1	1.4	1.6	2.0	0.6	
Pregnancy complications, %						
Pre-eclampsia	2.1	1.6	2.6	3.2	4.7	0.02
Pregnancy-induced hypertension	2.1	3.2	5.1	8.7	17.1	<0.001

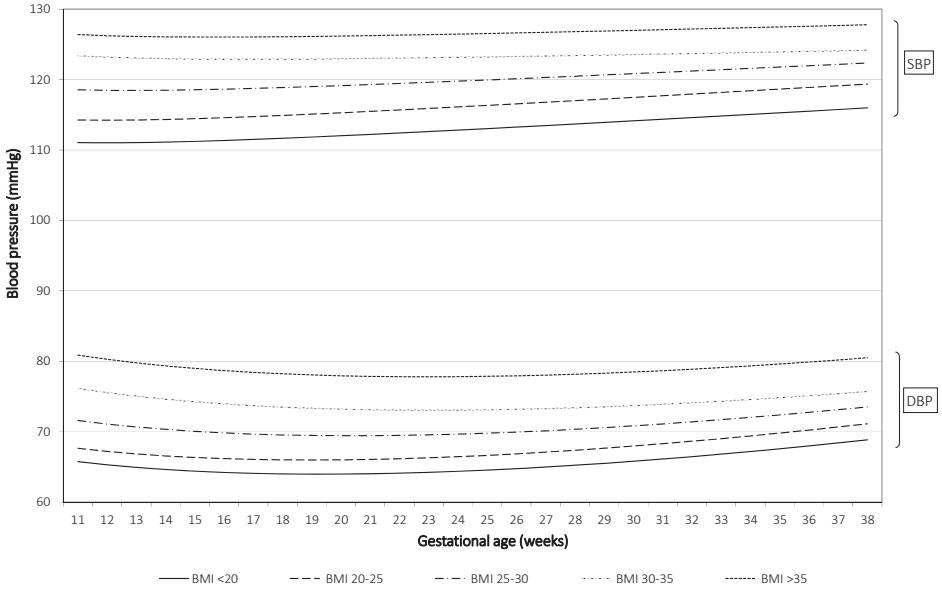
[1]Values are means (standard deviation) or percentages. [2]Median (95% range). [3]Differences in subject characteristics between the groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.

Maternal body mass index and longitudinally measured blood pressure

Figure 2.5.2 shows the blood pressure development during pregnancy for lean, normal weight, overweight, obese and morbidly obese women. Obese and morbidly obese mothers had the highest first trimester systolic blood pressure, but the lowest increase thereafter. Diastolic blood pressure showed a mid-pregnancy dip, with an increase thereafter for all groups of women. Diastolic blood pressure was the highest for morbidly obese women. The exact regression coefficients for gestational age-independent (intercept) and gestational age-dependent differences (interaction prepregnancy body mass index and gestational age) are given in Supplementary Table S2.5.1.

Table 2.5.2 gives the associations of prepregnancy body mass index with blood pressure in first, second and third trimester using multiple linear regression models. The trend analyses showed that higher prepregnancy body mass index was associated with higher systolic blood pressure in first, second and third trimester (differences for first, second and third trimester: 1.30 mmHg (95% Confidence Interval (CI): 0.95, 1.10), 0.98 mmHg (95% CI: 0.91, 1.04) and 0.89 mmHg (95% CI: 0.83, 0.96) per body mass index unit (P-value <0.001)). The highest systolic blood pressure levels were observed in morbidly obese women. Similar associations of prepregnancy body mass index and diastolic blood pressure were observed for all trimesters (differences for first, second and third trimester: 0.83 mmHg (95% CI: 0.77, 0.88), 0.81 mmHg (95% CI: 0.76, 0.86) and 0.74 mmHg (95% CI: 0.69, 0.79) per body mass index unit (P-value <0.001)).

Figure 2.5.2. Blood pressure patterns in different prepregnancy body mass index categories



Change in systolic and diastolic blood pressure in mmHg for lean, overweight, obese and morbidly obese women, compared to women with a normal body mass index based on repeated measurement analysis. Systolic blood pressure (SBP) = $ß_0 + ß_1 \times$ body mass index + $ß_2 \times$ gestational age + $ß_3 \times$ gestational age^{-2} + $ß_4 \times$ body mass index × gestational age. Diastolic blood pressure (DBP) = $ß_0 + ß_1 \times$ body mass index + $ß_2 \times$ gestational age + $ß_3 \times$ gestational age$^{0.5}$ + $ß_4 \times$ body mass index × gestational age. In these models, '$ß_0 + ß_1 \times$ body mass index' reflects the intercept and '$ß_2 \times$ gestational age + $ß_3 \times$ gestational age^{-2}' reflects the slope of change in blood pressure per week for systolic blood pressure, and '$ß_2 \times$ gestational age + $ß_3 \times$ gestational age$^{0.5}$' reflects the slope of change in blood pressure per week for diastolic blood pressure. Our term of interest is $ß_4$ which reflects the difference in change in blood pressure per week per body mass index category. Estimates and P-values are given in **Supplementary Table S2.5.1.**

Table 2.5.2. Associations of prepregnancy body mass index with systolic and diastolic blood pressure (*n = 6902*)[1]

Body mass index	First trimester[2] *n = 5295*	Second trimester[2] *n = 6500*	Third trimester[2] *n = 6570*
	Difference in systolic blood pressure (mmHg)		
Lean (<20 kg/m^2)	-3.28 (-4.14, -2.41)*	-3.73 (-4.49, -2.98)*	-3.49 (-4.24, -2.73)*
	n = 866	*n = 1053*	*n = 1062*
Normal (20-24.9 kg/m^2)	*Reference*	*Reference*	*Reference*
	n = 2998	*n = 3667*	*n = 3711*
Overweight (25-29.9 kg/m^2)	5.46 (4.63, 6.29)*	5.16 (4.44, 5.89)*	4.78 (4.06, 5.50)*
	n = 1007	*n = 1232*	*n = 1262*
Obese (30-34.9 kg/m^2)	10.80 (9.44, 12.17)*	9.46 (8.30, 10.62)*	8.60 (7.41, 9.79)*
	n = 309	*n = 400*	*n = 386*
Morbidly obese (≥35 kg/m^2)	13.07 (10.91, 15.23)*	14.37 (12.53, 16.21)*	12.63 (10.78, 14.48)*
	n = 115	*n = 148*	*n = 149*
Trend[3]	*1.30 (0.95, 1.10)**	*0.98 (0.91, 1.04)**	*0.89 (0.83, 0.96)**
	Difference in diastolic blood pressure (mmHg)		
Lean (<20 kg/m^2)	-1.71 (-2.38, -1.04)*	-2.46 (-3.06, -1.87)*	-2.17 (-2.76, -1.58)*
	n = 866	*n = 1053*	*n = 1062*
Normal (20-24.9 kg/m^2)	*Reference*	*Reference*	*Reference*
	n = 2998	*n = 3667*	*n = 3711*
Overweight (25-29.9 kg/m^2)	4.49 (3.85, 5.14)*	3.76 (3.19, 4.33)*	3.78 (3.23, 4.34)*
	n = 1007	*n = 1232*	*n = 1262*
Obese (30-34.9 kg/m^2)	8.69 (7.63, 9.74)*	8.50 (7.58, 9.41)*	*7.03 (6.10, 7.95)**
	n = 309	*n = 400*	*n = 386*
Morbidly obese (≥35 kg/m^2)	13.12 (11.44, 14.79)*	13.57 (12.12, 15.02)*	11.92 (10.48, 13.36)*
	n = 115	*n = 148*	*n = 149*
Trend[3]	*0.83 (0.77, 0.88)**	*0.81 (0.76, 0.86)**	*0.74 (0.69, 0.79)**

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in blood pressure in mmHg per body mass index group compared to the reference group of women with a normal body mass index, 20-24.9 kg/m^2. Estimates are from multiple imputed data. [2]Models are adjusted for gestational age at visit, maternal age, gestational weight gain, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, and maternal stress. [3]Tests for trend were based on multiple linear regression models with body mass index as a continuous variable. *P-value <0.001.

Body mass index, gestational weight gain and risks of pregnancy-induced hypertension and pre-eclampsia

As compared to normal weight women, those with overweight, obesity and morbid obesity had a higher risk of pregnancy-induced hypertension (Odds Ratio (OR) 2.12 (95% CI: 1.54, 2.91), OR 4.67 (95% CI: 3.07, 7.09) and OR 11.34 (95% CI: 6.80, 18.86), respectively) and pre-eclampsia (OR 1.82 (95% CI: 1.16, 2.83), OR 2.49 (95% CI: 1.29, 4.78) and OR 3.40 (95% CI: 1.39, 8.28), respectively) (Table 2.5.3). A positive trend was observed for each model (P-value <0.001). Gestational weight gain was associated with an increased risk of pregnancy-induced hypertension. As compared to the reference group (gestational weight gain <7 kg), women who gained 7 – 11.9 kg and women who gained at least 12 kg had a higher risk of pregnancy-induced hypertension (OR 1.50 (95% CI: 1.40, 2.16) and 1.86 (95% CI: 1.27, 2.70), respectively). No association between gestational weight gain and the risk of pre-eclampsia was observed.

Table 2.5.3. Prepregnancy body mass index, gestational weight gain and risks of pregnancy-induced hypertension and pre-eclampsia (*n = 6902*)[1]

Body mass index	Pregnancy-induced hypertension[2,3] (*n = 264*)	Pre-eclampsia[2,3] (*n = 131*)
Lean (<20 kg/m^2)	0.66 (0.42, 1.03)	1.22 (0.74, 2.00)
	n_{cases}= *23*	n_{cases}= *22*
Normal (20-24.9 kg/m^2)	*Reference*	*Reference*
	n_{cases}= *117*	n_{cases}= *60*
Overweight (25-29.9 kg/m^2)	2.12 (1.54, 2.91)**	1.82 (1.16, 2.83)*
	n_{cases}= *64*	n_{cases}= *31*
Obese (30-34.9 kg/m^2)	4.67 (3.07, 7.09)**	2.49 (1.29, 4.78)*
	n_{cases}= *35*	n_{cases}= *12*
Morbidly obese (≥35 kg/m^2)	11.34 (6.80, 18.86)**	3.40 (1.39, 8.28)*
	n_{cases}= *25*	n_{cases}= *6*
Trend[5]	*1.16 (1.13, 1.19)***	*1.08 (1.04, 1.12)***
Gestational weight gain	**Pregnancy-induced hypertension[2,4]** (*n = 252*)	**Pre-eclampsia[2,4]** (*n = 121*)
<7 kg	*Reference*	*Reference*
	n_{cases} = *55*	n_{cases} = *36*
7-11.9 kg	1.50 (1.04, 2.16)*	0.78 (0.48, 1.25)
	n_{cases} = *103*	n_{cases} = *42*
≥12 kg	1.86 (1.27, 2.70)*	1.08 (0.67, 1.74)
	n_{cases} = *94*	n_{cases} = *43*
Trend[5]	*1.06 (1.13, 1.19)***	*1.02 (0.99, 1.05)*

[1]Values are Odds Ratios (95% Confidence Interval) that reflect the difference in risks of pregnancy-induced hypertension and pre-eclampsia in different body mass index groups compared to women with a normal body mass index, 20-24.9 kg/m^2 and in different gestational weight gain groups compared to women with a gestational weight gain <7 kg. Estimates are from multiple imputed data. [2]Model is adjusted for maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, maternal stress. [3]Model is also adjusted for gestational weight gain. [4]Model is also adjusted for prepregnancy body mass index. [5]Tests for trend were based on multiple logistic regression models with body mass index and gestational weight gain as a continuous variable. *P-value <0.05. **P-value <0.001.

Discussion

Results from this prospective cohort study showed that higher prepregnancy body mass index is associated with both higher systolic and diastolic blood pressure in all trimesters. The difference in blood pressure between body mass index groups is already present from first trimester onwards and remains stable throughout pregnancy. Overweight, obesity and morbid obesity are also associated with increased risks of gestational hypertensive disorders. Higher gestational weight gain was associated with a higher risk of pregnancy-induced hypertension, but not with pre-eclampsia.

Some methodological issues need to be considered. One of the strengths of this study was the prospective data collection from early pregnancy onwards. We had a large sample size of 6902 participants with 18.365 blood pressure measurements. The response rate at baseline for participation in the Generation R Study cohort was 61%. The nonresponse would lead 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 mainly arise from loss to follow-up rather than from nonresponse at baseline.[19] Detailed information about a large

number of potential confounding factors was available in this study. However, because of the observational design, residual confounding due to other socio-demographic and lifestyle-related determinants might still be an issue. In addition, information on many covariates in this study was self-reported, which may have resulted in underreporting of certain adverse lifestyle-related determinants. Information on maternal prepregnancy weight was self-reported as well. Self-reported weight tends to be underestimated, so some misclassification might have occurred. However, self-reported prepregnancy weight and weight measured at intake were highly correlated in our study. Finally, we had a relatively small number of pregnancy-induced hypertension cases (*n = 264*) and pre-eclampsia cases (*n = 131*), which might have led to lack of power to assess associations with gestational hypertensive complications and the lower body mass index category and gestational weight gain. The relatively small number of cases of gestational hypertensive complications might also indicate a selection towards a relatively healthy, low-risk population.

Overweight and obesity are a major public health concern and have been associated with adverse pregnancy-related outcomes for mother and offspring. Within the Generation R study, it has already been shown that maternal prepregnancy body mass index is positively associated with fetal growth from second trimester onwards.[5] Furthermore, women within the highest quintile of prepregnancy body mass index and gestational weight gain were at increased risk of delivering large size for gestational age infants. Gestational weight gain modified the effect of prepregnancy body mass index. Women with the highest prepregnancy body mass index and the highest gestational weight gain had the highest risk of large size for gestational age infants.[5]

In this present study, we have shown that prepregnancy body mass index influences systolic and diastolic blood pressure levels during pregnancy, already from first trimester onwards. We observed significant differences in systolic and diastolic blood pressure between the several body mass index categories in each trimester. This is in line with observations in previous studies.[4,10,20] A study among 1733 women observed a positive association of prepregnancy body mass index with systolic and diastolic blood pressure in each trimester.[10] Another study among 166 women suggested that the effect of body mass index on diastolic blood pressure in the third trimester was only present in women without previous pregnancies.[20] In our study, we observed little difference between nulliparous and multiparous women for the associations between prepregnancy body mass index and blood pressure (data not shown). Furthermore, the study among 1733 women observed an increase in blood pressure with increasing body mass index categories at any gestational age, but the increase in blood pressure was attenuated with higher body mass index levels in later pregnancy.[11] Especially, for systolic blood pressure, this attenuation occurred. We observed that the two highest BMI categories had the smallest increase of systolic blood pressure throughout pregnancy, slightly decreasing the difference in systolic blood pressure between the different body mass index categories.

Multiple previous studies have examined the relationship between obesity and pre-eclampsia.[1,6,21–25] A review of 13 cohort studies observed a strong positive association

between prepregnancy body mass index and the risk of pre-eclampsia.[26] In line with this review, we observed that higher prepregnancy body mass index was associated with a higher risk of pre-eclampsia, with the highest risk among morbidly obese women. A limited number of studies have assessed the associations between underweight and the risk of hypertensive disorders during pregnancy. A study among 24.241 primigravid women observed a protective effect of underweight, body mass index less than 19.9 kg/m^2, for both the risk of pregnancy-induced hypertension and pre-eclampsia.[6] A study among Chinese women observed a protective effect of underweight against pregnancy-induced hypertension but not against pre-eclampsia.[1] We did not observe a protective effect of underweight, defined as a body mass index below 20 kg/m^2, against hypertensive complications during pregnancy. The difference might be due to a small number of cases, differences in study population and adjustment for possible covariates.

Gestational weight gain might also influence the risk of gestational hypertensive disorders. A study among 854.085 American women observed that the incidence of pre-eclampsia increased with an increasing rate of weight gain in both women with a normal prepregnancy body mass index and in obese women.[21] We also observed a higher risk of pregnancy-induced hypertension with higher gestational weight gain. However, we did not observe an association between gestational weight gain and pre-eclampsia. This might be due to a small number of cases of pre-eclampsia in our analysis.

The mechanisms by which higher body mass index may lead to higher blood pressure levels and increase the risk of gestational hypertensive disorders are not clear. It has been suggested that an imbalance in the autonomic function, especially hyperactivity of the sympathetic nervous system, might be a mechanism that can explain the observed associations.[4,10,11,27] Another hypothesis that has been suggested is that adiposity-related insulin resistance might indirectly influence blood pressure.[10,11,27] Furthermore, obesity is known to cause chronic inflammation and oxidative stress. It has been shown that there is increased systemic inflammation in first trimester in overweight and obese women with pre-eclampsia.[28,29] This might also explain part of the pathway of obesity with pre-eclampsia. Further research is necessary to explore these complex mechanisms underlying the relationship between obesity and blood pressure level and gestational hypertensive disorders. As the effect of prepregnancy body mass index on blood pressure is already present in first trimester, future preventive strategies should be focused on the preconceptional period.

In conclusion, overweight, obesity and morbid obesity are associated with both higher systolic and diastolic blood pressure levels in first, second and third trimester, and increased risks of gestational hypertensive disorders. Higher gestational weight gain is associated with an increased risk of pregnancy-induced hypertension, but not with pre-eclampsia. Preconception strategies to prevent obesity in women of reproductive age might lead to less hypertensive complications during pregnancy.

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Supplementary Material

Supplementary Table S2.5.1. Longitudinal associations between prepregnancy body mass index and systolic and diastolic blood pressure[1]

Prepregnancy body mass index	Intercept	P-value[2]	Slope (mmHg (95% CI))	P-value[2]
	Difference in systolic blood pressure			
Lean (<20 kg/m^2)	106.68	<0.001	-0.007 (-0.06, 0.04)	0.79
Normal (20-24.9 kg/m^2)	109.81	<0.001	*Reference*	
Overweight (25-29.9 kg/m^2)	114.63	<0.001	-0.048 (-0.10, -0.001)	<0.05
Obese (30-34.9 kg/m^2)	120.70	<0.001	-0.16 (-0.24, -0.08)	<0.001
Morbidly obese (≥35 kg/m^2)	123.46	<0.001	-0.14 (-0.26, -0.01)	<0.05
	Difference in diastolic blood pressure			
Lean (<20 kg/m^2)	93.80	<0.001	-0.014 (-0.05, 0.03)	0.49
Normal (20-24.9 kg/m^2)	95.55	<0.001	*Reference*	
Overweight (25-29.9 kg/m^2)	100.11	<0.001	-0.057 (-0.09, -0.02)	<0.05
Obese (30-34.9 kg/m^2)	105.62	<0.001	-0.14 (-0.21, -0.08)	<0.001
Morbidly obese (≥35 kg/m^2)	110.33	<0.001	-0.14 (-0.24, -0.05)	<0.05

[1]Values are based on repeated non-linear regression models and reflect the change in blood pressure in mmHg per body mass index category compared to the reference group of women with a normal body mass index , 20-24.9 kg/m^2. [2]P-value reflects the significance level of the estimate.

Chapter 2.6

Childhood cardiovascular outcomes of maternal obesity

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Abstract

Background: Maternal prepregnancy obesity is associated with impaired cardio-metabolic health in offspring. Whether these associations reflect direct intrauterine causal mechanisms remains unclear.

Methods: In a population-based prospective cohort study among 4871 mothers, fathers, and their children, we examined the associations of both maternal and paternal pre-pregnancy body mass index with childhood body fat distribution and cardio-metabolic outcomes and explored whether any association was explained by pregnancy, birth, and childhood factors. We measured childhood body mass index, total body and abdominal fat distribution, blood pressure, and blood levels of lipids, insulin, and C-peptide at the age of 6 years.

Results: We observed that higher maternal and paternal prepregnancy body mass index were associated with higher childhood body mass index, total body and abdominal fat mass measures, systolic blood pressure, and insulin levels and lower high-density lipoprotein cholesterol levels (P-value <0.05). Stronger associations were present for maternal than paternal body mass index, with statistical support for heterogeneity between these associations. The associations for childhood fat mass and cardio-metabolic outcomes attenuated after adjustment for childhood current body mass index. Compared with children from normal-weight mothers, those from obese mothers had increased risks of childhood overweight (Odds Ratio (OR) 3.84 (95% Confidence Interval (CI): 3.01, 4.90)) and clustering of cardio-metabolic risk factors (OR 3.00 (95% CI: 2.09, 4.34)). Smaller effect estimates for these outcomes were observed for paternal obesity.

Conclusions: Higher maternal and paternal prepregnancy body mass index were associated with an adverse cardio-metabolic profile in offspring, with stronger associations present for maternal prepregnancy body mass index. These findings suggest that maternal prepregnancy body mass index may influence the cardio-metabolic health of offspring through direct intrauterine mechanisms.

Introduction

Maternal obesity during pregnancy is associated with an adverse cardio-metabolic risk profile in childhood and adulthood.[1–4] The mechanisms underlying these associations might involve increased placental transfer of nutrients during fetal development, which may cause permanent adaptations in appetite, energy metabolism, and neuroendocrine function in offspring, which predispose individuals to a greater risk of cardio-metabolic disease in later life.[5] However, these associations might also reflect shared family-based, lifestyle-related characteristics or genetic factors.[5] Comparing the strength of associations of prepregnancy body mass index from both mother and father with childhood outcomes could help in disentangling underlying mechanisms.[6,7] Stronger associations for maternal body mass index suggest direct intrauterine mechanisms, whereas similar or stronger associations for paternal body mass index suggest a role for shared family-based, lifestyle-related characteristics or genetic factors. To date, studies comparing associations of maternal and paternal body mass index with childhood body mass index have shown conflicting results.[5,8–11] Also, most previous studies did not explore associations of parental body mass index with detailed childhood body and abdominal fat distribution and cardio-metabolic outcomes. It further remains unclear whether differences in magnitude of associations of parental body mass index with childhood outcomes are present across the full range of body mass index or confined to parental obesity only.

Therefore, in a population-based prospective cohort study among 4871 children and their parents, we examined the associations of maternal and paternal prepregnancy body mass index with childhood body mass index, total body and abdominal fat distribution, and cardio-metabolic risk factors. We also explored whether these associations are present across the full range of body mass index and explained by pregnancy, birth, or childhood characteristics.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onward in Rotterdam, The Netherlands.[12] The local medical ethical committee approved the study. Written informed consent was obtained from all mothers. In total, 6954 mothers had information about prepregnancy body mass index available and gave birth to singleton live-born children. Missing information about prepregnancy body mass index was mainly because of later enrollment in the study and nonparticipation in the first questionnaire. We excluded mothers and their children without follow-up data available. The population for analysis included 4871 (70%) children and their parents (flow chart given in **Supplementary Figure S2.6.1**).

Parental anthropometrics

At enrollment, we measured maternal and paternal height (cm) and weight (kg) without shoes and heavy clothing. Body mass index (kg/m^2) was calculated. Information about maternal weight just before pregnancy was obtained by questionnaire. In our population for analysis, 52.3% of all mothers were enrolled before a gestational age of 14 weeks. Correlation of prepregnancy weight, obtained by questionnaire, and weight measured at enrollment was 0.94 (P-value <0.001). Prepregnancy maternal and paternal body mass index were categorized into 4 categories (underweight [<20 kg/m^2], normal weight [20 – 24.9 kg/m^2], overweight [25 – 29.9 kg/m^2], and obese [≥30 kg/m^2]).

Childhood body fat and cardio-metabolic outcomes

All children were invited to participate in detailed body fat and cardio-metabolic follow-up measurements at the age of 6 years. We measured height and weight without shoes and heavy clothing and calculated body mass index. Childhood underweight, normal weight, overweight, and obesity were defined by the International Obesity Task Force cutoffs.[13] Body fat was measured by dual-energy x-ray absorptiometry (iDXA; General Electrics–Lunar, 2008, Madison, WI).[14] Total fat mass was calculated as percentage of total body weight measured by dual-energy x-ray absorptiometry. We calculated android/gynoid fat mass ratio.[14] We performed abdominal ultrasound examinations as described previously.[15,16] Subcutaneous and preperitoneal fat mass areas were measured as areas of 2 cm length along the midline starting from the reference point in the direction of the navel. Systolic and diastolic blood pressures were measured at the right brachial artery, 4× with 1-minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus TM (Paramus, NJ).[17] We used the mean systolic and diastolic blood pressure values using the last 3 blood pressure measurements. We obtained 30-minute fasting venous blood samples and measured total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, insulin, and C-peptide levels. In line with previous definitions used among pediatric populations to define childhood metabolic syndrome–like phenotype,[18] we defined clustering of cardio-metabolic risk factors as having any of the 3 or more following components: android fat mass percentage ≥75th percentile; systolic or diastolic blood pressure ≥75th percentile; high-density lipoprotein cholesterol ≤25th percentile or triglycerides ≥75th percentile; and insulin level ≥75th percentile. We used android fat mass as percentage of total body fat mass, which was used as proxy for waist circumference because waist circumference was not available.

Covariates

Information on maternal and paternal age, education level, ethnicity, and maternal folic acid supplement use was obtained at enrollment.[12] Information on maternal smoking and alcohol consumption was assessed by questionnaires during pregnancy. First

trimester maternal nutritional information was obtained by food frequency questionnaire.[19] Maternal weight gain until a gestational age of 30 weeks (median, 30.2 weeks; 95% range, 28.5 – 32.9) was measured. Information about pregnancy complications, mode of delivery and childhood sex, gestational age, and weight and length at birth was obtained from medical records.[20,21] Early childhood growth was measured at community health centers at 24 months. Information about breastfeeding, timing of introduction of solid foods, and average television-watching time was obtained by questionnaires.[12]

Statistical analysis

First, differences in subject characteristics between maternal body mass index categories were examined with 1-way ANOVA tests and χ^2 tests. Second, we examined the associations of maternal and paternal body mass index singularly and simultaneously with childhood outcomes in 4 linear regression models: (1) a basic model including child's age and sex; (2) a confounder model, which additionally included covariates selected on the basis of their associations with the outcomes of interest based on previous studies or a change in effect estimate >10%. We included childhood height as covariate in all models focused on fat mass outcomes; (3) an intermediate model, which additionally included maternal pregnancy complications, weight gain during pregnancy, gestational age and weight at birth, infant growth until 2 years of age, and current childhood body mass index; and (4) a fully adjusted model including all covariates. The confounder model was considered as the main model. Third, we examined the associations of maternal and paternal underweight, normal weight, overweight, and obesity with childhood cardio-metabolic outcomes using linear regression models and with the risks of childhood overweight and childhood clustering of cardio-metabolic risk factors using logistic regression models. For all analyses, we transformed non-normally distributed childhood outcome variables. We constructed standard deviation scores (SDS) values [(observed value−mean)/SD] for parental body mass index and childhood outcomes to enable comparison of effect estimates. We examined potential interactions between maternal body mass index and paternal body mass index, gestational weight gain, sex, ethnicity, gestational-age-adjusted birth weight, and childhood body mass index for these associations, but after taking multiple testing into account, no significant interactions were present, and no further stratified analyses were performed. Missing data of covariates were imputed using multiple imputations. All analyses were performed using Statistical Package of Social Sciences version 17.1 for Windows (SPSS Inc, Chicago, IL).

Results

Subject characteristics

Characteristics of the included mothers, fathers, and children are given in **Table 2.6.1.** Correlation coefficients among maternal, paternal, and childhood cardio-metabolic outcomes are shown in **Supplementary Table S2.6.1**. **Supplementary Table S2.6.2** shows that mothers without offspring follow-up data were more likely to be less educated and from non-European descent.

Parental body mass index and childhood cardio-metabolic outcomes

Table 2.6.2 shows the associations of parental body mass index with childhood outcomes per SDS change and the role of potential intermediates. In the confounder model, 1-SDS higher maternal and paternal body mass index were associated with 0.25-SDS (95% Confidence Interval (CI): 0.23, 0.28) and 0.22-SDS (95% CI: 0.19, 0.24) higher childhood body mass index, respectively (P-value for statistical difference between these associations <0.05). Including both maternal and paternal body mass index in the same model only slightly attenuated these effect estimates. The association of maternal body mass index with childhood body mass index was not explained by pregnancy complications and gestational weight gain. The associations of both maternal and paternal body mass index with childhood body mass index slightly attenuated after adjustment for birth characteristics and infant growth. In the fully adjusted model, both maternal and paternal body mass index remained significantly associated with childhood body mass index, with a significantly stronger association for maternal body mass index.

Similar patterns were present for the associations of parental body mass index with childhood total body and abdominal fat mass measures. Compared with paternal body mass index, maternal body mass index was more strongly associated with all childhood total body and abdominal fat mass measures. However, differences in the magnitude of effect estimates of maternal–offspring and paternal–offspring associations for android/gynoid fat mass ratio and abdominal preperitoneal fat mass were not statistically significant. After adjustment of these associations for current childhood body mass index, only the association of maternal BMI with childhood total body fat mass remained significant.

Table 2.6.1. Characteristics of mothers, fathers and their children (*n = 4871*)[1]

Characteristics	Total group *n = 4871*	Maternal underweight *n = 738*	Maternal normal weight *n = 2789*	Maternal overweight *n = 940*	Maternal obesity *n = 404*	P-value[2]
Maternal Characteristics						
Age, median (95% range), yrs	30.9 (19.9, 39.4)	30.4 (19.1, 38.7)	31.2 (19.8, 39.6)	30.8 (20.7, 39.5)	30.3 (20.6, 39.4)	<0.01
Gestational age at intake, median (95% range), wks	13.8 (9.9, 24.2)	13.8 (9.8, 24.1)	13.8 (10.0, 24.1)	13.9 (9.8, 24.1)	14.2 (10.1, 24.9)	0.38
BMI, mean (SD), kg/m^2	23.6 (4.2)	18.9 (0.9)	22.3 (1.4)	26.9 (1.4)	33.9 (3.7)	<0.01
Weight gain during pregnancy, mean (SD), kg	10.5 (4.9)	11.1 (3.9)	11.0 (4.4)	9.8 (5.2)	7.2 (7.0)	<0.01
Education, No. higher education (%)	2198 (46.3)	355 (49.0)	1422 (51.0)	336 (37.0)	85 (22.4)	<0.01
Race / Ethnicity, No. Dutch or European (%)	2985 (61.5)	481 (65.4)	1826 (65.7)	483 (51.5)	195 (48.9)	<0.01
Marital status, No. Married (%)	2379 (50.0)	327 (45.2)	1330 (48.5)	511 (56.2)	211 (54.8)	<0.01
Parity, No. nulliparous (%)	2833 (58.2)	472 (64.0)	1711 (61.3)	470 (50.0)	180 (44.6)	<0.01
Total energy intake, mean (SD), kcal	2052 (551)	2110 (562)	2075 (542)	1996 (541)	1907 (583)	<0.01
Folic acid supplement use, No. Yes (%)	3040 (75.1)	453 (76.7)	1837 (78.2)	537 (69.2)	213 (63.9)	<0.01
Smoking during pregnancy, No. Yes (%)	1187 (25.5)	199 (28.0)	660 (24.7)	226 (25.5)	102 (26.5)	0.31
Alcohol consumption during pregnancy, No. Yes (%)	2509 (54.0)	417 (59.0)	1550 (57.9)	411 (46.5)	131 (34.3)	<0.01
Maternal pregnancy complications						
Gestational hypertension, No. (%)	206 (4.4)	15 (2.1)	90 (3.3)	57 (6.1)	44 (10.9)	<0.01
Pre-eclampsia, No. (%)	86 (1.9)	12 (1.7)	35 (1.3)	22 (2.6)	17 (5.2)	<0.01
Gestational diabetes, No. (%)	51 (1.1)	3 (0.4)	12 (0.4)	23 (2.6)	13 (3.3)	<0.01
Paternal characteristics						
Age, median (95% range), yrs	33.2 (22.3, 46.1)	32.8 (21.7, 45.8)	33.2 (22.3, 45.9)	33.1 (22.3, 46.0)	32.8 (24.1, 48.9)	0.26
BMI, mean (SD), kg/m^2	25.3 (3.4)	24.3 (3.0)	25.1 (3.2)	26.1 (3.6)	27.0 (4.5)	<0.01
Paternal education, No. higher education (%)	1738 (52.4)	278 (55.9)	1124 (55.9)	269 (45.9)	67 (29.9)	<0.01
Race / Ethnicity, No. Dutch or European (%)	2620 (71.0)	396 (70.6)	1624 (73.6)	433 (65.7)	167 (63.7)	<0.01
Birth and infant characteristics						
Males, No. (%)	2444 (50.2)	389 (52.7)	1399 (50.2)	460 (48.9)	196 (48.5)	0.40
Gestational age at birth, median (95% range), wks	39.9 (35.9, 42.3)	40.0 (35.6, 42.1)	40.1 (36.1, 42.3)	40.3 (36.0, 42.4)	40.0 (34.4, 42.4)	<0.01
Birth weight, mean (SD), g	3435 (545)	3275 (513)	3445 (539)	3500 (543)	3504 (589)	<0.01
Caesarean delivery, No. (%)	539 (12.1)	71 (10.6)	289 (11.4)	126 (14.9)	53 (13.8)	0.02
Ever breastfeeding, No. Yes (%)	3566 (92.7)	545 (93.3)	2095 (93.4)	655 (92.5)	271 (87.4)	<0.01
Breastfeeding duration, median (95% range), mo	3.5 (0.5, 12.0)	3.5 (0.5, 12.0)	3.8 (0.5, 12.0)	3.5 (0.5, 12.0)	2.5 (0.5, 12.0)	<0.01
Introduction of solid foods, No. before 6 months (%)	2687 (89.5)	376 (87.9)	1613 (89.5)	490 (90.4)	208 (90.8)	<0.01
Television watching, No. More than 2 hours/day (%)	747 (19.3)	105 (17.7)	356 (15.8)	192 (26.3)	94 (33.2)	<0.01

Table 2.6.1. Characteristics of mothers, fathers and their children (*n = 4871*)[1] (*continued*)

Characteristics	Total group *n = 4871*	Maternal underweight *n = 738*	Maternal normal weight *n = 2789*	Maternal overweight *n = 940*	Maternal obesity *n = 404*	P-value[2]
Childhood characteristics						
Age at follow up, median (95% range), yrs	6.0 (5.6, 8.0)	6.0 (5.6, 8.0)	6.0 (5.6, 7.9)	6.0 (5.6, 7.9)	6.1 (5.6, 8.1)	<0.01
BMI, mean (SD), kg/m^2	16.2 (1.9)	15.6 (1.5)	16.1 (1.6)	16.6 (2.0)	17.7 (2.8)	<0.01
Overweight or obesity, No. (%)	866 (17.8)	57 (7.8)	393 (14.1)	245 (26.1)	171 (42.3)	<0.01
Total fat mass, mean (SD), %	25.0 (5.7)	23.3 (4.9)	24.4 (5.3)	26.0 (5.8)	28.8 (7.0)	<0.01
Android/gynoid fat mass ratio, mean (SD)	0.25 (0.06)	0.24 (0.06)	0.25 (0.06)	0.26 (0.07)	0.29 (0.08)	<0.01
Systolic blood pressure, mean (SD), mmHg	102.8 (8.2)	102.0 (7.9)	102.4 (8.1)	103.6 (8.4)	104.9 (8.4)	<0.01
Diastolic blood pressure, mean (SD), mmHg	60.8 (6.9)	60.7 (6.7)	60.6 (6.8)	60.8 (6.8)	61.8 (7.2)	0.02
Total-cholesterol, mean (SD), mmol/L	4.2 (0.6)	4.2 (0.6)	4.2 (0.6)	4.2 (0.6)	4.2 (0.7)	0.42
HDL – cholesterol, mean (SD), mmol/L	1.3 (0.3)	1.3 (0.3)	1.4 (0.3)	1.3 (0.3)	1.3 (0.3)	0.80
LDL – cholesterol, mean (SD), mmol/L	2.4 (0.6)	2.3 (0.6)	2.3 (0.6)	2.4 (0.6)	2.4 (0.5)	0.23
Triglycerides, median (95% range), mmol/L	1.0 (0.4, 2.4)	0.9 (0.4, 2.4)	1.0 (0.4, 2.3)	0.9 (0.4, 2.5)	1.0 (0.4, 2.6)	0.11
Insulin, median (95% range), pmol/L	117.0 (16.1, 405.8)	121.9 (13.0, 357.7)	117.4 (15.8, 394.3)	109.7 (17.7, 424.4)	123.5 (14.5, 493.8)	0.04
C-peptide, median (95% range), nmol/L	1.0 (0.3, 2.2)	1.0 (0.3, 2.0)	1.0 (0.3, 2.1)	1.01 (0.3, 2.3)	1.1 (0.3, 2.6)	0.04
Cardio-metabolic risk factor clustering, No. (%)	314 (10.4)	39 (8.5)	144 (8.3)	77 (12.9)	54 (22.4)	<0.01

[1]Values represent means (SD), medians (95% range) or numbers of subjects (valid %). [2]Differences in subject characteristics between groups were evaluated using one-way-ANOVA-tests for continuous variables and Chi-square tests for proportions.

Table 2.6.2. Parental body mass index and childhood fat mass measures (*n = 4871*)

Model	Body mass index	Total fat mass	Android/gynoid fat mass ratio	Subcutaneous abdominal fat area	Preperitoneal abdominal fat area
Maternal model					
Basic model[1]	0.27 (0.24, 0.29)**	0.25 (0.22, 0.27)**	0.18 (0.15, 0.21)	0.22 (0.19, 0.25) **	0.17 (0.14, 0.19)
Confounder model[2]	0.25 (0.23, 0.28)**	0.19 (0.17, 0.21)**	0.15 (0.12, 0.18)	0.16 (0.14, 0.19)	0.12 (0.09, 0.15)
Mediator models[3]					
Pregnancy complications	0.25 (0.23, 0.28)	0.19 (0.16, 0.21)	0.15 (0.12, 0.18)	0.17 (0.14, 0.19)	0.12 (0.09, 0.15)
Maternal gestational weight gain	0.28 (0.25, 0.31)	0.20 (0.18, 0.23)	0.16 (0.13, 0.19)	0.17 (0.14, 0.20)	0.13 (0.10, 0.16)
Birth characteristics	0.23 (0.20, 0.25)	0.19 (0.17, 0.22)**	0.15 (0.13, 0.18)	0.16 (0.14, 0.19)	0.12 (0.09, 0.15)
Infant growth	0.25 (0.22, 0.27)**	0.19 (0.16, 0.21)**	0.15 (0.12, 0.18)	0.16 (0.13, 0.19) **	0.12 (0.09, 0.15)
Childhood BMI	-	0.02 (0, 0.04)	-0.02 (-0.04, 0.01)	0 (-0.02, 0.03)	0 (-0.03, 0.03)
Fully adjusted model[4]	0.19 (0.16, 0.22)**	0.03 (0.01, 0.05)	-0.02 (-0.04, 0.01)	0.01 (-0.02, 0.03)	0 (-0.03, 0.03)
Paternal model					
Basic model[1]	0.22 (0.19, 0.24)**	0.18 (0.15, 0.20)**	0.14 (0.11, 0.17)	0.16 (0.12, 0.19)**	0.12 (0.09, 0.16)
Confounder model[2,5]	0.22 (0.19, 0.24)**	0.15 (0.12, 0.17)**	0.13 (0.10, 0.16)	0.13 (0.10, 0.16)	0.10 (0.07, 0.13)
Mediator models[3]					
Birth characteristics	0.20 (0.18, 0.23)	0.15 (0.12, 0.17)**	0.13 (0.10, 0.16)	0.12 (0.09, 0.15)	0.10 (0.06, 0.13)
Infant growth	0.19 (0.16, 0.22)**	0.13 (0.11, 0.16)**	0.12 (0.09, 0.15)	0.12 (0.09, 0.15)**	0.09 (0.06, 0.12)
Childhood BMI	-	0.01 (-0.01, 0.03)	0 (-0.03, 0.02)	0 (-0.02, 0.03)	0 (-0.03, 0.03)
Fully adjusted model[6]	0.14 (0.11, 0.16)**	0.01 (-0.01, 0.03)	0 (-0.02, 0.03)	0 (-0.02, 0.03)	0.01 (-0.03, 0.04)
Combined maternal and paternal model					
Basic model[1]					
Maternal BMI	0.21 (0.18, 0.24)**	0.20 (0.17, 0.23)**	0.14 (0.11, 0.17)	0.18 (0.15, 0.21) **	0.13 (0.09, 0.16)
Paternal BMI	0.17 (0.15, 0.20)**	0.13 (0.11, 0.16)**	0.11 (0.08, 0.14)	0.12 (0.09, 0.15) **	0.10 (0.07, 0.13)
Confounder model[2,7]					
Maternal BMI	0.21 (0.18, 0.24)	0.16 (0.13, 0.19)**	0.12 (0.09, 0.15)	0.14 (0.11, 0.18) **	0.10 (0.06, 0.13)
Paternal BMI	0.18 (0.15, 0.21)	0.12 (0.09, 0.14)**	0.11 (0.08, 0.14)	0.10 (0.07, 0.13) **	0.08 (0.05, 0.11)
Fully adjusted model[8]					
Maternal BMI	0.16 (0.13, 0.19)**	0.03 (0.01, 0.05)	-0.02 (-0.05, 0.01)	0.02 (-0.01, 0.05)	0.01 (-0.03, 0.05)
Paternal BMI	0.11 (0.09, 0.14)**	0.01 (-0.01, 0.03)	0 (-0.02, 0.03)	0 (-0.03, 0.02)	0 (-0.03, 0.04)

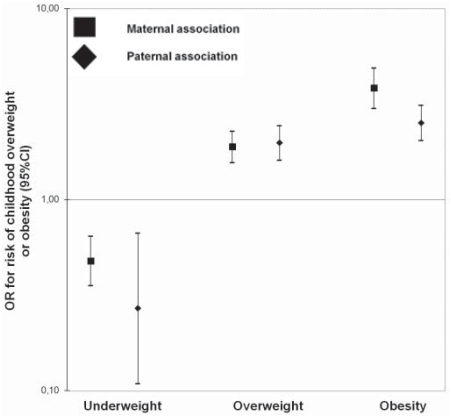
Values are regression coefficients (95% CI) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal and paternal prepregnancy body mass index. Estimates are based on multiple imputed data. [1]Basic model includes child's sex and age at outcome measurements. [2]Confounder model includes maternal age, educational level, ethnicity, parity, smoking and alcohol consumption during pregnancy and folic acid supplement use, total calorie intake during pregnancy, caesarean delivery, breastfeeding duration, timing of introduction of solid foods, child average duration of tv watching, childhood height (for fat mass outcomes only). [3]Intermediate models are confounder models additionally adjusted for each potential intermediate. [4]Fully adjusted model includes all potential confounders and intermediates.[5]Paternal confounder model includes paternal age, paternal educational level and paternal ethnicity instead of maternal age, maternal educational level and maternal ethnicity. [6]Fully adjusted paternal model includes all potential confounders and intermediates. [7]Maternal and paternal combined confounder model includes both maternal and paternal confounders.[8]Combined fully adjusted model includes all potential maternal and paternal confounders and intermediates. **P-value <0.05 for heterogeneity between maternal and paternal associations.

Table 2.6.3. Parental body mass index and childhood cardio-metabolic risk factors (*n = 4871*)

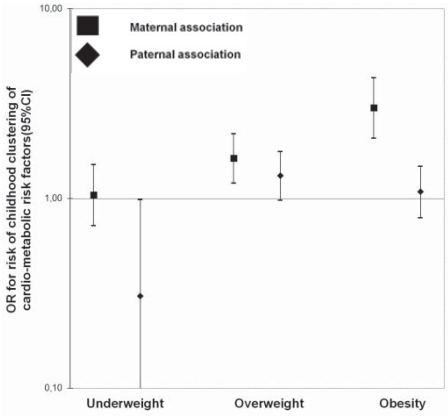
Model	Systolic blood pressure	Diastolic blood pressure	Total-cholesterol	HDL-cholesterol	LDL-cholesterol	Triglycerides	Insulin
Maternal model							
Basic model[1]	0.10 (0.07, 0.12)	0.04 (0.01, 0.07)	0.01 (-0.03, 0.04)	-0.02 (-0.05, 0.02)	0.02 (-0.02, 0.05)	0.02 (-0.01, 0.06)	0.04 (0, 0.07)
Confounder model[2]	0.08 (0.05, 0.11)	0.02 (-0.01, 0.05)	-0.01 (-0.05, 0.03)	-0.04 (-0.08, 0)	0 (-0.03, 0.04)	0.03 (-0.01, 0.06)	0.05 (0.01, 0.08)
Mediator models[3]							
Pregnancy complications	0.07 (0.04, 0.10)	0.01 (-0.02, 0.04)	-0.01 (-0.05, 0.03)	-0.04 (-0.07, 0)	0 (-0.04, 0.04)	0.03 (-0.01, 0.06)	0.04 (0.01, 0.08)
Maternal gestational weight gain	0.09 (0.06, 0.12)	0.02 (-0.01, 0.05)	-0.01 (-0.05, 0.02)	-0.05 (-0.08, -0.01)	0.01 (-0.03, 0.04)	0.03 (-0.01, 0.07)	0.05 (0.01, 0.09)
Birth characteristics	0.08 (0.05, 0.11)	0.03 (0, 0.06)	-0.01 (-0.04, 0.03)	-0.04 (-0.08, 0)	0.01 (-0.03, 0.04)	0.03 (-0.01, 0.07)	0.05 (0.02, 0.09)
Infant growth	0.08 (0.05, 0.11)	0.02 (-0.01, 0.05)	-0.01 (-0.05, 0.03)	-0.04 (-0.08, 0)	0 (-0.03, 0.04)	0.03 (-0.01, 0.06)	0.05 (0.01, 0.09)
Childhood BMI	0.02 (-0.01, 0.05)	0 (-0.03, 0.03)	-0.04 (-0.08, 0)	-0.02 (-0.05, 0.02)	-0.02 (-0.06, 0.02)	-0.01 (-0.05, 0.03)	-0.01 (-0.04, 0.03)
Fully adjusted model[4]	0.04 (0.01, 0.07)	0.01 (-0.03, 0.04)	-0.04 (-0.08, 0)	-0.02 (-0.06, 0.03)	-0.02 (-0.06, 0.02)	-0.01 (-0.05, 0.03)	0 (-0.04, 0.04)
Paternal model							
Basic model[1]	0.07 (0.04, 0.10)	0.03 (0, 0.06)	0 (-0.04, 0.04)	-0.03 (-0.07, 0.01)	0.01 (-0.03, 0.05)	0.02 (-0.02, 0.06)	0.01 (-0.03, 0.05)
Confounder model[2,5]	0.06 (0.03, 0.09)	0.02 (-0.01, 0.05)	-0.01 (-0.05, 0.03)	-0.05 (-0.08,-0.01)	0.01 (-0.03, 0.05)	0.02 (-0.03, 0.06)	0.01 (-0.03, 0.05)
Mediator models							
Birth characteristics	0.06 (0.03, 0.09)	0.02 (-0.01, 0.06)	-0.01 (-0.05, 0.04)	-0.04 (-0.08, 0)	0.01 (-0.03, 0.05)	0.02 (-0.02, 0.06)	0.01 (-0.03, 0.05)
Infant growth	0.05 (0.02, 0.08)	0.02 (-0.02, 0.05)	-0.01 (-0.05, 0.03)	-0.04 (-0.08, 0)	0.01 (-0.03, 0.05)	0.02 (-0.02, 0.06)	0 (-0.04, 0.04)
Childhood BMI	0.02 (-0.02, 0.05)	0.01 (-0.02, 0.04)	-0.02 (-0.07, 0.02)	-0.02 (-0.06, 0.02)	-0.01 (-0.05, 0.03)	-0.02 (-0.06, 0.03)	-0.04 (-0.08, 0)
Fully adjusted model[6]	0.01 (-0.02, 0.05)	0.01 (-0.03, 0.04)	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.02)	-0.01 (-0.05, 0.03)	-0.01 (-0.05, 0.03)	-0.04 (-0.08, 0)
Combined maternal and paternal model							
Basic model[1]							
Maternal BMI	0.08 (0.05, 0.12)	0.03 (0, 0.07)	0.01 (-0.03, 0.05)	-0.02 (-0.07, 0.02)	0.03 (-0.01, 0.07)	0.02 (-0.02, 0.07)	0.02 (-0.02, 0.07)
Paternal BMI	0.05 (0.02, 0.09)	0.03 (-0.01, 0.06)	0 (-0.04, 0.04)	-0.03 (-0.07, 0.01)	0.01 (-0.04, 0.05)	0.01 (-0.03, 0.05)	0 (-0.04, 0.05)
Confounder model[2,7]							
Maternal BMI	0.07 (0.04, 0.10)	0.02 (-0.02, 0.05)	-0.01 (-0.05, 0.04)	-0.04 (-0.08, 0.01)	0.02 (-0.03, 0.06)	0.03 (-0.02, 0.07)	0.03 (-0.01, 0.08)
Paternal BMI	0.05 (0.01, 0.08)	0.02 (-0.02, 0.05)	-0.01 (-0.05, 0.04)	-0.04 (-0.08, 0)	0 (-0.04, 0.04)	0.01 (-0.03, 0.06)	0 (-0.04, 0.04)
Fully adjusted model[8]							
Maternal BMI	0.05 (0.01, 0.08)	0.01 (-0.03, 0.05)	-0.04 (-0.08, 0.01)	-0.01 (-0.06, 0.04)	-0.01 (-0.06, 0.04)	-0.01 (-0.05, 0.04)	-0.02 (-0.07, 0.03)
Paternal BMI	0.01 (-0.03, 0.03)	0 (-0.03, 0.04)	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.02)	-0.01 (-0.05, 0.03)	-0.01 (-0.05, 0.03)	-0.04 (-0.08, 0.01)

Values are regression coefficients (95% CI) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal and paternal prepregnancy body mass index. Estimates are based on multiple imputed data. [1]Basic model includes child's sex and age at outcome measurements. [2] Confounder model includes maternal age, educational level, ethnicity, parity, smoking and alcohol consumption during pregnancy and folic acid supplement use, total calorie intake during pregnancy, caesarean delivery, breastfeeding duration, timing of introduction of solid foods, child average duration of tv watching. [3]Intermediate models are confounder models additionally adjusted for each potential intermediate. [4]Fully adjusted model includes all potential confounders and intermediates. [5]Paternal confounder model includes paternal age, paternal educational level and paternal ethnicity instead of maternal age, maternal educational level and maternal ethnicity. [6]Fully adjusted paternal model includes all potential confounders and intermediates. [7]Maternal and paternal combined confounder model includes both maternal and paternal confounders. [8]Combined fully adjusted model includes all potential maternal and paternal confounders and intermediates. **All P-values for heterogeneity between maternal and paternal associations not significant.

Figure 2.6.1. Associations of maternal and paternal underweight, overweight and obesity with the risks of childhood overweight and childhood clustering of cardio-metabolic risk factors (*n = 4871*)[1]



2.6.1a. Childhood overweight and obesity



2.6.1b. Childhood clustering of cardio-metabolic risk factors

[1]Values are Odds Ratios (95% CI) from logistic regression models that reflect the risks of childhood overweight and obesity and childhood clustering of cardio-metabolic risk factors for maternal and paternal underweight, overweight and obesity as compared to the reference group (maternal and paternal normal weight). Estimates are based on multiple imputed data. Maternal models include child's sex and age at outcome measurements, maternal age, educational level, ethnicity, parity, smoking and alcohol consumption during pregnancy and folic acid supplement use, total calorie intake during pregnancy, caesarean delivery, breastfeeding duration, timing of introduction of solid foods, child average duration of tv watching. Paternal models include paternal age, paternal educational level and paternal ethnicity instead of maternal age, maternal educational level and maternal ethnicity.

Table 2.6.3 shows that higher maternal and paternal body mass index were associated with a higher childhood systolic blood pressure (differences: 0.08 SDS (95% CI: 0.05, 0.11) and 0.06 SDS (95% CI: 0.03, 0.09) per SDS change in maternal and paternal body mass index, respectively) but not diastolic blood pressure. These associations were largely explained by childhood body mass index. In the fully adjusted model, maternal body mass index, but not paternal body mass index, was still associated with a higher childhood systolic blood pressure (P-value <0.05). In the confounder model, a higher maternal body mass index was associated with lower childhood high-density lipoprotein cholesterol and higher insulin levels (P-value <0.05) but not with childhood total cholesterol, low-density lipoprotein cholesterol, triglycerides, and C-peptide levels (results for C-peptide not shown). Higher paternal body mass index was only associated with lower childhood high-density lipoprotein cholesterol (P-value <0.05), with similar effect estimates as for maternal body mass index. These associations were fully explained by childhood body mass index.

Parental obesity and childhood cardio-metabolic outcomes

Figure S2.6.2 shows that compared with maternal normal weight, maternal obesity was associated with a higher childhood body mass index, total body and abdominal fat mass measures, systolic blood pressure, triglycerides, insulin, and C-peptide levels (all

P-values <0.05). Similar, but weaker, associations were present for paternal obesity. Figure 2.6.1 shows that compared with maternal normal weight, maternal obesity was associated with increased risks of childhood overweight (Odds Ratio (OR) 3.84 (95% CI: 3.01, 4.90)) and clustering of cardio-metabolic risk factors (OR 3.00 (95% CI: 2.09, 4.34)). Compared with children from normal-weight fathers, children from obese fathers also had an increased risk of childhood overweight (OR 2.52 (95% CI: 2.04, 3.12)) but not of clustering of childhood cardio-metabolic risk factors.

Discussion

We observed that higher maternal and paternal prepregnancy body mass index were associated with increased adiposity levels and an adverse cardio-metabolic profile in their children. Associations of maternal prepregnancy body mass index with childhood outcomes tended to be stronger compared with associations of paternal body mass index.

Methodological considerations

Strengths of this study were the prospective data collection from early pregnancy onward, large sample size, and detailed childhood body fat and cardio-metabolic measurements. Follow-up data were available in 70% of our study population. The nonresponse could lead to biased effect estimates if associations of parental body mass index with childhood adiposity and cardio-metabolic measures would be different between children included and not included in the analyses. Assuming that parents and children with a higher body mass index are less likely to participate in detailed adiposity and cardio-metabolic follow-up, our estimates may be underestimated. Information on maternal prepregnancy weight was self-reported, which might have led to misclassification and underestimation of the observed effects. However, we observed similar results when we used maternal weight measured at enrollment in the study (results not shown). No information about maternal and paternal insulin–glucose status was available in our study cohort. To obtain further insight into the potential underlying mechanisms, it is of interest to perform similar analyses taking into account parental insulin and glucose levels. We had detailed information about potential confounding factors available in this study. However, because of the observational design, residual confounding because of other lifestyle-related variables, such as parental and childhood nutritional intake, might still be an issue.

Interpretation of main findings

Previous studies showed that maternal obesity is associated with offspring obesity and an adverse cardio-metabolic profile.[22] These associations may be explained by direct intrauterine mechanisms or shared environmental, lifestyle-related, or genetic

characteristics. By comparing maternal-offspring and paternal-offspring associations, underlying mechanisms may be further elucidated.[6,7]

Previous studies examining the strengths of associations of both maternal and paternal prepregnancy body mass index with childhood outcomes have mainly focused on childhood body mass index and have reported inconsistent results.[5,8–11,23–25] Most studies reported no differences in the magnitude of parental associations with offspring body mass index.[8–11,24] However, in childhood, BMI might not be an appropriate measure of fat mass. A study among 4091 UK parent-offspring trios reported that maternal prepregnancy body mass index was more strongly associated with childhood fat mass, whereas in the same sample, similar effect estimates for the associations of maternal and paternal body mass index with childhood body mass index were reported.[8,9] A study among 89 parent–offspring pairs showed that maternal, but not paternal, body mass index was an important determinant of childhood total fat mass.[26] Compared with paternal body mass index, we observed that maternal body mass index tended to be more strongly associated with childhood body mass index, total body fat mass, android/gynoid fat mass ratio, abdominal subcutaneous and preperitoneal fat mass, which is a measure of visceral fat mass. In addition, the association of maternal, but not paternal, prepregnancy body mass index with childhood total body fat mass was independent of childhood current body mass index. Thus, our results suggest that children from mothers with a higher prepregnancy body mass index have a higher total body fat mass, independent of their body mass index, and relatively more abdominal fat mass. These specific total body and abdominal fat distribution measures are related to adult cardio-metabolic disease and risk of mortality.[27,28]

Parental body mass index has also been associated with separate cardio-metabolic risk factors and clustering of these risk factors in offspring. A study among 3864 UK children showed that maternal and paternal prepregnancy body mass index were significantly associated with offspring systolic blood pressure at 5 years in the fully adjusted models.[29] Another study among 9328 parents and their children reported that only maternal body mass index was significantly associated with offspring systolic blood pressure, whereas both maternal and paternal body mass index were associated with offspring lipid levels and inflammatory markers, with similar effect estimates. These associations were modified by offspring body mass index, and after adjustment for offspring adiposity levels, most associations attenuated or reversed.[30] Among 940 Swedish children and 873 adolescents, it was shown that only maternal weight status influenced offspring cardiorespiratory fitness, after taking offspring fatness into account.[25] A study among 599 US children and their parents showed that both maternal and paternal body mass index were associated with offspring risk of clustering of cardiovascular risk factors.[31] In this study, stronger associations for maternal body mass index tended to be present. We observed that only higher maternal prepregnancy body mass index was associated with higher childhood systolic blood pressure. No associations of parental body mass index with childhood metabolic measures were present after adjustment for childhood body mass index. Maternal and paternal body mass index were associated

with the risk of childhood overweight, whereas only maternal body mass index was associated with the risk of clustering of cardio-metabolic risk factors.

The associations of maternal prepregnancy body mass index with these childhood body fat distribution and cardio-metabolic outcomes were strongest for maternal obesity, but were also present across the full range. Although the observed effect estimates were small to moderate, these childhood cardio-metabolic risk factors tend to track from childhood into adulthood and are associated with cardiovascular disease in later life.[32–36] Thus, these results suggest that especially maternal prepregnancy body mass index may be a critical factor for offspring cardio-metabolic health in later life.

The associations of maternal prepregnancy body mass index with childhood outcomes may be explained by several mechanisms. Shared family-based, lifestyle-related characteristics and genetic factors are likely to explain part of the associations. Previously, we have shown that overweight and obese mothers differ from normal-weight mothers in socio-demographic and lifestyle-related characteristics.[37] However, for all childhood adiposity outcomes, systolic blood pressure, insulin, and clustering of cardio-metabolic risk factors associations of maternal prepregnancy body mass index tended to be stronger than associations of paternal body mass index. In addition, extensive adjustment for socio-demographic and lifestyle-related characteristics did not explain our findings. The observed effects were also not mediated by pregnancy complications, birth characteristics, or infant growth, which are all identified risk factors related to both maternal prepregnancy body mass index and health of offspring.[38–41] Thus, our findings suggest that associations of maternal prepregnancy body mass index with offspring cardio-metabolic health outcomes may, at least partly, be explained by direct intrauterine mechanisms. This may include higher maternal plasma concentrations and placental transfer of glucose, amino acids, and free fatty acids during pregnancy, which may influence programming of offspring adiposity and an adverse cardio-metabolic profile in later life.[22,42] Further research is needed to obtain further insight into the causality and underlying mechanisms of these associations.

Perspectives

Both maternal and paternal prepregnancy body mass index are associated with increased adiposity levels and an adverse cardio-metabolic profile in offspring, with stronger associations present for maternal prepregnancy body mass index. Preventive strategies that focus on reduction of obesity in pregnant women may lead to better cardio-metabolic health in their offspring.

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Supplementary Material

Supplementary Table S2.6.1. Correlation coefficients between maternal, paternal and offspring weight and cardio-metabolic measures[1]

	Maternal measures	Paternal measures	Offspring measures												
	BMI	BMI	BMI	TFM	AGFM	SFM	PFM	SBP	DBP	Cholesterol	HDL	LDL	Triglycerides	Insulin	C-peptide
Maternal measures															
BMI	1	**0.22**	**0.31**	**0.26**	**0.19**	**0.21**	**0.14**	**0.10**	**0.05**	0.02	-0.02	0.02	0	0.01	-0.01
Paternal measures															
BMI	**0.22**	1	**0.25**	**0.20**	**0.15**	**0.16**	**0.13**	**0.08**	**0.04**	0.01	-0.03	0.02	0.01	0	0
Offspring measures															
BMI	**0.31**	**0.25**	1	**0.71**	**0.64**	**0.60**	**0.42**	**0.24**	**0.09**	**0.10**	**-0.05**	**0.08**	**0.06**	**0.14**	**0.10**
TFM	**0.26**	**0.20**	**0.71**	1	**0.56**	**0.81**	**0.56**	**0.21**	**0.13**	**0.15**	**-0.08**	**0.18**	**0.07**	**0.10**	**0.07**
AGFM	**0.19**	**0.15**	**0.64**	**0.68**	1	**0.57**	**0.40**	**0.16**	**0.07**	**0.09**	**-0.10**	**0.08**	**0.14**	**0.09**	**0.09**
SFM	**0.21**	**0.16**	**0.60**	**0.81**	**0.57**	1	**0.66**	**0.19**	**0.10**	**0.14**	-0.04	**0.16**	**0.07**	**0.10**	**0.06**
PFM	**0.14**	**0.13**	**0.42**	**0.56**	**0.40**	**0.66**	1	**0.12**	**0.05**	**0.16**	0.03	**0.15**	**0.08**	**0.11**	**0.07**
SBP	**0.10**	**0.08**	**0.24**	**0.21**	**0.16**	**0.19**	**0.12**	1	**0.62**	**0.07**	**0.07**	0.03	-0.01	**0.08**	**0.04**
DBP	**0.05**	**0.04**	**0.09**	**0.13**	**0.07**	**0.10**	**0.05**	**0.62**	1	**0.04**	**0.06**	0.02	-0.03	-0.03	**-0.05**
Cholesterol	0.02	0.01	**0.10**	**0.15**	**0.09**	**0.14**	**0.16**	**0.07**	**0.04**	1	**0.30**	**0.86**	**0.16**	0	-0.03
HDL	-0.02	-0.03	**-0.05**	**-0.08**	**-0.10**	-0.04	0.03	**0.07**	**0.06**	**0.30**	1	**-0.05**	**-0.38**	**-0.06**	**-0.09**
LDL	0.02	0.02	**0.08**	**0.16**	**0.08**	**0.16**	**0.15**	0.03	0.02	**0.86**	**-0.05**	1	**0.12**	**-0.04**	**-0.06**
Triglycerides	0	0.01	**0.06**	**0.07**	**0.14**	**0.07**	**0.08**	-0.01	-0.03	**0.16**	**-0.38**	**0.12**	1	**0.19**	**0.20**
Insulin	0.01	0	**0.14**	**0.10**	**0.09**	**0.10**	**0.11**	0.08	-0.03	0	**-0.06**	**-0.04**	**0.19**	1	**0.88**
C-peptide	-0.01	0	**0.10**	**0.07**	**0.09**	**0.06**	**0.07**	**0.04**	**-0.05**	-0.03	**-0.09**	**-0.06**	**0.20**	**0.88**	1

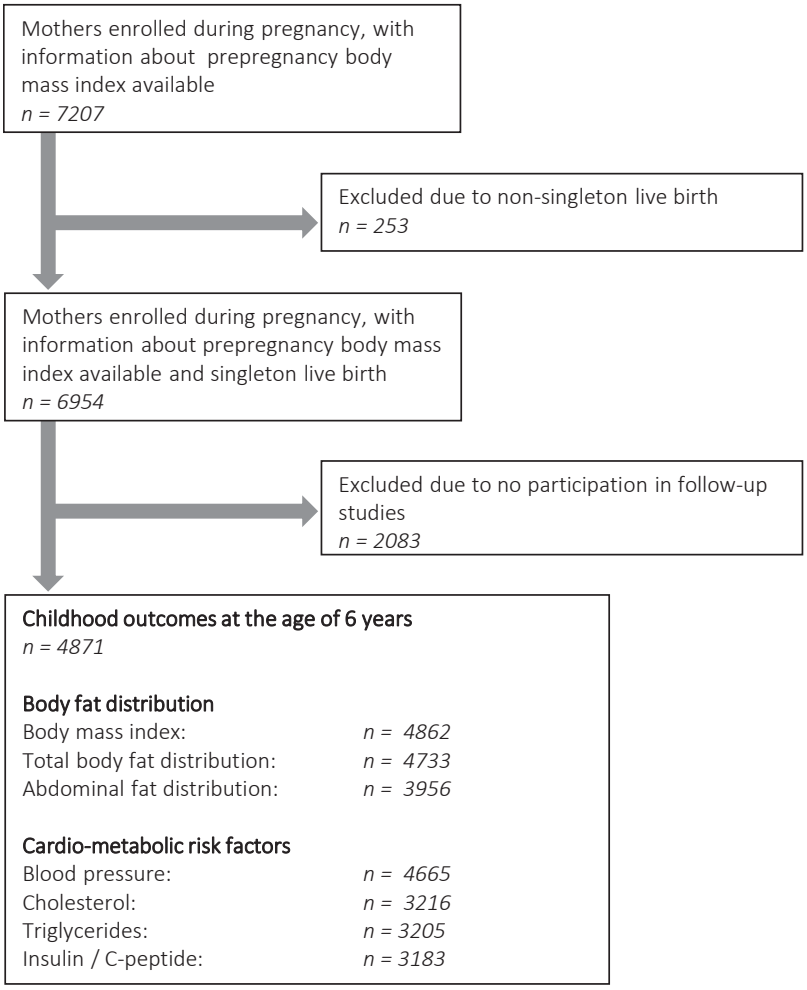
[1]Values are Pearson's or Spearman rank correlation coefficients. Bold values are significant. Abbreviations: TFM: total fat mass; AGFM: android/gynoid fat mass ratio; SFM: abdominal subcutaenous fat mass; PFM: abdominal preperitoneal fat mass; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Supplementary Table S2.6.2. Non-response analysis for childhood follow-up data at 6 years (*n = 6954*) [1]

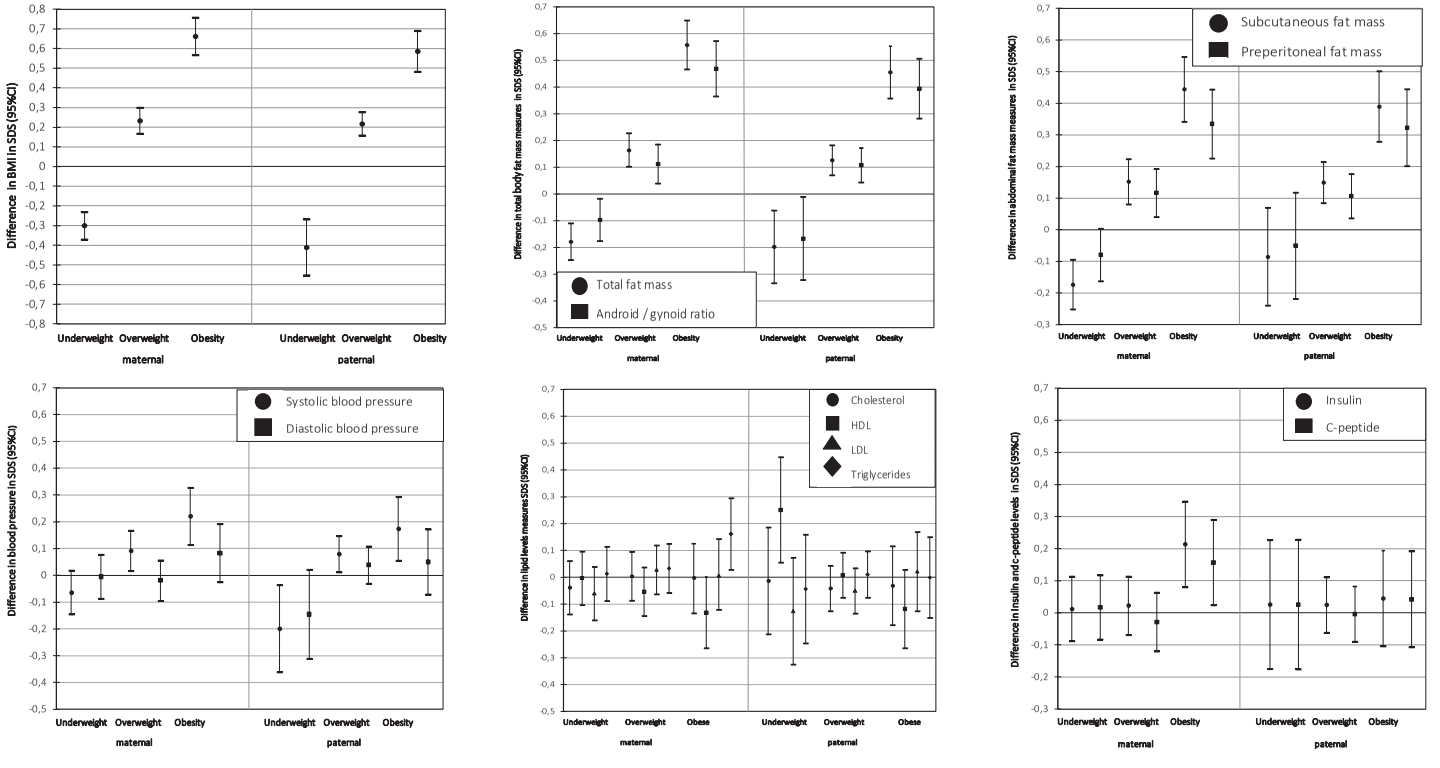
Characteristics	Follow-up at 6 years *n = 4871*	Loss to follow-up at 6 years *n = 2083*	P-value[4]
Maternal characteristics			
Age, mean (SD), years	30.4 (5.1)	28.3 (5.4)	<0.01
Height, mean (SD), cm	167.8 (7.4)	166.6 (7.3)	<0.01
Prepregnancy weight, mean (SD), kg	66.5 (12.6)	65.8 (13.4)	0.05
Prepregnancy body mass index, mean (SD), kg/m^2	23.6 (4.2)	23.7 (4.7)	0.41
Gestational weight gain, mean (SD), kg	10.5 (4.9)	10.3 (5.1)	0.19
Gestational age at intake, median (95% range), weeks[2]	13.8 (9.9, 24.2)	14.2 (9.9, 24.5)	<0.01
Parity, nulliparous, No. (%)[3]	2833 (58.2)	1124 (54.0)	<0.01
Education, No. higher education (%)[3]	2198 (46.3)	652 (32.9)	<0.01
Race / Ethnicity, No. European (%)[3]	2985 (61.5)	987 (48.7)	<0.01
Smoking habits during pregnancy, No. Yes (%)[3]	1187 (25.5)	580 (28.8)	<0.01
Alcohol consumption during pregnancy, No. Yes (%)[3]	2509 (54.0)	842 (42.2)	<0.01
Folic acid supplement use, No. (%)[3]			
No use	1009 (24.9)	656 (38.6)	<0.01
First 10 weeks use	1310 (32.4)	526 (31.0)	
Preconception use	1730 (42.7)	517 (30.4)	
Maternal pregnancy complications			
Gestational hypertension, No. (%)[3]	206 (4.4)	58 (3.0)	<0.01
Pre-eclampsia, No. (%)[3]	86 (1.9)	47 (2.5)	0.15
Gestational diabetes, No. (%)[3]	51 (1.1)	19 (1.0)	0.79
Paternal characteristics			
Age, mean (SD), years	33.2 (5.7)	31.5 (5.9)	<0.01
Body mass index, mean (SD), kg/m^2	25.3 (3.4)	25.4 (3.7)	0.45
Paternal education, No. higher education (%)[3]	1738 (52.4)	483 (46.1)	<0.01
Race / Ethnicity, No. Dutch or European (%)[3]	2620 (71.0)	797 (62.1)	<0.01
Birth and infant characteristics			
Males, No. (%)[3]	2444 (50.2)	1074 (51.6)	0.28
Gestational age at birth, median (95% range), weeks[2]	40.1 (35.9, 42.3)	40.0 (34.7, 42.4)	<0.01
Birth weight, mean (SD), g	3435 (545)	3385 (582)	<0.01
Caesarean delivery, No. (%)[3]	539 (12.1)	238 (12.8)	0.48
Ever breastfeeding, No. Yes (%)[3]	3566 (92.7)	1020 (90.1)	<0.01
Breastfeeding duration, median (95% range), months[2]	3.5 (0.5, 12.0)	2.5 (0.5, 12.0)	<0.01

[1]Values are means (standard deviation). [2]Values are medians (95% range). [3]Values are observed numbers (valid percentages). [4]Differences in subject characteristics between the groups were evaluated using one-way ANOVA tests for continuous variables and Chi-square tests for proportions.

Supplementary Figure S2.6.1. Participants flow chart in the Generation R Study, Rotterdam, the Netherlands



Supplementary Figure S2.6.2. Associations of maternal and paternal underweight, overweight and obesity with childhood adiposity measures and cardio-metabolic outcomes (*n = 4871*)[1]



[1]Values are regression coefficients (95% Confidence Interval) from linear regression models that reflect differences in childhood outcomes in SDS for maternal and paternal underweight, overweight and obesity as compared to the reference group (maternal and paternal normal weight). Estimates are based on multiple imputed data. Maternal models are adjusted for child's sex and age at outcome measurements and childhood height (for fat mass outcomes only), maternal age, educational level, ethnicity, parity, smoking during pregnancy, alcohol consumption during pregnancy and folic acid supplement use, total calorie intake during pregnancy, caesarean delivery, breastfeeding duration, timing of introduction of solid foods, child average duration of tv watching. Paternal models are adjusted for paternal age, paternal educational level and paternal ethnicity, instead of maternal age, maternal educational level and maternal ethnicity.

Chapter 2.7

Maternal weight gain in different trimesters and childhood cardiovascular risk factors

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Submitted

Abstract

Background: Excessive gestational weight gain seems to be associated with offspring cardio-metabolic risk factors. Not much is known about critical periods of gestational weight gain.

Objective: We examined the associations of maternal weight gain in different periods of pregnancy with childhood cardio-metabolic risk factors.

Design: In a population-based prospective cohort study from early pregnancy onwards among 5908 mothers and their children, we obtained maternal prepregnancy weight and weight in early-, mid- and late-pregnancy. At the age of 6 years, we measured childhood body mass index, total body and abdominal fat distribution, blood pressure and blood levels of lipids, insulin and C-peptide.

Results: Independent from maternal prepregnancy weight and weight gain in other periods, higher weight gain in early pregnancy was associated with higher childhood body mass index, total fat mass, android/gynoid fat mass ratio, abdominal subcutaneous and preperitoneal fat mass, systolic blood pressure, insulin and C-peptide (P-values <0.05). Higher weight gain in mid-pregnancy was independently associated with higher childhood body mass index, total and abdominal subcutaneous fat mass and systolic blood pressure (P-values <0.05). The associations for childhood cardio-metabolic outcomes attenuated after adjustment for childhood body mass index. Weight gain in late-pregnancy was not associated with childhood outcomes. Higher weight gain in early, but not in mid- or late-pregnancy, was associated with increased risks of childhood overweight and clustering of cardio-metabolic risk factors (Odds Ratio (OR) 1.19 (95% Confidence Interval (CI): 1.10, 1.29) and OR 1.20 (95% CI: 1.07, 1.35) per standard deviation increase in early-gestational weight gain, respectively).

Conclusions: Higher weight gain in early pregnancy is associated with an adverse cardio-metabolic profile in offspring. This association is largely mediated by childhood adiposity.

Introduction

Increased maternal gestational weight gain may influence long-term cardio-metabolic health of offspring.[1] The mechanisms underlying these associations are not known, and may depend upon the timing of gestational weight gain.[1-4] Gestational weight gain is a complex trait. Maternal gestational weight gain in early-pregnancy largely reflects maternal fat deposition, whereas gestational weight gain in mid- and late-pregnancy largely reflect maternal and amniotic fluid expansion, and growth of fetus, placenta and uterus.[5] Higher placental transfer of nutrients, such as glucose and free fatty acids in mothers with increased gestational weight gain, may lead to permanent fetal and childhood adaptations in appetite, energy metabolism and neuro-endocrine function, and predispose individuals to a greater risk of cardio-metabolic disease in later life.[1,6] Previous studies suggested that weight gain in early-pregnancy is associated with offspring body mass index, whereas weight gain in mid-pregnancy tended to be associated with offspring metabolic and inflammatory biomarkers.[4,7,8] It is not known whether these associations are independent from other periods of gestational weight gain or explained by pregnancy complications and infant growth characteristics. Also, previous studies did not examine associations of specific periods of gestational weight gain with detailed childhood body fat distribution and cardio-metabolic measures.

Therefore, we aimed to identify critical periods of maternal gestational weight gain for cardio-metabolic health in offspring. In a population-based prospective cohort study among 5908 mothers and their children, we examined the associations of specific periods of gestational weight gain and excessive gestational weight gain with childhood cardio-metabolic risk factors. We also examined whether these associations are independent from gestational weight gain in other periods or explained by pregnancy, birth and infant characteristics.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[9] The local Medical Ethical Committee approved the study. Written informed consent was obtained from all mothers. In total, 8614 mothers had information about at least one maternal weight measurement during pregnancy available and gave birth to singleton live born children. We excluded children without follow-up data available. The population for analysis included 5908 (69%) mothers and their children (Flow chart is given in **Supplementary Figure S2.7.1**).

Maternal anthropometrics and gestational weight gain

At enrolment, we measured maternal height (cm) and weight (kg) without shoes and heavy clothing and calculated body mass index (kg/m^2). Information about maternal weight just before pregnancy was obtained by questionnaire. Prepregnancy body mass index was categorized in 4 categories (underweight (<20 kg/m^2), normal weight (20 - 24.9 kg/m^2), overweight (25 - 29.9 kg/m^2) and obese (≥30 kg/m^2)). Maternal weight was assessed in early-, mid- and late-pregnancy. Early-, mid- and late-gestational weight gain were defined as: start of pregnancy until 13 weeks of gestation (median 13.4 wks, 95% range 9.9-18.9); from 13 until 26 weeks of gestation (median 29.9 wks, 95% range 20.5, 31.4); from 26 until 40 weeks of gestation (median 39.0 wks, 95% range 32.8, 42.0), respectively. These periods were defined based on data collection within our study cohort. When we used narrower ranges to define specific periods of gestational weight gain, conclusions were similar (results not shown). Gestational weight gain until a gestational age of 30 weeks (median 30.2, 95% range 28.5, 32.8) was measured and available for 5678 mothers. Information about maximum weight during pregnancy was assessed by questionnaire 2 months after delivery in a subgroup of 3118 mothers. Maximum weight from questionnaire and weight measured at 30 weeks were strongly correlated (r = 0.88 (P-value <0.001)). Among this subgroup of mothers, we defined excessive gestational weight gain in relation to maternal prepregnancy body mass index according to the Institute of Medicine (IOM) guidelines (for underweight and normal weight mothers: total weight gain >16 kg; for overweight mothers: total weight gain >11.5 kg; for obese mothers: total weight gain >9 kg).[5]

Childhood body fat and cardio-metabolic outcomes

All children were invited to participate in detailed body fat and cardio-metabolic follow-up measurements at the age of 6 years.

We measured height and weight without shoes and heavy clothing and calculated body mass index. Childhood underweight, normal weight, overweight and obesity were defined by the International Obesity Task Force cut offs.[10] Body fat was measured by Dual-Energy X-ray absorptiometry (DXA) (iDXA, General Electrics–Lunar, 2008, Madison, WI, USA).[11] Total fat mass was calculated as percentage of total body weight measured by DXA. We calculated the android/gynoid fat mass ratio. As described previously, we performed abdominal ultrasound examinations to measure preperitoneal and subcutaneous abdominal fat thicknesses.[12] Preperitoneal and subcutaneous fat mass areas were measured as areas of 2 cm length along the midline starting from the reference point in direction of the navel.

Systolic and diastolic blood pressure was measured at the right brachial artery, four times with one-minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus TM (Paramus, NJ, USA).[13] We calculated mean systolic and diastolic blood pressure values using the last three blood pressure measurements.

We obtained thirty-minutes fasting venous blood samples and measured total-cholesterol, Low-Density Lipoprotein (LDL)-cholesterol, High-Density Lipoprotein (HDL)-cholesterol, triglycerides, insulin and C-peptide levels.

In line with previous definitions used among paediatric populations to define childhood metabolic-syndrome-like-phenotype,[14] we defined clustering of cardio-metabolic risk factors as having any of the 3 or more following components: android fat mass percentage ≥75th percentile; systolic or diastolic blood pressure ≥75th percentile; HDL-cholesterol ≤25th percentile or triglycerides ≥75th percentile; and insulin level ≥75th percentile. We used android fat mass as percentage of total body fat mass as proxy for waist circumference since waist circumference was not available.

Covariates

Maternal age was assessed at intake.[9] Information on maternal education level, ethnicity, folic acid supplementation use, smoking and alcohol consumption was assessed by questionnaires during pregnancy. First-trimester nutritional intake was obtained by food frequency questionnaire. We used medical records to collect information about pregnancy complications and mode of delivery.[15] Information about childhood sex, gestational age, weight and length at birth was available.[16,17] Infant growth was measured at community health centers according to standardized procedures at 24 months. Information about breastfeeding, timing of introduction of solid foods and average television watching time was obtained by questionnaires.

Statistical analysis

First, since maternal weight measurements throughout pregnancy are strongly correlated, we performed conditional regression analyses to explore the independent associations of maternal prepregnancy weight and weight gain in each pregnancy period, taking account for their correlations, with childhood outcomes (Correlation coefficients between maternal gestational weight measures shown in **Supplementary Table S2.7.1**).[18] We constructed maternal weight gain variables for each period, which are statistically independent from each other, by using standardized residuals obtained from regression of maternal weight at a specific time point on prior maternal weight measurements. Second, we examined associations of maternal gestational weight gain in early-,mid- and late-pregnancy with childhood outcomes separately and the role of potential mediators using linear regression models. For these analyses, we used 4 linear regression models; (1) a basic model including child's age and sex; (2) a confounder model, which additionally included covariates selected on their associations with the outcomes of interest or a change in effect estimate of >10%. We included childhood height as covariate in all models focused on fat mass outcomes; (3) an intermediate model, which additionally included potential intermediates (maternal pregnancy complications, gestational age and weight at birth, infant growth from birth until 2 years of age, and current childhood body mass index); and (4) a fully adjusted model including all confounders

and potential mediators. The confounder model was considered as main model. Third, we examined the associations of total and excessive gestational weight gain according to the IOM criteria with childhood outcomes using linear regression models. Finally, we examined the associations of specific periods of gestational weight gain and excessive gestational weight gain with the risks of childhood overweight and clustering of cardio-metabolic risk factors using logistic regression models. For all analyses, not normally distributed childhood outcome variables were log-transformed. We constructed standard deviation scores (SDS) values ((observed value - mean)/SD) for gestational weight gain variables and childhood outcomes to enable comparison of effect estimates. We examined potential interactions between maternal prepregnancy body mass index and gestational weight gain in each period and total gestational weight gain. We also explored potential interactions of gestational weight gain with sex, ethnicity, gestational-age-adjusted birth weight and childhood body mass index for these associations. After taking multiple testing into account no significant interactions were present, and no further stratified analyses were performed. Missing data of maternal weight variables (for conditional analyses only) and covariates were imputed using multiple imputation. Sensitivity analyses among mothers with all three weight measurements available were performed. All analyses were performed using Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Table 2.7.1 shows participants characteristics. Correlation coefficients between maternal gestational weight measures, birth weight and childhood cardio-metabolic outcomes are shown in Supplementary Table S2.7.1. Supplementary Table S2.7.2 and S2.7.3 show that mothers without maximum gestational weight gain and childhood follow-up data available were more often lower educated and from non-European descent.

Gestational weight gain in different periods of pregnancy

Figure 2.7.1 shows the independent associations of maternal prepregnancy weight and gestational weight gain in early-, mid- and late-pregnancy with childhood outcomes from conditional analyses. Maternal prepregnancy weight was associated with childhood body mass index, body fat distribution measures, systolic blood pressure, HDL-cholesterol and insulin and C-peptide levels (all P-values <0.05 in confounder model). The associations of maternal prepregnancy weight with childhood outcomes were stronger than associations of maternal gestational weight gain. Independent from maternal prepregnancy weight and weight gain in other periods, higher maternal gestational weight gain in early-pregnancy was associated with higher levels of childhood

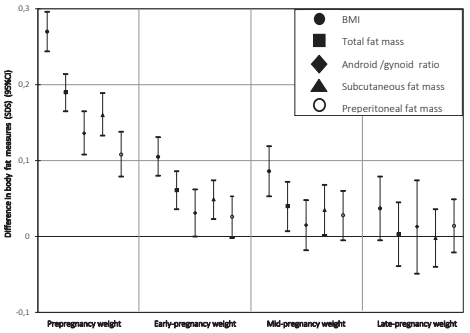
body mass index, total fat mass, android/gynoid fat mass ratio, abdominal subcutaneous fat mass and systolic blood pressure (all P-values <0.05).

Table 2.7.1. Characteristics of mothers and their children (*n = 5908*)[1]

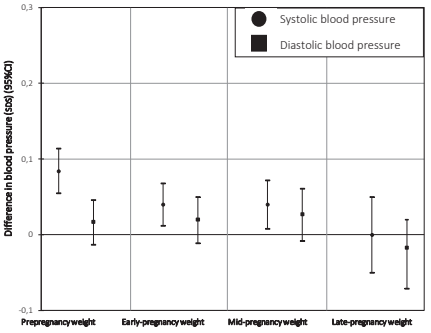
Characteristics	Value
Maternal Characteristics	
Age, mean (SD), years	30.3 (5.1)
Height, mean (SD), cm	167.5 (7.4)
Weight, mean (SD), kg	66.5 (12.6)
Prepregnancy body mass index, mean (SD), kg/m^2	23.6 (4.3)
Maximum weight gain, mean (SD), kg	14.9 (5.8)
Weight gain in early pregnancy, mean (SD), kg/week	0.17 (0.16)
Weight gain in mid-pregnancy, mean (SD), kg/week	0.49 (0.24)
Weight gain in late-pregnancy, mean (SD), kg/week	0.55 (0.39)
Excessive gestational weight gain (IOM criteria), No.(%)	1206 (45.7)
Gestational age at intake, median (95% range), weeks	13.9 (9.9, 24.4)
Education, No. Higher (%)	2435 (45.8)
Ethnicity, No. Dutch or European (%)	3501 (60.6)
Parity, No. Nulliparous (%)	3350 (56.7)
Total energy intake, mean (SD), kcal	2047 (558)
Folic acid supplement use, No. Yes (%)	1937 (43.1)
Smoking during pregnancy, No. Yes (%)	1369 (26.1)
Alcohol consumption during pregnancy, No. Yes (%)	2797 (54.0)
Maternal pregnancy complications	
Gestational hypertension, No. (%)	233 (4.1)
Pre-eclampsia, No. (%)	106 (1.9)
Gestational diabetes, No. (%)	59 (1.0)
Birth and infant characteristics	
Males, No. (%)	2949 (49.9)
Gestational age at birth, median (95% range), weeks	40.1 (35.9, 42.3)
Birth weight, mean (SD), g	3426 (550)
Caesarean delivery, No. (%)	655 (12.2)
Ever breastfeeding, Yes, No. (%)	4283 (92.6)
Breastfeeding duration, mean (SD), months	3.5 (0.5, 12.0)
Introduction of solid foods, No. Before 6 months (%)	3198 (89.6)
Television watching, No. More than 2 hours/day (%)	902 (19.8)
Childhood characteristics	
Age at follow up, median (95% range), years	6.0 (5.6, 8.0)
Height, mean (SD), cm	119.5 (6.1)
Weight, mean (SD), kg	23.4 (4.3)
Body mass index, mean (SD), kg/m^2	16.3 (1.9)
Overweight or obesity, No. (%)	1075 (18.2)
Total fat mass, mean (SD), %	25.1 (5.7)
Android/gynoid fat mass ratio, mean (SD)	0.25 (0.07)
Abdominal subcutaneous fat mass area, median (95% range), cm^2	0.49 (0.18, 1.94)
Abdominal preperitoneal fat mass area, median (95% range), cm^2	0.40 (0.16, 1.21)
Systolic blood pressure, mean (SD), mmHg	102.9 (8.2)
Diastolic blood pressure, mean (SD), mmHg	60.8 (6.9)
Total-cholesterol, mean (SD), mmol/L	4.2 (0.6)
HDL– cholesterol, mean (SD), mmol/L	1.3 (0.3)
LDL – cholesterol, mean (SD), mmol/L	2.4 (0.6)
Triglycerides, median (95% range), mmol/L	0.9 (0.4, 2.4)
Insulin, median (95% range), pmol/L	113.9 (16.9, 403.2)
C-peptide, median (95% range), nmol/L	1.0 (0.3, 2.1)
Cardio-metabolic risk factor clustering, No. (%)	384 (10.4)

[1]Values represent means (SD), median (95% range) or number of subjects (valid %).

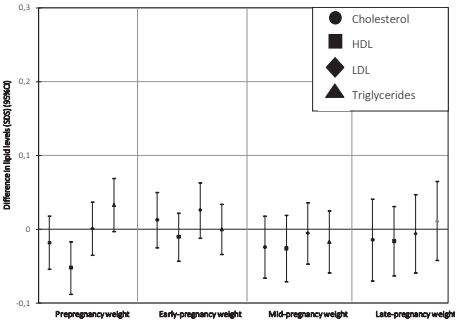
Figure 2.7.1. Associations of maternal prepregnancy weight and weight gain in each period of pregnancy with childhood cardio-metabolic outcomes from conditional analyses (*n = 5735*)



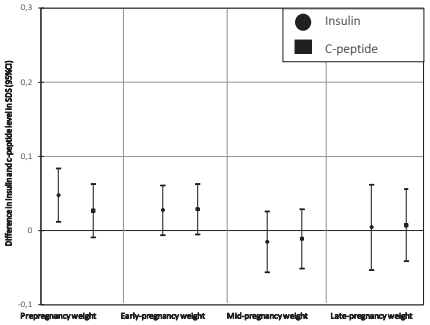
2.7.1.a. Childhood body fat mass measures



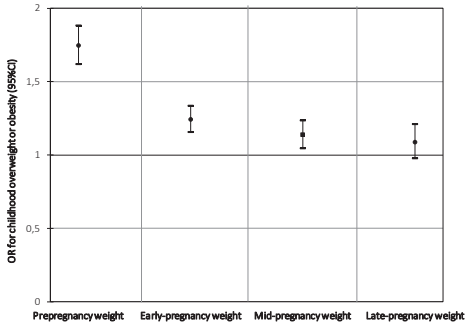
2.7.1b. Childhood blood pressure



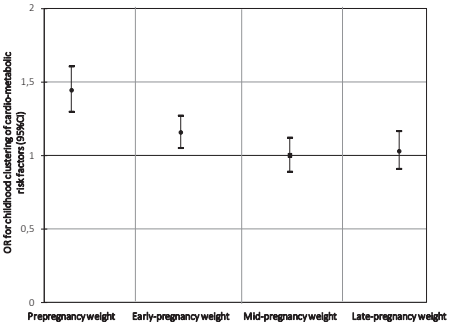
2.7.1.c. Childhood lipid levels



2.7.1.d. Childhood insulin and C-peptide



2.7.1.e. Childhood overweight



2.7.1.f. Childhood clustering of cardio-metabolic risk factors

Values are regression coefficients (95% Confidence Interval) from linear and logistic regression models that reflect the difference in childhood outcomes per SDS change in maternal prepregnancy weight and per SDS change in standardised residual change in maternal weight in early, mid and late-pregnancy from conditional regression analyses. Models were adjusted for child's sex and age at outcome measurements, maternal age, educational level, ethnicity, parity, height at intake, smoking and alcohol consumption during pregnancy and folic acid supplement use, total calorie intake during pregnancy, delivery mode, breastfeeding duration, timing of introduction of solid foods and average duration of tv-watching. Models focused on fat mass outcomes were also adjusted for childhood height. Models additionally adjusted for potential intermediates (pregnancy complications, birth characteristics, childhood size) are shown in the **Supplementary Tables S2.7.4** and **S2.7.5.**

Associations of maternal gestational weight gain in early-pregnancy with childhood abdominal preperitoneal fat mass, insulin and C-peptide were of borderline significance. Higher maternal gestational weight gain in mid-pregnancy was independently associated with higher childhood body mass index, total fat mass and abdominal subcutaneous fat mass and systolic blood pressure (all P-values <0.05). No independent associations were present for maternal gestational weight gain in late-pregnancy. Only maternal prepregnancy weight and gestational weight gain in early-pregnancy were independently associated with the risks of childhood overweight and clustering of cardio-metabolic risk factors (all P-values <0.05) (Figures 2.7.1E-F). When we restricted analyses to mothers with all three gestational weight measurements available, findings were similar (results not shown).

Role of maternal pregnancy complications, birth outcomes and childhood size

Table 2.7.2 shows that associations of maternal gestational weight gain in early-pregnancy with childhood body mass index, total fat mass, android/gynoid fat mass ratio and abdominal subcutaneous fat mass were not explained by pregnancy complications, gestational age and weight at birth or infant growth. The associations of maternal gestational weight gain in early-pregnancy with childhood fat mass outcomes attenuated towards non-significant after adjustment for childhood body mass index. The association of maternal gestational weight gain in mid-pregnancy with offspring body mass index was partly explained by birth characteristics. Maternal gestational weight gain in late-pregnancy was associated with childhood body mass index only, but this association was fully explained by birth characteristics.

Table 2.7.3 shows that specific periods of maternal gestational weight gain were not significantly associated with childhood cardio-metabolic outcomes. Maternal gestational weight gain in early- and mid-pregnancy tended to be associated with childhood systolic blood pressure, but this association was explained by childhood body mass index. Results for conditional weight gain models additionally adjusted for potential intermediates were similar and are given in Supplementary Table S2.7.4 and S2.7.5. Associations of total gestational weight gain with childhood cardio-metabolic outcomes are shown in Supplementary Table S2.7.6. Total gestational weight gain was associated with offspring body mass index only.

Figure 2.7.2A shows that higher gestational weight gain in early- and mid-, but not late-pregnancy, were associated with increased risks of childhood overweight (OR 1.19 (95% CI: 1.10, 1.29) and OR 1.09 (95% CI: 1.01, 1.18), per SD increase in early- and mid-gestational weight gain, respectively). The association for gestational weight gain in mid-pregnancy was explained by birth characteristics. Only higher gestational weight gain in early-pregnancy was associated with an increased risk of childhood clustering of cardio-metabolic risk factors (OR 1.20 (95% CI: 1.07, 1.35) per SD increase, respectively). This association was not explained by potential intermediates (Figure 2.7.2B). Figures 2.7.2A and 2.7.2B also show that children from mothers with excessive gestational weight gain had increased risks of childhood overweight (OR 1.54 (95% CI: 1.22, 1.96))

and clustering of cardio-metabolic risk factors (OR 1.68 (95% CI: 1.17, 2.41)), independent of potential intermediates. Associations of excessive gestational weight gain with separate childhood cardio-metabolic outcomes are shown in Supplementary Table S2.7.7.

Table 2.7.2. Weight gain in early-, mid- and late-pregnancy and childhood body composition

	BMI (SDS)	Total fat mass (SDS)	Android/gynoid fat mass ratio (SDS)	Subcutaneous abdominal fat area (SDS)	Preperitoneal abdominal fat area (SDS)
Early-pregnancy					
Basic model[1]	0.07 (0.04, 0.10)*	0.05 (0.02, 0.08)*	0.05 (0.02, 0.08)*	0.04 (0.01, 0.07)*	0.03 (-0.01, 0.06)
Confounder model[2]	0.09 (0.06, 0.12)*	0.05 (0.03, 0.08)*	0.05 (0.02, 0.08)*	0.05 (0.02, 0.08)*	0.03 (0, 0.06)
Mediator models[3]					
Pregnancy complications	0.09 (0.06, 0.12)*	0.05 (0.03, 0.08)*	0.05 (0.02, 0.08)*	0.05 (0.02, 0.08)*	0.03 (0, 0.06)
Birth characteristics	0.08 (0.05, 0.11)*	0.06 (0.03, 0.08)*	0.05 (0.02, 0.08)*	0.05 (0.02, 0.08)*	0.03 (-0.01, 0.06)
Infant growth	0.09 (0.07, 0.12)*	0.06 (0.03, 0.08)*	0.06 (0.03, 0.09)*	0.05 (0.02, 0.08)*	0.03 (0, 0.06)
Childhood BMI	-	0 (-0.02, 0.02)	0 (-0.02, 0.03)	0 (-0.02, 0.02)	-0.01 (-0.04, 0.02)
Fully adjusted model[4]	0.06 (0.04, 0.09)*	0 (-0.01, 0.02)	0.01 (-0.02, 0.03)	0 (-0.02, 0.03)	-0.01 (-0.04, 0.02)
Mid-pregnancy					
Basic model[1]	0.02 (-0.01, 0.05)	-0.02 (-0.04, 0.01)	-0.01 (-0.04, 0.02)	-0.01 (-0.04, 0.02)	0 (-0.03, 0.03)
Confounder model[2]	0.07 (0.04, 0.09)*	0.03 (0, 0.05)*	0.01 (-0.02, 0.04)	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)
Mediator models[3]					
Pregnancy complications	0.07 (0.04, 0.09)*	0.03 (0, 0.05)*	0.01 (-0.02, 0.04)	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)
Birth characteristics	0.04 (0.02, 0.07)*	0.03 (0, 0.05)*	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)
Infant growth	0.08 (0.06, 0.11)*	0.04 (0.01, 0.06)*	0.02 (-0.01, 0.05)	0.03 (0, 0.06)*	0.02 (-0.01, 0.05)
Childhood BMI	-	-0.01 (-0.03, 0.01)	-0.02 (0.05, 0)	-0.01 (-0.03, 0.01)	-0.01 (-0.04, 0.02)
Fully adjusted model[4]	0.03 (0.01, 0.05)*	0 (-0.02, 0.02)	-0.01 (-0.03, 0.02)	0 (-0.02, 0.02)	0 (-0.03, 0.03)
Late-pregnancy					
Basic model[1]	0.02 (-0.01, 0.05)	0 (-0.03, 0.03)	0.02 (-0.01, 0.05)	-0.01 (-0.05, 0.02)	0 (-0.04, 0.03)
Confounder model[2]	0.03 (0, 0.06)*	0 (-0.03, 0.03)	0.02 (-0.01, 0.05)	-0.02 (-0.05, 0.02)	0 (-0.04, 0.03)
Mediator models[3]					
Pregnancy complications	0.03 (0, 0.06)*	0 (-0.03, 0.03)	0.02 (-0.02, 0.05)	-0.02 (-0.05, 0.02)	0 (-0.04, 0.03)
Birth characteristics	0.01 (-0.02, 0.05)	0 (-0.03, 0.03)	0.02 (-0.02, 0.05)	-0.02 (-0.05, 0.02)	0 (-0.04, 0.03)
Infant growth	0.05 (0.02, 0.08)*	0.01 (-0.02, 0.04)	0.02 (-0.01, 0.05)	-0.01 (-0.04, 0.03)	0.01 (-0.03, 0.04)
Childhood BMI	-	-0.01 (0.03, 0.01)	0 (-0.02, 0.03)	-0.02 (-0.05, 0)*	-0.01 (-0.04, 0.02)
Fully adjusted model[4]	0.01 (-0.02, 0.04)	-0.01 (-0.03, 0.02)	0.01 (-0.02, 0.04)	-0.02 (-0.05, 0.01)	0 (-0.04, 0.03)

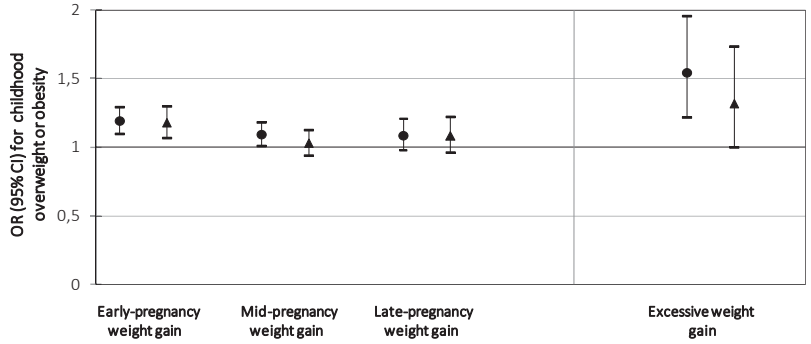
Values are regression coefficients (95% Confidence Interval) that reflect the difference in childhood outcomes per SDS change in gestational weight gain in early-, mid- and late-pregnancy. [1]Basic model is adjusted for child's sex and age at outcome measurements. [2]Confounder models include maternal age, educational level, ethnicity, prepregnancy body mass index, parity, smoking, alcohol consumption, folic acid supplement use, total calorie intake, delivery mode, breastfeeding, age at introduction of solid foods, and tv watching. Models for fat mass outcomes are additionally adjusted for childhood height. [3]Intermediate models are additionally adjusted for each potential intermediate. [4]Fully adjusted models include all potential confounders and intermediates. *P-value <0.05.

Table 2.7.3. Weight gain in early-, mid- and late-pregnancy and childhood cardio-metabolic outcomes

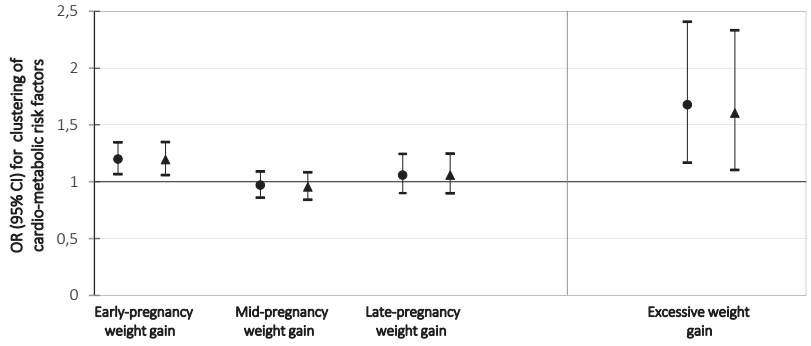
	Systolic blood pressure (SDS)	Diastolic blood pressure (SDS)	Total-cholesterol (SDS)	HDL-cholesterol (SDS)	LDL-cholesterol (SDS)	Triglycerides (SDS)	Insulin[5] (SDS)
Early-pregnancy							
Basic model[1]	0.02 (-0.01, 0.05)	0.01 (-0.02, 0.04)	0.02 (-0.02, 0.06)	0.01 (-0.03, 0.04)	0.03 (0, 0.07)	-0.01 (-0.05, 0.03)	0.02 (-0.02, 0.06)
Confounder model[2]	0.03 (0, 0.06)	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.05)	0 (-0.04, 0.04)	0.03 (-0.01, 0.07)	0 (-0.04, 0.04)	0.03 (-0.01, 0.06)
Mediator models[3]							
Pregnancy complications	0.03 (-0.01, 0.06)	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.05)	0 (-0.04, 0.04)	0.03 (-0.01, 0.07)	0 (-0.04, 0.03)	0.02 (-0.02, 0.06)
Birth characteristics	0.03 (0, 0.06)	0.01 (-0.02, 0.05)	0.02 (-0.02, 0.05)	0 (-0.04, 0.04)	0.03 (-0.01, 0.07)	0 (-0.04, 0.04)	0.03 (-0.01, 0.07)
Infant growth	0.03 (0, 0.06)	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.05)	0 (-0.04, 0.04)	0.03 (-0.01, 0.07)	0 (-0.04, 0.04)	0.03 (-0.01, 0.07)
Childhood BMI	0.01 (-0.02, 0.04)	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.04)	0.02 (-0.02, 0.06)	-0.02 (-0.05, 0.02)	0.01 (-0.03, 0.05)
Fully adjusted model[4]	0.01 (-0.02, 0.04)	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)	0.02 (-0.02, 0.06)	-0.02 (-0.05, 0.02)	0.01 (-0.03, 0.05)
Mid-pregnancy							
Basic model[1]	0.02 (-0.01, 0.05)	-0.01 (-0.04, 0.02)	-0.02 (-0.06, 0.01)	-0.02 (-0.06, 0.02)	-0.01 (-0.05, 0.02)	-0.01 (-0.05, 0.02)	-0.03 (-0.07, 0.01)
Confounder model[2]	0.02 (-0.01, 0.05)	0 (-0.03, 0.03)	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.01)	-0.01 (-0.04, 0.03)	-0.01 (-0.05, 0.03)	-0.03 (-0.07, 0.01)
Mediator models[3]							
Pregnancy complications	0.02 (-0.01, 0.05)	0 (-0.03, 0.03)	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.02)	-0.01 (-0.04, 0.03)	-0.01 (-0.05, 0.03)	-0.03 (-0.07, 0.01)
Birth characteristics	0.03 (0, 0.06)	0.01 (-0.03, 0.04)	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.01)	0 (-0.04, 0.04)	-0.01 (-0.05, 0.03)	-0.03 (-0.06, 0.01)
Infant growth	0.03 (0, 0.06)*	0.01 (-0.02, 0.04)	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.01)	0 (-0.04, 0.03)	-0.01 (-0.05, 0.03)	-0.02 (-0.06, 0.02)
Childhood BMI	0.01 (-0.02, 0.04)	-0.01 (-0.04, 0.02)	-0.03 (-0.06, 0.01)	-0.02 (-0.06, 0.02)	-0.01 (-0.05, 0.03)	-0.02 (-0.06, 0.02)	-0.04 (-0.08, 0)*
Fully adjusted model[4]	0.02 (-0.01, 0.04)	0 (-0.03, 0.03)	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.02)	-0.01 (-0.04, 0.03)	-0.02 (-0.05, 0.02)	-0.03 (-0.07, 0.01)
Late-pregnancy							
Basic model[1]	-0.01 (-0.04, 0.03)	-0.01 (-0.05, 0.03)	0 (-0.04, 0.05)	-0.03 (-0.07, -0.01)	0.03 (-0.02, 0.07)	0.01 (-0.03, 0.06)	0.01 (-0.03, 0.06)
Confounder model[2]	-0.01 (-0.04, 0.03)	-0.02 (-0.05, 0.02)	0 (-0.04, 0.05)	-0.02 (-0.07, 0.02)	0.02 (-0.03, 0.06)	0.01 (-0.03, 0.06)	0.01 (-0.04, 0.06)
Mediator models[3]							
Pregnancy complications	-0.01 (-0.05, 0.02)	-0.02 (-0.06, 0.01)	0 (-0.04, 0.05)	-0.02 (-0.07, 0.02)	0.02 (-0.03, 0.06)	0.02 (-0.03, 0.07)	0.01 (-0.04, 0.06)
Birth characteristics	-0.01 (-0.05, 0.03)	-0.02 (-0.05, 0.02)	0 (-0.05, 0.05)	-0.03 (-0.07, 0.02)	0.02 (-0.03, 0.06)	0.02 (-0.03, 0.07)	0.01 (-0.04, 0.06)
Infant growth	0 (-0.04, 0.04)	-0.01 (-0.05, 0.02)	0 (-0.04, 0.05)	-0.02 (-0.07, 0.02)	0.02 (-0.03, 0.06)	0.01 (-0.03, 0.06)	0.01 (-0.03, 0.06)
Childhood BMI	-0.01 (-0.05, 0.02)	-0.02 (-0.06, 0.02)	0 (-0.04, 0.05)	-0.02 (-0.07, 0.03)	0.02 (-0.03, 0.06)	0.01 (-0.03, 0.06)	0.01 (-0.04, 0.05)
Fully adjusted model[4]	-0.01 (-0.05, 0.02)	-0.02 (-0.06, 0.02)	0 (-0.04, 0.05)	-0.03 (-0.07, 0.02)	0.02 (-0.03, 0.06)	0.03 (-0.02, 0.07)	0.01 (-0.04, 0.06)

Values are regression coefficients (95% Confidence Interval) that reflect the difference in childhood outcomes per SDS change in gestational weight gain in early-, mid- and late-pregnancy. [1]Basic model is adjusted for child's sex and age at outcome measurements. [2]Confounder models include maternal age, educational level, ethnicity, prepregnancy body mass index, parity, smoking, alcohol consumption, folic acid supplement use, and total calorie intake, delivery mode, breastfeeding, age at introduction of solid foods, and tv watching. [3]Intermediate models are additionally adjusted for each potential intermediate. [4]Fully adjusted models include all potential confounders and intermediates. [5]Results for C-peptide were similar as for insulin levels (results not shown). *P-value <0.05.

Figure 2.7.2. Associations of gestational weight gain with the risk of childhood overweight and clustering of cardio-metabolic risk factors (*n = 5908*)



2.7.2a. Childhood overweight



2.7.2b. Childhood clustering of cardio-metabolic risk factors

Values are Odds Ratios (95% Confidence Interval) from logistic regression models that reflect the risks of childhood overweight and obesity and childhood clustering of cardio-metabolic risk factors per SDS change in early-, mid- and late-gestational weight gain, and for excessive gestational weight gain as compared to the reference group (non-excessive gestational weight gain). Confounder models (represented by circle) are adjusted for child's sex and age at outcome measurements, maternal age, educational level, ethnicity, prepregnancy body mass index (period-specific gestational weight gain models), parity, smoking and alcohol consumption during pregnancy and folic acid supplement use, total calorie intake during pregnancy, delivery mode, breastfeeding duration, timing of introduction of solid foods, and tv watching. Full models (represented by triangle) are additionally adjusted for pregnancy complications, gestational age and weight at birth, infant length and weight growth.

Discussion

We observed that higher maternal gestational weight gain in early-, but not in mid- and late-, pregnancy is associated with increased adiposity levels and an adverse cardio-metabolic profile in childhood. These associations were independent from maternal prepregnancy weight and weight gain in other periods, and not explained by pregnancy complications or birth and infant growth characteristics. The associations of weight gain later in pregnancy with childhood outcomes seem to be partly explained by birth characteristics.

Methodological considerations

Strengths of this study were the prospective data collection from early pregnancy onwards, large sample size and multiple maternal weight measurements throughout pregnancy. Follow-up data were available in 69% of our study population. The non-response could lead to biased effect estimates if associations would be different between mothers and children included and not included in the analyses. Assuming that mothers and children with higher weights are less likely to participate in detailed follow-up studies, our estimates may be underestimated. Furthermore, not all gestational weight measurements were available for all mothers due to later enrolment in the study or non-participation in physical examinations or questionnaires. To avoid bias related to a complete-case analysis and to maintain statistical power, we used multiple imputations for missing information of maternal weight measurements for conditional analysis.[19] Observed differences in significance between conditional and regular linear regression analyses are partly due to smaller numbers in regular analyses. Compared to the complete-case analysis, effect estimates changed slightly after using multiple imputations for missing values (results not shown), but conclusions were similar. Information on maternal prepregnancy weight and maximum gestational weight was self-reported. Self-reported weight tends to be underestimated especially in case of higher maternal weight, which might have led to an underestimation of observed effects for maximum gestational weight gain and an overestimation for gestational weight gain in early pregnancy. Finally, although information about a large number of potential confounding factors was available, because of the observational design, residual confounding might still be an issue.

Interpretation of main findings

Previous studies have shown that gestational weight gain, especially in mid- and late-pregnancy, is associated with birth weight.[20,21] In the same population as the present study, we previously observed that specific periods of gestational weight gain are associated with risks of adverse pregnancy outcomes.[21] In the current study, we aimed to identify critical periods of gestational weight gain for cardio-metabolic health in offspring. An accumulating body of evidence suggests that gestational weight gain might influence offspring cardio-metabolic health in later life.[4,6-8,22-28] The effects of gestational weight gain on childhood outcomes may depend upon timing of gestational weight gain. A study performed among 5154 UK mother-offspring pairs showed that gestational weight gain in the first 14 weeks tended to be incrementally associated with offspring BMI, waist circumference and fat mass at 9 years, but after 14 weeks of gestation, only high levels of gestational weight gain were associated with offspring adiposity measures.[4] No associations of trimester-specific weight gain with blood pressure were present, whereas weight gain from 14 to 36 weeks of gestation tended to be linearly associated with HDL-cholesterol, triglycerides and inflammatory markers. In these analyses, only maternal estimated prepregnancy weight and gestational weight gain in the

previous period were taken into account. Another study among 3015 US mothers and their children showed that only first-trimester weight gain was associated with childhood body mass index, and suggested that maternal prepregnancy body mass index modified this association.[7] These models were not adjusted for weight gain in other trimesters. A Finish study among 6637 mothers and their adolescent offspring showed that weight gain of >7 kg in the first 20 weeks of gestation was associated with offspring overweight and higher waist-circumference, but this study did not study whether these effects were independent from weight gain in later pregnancy.[8]

In line with these studies, we observed that maternal gestational weight gain in early- and mid-pregnancy was independently associated with childhood body mass index. Additionally, we observed associations of maternal gestational weight gain in early-pregnancy with childhood total fat mass, android/gynoid fat mass ratio and abdominal subcutaneous fat mass. Thus, our results suggest that higher maternal gestational weight gain in early-pregnancy leads to higher body mass index, higher total fat mass and relatively more abdominal fat mass in childhood. Next to body mass index, these specific total body and abdominal fat distribution measures are related to the risk of cardio-metabolic disease and mortality in later life.[29,30] We also observed that maternal gestational weight gain in early-pregnancy tended to be independently associated with childhood systolic blood pressure, insulin and C-peptide levels and clustering of cardio-metabolic risk factors, but no independent associations with lipid levels were present. The associations of maternal gestational weight gain in early-pregnancy with specific body fat mass measures and cardio-metabolic risk factors were largely mediated by childhood body mass index. Although observed effect estimates were small, these childhood cardio-metabolic risk factors track from childhood into adulthood and are associated with cardiovascular disease in later life.[31-34] The associations of maternal prepregnancy weight with childhood cardio-metabolic outcomes were stronger than those for gestational weight gain, but did not explain or modify the associations of gestational weight gain with these outcomes.

Increased total weight gain and excessive gestational weight gain according to IOM criteria have been associated with increased risks of offspring obesity, independent from maternal prepregnancy body mass index.[4,6,22-26,28,35] Associations of increased gestational weight gain with offspring blood pressure, lipid profile and inflammatory markers are less consistent and seem to be mainly driven by offspring adiposity.[4,23,25] Accordingly, we observed associations of total and excessive gestational weight gain with increased childhood adiposity levels and increased risks of childhood overweight. Total and excessive gestational weight gain were also associated with the risk of childhood clustering of cardio-metabolic risk factors, but no significant associations of these measures with separate cardio-metabolic risk factors were present. This may be due to lack of statistical power as a smaller number of mothers had information about total gestational weight gain available.

The associations of gestational weight gain and childhood adiposity and related cardio-metabolic outcomes may be explained by several factors. Mothers who gain a large amount of weight during pregnancy are likely to have different socio-demographic and

lifestyle characteristics as compared to mothers who gain recommended amounts of weight.[21] These factors may account for the observed effects. However, extensive adjustment for socio-demographic and lifestyle factors did not explain our findings. The observed effects of gestational weight gain in each pregnancy period were not mediated by maternal pregnancy complications or infant growth characteristics.[36-38] Weight gain during pregnancy, especially in later pregnancy, might also just reflect higher fetal weight and birth weight, which are known to be associated with obesity in later life.[39] In line with this hypothesis, we observed that associations of gestational weight gain later in pregnancy with childhood outcomes were partly explained by birth characteristics. Thus, the effects of gestational weight gain on childhood outcomes may vary during pregnancy, and our results suggest that especially early-pregnancy might be a specific and independent critical period for gestational weight gain. The mechanisms by which maternal gestational weight gain in early-pregnancy lead to an adverse childhood cardio-metabolic profile are not known, but may include increased placental transfer of maternal levels of glucose, free fatty acids and amino-acids and subsequent programming of adiposity and an adverse cardio-metabolic profile in later life.[40] Further mechanistic studies are needed to obtain further insight in the underlying mechanisms.

Conclusion

We observed that increased maternal weight gain in early pregnancy is associated with an adverse cardio-metabolic profile in childhood. This association is largely mediated by childhood body mass index. Future preventive strategies focused on reduction of excessive maternal weight gain, especially in early pregnancy, may lead to better cardio-metabolic health in offspring.

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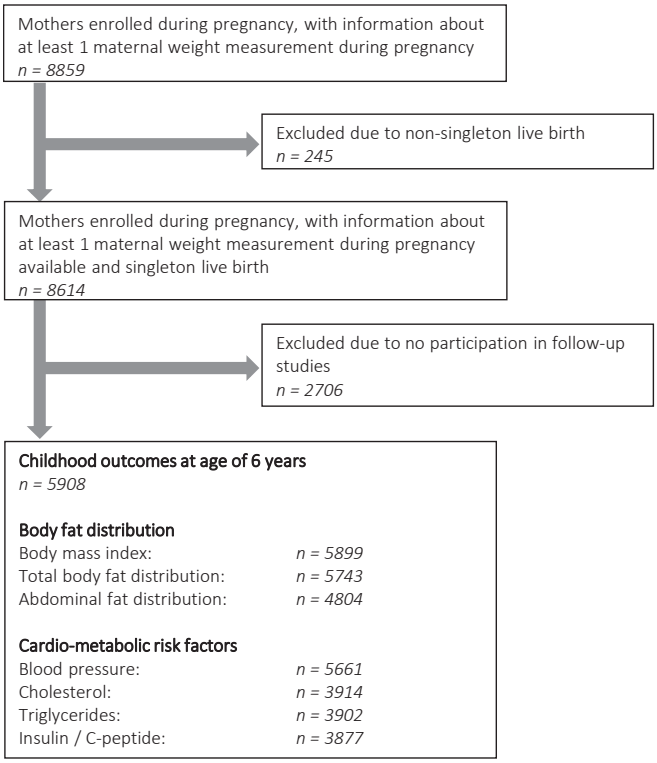
Supplementary Material

Supplementary Figure S2.7.1. Participants flow chart in the Generation R Study, Rotterdam, the Netherlands



Supplementary Table S2.7.1. Correlation coefficients between maternal and offspring weight and cardio-metabolic measures[1]

	Maternal measures					Offspring measures												
	PPW	Early-GWG	Mid-GWG	Late-GWG	Maximum GWG	Birth weight	BMI	TFM	AGFM	SFM	PFM	SBP	DBP	Cholesterol	HDL	LDL	TG	Insulin	C-peptide
Maternal measures																			
PPW	1	**0.96**	**0.92**	**0.90**	**0.89**	**0.21**	**0.24**	**0.18**	**0.12**	**0.12**	**0.08**	**0.08**	0.01	-0.02	**-0.05**	0.01	0.02	**0.04**	**0.04**
Early-GWG	**0.96**	1	**0.95**	**0.92**	**0.92**	**0.21**	**0.25**	**0.19**	**0.11**	**0.14**	**0.08**	**0.08**	0.02	0	**-0.06**	0.03	0.01	**0.05**	**0.04**
Mid-GWG	**0.92**	**0.95**	1	**0.95**	**0.95**	**0.25**	**0.26**	**0.18**	**0.11**	**0.13**	**0.09**	**0.09**	0.01	-0.02	**-0.07**	0.02	0.01	**0.05**	**0.05**
Late-GWG	**0.90**	**0.92**	**0.95**	1	**0.99**	**0.30**	**0.24**	**0.17**	**0.11**	**0.11**	**0.08**	**0.06**	0.02	-0.03	-0.04	0.01	-0.01	0.03	0.02
Maximum GWG	**0.89**	**0.92**	**0.95**	**0.99**	1	**0.29**	**0.23**	**0.16**	**0.11**	**0.10**	**0.07**	**0.07**	0.02	-0.02	-0.04	0.01	0	0.04	0.03
Offspring measures																			
Birth weight	**0.21**	**0.21**	**0.25**	**0.30**	**0.29**	1	**0.16**	**-0.05**	**-0.04**	-0.01	-0.02	**-0.05**	**-0.08**	**-0.05**	-0.02	**-0.04**	-0.01	0.02	0.01
BMI	**0.31**	**0.25**	**0.26**	**0.25**	**0.24**	**0.16**	1	**0.71**	**0.61**	**0.62**	**0.43**	**0.25**	**0.10**	**0.10**	**-0.04**	**0.09**	**0.06**	**0.13**	**0.09**
TFM	**0.26**	**0.19**	**0.18**	**0.17**	**0.17**	**-0.05**	**0.71**	1	**0.65**	**0.82**	**0.56**	**0.21**	**0.14**	**0.16**	**-0.08**	**0.17**	**0.08**	**0.09**	**0.06**
AGFM	**0.19**	**0.13**	**0.12**	**0.12**	**0.13**	**-0.04**	**0.61**	**0.65**	1	**0.57**	**0.40**	**0.16**	**0.08**	**0.09**	**-0.10**	**0.10**	**0.14**	**0.09**	**0.09**
SFM	**0.21**	**0.14**	**0.13**	**0.10**	**0.10**	-0.01	**0.62**	**0.82**	**0.57**	1	**0.66**	**0.20**	**0.11**	**0.14**	-0.03	**0.16**	**0.06**	**0.09**	**0.06**
PFM	**0.14**	**0.08**	**0.08**	**0.08**	**0.07**	-0.02	**0.43**	**0.56**	**0.40**	**0.66**	1	**0.14**	**0.07**	**0.16**	0.02	**0.14**	**0.08**	**0.09**	**0.06**
SBP	**0.08**	**0.08**	**0.09**	**0.06**	**0.07**	**-0.05**	**0.25**	**0.21**	**0.16**	**0.20**	**0.14**	1	**0.62**	**0.07**	**0.06**	**0.04**	-0.02	**0.08**	**0.04**
DBP	0.01	0.02	0.01	0.02	0.02	**-0.08**	**0.10**	**0.14**	**0.08**	**0.11**	**0.07**	**0.62**	1	**0.04**	**0.04**	**0.04**	-0.02	-0.02	**-0.05**
Cholesterol	-0.02	0	0.02	-0.03	-0.02	**-0.05**	**0.10**	**0.16**	**0.09**	**0.14**	**0.16**	**0.07**	**0.04**	1	**0.30**	**0.85**	**0.16**	-0.02	**-0.04**
HDL	**-0.05**	**-0.06**	**-0.07**	-0.04	-0.04	-0.02	**-0.04**	**-0.08**	**-0.10**	-0.03	0.02	**0.06**	**0.04**	**0.30**	1	**-0.05**	**-0.39**	-0.07	**-0.10**
LDL	0.01	0.03	0.02	0.01	0.01	**-0.04**	**0.09**	**0.17**	**0.10**	**0.16**	**0.14**	**0.04**	**0.04**	**0.85**	**-0.05**	1	**0.12**	**-0.05**	**-0.07**
TG	0.02	0.01	0.01	-0.01	0	-0.01	**0.06**	**0.08**	**0.14**	**0.06**	**0.08**	-0.02	-0.02	**0.16**	**-0.39**	**0.12**	1	**0.20**	**0.20**
Insulin	**0.04**	**0.05**	**0.05**	0.03	0.04	0.02	**0.13**	**0.09**	**0.09**	**0.09**	**0.09**	**0.08**	-0.02	-0.02	-0.07	**-0.05**	**0.20**	1	**0.88**
C-peptide	**0.04**	**0.04**	**0.05**	0.02	0.03	0.01	**0.09**	**0.06**	**0.09**	**0.06**	**0.06**	**0.04**	**-0.05**	**-0.04**	**-0.10**	**-0.07**	**0.20**	**0.88**	1

[1]Values are Pearson's or Spearman rank correlation coefficients. Bold values are significant. Abbreviations: PPW: prepregnancy weight; GWG: gestatioanl weight gain; TFM: total fat mass; AGFM: android/gynoid fat mass ratio; SFM: abdominal subcutaenous fat mass; PFM: abdominal preperitoneal fat mass; SBP: systolic blood pressure; DBP: diastolic blood pressure; TG: triglycerides.

Supplementary Table S2.7.2. Non-response analysis for availability of maximum weight data from questionnaire (*n = 5908*)[1]

	Information about maximum gestational weight gain *n = 3118*	No information about maximum gestational weight gain *n = 2790*	P-value[4]
Maternal characteristics			
Age, mean (SD), years	31.2 (4.6)	29.2 (5.4)	<0.01
Height, mean (SD), cm	168.8 (7.2)	166.1 (7.4)	<0.01
Prepregnancy weight, mean (SD), kg	66.4 (11.9)	66.5 (13.4)	0.77
Prepregnancy body mass index, mean (SD), kg/m^2	23.3 (3.9)	24.0 (4.5)	<0.01
Gestational age at intake, median (95% range), weeks[2]	13.5 (9.9, 23.6)	14.5 (10.1, 25.1)	<0.01
Parity, No. nulliparous (%)[3]	1872 (60.0)	1478 (53.0)	<0.01
Education, No. higher education (%)[3]	1766 (58.2)	768 (30.8)	<0.01
Race / Ethnicity, No. European (%)[3]	2249 (72.5)	1252 (46.8)	<0.01
Smoking habits during pregnancy, No. Yes (%)[3]	664 (23.5)	705 (29.2)	<0.01
Alcohol consumption during pregnancy, No. Yes (%)[3]	1737 (62.1)	1060 (44.5)	<0.01
Folic acid supplement use, No. (%)[3]			
No use	408 (16.6)	722 (35.5)	<0.01
First 10 weeks use	798 (32.5)	624 (30.7)	
Preconception use	1251 (50.9)	686 (33.8)	
Maternal pregnancy complications			
Gestational hypertension, No. (%)[3]	129 (4.3)	104 (3.7)	0.28
Pre-eclampsia, No. (%)[3]	57 (2.0)	49 (1.9)	0.49
Gestational diabetes, No. (%)[3]	21 (0.7)	38 (1.4)	<0.01
Birth and infant characteristics			
Males, No. (%)[3]	1545 (49.6)	1404 (50.3)	0.29
Gestational age at birth, median (95% range), weeks[2]	40.1 (36.1, 42.3)	40.1 (35.4, 42.3)	<0.01
Birth weight, mean (SD), g	3465 (524)	3384 (576)	<0.01
Caesarean delivery, No. (%)[3]	352 (12.2)	303 (12.1)	0.47
Ever breastfeeding, No. Yes (%)[3]	2894 (92.8)	1389 (92.2)	0.23
Breastfeeding duration, median (95% range), months[2]	3.5 (0.5, 12.0)	3.5 (0.5, 12.0)	0.11
Childhood characteristics			
Age at follow up, median (95% range), years[2]	6.0 (5.9, 7.1)	6.2 (5.7, 8.2)	<0.01
Height, mean (SD), cm	118.7 (5.5)	120.4 (6.6)	<0.01
Weight, mean (SD), kg	22.6 (3.5)	24.2 (5.0)	<0.01
Body mass index, mean (SD), kg/m^2	16.0 (1.6)	16.6 (2.2)	<0.01
Total fat mass, mean (SD), %	24.3 (5.1)	25.8 (6.3)	<0.01
Android/gynoid fat mass ratio, mean (SD)	0.25 (0.06)	0.26 (0.08)	<0.01
Systolic blood pressure, mean (SD), mmHg	102.2 (8.0)	103.6 (8.4)	<0.01
Diastolic blood pressure, mean (SD), mmHg	60.4 (6.7)	61.2 (7.0)	<0.01
Total-cholesterol, mean (SD), mmol/L	4.2 (0.6)	4.2 (0.6)	0.47
HDL-cholesterol, mean (SD), mmol/L	1.3 (0.3)	1.4 (0.3)	<0.01
LDL-cholesterol, mean (SD), mmol/L	2.4 (0.6)	2.3 (0.6)	0.15
Triglycerides, median (95% range), mmol/L[2]	0.9 (0.4, 2.3)	0.9 (0.4, 2.4)	0.59
Insulin, median (95% range), pmol/L[2]	117.9 (17.7, 408.4)	109.1 (15.6, 397.3)	0.39
C-peptide, median (95% range), nmol/L[2]	1.0 (0.3, 2.1)	0.9 (0.3, 2.2)	0.14

[1]Values are means (standard deviation). [2]Values are medians (95% range). [3]Values are observed numbers and valid percentages. [4]Differences in subject characteristics between the groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.

Supplementary Table S2.7.3. Non-response analysis for childhood follow-up data at 6 years (*n = 8614*)[1]

	Follow-up at 6 years *n = 5908*	Loss to follow-up at 6 years *n = 2706*	P-value[4]
Maternal characteristics			
Age, mean (SD), years	30.3 (5.1)	28.2 (5.5)	<0.01
Height, mean (SD), cm	167.5 (7.4)	166.2 (7.4)	<0.01
Prepregnancy weight, mean (SD), kg	66.5 (12.6)	65.8 (13.5)	0.05
Prepregnancy body mass index, mean (SD), kg/m^2	23.6 (4.2)	23.7 (4.7)	0.38
Gestational age at intake, median (95% range), weeks[2]	13.9 (9.9, 24.4)	14.5 (9.9, 27.8)	<0.01
Parity, No. nulliparous, (%)[3]	3350 (56.7)	1388 (51.3)	<0.01
Education, No. higher education (%)[3]	2534 (45.8)	750 (32.5)	<0.01
Race / Ethnicity, No. European (%)[3]	3501 (60.6)	1130 (47.8)	<0.01
Smoking habits during pregnancy, No. Yes (%)[3]	1369 (26.1)	679 (29.8)	<0.01
Alcohol consumption during pregnancy, No. Yes (%)[3]	2797 (54.0)	933 (41.6)	<0.01
Folic acid supplement use, No. (%)[3]			
No use	1130 (25.2)	745 (39.4)	<0.01
First 10 weeks use	1422 (31.7)	563 (29.8)	
Preconception use	1937 (43.1)	582 (30.8)	
Maternal pregnancy complications			
Gestational hypertension, No. (%)[3]	233 (4.1)	76 (3.1)	0.01
Pre-eclampsia, No. (%)[3]	106 (1.9)	66 (2.7)	0.02
Gestational diabetes, No. (%)[3]	59 (1.0)	30 (1.2)	0.32
Birth and infant characteristics			
Males, No. (%)[3]	2949 (49.9)	1401 (51.8)	0.06
Gestational age, median (95% range), weeks[2]	40.1 (35.9, 42.3)	40.0 (34.7, 42.4)	<0.01
Birth weight, mean (SD), g	3426 (550)	3377 (583)	<0.01
Caesarean delivery, No. (%)[3]	655 (12.2)	310 (12.9)	0.20
Ever breastfeeding, No. Yes (%)[3]	4283 (92.6)	1267 (90.5)	<0.01
Breastfeeding duration, median (95% range), months[2]	3.5 (0.5, 12.0)	2.5 (0.5, 12.0)	<0.01

[1]Values are means (standard deviation). [2]Median (95% range). [3]Values are observed numbers and valid percentages. [4]Differences in subject characteristics between the groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.

Supplementary Table S2.7.4. Associations of prepregnancy weight and weight gain in each period of pregnancy with childhood body fat outcomes from conditional analyses (intermediate and full models)[1,2]

	Body mass index (SDS)	**Total fat mass** (SDS)	**Android/gynoid fat mass ratio** (SDS)	**Subcutaneous abdominal fat area** (SDS)	**Preperitoneal abdominal fat area** (SDS)
Prepregnancy weight					
Mediator models[3]					
Pregnancy complications	0.27 (0.25, 0.30)*	0.19 (0.17, 0.22)*	0.14 (0.11, 0.16)*	0.17 (0.14, 0.19)*	0.11 (0.08, 0.14)*
Birth characteristics	0.24 (0.21, 0.27)*	0.19 (0.17, 0.22)*	0.14 (0.11, 0.17)*	0.16 (0.13, 0.19)*	0.11 (0.08, 0.14)*
Infant growth	0.27 (0.24, 0.30)*	0.19 (0.16, 0.22)*	0.14 (0.11, 0.17)*	0.16 (0.13, 0.19)*	0.11 (0.08, 0.14)*
Childhood BMI	-	0.01 (-0.01, 0.03)	-0.03 (-0.05, -0.01)*	0 (-0.02, 0.02)	-0.01 (-0.04, 0.02)
Fully adjusted model[4]	0.18 (0.15, 0.21)*	0.02 (0, 0.04)*	-0.02 (-0.05, 0)	0.01 (-0.01, 0.03)	0 (-0.03, 0.02)
Early-pregnancy weight					
Mediator models[3]					
Pregnancy complications	0.11 (0.08, 0.13)*	0.06 (0.04, 0.09)*	0.03 (0, 0.06)*	0.05 (0.02, 0.08)*	0.03 (0, 0.05)
Birth characteristics	0.09 (0.07, 0.12)*	0.06 (0.04, 0.09)*	0.03 (0, 0.07)*	0.05 (0.02, 0.07)*	0.03 (0, 0.05)
Infant growth	0.11 (0.09, 0.13)*	0.07 (0.04, 0.09)*	0.04 (0.01, 0.06)*	0.05 (0.03, 0.08)*	0.03 (0, 0.06)*
Childhood BMI	-	-0.01 (-0.02, 0.01)	-0.03 (-0.06, -0.01)*	-0.01 (-0.03, 0.01)	-0.02 (-0.04, 0.01)
Fully adjusted model[4]	0.07 (0.05, 0.09)*	0 (-0.02, 0.02)	-0.03 (-0.05, 0)	0 (-0.02, 0.02)	-0.01 (-0.04, 0.01)
Mid-pregnancy weight					
Mediator models[3]					
Pregnancy complications	0.09 (0.05, 0.12)*	0.04 (0.01, 0.07)*	0.01 (-0.02, 0.05)	0.04 (0, 0.07)*	0.03 (0, 0.06)
Birth characteristics	0.06 (0.03, 0.09)*	0.04 (0.01, 0.08)*	0.02 (-0.01, 0.05)	0.03 (0, 0.07)*	0.03 (0, 0.06)
Infant growth	0.10 (0.07, 0.13)	0.05 (0.02, 0.08)*	0.02 (-0.01, 0.06)	0.04 (0.01, 0.08)*	0.03 (0, 0.07)
Childhood BMI	-	-0.01 (-0.03, 0.01)	-0.03 (-0.06, 0)	-0.01 (-0.03, 0.02)	0 (-0.03, 0.03)
Fully adjusted model[4]	0.04 (0.02, 0.07)*	0.01 (-0.02, 0.03)	-0.02 (-0.04, 0.01)	0 (-0.02, 0.03)	0.01 (-0.03, 0.04)
Late-pregnancy weight					
Mediator models[3]					
Pregnancy complications	0.04 (0, 0.08)	0 (-0.04, 0.05)	0.01 (-0.05, 0.07)	0 (-0.04, 0.04)	0.02 (-0.02, 0.05)
Birth characteristics	0.02 (-0.03, 0.06)	0.01 (-0.04, 0.05)	0.02 (-0.05, 0.08)	0 (-0.04, 0.04)	0.02 (-0.02, 0.05)
Infant growth	0.06 (0.03, 0.10)	0.02 (-0.02, 0.05)	0.03 (-0.03, 0.08)	0.01 (-0.02, 0.05)	0.03 (-0.01, 0.06)
Childhood BMI	-	-0.01 (-0.04, 0.01)	0 (-0.04, 0.04)	-0.02 (-0.05, 0.01)	0 (-0.03, 0.03)
Fully adjusted model[4]	0.02 (-0.01, 0.06)	-0.01 (-0.03, 0.02)	0 (-0.04, 0.04)	-0.01 (-0.04, 0.02)	0.01 (-0.03, 0.04)

[1]Values are regression coefficients (95% Confidence Interval) from linear regression models that reflect the difference in childhood body fat outcomes per SDS change in maternal pregnancy weight and per SDS change in standardised residual change in maternal weight in early-, mid- and late-pregnancy from conditional regression analyses. Estimates are based on multiple imputed data. [2]Models are adjusted for child's sex and age at outcome measurements, maternal age, educational level, ethnicity, parity, height at intake, smoking during pregnancy, alcohol consumption during pregnancy and folic acid supplement use, total calorie intake during pregnancy, delivery mode, breastfeeding duration, timing of introduction of solid foods and average duration of tv-watching. Models focused on fat mass outcomes were also adjusted for childhood height. [3]Intermediate models are additionally adjusted for each potential intermediate. [4]Fully adjusted models include all potential confounders and intermediates. *P-value <0.05.

Supplementary Table S2.7.5. Associations of prepregnancy weight and weight gain in each period of pregnancy with childhood cardio-metabolic outcomes from conditional analyses (intermediate and full models)[1,2]

	Systolic blood pressure (SDS)	**Diastolic blood pressure** (SDS)	**Total-cholesterol** (SDS)	**HDL-cholesterol** (SDS)	**LDL-cholesterol** (SDS)	**Triglycerides** (SDS)	**Insulin** (SDS)	**C-peptide** (SDS)
Prepregnancy weight								
Mediator models[3]								
Pregnancy complications	0.08 (0.05, 0.11)*	0.01 (-0.02, 0.04)	-0.02 (-0.05, 0.02)	-0.05 (-0.08, -0.02)*	0 (-0.04, 0.04)	0.04 (0, 0.07)	0.05 (0.01, 0.08)*	0.02 (-0.01, 0.06)
Birth characteristics	0.09 (0.06, 0.12)*	0.03 (0, 0.06)	-0.02 (-0.05, 0.02)	-0.05 (-0.09, -0.02)*	0 (-0.03, 0.04)	0.04 (0, 0.08)*	0.06 (0.02, 0.09)*	0.04 (0, 0.07)
Infant growth	0.09 (0.06, 0.12)*	0.02 (-0.01, 0.05)	-0.02 (-0.05, 0.02)	-0.05 (-0.09, -0.02)*	0 (-0.04, 0.04)	0.03 (0, 0.07)	0.05 (0.01, 0.09)*	0.03 (-0.01, 0.06)
Childhood BMI	0.03 (0, 0.06)	0 (-0.03, 0.03)	-0.05 (-0.08, -0.01)*	-0.03 (-0.07, 0.01)	-0.03 (-0.07, 0.01)	0 (-0.04, 0.04)	-0.01 (0.04, 0.03)	-0.02 (-0.06, 0.02)
Fully adjusted model[4]	0.04 (0, 0.07)*	0 (-0.03, 0.03)	-0.05 (-0.08, -0.01)*	-0.03 (-0.06, 0.01)	-0.03 (-0.07, 0.01)	0 (-0.04, 0.04)	-0.01 (0.04, 0.03)	-0.01 (-0.05, 0.03)
Early-pregnancy weight								
Mediator models[3]								
Pregnancy complications	0.04 (0.01, 0.07)*	0.02 (-0.01, 0.05)	0.01 (-0.03, 0.05)	-0.01 (-0.04, 0.02)	0.03 (-0.01, 0.06)	0 (-0.03, 0.04)	0.03 (-0.01, 0.06)	0.03 (-0.01, 0.06)
Birth characteristics	0.04 (0.02, 0.07)*	0.02 (-0.01, 0.06)	0.01 (-0.02, 0.05)	-0.01 (-0.04, 0.02)	0.03 (-0.01, 0.07)	0 (-0.03, 0.04)	0.03 (0, 0.07)	0.03 (0, 0.07)
Infant growth	0.04 (0.02, 0.07)*	0.02 (-0.01, 0.05)	0.01 (-0.03, 0.05)	-0.01 (-0.04, 0.02)	0.03 (-0.01, 0.06)	0 (-0.03, 0.03)	0.03 (-0.01, 0.06)	0.03 (0, 0.06)
Childhood BMI	0.02 (-0.01, 0.05)	0.01 (-0.02, 0.04)	0 (-0.04, 0.04)	0 (-0.04, 0.03)	0.02 (-0.02, 0.05)	-0.01 (-0.05, 0.02)	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.06)
Fully adjusted model[4]	0.02 (-0.01, 0.05)	0.02 (-0.02, 0.05)	0 (-0.03, 0.04)	0 (-0.04, 0.03)	0.02 (-0.02, 0.05)	-0.01 (-0.05, 0.02)	0.01 (-0.02, 0.04)	0.02 (-0.02, 0.05)
Mid-pregnancy weight								
Mediator models[3]								
Pregnancy complications	0.04 (0.01, 0.07)*	0.02 (-0.01, 0.06)	-0.02 (-0.07, 0.02)	-0.02 (-0.07, 0.02)	-0.01 (-0.05, 0.04)	-0.02 (-0.06, 0.03)	-0.02 (-0.06, 0.03)	-0.01 (-0.05, 0.03)
Birth characteristics	0.05 (0.01, 0.08)*	0.03 (0, 0.07)	-0.02 (-0.06, 0.02)	-0.03 (-0.07, 0.02)	0 (-0.05, 0.04)	-0.01 (-0.06, 0.03)	-0.01 (-0.05, 0.03)	-0.01 (-0.05, 0.04)
Infant growth	0.05 (0.02, 0.08)*	0.03 (0, 0.07)	-0.02 (-0.06, 0.02)	-0.03 (-0.07, 0.02)	0 (-0.05, 0.04)	-0.02 (-0.06, 0.03)	-0.01 (-0.05, 0.03)	-0.01 (-0.05, 0.03)
Childhood BMI	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.06)	-0.03 (-0.07, 0.01)	-0.02 (-0.07, 0.03)	-0.01 (-0.05, 0.03)	-0.03 (-0.07, 0.02)	-0.03 (-0.07, 0.01)	-0.02 (-0.06, 0.02)
Fully adjusted model[4]	0.03 (0, 0.06)	0.03 (-0.01, 0.06)	-0.03 (-0.07, 0.01)	-0.02 (-0.07, 0.03)	-0.01 (-0.05, 0.03)	-0.02 (-0.06, 0.02)	-0.03 (-0.07, 0.01)	-0.02 (-0.06, 0.03)
Late-pregnancy weight								
Mediator models[3]								
Pregnancy complications	0 (-0.05, 0.05)	-0.02 (-0.08, 0.03)	-0.01 (-0.07, 0.04)	-0.01 (-0.06, 0.03)	-0.01 (-0.06, 0.05)	0.01 (-0.04, 0.07)	0 (-0.06, 0.06)	0.01 (-0.05, 0.06)
Birth characteristics	0 (-0.05, 0.06)	-0.01 (-0.07, 0.04)	-0.01 (-0.07, 0.05)	-0.02 (-0.07, 0.03)	0 (-0.05, 0.05)	0.02 (-0.04, 0.07)	0.01 (-0.05, 0.07)	0.01 (-0.04, 0.06)
Infant growth	0.02 (-0.03, 0.07)	-0.01 (-0.06, 0.04)	-0.01 (-0.07, 0.04)	-0.02 (-0.07, 0.03)	0 (-0.06, 0.05)	0.01 (-0.04, 0.07)	0.01 (-0.04, 0.07)	0.01 (-0.04, 0.06)
Childhood BMI	-0.01 (-0.06, 0.05)	-0.02 (-0.07, 0.04)	-0.02 (-0.07, 0.04)	-0.01 (-0.06, 0.03)	-0.01 (-0.06, 0.04)	0.01 (-0.05, 0.06)	0 (-0.06, 0.05)	0 (-0.05, 0.05)
Fully adjusted model[4]	0 (-0.05, 0.06)	-0.02 (-0.07, 0.04)	-0.02 (-0.07, 0.04)	-0.01 (-0.06, 0.04)	-0.01 (-0.06, 0.05)	0.01 (-0.04, 0.07)	0 (-0.06, 0.05)	0.01 (-0.04, 0.05)

[1]Values are regression coefficients (95% Confidence Interval) from linear regression models that reflect the difference in childhood cardio-metabolic outcomes per SDS change in maternal prepregnancy weight and per SDS change in standardised residual change in maternal weight in early-, mid- and late-pregnancy from conditional regression analyses. Estimates are based on multiple imputed data.[2]Models are adjusted for child's sex and age at outcome measurements, maternal age, educational level, ethnicity, parity, height at intake, smoking during pregnancy, alcohol consumption during pregnancy and folic acid supplement use, total calorie intake during pregnancy, delivery mode, breastfeeding duration, timing of introduction of solid foods and average duration of tv-watching. [3]Intermediate models are additionally adjusted for each potential intermediate. [4] Fully adjusted models include all potential confounders and intermediates.*P-value <0.05.

Supplementary Table S2.7.6. Maximum weight gain during pregnancy and childhood body composition and cardio-metabolic outcomes[1]

Childhood outcomes	Change in maximum gestational weight gain (SDS)
Difference in childhood body fat outcomes	
Body mass index (SDS)	0.09 (0.06, 0.13)**
Total fat mass (SDS)	0.02 (-0.01, 0.05)
Android/gynoid fat mass ratio (SDS)	0.03 (-0.01, 0.06)
Abdominal subcutaneous fat area (SDS)	0 (-0.03, 0.04)
Abdominal preperitoneal fat area (SDS)	0.01 (-0.03, 0.05)
Differences in childhood cardio-metabolic outcomes	
Systolic blood pressure (SDS)	0.01 (-0.03, 0.04)
Diastolic blood pressure (SDS)	0.01 (-0.03, 0.05)
Total cholesterol (SDS)	-0.02 (-0.07, 0.03)
HDL-cholesterol (SDS)	-0.04 (-0.09, 0.01)
LDL-cholesterol (SDS)	0.02 (-0.03, 0.07)
Triglyceride (SDS)	0.01 (-0.04, 0.06)
Insulin (SDS)	0.02 (-0.03, 0.07)
C-peptide (SDS)	0.01 (-0.04, 0.06)

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in childhood outcomes per SDS change in maximum weight gain during pregnancy. Estimates are based on multiple imputed data. Model is adjusted for child's sex, age and height (body fat outcomes only) at measurement, maternal age, educational level, ethnicity, pre-pregnancy body mass index, parity, smoking during pregnancy, alcohol consumption during pregnancy and folic acid supplement use, total calorie intake, caesarean delivery, breastfeeding duration, timing of introduction of solid foods, average duration of tv watching. Additional adjustment for potential intermediates (pregnancy complications, birth characteristics, childhood size) partly attenuated the effect estimates (results not shown). **P-value <0.01.

Supplementary Table S2.7.7. Excessive weight gain during pregnancy and childhood body composition and cardio-metabolic outcomes[1]

Childhood outcomes	Excessive gestational weight gain according to IOM criteria
Difference in childhood body fat outcomes	
Body mass index (SDS)	0.20 (0.13, 0.26)**
Total fat mass (SDS)	0.08 (0.02, 0.14)**
Android/gynoid fat mass ratio (SDS)	0.07 (0.01, 0.14)*
Abdominal subcutaneous fat area (SDS)	0.05 (-0.02, 0.12)
Abdominal preperitoneal fat area (SDS)	0.03 (-0.05, 0.11)
Difference in childhood cardio-metabolic outcomes	
Systolic blood pressure (SDS)	0.01 (-0.07, 0.08)
Diastolic blood pressure (SDS)	0.05 (-0.03, 0.13)
Total cholesterol (SDS)	-0.03 (-0.13, 0.06)
HDL-cholesterol (SDS)	-0.06 (-0.15, 0.04)
LDL-cholesterol (SDS)	0.01 (-0.09, 0.11)
Triglyceride (SDS)	0.02 (-0.08, 0.11)
Insulin (SDS)	0.03 (-0.07, 0.12)
C-peptide (SDS)	-0.02 (-0.11, 0.08)

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in childhood outcomes for mothers with excessive gestational weight gain as compared to mothers with non-excessive gestational weight gain. Excessive gestational weight gain is defined according to the IOM criteria dependent on mother's prepregnancy body mass index. Estimates are based on multiple imputed data. Model is adjusted for child's sex, age and height (body fat outcomes only) at measurement, maternal age, educational level, ethnicity, parity, smoking during pregnancy, alcohol consumption during pregnancy and folic acid supplement use, total calorie intake, caesarean delivery, breastfeeding duration, timing of introduction of solid foods, average duration of tv watching. Additional adjustment for potential intermediates (pregnancy complications, birth characteristics, childhood size) partly attenuated the effect estimates (results not shown). *P-value <0.05. **P-value <0.01.

Chapter 3

Placental and fetal influences

Chapter 3.1

Placental haemodynamics and the risks of pregnancy complications

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Abstract

Background: Characteristics of the uterine and umbilical artery blood flow patterns are indirect measures of uteroplacental circulation. We examined whether uterine and umbilical artery resistance indices are influenced by maternal demographic and lifestyle characteristics, track from the second trimester to the third, and are associated with the risk of pregnancy complications.

Methods: This analysis was embedded among 7660 pregnant women in the Generation R Study (Rotterdam, the Netherlands, 2001–2005). Placental resistance indices were assessed in the second and third trimesters. Information about pregnancy outcomes was obtained from medical records.

Results: Maternal characteristics affected second- and third-trimester placental resistance indices. Correlation coefficients for correlation between the second and third trimesters were 0.50 and 0.32 for uterine artery resistance index and umbilical artery pulsatility index, respectively. Higher placental resistance indices in the second and third trimesters and persistence in the highest tertile of uterine artery resistance index from the second trimester to the third were associated with the risks of pre-eclampsia, preterm birth, and small size for gestational age at birth (all P-values <0.05).

Conclusions: Our study shows that placental resistance indices are influenced by maternal demographic and lifestyle characteristics and track moderately from the second trimester to the third. Increased placental resistance indices in the second and third trimesters are associated with increased risks of adverse pregnancy outcomes.

Introduction

Gestational hypertensive disorders and fetal growth restriction are important causes of maternal and fetal morbidity and mortality.[1–3] Abnormal early placentation might be involved in the underlying mechanisms.[4] Abnormal early placentation can lead to higher uterine and umbilical artery resistance patterns, which can be measured by Doppler wave forms.[5–7] Abnormal uterine artery and umbilical artery wave forms in midpregnancy indicate impaired uteroplacental and fetoplacental circulation and have been associated with pre-eclampsia and fetal growth retardation.[6–9] However, some studies have suggested that uteroplacental blood flow and resistance patterns change during the second and third trimesters and that assessment of uteroplacental circulation early in the third trimester might be more important for prediction of pregnancy outcomes.[10,11] Thus far, not much is known about the development of placental resistance indices from the second trimester onwards or whether this is influenced by maternal characteristics. In addition, it is not known whether placental resistance indices track during pregnancy. Tracking can be used to describe the longitudinal development of a variable and focuses on the maintenance of one's relative position in a distribution of values over time.[12]

Therefore, in a population-based prospective cohort study among 7660 pregnant women, we examined whether uterine and umbilical artery resistance indices are influenced by maternal demographic and lifestyle characteristics, whether they track from the second trimester to the third, and whether they are associated with the risk of maternal and fetal pregnancy complications.

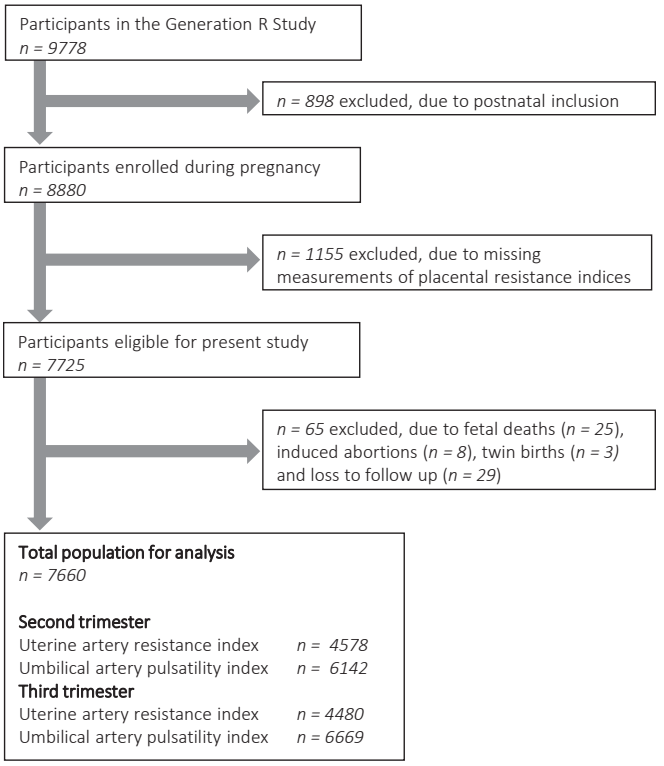
Materials and Methods

Study design

This analysis was embedded in the Generation R Study, a population-based prospective cohort study that included women from early pregnancy onwards in Rotterdam, the Netherlands.[13] The study was approved by the Medical Ethical Committee of Erasmus Medical Center in Rotterdam. Written consent was obtained from all participating women. All pregnant women were enrolled between 2001 and 2005. The Generation R Study is a birth cohort study involving prenatal recruitment. Estimation of the precise number of eligible pregnant women is difficult, since there is no satisfactory registry of pregnancies. Therefore, the overall response rate of the study was calculated at birth, as the children formed a prenatally recruited birth cohort. The response rate at birth was 61% and reflected the number of children born to mothers living in the study area at their delivery date and participating in the study as a percentage of the total number of children born to mothers who fulfilled these eligibility criteria. In total, 9778 women were enrolled in the study. Of these women, 91% (*n = 8880*) were enrolled during pregnancy. For the present study, we excluded women without any placental resistance

index measurements (*n = 1155*) and restricted our analyses to low-risk pregnancies. Thus, the cohort for analysis comprised 7660 pregnant women (**Figure 3.1.1**).

Figure 3.1.1. Flow chart of the participants



Maternal socio-demographic and lifestyle-related variables

Gestational age was established by fetal ultrasonography during the first ultrasound visit.[13] Maternal age was assessed at enrollment. During all prenatal visits (all 3 trimesters), maternal anthropometric characteristics were measured at one of the research centers. Height (cm) and weight (kg) were measured without shoes and heavy clothing, and body mass index (weight (kg)/height $(m)^2$) was calculated for each pregnancy period. We defined gestational weight gain as the difference between weight before pregnancy and weight in the third trimester. Information on educational level, ethnicity, parity, and use of folic acid supplements was obtained at enrollment. Information about smoking and alcohol consumption was assessed by questionnaire in each trimester.[13]

Placental hemodynamic function

Placental vascular resistance was evaluated with recorded flow velocity wave forms from the uterine and umbilical arteries in the second and third trimesters.[14] A raised uterine artery resistance index and umbilical artery pulsatility index indicate increased placental resistance.[7] Uterine artery resistance index was measured in the uterine arteries near the crossover with the external iliac artery. Umbilical artery pulsatility index was measured in a free-floating loop of the umbilical cord. For each measurement, 3 consecutive uniform wave forms were recorded by pulsed Doppler ultrasound, during fetal apnea and without fetal movement. The mean of 3 measurements was used for further analysis. The presence of notching was assessed in the uterine arteries and reflects an abnormal wave form resulting from increased blood flow resistance. Ultrasound measurements were performed in a blinded fashion with regard to previous measurements and pregnancy outcomes. Since placental resistance indices were measured at only one of the 2 dedicated research centers, placental resistance index measurements were available for a subgroup of women. Of the 8880 prenatally enrolled women, 7725 (87%) women received placental resistance index measurements.

Gestational hypertension and pre-eclampsia

Information on pregnancy complications was obtained from medical records. For women who had suspected pregnancy complications on the basis of these records, the records were cross-checked with the original hospital charts. These procedures have been described in detail elsewhere.[15] Briefly, gestational hypertension was defined as development of systolic blood pressure ≥140 mmHg and/ or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation in previously normotensive women. These criteria, plus the presence of proteinuria (defined as 2 or more dipstick readings of 2+ or greater, 1 catheter sample reading of 1+ or greater, or a 24-hour urine collection containing at least 300 mg of protein), were used to identify women with pre-eclampsia.[16]

Delivery and birth complications

Gestational age at birth, birth weight, and offspring sex were obtained from midwife and hospital registries at birth.[13] Preterm birth was defined as a gestational age of <37 weeks at birth (median: 35.7 weeks; range: 24.9 – 36.8 weeks). Gestational-age-adjusted standard deviation scores for birth weight were constructed using growth standards from Niklasson et al.[17] Small size for gestational age at birth was defined as a gestational-age-adjusted birth weight below the fifth percentile in the study cohort (less than −1.77 standard deviations).[13]

Statistical analysis

We performed a nonresponse analysis to examine whether maternal characteristics differed among women with and without placental resistance index measurements. Using unbalanced repeated measurement regression models, we analyzed the longitudinal uterine artery and umbilical artery resistance patterns in women with uncomplicated pregnancies and women with complicated pregnancies. For this analysis, we defined a complicated pregnancy as a pregnancy complicated by either gestational hypertensive disorders or preterm delivery or delivery of a small for gestational age (SGA) infant. The models are described in detail in the Supplementary Material. Next, we examined the associations of maternal characteristics with uterine artery resistance index and umbilical artery pulsatility index using multivariate linear regression models and with the presence of third-trimester notching using logistic regression models. For these analyses, we standardized uterine artery resistance index and umbilical artery pulsatility index values by dividing the original values by their corresponding standard deviations. To examine whether placental resistance indices track from the second trimester to the third, we estimated Pearson's correlation coefficients. We subsequently categorized uterine artery resistance index and umbilical artery pulsatility index in tertiles in the second and third trimesters and used logistic regression models to calculate the odds ratio for remaining in the same placental resistance index tertile from the second trimester to the third. In these analyses, the third-trimester placental resistance index tertile was the dependent variable and the second-trimester placental resistance index tertile was the independent variable. We further examined the associations of placental resistance indices in the second and third trimesters, the change in these indices during this period, and the presence of notching with the risks of gestational hypertension, pre-eclampsia, preterm birth, and delivering an SGA infant using multiple logistic regression models. These models were adjusted for potential confounders.

Missing data on the covariates were imputed using multiple imputation. The percentages of missing values within the population for analysis were lower than 20%, except for gestational weight gain (21.8%) and use of folic acid supplements (25.4%). The repeated-measurement analysis was performed using the Statistical Analysis System, version 9.2 (SAS Institute Inc., Cary, North Carolina), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were performed using the Statistical Package for the Social Sciences, version 17.0 for Windows (SPSS Inc., Chicago, Illinois).

Results

Subject characteristics

Characteristics of the included women are shown in **Table 3.1.1**. In total, 271 (3.5%) and 150 (2.0%) women developed gestational hypertension and pre-eclampsia, respectively,

and 382 (5.0%) and 380 (5.0%) children were born preterm and SGA, respectively. Non-response analysis showed that women without placental resistance index measurements weighed slightly more and were more frequently less educated and of non-European descent (**Supplementary Table S3.1.1**). Uterine artery resistance index and umbilical artery pulsatility index decreased from the second trimester onwards in both uncomplicated and complicated pregnancies (**Supplementary Figure S3.1.1**).

Table 3.1.1. Characteristics of mothers and their children in the Generation R Study (*n = 7660*)[1]

Characteristics	Mean (SD)	Median (90% range)	No.	%
Maternal characteristics				
Age, years		30.3 (20.4, 37.9)		
Height, cm	167.1 (7.4)			
Prepregnancy weight, kg	66.1 (12.7)			
Prepregnancy Body Mass Index, kg/m^2	23.6 (4.3)			
Gestational age at subject's enrolment, weeks		14.2 (10.9, 22.9)		
Education				
Primary			790	11.2
Secondary			3239	46.1
Higher			3006	42.7
Race / Ethnicity				
Dutch or European			4163	58.2
Non-European			2991	41.8
Parity, No. nulliparous			4257	56.2
Folic acid supplement use				
Yes			4093	71.6
No			1627	28.4
Smoking				
Yes			1702	25.8
No			4906	74.2
Alcohol consumption				
Yes			3395	51.1
No			3251	48.9
Mean uterine artery resistance index				
Second trimester	0.54 (0.09)			
Third trimester	0.49 (0.08)			
Mean umbilical artery pulsatility index				
Second trimester	1.20 (0.19)			
Third trimester	0.98 (0.17)			
Maternal pregnancy complications				
Gestational hypertension			271	3.5
Pre-eclampsia			150	2.0
Delivery and child characteristics				
Males			3895	50.8
Gestational age, weeks		40.1 (36.9, 42.0)		
Preterm birth			382	5.0
Birth Weight, g	3416 (558)			
Small for gestational age			380	5.0

Abbreviations: SD, standard deviation; No., number of subjects.
[1]Values represent mean (SD), median (90% range) or number of subjects (%).

Table 3.1.2. Maternal characteristics and placental resistance indices in the Generation R Study (*n = 7660*)

	Uterine artery RI (SD)[1]				Umbilical artery PI (SD)[1]				Notching[2]	
	Second trimester		Third trimester		Second trimester		Third trimester		Third trimester	
	n = 4578		*n = 4480*		*n = 6142*		*n = 6669*		*n = 4738*	
Maternal risk factors	Beta	95% CI	Beta	95% CI	Beta	95% CI	Beta	95% CI	OR	95% CI
Age[3], yrs	0.03	-0.01, 0.06	0.10*	0.07, 0.14	-0.03	-0.06, 0.001	0.11	-0.02, 0.04	0.81*	0.72, 0.91
Prepregnancy body mass index[3], kg/m^2 (SD)	0.01	-0.03, 0.05	0.04*	0.01, 0.07	0.03	0, 0.06	0.01	-0.02, 0.03	0.99	0.88, 1.10
Gestational weight gain[3], kg	-0.02	-0.05, 0.001	0.01	-0.02, 0.03	0.002	-0.02, 0.02	-0.02*	-0.04, 0	0.94	0.87, 1.01
Education										
Primary	0.11	-0.004, 0.23	0.15*	0.02, 0.28	0.02	-0.08, 0.12	0.05	-0.06, 0.15	1.14	0.77, 1.67
Secondary	0.06	-0.02, 0.13	0.06	-0.02, 0.13	0.07*	0.01, 0.13	0.05	-0.02, 0.11	0.98	0.76, 1.26
Higher	*Reference*		*Reference*		*Reference*		*Reference*		*Reference*	
Ethnicity										
Dutch or European	*Reference*		*Reference*		*Reference*		*Reference*		*Reference*	
Non-European	0.03	-0.04, 0.10	0.05	-0.03, 0.13	0.07*	0.01, 0.13	0.01	-0.06, 0.07	1.30*	1.02, 1.67
Parity										
Nulliparous	*Reference*		*Reference*		*Reference*		*Reference*		*Reference*	
Multiparous	0.07*	0.01, 0.13	0.09*	0.02, 0.15	-0.13*	-0.18, -0.08	-0.07*	-0.13, -0.02	0.66*	0.53, 0.83
Folic acid supplement use										
No	*Reference*		*Reference*		*Reference*		*Reference*		*Reference*	
Yes	-0.19*	-0.27, -0.10	-0.10*	-0.19, -0.02	-0.06	-0.12, 0.01	-0.03	-0.10, 0.04	0.81	0.62, 1.05
Smoking										
No	*Reference*		*Reference*		*Reference*		*Reference*		*Reference*	
Yes	0.02	-0.06, 0.10	0.08	0, 0.16	0.14*	0.08, 0.20	0.13*	0.08, 0.19	0.95	0.75, 1.20
Alcohol										
No	*Reference*		*Reference*		*Reference*		*Reference*		*Reference*	
Yes	0.04	-0.03, 0.11	-0.02	-0.09, 0.05	0.01	-0.05, 0.07	-0.04	-0.10, 0.01	1.11	0.87, 1.40

Abbreviations: OR; Odds Ratio, CI; Confidence Interval; SD, standard deviation; RI, resistance index; PI, pulsatility index.

[1]Values are multivariate linear regression coefficients and 95% Confidence Intervals. For continuous variables, estimates reflect the difference in the placental resistance indices (in standard deviation) per standard deviation change of the risk factor. For categorical variables or dichotomous variables, the effect estimates represent the difference in placental resistance indices (in standard deviation), compared to reference group. Estimates are based on multiple imputed data. [2]Values are Odds Ratios and 95% Confidence Intervals. For continuous variables, estimates reflect the difference in risk of notching per standard deviation change of the risk factor. For categorical variables or dichotomous variables, the effect estimates reflect difference in risk of notching, compared to reference group. Estimates are based on multiple imputed data. [3]A 1 standard deviation change in maternal age corresponded to a change of 5.3 years. A 1 standard deviation change in prepregnancy body mass index corresponded to a change of 4.3 units. A 1 standard deviation change in gestational weight gain corresponded to a change of 5.1 kg. *P <0.05.

Maternal characteristics and placental hemodynamic indices

In the multivariate analyses, multiparity and no folic acid supplement use were associated with a slightly higher second-trimester uterine artery resistance index (Table 3.1.2). Higher maternal age, higher prepregnancy body mass index, lower maternal educational level, multiparity, and no folic acid supplement use were associated with a slightly higher third-trimester uterine artery resistance index (all P-values <0.05). Lower maternal educational level, non-European ethnicity, nulliparity, and maternal smoking were associated with higher second-trimester umbilical artery pulsatility index, whereas lower gestational weight gain, nulliparity, and maternal smoking during pregnancy were associated with a higher third-trimester umbilical artery pulsatility index (all P-values <0.05). Higher maternal age and multiparity were associated with a lower risk of notching (Odds Ratio (OR) 0.81 (95% Confidence Interval (CI): 0.72, 0.91) per standard-deviation change in maternal age and OR 0.66 (95% CI: 0.53, 0.83) for multiparity as compared with nulliparity).

Table 3.1.3. Tracking of placental resistance indices from second to third trimester in the Generation R Study[1,2]

	Third trimester tertiles												
Second	First				Second				Third				
trimester	*No.*				*No.*				*No.*				*Total*
tertiles	*tertiles*	%	OR	95% CI	*tertiles*	%	OR	95% CI	*tertiles*	%	OR	95% CI	*n*
Uterine artery RI													
First	*567*	57.6	4.47*	3.78, 5.28	*286*	29.1	0.72*	0.61, 0.85	*131*	13.3	0.23*	0.19, 0.28	*984*
Second	*309*	33.5	0.90	0.77, 1.06	*385*	41.8	1.72*	1.47, 2.04	*228*	24.7	0.62*	0.52, 0.74	*922*
Third	*124*	13.4	0.18*	0.14, 0.22	*280*	30.3	0.79*	0.67, 0.94	*520*	56.3	5.72*	4.79, 6.83	*924*
Total n	*1000*				*951*				*879*				*2830*
Umbilical artery PI													
First	*879*	48.4	2.63*	2.32, 2.96	*574*	31.6	0.95	0.84, 1.08	*364*	20.0	0.36*	0.31, 0.41	*1817*
Second	*544*	32.2	0.88*	0.78, 0.99	*567*	33.6	1.08	0.95, 1.22	*575*	34.1	1.06	0.94, 1.20	*1686*
Third	*360*	21.1	0.39*	0.34, 0.44	*551*	32.3	0.98	0.86, 1.10	*797*	46.7	2.44*	2.16, 2.76	*1708*
Total n	*1783*				*1692*				*1736*				*5211*

Abbreviations: No. tertiles, number of women that remain in the same tertile; %, percentage of women that remain in the same tertile; OR, Odds Ratio; CI, Confidence Interval; RI, resistance index; PI, pulsatility index.

[1]Values are Odds Ratio (95% Confidence Interval) (number and percentage of women that remain in the same tertile) to remain in the same tertile of uterine artery resistance index and umbilical artery pulsatility index from second to third trimester. Estimates are from multiple imputed data. [2]Model was adjusted for gestational age at subject's enrolment, gestational age in each pregnancy period, maternal age, educational level, parity, ethnicity, prepregnancy body mass index, smoking habits, alcohol consumption and folic acid supplement use. *P-value <0.05.

Tracking of placental hemodynamic indices

Pearson's correlation coefficients for correlation between the second trimester and the third trimester were 0.50 and 0.32 for uterine artery resistance index and umbilical artery pulsatility index, respectively. We observed similar effect estimates when we calculated the correlation based on intraclass correlation coefficients from the longitudinal models (0.49 and 0.31 for uterine artery resistance index and umbilical artery pulsatility index, respectively). The specific scatterplots are given in Supplementary

Figure S3.1.2. Table 3.1.3 shows that for uterine artery resistance index, approximately 56% of the women who were in the highest tertile in the second trimester remained in the highest tertile in the third trimester, while approximately 30% and 13% ended up in the middle and lowest tertiles, respectively. Fewer women remained in the same tertile of umbilical artery pulsatility index. The ORs for staying in the upper tertile from the second trimester to the third trimester were 5.72 (95% CI: 4.79, 6.83) for uterine artery resistance index and 2.44 (95% CI: 2.16, 2.76) for umbilical artery pulsatility index.

Table 3.1.4. Placental resistance indices and the risks of maternal and fetal pregnancy complications in the Generation R Study (*n = 7432*)[1,2]

	Gestational hypertension		Pre-eclampsia		Preterm birth		Small for gestational age	
Pregnancy period	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Second trimester								
Uterine artery RI	1.02	0.85, 1.21	1.61*	1.32, 1.96	1.67*	1.46, 1.90	1.45*	1.27, 1.65
Umbilical artery PI	1.16*	1.00, 1.33	1.22*	1.02, 1.46	1.10	0.97, 1.23	1.28*	1.14, 1.43
Third trimester								
Uterine artery RI	0.93	0.78, 1.10	1.87*	1.54, 2.27	1.84*	1.61, 2.09	1.66*	1.46, 1.89
Umbilical artery PI	1.03	0.89, 1.18	1.38*	1.17, 1.62	1.22*	1.09, 1.36	1.56*	1.40, 1.73
Unilateral notching	1.51	0.85, 2.68	3.15*	1.67, 5.91	3.12*	2.10, 4.61	3.43*	2.36, 4.97
Bilateral notching	3.90*	2.07, 7.30	8.51*	4.46, 16.19	4.23*	2.56, 6.97	4.17*	2.54, 6.82
Second to third trimester change								
Uterine artery RI	1.11	0.88, 1.38	1.21	0.91, 1.61	1.14	0.94, 1.38	1.07	0.86, 1.25
Umbilical artery PI	0.94	0.80, 1.10	1.22	0.99, 1.50	1.05	0.92, 1.21	1.13	0.99, 1.28

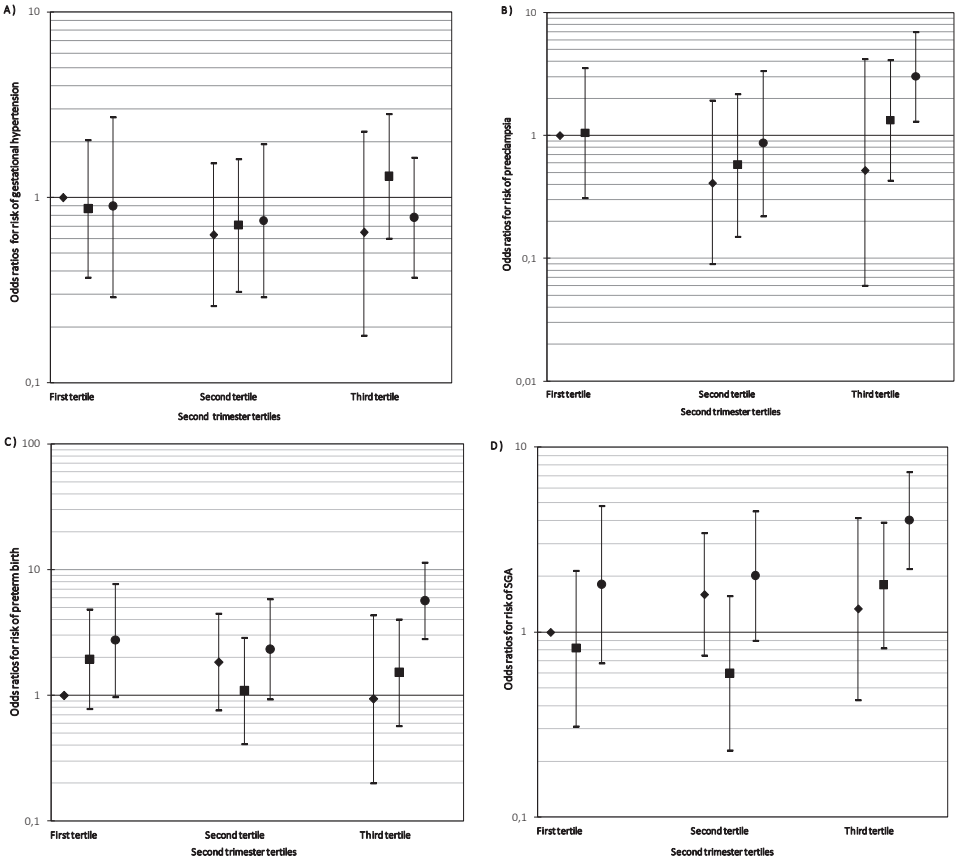
Abbreviations: OR, Odds Ratio; CI, Confidence Interval; RI, resistance index, PI; pulsatility index.
[1]Values are Odds Ratios and 95% Confidence Intervals that reflect the difference in risks of pregnancy complications per standard deviation change in placental resistance indices in second and third trimester and between the two trimesters. For notching, Odds Ratios and 95% Confidence Interval reflect the difference in risks of pregnancy complication, as compared to no notching. Estimates are from multiple imputed data. [2]Model was adjusted for gestational age at subject's enrolment, gestational age at each pregnancy period, maternal age, educational level, ethnicity, parity, prepregnancy body mass index, smoking habits, alcohol consumption and folic acid supplement use. *P-value <0.05.

Placental hemodynamic indices and pregnancy complications

Table 3.1.4 shows that second- and third-trimester uterine artery resistance indices were not associated with the risk of gestational hypertension. Second-trimester uterine artery resistance index and umbilical artery pulsatility index were associated with the risk of pre-eclampsia (OR 1.61 (95% CI: 1.32, 1.96) and OR 1.22 (95% CI: 1.02, 1.46) per standard-deviation change in resistance index, respectively). Second-trimester uterine artery resistance index, but not umbilical artery pulsatility index, was associated with the risk of preterm birth (OR 1.67 (95% CI: 1.46, 1.90) per standard-deviation change in resistance index). Second-trimester uterine artery resistance index and umbilical artery pulsatility index were associated with the risk of delivering an SGA infant (OR 1.45 (95% CI: 1.27, 1.65) and OR 1.28 (95% CI: 1.14, 1.43) per standard-deviation change in resistance index, respectively). Stronger associations were observed for all outcomes in the third trimester.

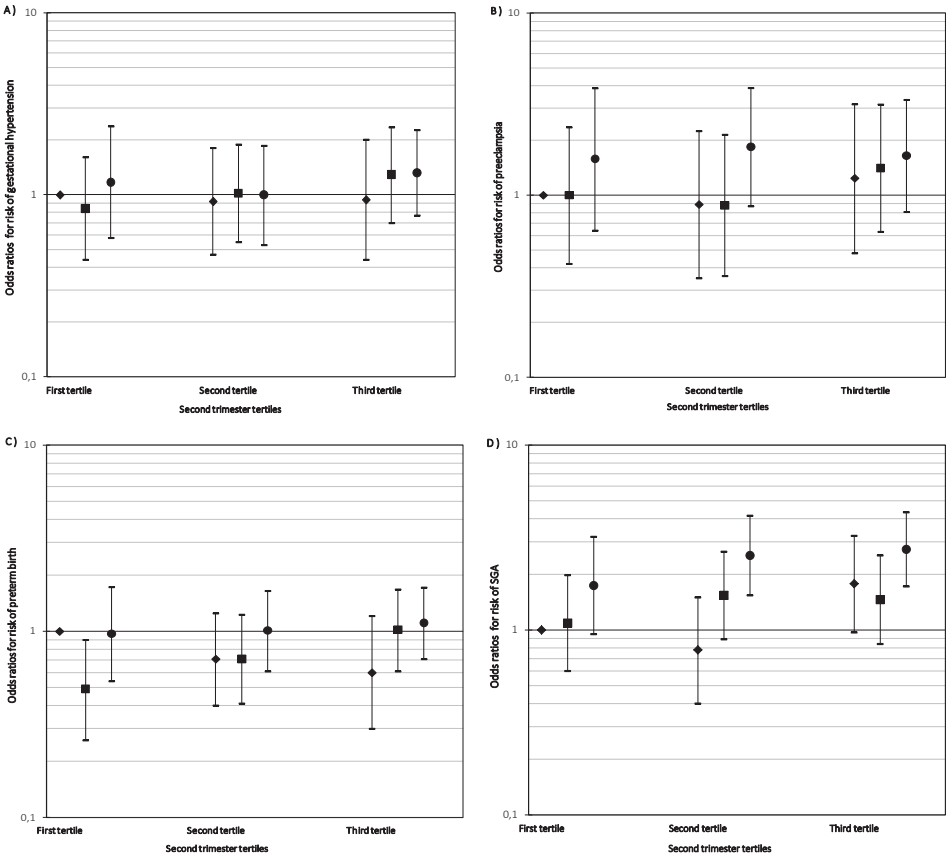
Women who remained in the highest uterine artery resistance index tertile from the second trimester to the third trimester had the highest risk of all adverse pregnancy outcomes (**Figure 3.1.2**). Persistence in the highest umbilical artery pulsatility index tertile from the second trimester to the third was associated with risk of delivering an SGA infant (**Figure 3.1.3**). As compared with no third-trimester notching, unilateral notching was associated with the risks of pre-eclampsia, preterm birth, and delivering an SGA infant (OR 3.15 (95% CI: 1.67, 5.91), OR 3.12 (95% CI: 2.10, 4.61), and OR 3.43 (95% CI: 2.36, 4.97), respectively) (**Table 3.1.4**). Bilateral notching was more strongly associated with the risks of these pregnancy complications (all P-values <0.01).

Figure 3.1.2. Association between change in uterine artery resistance index tertile from the second trimester to the third and the risk of adverse pregnancy outcomes in the Generation R Study



Odds Ratios (95% Confidence Intervals) reflect the risks of gestational hypertension (A), pre-eclampsia (B), preterm birth (C), and delivering a small for gestational age (SGA) infant (D) for each change in uterine artery resistance index tertile from the second trimester to the third trimester, as compared with persistence in the lowest uterine artery resistance index tertile from the second trimester to the third. Diamonds represent the first tertile of third-trimester uterine artery resistance index, squares represent the second tertile, and circles represent the third tertile. Estimates were derived from multiple imputed data. Models included adjustment for gestational age at subject's enrollment, gestational age in each pregnancy period, maternal age, educational level, ethnicity, parity, prepregnancy body mass index, smoking habits, alcohol consumption, and use of folic acid supplements.

Figure 3.1.3. Association between change in umbilical artery pulsatility index tertile from the second trimester to the third and the risk of adverse pregnancy outcomes in the Generation R Study



Odds Ratios (95% Confidence Intervals) reflect the risks of gestational hypertension (A), pre-eclampsia (B), preterm birth (C), and delivering a small for gestational age (SGA) infant (D) for each change in umbilical artery pulsatility index tertile from the second trimester to the third trimester, as compared with persistence in the lowest umbilical artery pulsatility index tertile from the second trimester to the third. Diamonds represent the first tertile of third-trimester umbilical artery pulsatility index, squares represent the second tertile, and circles represent the third tertile. Estimates were derived from multiple imputed data. Models included adjustment for gestational age at subject's enrollment, gestational age in each pregnancy period, maternal age, educational level, ethnicity, parity, prepregnancy body mass index, smoking habits, alcohol consumption, and use of folic acid supplements.

Discussion

Results from this prospective population-based cohort study showed that placental resistance indices are influenced by maternal demographic and lifestyle characteristics. Uterine artery resistance index tracks moderately from the second trimester to the third trimester, whereas umbilical artery pulsatility index tracks poorly from the second

trimester to the third. Increased placental resistance indices in the second and third trimesters are associated with increased risks of adverse pregnancy outcomes.

Methodological limitations

One of the strengths of this study was the prospective data collection from early pregnancy onwards. We had a large sample size of 7660 participants with 9058 uterine artery resistance index measurements and 12.811 umbilical artery pulsatility index measurements. The response rate at baseline for participation in the study was 61%. The response rate reflects the number of children born to mothers living in the study area on their delivery date and participating in the study as a percentage of the total number of children born to mothers who fulfilled these eligibility criteria. The percentages of women from ethnic minority groups and of lower socioeconomic status were slightly lower than expected from the population figures in Rotterdam.[13] Furthermore, placental resistance index measurements were performed in 87% of prenatally enrolled women, as measurements were performed at only 1 of the 2 research centers. Non-response analyses showed that women without placental resistance index measurements tended to be less educated and of non-European descent and had higher body mass indices. Additionally, adverse pregnancy outcomes were more often present in women without placental resistance index measurements. Non-response would lead to biased effect estimates if the selection mechanisms were related to both the determinant and the outcome, and the associations would be different between persons included in the analyses and those not included. However, this seems unlikely, as selection on outcome is unlikely because of the prospective nature of this study and because biased estimates in large cohort studies arise mainly from loss to follow-up rather than from non-response at baseline.[18] The non-response might have led to selection of a more affluent and relatively healthy population and might have affected the generalizability of our results. Furthermore, we had a relatively small number of cases of gestational hypertension, pre-eclampsia and preterm birth, which might indicate selection towards a healthy, low-risk population. This might influence the generalizability of our results to the general population. It might be of interest to perform similar analyses in a high-risk population. Detailed information about a large number of maternal socio-demographic and lifestyle-related factors was available in this study. However, because of the observational design, residual confounding due to other socio-demographic and lifestyle-related determinants might still be an issue. Ultrasound measurements were performed in a blinded fashion with regard to previous measurements. In addition, because of the prospective nature of the study, pregnancy outcomes were not known at the time of the ultrasound measurements. Therefore, it is unlikely that there was information bias due to knowledge of prior measurements or pregnancy outcome. Finally, several different outcomes were studied: gestational hypertension, pre-eclampsia, preterm birth, and SGA birth. Since these outcomes are strongly related, we did not perform adjustment for multiple testing.

Influence of maternal characteristics on development of placental resistance indices

Characteristics of uterine artery and umbilical artery blood flow patterns are indirect measures of uteroplacental circulation. During the first half of pregnancy, there is a linear decrease in uterine artery and umbilical artery resistance and a significant decrease in the prevalence of notching, which is in line with the physiological changes that occur in early and mid-pregnancy during placentation.[7,19,20] Few studies have examined the development of placental resistance indices from the second trimester onwards. Our study shows that the uterine artery resistance index and umbilical artery pulsatility index decrease from the second trimester onwards, which is in line with the previous findings.[11,21,22] The continued decline in placental resistance indices might be explained by slowly continued trophoblastic invasion and maternal hemodynamic adaptations.[11,21,23]

Several maternal demographic and lifestyle characteristics have been associated with adverse pregnancy outcomes. The influence of these characteristics on uteroplacental circulation might partly explain the suggested associations. Higher maternal age, lower maternal educational level, and non-European descent tended to be associated with slightly higher uterine artery and umbilical artery resistance indices from the second trimester onwards, but results were not consistent. Furthermore, we observed that parity, use of folic acid supplements, and maternal smoking during pregnancy consistently influenced placental resistance indices. Multiparity was associated with higher second- and third-trimester uterine artery resistance indices but with lower umbilical artery pulsatility indices. The mechanisms explaining these associations are not known. Use of folic acid supplements during pregnancy was associated with lower second- and third-trimester uterine artery resistance indices, but not with umbilical artery pulsatility indices. It has been suggested that folate might influence trophoblastic invasion of the spiral arteries and placentation,[24,25] which might partly explain the observed lower impedance in uterine arteries among women who used folic acid supplements.[24] In our study, maternal smoking was not associated with uterine artery resistance index when corrected for the other maternal characteristics, but it was associated with higher umbilical artery pulsatility indices in the second and third trimesters. Similarly, in a study among 2459 nulliparous women, Kho et al.[26] reported that maternal smoking during pregnancy was associated with higher umbilical artery pulsatility index levels in the second trimester but not with uterine artery resistance index levels after adjustment for confounders. It has been suggested that maternal smoking might have a larger influence on vasculature in the placental villi and a smaller impact on the uteroplacental blood supply, which could partly explain the observed effects.[26]

Tracking of placental resistance indices and the risk of adverse pregnancy outcomes

Many studies have examined the predictive accuracy of placental resistance indices for the prediction of gestational hypertensive disorders and fetal growth restriction.[8,27] Our study shows that uterine artery resistance index tracks moderately from the second

trimester to the third trimester, whereas umbilical artery pulsatility index tracks poorly from the second trimester to the third. Accordingly, a study of 3107 pregnancies showed that among normal pregnancies starting with a high uterine artery pulsatility index in the first trimester, there is normalization of the pulsatility index with advancing gestation, indicating that uterine artery pulsatility index tracks poorly in normal pregnancies.[28] In this latter study, the uterine artery pulsatility index remained high from the first trimester to the second trimester only among pregnancies leading to preeclampsia.[28]

Several studies have shown that increased placental resistance indices measured in either the first, second, or third trimester are associated with increased risks of adverse pregnancy outcomes.[10,29–33] These studies used cutoff values for abnormal placental resistance indices and did not assess the associations of small variations in placental resistance indices with adverse outcomes. We found that already small variations in placental resistance indices in a low-risk population are associated with the risk of adverse pregnancy outcomes. In addition, it has been shown that persistence of the placental resistance indices above the 90th or 95th percentile from the first trimester to the second or from the second trimester to the third is associated with the risk of preeclampsia and fetal growth restriction, which is in line with observations in our study.[19,33] The association between placental resistance indices and the risk of gestational hypertension remains controversial, as only some studies have found an association.

Several authors have reported that women with unilateral and bilateral notches in the second trimester have a high risk of developing pregnancy complications.[29,34] A study among 1022 women that assessed notching at approximately 20 weeks of gestation showed that women with bilateral notches have a strongly increased risk of preeclampsia, preterm delivery, and delivering an SGA infant.[29] We assessed notching early in the third trimester and found strong associations of unilateral and bilateral notching with the risk of these adverse pregnancy outcomes.

Most studies that examined the predictive accuracy of uterine artery resistance index and umbilical artery pulsatility index have suggested that among low-risk populations, the predictive accuracy of placental resistance measurements is not sufficient for clinical practice.[9,27,35] In line with these findings, we observed moderate tracking of the placental resistance indices in our study population. As compared with umbilical artery pulsatility index, uterine artery resistance index tracked better and was more strongly associated with the risk of adverse pregnancy outcomes. Therefore, uterine artery resistance index might be a more useful measurement for the prediction of adverse pregnancy outcomes. In addition, notching in the uterine artery might be a good measure for prediction of adverse pregnancy outcomes. Further research to examine tracking of the placental resistance indices among high-risk populations and to examine the predictive value of notching is necessary.

Conclusion

This study showed that uterine artery resistance index tracks moderately from the second trimester to the third trimester, whereas umbilical artery pulsatility index tracks poorly from the second trimester to the third. These placental resistance indices are influenced by maternal demographic and lifestyle characteristics and are associated with increased risks of adverse pregnancy outcomes. Further research is needed to assess the predictive accuracy of placental resistance index measurements and to assess the effects of small variations in placental resistance indices on fetal growth and childhood growth and development.

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Supplementary Material

Supplementary Methods S3.1.1. Placental resistance indices development in uncomplicated and complicated pregnancies

The associations between pregnancy complications and placental resistance indices were analysed using unbalanced repeated-measurements regression analysis assuming random effects for the intercept and slope (1,2). These regression models enable studies on repeatedly measured outcomes, taking account for the correlation between measurements and have an optimal use of available data. Both gestational age-independent (difference constant over time) and gestational age-dependent (difference not-constant over time) effects were assessed. We used compound symmetry covariance models (1,2). We constructed best-fitting models for placental resistance indices. We started with a linear model and examined whether adding second-degree fractional polynomial of gestational age improved the models by comparing the deviances and checking the goodness of fit (smallest –2 log likelihood). Since adding fractional polynomials of gestational age to the model did not improve the model fit, we did not include these fractional polynomials in the final models. Next, we added pregnancy complications as a categorical variable to the model as additional intercept and as an interaction term with gestational age. The final models including gestational age and pregnancy complications can be written as:

Uterine artery resistance index = $ß_0 + ß_1 \times$ pregnancy complication + $ß_2 \times$ gestational age + $ß_3 \times$ pregnancy complication × gestational age

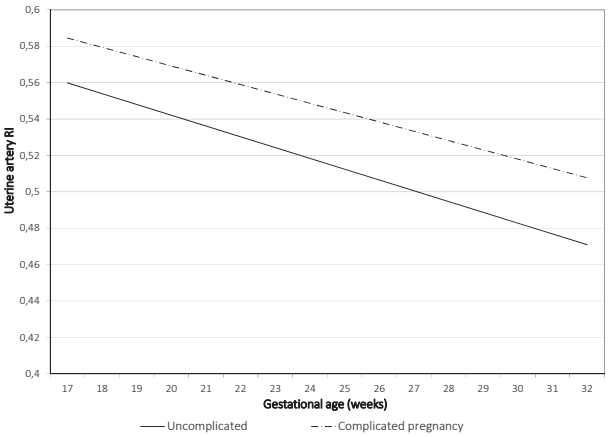
Umbilical artery pulsatility index = $ß_0 + ß_1 \times$ pregnancy complication + $ß_2 \times$ gestational age + $ß_3 \times$ pregnancy complication × gestational age

In these models, '$ß_0 + ß_1 \times$ pregnancy complication' reflects the intercept and '$ß_2 \times$ gestational age' reflects the slope of change in placental resistance index per week. Main interest was in the term '$ß_3 \times$ pregnancy complication × gestational age', which reflects the difference in change in placental resistance index per week for pregnancy complicated by adverse outcomes as compared to uncomplicated pregnancies. The uterine artery and umbilical artery resistance patterns in women with uncomplicated pregnancies and women with complicated pregnancies are shown in **Supplementary Figure S3.1.1a** and **1b** below.

References

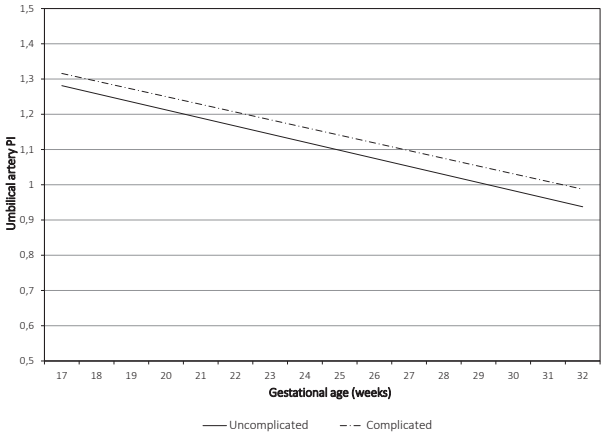
[1]. Goldstein H. Multilevel statistical methods. 2nd ed. Edward Arnold: London,1995.
[2] Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 1999;28:964–74.

Supplementary Figure S3.1.1. Placental resistance indices development in uncomplicated and complicated pregnancies



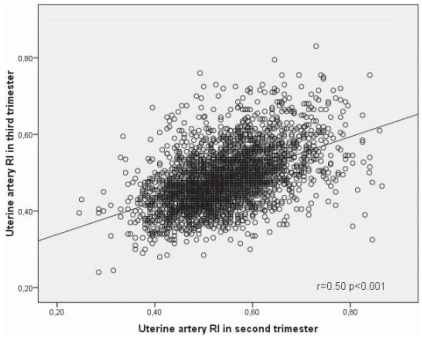
3.1.1a. Uterine artery resistance index development in uncomplicated and complicated pregnancy. Change in placental resistance indices measurements for women with a pregnancy complicated by adverse pregnancy outcomes compared to women with an uncomplicated pregnancy based on repeated measurement analysis. Uterine artery resistance index = $ß_0 + ß_1 \times$ pregnancy complication + $ß_2 \times$ gestational age + $ß_3 \times$ pregnancy complication × gestational age.

Supplementary Figure S3.1.1. Placental resistance indices development in uncomplicated and complicated pregnancies (*continued*)

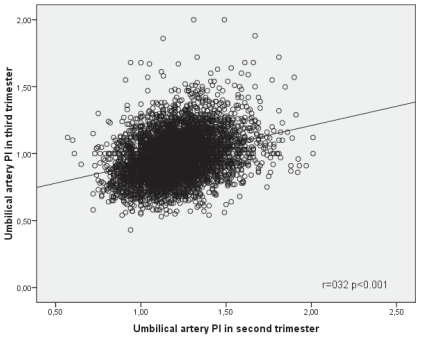


3.1.1b. Umbilical artery pulsatility index development in uncomplicated and complicated pregnancy. Change in placental resistance indices measurements for women with a pregnancy complicated by adverse pregnancy outcomes compared to women with an uncomplicated pregnancy based on repeated measurement analysis. Umbilical artery pulsatility index = $ß_0$ + $ß_1$ × pregnancy complication + $ß_2$ × gestational age + $ß_3$ × pregnancy complication × gestational age.

Supplementary Figure S3.1.2. Correlation of placental resistance indices between second and third trimester



3.1.2a. Correlation between second and third trimester uterine artery resistance index



3.1.2b. Correlation between second and third trimester umbilical artery pulsatility index

Supplementary Table S3.1.1. Characteristics of the women with and without placental resistance indices measurements[1,2]

	Women with placental resistance indices measurements *n = 7725*	Women without placental resistance indices measurements *n = 1155*	P-value
Maternal characteristics			
Age, median (90% range), years	30.3 (20.4, 37.9)	29.9 (19.7, 37.7)	<0.05
Height, mean (SD), cm	167.2 (7.4)	166.6 (7.5)	<0.01
Prepregnancy weight, mean (SD), kg	66.1 (12.7)	67.5 (13.8)	<0.05
Prepregnancy Body Mass Index, mean (SD), kg/m^2	23.6 (4.3)	24.2 (4.8)	<0.01
Gestational age at subject's enrolment, median (90% range), weeks	14.2 (10.9, 22.9)	15.9 (11.2, 24.8)	<0.01
Education, No. (%)			
Primary	797 (11.3)	143 (14.6)	<0.01
Secondary	3265 (46.1)	479 (49.8)	
Higher	3022 (42.7)	357 (36.5)	
Race / Ethnicity, No. (%)			
Dutch or European	4189 (58.1)	527 (52.9)	<0.01
Non-European	3017 (41.9)	469 (47.1)	
Parity, No.(%)			
Nulliparous	4287 (56.2)	575 (51.0)	<0.01
Multiparous	3342 (43.8)	553 (49.0)	
Folic acid supplement use, No. (%)			
Yes	4116 (71.4)	516 (64.6)	<0.01
No	1645 (28.6)	283 (35.4)	
Smoking, No. (%)			
Yes	1716 (25.8)	222 (23.7)	0.15
No	4942 (74.2)	714 (76.3)	
Alcohol consumption, No. (%)			
Yes	3414 (51.0)	417 (44.2)	0.01
No	3282 (49.0)	526 (55.8)	
Maternal pregnancy complications			
Gestational hypertension, No. (%)	273 (3.8)	45 (4.4)	0.33
Pre-eclampsia, No. (%)	152 (2.1)	35 (3.4)	0.01
Delivery and child characteristics			
Males, No. (%)	3897 (50.9)	470 (47.9)	0.08
Gestational age, median (90% range), weeks	40.1 (36.9, 42.0)	39.7 (34.9, 42.1)	0.01
Birth weight, mean (SD), g	3415 (559)	3361 (600)	0.01
Preterm birth, No. (%)	389 (5.1)	122 (11.4)	0.01
Small for gestational age, No. (%)	380 (5.0)	57 (6.0)	0.19

[1]Values represent mean (SD), median (90% range) or number of subjects (%). [2]Differences in subject characteristics between the groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.

Chapter 3.2

Placental vascular dysfunction, early growth and childhood cardiovascular risk factors

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Abstract

Background: Suboptimal fetal nutrition may influence early growth and cardiovascular development. We examined whether umbilical and uterine artery resistance indices, as measures of feto-placental and utero-placental vascular function, respectively, are associated with fetal and childhood growth and cardiovascular development.

Methods and results: This study was embedded in a population-based prospective cohort study among 6716 mothers and their children. Umbilical artery pulsatility index and uterine artery resistance index and fetal growth were measured in third trimester. Childhood growth was repeatedly assessed from birth to the age of 6 years. We measured body fat distribution, left ventricular mass, and blood pressure at the age of 6 years. Higher third trimester umbilical and uterine artery vascular resistance were associated with lower fetal length and weight growth in third trimester resulting in a smaller size at birth among boys and girls (P-values <0.05). These differences in length and weight growth became smaller from the age of 6 months onwards, but were still present at the age of 6 years. Higher third trimester umbilical artery vascular resistance, but not uterine artery vascular resistance, was associated with higher childhood body mass index, total fat mass, android/gynoid fat mass ratio, and systolic blood pressure, and with a lower left ventricular mass (P-values <0.05). These associations were not explained by birth weight. Stronger associations tended to be present among girls as compared with boys.

Conclusions: Higher third trimester feto-placental vascular resistance, but not utero-placental vascular resistance, was associated with slower fetal growth rates and cardiovascular adaptations in childhood.

Introduction

Low birth weight is associated with cardiovascular disease in adulthood.[1–3] These associations may be explained by developmental adaptations in early life, in response to suboptimal fetal nutrition.[3,4] These developmental adaptations may lead to fetal growth restriction and subclinical cardiovascular alterations, which predispose to cardiovascular disease in adulthood.[4] The placenta is a major determinant of the fetal supply line.[5] Suboptimal placental growth and function, which is unable to meet fetal nutrient requirements, may therefore lead to developmental adaptations with a persistent influence on growth and cardiovascular function in later life.[5–7] Previous studies among adults suggested that both low and high placental weight are associated with adverse cardio-metabolic outcomes in later life, but results are not consistent.[7] Placental weight is only a crude measure of placental growth and function and liable to measurement error.[5,7] More detailed measures of placental function, assessed during pregnancy, might give further insight in long-term consequences of placental dysfunction.

Placental vascular function can be assessed by Doppler ultrasound of the umbilical and uterine arteries throughout pregnancy, which reflect feto-placental vascular resistance and utero-placental vascular resistance, respectively.[8] Feto-placental vascular resistance is a parameter of the fetal circulation, and increased feto-placental vascular resistance may occur as a result of impaired placentation or suboptimal fetal vascular development.[6,8] Utero-placental vascular resistance, a parameter of the maternal circulation, may increase as a result of impaired placentation.[8]

Previously, we observed that third trimester small and subclinical variations in feto-placental vascular function correlate with fetal growth.[9] Also, we have shown that higher third trimester resistance of the feto-placental circulation and utero-placental circulation are associated with a higher risk of a small size for gestational age infant.[10] Thus far, it is not known whether normal variation in placental vascular function influences longitudinally measured fetal and childhood growth and childhood cardiovascular development. As the placental vascular bed forms an important component of the fetal vascular system, and the largest variation is expected in third trimester, we hypothesized that especially changes in third trimester feto-placental vascular resistance lead to growth and cardiovascular system adaptations.[9]

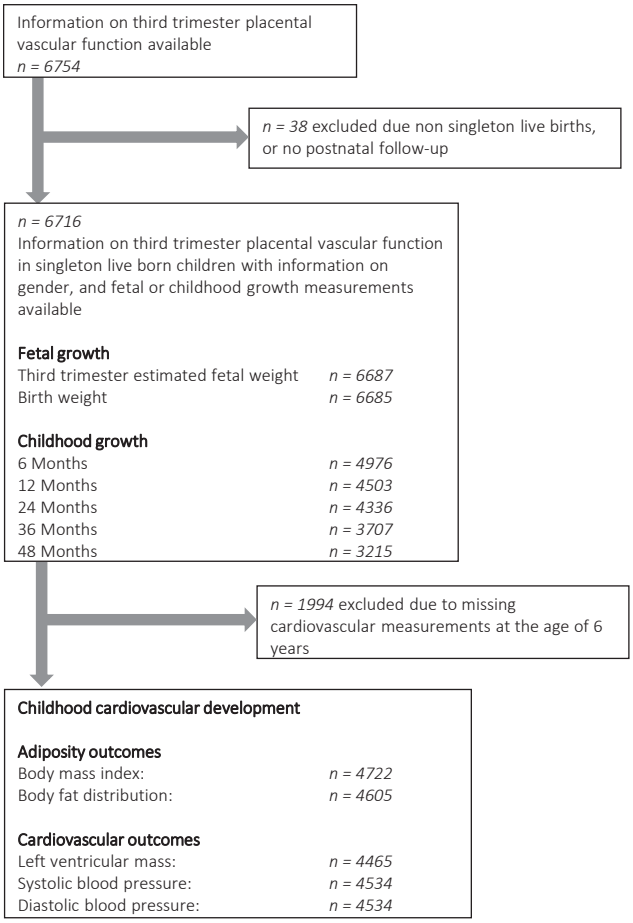
Therefore, in a population-based prospective cohort study among 6716 mothers and their children, we examined the associations of third trimester feto-placental and utero-placental vascular function with repeatedly measured fetal and childhood growth characteristics, and with cardiovascular development in childhood.

Methods

Study design

This study was embedded in the Generation R study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, The Netherlands.[11] The study has been approved by the medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Written consent was obtained from all participating women.[12] Response rate at birth was 61%. In total, 8880 mothers were enrolled during pregnancy. Because placental resistance indices were only measured in 1 of the 2 dedicated research centers, placental resistance indices were available in a subgroup of *n = 6754* mothers. We excluded pregnancies not leading to singleton live-born children and mothers without information about prenatal or postnatal offspring follow-up data available. Thus, our population for analysis involved 6716 mothers and their children (**Figure 3.2.1**).

Figure 3.2.1. Flow chart of the participants



Feto-placental and utero-placental vascular function measurements

Placental vascular resistance was evaluated with recorded flow velocity waveforms from the umbilical and uterine arteries in third trimester in a dedicated research center, as described previously.[10] Umbilical and uterine artery vascular resistance indices are parameters of the feto-placental circulation and utero-placental circulation, respectively.[8] A raised uterine artery resistance index and umbilical artery pulsatility index indicate increased placental resistance.[8] Umbilical artery pulsatility index was measured in a free-floating loop of the umbilical cord. Uterine artery resistance index was measured in the uterine arteries near the crossover with the external iliac artery. For each measurement, 3 consecutive uniform waveforms were recorded by pulsed Doppler ultrasound, during fetal apnea and without fetal movement. The mean of three measurements was used for further analysis. We assessed reproducibility of the ultrasound measurements in a subgroup and observed high intraclass correlation coefficient values (>0.80) with corresponding low coefficient of variation values (<10%), which indicates adequate reproducibility for these ultrasound measurements.[9]

Fetal and early childhood growth measurements

Fetal ultrasound examinations were performed in first (median: 13.5 weeks of gestation; 95% range: [defined as 2.5th and 97.5th percentile] 10.6, 17.5), second (median: 20.6 weeks of gestation; 95% range: 18.6, 23.4), and third trimester (median: 30.3 weeks of gestation; 95% range: 28.4, 33.0). We established gestational age by using data from the first fetal ultrasound examinations.[13] Third trimester fetal head circumference, abdominal circumference, and femur length were measured to the nearest millimeter using standardized ultrasound procedures.[14] Estimated fetal weight was calculated using the formula of Hadlock et al.[15] We constructed gestational age-adjusted standard deviation scores (SDS) for all fetal growth measurements.[13] Information about childhood sex, gestational age, weight, and length at birth was obtained from medical records. Gestational-age-adjusted SDS for birth weight and length were constructed using North-European growth standards.[16] These gestational-age-adjusted SDS for fetal growth and birth characteristics represent the equivalent of z-scores.

Well-trained staff in Community Health Centers obtained postnatal growth characteristics according to standard schedule and procedures at the ages of 6 months (median 6.2 mo; 95% range: 5.2, 8.2), 12 months (median: 11.1 mo; 95% range: 10.1, 12.5), 24 months (median: 24.8 mo; 95% range: 23.4, 28.2), 36 months (median: 36.7 mo; 95% range: 35.6, 40.9), and 48 months (median: 45.8 mo; 95% range: 44.4, 48.6). SDS for postnatal growth characteristics were obtained with Dutch growth reference Charts (Growth Analyzer 3.0; Dutch Growth Research Foundation, Rotterdam, The Netherlands).

Childhood adiposity and cardiovascular outcomes

At the age of 6 years, children visited a dedicated research center.[11] We measured children's height and weight without shoes and heavy clothing. Body mass index was calculated. Body composition was measured by dual-energy X-ray absorptiometry scan (iDXA, General Electrics – Lunar, 2008, Madison, WI). Total fat mass was calculated as percentage of total body weight measured by dual-energy X-ray absorptiometry. Android/gynoid fat mass ratio was calculated, and expressed as percentage.[17]

Two-dimensional M-mode echocardiographic measurements of the interventricular end-diastolic septal thickness, left ventricular end-diastolic diameter and left ventricular end-diastolic posterior wall thickness were performed using methods recommended by the American Society of Echocardiography, and left ventricular mass was calculated.[18,19] Systolic and diastolic blood pressure of the children were measured at the right brachial artery, 4 times with 1-minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus TM (Paramus, NJ). A cuff was selected with a cuff width ≈40% of the arm circumference and long enough to cover 90% of the arm circumference.[20] We calculated the mean value for systolic and diastolic blood pressure using the last 3 blood pressure measurements.

Covariates

Information on maternal age was assessed at intake.[11] Maternal weight and height were assessed at enrollment, and body mass index was calculated. Information about maternal parity, ethnicity, education level and folic acid supplementation use was obtained at enrollment. Information about maternal smoking was assessed by questionnaire during pregnancy. Information on pregnancy complications was obtained from medical records.[21] Information about breastfeeding was obtained by questionnaires.

Statistical analysis

First, we explored the associations of third trimester placental resistance indices with repeatedly measured fetal and childhood growth characteristics ([femur] length and [estimated fetal] weight) using unbalanced repeated measurement regression models. These models take the correlation between repeated measurements of the same subject into account and allow for incomplete outcome data.[22,23] For these analyses, we used growth characteristics in SDS. The effect estimates for these associations are shown per standard deviation change in placental resistance indices to enable comparison of effect estimates. Second, we examined the associations of third trimester placental vascular function with childhood body fat distribution and cardiovascular development using multivariate linear regression models. All models were adjusted for gestational age at enrollment and at placental vascular resistance measurement, maternal age, parity, ethnicity, educational level, prepregnancy body mass index, smoking during pregnancy, folic acid supplementation use, pregnancy complications, gestational age at

birth and child sex, infant breastfeeding, and age at outcome measurement. These covariates were selected based on their associations with the outcomes of interest based on previous studies or a change in effect estimate of >10%. Supplementary Tables S3.2.1-3.2.3 in the Supplementary Material show the associations of each covariate with the outcomes of interest. All body fat distribution outcomes were additionally adjusted for child's height, and all cardiovascular outcomes were additionally adjusted for child's body mass index. To explore whether birth weight explained these associations, analyses were additionally adjusted for gestational-age-adjusted birth weight. We tested potential interactions between placental vascular function and sex, and between placental vascular function and birth weight for the analyses focused on childhood outcomes. Because significant interactions with sex, but not with birth weight, were present, all analyses were performed for the total group and for boys and girls separately. Missing data of covariates were imputed using multiple imputations (details given in the Supplementary Material). The repeated measurement analysis was performed using the Statistical Analysis System version 9.2 (SAS, Institute Inc. Cary NC), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL).

Results

Subject characteristics

Characteristics of the participants are shown in Table 3.2.1. Supplementary Table S3.2.4 shows fetal and childhood growth characteristics. Supplementary Table S3.2.5 shows that mothers whose children participated in follow-up measurements were more often higher educated and from European descent as compared with mothers whose children did not participate.

Table 3.2.1. Maternal and childhood characteristics (*n = 6716*)[1]

Characteristics	Total Group *n = 6716*	Boys *n = 3424*	Girls *n = 3292*
Maternal characteristics			
Age, mean (SD), years	29.7 (5.3)	29.7 (5.3)	29.8 (5.3)
Height, mean (SD), cm	167.3 (7.4)	167.3 (7.3)	167.3 (7.5)
Prepregnancy weight, mean (SD), kg	66.1 (12.5)	66.0 (12.6)	66.2 (12.4)
Prepregnancy Body Mass Index, mean (SD), kg/m^2	23.5 (4.2)	23.5 (4.3)	23.6 (4.2)
Gestational age at intake, median (95% range), weeks	13.8 (9.8, 25.5)	13.8 (9.6, 24.9)	13.7 (9.8, 26.5)
Education, No. (%)			
Primary	676 (10.9)	341 (10.8)	335 (11.0)
Secondary	2833 (45.6)	1455 (45.8)	1378 (45.3)
Higher	2707 (43.5)	1375 (43.4)	1332 (43.7)
Ethnicity, No. (%)			
Dutch or European	3768 (58.6)	1904 (58.0)	1864 (59.2)
Non – European	2666 (41.4)	1381 (42.0)	1285 (40.8)
Parity, No. (%)			
Nulliparous	3767 (56.7)	1912 (56.4)	1855 (57.0)
Multiparous	2882 (43.3)	1480 (43.6)	1402 (43.0)
Folic acid supplement use, No. (%)			
Yes	3651 (72.3)	1819 (71.2)	1832 (73.5)
No	1397 (27.7)	736 (28.8)	661 (26.5)
Smoking, No. (%)			
Yes	1621 (27.3)	864 (28.6)	757 (26.0)
No	4316 (72.7)	2157 (71.4)	2159 (74.0)
Third trimester placental resistance indices			
Umbilical artery pulsatility index, mean (SD)	0.98 (0.17)	0.97 (0.17)	1.00 (0.17)
Uterine artery resistance index, mean (SD)	0.49 (0.08)	0.49 (0.08)	0.48 (0.08)
Pregnancy complications			
Gestational hypertension, No. (%), Yes	228 (3.6)	123 (3.8)	105 (3.4)
Pre-eclampsia, No. (%), Yes	130 (2.1)	63 (2.0)	67 (2.2)
Diabetes gravidarum, No. (%), Yes	58 (0.9)	32 (1.0)	26 (0.8)
Birth and infant characteristics			
Gestational age, median (95% range), weeks	40.1 (36.0, 42.3)	40.1 (36.0, 42.4)	40.1 (36.1, 42.2)
Birth weight, mean (SD), g	3430 (536)	3487 (547)	3370 (518)
Breastfeeding No. (%), Yes	4670 (91.9)	2358 (91.7)	2312 (92.2)
Childhood characteristics			
Age at follow up, median (95% range), years	6.0 (5.6, 7.7)	6.0 (5.6, 7.8)	6.0 (5.6, 7.7)
Length, mean (SD), cm	119.1 (5.8)	119.6 (5.8)	118.7 (5.8)
Weight, mean (SD), kg	23.1 (4.1)	23.3 (3.9)	23.0 (4.2)
Body mass index, mean (SD), kg/m^2	16.2 (1.8)	16.2 (1.7)	16.2 (1.9)
Total fat mass, mean (SD),%	24.8 (5.6)	22.6 (4.9)	27.1 (5.3)
Android/gynoid fat mass ratio, mean (SD),%	25.1 (6.3)	24.8 (5.8)	25.4 (6.8)
Left ventricular mass, mean (SD), g	53.4 (11.6)	55.9 (11.7)	50.7 (10.6)
Systolic blood pressure, mean (SD), mmHg	102.6 (8.1)	102.2 (7.9)	103.1 (8.4)
Diastolic blood pressure, mean (SD), mmHg	60.6 (6.8)	60.0 (6.8)	61.3 (6.8)

[1]Values are means (standard deviations) or medians (95% range) or observed numbers (valid percentages). Valid percentages represent the percentage of only non-missing cases in each category of categorical variables.

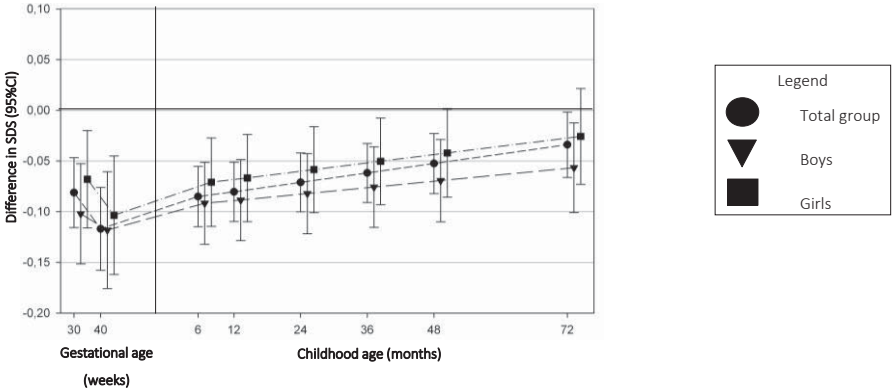
Placental vascular function and fetal and childhood growth characteristics

Higher third trimester umbilical artery pulsatility index and uterine artery resistance index were associated with lower third trimester fetal length and weight growth, resulting in a smaller size at birth among boys and girls (difference in birth length and birth weight for the total group: −0.12 SDS (95% Confidence Interval (CI): −0.16, −0.08), −0.17 SDS (95% CI: −0.20, −0.14) per SD change in umbilical artery pulsatility index, and −0.09 SDS (95% CI: −0.14, −0.04), −0.16 SDS (95% CI: −0.20, −0.12) per SD change in uterine artery resistance index, respectively; Figure 3.2.2A–D). The effect estimates for the associations of third trimester umbilical artery pulsatility index and uterine artery resistance index with childhood length and weight growth became smaller from the age of 6 months onwards among boys and girls. At the age of 6 years, higher third trimester umbilical artery pulsatility index and uterine artery resistance index were still associated with a shorter stature and lower weight among all children (difference in length and weight at the age of 6 years for the total group: −0.03 SDS (95% CI: −0.06, 0), −0.03 SDS (95% CI: −0.06, 0) per SD change in umbilical artery pulsatility index, and −0.06 SDS (95% CI: −0.10, −0.02), −0.07 SDS (95% CI: −0.10, −0.03) per SD change in uterine artery resistance index, respectively). The interaction term of third trimester umbilical artery pulsatility index with sex for weight growth was significant in the repeated measurement regression model. Among boys, higher third trimester umbilical artery pulsatility index was associated with lower childhood weight growth until the age of 6 years, whereas among girls these associations were no longer significant from the age of 3 years onwards.

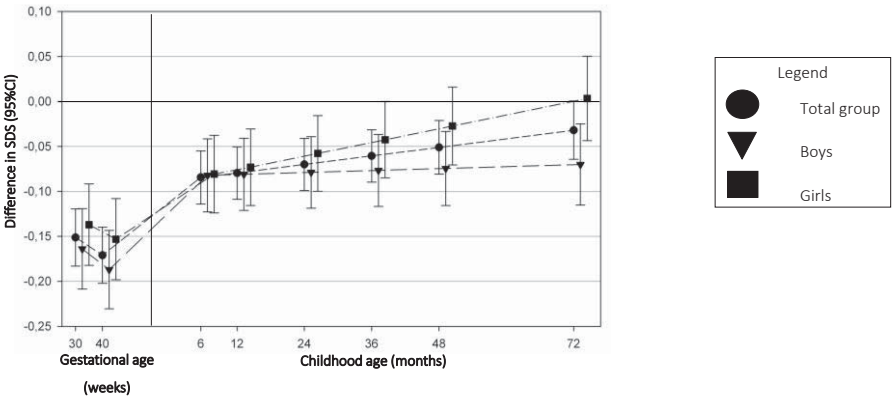
Placental vascular function and childhood cardiovascular risk factors

Table 3.2.2 shows the associations of third trimester umbilical artery vascular resistance with childhood cardiovascular outcomes at the age of 6 years, unadjusted and adjusted for gestational-age-adjusted birth weight, respectively. In the total group, we observed that, in the model unadjusted for birth weight, higher third trimester umbilical artery pulsatility index was associated with a lower childhood height and weight, but a higher total fat mass percentage and android/gynoid fat mass ratio (differences: −0.33 cm (95% CI: −0.48, −0.18); −0.14 kg (95% CI: −0.25, −0.04); 0.17 % (95% CI: 0.02, 0.31) and 0.26 % (95% CI: 0.08, 0.45) per SD change in third trimester umbilical artery pulsatility index, respectively). The associations of third trimester umbilical artery pulsatility index with childhood growth outcomes, but not body fat distribution outcomes, were largely explained by birth weight. Analysis stratified by sex, showed that a higher third trimester umbilical artery pulsatility index was associated with a higher childhood body mass index, total fat mass and android/gynoid fat mass ratio among girls (difference in body mass index, total fat mass percentage, android/gynoid fat mass ratio: 0.08 kg/m^2 (95% CI: 0.01, 0.16), 0.25 % (95% CI: 0.05, 0.46), and 0.43 % (95% CI: 0.15, 0.71) per SD change in third trimester umbilical artery pulsatility index in the fully adjusted model, respectively).

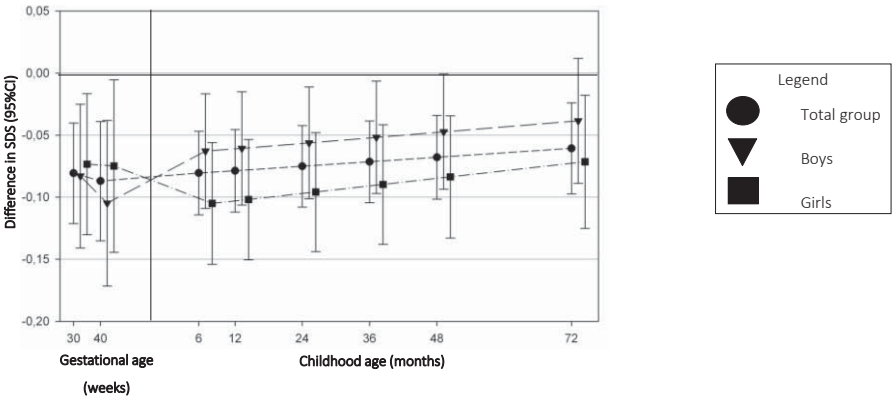
Figure 3.2.2. Associations of third trimester feto-placental vascular function and utero-placental vascular function with fetal and childhood growth characteristics



3.2.2a. Umbilical artery pulsatility index and length growth

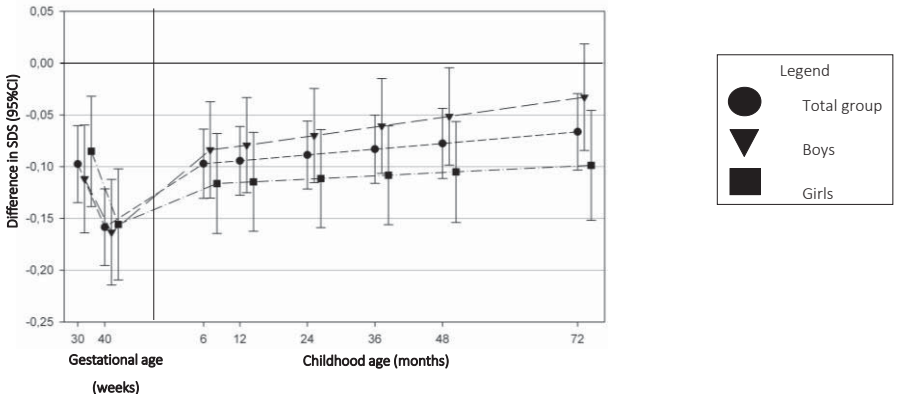


3.2.2b. Umbilical artery pulsatillity index and weight growth



3.2.2c. Uterine artery resistance index and length growth

Figure 3.2.2. Associations of third trimester feto-placental vascular function and utero-placental vascular function with fetal and childhood growth characteristics (*continued*)



3.2.2d. Uterine artery resistance index and weight growth

Fetal and childhood length and weight growth in SDS per SD change in third trimester umbilical artery pulsatility index and uterine artery resistance index. Results are based on repeated measurement regression models and reflect the differences in (gestational) age-adjusted standard deviation scores (SDS) of length and weight growth per SD change in third trimester umbilical artery vascular resistance and uterine artery vascular resistance at 30 weeks and 40 weeks of gestation prenatal and at 6 months, 12 months, 24 months, 36 months, 48 months and 72 months postnatal. All models are adjusted for gestational age at enrollment and at placental resistance index measurement, maternal age, parity, ethnicity, education, body mass index, smoking, folic acid supplementation use, and pregnancy complications. Total group analyses are additionally adjusted for child's sex. P-value for sex interaction <0.01 for model focused on third trimester umbilical artery vascular resistance and weight growth. Sex interaction terms were not significant in the other models.

A higher third trimester umbilical artery pulsatility index was associated with a lower childhood left ventricular mass and higher systolic blood pressure among all children (difference: -0.57 g (95% CI: −0.88, −0.25), 0.31 mmHg (95% CI: 0.07, 0.55) per SD change in third trimester umbilical artery pulsatility index, respectively). These associations were not explained by birth weight. Sex interaction terms were not significant, but a stronger association for systolic blood pressure tended to be present among girls.

Table 3.2.3 shows that a higher third trimester uterine artery resistance index was associated with a lower height and weight at the age of 6 years, but not with other cardiovascular risk factors. These associations were explained by birth weight.

Table 3.2.2. Associations of third trimester feto-placental vascular function with childhood anthropometrics, body fat distribution and cardiovascular development at 6 years of age[1]

	Difference (95% CI) in growth, body fat distribution and cardiovascular outcomes per SD-change of third trimester umbilical artery pulsatility index						
	Unadjusted for gestational-age-adjusted birth weight			Adjusted for gestational-age-adjusted birth weight[2]			*P-value for sex interaction*
	Total group[3]	Boys	Girls	Total group[3]	Boys	Girls	
Childhood growth							
Height (cm)	-0.33 (-0.48, -0.18)*	-0.48 (-0.69, -0.27)*	-0.18 (-0.39, 0.04)	-0.11 (-0.25, 0.04)	-0.21 (-0.42, -0.01)*	-0.01 (-0.22, 0.20)	*0.04*
Weight (kg)	-0.14 (-0.25, -0.04)*	-0.29 (-0.44, -0.15)*	0 (-0.15, 0.16)	0.01 (-0.10, 0.12)	-0.11 (-0.26, 0.03)	0.13 (-0.03, 0.28)	*0.004*
Body mass index (kg/m^2)	-0.02 (-0.07, 0.03)	-0.08 (-0.15, -0.01)*	0.04 (-0.03, 0.12)	0.03 (-0.02, 0.08)	-0.02 (-0.10, 0.05)	0.08 (0.01, 0.16)*	*0.01*
Body fat distribution[4]							
Total fat mass (%)	0.17 (0.02, 0.31)*	0.06 (-0.14, 0.27)	0.26 (0.06, 0.47)*	0.16 (0.01, 0.31)*	0.06 (-0.15, 0.26)	0.25 (0.05, 0.46)*	*0.04*
Android/gynoid fat mass ratio (%)	0.26 (0.08, 0.45)*	0.07 (-0.18, 0.32)	0.45 (0.17, 0.72)*	0.25 (0.06, 0.44)*	0.06 (-0.19, 0.32)	0.43 (0.15, 0.71)*	*0.02*
Cardiovascular outcomes[5]							
Left ventricular mass (g)	-0.57 (-0.88, -0.25)*	-0.67 (-1.15, -0.20)*	-0.48 (-0.90, -0.06)*	-0.32 (-0.63, 0.00)*	-0.36 (-0.84, 0.12)	-0.29 (-0.71, 0.13)	*0.17*
Systolic blood pressure (mmHg)	0.31 (0.07, 0.55)*	0.24 (-0.09, 0.57)	0.40 (0.04, 0.75)*	0.26 (0.02, 0.50)*	0.19 (-0.15, 0.53)	0.33 (-0.02, 0.69)	*0.23*
Diastolic blood pressure (mmHg)	-0.12 (-0.33, 0.08)	-0.20 (-0.49, 0.10)	-0.05 (-0.34, 0.24)	-0.19 (-0.40, 0.02)	-0.26 (-0.57, 0.04)	-0.10 (-0.40, 0.19)	*0.39*

[1]Values are regression coefficients (95% Confidence Interval) and reflect differences in childhood anthropometrics, body fat distribution and cardiovascular outcomes per standard deviation change of placental resistance index. All models are adjusted for gestational age at enrollment and at placental resistance index measurement, maternal age, parity, ethnicity, education, body mass index, smoking, folic acid supplementation use, pregnancy complications, gestational age at birth, infant breastfeeding, childhood age at measurement. [2]Model additionally adjusted for gestational-age-adjusted birth weight. [3]Models for total group are additionally adjusted for child's sex. [4]Models focused on body fat distribution are additionally adjusted for childhood height at measurement. [5]Models focused on cardiovascular outcomes are additionally adjusted for childhood body mass index at measurement. *P-value <0.05.

Table 3.2.3. Associations of third trimester utero-placental vascular function with childhood anthropometrics, body fat distribution and cardiovascular development at 6 years of age[1]

	Difference (95% CI) in growth, body fat distribution and cardiovascular outcomes per SD-change of third trimester uterine artery resistance index						
	Unadjusted for gestational-age-adjusted birth weight			Adjusted for gestational-age-adjusted birth weight[2]			*P-value for sex interaction*
	Total group[3]	Boys	Girls	Total group[3]	Boys	Girls	
Childhood growth							
Height (cm)	-0.27 (-0.45, -0.10)*	-0.24 (-0.49, 0.01)	-0.32 (-0.58, -0.07)*	-0.09 (-0.27, 0.08)	0 (-0.24, 0.24)	-0.19 (-0.43, 0.06)	*0.39*
Weight (kg)	-0.15 (-0.27, -0.02)*	-0.11 (-0.28, 0.06)	-0.20 (-0.38, -0.01)*	-0.02 (-0.14, 0.11)	0.04 (-0.13, 0.21)	-0.09 (-0.27, 0.09)	*0.24*
Body mass index (kg/m^2)	-0.03 (-0.09, 0.03)	-0.01 (-0.09, 0.07)	-0.06 (-0.14, 0.03)	0.01 (-0.05, 0.07)	0.03 (-0.05, 0.11)	-0.02 (-0.10, 0.07)	*0.28*
Body fat distribution[4]							
Total fat mass (%)	-0.02 (-0.18, 0.15)	-0.02 (-0.25, 0.22)	0 (-0.24, 0.23)	-0.02 (-0.19, 0.15)	-0.04 (-0.28, 0.20)	0 (-0.24, 0.24)	*0.74*
Android/gynoid fat mass ratio (%)	0.01 (-0.21, 0.23)	-0.03 (-0.33, 0.27)	0.06 (-0.26, 0.37)	0 (-0.21, 0.22)	-0.06 (-0.36, 0.25)	0.06 (-0.25, 0.38)	*0.86*
Cardiovascular outcomes[5]							
Left ventricular mass (g)	0 (-0.38, 0.38)	-0.06 (-0.63, 0.51)	0.07 (-0.43, 0.57)	0.20 (-0.17, 0.58)	0.25 (-0.32, 0.81)	0.20 (-0.30, 0.69)	*0.83*
Systolic blood pressure (mmHg)	0.13 (-0.15, 0.41)	0.25 (-0.14, 0.64)	-0.01 (-0.43, 0.40)	0.07 (-0.21, 0.36)	0.21 (-0.19, 0.60)	-0.07 (-0.49, 0.34)	*0.36*
Diastolic blood pressure (mmHg)	0.03 (-0.21, 0.28)	0.08 (-0.27, 0.43)	-0.10 (-0.36, 0.34)	-0.04 (-0.28, 0.21)	0.01 (-0.35, 0.36)	-0.07 (-0.41, 0.28)	*0.85*

[1]Values are regression coefficients (95% Confidence Interval) and reflect differences in childhood anthropometrics, body fat distribution and cardiovascular outcomes per standard deviation change of placental resistance index. All models are adjusted for gestational age at enrollment and at placental resistance index measurement, maternal age, parity, ethnicity, education, body mass index, smoking, folic acid supplementation use, pregnancy complications, gestational age at birth, infant breastfeeding, childhood age at measurement. [2]Model additionally adjusted for gestational-age-adjusted birth weight. [3]Models for total group are additionally adjusted for child's sex. [4]Models focused on body fat distribution are additionally adjusted for childhood height at measurement. [5]Models focused on cardiovascular outcomes are additionally adjusted for childhood body mass index at measurement. *P-value <0.05.

Discussion

In this population-based prospective cohort study, we observed that higher third trimester umbilical artery and uterine artery resistance indices were associated with lower fetal growth rates in third trimester, resulting in a smaller size at birth. Differences in length and weight growth characteristics became smaller from the age of 6 months onwards but persisted until the age of 6 years. Higher third trimester feto-placental vascular resistance, but not utero-placental vascular resistance, was associated with childhood cardiovascular adaptations. These associations were only partly explained by birth weight and appeared to be stronger among girls than among boys.

Methodological considerations

This study had a prospective data collection from early fetal life onwards. We had a large sample size of 6716 pregnant women and their children. To our knowledge, this is the first study that examined the associations of placental vascular resistance with repeatedly measured fetal and childhood growth characteristics and childhood cardiovascular risk factors. A potential limitation might be the response rate of 61%. Pregnant women who participated were higher educated, healthier, and more frequently of Dutch origin than were those who did not participate.[11] It is unlikely that this selective response at baseline has led to biased estimates.[24] Follow-up data at the age of 6 years were available in 70% of our study population. Mothers without offspring follow-up data available were more often lower educated and from non-European descent. The non-response would lead to biased effect estimates if the associations would be different between those included and not included in the analyses. This seems unlikely. However, non-response at baseline and at follow-up might affect the generalizability of our results. Because our study population is a relatively healthy, low-risk population, the variation in placental vascular function was small. It might be of interest to perform similar analyses among higher risk populations. Detailed information about a large number of potential confounding variables was available. Extensive adjustment for these socio-demographic and lifestyle-related determinants in our analyses did not explain the associations of placental vascular dysfunction with childhood outcomes. However, residual confounding attributable to other lifestyle-related variables, such as maternal and childhood nutritional intake and physical activity, might still be an issue, as in any observational study.

Interpretation of main findings

Developmental adaptations in response to suboptimal fetal nutrition may lead to fetal growth restriction and subclinical cardiovascular alterations on short term, and may predispose to obesity and cardiovascular disease in adulthood.[4] Because fetal nutrition largely depends on placental function, the placenta may play a key role in this

developmental origins hypothesis.[5,7] Placental dysfunction not only affects fetal nutrient supply, but may also directly affect the fetal cardiovascular system.[5,7,9]

We examined the associations of umbilical and uterine artery blood flow during pregnancy, as detailed measures of feto-placental and utero-placental vascular function, with fetal and childhood outcomes. On the fetal side, blood enters the placenta through the umbilical arteries, which form a capillary network in the terminal villi of the villous tree.[5,8] As the villous and capillary surface areas increase during pregnancy, the umbilical artery vascular resistance normally decreases throughout pregnancy.[5,8] On the other side, maternal blood enters the intervillious space in the placenta through the spiral arteries, which descend from the uterine arteries.[5,8] Normally, during early pregnancy, the spiral arteries are remodeled as a result of trophoblastic invasion, which changes the spiral arteries from narrow muscular vessels into wide non-muscular arteries, leading to the development of a high-flow and low-resistance circulation.[5,8] Both the umbilical and uterine artery vascular resistance provide information about the placental circulation, but assessment of vascular resistance in the umbilical artery may be more closely related to the fetal condition. Abnormalities in the umbilical artery vascular resistance are strongly related to intra-uterine fetal growth restriction and fetal distress.[25] Furthermore, endothelium in the feto-placental circulation forms a continuum with fetal endothelium, and higher umbilical artery vascular resistance may therefore also reflect fetal vascular adaptations.[26] Abnormal placentation may lead to fetal endothelial dysfunction and inflammatory responses, which may predispose the individual to development of arteriosclerosis and hypertension later in life.[26–28] In line with this hypothesis, we observed stronger associations of arterial vascular resistance variation with fetal and childhood outcomes in the umbilical artery than in the uterine artery.

Multiple studies have shown that abnormal umbilical artery and uterine artery resistance indices in each trimester of pregnancy are associated with the risk of small size for gestational age at birth.[29,30] These studies were mainly performed among high-risk populations and used cut-off values to define abnormal resistance indices. In line with these previous studies, we observed that also among a low risk population, small changes in third trimester feto-placental and utero-placental vascular resistance were associated with lower fetal growth characteristics from third trimester onwards. Not much is known about postnatal growth and cardiovascular consequences of placental vascular dysfunction. A study among 914 mother-neonate pairs showed that placental volume at 19 weeks of gestation was positively associated with neonatal fat mass assessed by dual-energy X-ray absorptiometry.[31] A study among 23.967 mother-child pairs observed that placental lateral growth measures, which are measures that give some information about the umbilical-chorionic vessels and the number of spiral arteries supplying the placenta, are related to childhood body mass index.[32] These associations were small and partly explained by birth weight. We observed that higher third trimester utero-placental vascular resistance was associated with lower childhood length and weight growth characteristics, but these associations were fully explained by birth weight. We further observed that higher third trimester feto-placental vascular resistance was associated with small differences in body mass index and fat mass levels at

the age of 6 years, especially among girls. These associations were independent of birth weight.

The feto-placental vascular resistance is related to fetal vascular function and an important determinant of fetal cardiac afterload. Changes in feto-placental vascular function may therefore be related to fetal cardiac and vascular development.[5,28,33,34] We observed that higher third trimester umbilical artery pulsatility index was associated with a lower left ventricular mass and higher systolic blood pressure in childhood. The mechanisms by which feto-placental vascular dysfunction may lead to smaller left ventricular mass have not been studied yet. Studies in sheep have shown that placental insufficiency is associated with a smaller fetal heart and lower number of immature cardiomyocytes.[34,35] It has also been suggested that mostly the right side of the fetal heart is affected by increased placental vascular resistance.[34,36] We had only information about left cardiac structures available. More detailed studies of the structure and function of both the left and right ventricles may provide more information about the persistent cardiac consequences of placental dysfunction. Our findings related to systolic blood pressure are in line with a study among 428 Jamaican mothers and their children, which reported an inverse association of placental volume assessed at 20 weeks of gestation and systolic blood pressure in early childhood.[37] Also, a study among 13.273 mothers and their children observed that higher placental weight and size were associated with lower infancy systolic blood pressure, but higher childhood systolic blood pressure. In the same study, placental vascular lesions, which may also reduce feto-placental blood flow, were associated with higher infancy systolic blood pressure.[38]

Sex-specific differences for the associations of third trimester feto-placental vascular function with childhood growth and body fat distribution, with stronger associations among girls, tended to be present. Previous studies have suggested that sex-specific fetal responses occur in response to an adverse prenatal environment.[39,40] Our observed sex-differences may partly be explained by sex-differences in childhood growth and body fat distribution, and differences in in-utero responses to an adverse environment. We did not observe significant sex-specific differences for the association of third trimester feto-placental vascular function with childhood systolic blood pressure, which is inconsistent with several other studies that examined associations of placental size at birth with blood pressure at older ages.[41–43] These suggested sex-differences related to blood pressure might become more apparent at later ages. We also explored whether associations of placental vascular resistance with childhood outcomes differed among birth weight categories, but no significant interaction terms were present. This suggests that associations of feto-placental vascular resistance with childhood cardiovascular development may be present across the full range of birth weight.

The observed effect estimates for the associations of feto-placental vascular resistance with childhood cardiovascular risk factors were small. Although they are important from a cardiovascular developmental perspective, their effects on the risk of cardiovascular disease should be further studied. However, previous studies have shown that childhood cardiovascular risk factors tend to track into adulthood. A study among 2204 subjects showed that childhood body mass index and blood pressure,

measured at 6 years of age, were correlated with these measures in adulthood.[44] A large meta-analyses in which tracking of blood pressure from children aged <18 years to adulthood was examined, showed that blood pressure tracking was already present from early childhood onwards.[45] A study among 4857 children and adolescents, aged 5 to 20 years, with a median age of 11 years, showed that childhood obesity and hypertension were associated with increased rates of premature death from endogenous causes.[46] Thus, these findings suggest that even subclinical differences in risk factors for cardiovascular disease in childhood are related to the development of cardiovascular disease in later life. Further studies are needed to gain further insight in the associations of placental vascular function with cardiovascular risk factors in childhood and adulthood, and to explore their potential underlying mechanisms.

Conclusion

Suboptimal fetal nutrition may lead to fetal growth and cardiovascular developmental adaptations, and subsequently to cardiovascular disease in adulthood. Our study shows that higher third trimester umbilical and uterine artery vascular resistance were associated with lower fetal growth characteristics and a smaller size at birth. Higher third trimester feto-placental vascular resistance, but not utero-placental vascular resistance, was associated with an adverse cardiovascular profile in childhood. These associations were only partly explained by birth weight. Further studies examining detailed measures of placental function, such as placental morphology, vascular function and nutrient transporter activity, and their associations with growth and cardio-metabolic outcomes in later life might provide further insight in underlying mechanisms.

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Supplementary Material

Supplementary Methods S3.2.1. Multiple imputations for missing data of covariates

We imputed missing data of the covariates using multiple imputations (1). The percentages of missing values for the covariates within the population for analysis were lower than 20% except for folic acid supplement use (24.8%) and breastfeeding (24.4%). For the multiple imputation, we used Fully Conditional Specification, an iterative of the Markov chain Monte Carlo approach. For each variable, the fully conditional specification method fits a model using all other available variables in the model as predictors, and then imputes missing values for the specific variable being fit. In the imputation model, we included all covariates, plus maternal height measured at enrolment, maternal weight and blood pressure measured in first, second and third trimester, second trimester uterine and umbilical artery vascular resistance, placental weight, household income and breastfeeding duration. Furthermore, we additionally added the studied determinants and outcomes in the imputation model as prediction variables only; they were not imputed themselves. Five imputed datasets were created and analysed together.

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Supplementary Table S3.2.1. Associations of covariates with childhood anthropometric measures[1]

	Childhood anthropometric measures		
Covariates included in the models	Height (cm)	Weight (kg)	Body mass index (kg/m^2)
Gestational age at intake (wks)	0.06 (0.02, 0.10)*	0.08 (0.05, 0.11)*	0.04 (0.02, 0.05)*
Gestational age at placental resistance measurement (wks)	0.06 (-0.13, 0.25)	-0.02 (-0.16, 0.11)	-0.03 (-0.09, 0.04)
Maternal age (yr)	-0.06 (-0.09, -0.03)*	-0.09 (-0.11, -0.07)*	-0.04 (-0.06, -0.03)*
Parity			
Nulliparous	*Reference*	*Reference*	*Reference*
Multiparous	-0.25 (-0.58, 0.09)	0.05 (-0.19, 0.29)	0.09 (-0.01, 0.20)
Ethnicity			
Dutch or European	*Reference*	*Reference*	*Reference*
Non – European	0.05 (-0.30, 0.39)	1.08 (0.84, 1.31)*	0.70 (0.59, 0.80)*
Education			
Primary	0.30 (-0.31, 0.92)	1.66 (1.24, 2.08)*	1.01 (0.82, 1.20)*
Secondary	0.42 (0.06, 0.77)*	1.01 (0.77, 1.26)*	0.54 (0.43, 0.65)*
Higher	*Reference*	*Reference*	*Reference*
Prepregnancy body mass index (kg/m^2)	0.12 (0.08, 0.17)*	0.25 (0.22, 0.28)	0.13 (0.12, 0.15)*
Smoking			
Yes	0.06 (-0.34, 0.46)	0.53 (0.25, 0.80)*	0.32 (0.20, 0.45)
No	*Reference*	*Reference*	*Reference*
Folic acid supplement use			
Yes	*Reference*	*Reference*	*Reference*
No	0.54 (0.16, 0.92)*	1.05 (0.79, 1.32)*	0.54 (0.43, 0.66)*
Maternal pregnancy complications			
Yes	0.60 (-0.09, 1.29)	0.60 (0.12, 1.08)*	0.25 (0.03, 0.47)*
No	*Reference*	*Reference*	*Reference*
Child's sex			
Boys	*Reference*	*Reference*	*Reference*
Girls	-0.91 (-1.25, -0.58)*	-0.30 (-0.53, -0.06)*	0.03 (-0.08, 0.13)
Gestational age at birth (wks)	0.08 (-0.03, 0.18)	0.01 (-0.07, 0.08)	-0.01 (-0.04, 0.02)
Breastfeeding			
Yes	0.44 (-0.23, 1.10)	0.08 (-0.37, 0.53)	-0.03 (-0.24, 0.17)
No	*Reference*	*Reference*	*Reference*
Childhood age at outcome measurement (yr)	6.10 (5.81, 6.40)*	3.63 (3.41, 3.85)*	0.73 (0.62, 0.83)*

[1]Values are regression coefficients (95% Confidence Interval) from univariate regression models and reflect differences in childhood anthropometric measures per unit change of each covariate and for different categories of each covariate as compared to the reference group. *P-value <0.05.

Supplementary Table S3.2.2. Associations of covariates with childhood body fat distribution outcomes[1]

	Childhood body fat mass measures	
Covariates included in the models	Total body fat mass (%)	Android/gynoid fat mass ratio (%)
Gestational age at intake (wks)	0.08 (0.04, 0.12)*	0.06 (0.01, 0.10)*
Gestational age at placental resistance measurement (wks)	-0.27 (-0.46, -0.09)*	-0.16 (-0.37, 0.05)
Maternal age (yr)	-0.15 (-0.18, -0.12)*	-0.13 (0.16, -0.09)*
Parity		
Nulliparous	*Reference*	*Reference*
Multiparous	-0.14 (-0.47, 0.19)	-0.12 (-0.49, 0.25)
Ethnicity		
Dutch or European	*Reference*	*Reference*
Non – European	2.17 (1.84, 1.50)*	1.59 (1.22, 1.97)*
Education		
Primary	3.38 (2.80, 3.95)*	2.24 (1.58, 2.91)*
Secondary	2.16 (1.83, 2.50)*	1.40 (1.02, 1.79)*
Higher	*Reference*	*Reference*
Prepregnancy body mass index (kg/m^2)	0.35 (0.31, 0.39)*	0.26 (0.21, 0.31)*
Smoking		
Yes	0.79 (0.41, 1.18)*	1.47 (1.04, 1.91)*
No	*Reference*	*Reference*
Folic acid supplement use		
Yes	*Reference*	*Reference*
No	1.56 (1.20, 1.92)*	1.29 (0.88, 1.69)*
Maternal pregnancy complications		
Yes	0.94 (0.27, 1.61)*	0.65 (-0.11, 1.41)
No	*Reference*	*Reference*
Child's sex		
Boys	*Reference*	*Reference*
Girls	4.44 (4.14, 4.74)*	0.61 (0.25, 0.98)*
Gestational age at birth (wks)	-0.21 (-0.32, -0.11)*	-0.21 (-0.33, -0.09)*
Breastfeeding		
Yes	-0.31 (-0.96, 0.33)	0.11 (-0.61, 0.83)
No	*Reference*	*Reference*
Childhood age at outcome measurement (yr)	1.31 (0.97, 1.65)*	1.40 (1.02, 1.79)*

[1]Values are regression coefficients (95% Confidence Interval) from univariate regression models and reflect differences in childhood body fat distribution outcomes per unit change of each covariate and for different categories of each covariate as compared to the reference group. *P-value <0.05.

Supplementary Table S3.2.3. Associations of covariates with childhood cardiovascular outcomes[1]

	Childhood cardiovascular outcomes		
Covariates included in the models	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Left ventricular mass (g)
Gestational age at intake (wks)	0.06 (0, 0.12)*	0.03 (-0.02, 0.08)	0.05 (-0.03, 0.14)
Gestational age at placental resistance measurement (wks)	-0.18 (-0.46, 0.09)	-0.17 (-0.40, 0.06)	0.40 (0.01, 0.80)*
Maternal age (yr)	-0.12 (-0.16, -0.07)*	-0.11 (-0.15, -0.07)*	-0.04 (-0.11, 0.03)
Parity			
Nulliparous	*Reference*	*Reference*	*Reference*
Multiparous	-0.30 (-0.78, 0.19)	-0.42 (-0.82, -0.01)*	0.32 (-0.36, 1.00)
Ethnicity			
Dutch or European	*Reference*	*Reference*	*Reference*
Non – European	1.42 (0.93, 1.91)*	1.13 (0.72, 1.55)*	-0.87 (-1.57, -0.17)*
Education			
Primary	2.69 (1.81, 3.56)*	1.89 (1.15, 2.63)*	-0.15 (-1.40, 1.10)
Secondary	1.67 (1.16, 2.18)*	1.33 (0.90, 1.76)*	-0.07 (-0.79, 0.66)
Higher	*Reference*	*Reference*	*Reference*
Prepregnancy body mass index (kg/m^2)	0.21 (0.14, 0.27)*	0.07 (0.02, 0.13)*	0.23 (0.14, 0.32)*
Smoking			
Yes	0.76 (0.18, 1.33)*	0.65 (0.17, 1.13)*	0.57 (-0.24, 1.38)
No	*Reference*	*Reference*	*Reference*
Folic acid supplement use			
Yes	*Reference*	*Reference*	*Reference*
No	1.13 (0.58, 1.67)*	0.39 (-0.06, 0.85)	0.11 (-0.67, 0.88)
Maternal pregnancy complications			
Yes	1.02 (0.05, 2.00)*	1.26 (0.44, 2.08)*	2.12 (0.72, 3.52)*
No	*Reference*	*Reference*	*Reference*
Child's sex			
Boys	*Reference*	*Reference*	*Reference*
Girls	0.86 (0.39, 1.33)*	1.30 (0.90, 1.70)*	-5.13 (-5.79, -4.48)*
Gestational age at birth (wks)	-0.30 (-0.45, -0.14)*	-0.19 (-0.32, -0.06)*	0.30 (0.08, 0.51)*
Breastfeeding			
Yes	-0.41 (-1.39, 0.57)	-0.56 (-1.39, 0.26)	1.12 (-0.27, 2.51)
No	*Reference*	*Reference*	*Reference*
Childhood age at outcome measurement (yr)	2.39 (1.90, 2.88)*	1.19 (0.77, 1.60)*	5.19 (4.52, 5.89)*

[1]Values are regression coefficients (95% Confidence Interval) from univariate regression models and reflect differences in childhood cardiovascular outcomes per unit change of each covariate and for different categories of each covariate as compared to the reference group. *P-value <0.05.

Supplementary Table S3.2.4. Fetal and childhood growth characteristics (*n = 6716*)[1]

Growth characteristics	Total *n = 6716*	Boys *n = 3424*	Girls *n = 3292*
Fetal growth characteristics			
Third trimester			
Gestational age, median (90% range), weeks	30.3 (28.8, 32.2)	30.5 (28.9, 32.3)	30.3 (28.8, 32.2)
Femur length, mean (SD), mm	57 (44)	57 (44)	58 (44)
Estimated fetal weight, mean (SD), g	1613 (253)	1623 (252)	1603 (254)
Birth			
Gestational age, median (90% range), weeks	39.9 (37.0, 42.0)	39.9 (36.9, 42.1)	39.8 (37.0, 42.0)
Birth length, mean (SD), cm	50.2 (2.4)	50.5 (2.4)	49.8 (2.2)
Birth weight, mean (SD), g	3417 (559)	3476 (574)	3358 (536)
Childhood growth characteristics			
6 months			
Age at follow up, median (90% range), months	6.2 (5.4, 7.5)	6.2 (5.4, 7.5)	6.2 (5.4, 7.5)
Length, mean (SD), cm	67.6 (2.6)	68.6 (2.5)	66.7 (2.5)
Weight, mean (SD), kg	7.9 (0.9)	8.2 (0.9)	7.6 (0.8)
12 months			
Age at follow up, median (90% range), months	11.1 (10.2, 12.3)	11.1 (10.2, 12.3)	11.1 (10.2, 12.3)
Length, mean (SD), cm	74.4 (2.7)	75.2 (2.6)	73.5 (2.6)
Weight, mean (SD), kg	9.8 (1.1)	10.0 (1.1)	9.3 (1.0)
24 months			
Age at follow up, median (90% range), months	24.8 (23.5, 27.5)	24.7 (23.5, 27.6)	24.8 (23.6, 27.4)
Height, mean (SD), cm	88.3 (3.5)	88.9 (3.4)	87.7 (3.5)
Weight, mean (SD), kg	13.0 (1.5)	13.2 (1.5)	12.7 (1.5)
36 months			
Age at follow up, median (90% range), months	36.7 (35.6, 39.8)	36.7 (35.6, 40.0)	36.7 (35.6, 39.6)
Height, mean (SD), cm	97.4 (3.8)	97.9 (3.8)	96.8 (3.8)
Weight, mean (SD), kg	15.3 (1.9)	15.5 (1.8)	15.0 (1.9)
48 months			
Age at follow up, median (90% range), months	45.8 (44.7, 48.0)	45.8 (44.7, 48.1)	45.8 (44.7, 47.9)
Height, mean (SD), cm	103.2 (4.1)	103.7 (4.1)	102.7 (4.2)
Weight, mean (SD), kg	17.0 (2.2)	17.2 (2.2)	16.7 (2.2)
72 months			
Age at follow up, median (90% range), months	72.1 (69.0, 86.1)	72.2 (68.9, 85.5)	72.2 (68.9, 85.6)
Height, mean (SD), cm	119.1 (5.8)	119.6 (5.8)	118.7 (5.8)
Weight, mean (SD), kg	23.1 (4.1)	23.3 (3.9)	23.0 (4.2)

[1]Values represent means (SD) and medians (90% range).

Supplementary Table S3.2.5. Non-response analysis (*n = 6716*) [1]

Characteristics	Cardiovascular follow-up at 6 years *n = 4722*	No cardiovascular follow-up at 6 years *n = 1994*	P-value[4]
Maternal characteristics			
Age, mean (SD), yr	30.4 (5.1)	28.3 (5.4)	<0.01
Height, mean (SD), cm	167.8 (7.4)	166.4 (7.4)	<0.01
Prepregnancy weight, mean (SD), kg	66.3 (12.3)	65.7 (13.0)	0.11
Prepregnancy body mass index, mean (SD), kg/m^2	23.5 (4.1)	23.6 (4.5)	0.51
Gestational age at intake, median (90% range), weeks[2]	13.6 (10.6, 22.2)	14.1 (10.6, 23.7)	<0.01
Parity, nulliparous, No. (%)[3]	2723 (58.0)	1045 (53.5)	<0.01
Education, No. (%)[3]			
Primary or secondary school	2339 (52.4)	1171 (66.8)	<0.01
Higher education	2124 (47.6)	583 (33.2)	
Race / Ethnicity, No. (%)[3]			
Dutch, other European	2883 (62.1)	885 (49.4)	<0.01
Non-European	1759 (37.9)	908 (50.6)	
Smoking habits, No. (%)[3]			
None	3112 (73.9)	1205 (69.7)	<0.01
Yes	1099 (26.1)	523 (30.3)	
Folic acid supplement use, No. (%)[3]			
No	846 (23.4)	552 (38.5)	<0.01
Yes	2769 (76.6)	882 (61.5)	
Uterine artery resistance index, mean (SD)			
Third trimester	0.48 (0.08)	0.49 (0.08)	0.03
Umbilical artery pulsatility index, mean (SD)			
Third trimester	0.98 (0.17)	0.99 (0.17)	0.06
Maternal pregnancy complications			
Gestational hypertension, No. (%), Yes[3]	175 (3.9)	53 (2.9)	0.03
Pre-eclampsia, No. (%), Yes[3]	81 (1.8)	49 (2.7)	0.03
Diabetes gravidarum, No. (%), Yes[3]	38 (0.8)	20 (1.1)	0.23
Birth and infant characteristics			
Males, No. (%)[3]	2383 (50.4)	1040 (52.3)	0.08
Gestational age at birth, median (90% range), weeks[2]	40.1 (37.1, 42.0)	40.1 (36.9, 42.1)	0.02
Birth weight, mean (SD), g	3448 (525)	3389 (560)	<0.01
Ever breastfeeding, No.(%), Yes[3]	3626 (92.5)	1044 (89.9)	<0.01

[1]Values are means (standard deviation). [2]Medians (90% range) [3]Values are observed numbers (valid percentages). Valid percentages represent the percentage of only non-missing cases in each category of categorical variables. [4]Differences in subject characteristics between the groups were evaluated using two-sample t test for unequal variances for normally distributed continuous variables, Wilcoxon rank sum test for not normally distributed continuous variables and chi-square tests for proportions.

Chapter 3.3

Tracking of fetal growth characteristics and adverse birth outcomes

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Abstract

Background: Fetal growth characteristics are used to identify influences of several maternal characteristics and to identify individuals at increased risk of adverse outcomes. The extent to which fetal growth characteristics track in different trimesters is not known.

Methods: In a population-based prospective cohort study among 8636 pregnant women, we examined the extent to which fetal growth characteristics track, are influenced by maternal socio-demographic and lifestyle-related determinants and are associated with birth outcomes. Fetal growth was assessed in each trimester and at birth.

Results: Correlation coefficient between first trimester crown-rump length and birth weight was r = 0.12 (P-value <0.05). Correlation coefficients for fetal head circumference, (femur) length and (estimated) fetal weight ranged from r = 0.16 to r = 0.30 (all p values <0.05) between second trimester and birth and from r = 0.36 to r = 0.58 (all p values <0.05) between third trimester and birth, and were highest for estimated fetal weight. Correlation coefficients for estimated fetal weight tended to be lower among overweight mothers, as compared to normal weight mothers, but were not influenced by other maternal characteristics. First, second and third trimester fetal growth characteristics were associated with risks of preterm birth and small size for gestational age at birth, with the strongest associations present in third trimester.

Conclusions: Fetal growth characteristics track moderately throughout gestation, with stronger tracking coefficients present in later pregnancy. Tracking coefficients were not materially influenced by maternal socio-demographic and lifestyle characteristics. First, second and third trimester fetal growth characteristics were associated with the risk of adverse birth outcomes.

Introduction

Fetal growth assessment is important for prenatal care.[1-3] Fetal growth is influenced by several maternal socio-demographic and lifestyle-related characteristics.[1,3-8] Poor second and third trimester fetal growth has been associated with increased risks of preterm birth and low birth weight, and long-term adverse health outcomes.[9-11] Recent studies also observed associations of first trimester fetal growth restriction with the risk of small size for gestational age at birth.[12-14] Most previous studies used fetal growth measured once during pregnancy to examine associations of fetal growth with the risk of adverse birth outcomes.

Studies using longitudinal fetal growth data are scarce. Repeatedly measured fetal growth characteristics enable tracking analyses, which can be used to describe the longitudinal development of individual characteristics. Tracking analyses focus on the maintenance of one's relative position in a population distribution of values over time.[15] Tracking has been described for various health outcomes, such as body mass index and cardiovascular risk factors.[16-19] Tracking of risk factors throughout the life course helps to understand the stability of risk factors across a longer age window, to get further insight in the early origins of adverse outcomes, and may ultimately be relevant for developing clinical prediction models.[15,19] To our knowledge, there are no studies yet that examined tracking of fetal growth characteristics during different periods of gestation in low-risk populations. Examining the extent of tracking of longitudinal fetal growth characteristics might give further insight in the correlations of fetal ultrasound measurements with adverse birth outcomes. These correlations are primarily of interest from an etiological perspective, but may also help to improve future prediction models based on fetal ultrasound measurements.

Therefore, we examined in a population-based prospective cohort study among 8636 pregnant women the extent of tracking of different fetal growth characteristics, and whether this tracking is influenced by maternal socio-demographic and lifestyle-related characteristics and associated with the risk of adverse birth outcomes.

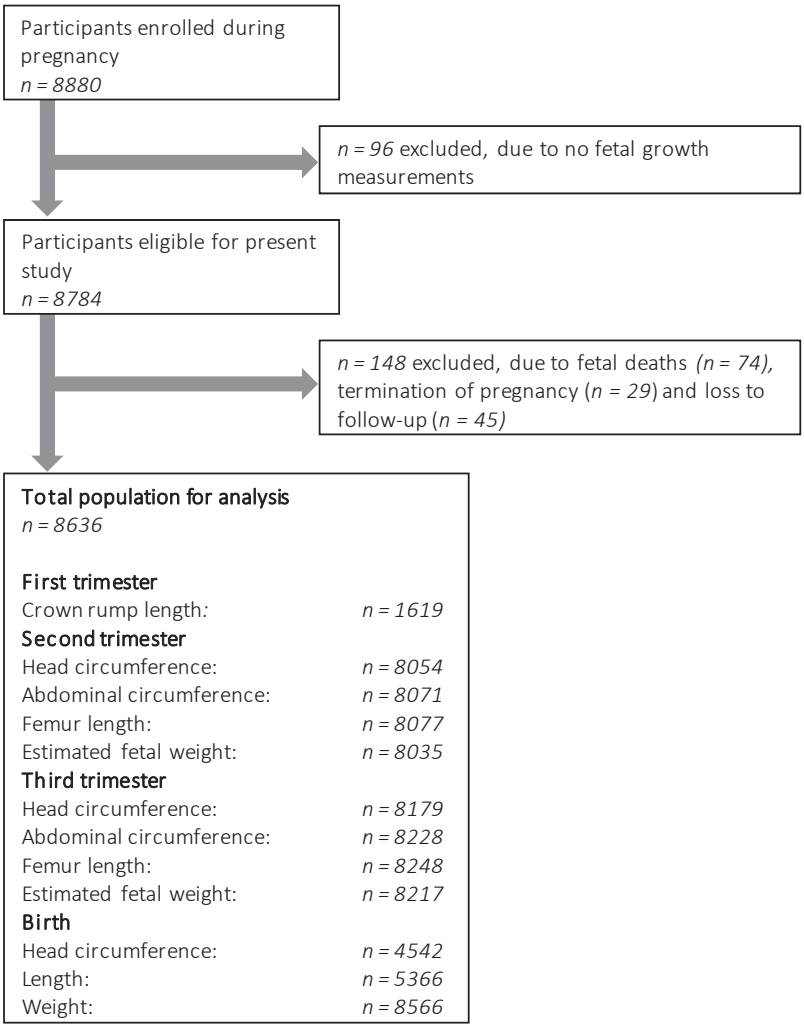
Subjects and methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[20] The study has been approved by the local Medical Ethical Committee (MEC 198.782/2001/31). Written consent was obtained from all participating women. All pregnant women were enrolled between 2001 and 2005. Response rate at birth was 61%. In total, 8880 women were enrolled during pregnancy. For the present study, we excluded women without any fetal growth measurements (*n = 96*). We also excluded pregnancies leading to fetal death (*n = 74*), termination of pregnancy (*n = 29*) and loss to follow up at birth (*n = 45*).

Thus, the cohort for analysis comprised 8636 pregnant women (**Figure 3.3.1**). Additionally, we restricted the analyses focused on first trimester fetal growth to women with a known last menstrual date and enrolment before 14 weeks of gestation (*n = 1619*), as described previously.[12]

Figure 3.3.1. Flow chart of the participants in the Generation R Study, 2001-2005



Fetal growth measurements

Fetal ultrasound examinations were carried out in two dedicated research centers in first (median 12.4 weeks of gestation, 95% range 10.7-13.9), second (median 20.5 weeks of gestation, 95% range 18.5-23.5) and third trimester (median 30.3 weeks of gestation, 95% range 28.3-33.0).

In first trimester, we used crown-rump length to assess fetal growth only in mothers with a known and reliable first day of the last menstrual period, a regular menstrual cycle of 28 days (range 24 – 32 days) and who had fetal crown-rump length measured between a gestational age of 10 weeks 0 days and 13 weeks 6 days.[12] The first day of the last menstrual period was obtained from the referring letter from the community midwife or hospital. This date was confirmed with the subjects at the ultrasound visit, and additional information on the regularity and duration of the menstrual cycle was obtained.[12]

For mothers without this information, gestational age was established by first trimester fetal ultrasound examination. This strategy was performed because of the large number of mothers who do not know their exact date of their last menstrual period or have irregular menstrual cycles.[21] Subsequently, in the second and third trimester, we measured fetal head circumference, abdominal circumference and femur length to the nearest millimeter using standardized ultrasound procedures. Estimated fetal weight was subsequently calculated using the formula of Hadlock et al.[22] Longitudinal growth curves and gestational-age-adjusted standard deviation scores (SDS) were constructed for all fetal growth measurements.[21] These gestational-age-adjusted SDS were based on reference growth curves from the whole study population, and represent the equivalent of z-scores.[21]

Birth outcomes

Information about offspring sex, gestational age, weight, length and head circumference at birth was obtained from medical records.[20] Because head circumference and length were not routinely measured at birth fewer measurements were available (*n = 4542* and *n = 5366* for head circumference and length at birth, respectively). Gestational-age-adjusted SDS for birth weight, length and head circumference were constructed using North-European growth standards.[23] Preterm birth was defined as a gestational age of <37 weeks at birth. Postterm birth was defined as a gestational age of >42 weeks at birth. Information about spontaneous and medically-induced birth was available from medical records.[20] Small size for gestational age at birth and large size for gestational age at birth were defined as a gestational-age-adjusted birth weight below the 5th percentile (-1.78 SD) and above the 95th percentile (1.57 SD) in the study cohort, respectively.

Maternal socio-demographic and lifestyle characteristics

Maternal age was assessed at enrolment. Maternal height (cm) and weight (kg) were measured without shoes and heavy clothing at enrolment and body mass index (kg/m^2) was calculated. Information on educational level, ethnicity, parity and folic acid supplement use was obtained at enrolment by questionnaire. Information about smoking and alcohol consumption was assessed by questionnaires in each trimester.[20]

Statistical analysis

First, we estimated the extent of tracking of fetal growth characteristics SDS from second to third trimester and from second and third trimester to birth, using Pearson's correlation coefficients. Next, we categorized second and third trimester fetal head circumference, femur length and estimated fetal weight SDS in tertiles and used linear regression models to calculate the differences in growth characteristics at birth for the lower and upper tertiles, as compared to the middle tertile. We used similar models to examine tracking of fetal growth characteristics from second to third trimester. Subsequently, we also categorized head circumference, length and weight SDS at birth in tertiles and used logistic regression models to calculate the Odds Ratio (OR) to remain in the same fetal growth characteristic tertile from second to third trimester and from second and third trimester to birth. Similar analyses were performed among a subgroup of women with a known menstrual date, to examine tracking of fetal growth characteristics from first trimester to birth.

Second, we examined whether maternal socio-demographic and lifestyle-related characteristics influence tracking of fetal growth characteristics in different periods of pregnancy. For these analyses, we examined potential interactions between maternal socio-demographic and lifestyle characteristics with fetal growth characteristics. Additionally, we categorized each maternal characteristic, and for each category of that specific maternal characteristic, we estimated fetal growth tracking coefficients using linear regression models.

Third, we used unbalanced repeated measurement regression models to examine whether longitudinal fetal growth patterns are associated with the risk of preterm and postterm birth and small size for gestational age at birth and large size for gestational age at birth. These models take the correlation between repeated measurements of the same subject into account, and allow for optimal use of available data. For these analyses, we used gestational-age-adjusted SDS for each fetal growth characteristic. We categorized children into 3 categories for gestational age at birth: born preterm, born a term and born postterm, and in 3 categories for gestational-age-adjusted size at birth: small size for gestational age, appropriate size for gestational age and large size for gestational age. These categories were included in the models as intercept and as interaction term with gestational age, to study the gestational age-independent effects (difference constant over time) as well as gestational age-dependent effects (difference

non-constant over time), respectively. The actual models are described in detail in the Supplementary Material.

Fourth, we further examined the associations of each fetal growth characteristic SDS in first, second and third trimester and the change of fetal growth characteristics SDS from second to third trimester with the risks of adverse birth outcomes using multiple logistic regression models. These models were adjusted for gestational age at intake, gestational age at each pregnancy period, maternal age, educational level, parity, ethnicity, prepregnancy body mass index, smoking habits, alcohol consumption and folic acid supplement use. Sensitivity analyses among women with a known menstrual date were performed. Missing data of the covariates were imputed using multiple imputations. The percentages of missing values within the population for analysis were lower than 19.4% except for folic acid supplement use (26.0%). We used Markov chain Monte Carlo approach for multiple imputation of missing values in the covariates. Five imputed datasets were created and analyzed together. The repeated measurement analysis was performed using the Statistical Analysis System version 9.2 (SAS, Institute Inc. Cary NC, USA), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Table 3.3.1 shows the participants characteristics. In total, 464 children were born preterm, 428 children were born postterm, 427 were small size for gestational age at birth and 427 were large size for gestational age at birth.

Tracking of fetal growth characteristics

Correlation coefficients for fetal head circumference, (femur) length and (estimated) fetal weight were r = 0.16, r = 0.20, r = 0.30 between second trimester and birth and r = 0.38, r = 0.36, r = 0.58 (all P-values <0.05) between third trimester and birth, respectively. The corresponding data and scatterplots are given in Table 3.3.2 and Supplementary Figure S3.3.1.

Table 3.3.3 shows that the differences in head circumference, length and weight at birth for fetuses who were in the upper tertile of each corresponding fetal growth characteristic in second trimester were 0.18 SDS (95% Confidence Interval (CI): 0.10, 0.26), 0.20 SDS (95% CI: 0.12, 0.27) and 0.31 SDS (95% CI: 0.26, 0.36), respectively as compared to fetuses who were in the middle tertile in second trimester. Stronger effect estimates were observed for the associations of third trimester fetal growth characteristics with birth measures (Table 3.3.3). The corresponding histograms are given in Supplementary Figure S3.3.2. Also, Supplementary Tables S3.3.1 and S3.3.2 show that the

ORs for staying in the upper tertile from second trimester to birth and third trimester to birth were strongest for weight (OR 2.22 (95% CI: 2.01, 2.46) and OR 5.29 (95% CI: 4.77, 5.87), for staying in the upper fetal weight tertile from second trimester to birth and third trimester to birth, respectively). As compared with these effect estimates, the ORs for staying in the upper tertile from second to third trimester were stronger.

Within a subgroup of women with a known menstrual date, we examined tracking of fetal growth characteristics from first trimester to birth. Correlation coefficients between crown-rump length in first trimester and head circumference, length and weight at birth were r = 0.10, r = 0.14, and r = 0.12 (P-values <0.05), respectively (Table 3.3.2). Difference in weight at birth for fetuses in the upper tertile of crown-rump length in first trimester was 0.12 SDS (95% CI: 0, 0.25), as compared to fetuses in the middle tertile of crown-rump length (Supplementary Table S3.3.3). The ORs for staying in the upper tertile from crown-rump length in first trimester to weight at birth was 1.29 (95% CI: 1.00, 1.65) (Supplementary Table S3.3.4). When we assessed tracking of fetal growth characteristics from second and third trimester to birth among women with a known menstrual date, results only changed slightly (results not shown).

Maternal socio-demographic and lifestyle characteristics and tracking of fetal growth

Table 3.3.4 shows that maternal height, parity, educational level, ethnic background, alcohol consumption during pregnancy, smoking during pregnancy and folic acid supplement use did not influence tracking coefficients for (estimated) fetal weight. Tracking coefficients for (estimated) fetal weight from second to third trimester and third trimester to birth were lower among overweight and obese mothers, as compared to normal weight mothers. Among younger mothers, tracking coefficients for (estimated) fetal weight from third trimester to birth were lower, as compared to older mothers. After adjusting for multiple testing, these interaction terms were no longer significant. The influences of maternal characteristics on tracking coefficients for head circumference and length during different periods of gestation are given in the Supplementary Table S3.3.5 and S3.3.6.

Fetal growth and the risk of adverse birth outcomes

Figure 3.3.2 shows that as compared to children born term, children born preterm had smaller fetal head circumference, length and weight growth from third trimester onwards, whereas children born after 42 weeks of gestation had smaller fetal growth characteristics in second trimester and higher fetal growth characteristics thereafter. As compared to children born with an appropriate size for gestational age, children born small for gestational age had lower fetal growth rates from second trimester onwards, whereas children born large for gestational age had higher fetal growth rates from second trimester onwards. The fetal growth variation was more strongly associated with size at birth as compared to gestational age at birth.

Table 3.3.1. Characteristics of mothers and their children in the Generation R Study, 2001-2005 (*n = 8636*)[1]

Characteristics	Value
Maternal characteristics	
Age, median (95% range), years	30.2 (19.2, 39.2)
Height, mean (SD), cm	167.1 (7.4)
Prepregnancy weight, mean (SD), kg	66.2 (12.8)
Prepregnancy Body Mass Index, mean (SD), kg/m^2	23.6 (4.4)
Gestational age at intake, median (95% range), weeks	14.4 (10.4, 28.9)
Education, No. (%)	
Primary	918 (11.7)
Secondary	3648 (46.4)
Higher	3289 (41.9)
Race / Ethnicity, No. (%)	
Dutch or European	4588 (57.4)
Non-European	3400 (42.6)
Parity, No. nulliparous (%)	4749 (55.7)
Folic acid supplement use, No. (%)	
Yes	4508 (70.5)
No	1882 (29.5)
Smoking, No. (%)	
Yes	1885 (25.5)
No	5508 (74.5)
Alcohol consumption, No. (%)	
Yes	3735 (50.2)
No	3702 (49.8)
Fetal growth characteristics	
First trimester	
Gestational age, median (95% range), weeks	12.4 (10.7, 13.9)
Crown-rump length, mean (SD), mm	61 (11)
Second trimester	
Gestational age, median (95% range), weeks	20.5 (18.5, 23.5)
Head circumference, mean (SD), mm	180 (15)
Abdominal circumference, mean (SD), mm	157 (15)
Femur length, mean (SD), mm	34 (4)
Estimated fetal weight, mean (SD), g	383 (96)
Third trimester	
Gestational age, median (95% range), weeks	30.3 (28.3, 33.0)
Head circumference, mean (SD), mm	285 (13)
Abdominal circumference, mean (SD), mm	264 (17)
Femur length, mean (SD), mm	57 (3)
Estimated fetal weight, mean (SD), g	1616 (266)
Birth characteristics	
Males, No. (%)	4361 (50.5)
Gestational age, median (95% range), weeks	40.1 (35.4, 42.3)
Birth head circumference, mean (SD), cm	33.8 (1.7)
Birth length, mean (SD), cm	50.2 (2.4)
Birth weight, mean (SD), g	3410 (562)
Overall preterm birth, No. (%)	464 (5.4)
Spontaneous preterm birth, No. (%)	296 (3.5)
Postterm birth, No. (%)	428 (5.2)
Small for gestational age, No. (%)	427 (5.0)
Large for gestational age, No. (%)	427 (5.0)

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

Table 3.3.2. Correlation of fetal growth characteristics from first, second and third trimester to birth in the Generation R Study, 2001-2005[1]

	Second trimester				Third trimester				Birth		
Measurements	Head circumference	Abdominal circumference	Femur length	Estimated fetal weight	Head circumference	Abdominal circumference	Femur length	Estimated fetal weight	Head circumference	Length	Weight
First trimester											
Crown rump length	-0.11 (-0.16, -0.05)	-0.09 (-0.14, -0.04)	-0.08 (-0.12, -0.03)	-0.10 (-0.15, -0.06)	0.06 (0.01, 0.11)	0.06 (0.01, 0.11)	0.00 (-0.05, 0.05)	0.04 (-0.01, 0.09)	0.10 (0.02, 0.18)	0.14 (0.07, 0.21)	0.12 (0.07, 0.17)
Second trimester											
Head circumference	-	0.45 (0.43, 0.47)	0.41 (0.39, 0.43)	0.52 (0.50, 0.54)	0.43 (0.41, 0.45)	0.31 (0.29, 0.33)	0.25 (0.23, 0.27)	0.36 (0.34, 0.38)	0.16 (0.13, 0.20)	0.17 (0.14, 0.20)	0.22 (0.20, 0.24)
Abdominal circumference	-	-	0.35 (0.32, 0.37)	0.86 (0.85, 0.88)	-	0.44 (0.42, 0.46)	0.24 (0.22, 0.26)	0.46 (0.44, 0.48)	0.15 (0.12, 0.19)	0.21 (0.18, 0.24)	0.30 (0.28, 0.32)
Femur length	-	-	-	0.74 (0.73, 0.76)	-	-	0.53 (0.51, 0.55)	0.41 (0.39, 0.43)	0.09 (0.05, 0.12)	0.20 (0.17, 0.23)	0.18 (0.16, 0.21)
Estimated fetal weight	-	-	-	-	-	-	-	0.53 (0.51, 0.55)	0.15 (0.11, 0.18)	0.25 (0.21, 0.28)	0.30 (0.28, 0.32)
Third trimester											
Head circumference	-	-	-	-	-	0.45 (0.43, 0.47)	0.25 (0.23, 0.27)	0.47 (0.45, 0.49)	0.38 (0.34, 0.41)	0.28 (0.25, 0.31)	0.40 (0.38, 0.42)
Abdominal circumference	-	-	-	-	-	-	0.33 (0.30, 0.35)	0.92 (0.91, 0.93)	0.31 (0.28, 0.34)	0.39 (0.36, 0.42)	0.57 (0.55, 0.58)
Femur length	-	-	-	-	-	-	-	0.67 (0.65, 0.69)	0.20 (0.17, 0.23)	0.36 (0.33, 0.39)	0.35 (0.33, 0.37)
Estimated fetal weight	-	-	-	-	-	-	-	-	0.32 (0.29, 0.35)	0.45 (0.42, 0.48)	0.58 (0.57, 0.60)
Birth											
Head circumference	-	-	-	-	-	-	-	-	-	0.44 (0.41, 0.47)	0.42 (0.40, 0.46)
Length	-	-	-	-	-	-	-	-	-	-	0.50 (0.48, 0.52)

[1]Values are regression coefficients (95% Confidence Interval) from linear regression models reflecting correlations of fetal growth characteristics in SDS from first, second and third trimester to birth.

Table 3.3.3. Tracking of fetal growth characteristics during different periods of gestation in the Generation R Study, 2001-2005[1,2]

	Second to third trimester[3]	Second trimester to birth[4]	Third trimester to birth[5]
	Head circumference (SDS)		
Tertiles of head circumference			
Lowest	-0.48 (-0.53, -0.43)**	-0.17 (-0.25, -0.09)**	-0.42 (-0.50, -0.34)**
Middle	*Reference*	*Reference*	*Reference*
Highest	0.46 (0.41, 0.51)**	0.18 (0.10, 0.26)**	0.34 (0.27, 0.42)**
	(Femur) length (SDS)		
Tertiles of femur length			
Lowest	-0.61 (-0.66, -0.57)**	-0.23 (-0.31, -0.16)**	-0.39 (-0.46, -0.32)**
Middle	*Reference*	*Reference*	*Reference*
Highest	0.55 (0.51, 0.60)**	0.20 (0.12, 0.27)**	0.37 (0.29, 0.44)**
	(Estimated or birth) weight (SDS)		
Tertiles of estimated weight			
Lowest	-0.55 (-0.60, -0.50)**	-0.34 (-0.39, -0.29)**	-0.61 (-0.66,- 0.56)**
Middle	*Reference*	*Reference*	*Reference*
Highest	0.57 (0.53, 0.62)**	0.31 (0.26, 0.36)**	0.60 (0.55, 0.64)**

[1]Values are differences (95% Confidence Interval) in fetal growth characteristics in SDS from linear regression models. Estimates are from multiple imputed data. Corresponding histograms are given in **Supplementary Figure S3.3.2**. [2]Models are adjusted for gestational age at intake, gestational age during each pregnancy period, maternal age, educational level, parity, ethnicity, prepregnancy body mass index, smoking habits during pregnancy, alcohol consumption during pregnancy and folic acid supplement use during pregnancy. [3]Values are differences in head circumference, length and weight in SDS in third trimester for fetuses who were in the lower and upper tertile of each fetal growth characteristic in second trimester as compared to fetuses who were in the middle tertile in second trimester. [4]Values are differences in head circumference, length and weight in SDS at birth for fetuses who were in the lower and upper tertile of each fetal growth characteristic in second trimester as compared to fetuses who were in the middle tertile in second trimester. [5]Values are differences in head circumference, length and weight in SDS at birth for fetuses who were in the lower and upper tertile of each fetal growth characteristic in third trimester as compared to fetuses who were in the middle tertile in third trimester. **P-value <0.01.

Table 3.3.5 shows that larger first trimester crown-rump length was associated with a lower risk of overall preterm birth and small size for gestational age at birth (OR for overall preterm birth and small size for gestational age at birth 0.71 (95% CI: 0.55, 0.92) and 0.72 (95% CI: 0.55, 0.92) per SDS change in crown-rump length, respectively). The results for preterm birth were not materially affected when we restricted analyses to spontaneous preterm birth only. Larger second trimester fetal growth characteristics were associated with a lower risk of postterm birth and small size for gestational age at birth and a higher risk of large size for gestational age at birth (P-values <0.05), but were not associated with the risk of overall preterm birth. Stronger associations were present for all third trimester fetal growth characteristics, with the strongest effect estimates for estimated fetal weight (OR for the risk of overall preterm birth (OR 0.82 (95% CI: 0.73, 0.91), postterm birth (OR 0.83 (95% CI: 0.75, 0.92), small size for gestational age at birth (OR 0.19 (95% CI: 0.16, 0.22) and large size for gestational age at birth (OR 3.17 (95% CI: 2.84, 3.53) per SDS change in third trimester estimated fetal weight, respectively). When we restricted analyses to spontaneous preterm birth only, larger second trimester fetal growth characteristics were associated with a higher risk of spontaneous preterm birth, whereas the associations of third trimester fetal growth characteristics with spontaneous preterm birth attenuated. Additional adjustment for maternal pregnancy complications and mode of delivery did not materially change the effect

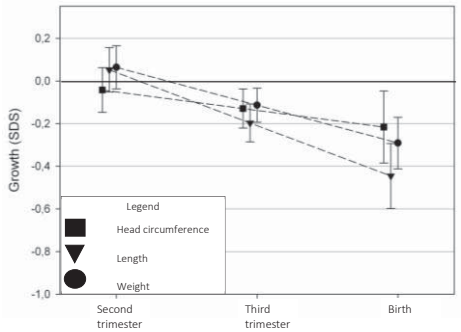
estimates (results not shown). Results were similar when analyses were performed among women with a known last menstrual date (results not shown).

Table 3.3.4. Maternal characteristics and fetal weight tracking coefficients during different periods of gestation in the Generation R Study, 2001-2005[1]

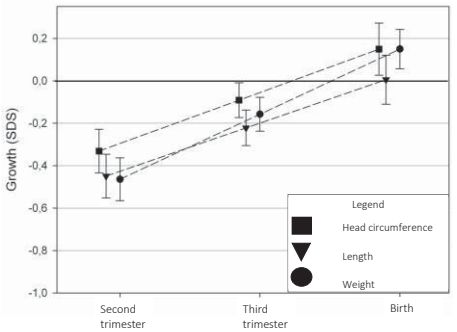
	Second to third trimester		Second trimester to birth		Third trimester to birth	
Maternal characteristics	*n*	Regression coefficient (95% CI)	*n*	Regression coefficient (95% CI)	*n*	Regression coefficient (95% CI)
Age (yrs)						
< 25 yrs	*1590*	0.52 (0.48, 0.56)	*1645*	0.27 (0.23, 0.31)	*1692*	0.53 (0.49, 0.57)
25-35 yrs	*5001*	0.54 (0.52, 0.56)	*5147*	0.31 (0.28, 0.34)	*5259*	0.59 (0.56, 0.61)
>35 yrs	*1129*	0.48 (0.43, 0.53)	*1171*	0.28 (0.22, 0.34)	*1216*	0.59 (0.55, 0.64)
		Interaction P=0.57		*Interaction P=0.34*		*Interaction P=0.01*
Height (cm)						
< 165 cm	*3284*	0.53 (0.51, 0.56)	*3414*	0.31 (0.27, 0.34)	*3515*	0.59 (0.56, 0.62)
165-175 cm	*3363*	0.52 (0.49, 0.55)	*3450*	0.26 (0.23, 0.29)	*3531*	0.55 (0.53, 0.58)
>175 cm	*1073*	0.49 (0.46, 0.52)	*1099*	0.28 (0.23, 0.34)	*1121*	0.53 (0.48, 0.58)
		Interaction P=0.12		*Interaction P=0.18*		*Interaction P=0.16*
Prepregnancy weight (kg)						
<65 kg	*4126*	0.52 (0.49, 0.55)	*4249*	0.29 (0.26, 0.32)	*4344*	0.58 (0.55, 0.60)
65-75 kg	*2454*	0.52 (0.48, 0.55)	*2539*	0.28 (0.24, 0.32)	*2615*	0.56 (0.52, 0.59)
>75 kg	*1139*	0.51 (0.45, 0.56)	*1175*	0.26 (0.20, 0.31)	*1209*	0.55 (0.51, 0.60)
		Interaction P=0.42		*Interaction P=0.03*		*Interaction P=0.02*
Prepregnancy body mass index (kg/m^2)						
Normal	*5499*	0.52 (0.50, 0.55)	*5667*	0.30 (0.27, 0.33)	*5786*	0.58 (0.56, 0.60)
Overweight	*1678*	0.53 (0.48, 0.58)	*1730*	0.29 (0.24, 0.34)	*1802*	0.58 (0.54, 0.62)
Obesity	*543*	0.51 (0.43, 0.59)	*566*	0.25 (0.17, 0.34)	*579*	0.57 (0.51, 0.64)
		Interaction P=0.95		*Interaction P=0.22*		*Interaction P=0.32*
Parity						
Nulliparous	*4373*	0.53 (0.50, 0.55)	*4468*	0.31 (0.28, 0.33)	*4581*	0.58 (0.55, 0.60)
Multiparous	*3347*	0.52 (0.49, 0.55)	*3495*	0.27 (0.24, 0.31)	*3586*	0.56 (0.54, 0.59)
		Interaction P=0.71		*Interaction P=0.14*		*Interaction P=0.50*
Highest education						
Primary school	*899*	0.57 (0.51. 0.63)	*943*	0.28 (0.21, 0.35)	*984*	0.52 (0.47, 0.58)
Secondary school	*3612*	0.52 (0.50, 0.55)	*3736*	0.30 (0.27, 0.33)	*3840*	0.58 (0.56, 0.61)
Higher education	*3210*	0.52 (0.49, 0.50)	*3284*	0.30 (0.27, 0.34)	*3343*	0.59 (0.56, 0.62)
		Interaction P=0.21		*Interaction P=0.58*		*Interaction P=0.03*
Ethnicity						
European	*4423*	0.52 (0.50, 0.55)	*4535*	0.30 (0.27, 0.32)	*4612*	0.58 (0.55, 0.60)
Non-European	*3297*	0.53 (0.50, 0.56)	*3428*	0.29 (0.26, 0.33)	*3555*	0.58 (0.55, 0.61)
		Interaction P=0.65		*Interaction P=0.92*		*Interaction P=0.58*
Alcohol consumption						
No	*3802*	0.52 (0.50, 0.55)	*3946*	0.28 (0.25, 0.31)	*4105*	0.57 (0.54, 0.60)
Yes	*3918*	0.53 (0.51, 0.56)	*4017*	0.32 (0.29, 0.35)	*4062*	0.60 (0.57, 0.62)
		Interaction P=0.63		*Interaction P=0.12*		*Interaction P=0.16*
Smoking habits						
None	*5741*	0.53 (0.50, 0.55)	*5930*	0.29 (0.27, 0.32)	*6092*	0.58 (0.56, 0.60)
Yes	*1978*	0.53 (0.49, 0.57)	*2033*	0.31 (0.27, 0.36)	*2075*	0.58 (0.55, 0.62)
		Interaction P=0.81		*Interaction P=0.47*		*Interaction P=0.89*
Folic acid supplement use						
Preconception	*2990*	0.51 (0.48, 0.54)	*3065*	0.32 (0.28, 0.36)	*3107*	0.59 (0.56, 0.63)
First 10 weeks	*2430*	0.55 (0.51, 0.58)	*2488*	0.28 (0.24, 0.33)	*2537*	0.56 (0.52, 0.60)
None	*2300*	0.53 (0.49, 0.56)	*2410*	0.29 (0.25, 0.34)	*2523*	0.58 (0.55, 0.61)
		Interaction P=0.57		*Interaction P=0.48*		*Interaction P=0.51*

[1]Values are regression coefficients (95% Confidence Interval) for fetal weight among different maternal characteristic categories during different periods of gestation from linear regression models. Estimates are from multiple imputed data.

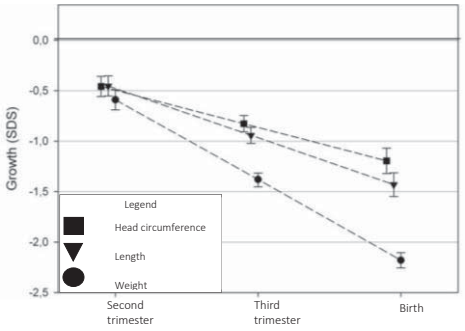
Figure 3.3.2. Longitudinal fetal growth patterns and the risks of adverse birth outcomes in the Generation R Study, 2001-2005 (*n = 8636*)[1]



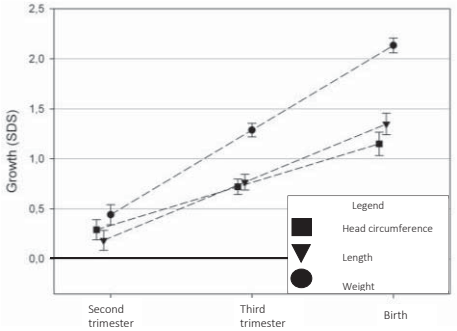
3.3.2a. Fetal growth among preterm born infants as compared to term born infants



3.3.2b. Fetal growth among postterm born infants as compared to term born infants



3.3.2c. Fetal growth among small for gestational age infants as compared to appropriate size for gestational age infants



3.3.2d. Fetal growth among large for gestational age infants as compared to appropriate size for gestational age infants

[1]Fetal growth among preterm born infants (A), fetal growth among postterm born infants (B), fetal growth among small for gestational age infants (C), fetal growth among large for gestational age infants (D). 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 preterm born infants and postterm born infants compared to term born infants in second trimester, third trimester and at birth and for small for gestational age infants and large for gestational age infants as compared to appropriate size for gestational age infants in second trimester, third trimester and at birth, respectively (reference group represented as zero line). Results for spontaneous preterm birth were similar (not shown). Head circumference, length and weight growth characteristics used in the models in the fetal period: second and third trimester: head circumference, femur length and estimated fetal weight; at birth: birth head circumference, birth length and birth weight. Model information is given in the Supplementary Material.

Table 3.3.5. Associations of fetal growth characteristics with the risks of adverse birth outcomes in the Generation R Study, 2001-2005 (*n = 8636*)[1,2]

Pregnancy period	Preterm birth	Spontaneous preterm birth	Postterm birth	Small for gestational age	Large for gestational age
First trimester ultrasound					
CRL[3]	0.71 (0.55, 0.92)*	0.75 (0.55, 1.02)	1.14 (0.89, 1.46)	0.72 (0.55, 0.92)*	1.27 (1.01, 1.60)*
Second trimester ultrasound					
HC	1.10 (1.00, 1.21)	1.15 (1.02, 1.29)**	0.71 (0.64, 0.79)**	0.63 (0.57, 0.70)**	1.35 (1.22, 1.49)**
AC	1.05 (0.96, 1.15)	1.13 (1.00, 1.27)*	0.73 (0.66, 0.81)**	0.51 (0.45, 0.56)**	1.58 (1.42, 1.74)**
FL	1.07 (0.97, 1.17)	1.17 (1.04, 1.31)*	0.64 (0.58, 0.71)**	0.60 (0.54, 0.66)**	1.34 (1.21, 1.58)**
EFW	1.09 (0.99, 1.20)	1.19 (1.06, 1.33)**	0.62 (0.55, 0.69)**	0.44 (0.39, 0.50)**	1.54 (1.40, 1.69)**
Third trimester ultrasound					
HC	0.74 (0.67, 0.82)**	0.77 (0.68, 0.88)**	0.96 (0.87, 1.07)	0.42 (0.37, 0.47)**	2.14 (1.92, 2.38)**
AC	0.81 (0.73, 0.90)**	0.97 (0.85, 1.10)	0.91 (0.82, 1.01)	0.25 (0.22, 0.29)**	3.26 (2.90, 3.67)**
FL	0.82 (0.74, 0.91)**	0.93 (0.82, 1.05)	0.79 (0.71, 0.87)**	0.38 (0.34, 0.43)**	1.96 (1.76, 2.17)**
EFW	0.82 (0.73, 0.91)**	0.98 (0.86, 1.11)	0.83 (0.75, 0.92)**	0.19 (0.16, 0.22)**	3.17 (2.84, 3.53)**
Second to third trimester change[4]					
HC	0.71 (0.64, 0.79)**	0.73 (0.64, 0.82)**	1.35 (1.22, 1.50)**	0.73 (0.65, 0.84)**	1.43 (1.29, 1.58)**
AC	0.76 (0.64, 0.84)**	0.87 (0.77, 0.98)*	1.21 (1.10, 1.33)**	0.61 (0.55, 0.67)**	1.76 (1.59, 1.94)**
FL	0.75 (0.67, 0.83)**	0.77 (0.68, 0.88)**	1.25 (1.12, 1.39)**	0.66 (0.59, 0.73)**	1.43 (1.29, 1.59)**
EFW	0.71 (0.64, 0.80)**	0.81 (0.71, 0.93)**	1.31 (1.18, 1.46)**	0.55 (0.49, 0.61)**	2.09 (1.87, 2.33)**

Abbreviations: CRL, crown-rump length; HC, head circumference; AC, abdominal circumference; FL, femur length; EFW, estimated fetal weight

[1]Values are Odds Ratios (95% Confidence Interval) that reflect the difference in risks of pregnancy complications per standard deviation score (SDS) change in fetal growth characteristic in first, second and third trimester and between second and third trimester. Estimates are from multiple imputed data. [2]Models are adjusted for gestational age at intake, maternal age, educational level, ethnicity, parity, prepregnancy body mass index, smoking habits during pregnancy, alcohol consumption during pregnancy and folic acid supplement use during pregnancy. [3]Model is additionally adjusted for duration of menstrual cycle. [4]Model is additionally adjusted for gestational age in pregnancy period. *P-value <0.05. **P-value <0.01.

Discussion

Results from this population-based prospective cohort study showed that fetal growth characteristics track moderately during pregnancy. Tracking coefficients were strongest in late pregnancy and were not materially influenced by maternal socio-demographic and lifestyle characteristics. First, second and third trimester fetal growth characteristics were associated with the risk of adverse birth outcomes, with the strongest associations present for third trimester abdominal circumference and estimated fetal weight.

Methodological considerations

We had a prospective data collection from early pregnancy onwards and a large sample size of 8636 participants with fetal growth measurements available in each trimester. The response rate at baseline for participation in the study was 61%. The non-response at baseline would lead to biased effect estimates if associations would be different between those included and not included in the analyses. However, this seems unlikely.[24] The non-response might have led to a selection of a more healthy population, and might affect the generalizability of our results. Furthermore, we had a relative small number of cases of adverse birth outcomes which might also indicate a selection

towards a healthy, low-risk population. Pregnancy dating for most women was performed using ultrasound measurements of crown-rump length or biparietal diameter at the first visit. This method might be better than dating by last menstrual period, but neglects variation in early fetal growth. As a consequence, growth variation in second and third trimester might be underestimated. However, when we assessed tracking of fetal growth characteristics throughout gestation among women with a known menstrual date, conclusions were similar. Although, we observed a high reproducibility of the fetal ultrasound measurements,[21] ultrasound assessment of fetal growth, especially in early pregnancy, may be liable to measurement error. Growth measures at birth, especially head circumference, may be also prone to imprecision and inaccuracy. The tracking correlation of estimated fetal weight may especially be affected by measurement error, as this is calculated from multiple fetal biometry measures. In addition, it needs to be taken into account that measurement error could be related to fetal health status. Thus, our observed tracking correlation coefficients may be underestimated due to measurement error of the ultrasound assessment and at birth. The absolute measurement error is expected to be stable throughout pregnancy. However, because of the smaller fetal size, the relative measurement error might be larger in early pregnancy than in late pregnancy. This might have led to an underestimation of especially the correlation coefficients in early pregnancy.

Interpretation of main findings

Fetal ultrasound measurements are important examinations during pregnancy. However, not much is known about the stability of fetal growth characteristics throughout pregnancy. We examined tracking of fetal growth characteristics throughout gestation in a low-risk population. We observed low to moderate correlation coefficients between first, second and third trimester fetal growth characteristics and size at birth, but some tracking of fetal growth characteristics was present already from first trimester onwards. The observed correlation coefficients of fetal growth characteristics between different trimesters were stronger in late pregnancy. The strongest correlation coefficients of fetal growth characteristics were observed between third trimester and birth. Compared with the other fetal growth characteristics, abdominal circumference and estimated fetal weight tracked most strongly during different periods of gestation and were most strongly correlated with birth weight. These findings are in line with a previous study performed among 1650 low-risk British pregnancies, which reported that the correlation of fetal growth measures between 20 and 30 weeks of gestation and birth was generally poor, but the correlation of fetal size at 30 weeks of gestation with size at birth was better than the 20 weeks correlation.[25] Furthermore, a study among 625 low-risk fetuses examining tracking of femur length throughout gestation reported that deviation from tracking of femur length, defined as deviation from the fetus's original femur length quartile, occurred in 87% of fetuses.[26] Thus, results from both our and previous studies suggest moderate tracking of fetal growth characteristics throughout pregnancy among low-risk populations.

Fetal growth charts, which show intrauterine growth as a smooth continuous process, suggest that a fetus's growth characteristics track relative to growth characteristics of other fetuses. Poor tracking of fetal growth characteristics from early pregnancy to birth may partly be due to measurement error related to fetal ultrasound assessment, but may also suggest that a fetus does not have a stable growth trajectory from early pregnancy onwards. Generally, it is assumed that each fetus maintains its own growth percentile. Whether deviation from this percentile occurs due to genetic influences or due to environmental influences on fetal growth remains unclear.[25] The study from Bjornerem et al focused on tracking of femur length throughout gestation among low-risk fetuses suggested that placental weight, maternal height and weight contributed to deviation from tracking.[26] We observed that tracking of fetal length and weight growth characteristics from second and third trimester to birth tended to be lower among overweight or obese mothers, but after taking multiple testing into account these findings were no longer significant. Lower correlation coefficients between fetal growth characteristics among overweight and obese women might also be explained by a higher amount of measurement error of fetal ultrasound measures among these women. We observed no other consistent influences of maternal characteristics on tracking coefficients of fetal growth. This does not suggest that these maternal characteristics do not affect fetal growth. Many studies, of which several from the same cohort as in the present study, have shown that both non-pathological and pathological maternal and fetal characteristics influence fetal growth.[4-8] However, the effect estimates for these associations are small to moderate. The lack of associations of various maternal socio-demographic and lifestyle-related variables with fetal growth tracking coefficients may be due to the relatively small influences of these maternal characteristics on fetal growth, but may also suggest that potential growth adaptations are already occurring in early pregnancy. If the growth trajectory is already changed early in pregnancy, the tracking coefficients later in pregnancy are not modified. We have previously observed that various maternal socio-demographic and lifestyle-related variables affect fetal growth in first trimester.[12]

We observed that higher first trimester fetal crown-rump length was associated with lower risks of preterm birth and small size for gestational age at birth. Previously, we observed in the same cohort as used in the present study, that first trimester fetal growth restriction is associated with higher risks of accelerated postnatal growth and adverse cardiovascular outcomes in childhood.[12,27] A study among 976 pregnant women after assisted reproductive therapy reported that fetal size in first trimester was positively associated with birth weight.[13] Another study among 4229 women reported that suboptimal first trimester fetal growth was associated with low birth weight and premature delivery.[28] However, a Dutch case-control study among 129 women observed no association between shorter first trimester crown-rump length and spontaneous preterm birth <32 weeks of gestation.[29] The authors from this latter study suggested that shorter fetal crown-rump length may be associated with medically-induced preterm birth due to fetal growth restriction.[29] However, in our study we observed that lower first trimester fetal growth tended to be associated with increased risks of both overall

and spontaneous preterm birth. It has been suggested that variation in first-trimester fetal size largely reflects variation in timing of ovulation and implantation, and that this variation may partly explain these associations.[30] We were unable to measure timing of ovulation and implantation. However, all analyses focused on first trimester fetal growth and birth outcomes were adjusted for duration of last menstrual cycle, which is strongly associated with the timing of ovulation. Still, even with a known and reliable last menstrual period, a certain fraction of women with regular cycles have early or delayed ovulation. We performed a sensitivity analysis with a restriction to participants who had a gestational age based on last menstruation within 7 days of a gestational age based on crown-rump length (93%). This analysis did not materially change our effect estimates focused on birth outcomes.

Larger second and third trimester fetal growth characteristics were associated with a lower risk of postterm birth, small size for gestational age at birth and a higher risk of large size for gestational age at birth. Associations of second and third trimester fetal growth characteristics with adverse outcomes were stronger as compared to associations of first trimester fetal growth, which is in line with our observed stronger tracking coefficients in later pregnancy. Strongest associations with adverse outcomes were present for third trimester abdominal circumference and estimated fetal weight. Accordingly, previous studies suggested that abnormal abdominal circumference and estimated fetal weight are most predictive of adverse birth outcomes and ultrasound assessment performed closer to delivery tends to have a higher predictive ability as compared to ultrasound assessment performed in early gestation.[9,31-33]

Several studies have reported that smaller third trimester fetal growth characteristics are associated with a higher risk of overall and spontaneous preterm birth.[9,34] We observed that smaller third trimester fetal growth characteristics were associated with a higher risk of overall preterm birth. The smaller effect estimates after exclusion of non-spontaneous preterm births, suggest that at least part of this association is explained by medically-induced preterm delivery. Less is known about the associations of second trimester fetal growth with preterm birth. Reported associations of second trimester fetal growth characteristics with preterm birth are less consistent.[10,35-38] A study among 290 young, primarily minority US women reported no differences in second trimester fetal growth characteristics between preterm and term born children, but third trimester fetal growth characteristics and rates of fetal growth between 20-32 weeks were lower among preterm born children.[35] Another study among 541 low-risk women with a spontaneous delivery showed that small second trimester fetal size was associated with a lower birth weight and longer pregnancy duration.[36] In line with this latter study, our longitudinal analyses showed that spontaneous preterm born children tended to be larger in second trimester and became smaller from third trimester onwards, whereas postterm born children were smaller in second trimester and became larger from third trimester onwards. It has been suggested that fetuses who are smaller within the normal biological variation, but not severely growth restricted, have a longer pregnancy duration.[36] However, the associations of fetal growth characteristics with gestational age at birth may also be explained by misclassification of gestational age. Also,

the associations of fetal growth characteristics with the risk of preterm birth may be different for different aetiologies of preterm birth.[35,39] Further research to assess associations of fetal growth characteristics with the risks of various types of preterm birth is necessary. Our findings related to the associations of second and third trimester fetal growth characteristics with the risks of small- and large size for gestational age at birth are in line with previous studies performed among both high-risk and low-risk populations.[37,40-43]

The use of a single measurement of fetal growth for identification of fetuses at risk of adverse birth outcomes has important limitations.[44] Compared to a single fetal growth measurement, longitudinal growth measurements may provide additional information for identification of fetuses at risk. We observed specific longitudinal growth patterns from second trimester onwards that were associated with adverse birth outcomes. A study performed among a high-risk population of 321 women showed that fetuses with inadequate growth between two ultrasound assessments, defined as estimated fetal weight growth below or at the 10th percentile, were more likely to be small for their gestational age at birth and to be born preterm, as compared to normal growing fetuses.[44]

Most studies that examined the predictive accuracy of a single fetal ultrasound examination have suggested that among low-risk populations the predictive accuracy for adverse birth outcomes is moderate, especially for fetal ultrasound performed in early pregnancy.[31,45-48] In line with these findings, we observed moderate tracking of fetal growth characteristics throughout pregnancy. Further research is necessary to examine whether serial assessment of fetal growth, in addition to individual characteristics or specific biomarkers, improve the prediction of adverse birth outcomes, especially among lower risk populations.

Conclusion

Our study showed that fetal growth characteristics track moderately during pregnancy and fetal growth tracking coefficients are not materially influenced by maternal characteristics. First, second and third trimester fetal growth characteristics are associated with the risk of adverse birth outcomes, with the strongest associations present in third trimester. Further studies are needed focused on the predictive value of different and combined fetal ultrasound examinations for the prediction of adverse birth outcomes, especially among low-risk populations.

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Supplementary Material

Supplementary Methods S3.3.1. Unbalanced repeated measurement regression models

Using unbalanced repeated measurement regression models, we analyzed the longitudinal fetal growth patterns among term, preterm and postterm born infants and among appropriate size for gestational age, small for gestational age and large for gestational age infants. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data.[1,2] These models can be written as:

Head circumference (SDS) = $ß_0 + ß_1 \times$ gestational age at birth category + $ß_2 \times$ gestational age + $ß_3 \times$ gestational age at birth category × gestational age

Head circumference (SDS) = $ß_0 + ß_1 \times$ size at birth category + $ß_2 \times$ gestational age + $ß_3 \times$ size at birth category × gestational age

Length (SDS) = $ß_0 + ß_1 \times$ gestational age at birth category + $ß_2 \times$ gestational age + $ß_3 \times$ gestational age at birth category × gestational age

Length (SDS) = $ß_0 + ß_1 \times$ size at birth category + $ß_2 \times$ gestational age + $ß_3 \times$ size at birth category × gestational age

Weight (SDS) = $ß_0 + ß_1 \times$ gestational age at birth category + $ß_2 \times$ gestational age + $ß_3 \times$ gestational age at birth category × gestational age

Weight (SDS) = $ß_0 + ß_1 \times$ size at birth category + $ß_2 \times$ gestational age + $ß_3 \times$ size at birth category × gestational age

In these models, '$ß_0 + ß_1 \times$ gestational age/size at birth category' reflects the intercept. The intercept reflects the mean fetal growth characteristic value in SDS for each birth outcome category. The term '$ß_3 \times$ gestational age/size at birth category × gestational age', reflects the difference in change in fetal growth characteristic per week between the different birth outcome categories.

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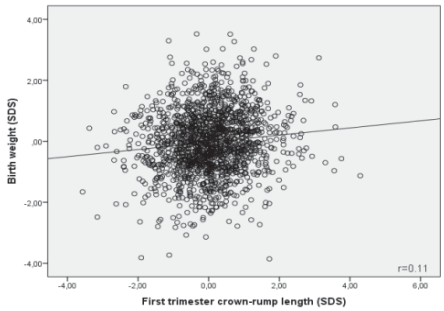
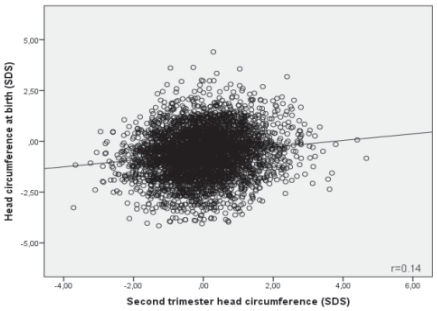
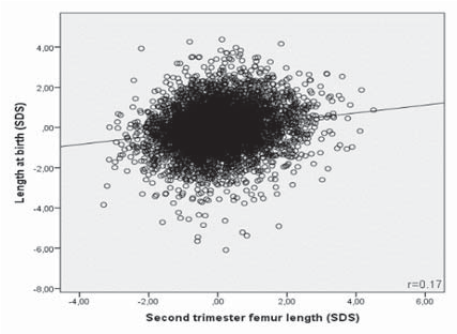
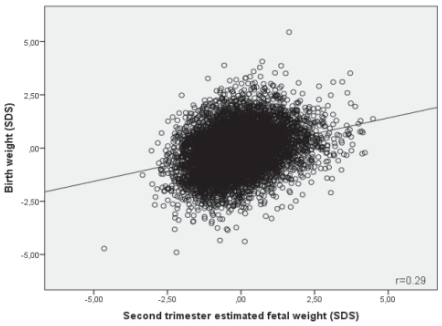
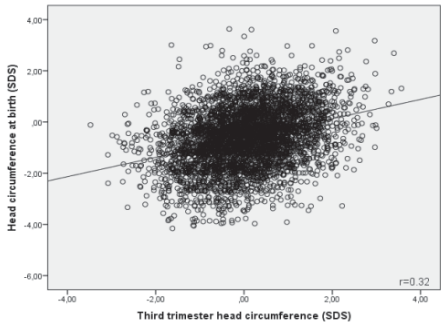
Supplementary Figure S3.3.1. Correlation of fetal growth characteristics between first, second and third trimester and birth in the Generation R Study, 2001-2005

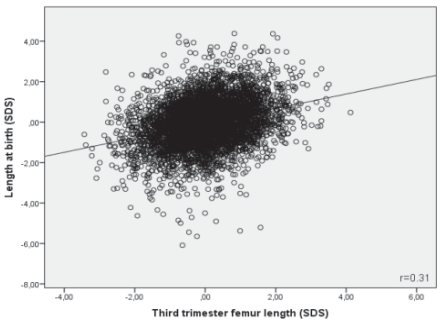


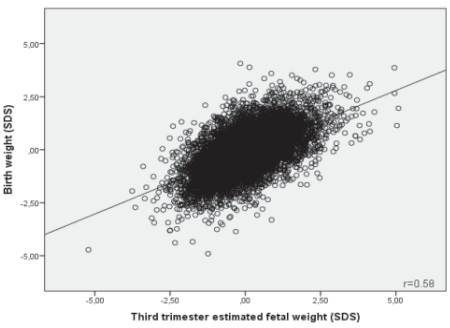






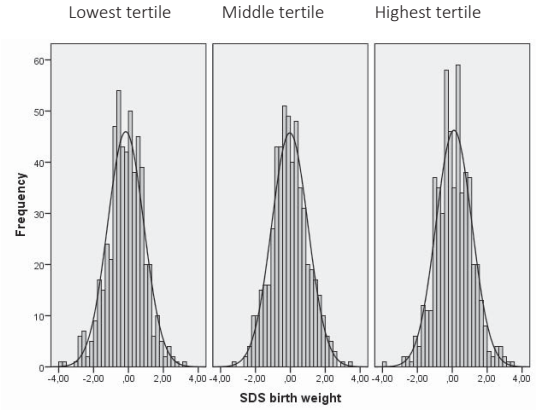




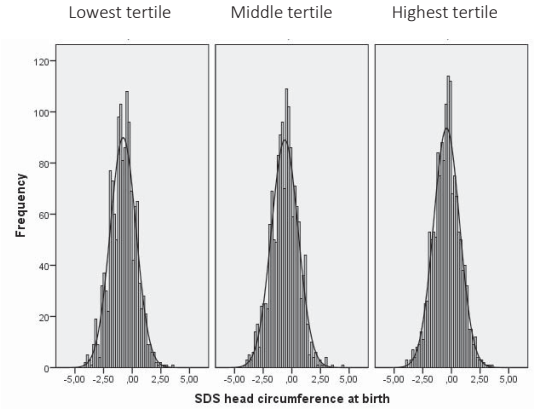


Supplementary Figure S3.3.2. Histograms of fetal growth characteristics at birth in tertiles of first, second and third trimester fetal growth characteristics in the Generation R Study, 2001-2005

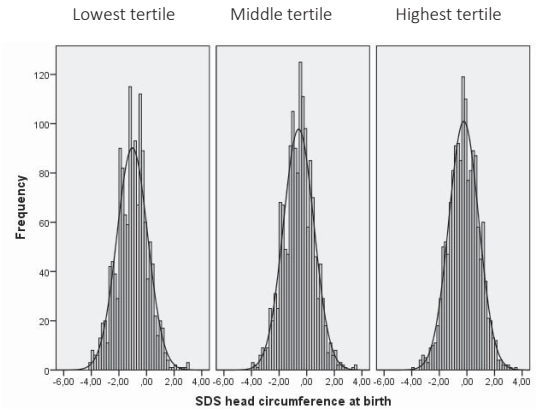
3.3.2a. Birth weight distribution in tertiles of first trimester crown-rump-length



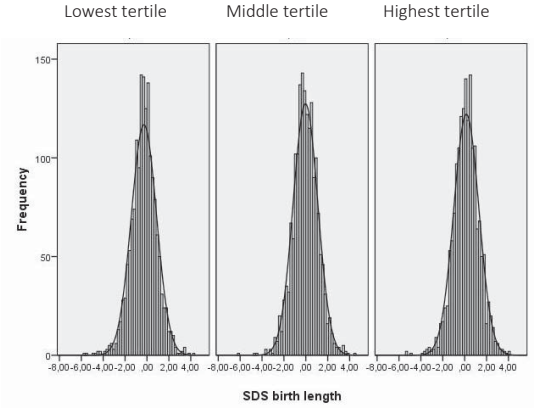
3.3.2b. Head circumference at birth distribution in tertiles of second trimester head circumference



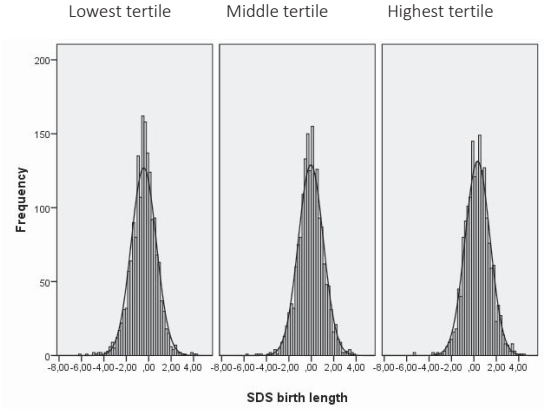
3.3.2c. Head circumference at birth distribution in tertiles of third trimester head circumference



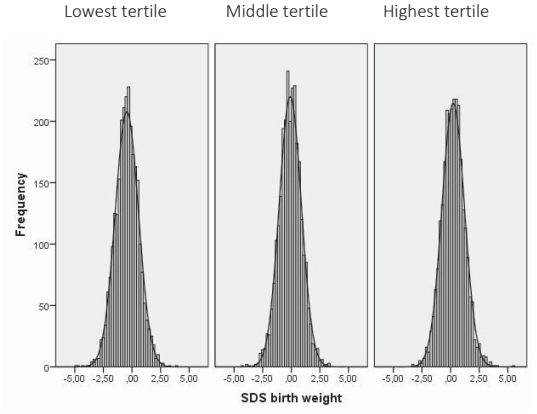
3.3.2d. Length at birth distribution in tertiles of second trimester femur length



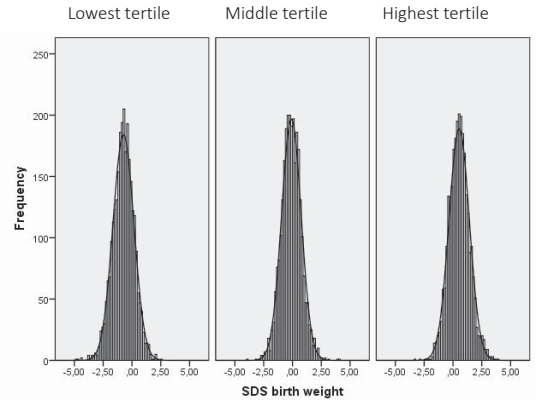
3.3.2e. Length at birth distribution in tertiles of third trimester femur length



3.3.2f. Birth weight distribution in tertiles of second trimester estimated fetal weight



3.3.2g. Birth weight distribution in tertiles of third trimester estimated fetal weight



Supplementary Table S3.3.1. Tracking of fetal growth characteristics from second and third trimester to birth in the Generation R Study, 2001-2005[1,2]

Second trimester tertiles	Tertiles birth head circumference			
Head circumference	Lowest	Middle	Highest	*n*
Lowest	1.46 (1.27, 1.68)**	1.03 (0.93, 1.21)	0.65 (0.56, 0.75)**	*1388*
	n = 545	*n = 474*	*n = 369*	
	(39.3%)	(34.1%)	(26.6%)	
Middle	0.97 (0.85, 1.16)	1.02 (0.90, 1.17)	1.00 (0.87, 1.15)	*1426*
	n = 471	*n = 485*	*n = 470*	
	(33.0%)	(34.0%)	(33.0%)	
Highest	0.70 (0.61, 0.80)**	0.96 (0.84, 1.09)	1.48 (1.29, 1.69)**	*1463*
	n = 409	*n = 481*	*n = 573*	
	(28.0%)	(32.9%)	(39.2%)	
n	*1425*	*1440*	*1412*	*4277*
	Tertiles birth length			
Femur length	Lowest	Middle	Highest	*n*
Lowest	1.61 (1.42, 1.82)**	1.07 (0.94, 1.21)	0.56 (0.49, 0.64)**	*1620*
	n = 656	*n = 558*	*n = 406*	
	(40.5%)	(34.4%)	(25.1%)	
Middle	1.01 (0.89, 1.14)	0.96 (0.85, 1.09)	1.03 (0.91, 1.17)	*1723*
	n = 570	*n = 565*	*n = 588*	
	(33.1%)	(32.8%)	(34.1%)	
Highest	0.61 (0.53, 0.69)**	0.97 (0.86, 1.10)	1.65 (1.46, 1.87)**	*1690*
	n = 449	*n = 556*	*n = 685*	
	(26.6%)	(32.9%)	(40.5%)	
n	*1675*	*1679*	*1679*	*5033*
	Tertiles birth weight			
Estimated fetal weight	Lowest	Middle	Highest	*n*
Lowest	2.43 (2.20, 2.68)**	0.87 (0.79, 0.96)**	0.44 (0.39, 0.49)**	*2652*
	n = 1238	*n = 836*	*n = 578*	
	(46.7%)	(31.5%)	(21.8%)	
Middle	0.90 (0.81, 0.99)*	1.16 (1.05, 1.29)**	0.96 (0.86, 1.06)	*2659*
	n = 838	*n = 946*	*n = 875*	
	(31.5%)	(35.6%)	(32.9%)	
Highest	0.42 (0.37, 0.46)**	0.99 (0.89, 1.09)	2.22 (2.01, 2.46)**	*2652*
	n = 571	*n = 881*	*n = 1200*	
	(21.5%)	(33.2%)	(45.2%)	
n	*2647*	*2663*	*2653*	*7963*

Supplementary Table S3.3.1. Tracking of fetal growth characteristics from second and third trimester to birth in the Generation R Study, 2001-2005[1,2] (*continued*)

Third trimester tertiles	**Tertiles birth head circumference**			
Head circumference	Lowest	Middle	Highest	*n*
Lowest	2.49 (2.17, 2.86)**	0.95 (0.83, 1.09)	0.37 (0.32, 0.44)**	*1386*
	n = 661	*n = 458*	*n = 267*	
	(47.7%)	(33.0%)	(19.3%)	
Middle	0.92 (0.80, 1.05)	1.09 (0.96, 1.24)	0.99 (0.87, 1.14)	*1491*
	n = 477	*n = 518*	*n = 496*	
	(32.0%)	(34.7%)	(33.3%)	
Highest	0.42 (0.36, 0.48)**	0.96 (0.84, 1.10)	2.32 (2.03, 2.64)**	*1506*
	n = 319	*n = 493*	*n = 694*	
	(21.2%)	(32.7%)	(46.1%)	
n	*1457*	*1469*	*1457*	*4383*
	Tertiles birth length			
Femur length	Lowest	Middle	Highest	*n*
Lowest	2.35 (2.08, 2.66)**	0.99 (0.88, 1.12)	0.39 (0.34, 0.45)**	*1705*
	n = 789	*n = 567*	*n = 349*	
	(46.3%)	(33.3%)	(20.5%)	
Middle	0.91 (0.81, 1.04)	1.15 (1.02, 1.30)*	0.95 (0.83, 1.07)	*1731*
	n = 555	*n = 611*	*n = 565*	
	(32.1%)	(35.3%)	(32.6%)	
Highest	0.44 (0.38, 0.50)**	0.88 (0.77, 0.99)*	2.45 (2.17, 2.77)**	*1754*
	n = 386	*n = 549*	*n = 819*	
	(22.0%)	(31.3%)	(46.7%)	
n	*1730*	*1727*	*1733*	*5190*
	Tertiles birth weight			
Estimated fetal weight	Lowest	Middle	Highest	*n*
Lowest	5.53 (4.98, 6.13)**	0.79 (0.72, 0.87)**	0.15 (0.13, 0.18)**	*2725*
	n = 1608	*n = 825*	*n = 292*	
	(59.0%)	(30.3%)	(10.7%)	
Middle	0.81 (0.73, 0.89)**	1.46 (1.32, 1.60)**	0.83 (0.75, 0.91)**	*2720*
	n = 820	*n = 1061*	*n = 839*	
	(30.1%)	(39.0%)	(30.8%)	
Highest	0.15 (0.13, 0.17)**	0.85 (0.77, 0.94)**	5.29 (4.77, 5.87)**	*2722*
	n = 277	*n = 838*	*n = 1607*	
	(10.2%)	(30.8%)	(59.0%)	
n	*2705*	*2724*	*2738*	*8167*

[1]Values are Odds Ratios (95% Confidence Interval) (numbers and percentages of fetuses that remain in the same tertile) to remain in the same tertile of each fetal growth characteristic from second and third trimester to birth. Estimates are from multiple imputed data. [2]Model is adjusted for gestational age at intake, gestational age during each pregnancy period, maternal age, educational level, parity, ethnicity, prepregnancy body mass index, smoking habits during pregnancy, alcohol consumption during pregnancy and folic acid supplement use during pregnancy. *P-value <0.05. **P-value <0.01.

Supplementary Table S3.3.2. Tracking of fetal growth characteristics from second to third trimester in the Generation R Study, 2001-2005[1,2]

	Tertiles third trimester			
Tertiles second trimester	Lowest	Middle	Highest	*n*
Head circumference				
Lowest	3.48 (3.14, 3.85)**	0.91 (0.82, 1.01)	0.26 (0.23, 0.29)**	*2562*
	n = 1338	*n = 823*	*n = 401*	
	(52.2%)	(32.1%)	(15.7%)	
Middle	0.81 (0.74, 0.90)**	1.39 (1.26, 1.54)**	0.87 (0.79, 0.96)**	*2594*
	n = 787	*n = 999*	*n = 808*	
	(30.3%)	(38.5%)	(31.1%)	
Highest	0.30 (0.26, 0.33) **	0.77 (0.70, 0.86) **	3.66 (3.31, 4.05) **	*2553*
	n = 439	*n = 762*	*n = 1352*	
	(17.2%)	(29.8%)	(52.9%)	
n	*2564*	*2584*	*2561*	*7709*
Abdominal circumference				*n*
Lowest	3.48 (3.15, 3.86)**	0.85 (0.77, 0.84)**	0.28 (0.25, 0.32)**	*2583*
	n = 1348	*n = 804*	*n = 431*	
	(52.2%)	(31.1%)	(16.6%)	
Middle	0.88 (0.78, 0.96)**	1.34 (1.22, 1.49)**	0.85 (0.76, 0.94)**	*2579*
	n = 801	*n = 982*	*n = 796*	
	(31.1%)	(38.1%)	(30.8%)	
Highest	0.28 (0.25, 0.31)**	0.87 (0.79, 0.96)**	3.47 (3.13, 3.84)**	*2603*
	n = 421	*n = 819*	*n = 1363*	
	(16.2%)	(31.5%)	(52.4%)	
n	*2570*	*2605*	*2590*	*7765*
Femur length				*n*
Lowest	5.25 (4.73, 5.81)**	0.73 (0.66, 0.81)**	0.19 (0.74, 0.96)**	*2604*
	n = 1517	*n = 760*	*n = 327*	
	(58.3%)	(29.2%)	(12.6%)	
Middle	0.75 (0.67, 0.82)**	1.61 (1.46, 1.77)**	0.82 (0.74, 0.90)**	*2592*
	n = 760	*n = 1058*	*n = 774*	
	(29.3%)	(40.8%)	(29.9%)	
Highest	0.19 (0.17, 0.21)**	0.83 (0.75, 0.92)**	4.77 (4.30, 5.29)**	*2592*
	n = 329	*n = 797*	*n = 1466*	
	(12.7%)	(30.7%)	(56.6%)	
n	*2606*	*2615*	*2567*	*7788*
Estimated fetal weight				*n*
Lowest	4.49 (4.05, 4.98)**	0.84 (0.76, 0.93)**	0.20 (0.17, 0.23)**	*2571*
	n = 1435	*n = 799*	*n = 337*	
	(55.8%)	(31.2%)	(13.1%)	
Middle	0.82 (0.74, 0.91)**	1.53 (1.38, 1.69)**	0.78 (0.70, 0.87)**	*2572*
	n = 779	*n = 1028*	*n = 765*	
	(30.3%)	(40.0%)	(29.7%)	
Highest	0.21 (0.18, 0.24)**	0.77 (0.70, 0.85)**	4.86 (4.38, 5.39)**	*2577*
	n = 351	*n = 763*	*n = 1463*	
	(13.6%)	(29.6%)	(56.8%)	
n	*2565*	*2590*	*2565*	*7720*

[1]Values are Odds Ratios (95% Confidence Interval) (numbers and percentages of fetuses that remain in the same tertile) to remain in the same tertile of each fetal growth characteristic from second to third trimester. Estimates are from multiple imputed data. [2]Model is adjusted for gestational age at intake, gestational age during pregnancy period, maternal age, educational level, parity, ethnicity, prepregnancy body mass index, smoking habits during pregnancy, alcohol consumption during pregnancy and folic acid supplement use during pregnancy. **P-value <0.01.

Supplementary Table S3.3.3. Associations of first trimester fetal crown-rump length with differences in fetal growth characteristics at birth in the Generation R Study, 2001-2005[1,2]

Tertiles of first trimester crown-rump length	Birth measures
	Head circumference at birth (SDS)
Lowest	-0.03 (-0.22, 0.17)
Middle	*Reference*
Highest	0 (-0.19, 0.19)
	Length at birth (SDS)
Lowest	-0.17 (-0.35, 0)
Middle	*Reference*
Highest	0.07 (-0.10, 0.23)
	Weight at birth (SDS)
Lowest	-0.10 (-0.23, 0.03)
Middle	*Reference*
Highest	0.12 (0, 0.25)

[1]Values are differences (95% Confidence Interval) in head circumference, length and weight at birth in SDS for fetuses who were in the lower and upper tertile of first trimester fetal crown-rump-length as compared to fetuses who were in the middle tertile of first trimester fetal crown-rump-length. Estimates are from multiple imputed data. Corresponding histograms are given in **Supplementary Figure S3.3.2**. [2]Model is adjusted for gestational age at intake, gestational age during pregnancy period, maternal age, educational level, parity, ethnicity, prepregnancy body mass index, smoking habits during pregnancy, alcohol consumption during pregnancy and folic acid supplement use during pregnancy.

Supplementary Table S3.3.4. Tracking of fetal growth characteristics from first trimester to birth in the Generation R Study, 2001-2005[1,2]

Tertiles	**Tertiles birth head circumference**			
First trimester CRL	Lowest	Middle	Highest	*n*
Lowest	1.08 (0.96, 1.82)	1.05 (0.75, 1.46)	0.88 (0.62, 1.23)	*306*
	n = 101	*n = 112*	*n = 93*	
	(33.0%)	(36.6%)	(30.4%)	
Middle	0.97 (0.72, 1.30)	1.09 (0.82, 1.47)	0.96 (0.72, 1.28)	*308*
	n = 97	*n = 104*	*n = 107*	
	(31.5%)	(33.8%)	(34.7%)	
Highest	0.97 (0.69, 1.35)	0.85 (0.61, 1.19)	1.19 (0.86, 1.64)	*307*
	n = 95	*n = 87*	*n = 125*	
	(30.9%)	(28.3%)	(40.7%)	
n	*293*	*303*	*325*	*921*
Tertiles	**Tertiles birth length**			
First trimester CRL	Lowest	Middle	Highest	*n*
Lowest	1.32 (0.96, 1.82)	1.06 (0.77, 1.46)	0.72 (0.52, 0.98)*	*343*
	n = 123	*n = 113*	*n = 107*	
	(35.9%)	(32.9%)	(31.2%)	
Middle	0.81 (0.61, 1.07)	1.18 (0.90, 1.55)	1.05 (0.80, 1.36)	*372*
	n = 105	*n = 125*	*n = 142*	
	(28.2%)	(33.6%)	(38.2%)	
Highest	1.00 (0.73, 1.36)	0.76 (0.56, 1.04)	1.28 (0.95, 1.73)	*358*
	n = 107	*n = 101*	*n = 150*	
	(29.9%)	(28.2%)	(41.9%)	
n	*335*	*339*	*399*	*1073*
Tertiles	**Tertiles birth weight**			
First trimester CRL	Lowest	Middle	Highest	*n*
Lowest	1.37 (1.05, 1.78)*	0.90 (0.70, 1.17)	0.82 (0.63, 1.06)	*538*
	n = 196	*n = 166*	*n = 176*	
	(36.4%)	(30.9%)	(32.7%)	
Middle	1.02 (0.81, 1.28)	1.05 (0.84, 1.31)	0.95 (0.76, 1.19)	*537*
	n = 168	*n = 179*	*n = 190*	
	(31.3%)	(33.3%)	(35.4%)	
Highest	0.72 (0.56, 0.94)*	1.04 (0.81, 1.33)	1.29 (1.00, 1.65)*	*537*
	n = 143	*n = 180*	*n = 214*	
	(26.6%)	(33.5%)	(39.9%)	
n	*507*	*525*	*580*	*1612*

Abbreviations: CRL, crown-rump length.

[1]Values are Odds Ratios (95% Confidence Interval) (numbers and percentages of fetuses that remain in the same tertile) to remain in the same tertile of each fetal growth characteristic from first trimester to birth. Estimates are from multiple imputed data. [2]Model is adjusted for gestational age at intake, gestational age during pregnancy period, maternal age, educational level, parity, ethnicity, prepregnancy body mass index, smoking habits during pregnancy, alcohol consumption during pregnancy and folic acid supplement use during pregnancy. *P-value <0.05.

Supplementary Table S3.3.5. Maternal characteristics and head circumference tracking coefficients during different periods of gestation in the Generation R Study, 2001-2005[1]

	Second to third trimester		Third trimester to birth		Second trimester to birth	
Maternal characteristics	*n*	Regression coefficient (95% CI)	*n*	Regression coefficient (95% CI)	*n*	Regression coefficient (95% CI)
Age (yrs)						
< 25 yrs	*1580*	0.38 (0.34, 0.42)	*911*	0.34 (0.27, 0.42)	*887*	0.13 (0.06, 0.20)
25-35 yrs	*4997*	0.45 (0.42, 0.47)	*2825*	0.37 (0.33, 0.41)	*2761*	0.17 (0.13, 0.21)
>35 yrs	*1132*	0.43 (0.38, 0.48)	*647*	0.42 (0.34, 0.50)	*629*	0.15 (0.07, 0.24)
		Interaction P=0.04		*Interaction P=0.24*		*Interaction P=0.38*
Height (cm)						
< 165 cm	*3295*	0.44 (0.41, 0.47)	*1790*	0.39 (0.34, 0.44)	*1725*	0.19 (0.14, 0.25)
165-175 cm	*3356*	0.40 (0.37, 0.42)	*1943*	0.35 (0.30, 0.40)	*1913*	0.12 (0.07, 0.17)
>175 cm	*1058*	0.47 (0.41, 0.52)	*650*	0.30 (0.22, 0.38)	*639*	0.12 (0.03, 0.20)
		Interaction P=0.45		*Interaction P=0.11*		*Interaction P=0.05*
Prepregnancy weight (kg)						
< 65 kg	*4112*	0.42 (0.40, 0.45)	*2317*	0.38 (0.32, 0.43)	*2280*	0.17 (0.13, 0.22)
65-75 kg	*2446*	0.42 (0.38, 0.46)	*1438*	0.37 (0.30, 0.45)	*1391*	0.17 (0.10, 0.23)
>75 kg	*1151*	0.45 (0.40, 0.50)	*628*	0.34 (0.26, 0.43)	*606*	0.08 (0.01, 0.18)
		Interaction P=0.88		*Interaction P=0.49*		*Interaction P=0.31*
Prepregnancy body mass index (kg/m^2)						
Normal	*5472*	0.42 (0.40, 0.44)	*3161*	0.38 (0.34, 0.42)	*3099*	0.17 (0.13, 0.21)
Overweight	*1685*	0.45 (0.41, 0.50)	*926*	0.41 (0.33, 0.50)	*894*	0.15 (0.07, 0.23)
Obesity	*552*	0.48 (0.41, 0.55)	*296*	0.26 (0.14, 0.38)	*283*	0.15 (0.02, 0.27)
		Interaction P=0.41		*Interaction P=0.92*		*Interaction P=0.94*
Parity						
Nulliparous	*4337*	0.42 (0.39, 0.44)	*2329*	0.37 (0.33, 0.42)	*2275*	0.15 (0.10, 0.20)
Multiparous	*3372*	0.45 (0.42, 0.48)	*2054*	0.37 (0.33, 0.42)	*2002*	0.18 (0.13, 0.23)
		Interaction P=0.10		*Interaction P=0.99*		*Interaction P=0.43*
Highest education						
Primary school	*895*	0.45 (0.39, 0.50)	*496*	0.28 (0.19, 0.38)	*466*	0.14 (0.03, 0.25)
Secondary school	*3610*	0.42 (0.39, 0.45)	*2056*	0.39 (0.34, 0.44)	*2007*	0.16 (0.11, 0.21)
Higher education	*3204*	0.43 (0.40, 0.46)	*1831*	0.38 (0.32, 0.43)	*1804*	0.16 (0.11, 0.22)
		Interaction P=0.76		*Interaction P=0.25*		*Interaction P=0.73*
Ethnicity						
European	*4424*	0.43 (0.40, 0.45)	*2550*	0.36 (0.31, 0.40)	*2518*	0.13 (0.09, 0.17)
Non-European	*3285*	0.43 (0.40, 0.46)	*1833*	0.39 (0.34, 0.44)	*1759*	0.19 (0.14, 0.25)
		Interaction P=0.89		*Interaction P=0.31*		*Interaction P=0.07*
Alcohol consumption						
No	*3799*	0.42 (0.39, 0.45)	*2175*	0.32 (0.27, 0.37)	*2088*	0.14 (0.08, 0.19)
Yes	*3910*	0.45 (0.42, 0.47)	*2208*	0.43 (0.38, 0.48)	*2189*	0.19 (0.14, 0.24)
		Interaction P=0.17		*Interaction P=0.004*		*Interaction P=0.18*
Smoking habits						
None	*5746*	0.44 (0.42, 0.46)	*3307*	0.37 (0.33, 0.41)	*3219*	0.17 (0.13, 0.21)
Yes	*1963*	0.40 (0.36, 0.44)	*1076*	0.39 (0.32, 0.46)	*1058*	0.14 (0.07, 0.21)
		Interaction P=0.10		*Interaction P=0.56*		*Interaction P=0.52*
Folic acid supplement use						
Preconception	*2989*	0.44 (0.41, 0.48)	*1694*	0.36 (0.30, 0.41)	*1680*	0.13 (0.07, 0.19)
First 10 weeks	*2426*	0.43 (0.39, 0.46)	*1385*	0.42 (0.36, 0.48)	*1356*	0.18 (0.11, 0.26)
None	*2292*	0.41 (0.38, 0.45)	*1304*	0.35 (0.28, 0.42)	*1242*	0.17 (0.09, 0.25)
		Interaction P=0.26		*Interaction P=0.94*		*Interaction P=0.43*

[1]Values are regression coefficients (95% Confidence Interval) for head circumference during different periods of gestation. Estimates are based on multiple imputed data.

Supplementary Table S3.3.6. Maternal characteristics and length tracking coefficients during different periods of gestation in the Generation R Study, 2001-2005[1]

	Second to third trimester		Third trimester to birth		Second trimester to birth	
Maternal characteristics	*n*	Regression coefficient (95% CI)	*n*	Regression coefficient (95% CI)	*n*	Regression coefficient (95% CI)
Age (yrs)						
< 25 yrs	*1603*	0.54 (0.51, 0.58)	*1053*	0.34 (0.28, 0.41)	*1010*	0.22 (0.16, 0.29)
25-35 yrs	*5047*	0.54 (0.51, 0.56)	*3369*	0.37 (0.34, 0.41)	*3285*	0.19 (0.15, 0.23)
>35 yrs	*1138*	0.49 (0.45, 0.54)	*768*	0.34 (0.27, 0.41)	*738*	0.20 (0.12, 0.28)
		Interaction P=0.18		*Interaction P=0.30*		*Interaction P=0.62*
Height (cm)						
< 165 cm	*3313*	0.55 (0.53, 0.58)	*2105*	0.35 (0.30, 0.39)	*2024*	0.22 (0.17, 0.26)
165-175 cm	*3393*	0.52 (0.49, 0.55)	*2311*	0.34 (0.29, 0.38)	*2256*	0.17 (0.12, 0.21)
>175 cm	*1082*	0.47 (0.42, 0.52)	*775*	0.33 (0.29, 0.37)	*753*	0.18 (0.13, 0.22)
		Interaction P=0.01		*Interaction P=0.94*		*Interaction P=0.19*
Prepregnancy weight (kg)						
<65 kg	*4163*	0.53 (0.51, 0.56)	*2742*	0.36 (0.32, 0.41)	*2672*	0.21 (0.16, 0.25)
65-75 kg	*2472*	0.53 (0.49, 0.57)	*1680*	0.33 (0.27, 0.39)	*1623*	0.17 (0.11, 0.24)
>75 kg	*1153*	0.48 (0.43, 0.52)	*768*	0.35 (0.27, 0.42)	*738*	0.12 (0.04, 0.20)
		Interaction P=0.03		*Interaction P=0.31*		*Interaction P=0.01*
Prepregnancy body mass index (kg/m^2)						
Normal	*5545*	0.53 (0.51, 0.55)	*3731*	0.36 (0.33, 0.40)	*3634*	0.21 (0.18, 0.25)
Overweight	*1694*	0.54 (0.50, 0.58)	*1094*	0.35 (0.28, 0.42)	*1049*	0.16 (0.09, 0.23)
Obesity	*549*	0.50 (0.43, 0.57)	*365*	0.35 (0.24, 0.45)	*351*	0.13 (0.03, 0.24)
		Interaction P=0.60		*Interaction P=0.42*		*Interaction P=0.11*
Parity						
Nulliparous	*4409*	0.52 (0.49, 0.54)	*2858*	0.37 (0.33, 0.41)	*2765*	0.17 (0.13, 0.21)
Multiparous	*3379*	0.55 (0.52, 0.58)	*2332*	0.34 (0.30, 0.39)	*2268*	0.22 (0.18, 0.27)
		Interaction P=0.06		*Interaction P=0.47*		*Interaction P=0.13*
Highest completed education						
Primary school	*908*	0.58 (0.52, 0.63)	*559*	0.37 (0.28, 0.46)	*526*	0.25 (0.16, 0.35)
Secondary school	*3642*	0.53 (0.51, 0.56)	*2459*	0.35 (0.31, 0.40)	*2370*	0.19 (0.15, 0.23)
Higher education	*3238*	0.52 (0.49, 0.55)	*2172*	0.37 (0.32, 0.42)	*2137*	0.20 (0.15, 0.25)
		Interaction P=0.10		*Interaction P=0.80*		*Interaction P=0.58*
Ethnicity						
European	*4465*	0.52 (0.49, 0.54)	*3091*	0.39 (0.35, 0.43)	*3018*	0.21 (0.17, 0.25)
Non-European	*3323*	0.55 (0.53, 0.58)	*2099*	0.33 (0.28, 0.37)	*2015*	0.19 (0.14, 0.24)
		Interaction P=0.04		*Interaction P=0.03*		*Interaction P=0.57*
Alcohol consumption						
No	*3839*	0.54 (0.51, 0.56)	*2552*	0.34 (0.30, 0.39)	*2431*	0.21 (0.16, 0.26)
Yes	*3949*	0.53 (0.50, 0.56)	*2638*	0.38 (0.34, 0.43)	*2602*	0.19 (0.14, 0.24)
		Interaction P=0.72		*Interaction P=0.21*		*Interaction P=0.56*
Smoking habits						
None	*5796*	0.53 (0.51, 0.56)	*3888*	0.34 (0.31, 0.38)	*3766*	0.19 (0.15, 0.22)
Yes	*1992*	0.52 (0.48, 0.56)	*1302*	0.39 (0.33, 0.45)	*1267*	0.21 (0.14, 0.27)
		Interaction P=0.61		*Interaction P=0.17*		*Interaction P=0.61*
Folic acid supplement use						
Preconception	*3019*	0.51 (0.48, 0.54)	*2061*	0.35 (0.29, 0.41)	*2025*	0.20 (0.14, 0.25)
First 10 weeks	*2444*	0.53 (0.50, 0.57)	*1642*	0.38 (0.31, 0.44)	*1596*	0.19 (0.13, 0.25)
None	*2325*	0.56 (0.53, 0.59)	*1488*	0.36 (0.30, 0.42)	*1412*	0.21 (0.16, 0.27)
		Interaction P=0.03		*Interaction P=0.73*		*Interaction P=0.73*

[1]Values are regression coefficients (95% confidence interval) for length during different periods of gestation. Estimates are based on multiple imputed data.

Chapter 3.4

First trimester fetal growth and cardiovascular risk factors in childhood

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Abstract

Objective: To examine whether first trimester fetal growth restriction correlates with cardiovascular outcomes in childhood.

Design: Population based prospective cohort study.

Setting: City of Rotterdam, the Netherlands.

Participants: 1184 children with first trimester fetal crown to rump length measurements, whose mothers had a reliable first day of their last menstrual period and a regular menstrual cycle.

Main outcomes measures: Body mass index, total and abdominal fat distribution, blood pressure, and blood concentrations of cholesterol, triglycerides, insulin, and C-peptide at the median age of 6.0 (90% range 5.7-6.8) years. Clustering of cardiovascular risk factors was defined as having three or more of: high android fat mass; high systolic or diastolic blood pressure; low high density lipoprotein cholesterol or high triglycerides concentrations; and high insulin concentrations.

Results: One standard deviation score greater first trimester fetal crown to rump length was associated with a lower total fat mass (−0.30 % (95% Confidence Interval (CI): −0.57, −0.03)), android fat mass (−0.07 % (95% CI: −0.12, −0.02)), android/gynoid fat mass ratio (−0.53 (95% CI: −0.89, −0.17)), diastolic blood pressure (−0.43 mmHg (95% CI: −0.84, −0.01)), total cholesterol (−0.05 mmol/L (95% CI: −0.10, 0)), low density lipoprotein cholesterol (−0.04 mmol/L (95% CI: −0.09, 0) and risk of clustering of cardiovascular risk factors (relative risk: 0.81 (95% CI: 0.66, 1.00)) in childhood. Additional adjustment for gestational age and weight at birth changed these effect estimates only slightly. Childhood body mass index fully explained the associations of first trimester fetal crown to rump length with childhood total fat mass. First trimester fetal growth was not associated with other cardiovascular outcomes. Longitudinal growth analyses showed that compared with school age children without clustering of cardiovascular risk factors, those with clustering had a smaller first trimester fetal crown to rump length and lower second and third trimester estimated fetal weight but higher weight growth from the age of 6 months onwards.

Conclusions: Impaired first trimester fetal growth is associated with an adverse cardiovascular risk profile in school age children. Early fetal life might be a critical period for cardiovascular health in later life.

Introduction

Fetal developmental adaptations in response to adverse environmental exposures may permanently affect the structure and function of cardiovascular organs.[1] These adaptations may lead to increased risks of cardiovascular disease in adulthood.[1] Human development rates are highest during the first trimester of pregnancy.[2] This period includes the embryonic phase and is essential for development of fetal cardiovascular and metabolic organs.[3] Therefore, the first trimester of pregnancy may be a critical period for cardiovascular health in childhood and adulthood.

In obstetric care practice, first trimester fetal crown to rump length is commonly used for dating pregnancy, assuming no growth variation.[3] However, among pregnant women with a known first day of the last menstrual period and a regular cycle, fetal crown to rump length can be used as a first trimester growth outcome.[4] First trimester fetal growth seems to be influenced by maternal age, ethnicity, parity, blood pressure, haemoglobin concentrations, smoking, and folic acid supplement use and is associated with increased risks of adverse birth outcomes.[4-8] Whether first trimester fetal growth restriction is associated with risk factors for cardiovascular disease in later life remains unknown.

In a population based prospective cohort study among 1184 mothers with a known first day of the last menstrual period and a regular cycle, and their children, we examined the associations of first trimester fetal crown to rump length with cardiovascular risk factors in childhood. Cardiovascular outcomes of interest included body mass index, body fat distribution, blood pressure, lipid concentrations, and insulin measures, which are known risk factors for cardiovascular disease in adulthood and track from childhood to adulthood.[9,10]

Methods

Design and population

This study was nested in the Generation R Study, a population based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[11] Participating mothers gave written consent.[12] Enrolment in the full Generation R Study was aimed at early pregnancy but allowed until birth. In total, 8880 mothers were enrolled in the full study during pregnancy. Of these mothers, 4685 did not have a fetal crown to rump length measurement, mainly because of a later enrolment in the study.[4,10] Of all 4195 mothers with a fetal crown to rump length measurement, 2576 were not eligible for the nested study because their fetal crown to rump length measurements were not within the range of 10 weeks 0 days to 13 weeks 6 days or they had an unknown first day of last menstrual period or an irregular menstrual cycle.[4] Of the remaining 1619 eligible mothers who had a first trimester crown to rump length measurement, had a known gestational age based on the last menstrual period, and gave birth to a singleton live

born child, 1184 participated with their children in detailed follow-up measurements at the age 6 years (**Supplementary Figure S3.4.1**).

First trimester fetal crown to rump length

We measured first trimester fetal crown to rump length in the gestational age range of 10 weeks 0 days to 13 weeks 6 days in a true mid-sagittal plane with the genital tubercle and the fetal spine longitudinally in view.[4,13] First day of the last menstrual period came from the referring letter from the community midwife or hospital.[4] We confirmed this date with the mother at the ultrasound visit and obtained additional information on the regularity and duration of the menstrual cycle. Intra-class correlation coefficients for intra-observer and inter-observer reproducibility of crown to rump length measurements were 0.998 and 0.995.[14] As previously described, we constructed gestational age adjusted standard deviation scores for first trimester fetal crown to rump length.[4]

Fetal and childhood growth

We measured second and third trimester fetal head circumference, abdominal circumference, and femur length to the nearest millimetre by using standardised ultrasound procedures.[15] We used the Hadlock formula to calculate estimated fetal weight.[16] Sex, date of birth, and birth anthropometrics (length, weight) came from registries. Well trained staff in community health centres measured childhood growth characteristics (weight, length) by using standardised procedures at the ages of 6, 12, 24, 36, and 48 months.[9] For all fetal, birth, and childhood growth characteristics, we used reference growth charts to construct standard deviation score values with a commercially available package (Growth Analyser 3.0, Dutch Growth Research Foundation, Rotterdam, Netherlands).[15,17]

Childhood cardiovascular outcomes

We invited all children to a dedicated research facility in the Erasmus University Medical Center, Sophia Children's Hospital for detailed measurements at the age of 6 years. We measured height and weight and calculated body mass index. We measured body fat by dual energy x ray absorptiometry (iDXA, General Electrics, 2008, Madison, WI, USA). We calculated total fat mass as a percentage of total body weight measured by absorptiometry. We calculated android and gynoid fat mass as a percentage of total fat mass, as well as their ratio.[18] We used the android/gynoid fat mass ratio as a measure of body fat distribution, as we did not measure waist/hip ratio. Higher waist/hip ratio and android/gynoid fat mass ratio reflect an adverse body fat distribution and are associated with mortality in adults and insulin resistance in children, respectively.[19,20] We measured systolic and diastolic blood pressure at the right brachial artery, four times at one minute intervals, by using the validated automatic sphygmomanometer Datascope Accutor Plus (Paramus, NJ, USA).[21] We selected a cuff with a width approximately 40%

of the arm circumference and long enough to cover 90% of the arm circumference. We obtained venous blood samples after 30 minutes' fasting from the children and measured total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglycerides, insulin, and C-peptide concentrations.

We used the previously described definition of childhood metabolic syndrome phenotype to define children with clustering of cardiovascular risk factors.[22] We defined children with clustering of cardiovascular risk factors as those with three or more of the following components: android fat mass percentage 75th centile or above, systolic or diastolic blood pressure 75th centile or above; high density lipoprotein cholesterol 25th centile or below or triglycerides 75th centile or above, and insulin concentration 75th centile or above. We used android fat mass percentage as proxy for waist circumference, as waist circumference was not available.[20]

Covariates

We obtained information on maternal age, ethnicity, educational level, parity, folic acid supplementation, and smoking by questionnaire at enrolment.[11] Maternal height and weight were measured and body mass index was calculated at enrolment. We measured maternal blood pressure with the validated oscillometric sphygmomanometer (OMRON Healthcare Europe B V, Hoofddorp, Netherlands) and documented the mean value of two blood pressure readings.[23]

Statistical analysis

Firstly, we used first trimester fetal crown to rump length standard deviation scores as a continuous variable, to analyse the linear associations of first trimester fetal crown to rump length with childhood outcomes. Using mean plots and one way analysis of variance tests, we observed that the best fitting trend lines for these associations were linear. The model fit and explained variance did not improve with addition of a quadratic term to the multivariate regression models. To further explore non-linearity and for presentation purposes, we also categorized first trimester fetal crown to rump length in fifths and examined the associations of fifths of first trimester fetal crown to rump length standard deviation score with childhood outcomes by using multivariate regression models. For these analyses, we constructed standard deviation score values ((observed value−mean)/SD) for the childhood outcome measures to enable comparison of effect estimates for the different outcomes. We did not create age adjusted standard deviation scores, as the childhood outcomes were measured in a small age range without changes in standard deviation.

Secondly, we used different linear regression models to examine the associations of first trimester fetal crown to rump length standard deviation score with childhood outcomes and the role of fetal and childhood growth in these associations. We used four different models. The basic model was adjusted for duration of last menstrual cycle and child's sex and age at outcome measurement. Childhood height was included in all

models on fat mass outcomes to take account of skeletal growth.[24] The confounder model was additionally adjusted for maternal and childhood covariates including maternal age, educational level, ethnicity, parity, prepregnancy body mass index, diastolic blood pressure, smoking during pregnancy, folic acid supplement use, and duration of breastfeeding. We selected these confounders on the basis of their associations with the outcomes of interest or a change in effect estimate of more than 10%. **Supplementary Tables S3.4.1-S3.4.3** show the associations of each confounder with the outcomes of interest. We considered the confounder models to be the main models. Next, these models were additionally adjusted for gestational age at birth and birth weight to explore whether any association was explained by later fetal growth (fetal pathway model) and for child's current body mass index to explore whether any association was explained by current childhood size (childhood pathway model).

Thirdly, we examined the association of first trimester fetal crown to rump length standard deviation scores continuously and in fifths with the risk of childhood clustering of cardiovascular risk factors. We used a multivariate generalised linear model with a Poisson assumption, log linear link function, and robust standard errors estimation to calculate adjusted relative risks.[25] Subsequently, we explored the longitudinal length and weight growth patterns from first trimester onwards until the age of 6 years for children with and without clustering of cardiovascular risk factors. For this analysis, we used repeated measurement regression models, which take into account the correlation between repeated growth measurements of the same participant.[26,27]

For all analyses, the percentages of missing values of covariates were lower than 20%. We imputed missing data of the covariates by using multiple imputations.[28] Five datasets were created and analysed together. We used SAS version 9.2 for the repeated measurement analysis and SPSS 17.0 for other analyses.

Results

Participants' characteristics and non-response

Table 3.4.1 shows the maternal, fetal, and childhood characteristics. The specific fetal and childhood growth characteristics are shown in **Supplementary Table S3.4.4**. As only mothers with fetal crown to rump length measurement between 10 and 14 weeks of gestation and a known and reliable first day of last menstrual period were eligible for this analysis, we did several non-response analyses. **Supplementary Tables S3.4.5-S3.4.7** show results from these analyses. Compared with mothers with a first trimester fetal crown to rump length measurement (*n = 4195*), those without this measurement (*n = 4685*) were on average younger, shorter, and heavier; had a lower blood pressure; and were less frequently highly educated and European. We found no difference in birth weight (**Supplementary Table S3.4.5**).

Among mothers with a first trimester fetal crown to rump length measurement, we found similar differences between those without (*n = 2576*) and with (*n = 1619*)

information about their known last menstruation (**Supplementary Table S3.4.6**). Of the eligible group of 1619 mothers and children, 1184 participated in the follow-up studies at the age of 6 years. Mothers of children not participating in these follow-up studies (*n = 435*) were on average younger, were less frequently higher educated and European, and less frequently used folic acid supplements. Their children were more frequently breast fed. We found no differences in gestational age and weight at birth (**Supplementary Table S3.4.7**).

Table 3.4.1. Maternal, fetal and childhood characteristics (*n = 1184*)[1]

Characteristic	Value
Maternal characteristics	
Age, median (90% range), yr	31.3 (22.7, 38.1)
Height, mean (SD), cm	168.8 (7.0)
Prepregnancy weight, mean (SD), kg	66.9 (11.8)
Prepregnancy body mass index, mean (SD), kg/m^2	23.4 (3.9)
Gestational age at intake, median (90% range), wks	12.4 (10.5, 13.9)
Systolic blood pressure, mean (SD), mmHg	116.7 (12.4)
Diastolic blood pressure, mean (SD), mmHg	69.1 (9.4)
Parity, nulliparous, No. (%)	717/1179 (60.8)
Education, No. (%)	
Primary or secondary school	507/1155 (43.9)
Higher education	648/1155 (56.1)
Race / Ethnicity, No. (%)	
Dutch, other European	855/1175 (72.8)
Non-European	320/1175 (27.2)
Smoking habits, No. (%)	
None	820/1060 (77.4)
Yes	240/1060 (22.6)
Folic acid supplement use, No. (%)	
No use	119/949 (12.5)
First 10 weeks use	294/949 (31.0)
Preconception use	536/949 (56.5)
Fetal characteristics	
Gestational age at fetal crown to rump length measurement, median (90% range), wks	12.4 (11.0, 13.9)
First trimester fetal crown to rump length, mean (SD), mm	61 (11)
Birth and infant characteristics	
Males, No. (%)	575 (48.6)
Gestational age at birth, median (90% range), wks	40.1 (37.0, 42.0)
Birth weight, mean (SD), g	3456 (551)
Ever breastfeeding, No. (%)	
No	80/1046 (7.6)
Yes	966/1046 (92.4)
Breastfeeding duration, mean (SD), months	5.3 (3.8)
Childhood characteristics	
Age at follow up, median (90% range), yr	6.0 (5.7, 6.8)
Height, mean (SD), cm	119.0 (5.5)
Weight, mean (SD), kg	22.8 (3.7)
Body mass index, mean (SD), kg/m^2	16.1 (1.7)
Total fat mass, mean (SD), %	24.6 (5.2)
Android fat mass, mean (SD), %	3.8 (0.9)
Gynoid fat mass, mean (SD), %	15.3 (1.6)
Android/gynoid fat mass ratio, mean (SD)	0.25 (0.1)
Systolic blood pressure, mean (SD), mmHg	102.6 (8.1)
Diastolic blood pressure, mean (SD), mmHg	60.7 (6.8)
Cholesterol, mean (SD), mmol/L	4.2 (0.7)

Table 3.4.1. Maternal, fetal and childhood characteristics (*n = 1184*)[1] (*continued*)

Characteristic	Value
Low Density Lipoprotein cholesterol, mean (SD), mmol/L	2.4 (0.6)
High Density Lipoprotein cholesterol, mean (SD), mmol/L	1.3 (0.3)
High Density / Low Density Lipoprotein cholesterol, mean (SD)	0.6 (0.2)
Triglycerides, median (90% range), mmol/L	1.0 (0.4, 2.1)
Insulin, median (90% range), pmol/L	118.2 (25.9, 342.4)
C-peptide, median (90% range), nmol/L	1.0 (0.4, 1.9)
Cardiovascular risk factor clustering, No. (%)	81/745 (10.9)

[1]Values represent mean (SD), median (90% range) or number of subjects (valid %).

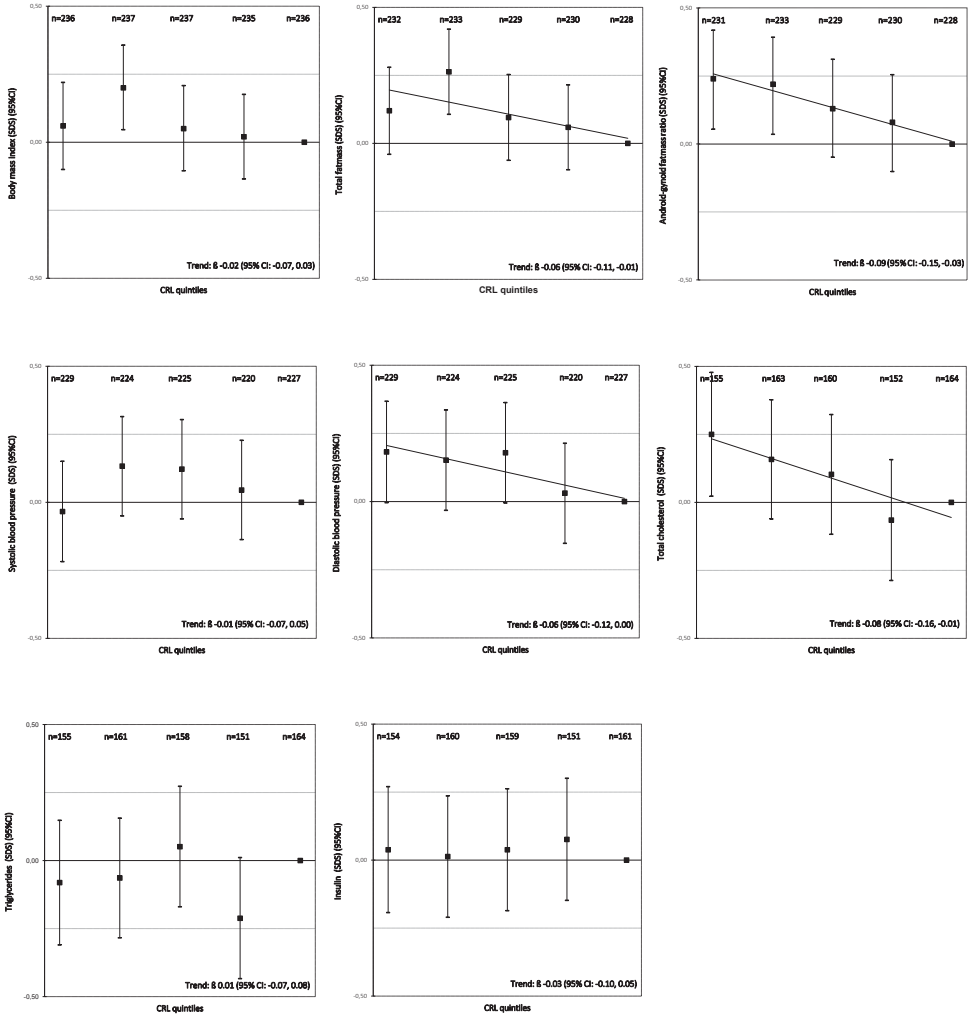
First trimester fetal crown to rump length and cardiovascular risk factors

Figure 3.4.1 shows that compared with children in the highest fifth of first trimester fetal crown to rump length, those in the lowest fifth tended to have higher total fat mass percentage, android/gynoid fat mass ratio, diastolic blood pressure, and total cholesterol (all P for trend <0.05). First trimester fetal crown to rump length was not associated with insulin or C-peptide concentrations. Results for C-peptide are not shown. Table 3.4.2 shows that in the confounder models, one standard deviation score greater first trimester fetal crown to rump length was associated with a lower total fat mass (−0.30 % (95% Confidence Interval (CI): −0.57, −0.03)), android fat mass (−0.07 % (95% CI: −0.12, −0.02)), android/gynoid fat mass ratio (−0.53 (95% CI: −0.89, −0.17)), diastolic blood pressure (−0.43 mmHg (95% CI: −0.84, −0.01)), total cholesterol (−0.05 mmol/L (95% CI: −0.10 to 0)), and low density lipoprotein cholesterol (−0.04 mmol/L (95% CI: −0.09 to 0)) in childhood. Additional adjustment for gestational age and weight at birth only slightly changed these effect estimates. Childhood body mass index fully explained the associations of first trimester fetal crown to rump length with childhood total fat mass. First trimester fetal crown to rump length was not associated with childhood body mass index, systolic blood pressure, or concentrations of triglycerides or insulin.

First trimester fetal crown to rump length and clustering of cardiovascular risk factors

One standard deviation score greater first trimester fetal crown to rump length was associated with a lower risk of clustering of cardiovascular risk factors (relative risk (RR): 0.81 (95% CI: 0.66, 1.00)) in childhood (Figure 3.4.2). When we compared fifths, we observed that compared with children in the lowest fifth of first trimester fetal crown to rump length, those in the highest fifth tended to have lower risks of clustering of cardiovascular risk factors (15.5% *v* 5.6% for lowest and highest fifth; RR: 0.50 (95% CI: 0.22, 1.10) (Figure 3.4.2). Adjustment for gestational age and weight at birth changed these effect estimates only slightly (Supplementary Figure S3.4.2).

Figure 3.4.1. First-trimester fetal growth and cardiovascular risk factors in childhood (*n = 1184*)



Values are linear regression coefficients (95% Confidence Interval) that reflect the difference in childhood outcomes expressed as standard deviation scores (SDS) between first-trimester fetal crown to rump length (CRL) quintiles as compared to the reference group (highest quintile). Estimates are based on multiple imputed data. Models were adjusted for child's sex and age at measurement, and for maternal duration of last menstrual cycle, age, educational level, ethnicity, parity, prepregnancy body mass index, diastolic blood pressure, smoking during pregnancy, folic acid supplement use and breastfeeding duration. Models for total fat mass and android/gynoid fat mass ratio were additionally adjusted for current childhood height. Trend lines are only given when P-value for linear trend <0.05.

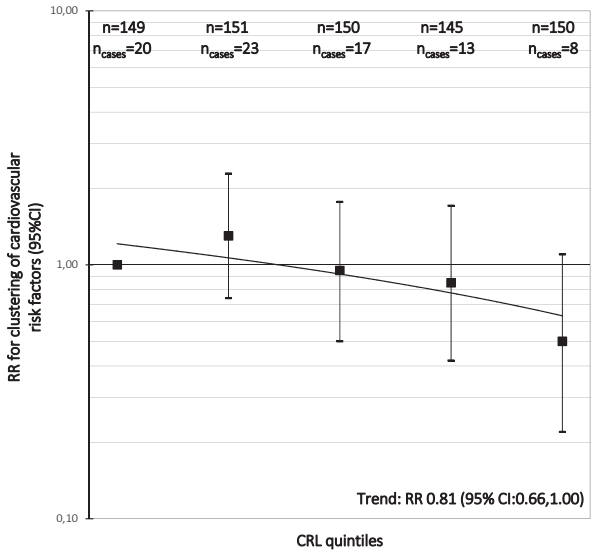
Table 3.4.2. First-trimester fetal growth and childhood cardiovascular risk factors (*n = 1184*)[1]

		Difference (95% Confidence Interval) in cardiovascular risk factors per SDS-change in first-trimester fetal crown to rump length							
	n	Basic model	P-value	Confounder model[2]	P-value	Fetal pathway model[3]	P-value	Childhood pathway model[4]	P-value
Body mass index (kg/m^2)	*1181*	-0.06 (-0.16, 0.04)	0.23	-0.04 (-0.14, 0.05)	0.38	-0.08 (-0.18, 0.02)	0.10	-	-
Total fat mass (%)	*1152*	-0.41 (-0.70, -0.13)	0.01	-0.30 (-0.57, -0.03)	0.03	-0.31 (-0.59, -0.04)	0.03	-0.16 (-0.36, 0.04)	0.11
Android fat mass (%)	*1151*	-0.08 (-0.14, -0.03)	<0.01	-0.07 (-0.12, -0.02)	0.01	-0.07 (-0.12, -0.01)	0.02	-0.04 (-0.08, 0)	0.07
Gynoid fat mass (%)	*1151*	0.05 (-0.04, 0.14)	0.27	0.05 (-0.04, 0.14)	0.32	0.05 (-0.04, 0.14)	0.25	0.06 (-0.03, 0.15)	0.17
Android/gynoid fat mass ratio (%)	*1151*	-0.62 (-0.98, -0.26)	<0.01	-0.53 (-0.89, -0.17)	<0.01	-0.52 (-0.88, -0.16)	<0.01	-0.38 (-0.68, -0.08)	0.01
Systolic blood pressure (mmHg)	*1125*	-0.22 (-0.70, 0.27)	0.39	-0.10 (-0.59, 0.39)	0.69	-0.09 (-0.58, 0.41)	0.74	-0.01 (-0.50, 0.47)	0.96
Diastolic blood pressure (mmHg)	*1125*	-0.51 (-0.92, -0.10)	0.02	-0.43 (-0.84, -0.01)	0.04	-0.42 (-0.84, 0)	0.05	-0.40 (-0.81, 0.02)	0.06
Total cholesterol (mmol/L)	*794*	-0.05 (-0.10, 0)	0.04	-0.05 (-0.10, 0)	0.04	-0.06 (-0.10, -0.01)	0.03	-0.05 (-0.10, 0)	0.04
HDL cholesterol (mmol/L)	*794*	-0.01 (-0.04, 0.01)	0.29	-0.01 (-0.04, 0.01)	0.27	-0.01 (-0.04, 0.01)	0.25	-0.01 (-0.04, 0.01)	0.23
LDL cholesterol (mmol/L)	*793*	-0.04 (-0.08, 0)	0.06	-0.04 (-0.09, 0)	0.06	-0.04 (-0.09, 0)	0.06	-0.04 (-0.09, 0)	0.06
HDL/LDL cholesterol ratio	*786*	0.01 (-0.01, 0.02)	0.20	0.01 (-0.01, 0.03)	0.21	0.01 (-0.01, 0.03)	0.19	0.01 (-0.01, 0.03)	0.22
Triglyceride (mmol/L)[5]	*789*	0.01 (-0.03, 0.04)	0.71	0.00 (-0.03, 0.04)	0.86	0.01 (-0.03, 0.04)	0.76	0.01 (-0.03, 0.04)	0.69
Insulin (pmol/L)[5]	*785*	-0.01 (-0.07, 0.05)	0.66	-0.02 (-0.08, 0.04)	0.49	-0.02 (-0.08, 0.04)	0.54	-0.01 (-0.07, 0.05)	0.63

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in childhood outcomes per standard deviation score first-trimester fetal crown to rump length. Basic model was adjusted for duration of last menstrual cycle, and child's sex and age at outcome measurements. Models for fat mass outcomes were additionally adjusted for current childhood height. [2]Confounders include maternal age, educational level, ethnicity, parity, prepregnancy body mass index, diastolic blood pressure, smoking during pregnancy and folic acid supplement use and breastfeeding duration. [3]Model additionally adjusted for gestational age and weight at birth. [4]Model additionally adjusted for childhood current body mass index. [5]Variables were log-transformed.

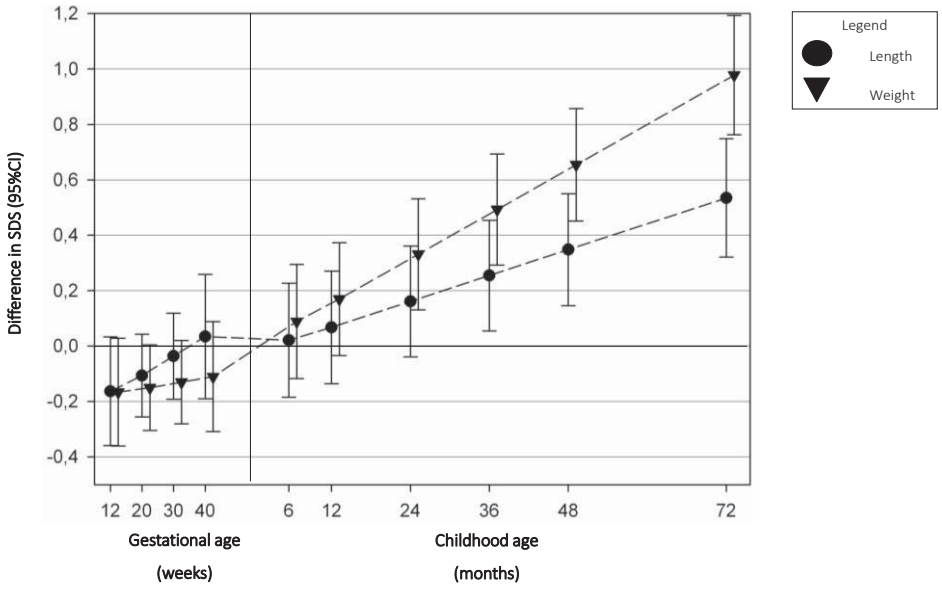
Figure 3.4.3 shows the longitudinal growth in fetal and childhood length and weight from first trimester fetal crown to rump length onwards in children with clustering of cardiovascular risk factors, compared with those without clustering of cardiovascular risk factors. First trimester fetal crown to rump length tended to be smaller in children with clustering of cardiovascular risk factors (difference −0.16 (95% CI: −0.36 to 0.03 standard deviation scores)). Estimated fetal weight, but not femur length, until birth tended to be smaller in children with clustering of cardiovascular risk factors. From the age of 6 months onwards, children with clustering of cardiovascular risk factors at age 6 years had a higher length and weight, with larger effect estimates for weight. The effect estimates were not materially affected by additional adjustment for potential confounders (Supplementary Table S3.4.8).

Figure 3.4.2. First-trimester fetal growth and clustering of cardiovascular risk factors (*n = 745*)



Values are Relative Risks (RR) (95% Confidence Interval) from generalized linear models that reflect the risk of childhood clustering of cardiovascular risk factors for first-trimester fetal crown to rump length quintiles, as compared to the reference group (lowest quintile). Estimates are based on multiple imputed data. Clustering of cardiovascular risk factors was defined as having 3 or more following components: android fat mass percentage ≥75th percentile; systolic or diastolic blood pressure ≥75th percentile; HDL-cholesterol ≤25th percentile or triglycerides ≥75th percentile; and insulin level ≥75th percentile [22]. Model was adjusted for child's sex and age at measurement, maternal duration of last menstrual cycle, age, educational level, ethnicity, parity, prepregnancy body mass index, diastolic blood pressure, smoking during pregnancy, folic acid supplement use, and breastfeeding duration.

Figure 3.4.3. Fetal and childhood length and weight growth from first trimester onwards in children with clustering of cardiovascular risk factors (*n = 745*)



Values are regression coefficients (95% Confidence Interval) that reflect the difference in length and weight standard deviation score (SDS) from first trimester onwards for children with clustering of cardiovascular risk factors, as compared to children without such clustering. Models were adjusted for maternal duration of last menstrual cycle, and child's sex and age at outcome measurements. Length and weight growth characteristics used in the models: Fetal period: first trimester: crown to rump length as both length and weight measure (starting point); second and third trimester: femur length and estimated fetal weight; At birth: birth length and birth weight; During childhood: length and weight. Clustering of cardiovascular risk factors was defined as having 3 or more following components: android fat mass percentage ≥75th percentile; systolic or diastolic blood pressure ≥75th percentile; HDL-cholesterol ≤25th percentile or triglycerides ≥75th percentile; and insulin level ≥75th percentile [22].

Discussion

We observed that smaller first trimester fetal size was associated with an adverse body fat distribution, higher diastolic blood pressure, and an adverse blood cholesterol profile in childhood. First trimester fetal growth restriction was also associated with an increased risk of clustering of these cardiovascular risk factors in childhood. These associations were not explained by maternal, birth, and childhood characteristics.

Interpretation of main findings

Adverse fetal exposures may lead to early developmental adaptations, including changes in the anatomy, physiology, and metabolism of various organ systems.[1] These adaptations may be beneficial for short term survival but may have adverse consequences at birth and in later life, such as increased risks of low birth weight and common diseases

in adulthood.[1] Studies showing consistent associations of low birth weight with increased risks of cardiovascular disease strongly support this hypothesis.[29,30] Clearly, low birth weight is not the causal factor leading to diseases in later life. Birth weight is merely an endpoint of different fetal exposures and growth patterns and the starting point of childhood growth. Most children with a low birth weight have a catch-up growth leading to a normal weight from the age of 2 years onwards.[31] Longitudinal studies also showed that the risk of cardiovascular disease is highest among adults born with a low birth weight who had a high postnatal weight gain.[32,33] These results suggest that a low birth weight as a result of restricted fetal environment may specifically lead to cardiovascular disease in later life, when postnatal life is characterized by a relatively high body mass index as a result of an affluent environment.[1] Not much is known about the specific fetal growth patterns leading to cardiovascular disease in later life.

Rates of growth and development are much higher in fetal life than in childhood. The highest development rates are in the first trimester of pregnancy, which includes the embryonic phase.[2] Studies in spontaneously conceived pregnancies and in pregnancies resulting from assisted reproductive technology observed that first trimester fetal growth restriction was associated with increased risks of prematurity and small size for gestational age at birth.[4,7,8] We also observed that smaller first trimester fetal crown to rump length led to compensatory accelerated childhood growth.[4] High rates of childhood weight gain may subsequently lead to development of cardiovascular risk factors in later life.

The study reported here shows for the first time that first trimester fetal crown to rump length is also associated with an adverse cardiovascular risk profile in childhood. Smaller first trimester fetal crown to rump length was associated with higher total fat mass percentage, android/gynoid fat mass ratio, diastolic blood pressure, and total cholesterol concentration in childhood. These associations were observed across the full range of first trimester fetal crown to rump length and not in the extremes only. Also, these associations were independent of potential maternal and childhood confounders and were changed only slightly by adjustment for gestational age and weight at birth and childhood body mass index. First trimester fetal growth was not associated with childhood body mass index, systolic blood pressure, or concentrations of triglycerides, insulin, or C-peptide. The observed associations suggest that the first trimester of pregnancy is a critical period for cardiovascular health in later life. Previous studies have shown that risk factors for cardiovascular disease in childhood track into adulthood and are related to development of cardiovascular disease in later life.[9,10,34] Thus, cardiovascular disease may have at least part of its origins in the first trimester of pregnancy or even the preconception period. The developmental mechanisms that explain the associations of first trimester fetal growth and risk factors for cardiovascular disease are not known, but they may include changes in methylation of DNA and expression of RNA in response to a suboptimal fetal environment.[1] More detailed first trimester ultrasound studies are needed to assess early cardiovascular and metabolic developmental adaptations.

The results from this study are important from an aetiological perspective. They suggest that the first trimester might be a critical period for cardiovascular and metabolic function. However, we acknowledge that the observed effect estimates were small and reflect subclinical changes in cardiovascular and metabolic function in school age children. None of the children had known cardiovascular disease. Previous longitudinal studies have shown tracking of cardiovascular and metabolic risk factors from childhood to adulthood.[9,10] Also, adiposity in school age children is related to cardiovascular disease in later life.[34] Further follow-up studies are needed to explore whether suboptimal first trimester development really is a risk factor for clinically manifest cardiovascular and metabolic disease in adulthood.

Strengths and limitations

This study was nested in a large population based prospective cohort study. In the full study, enrolment was aimed at early pregnancy but allowed until birth. As this study was specifically focused on the long term effects of variation in first trimester fetal growth, only a subgroup of mothers with a first trimester fetal crown to rump length measurement between 10 and 14 weeks of gestation and a known and reliable first day of last menstrual period was eligible. As a result of these necessary selection criteria, the eligible mothers reflect a small fraction of the full study population. Of all eligible mothers, 73% participated with their children in the follow-up studies at the age of 6 years. The non-response analyses showed that mothers not included in the analyses were on average younger, shorter, and heavier; had a lower blood pressure; were less frequently high educated and European; and less frequently used folic acid supplements. Their children were more frequently breastfed. Our effect estimates would be biased if the associations differ between participants included and not included in the analysis. Although this seems unlikely, we cannot exclude it. We found no differences in first trimester fetal crown to rump length or birth weight between children with and without participation in the follow-up studies. More importantly, the selection of the study sample might have affected the generalizability of the results. The study population is a rather healthy and relatively highly educated population. Whether the observed associations are similar in high risk populations should be studied further.

We tested the associations of first trimester fetal crown to rump length with several cardiovascular and metabolic outcomes that track from childhood to adulthood and are risk factors for cardiovascular disease in adulthood. The large number of statistical tests that we did may have led to false positive associations. However, because of the correlations between the cardiovascular and metabolic outcomes, we did not adjust the analyses for multiple testing. We measured first trimester fetal growth by fetal crown to rump length and used the first day of the last menstrual period to determine gestational age. Misclassification of gestational age might still be a problem, as the post-conception age depends on the timing of ovulation and implantation, which we were unable to measure.[35] Several maternal factors, such as maternal age and smoking, are associated with the duration of the follicular phase, after which ovulation occurs. Recall bias may

also affect the dating of the last menstrual period.[36] However, all analyses were adjusted for the duration of last menstrual cycle, which is strongly associated with the timing of ovulation. Even with a known and reliable date of last menstrual period, a certain fraction of women with regular cycles have early or delayed ovulation. We did a sensitivity analysis with a restriction to participants who had a gestational age based on last menstruation within seven days of a gestational age based on crown to rump length (93%). This analysis did not materially change our effect estimates for the childhood outcomes. The analyses were adjusted for several maternal and childhood confounders. Although we observed that stepwise adjustment for various different potential maternal and childhood confounders did not strongly change the effect estimates, residual confounding may still be a concern, as in any observational study.

Conclusions

These results suggest that the first trimester of pregnancy may be a critical period for development of cardiovascular risk factors in later life. The observed associations are primarily important from an aetiological perspective. Further studies are needed to identify the underlying causal biological mechanisms and long term consequences. Future strategies to improve cardiovascular health may start from early pregnancy onwards or even before conception.

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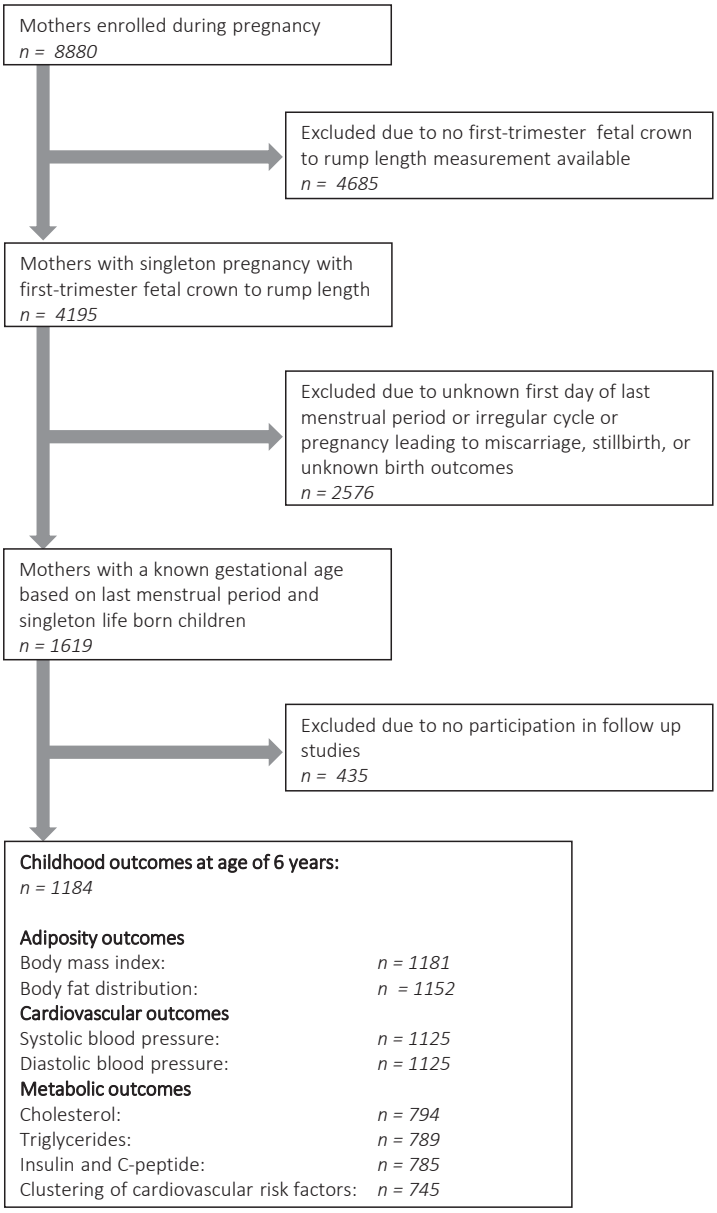
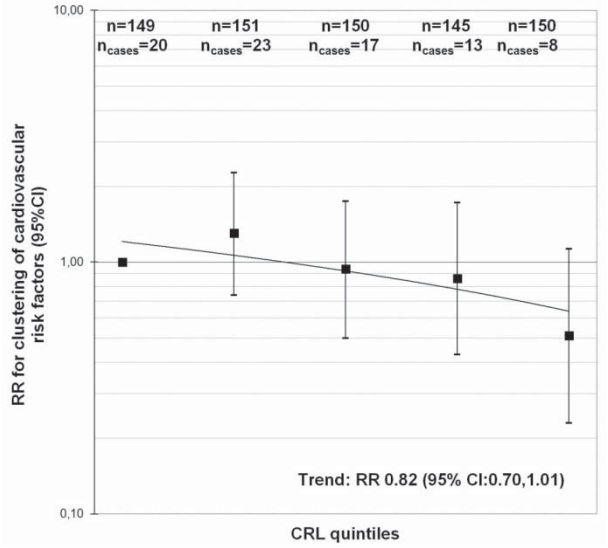
Supplementary Material

Supplementary Figure S3.4.1. Participants flow chart in the Generation R Study, Rotterdam, the Netherlands



Supplementary Figure S3.4.2. First-trimester fetal growth and clustering of cardiovascular risk factors adjusted for gestational age and weight at birth (*n = 745*)[1]



[1]Values are Relative Risks (95% Confidence Interval) from generalized linear models that reflect the risk of childhood clustering of cardiovascular risk factors for first-trimester fetal crown to rump length SDS quintiles, as compared to the reference group (lowest quintile). Model was adjusted for child's sex and age at measurement, maternal duration of last menstrual cycle, age, educational level, ethnicity, parity, prepregnancy body mass index, diastolic blood pressure, smoking during pregnancy, folic acid supplement use, breastfeeding duration and gestational age and weight at birth (fetal pathway model).

Supplementary Table S3.4.1. Associations of covariates with childhood body fat outcomes (*n = 1184*) [1]

	Body mass index (kg/m^2)	**Total fat mass** (%)	**Android/gynoid fat mass ratio** (%)
Duration of menstrual cycle, days	0.03 (-0.04, 0.10)	-0.08 (-0.30, 0.14)	0.03 (-0.23, 0.28)
Child sex			
Boys	*Reference*	*Reference*	*Reference*
Girls	-0.22 (-0.42, 0.03)*	4.19 (3.64, 4.75)*	0.39 (-0.30, 1.09)
Childhood age at outcome measurement,yr	0.63 (0.41, 0.85)*	1.43 (0.66, 2.20)*	1.41 (0.51, 2.31)*
Maternal age, yr	-0.03 (-0.05, -0.01)*	-0.16 (-0.22, -0.09)*	-0.13 (-0.21, -0.05)*
Education			
Primary	0.91 (0.45, 1.37)*	3.04 (1.69, 4.40)*	1.44 (-0.21, 3.09)
Secondary	0.37 (0.17, 0.57)*	1.95 (1.33, 2.57)*	1.48 (0.75, 2.21)*
Higher	*Reference*	*Reference*	*Reference*
Ethnicity			
Dutch or European	*Reference*	*Reference*	*Reference*
Non – European	0.40 (0.19, 0.62)*	1.44 (0.77, 2.11)*	0.90 (0.12, 1.68)*
Parity			
Nulliparous	*Reference*	*Reference*	*Reference*
Multiparous	0.02 (-0.17, 0.22)	-0.79 (-1.41, -0.18)*	-0.45 (-1.17, 0.26)
Prepregnancy body mass index, kg/m^2	0.13 (0.10, 0.15)*	0.33 (0.25, 0.41)*	0.27 (0.18, 0.36)*
Diastolic blood pressure, mmHg	0.01 (0, 0.02)*	0.04 (0.01, 0.08)*	0.02 (-0.02, 0.06)
Smoking			
Yes	0.34 (0.09, 0.58)*	1.16 (0.26, 2.06)*	1.85 (0.91, 2.79)*
No	*Reference*	*Reference*	*Reference*
Folic acid supplement use			
Yes	*Reference*	*Reference*	*Reference*
No	0.36 (0.15, 0.57)*	1.48 (0.84, 2.11)*	1.30 (0.56, 2.05)*
Breastfeeding duration, month	-0.02 (-0.05, 0.01)	-0.09 (-0.18, 0)	-0.08 (-0.18, 0.03)
Childhood height at outcome measurement, cm	-	0.13 (0.07, 0.18)*	0.11 (0.05, 0.18)*
Gestational age at birth, wks	-0.03 (-0.09, 0.03)	-0.12 (-0.30, 0.05)	-0.33 (-0.53, -0.13)*
Birth weight, SD	0.25 (0.15, 0.35)*	-0.17 (-0.47, 0.13)	-0.14 (-0.49, 0.21)
Childhood body mass index, kg/m^2	-	1.98 (1.84, 2.12)*	2.10 (1.93, 2.27)*

[1]Values are regression coefficients (95% Confidence Interval) from univariate regression models and reflect differences in childhood body fat distribution measures per unit change of each covariate and for different categories of each covariate as compared to the reference group. *P-value <0.05.

Supplementary Table S3.4.2. Associations of covariates with childhood blood pressure (*n = 1125*)[1]

	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Duration of menstrual cycle, days	-0.01 (-0.35, 0.34)	0.10 (-0.19, 0.39)
Child sex		
Boys	*Reference*	*Reference*
Girls	0.12 (-0.83, 1.07)	0.35 (-0.45, 2.14)
Childhood age at outcome measurement, yr	3.05 (1.89, 4.21)*	1.40 (0.41, 2.39) *
Maternal age, yr	-0.14 (-0.24, -0.03)*	-0.12 (-0.21, -0.03)*
Education		
Primary	2.65 (0.43, 4.87)*	3.25 (1.43, 5.07)*
Secondary	1.25 (0.26, 2.24)*	1.32 (0.49, 2.15)*
Higher	*Reference*	*Reference*
Ethnicity		
Dutch or European	*Reference*	*Reference*
Non – European	1.68 (0.62, 2.73)*	1.51 (0.63, 2.40)*
Parity		
Nulliparous	*Reference*	*Reference*
Multiparous	-0.69 (-1.66, 0.29)	-0.88 (-1.70, -0.06)*
Prepregnancy body mass index, kg/m^2	0.25 (0.12, 0.38)*	0.12 (0.01, 0.23)*
Diastolic blood pressure, mmHg	0.10 (0.05, 0.15)*	0.07 (0.02, 0.11)*
Smoking		
Yes	0.75 (-0.39, 1.88)	0.67 (-0.30, 1.65)
No	*Reference*	*Reference*
Folic acid supplement use		
Yes	*Reference*	*Reference*
No	1.61 (0.51, 2.71)*	0.56 (-0.35, 1.47)
Breastfeeding duration, month	-0.07 (-0.20, 0.06)	-0.09 (-0.20, 0.03)
Childhood height at outcome measurement, cm	-	-
Gestational age at birth, wks	-0.23 (-0.51, 0.04)	-0.03 (-0.26, 0.20)
Birth weight, SD	-0.35 (-0.82, 0.12)	-0.32 (-0.72, 0.08)
Childhood body mass index, kg/m^2	1.25 (0.98, 1.52)*	1.40 (0.42, 2.39)*

[1]Values are regression coefficients (95% Confidence Interval) from univariate regression models and reflect differences in childhood blood pressure per unit change of each covariate and for different categories of each covariate as compared to the reference group. *P-value <0.05.

Supplementary Table S3.4.3. Associations of covariates with childhood metabolic outcomes (*n = 789*)[1]

	Total cholesterol (mmol/l)	**Triglycerides**[2] (mmol/l)	**Insulin**[2] (pmol/l)
Duration of menstrual cycle, days	0.01 (-0.03, 0.04)	0.01 (-0.02, 0.03)	0.02 (-0.02, 0.06)
Child sex			
Boys	*Reference*	*Reference*	*Reference*
Girls	0.12 (0.03, 0.21)*	0.08 (0.02, 0.15)*	0.04 (-0.07, 0.15)
Childhood age at outcome measurement, yr	-0.01 (-0.12, 0.09)	-0.02 (-0.10, 0.05)	0.08 (-0.05, 0.21)
Maternal age, yr	0 (-0.01, 0.01)	0 (-0.01, 0.01)	-0.01 (-0.02, 0.01)
Education			
Primary	0 (-0.23, 0.23)	-0.04 (-0.13, 0.21)	0.09 (-0.21, 0.39)
Secondary	0.05 (-0.04, 0.15)	0.01 (-0.06, 0.08)	0 (-0.12, 0.12)
Higher	*Reference*	*Reference*	*Reference*
Ethnicity			
Dutch or European	*Reference*	*Reference*	*Reference*
Non – European	0.12 (0.01, 0.22)*	0 (-0.07, 0.08)	-0.09 (-0.22, 0.04)
Parity			
Nulliparous	*Reference*	*Reference*	*Reference*
Multiparous	-0.02 (-0.11, 0.08)	-0.01 (-0.08, 0.05)	0.05 (-0.06, 0.16)
Prepregnancy body mass index, kg/m^2	0 (-0.01, 0.01)	-0.01 (-0.02, 0)*	0 (-0.01, 0.02)
Diastolic blood pressure, mmHg	0 (-0.01, 0)	0 (-0.01, 0)	0 (-0.01, 0.01)
Smoking			
Yes	-0.03 (-0.17, 0.10)	-0.04 (-0.12, 0.05)	0.11 (-0.03, 0.25)
No	*Reference*	*Reference*	*Reference*
Folic acid supplement use			
Yes	*Reference*	*Reference*	*Reference*
No	0.04 (-0.06, 0.14)	0.04 (-0.03, 0.11)	-0.04 (-0.16, 0.08)
Breastfeeding duration, month	-0.01 (-0.02, 0)	0 (-0.01, 0.01)	-0.01 (-0.02, 0.01)
Childhood height at outcome measurement, cm	-	-	-
Gestational age at birth, wks	0.01 (-0.02, 0.04)	0 (-0.03, 0.02)	0.01 (-0.03, 0.05)
Birth weight, SD	0 (-0.05, 0.05)	-0.04 (-0.07, 0)	-0.02 (-0.06, 0.02)
Childhood body mass index, kg/m^2	0.03 (0, 0.06)*	0.02 (0, 0.04)	0.08 (0.05, 0.12)*

[1]Values are regression coefficients (95% Confidence Interval) from univariate regression models and reflect differences in childhood blood levels per unit change of each covariate and for different categories of each covariate as compared to the reference group. [2]Variables were log-transformed *P-value <0.05.

Supplementary Table S3.4.4. Fetal and childhood growth characteristics (*n = 1184*)[1]

Characteristics	Value
Fetal growth characteristics	
First trimester	
Gestational age, median (90% range), wks	12.4 (11.0, 13.9)
First trimester fetal crown to rump length, mean (SD), mm	61 (11)
Second trimester	
Gestational age, median (90% range), wks	20.4 (19.1, 22.2)
Femur length, mean (SD), mm	33 (3)
Estimated fetal weight, mean (SD), g	372 (74)
Third trimester	
Gestational age, median (90% range), wks	30.3 (29.1, 32.1)
Femur length, mean (SD), mm	58 (3)
Estimated fetal weight, mean (SD), g	1630 (241)
Birth	
Gestational age, median (90% range), wks	40.1 (37.0, 42.0)
Male sex, No (%)	575 (48.6)
Birth weight, mean (SD), g	3456 (551)
Childhood growth characteristics	
6 months	
Age at follow up, median (90% range), mo	6.2 (5.5, 7.3)
Height, mean (SD), cm	67.7 (2.5)
Weight, mean (SD), kg	7.8 (0.9)
12 months	
Age at follow up, median (90% range), mo	11.1 (10.2, 12.3)
Height, mean (SD), cm	74.4 (2.6)
Weight, mean (SD), kg	9.6 (1.1)
24 months	
Age at follow up, median (90% range), mo	25.0 (23.5, 27.3)
Height, mean (SD), cm	88.3 (3.4)
Weight, mean (SD), kg	12.9 (1.5)
36 months	
Age at follow up, median (90% range), mo	37.1 (35.5, 39.9)
Height, mean (SD), cm	97.3 (3.6)
Weight, mean (SD), kg	15.2 (1.8)
48 months	
Age at follow up, median (90% range), mo	46.0 (44.8, 47.9)
Height, mean (SD), cm	103.2 (4.1)
Weight, mean (SD), kg	16.9 (2.2)
72 months	
Age at follow up, median (90% range), mo	73.2 (68.9, 81.4)
Height, mean (SD), cm	119.0 (5.5)
Weight, mean (SD), kg	22.8 (3.7)

[1]Values represent mean (SD), median (90% range) or number of subjects (valid %).

Supplementary Table S3.4.5. Non-response analysis for first-trimester crown to rump length measurement in full cohort (*n = 8880*)[1]

	First trimester CRL measurement *n = 4195*	No first trimester CRL measurement *n = 4685*	P-value[4]
Maternal characteristics			
Age, median (90% range), yr	30.5 (21.1, 37.2)	29.9 (19.8, 38.4)	<0.01
Height, mean (SD), cm	167.8 (7.3)	167.1 (7.5)	<0.01
Prepregnancy weight, mean (SD), kg	66.4 (12.6)	66.2 (13.0)	0.64
Prepregnancy body mass index, mean (SD), kg/m^2	23.5 (4.2)	23.8 (4.5)	0.02
Gestational age at intake, median (90% range), wks[2]	12.5 (9.9, 14.9)	16.8 (12.6, 24.6)	<0.01
Systolic blood pressure, mean (SD), mmHg	116.2 (12.3)	114.7 (12.3)	<0.01
Diastolic blood pressure, mean (SD), mmHg	68.8 (9.6)	67.4 (9.5)	<0.01
Parity, nulliparous, No. (%)[3]	1743 (42.0)	2152 (46.7)	<0.01
Education, No. (%)[3]			
Primary or secondary school	2080 (53.3)	2604 (62.6)	<0.01
Higher education	1826 (46.7)	1553 (37.4)	
Race / Ethnicity, No. (%)[3]			
Dutch, other European	2563 (64.7)	2153 (50.8)	<0.01
Non-European	1397 (35.3)	2089 (49.2)	
Smoking habits, No. (%)[3]			
None	2715 (74.7)	2983 (76.1)	0.49
Yes	920 (25.3)	935 (23.9)	
Folic acid supplement use, No. (%)[3]			
No use	683 (21.4)	1245 (37.0)	<0.01
First 10 weeks use	1007 (31.5)	1033 (30.7)	
Preconception use	1504 (47.1)	1088 (32.3)	
Fetal characteristics			
Second trimester estimated fetal weight, mean (SD), g	369 (78)	396 (110)	<0.01
Third trimester estimated fetal weight, mean (SD), g	1607 (245)	1623 (285)	<0.01
Birth characteristics			
Males, No. (%)[3]	2072 (50.4)	2295 (50.6)	0.81
Gestational age, median (90% range), wks[2]	40.1 (36.9, 42.0)	40.0 (36.4, 42.1)	<0.01
Birth weight, mean (SD), g	3421 (566)	3398 (562)	0.08

[1]Values are mean (standard deviation). [2]Median (90% range). [3]Values are observed number and valid percentage. [4]Differences in subject characteristics between the groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.

Supplementary Table S3.4.6. Non-response analysis for first-trimester crown to rump length with and without information about last menstrual period and singleton live-born child (*n = 4195*)[1]

	Known last menstrual period and singleton live-born child *n = 1619*	Unknown last menstrual period and singleton live-born child *n = 2576*	P-value[4]
Maternal characteristics			
Age, median (90% range), yr	31.4 (21.9, 37.9)	29.8 (20.6, 37.0)	<0.01
Height, mean (SD), cm	168.7 (7.0)	167.2 (7.5)	<0.01
Prepregnancy weight, mean (SD), kg	66.9 (11.8)	66.2 (13.1)	0.22
Prepregnancy body mass index, mean (SD), kg/m^2	23.4 (3.8)	23.6 (4.5)	0.07
Gestational age at intake, median (90% range), wks[2]	12.4 (10.6, 13.9)	12.5 (9.6, 15.1)	0.01
Systolic blood pressure, mean (SD), mmHg	116.8 (12.5)	115.8 (12.2)	<0.01
Diastolic blood pressure, mean (SD), mmHg	69.1 (9.4)	68.7 (9.8)	0.16
Parity, nulliparous, No. (%)[3]	956 (59.4)	1451 (57.1)	0.15
Education, No. (%)[3]			
Primary or secondary school	703 (45.4)	1377 (58.4)	<0.01
Higher education	845 (54.6)	981 (41.6)	
Race / Ethnicity, No. (%)[3]			
Dutch, other European	1106 (70.7)	1457 (60.8)	<0.01
Non-European	458 (29.3)	939 (39.2)	
Smoking habits, No. (%)[3]			
None	1110 (76.8)	1605 (73.3)	0.13
Yes	336 (23.2)	584 (26.7)	
Folic acid supplement use, No. (%)[3]			
No use	192 (14.7)	491 (26.0)	<0.01
First 10 weeks use	415 (31.8)	592 (31.3)	
Preconception use	696 (53.4)	808 (42.7)	
Fetal characteristics			
Second trimester estimated fetal weight, mean (SD), g	371 (74)	368 (81)	0.33
Third trimester estimated fetal weight, mean (SD), g	1622 (240)	1598 (247)	<0.01
Birth characteristics			
Males, No. (%)[3]	802 (49.5)	1270 (50.9)	0.39
Gestational age, median (90% range), wks[2]	39.9 (37.1, 42.0)	39.8 (36.7, 42.0)	0.04
Birth weight, mean (SD), g	3453 (565)	3402 (564)	<0.01

[1]Values are mean (standard deviation). [2]Median (90% range). [3]Values are observed number and valid percentage. [4]Differences in subject characteristics between the groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.

Supplementary Table S3.4.7. Non-response analysis for loss to follow-up at the age of 6 years (*n = 1619*)[1]

	Follow-up at 6 years *n = 1184*	Loss to follow-up at 6 years *n = 435*	P-value[4]
Maternal characteristics			
Age, median (90% range), yr	31.3 (22.7, 38.1)	29.7 (5.0)	<0.01
Height, mean (SD), cm	168.8 (7.0)	168.5 (7.1)	0.43
Prepregnancy weight, mean (SD), kg	66.9 (11.8)	66.0 (11.9)	0.22
Prepregnancy body mass index, mean (SD), kg/m^2	23.4 (3.9)	23.2 (3.7)	0.29
Gestational age at intake, median (90% range), wks[2]	12.4 (10.5, 13.9)	12.4 (10.9, 13.9)	0.84
Systolic blood pressure, mean (SD), mmHg	116.7 (12.4)	116.8 (12.8)	0.87
Diastolic blood pressure, mean (SD), mmHg	69.1 (9.4)	69.1 (9.6)	0.93
Parity, nulliparous, No. (%)[3]	717 (60.8)	237 (55.2)	0.04
Education, No. (%)[3]			
Primary or secondary school	507 (43.9)	198 (49.5)	0.02
Higher education	648 (56.1)	202 (50.5)	
Race / Ethnicity, No. (%)[3]			
Dutch, other European	855 (72.8)	258 (63.1)	<0.01
Non-European	320 (27.2)	151 (36.9)	
Smoking habits, No. (%)[3]			
None	820 (77.4)	290 (75.1)	0.38
Yes	240 (22.6)	96 (24.9)	
Folic acid supplement use, No. (%)[3]			
No use	119 (12.5)	73 (20.6)	<0.01
First 10 weeks use	294 (31.0)	121 (34.2)	
Preconception use	536 (56.5)	160 (45.2)	
Fetal characteristics			
First trimester crown to rump length, mean (SD), mm	61 (11)	61 (11)	0.44
Second trimester estimated fetal weight, mean (SD), g	372 (74)	368 (73)	0.34
Third trimester estimated fetal weight, mean (SD), g	1630 (241)	1599 (238)	0.02
Birth and infant characteristics			
Males, No. (%)[3]	575 (48.6)	226 (52.1)	0.21
Gestational age, median (90% range), wks[2]	40.1 (37.0, 42.0)	40.3 (37.1, 42.1)	0.89
Birth weight, mean (SD), g	3456 (551)	3444 (602)	0.70
Ever breastfeeding, No. (%)			
No	80 (7.6)	36 (12.6)	0.01
Yes	966 (92.4)	250 (87.4)	
Breastfeeding duration, mean (SD), mo	5.3 (3.8)	4.6 (3.8)	0.02

[1]Values are mean (standard deviation). [2]Median (90% range). [3]Values are observed number and valid percentage. [4]Differences in subject characteristics between the groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.

Supplementary Table S3.4.8. Fetal and childhood length and weight growth in children with clustering of cardiovascular risk factors adjusted for confounders (*n = 745*)[1]

	SDS - difference (95% Confidence Interval) in growth characteristics in children with clustering of cardiovascular risk factors	
	Length (SDS)	Weight (SDS)
Gestational age		
12 weeks	-0.12 (-0.39, 0.15)	-0.08 (-0.35, 0.18)
20 weeks	-0.11 (-0.32, 0.10)	-0.15 (-0.36, 0.06)
30 weeks	-0.10 (-0.31, 0.12)	-0.24 (-0.44, -0.03)
40 weeks	-0.09 (-0.39, 0.22)	-0.32 (-0.59, -0.05)
Childhood age		
6 months	0.02 (-0.18, 0.23)	0.24 (-0.04, 0.51)
12 months	0.07 (-0.14, 0.27)	0.31 (0.03, 0.58)
24 months	0.16 (-0.04, 0.36)	0.45 (0.18, 0.72)
36 months	0.26 (0.05, 0.45)	0.59 (0.32, 0.86)
48 months	0.35 (0.15, 0.55)	0.73 (0.45, 1.00)
72 months	0.54 (0.32, 0.75)	1.09 (0.71, 1.31)

[1]Values are based on repeated linear regression models. Regression coefficients (95% Confidence Interval) reflect the difference in length and weight standard deviation score for children with clustering of cardiovascular risk factors, as compared to children without such clustering. Models are adjusted for duration of last menstrual cycle, child's sex and age at outcome measurements, maternal age, educational level, ethnicity, parity, prepregnancy body mass index, diastolic blood pressure, smoking during pregnancy and folic acid supplement use, and breastfeeding duration. Length and weight growth characteristics used in the models: Fetal period: first trimester: crown to rump length as both length and weight measure (starting point); second and third trimester: femur length and estimated fetal weight; At birth: birth length and birth weight; During childhood: length and weight. Clustering of cardiovascular risk factors, was defined as having 3 or more following components: android fat mass percentage ≥75th percentile; systolic or diastolic blood pressure ≥75th percentile; HDL-cholesterol ≤25th percentile or triglycerides ≥75th percentile; and insulin level ≥75th percentile [22].

Chapter 4

General discussion

Introduction

Cardiovascular disease is a major public health problem in the general adult population.[1] Cardiovascular and metabolic diseases have the largest clinical impact at older ages, and research into risk factors for cardiovascular and metabolic diseases has mostly been focused on adults. However, in the last decades, an accumulating body of evidence suggested that cardiovascular health in younger age groups also has major public health implications.

Cardiovascular health status of women of reproductive age may influence pregnancy outcomes. To meet the increasing metabolic demands of the mother and fetus, adaptions occur in the maternal circulation and metabolism during pregnancy. Normally, these adaptations lead to a better placental perfusion and nutrient supply to the fetus. Suboptimal adaptations may lead to increased risks of pregnancy complications[2], which may have long-term maternal and offspring consequences. Women who had pregnancy complications have higher risks of cardiovascular disease and type 2 diabetes many decades after their pregnancy.[3-5] Also, children born with a low and high birth weight have higher risks of obesity, cardiovascular disease and type 2 diabetes in adulthood.[6-10] Based on these findings, it has been hypothesized that adverse exposures, acting at different stages of fetal and early postnatal development, lead to permanent adaptations in the structure, physiology and function of various organ systems. This early programming contributes to short-term survival, but increases the susceptibility of cardiovascular and metabolic disease in later life.[11] Thus, cardiovascular health and disease in pregnant women and their children is important for short-term and long-term maternal and childhood health outcomes.

The aim of the studies presented in this thesis was to identify maternal physical factors at the start of pregnancy, placental and fetal factors and critical developmental periods during pregnancy associated with cardiovascular health outcomes in mothers and children. This chapter provides a general discussion of the main findings of the studies in this thesis, discusses general methodological issues and provides suggestions for future research.

Interpretation of main findings

Maternal influences

Various adverse maternal socio-demographic and lifestyle-related characteristics have been associated with the risk of adverse maternal and fetal pregnancy outcomes, but less is known about the role of maternal physical factors at the start of pregnancy. Identifying factors associated with cardiovascular health in pregnant women and their children, may help to develop future preventive strategies that improve pregnancy outcomes and maternal and offspring cardiovascular health outcomes.

Maternal blood pressure during pregnancy

Blood pressure is used as a screening method in obstetric care to detect or predict gestational hypertensive disorders, but the predictive accuracy of blood pressure measurement in early pregnancy remains unclear.[12] Tracking analyses focus on the maintenance of one's relative position in a population distribution of values over time, and can be used as a concept to examine the predictability of future values by early measurements.[13]

We examined whether maternal blood pressure tracks throughout pregnancy, whether this tracking is influenced by maternal characteristics and how it is associated with the risk of gestational hypertensive disorders. We observed moderate correlation coefficients between first and third trimester for systolic and diastolic blood pressure. Maternal age, height, gestational weight gain and ethnic background influenced these correlation coefficients. Furthermore, systolic and diastolic blood pressure changes from second to third trimester, but not from first to second trimester, were positively associated with the risks of gestational hypertension and pre-eclampsia. In this thesis, we did not study the associations of maternal blood pressure during pregnancy and gestational hypertensive disorders with childhood outcomes. A previous study performed within the same study cohort, has shown that higher maternal blood pressure levels during pregnancy were associated with impaired fetal growth from the third trimester onwards.[14] An increase in blood pressure from second trimester to third trimester was associated with an increased risk of adverse birth outcomes.[14] This latter study, as well as several other studies also showed that offspring from mothers who had pre-eclampsia had a higher risk of being born preterm and small for their gestational age and a higher risk of higher blood pressure in later life.[14-16] These findings suggest that maternal blood pressure development during pregnancy is important for both maternal and fetal pregnancy complications, and childhood cardiovascular outcomes.

- Maternal blood pressure tracks moderately during pregnancy and is influenced by maternal characteristics.
- Second to third trimester increases in systolic and diastolic blood pressure are associated with an increased risk of gestational hypertensive disorders.

Maternal parity

Background

There is a strong increase in the prevalence of one-child families in Western countries, which may partly be due to the decline in fertility rate and changes in social attitudes.[17] Although maternal parity cannot be modified, obtaining a better understanding of its impact on maternal and childhood outcomes is important for developing strategies for identification of individuals at high risk of adverse outcomes.

Parity and maternal outcomes

Maternal nulliparity is suggested to be an important risk factor for maternal pregnancy complications, including gestational hypertension and pre-eclampsia.[18,19] A systematic review of 52 observational studies showed that nulliparity is associated with an almost 3-times higher risk of pre-eclampsia.[18] Several studies have also suggested that blood pressure levels during pregnancy are higher among nulliparous women, but other studies observed no differences in blood pressure levels.[20-22] These differences may be explained by differences in study population, sample size, adjustment for confounding factors and the use of office or ambulatory blood pressure measurements. Inconsistent differences in placental vascular function among nulliparous and multiparous women have also been reported, with several studies suggesting that nulliparity is associated with an increased risk of uterine artery notching.[23,24] We observed that nulliparous pregnant women have higher blood pressure levels from first trimester onwards and higher risks of third trimester uterine artery notching, and gestational hypertensive disorders. The first pregnancy might thus be a major risk factor for maternal hemodynamic maladaptations and hypertensive complications.

Parity and childhood outcomes

Children from nulliparous mothers are generally smaller than children from multiparous mothers.[25] Differences in birth weight between firstborn and second-born children have been reported up to approximately 200 grams, which is of similar magnitude as the influence of maternal smoking during pregnancy on birth weight.[25-27] Among multiparous mothers only, there is a much smaller increase in birth weight with each following pregnancy.[26] Less is known about the associations of maternal parity with fetal growth in different trimesters of pregnancy. As low birth weight and small size for gestational age at birth are associated with increased risks of cardiovascular disorders in later life, it has been hypothesized that maternal parity may also be associated with long-term cardiovascular health in the offspring. A recent study among 1.065.710 Swedish men reported that birth order was negatively associated with body mass index in young-adulthood.[28] Next to the reported associations of maternal parity with body mass index in the offspring, it has also been shown that body fat mass level in early adulthood is influenced by maternal parity, independent of birth weight and current lifestyle-related factors.[29] However, these findings, as well as suggested associations of maternal parity with other cardiovascular risk factors, are inconsistent.[30-34] In our study, we observed that children of nulliparous mothers had slower fetal growth rates from third trimester onwards and accelerated infant growth rates. Maternal nulliparity was associated with increased risks of adverse birth outcomes, childhood adiposity and adverse childhood metabolic profile in the offspring. Among multiparous mothers only, increasing parity tended to be associated with a decreasing risk of adverse health outcomes in offspring. These associations of maternal parity with birth and childhood outcomes were not explained by socio-demographic and lifestyle-related factors, and associations with childhood outcomes were also independent of birth weight. These findings suggest that

the first pregnancy may have persistent growth and cardiovascular consequences for the offspring.

Underlying mechanisms

The mechanisms underlying these associations are largely unknown, but are likely to partly reflect maternal health status, social and behavioral factors which differ among nulliparous and multiparous women. However, next to these factors, biological mechanisms may also play a role, as associations with maternal, birth and early-childhood outcomes remained after adjustment for confounding factors. Biological mechanisms may involve persistent changes in the maternal vasculature following the first pregnancy, which leads to a more favorable environment for both placental development, fetal nutrition and fetal development in following pregnancies.[35] Further studies are needed to obtain further insight in these underlying mechanisms.

- Nulliparous pregnant women have higher blood pressure levels throughout pregnancy and higher risks of gestational hypertensive disorders.
- Maternal nulliparity is associated with increased risks of adverse birth outcomes, accelerated infant growth, childhood adiposity and an adverse childhood metabolic profile in the offspring.
- The first pregnancy might be an important risk factor for maternal and offspring cardiovascular health outcomes.

Maternal prepregnancy body mass index and gestational weight gain

Background

Worldwide, there is a strong increase in overweight and obesity prevalences among women of reproductive age.[36] Maternal prepregnancy obesity is an important risk factor for gestational hypertensive disorders, gestational diabetes and delivering large size for gestational age infants, but associations with other pregnancy complications are less consistent.[37] In addition, maternal prepregnancy obesity is strongly associated with the risk of obesity in the offspring.[38]

Next to maternal prepregnancy obesity, weight gain during pregnancy may also affect maternal and childhood outcomes.[39,40] The US Institute of Medicine (IOM) has established guidelines, which define optimal ranges of maternal weight gain during pregnancy, according to a mother's prepregnancy body mass index.[41] The guidelines have been established based on evidence from observational studies, relating gestational weight gain to maternal postpartum weight retention, infant size at birth and caesarean delivery, and childhood obesity.[41] Excessive gestational weight gain according to the IOM criteria is common, and associated with the risk of adverse pregnancy outcomes.[42] As the IOM gestational weight gain criteria combine prepregnancy body mass index and gestational weight gain, it is not possible to study the separate effects of maternal prepregnancy body mass index and gestational weight gain on different

maternal and childhood outcomes.[43] Total gestational weight gain reflects multiple components including actual maternal fat accumulation, but also pregnancy-related volume expansion and growth of the fetus, placenta and uterus. Gestational weight gain in different periods of pregnancy partly reflects different components.[41,44] Maternal gestational weight gain in early-pregnancy relatively largely reflects maternal fat deposition, whereas weight gain in mid- and late-pregnancy largely reflects maternal and amniotic fluid expansion, and growth of the fetus, placenta and uterus.[41,44] Examining the associations of critical periods of gestational weight gain with maternal and childhood outcomes may provide further insight in the underlying mechanisms.[45,46]

Prepregnancy body mass index, gestational weight gain and pregnancy outcomes

Results presented in this thesis showed that the risks of maternal prepregnancy overweight and obesity were higher among lower educated, non-European origin, and multiparous mothers and mothers with an obese partner. The risk of excessive gestational weight gain according to the IOM-criteria was higher among European origin, nulliparous, high dietary intake and smoking women, and among women having an obese partner. Maternal prepregnancy overweight and obesity were strongly associated with increased risks of gestational hypertensive disorders, gestational diabetes, caesarean delivery, large size for gestational age infants, and overweight and obesity in the offspring. Higher prepregnancy body mass index was also associated with both higher systolic and diastolic blood pressure in all trimesters. The difference in blood pressure between body mass index groups was already present from first trimester onwards and remained stable throughout pregnancy.

Excessive gestational weight gain according to the IOM criteria was associated with increased risks of gestational hypertension, caesarean delivery, large size for gestational age infants and overweight in the offspring. The risks of delivering a small size for gestational age or preterm born infant were lower among women who gained weight excessively. As compared to prepregnancy overweight and obesity, excessive gestational weight gain had a limited influence on adverse pregnancy outcomes.

Weight gain in early pregnancy was associated with the risk of gestational diabetes and gestational hypertension, whereas weight gain in late-pregnancy was associated with the risk of pre-eclampsia and gestational hypertension. Higher weight gain in early, mid- and late-pregnancy was associated with a lower risk of delivering a small size for gestational age infant, and a higher risk of delivering a large size for gestational age infant, with strongest effects for weight gain in mid- and late-pregnancy. Few studies have examined the associations of critical periods of gestational weight gain with pregnancy outcomes, and these studies have mainly focused on birth weight as an outcome.[47-49] Further research is important to explore reversed causation and to examine underlying mechanisms.

Prepregnancy body mass index and childhood outcomes

Maternal prepregnancy body mass index is strongly associated with body mass index in the offspring.[38] Whether these associations reflect direct intrauterine, causal

mechanisms remains unclear. An approach used in epidemiological studies to obtain further insight into causality is a sibling comparison study, which by design controls for environmental characteristics as well as maternal genotype that are similar within siblings.[50] Sibling studies among offspring from mothers who had high levels of prepregnancy weight loss due to gastrointestinal bypass surgery observed that the prevalence of overweight and obesity and adverse cardiovascular outcomes was higher in children born to mothers before surgery than those born to mothers after surgery. These findings suggest that some of the effect of maternal obesity on offspring outcomes may be through direct intra-uterine mechanisms.[51,52] However, it remains unclear whether this is similar across the whole distribution of maternal prepregnancy body mass index. A large study among 280.866 singleton-born Swedish men observed that a higher maternal body mass index in early pregnancy was associated with higher offspring body mass index at the age of 18 years in the whole cohort and between non-siblings, but not within-siblings, which suggests that the association may be explained by confounding environmental characteristics.[53]

Another approach to obtain further insight into causality of the associations of maternal prepregnancy body mass index with childhood cardiovascular outcomes involves comparing the strength of associations of prepregnancy body mass index from both mother and father with childhood outcomes.[54] Stronger associations for maternal prepregnancy body mass index suggest direct intra-uterine mechanisms may be involved, whereas similar or stronger associations for paternal body mass index suggest a role for shared family-based, lifestyle-related characteristics or genetic factors. Thus far, studies comparing associations of maternal and paternal body mass index with childhood body mass index have shown conflicting results.[55-62] In our contemporary study, we observed that both maternal and paternal prepregnancy body mass index were associated with increased adiposity levels and an adverse cardiovascular profile in offspring, with stronger associations present for maternal prepregnancy body mass index. These associations were not explained by maternal pregnancy complications, gestational weight gain or birth characteristics, but were largely mediated by childhood body mass index. These findings suggest that maternal prepregnancy body mass index is an important risk factor for cardiovascular health of offspring, and that at least part of the underlying mechanisms for this association may involve direct intra-uterine mechanisms.

Gestational weight gain and childhood outcomes

Increased maternal gestational weight gain may influence long-term cardiovascular health of offspring.[63] Most studies that reported these associations used the IOM criteria for excessive gestational weight gain or total gestational weight gain.[64-71] Studies using more detailed assessment methods for gestational weight gain have also suggested associations of gestational weight gain with offspring outcomes.[45,72,73] A sibling comparison study among 146.894 singleton-born Swedish men showed that among overweight and obese mothers, higher total gestational weight gain is associated with higher offspring body mass at the age of 18 years among siblings, suggesting a possible intra-uterine effect.[73] A study among 5154 UK mother-offspring pairs showed that

gestational weight gain in the first 14 weeks of pregnancy, when maternal fat gain is a relatively large component of gestational weight gain, tended to be positively associated with offspring body mass index, waist circumference and fat mass at 9 years, but after 14 weeks of gestation, only high levels of gestational weight gain were associated with offspring adiposity measures.[45] In this thesis, we observed that higher maternal gestational weight gain in early-pregnancy, but not in mid- and late-pregnancy, is associated with increased adiposity levels and an adverse cardiovascular profile in childhood. These associations were independent from maternal prepregnancy weight and weight gain in other periods, and not explained by pregnancy complications or birth and infant growth characteristics. Excessive gestational weight gain according to the IOM criteria was also associated with the risk of childhood overweight and clustering of cardiovascular risk factors. The associations of maternal prepregnancy weight with childhood adiposity and cardiovascular outcomes were stronger than those for gestational weight gain, but did not explain or modify the associations of gestational weight gain with these outcomes. These findings suggest that maternal weight gain in early pregnancy may be a critical period for childhood outcomes.

Underlying mechanisms

The possible mechanisms underlying the associations of maternal prepregnancy body mass index and gestational weight gain with childhood outcomes may involve increased placental transfer of nutrients to the developing fetus. This transfer may subsequently affect fetal development, fetal fat deposition and the development of the hypothalamic -endocrine system that controls appetite and energy metabolism.[74,75] Epigenetic mechanisms may also play an important role.[75] In animal models, effects of nutritional exposures on epigenetic changes have been shown.[75] Thus far, no large-scale studies among humans have been performed focused on epigenetic changes in response to maternal weight during pregnancy. It has been suggested that maternal prepregnancy obesity and excessive gestational weight gain may also have an intergenerational effect, in which these factors lead to an adverse in utero environment which may permanently affect growth and development of specifically female offspring, altering her metabolism in such a way as that she provides an adverse environment for her fetus.[76] Since an increasing number of women are overweight and obese at the start of pregnancy and gain an excessive amount of weight during pregnancy, their effect on offspring development in fetal and early postnatal life may contribute to the continuation of the obesity epidemic.[43]

- Maternal prepregnancy obesity and excessive gestational weight gain are associated with maternal socio-demographic, lifestyle, and genetic factors and with increased risks of adverse maternal, fetal and childhood outcomes.
- Both higher maternal and paternal prepregnancy body mass index are associated with increased adiposity levels and an adverse cardiovascular profile in offspring, with stronger associations present for maternal prepregnancy body mass index. These associations are largely mediated by childhood body mass index.
- Increased maternal weight gain in early pregnancy is associated with an adverse cardiovascular profile in childhood. This association is largely mediated by childhood body mass index.

Placental and fetal influences

Placental vascular function

Background

The placenta forms the active interface between the maternal and fetal blood circulations and regulates both maternal physiological changes during pregnancy as well fetal nutrient supply and fetal development.[77] The placenta is likely to play a key role in the development of maternal and fetal pregnancy complications.[78,79] The placenta may also play an important role in the developmental origins hypothesis.[77,80] Animals studies have shown that fetal growth restriction due to reduced uterine artery perfusion during late gestation is associated with an increased blood pressure and cardiovascular risk in later life.[81,82] Previous studies among adults suggested associations of both low and high placental weight with adverse cardiovascular outcomes in later life, but results are not consistent.[80] Placental weight is only a crude measure of placental growth and more detailed measures of placental function, assessed during pregnancy, might give further insight in long-term consequences of placental dysfunction.[77]

Abnormal early placentation can lead to higher uterine and umbilical artery resistance patterns, which can be measured by Doppler waveforms. Normally, during the first half of pregnancy, there is a decrease in uterine artery and umbilical artery resistance indices, which is in line with the physiological changes that occur during placentation.[83] Abnormal uterine artery and umbilical artery waveforms during pregnancy indicate an impaired uteroplacental and fetoplacental circulation. The utero-placental vascular resistance, a parameter primarily of the maternal circulation, may increase as a result of impaired placentation or impaired maternal hemodynamic adaptations.[83,84] Increased feto-placental vascular resistance, primarily a parameter of the fetal circulation, may occur as a result of impaired placentation or suboptimal fetal vascular development.[84] Studies, often performed among high-risk populations, have shown that abnormal mid-pregnancy uterine and umbilical artery resistance indices as well as uterine artery notching are associated with the risk of pre-eclampsia and fetal growth retardation.[84,85]

Placental vascular dysfunction and pregnancy outcomes

In this thesis, we examined whether uterine and umbilical artery resistance indices are influenced by maternal socio-demographic and lifestyle-related characteristics, whether they track from the second trimester to the third, and whether they are associated with the risk of maternal and fetal pregnancy complications. We observed that placental resistance indices are influenced by maternal parity, use of folic acid supplements and maternal smoking during pregnancy. The influence of these maternal characteristics on utero-placental and feto-placental circulation may be part of the underlying mechanisms that relate these maternal characteristics to the risk of adverse birth outcomes. We further showed that the uterine artery resistance index tracks moderately from the second trimester to the third, whereas the umbilical artery pulsatility index tracks poorly from the second trimester to the third. Already small variations in placental resistance indices in second and third trimester are associated with increased risks of adverse pregnancy outcomes. Thus, our findings suggest that among a low risk population, small variations in second and third trimester placental resistance indices are associated with increased risks of pregnancy complications.

Placental vascular dysfunction and childhood outcomes

We examined whether small variations in third trimester placental resistance indices are associated with fetal and childhood growth and cardiovascular development. As the placental vascular bed forms an important component of the fetal vascular system, and the largest variation is expected in third trimester, we hypothesized that especially changes in third trimester feto-placental vascular resistance may lead to fetal growth and cardiovascular system adaptations.[86] We observed that higher third trimester umbilical artery and uterine artery resistance indices were associated with lower fetal growth rates in third trimester, resulting in a smaller size at birth. Differences in length and weight growth characteristics became smaller from the age of 6 months onwards but persisted until the age of 6 years. Higher third trimester feto-placental vascular resistance, but not utero-placental vascular resistance, was associated with childhood cardiovascular adaptations. These associations were only partly explained by birth weight, and tended to be stronger among girls than among boys.

Underlying mechanisms

The mechanisms underlying these associations of impaired placentation and maternal and childhood outcomes are not well-understood. It has been suggested that oxidative stress due to impaired placental transfusion may be an important factor in the development of pregnancy complications.[78,87] Oxidative stress may also play a role in the observed long-term childhood cardiovascular consequences of impaired third trimester feto-placental vascular resistance.[88-90] Feto-placental vascular resistance is related to fetal vascular function and a determinant of fetal cardiac afterload.[77,91,92] Thus, alterations in feto-placental vascular function may affect fetal development and be a marker of fetal vascular adaptations. The observed associations tended to be stronger among girls than among boys. Sex differences in developmental programming of cardiovascular

risk factors in children merits further study, as findings are inconsistent.[93,94] Further studies with a longer follow-up are needed to examine whether feto-placental vascular resistance is also associated with cardiovascular adaptations at later ages, and whether these associations are different among boys and girls.

- Placental resistance indices are influenced by maternal socio-demographic and lifestyle characteristics and track moderately from the second trimester to the third.
- Increased placental resistance indices in the second and third trimesters are associated with increased risks of adverse pregnancy outcomes.
- Higher third trimester feto-placental vascular resistance, but not utero-placental vascular resistance, is associated with an adverse cardiovascular profile in childhood.

Fetal and early childhood growth

Background

In obstetric care, fetal ultrasound measurements are important examinations during pregnancy for identifying fetuses at risk of adverse outcomes. Poor fetal growth in second and third trimester of pregnancy is associated with increased risks of stillbirth, preterm birth, low birth weight, small size for gestational age at birth and long-term adverse health outcomes.[11,95] First trimester fetal growth is commonly used for pregnancy dating, assuming there is no growth variation in early fetal life. However, among pregnant women with a known first day of the last menstrual period and a regular cycle, fetal crown to rump length can also be used as a first trimester growth outcome.[96,97]

Recent studies observed associations of first trimester fetal growth restriction with the risk of preterm birth and small size for gestational age at birth.[96,98] Repeated ultrasound measurements in different trimesters of pregnancy enable tracking studies and identification of critical fetal periods for later development.

Fetal growth throughout gestation

Not much is known about the correlations of longitudinal fetal growth measurements from early pregnancy onwards with adverse birth outcomes, especially among low-risk populations. Tracking analyses can be used to assess the stability of fetal growth characteristics throughout pregnancy. Two previous studies performed among low-risk populations suggested that tracking of fetal growth characteristics is not common during pregnancy.[99,100] We observed that fetal growth characteristics track moderately throughout gestation, with stronger tracking coefficients present in later pregnancy. This lower tracking of fetal growth during early pregnancy as compared to late pregnancy may partly be due to measurement error related to fetal ultrasound assessment, which is relatively higher in early pregnancy, but may also suggests that a fetus does not have a stable growth trajectory from early pregnancy onwards. Maternal socio-

demographic and lifestyle-related characteristics did not materially influence fetal growth tracking coefficients, which may suggest that the influence of maternal characteristics on fetal growth trajectories is relatively small, or that potential growth adaptations due to maternal characteristics are already occurring in early pregnancy. First, second and third trimester fetal growth characteristics were associated with the risk of adverse birth outcomes, with the strongest associations present for third trimester fetal growth characteristics. Further studies among low-risk populations are needed to examine whether serial fetal ultrasound measures add to the prediction of adverse birth outcomes.

First trimester fetal growth and childhood outcomes

Impaired fetal growth may also be associated with increased susceptibility for cardiovascular disease in later life.[11] Both low and high birth weight are associated with cardiovascular disease in adulthood. Also, studies have shown that variation in fetal growth and early childhood growth is associated with differences in body composition, hemodynamic and metabolic adaptions.[101-104] Thus, these studies suggest that there may be critical periods of growth in early life that influence the development of cardiovascular disease in later life. In this thesis, we examined the associations of first trimester fetal growth with cardiovascular risk factors in childhood. The highest development rates are in the first trimester of pregnancy, which includes the embryonic phase.[105] Previously, we have already shown that first trimester fetal growth seems to be influenced by maternal socio-demographic and lifestyle-related characteristics and is associated with adverse birth outcomes and accelerated postnatal growth.[96] In this thesis, we observed that smaller first trimester fetal size was associated with an adverse body fat distribution, higher diastolic blood pressure, and an adverse blood cholesterol profile in childhood. First trimester fetal growth restriction was also associated with an increased risk of clustering of these cardiovascular risk factors in childhood. These associations were not explained by maternal, birth, and childhood characteristics. The observed associations suggest that the first trimester of pregnancy is a critical period for cardiovascular health in later life. The underlying mechanisms may include changes in methylation of DNA and expression of RNA in response to a suboptimal fetal environment.[11] More detailed first trimester fetal ultrasound studies are needed, that take into account timing of ovulation and implantation, to obtain further insight in these observed associations.[106]

- Fetal growth characteristics track moderately throughout gestation, with stronger tracking coefficients present in later pregnancy. Maternal socio-demographic and lifestyle-related characteristics do not materially influence fetal growth tracking coefficients.
- First, second and third trimester fetal growth characteristics are associated with the risk of adverse birth outcomes.
- Impaired first trimester fetal growth is associated with an adverse cardiovascular risk profile in school age children.

Methodological considerations

Specific strengths and limitations for the studies presented in this thesis have been described in Chapter 2 and Chapter 3 of this thesis. In the following paragraphs, general methodological considerations regarding selection bias, information bias and confounding are discussed.

Selection bias

Selection bias may occur if the association between the determinant and outcome of interest is different in subjects who participate in the study and those who were eligible for the study, but do not participate in the study. Of all children eligible at birth, the overall response to participate in the Generation R Study was 61%. The percentages of women from ethnic minority groups and of lower socioeconomic status were lower than expected from the population figures in Rotterdam.[107] Also, participating women had less pregnancy complications, such as gestational hypertensive disorders, preterm birth and low birth weight, which suggests a selection towards a relatively more affluent and healthy population. This selection towards a more affluent and healthy population may have led to lower prevalence rates, and subsequently reduced statistical power. Also, it may affect the generalizability of our findings to other, less healthy and affluent populations. However, several studies have shown that in cohort studies associations are not strongly influenced by selective non-participation at baseline, and we therefore consider it unlikely that our results are biased by selective non-response at baseline.[108,109]

Next to selective non-response at baseline, selection bias may also occur due to selective loss to follow-up. Loss to follow-up would lead to selection bias if associations would be different between those included in the analyses and those loss to follow-up. In the studies presented in this thesis, loss to follow-up at birth was low. At the age of 6 years, children and their mothers were invited to participate in detailed body fat and cardiovascular follow-up measurements. The response rate at this follow-up was approximately 70%. A lower percentage of children participated in blood sample measurements at the age of 6 years, which was mainly due to non-consent for venous puncture or crying of the child. Mothers from children who did not visit the research center more frequently had unhealthy lifestyle habits and were less well educated than the total study population. Overall, the selective loss to follow-up towards a more healthy population may have biased our effect estimates, but this bias is difficult to quantify.

Information bias

Information bias is a bias that arises in a study because of misclassification of determinant or outcome measurements.[110] Misclassification of either determinant or outcome can be classified as non-differential or differential. Non-differential misclassification involves misclassification where the determinant status is not related to the outcome status, and vice versa. Non-differential misclassification generally leads to an underestimation or dilution of the effect estimates. Differential misclassification involves

misclassification of determinant status related to the outcome status, and vice versa. Differential misclassification may lead to biased results, which can be either overestimated or underestimated.

Exposure data used in our studies were collected longitudinally and before assessment of the outcomes. Also, both parents as well as data collectors were unaware of the specific research questions under study. This makes differential misclassification of the exposure unlikely. However, non-differential misclassification might have occurred. Underreporting of adverse lifestyle-related factors might have occurred and led to an underestimation or overestimation of the observed effects. For example, in the studies presented in this thesis, information of maternal prepregnancy weight and maximum weight during pregnancy was self-reported. Self-reported weight tends to be underestimated especially in case of higher maternal weight, which might have led to an underestimation of observed effects for maternal prepregnancy body mass index and maximum gestational weight gain, but to an overestimation of the effect of gestational weight gain in early pregnancy. Also, pregnancy dating for most women was performed using ultrasound measurements of crown-rump length or biparietal diameter at the first visit. This method might be better than dating by last menstrual period, but neglects variation in early fetal growth. As a consequence, growth variation in second and third trimester might be underestimated and random measurement error in estimation of pregnancy duration may have occurred. Random measurement error in determinants may also have affected our tracking analyses. In most of our studies, the outcome was assessed using medical records, or standardized hands-on assessments of body composition and cardiovascular development. Furthermore, the observers were blinded to the exposure status, which makes differential misclassification of the outcomes less likely.

Confounding

A confounding factor is an extraneous variable associated with both the determinant and the outcome, and this variable is not an intermediate variable in the causal pathway between the exposure and the outcome.[110] If a confounding factor is not taken into account, this may lead to a biased effect estimate of the association between the determinant and the outcome. In this thesis, we used two approaches to deal with confounding in the studied associations. First, we adjusted all analyses for multiple potential confounders. We selected covariates based on previous studies, their associations with the outcomes of interest or a change in effect estimate of more than 10%. In most of the studies presented in this thesis, adjustment for potential confounders only moderately affected the effect estimates, which suggest that the observed associations are possibly true associations between the determinants and the outcomes. Although information about many potential confounders was available in the studies performed in this thesis, residual confounding may still be an issue, as in any observational study. Also, information about several confounding variables was self-reported and measurement error of the confounding variables might have occurred. Residual confounding may have led to an overestimation of the observed effect estimates. Second, we

assessed the associations of both maternal and paternal exposures during pregnancy. A similar effect size for the maternal and paternal association would suggest that the association of the maternal exposure with childhood outcomes is explained by unmeasured environmental factors, rather than direct intra-uterine mechanisms.

Future research

Maternal, placental and fetal exposures

We described associations of maternal, placental and fetal influences with maternal and childhood outcomes. Due to the observational design of our study, we cannot establish causality of the observed associations. A randomized controlled trial is the preferred study design to establish causality. Long-term follow-up studies of participants in ongoing or completed randomized controlled trials that aim to improve diet in overweight and obese pregnant women or aim to limit gestational weight gain are important.[43,111,112] These trials, that have originally been established to assess the influence of these interventions on perinatal outcomes, provide an unique opportunity to examine whether maternal obesity and gestational weight gain are causally related to childhood cardiovascular risk factors.[43] In addition, these trials provide further insight whether maternal lifestyle modification is effective for reducing long-term adverse cardiovascular outcomes in the offspring.[43] Thus far, these randomized controlled trials have suggested that dietary interventions during pregnancy may lead to a small reduction in the amount of gestational weight gain.[111,112] However, whether they also have a beneficial effect on maternal and childhood outcomes remains unclear. Many randomized controlled trials have started their interventions from the second trimester onwards.[111,112] Our findings in this thesis, as well as findings from other observational studies, highlight the importance for intervention trials also focused on the preconception or early pregnancy period.

Next to randomized controlled trials, which are not possible for all exposures studied in this thesis, long term follow-up of observational birth cohorts is necessary. Especially, observational studies that are able to use more sophisticated methods to obtain further insight into causality are important. Comparing effect size of maternal-offspring and paternal-offspring associations provides a method to separate intra-uterine mechanisms from associations explained by confounding familial and environmental characteristics.[54] Thus, future studies should aim to also include fathers in the study with similar assessment of exposures as among mothers, to allow these types of comparison studies. Mendelian randomization studies use genetic variants, which are robustly associated with the exposure of interest and not affected by confounding, as an instrumental variable for an specific exposure, to examine whether an exposure is causally related to the outcome.[113] Sibling comparison studies control for confounders as it is assumed that potential family-based confounders will be similar among siblings.[50] For the latter two methods, large sample sizes are necessary for sufficient statistical power, and

therefore collaborative efforts between multiple cohort studies in meta-analyses with sufficient large sample size are necessary.

More detailed assessment of the studied exposures might also provide further insight in the studied associations. To study the associations of maternal parity with childhood outcomes in further detail, studies with larger sample size are needed that can exclude only-child children from their analyses to distinguish effects of firstborn status and only-child status on childhood outcomes, and that can better explore the effect of family size. For gestational weight gain, studies are needed that have repeated maternal weight measurements during pregnancy available. More detailed measurement of the different components of gestational weight gain, including maternal fat accumulation, pregnancy-related hemodynamic adaptations and fetal growth, is necessary. The transfer of nutrients by the placenta does not only depend upon placental vascular function, but also on placental structure and function. It has been suggested that a 'placental phenotype', which constitutes of a combination of placental measures including placental morphology, blood flow and placental nutrient transporter activity and expression, provides a better proxy for the intrauterine environment than birth weight, and may give further insight in developmental programming of cardiovascular disease.[77] It is thus important that further studies also focus on other aspects of placental function, and the combination of these features. Fetal growth is the largest in absolute terms in third trimester, and this is the period when placental nutrient transfer must be sufficient to meet fetal requirements. As the placental function develops progressively during pregnancy, this placental capacity is already developed in earlier gestation. Maternal characteristics that affect the placenta in early pregnancy may thus have consequences for placental function in later pregnancy.[114] Further studies are needed that examine the influence of maternal exposures on placental function throughout gestation. Obtaining a better understanding of the role of the placenta in maternal and childhood outcomes, may also provide new targets for intervention. Due to strong advances in imaging techniques, it is possible to visualize embryonic development in further detail, which may provide further insight in our observed associations of first trimester fetal growth with childhood cardiovascular risk factors.[115]

Epigenetics is becoming increasingly of interest as an underlying mechanism in the developmental origins hypothesis.[116] Epigenetic mechanisms involve a range of modifications to DNA and associated proteins that together regulate gene activity. Environmental influences in early life may induce epigenetic changes, and thereby affect the risk of cardiovascular disease in later life.[116,117] Animals studies provide support for epigenetic modifications due to environmental exposures in early life.[116] In the placenta, it has been shown that there is an accumulation of environmentally induced changes in DNA methylation patterns throughout pregnancy.[118] Lower methylation of the IGF2 gene, an important factor in human growth and development, was found in adults exposed to undernutrition during the Dutch Famine.[119] These findings suggest that epigenetic modifications induced by early environmental factors may have phenotypic consequences throughout the life course. Future studies are needed to obtain further insight in the role of epigenetics, and critical periods for epigenetic variations, as

underlying mechanisms for associations of early life exposures and cardiovascular risk factors and disease in later life.

Maternal and childhood outcomes

In this thesis, we studied the associations of maternal, placental and fetal influences with several maternal and childhood cardiovascular outcomes. We studied the associations of several maternal risk factors with maternal blood pressure development during pregnancy, gestational hypertension and pre-eclampsia. However, we did not have information available about different sub-types of pre-eclampsia. As early-onset pre-eclampsia is strongly related to maternal and perinatal morbidity and mortality, it is of interest to examine the associations of maternal risk factors with different sub-types of pre-eclampsia in further detail.[120] Childhood outcomes studied in this thesis were childhood body composition, blood pressure, left ventricular mass, lipids and insulin levels. Further additional measurements of childhood body composition and cardiovascular development might provide further insight in the underlying mechanisms linking early life exposures to obesity and cardiovascular disease in later life. In the pathogenesis of atherosclerosis, endothelial dysfunction and impaired vascular reactivity induced by dyslipidemia play an important role.[121,122] Ultrasound assessment of endothelial function and intima media thickness may be used as preclinical markers of atherosclerosis. The microvasculature is an important component related to hypertension.[123] Using retinal vascular imaging the microvasculature in children can be studied. Also, imaging techniques, such as magnetic resonance imaging, are of interest to obtain further insight in detailed body fat distribution and cardiovascular development.

This thesis provides further insight in the associations of physical maternal factors, placental and fetal factors with maternal and childhood cardiovascular health outcomes. However, the significance of our findings with regard to the risk of cardiovascular disease in later life remains unclear. Multiple studies have shown that pregnancy complications, including gestational hypertensive disorders, gestational diabetes and fetal growth restriction are associated with an increased risk of chronic disease in women in later life.[124-128] However, the mechanisms underlying these associations remain unclear. It is likely that common predisposing risk factors associated with the risk of pregnancy complications and cardiovascular disease in later life partly explain the observed associations.[129] However, further studies are needed to examine whether there are additional factors during pregnancy that might partly explain these associations, whether pregnancy complications can be used as a screening method to identify women at increased risk of cardiovascular disease in later life, and possibilities for prevention among these women.[129]

The observed effect estimates for the associations of maternal, placental and fetal influences with childhood cardiovascular risk factors were small to moderate, and are mainly of interest from a cardiovascular developmental perspective. Previous studies have shown that childhood cardiovascular risk factors tend to track into adulthood.[130,131] Also, adiposity in school age children is related to cardiovascular disease in

later life.[104,132] Thus, these findings suggest that even subclinical differences in risk factors for cardiovascular disease in childhood are related to the development of cardiovascular disease in later life. However, their effects on the risk of cardiovascular disease should be further studied. To gain more insight in the longitudinal associations between maternal, placental and fetal influences and childhood adiposity and cardiovascular development, detailed body composition and cardiovascular measurements should be performed throughout the life course.

Clinical implications

We identified several maternal, placental and fetal factors associated with an increased risk of adverse maternal and childhood cardiovascular outcomes. These findings may be important for identification of high-risk individuals and for the development of preventive strategies or interventions already from early pregnancy onwards. Pregnancy is an important period where women are likely to be more motivated to make lifestyle changes. Based on our findings, early pregnancy seems to be a critical period for health outcomes in pregnant women and their children. Preventive strategies focused on improving maternal health status in the preconception period and in early pregnancy may help to improve maternal pregnancy outcomes and cardiovascular health status of the offspring. Further studies are needed to obtain improved guidelines for optimal amounts of weight gain during pregnancy for both maternal and childhood short-term and long-term outcomes.

Conclusion

Findings from this thesis suggest that maternal, placental and fetal influences are associated with maternal and childhood cardiovascular health outcomes. Although the observed associations were relatively small to moderate, they may be important for the burden of cardiovascular disease on a population level.
Health of the mother in early pregnancy may have important cardiovascular health consequences for mother and child.

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Chapter 5

Summary/Samenvatting

Summary

Chapter 1 describes the background and hypothesis for the studies presented in this thesis. Cardiovascular disease is a major public health problem in the general adult population. Because of the clinical impact that cardiovascular and metabolic diseases have at older ages, research into risk factors for cardiovascular and metabolic diseases has mostly been focused on adults. However, in the last decades, an accumulating body of evidence suggested that cardiovascular health in younger age groups also has major long-term public health implications. In women of reproductive age, cardiovascular health status may complicate pregnancy. Suboptimal maternal adaptions during pregnancy are related to the development of pregnancy complications, which may have long-term maternal and offspring cardiovascular health consequences. Large-scale epidemiological studies have shown that children born with a low and high birth weight have higher risks of cardiovascular disease and type 2 diabetes in adulthood. Based on these findings, it has been hypothesized that adverse exposures, acting at different stages of fetal and early postnatal development, lead to permanent adaptations in the structure, physiology and function of various organ systems. This early programming contributes to short-term survival, but increases the susceptibility of cardiovascular and metabolic disease in later life. Thus, cardiovascular health and disease in pregnant women and their children is important for clinically relevant, adverse short-term and long-term health outcomes. Identifying factors influencing cardiovascular health in pregnant women and their children, may help to develop future preventive strategies that improve cardiovascular health throughout the life course and in future generations. Therefore, studies presented in this thesis were designed to identify maternal, placental and fetal factors and critical developmental periods during pregnancy associated with cardiovascular health outcomes in mothers and children.

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 and genetic determinants of growth, development and health in fetal life and childhood.

In Chapter 2, studies on maternal influences on maternal and childhood outcomes are described. In Chapter 2.1, we found that maternal systolic and diastolic blood pressure tracked moderately during pregnancy. Blood pressure tracking coefficients were lower in younger, shorter, and non-European women and in women with higher gestational weight gain. Second to third trimester increases in systolic and diastolic blood pressure were associated with an increased risk of gestational hypertensive disorders.

In Chapter 2.2, we examined the associations of maternal parity with maternal pregnancy-related hemodynamic adaptations, placental vascular function and pregnancy complications. We observed that nulliparous pregnant women had a higher systolic and diastolic blood pressure level in each trimester of pregnancy, and a higher risk of third trimester uterine artery notching, which reflects an abnormal waveform resulting from increased blood flow resistance. Nulliparous women also had a higher risk of gestational

hypertensive disorders. The first pregnancy might thus be a major risk factor for maternal hemodynamic maladaptations and vascular complications. Next, we further explored the associations of maternal parity with childhood outcomes (Chapter 2.3). We observed that offspring from nulliparous mothers have lower fetal growth rates from third trimester onwards and increased risks of being born preterm and small for their gestational age, but a lower risk of being born large for their gestational age. Also, offspring from nulliparous mothers have accelerated infant growth rates and higher risks of childhood overweight and an adverse childhood metabolic profile. These findings suggest that maternal nulliparity may have persistent cardiovascular consequences for the offspring.

In Chapter 2.4 we described the associations of maternal prepregnancy body mass index and gestational weight gain with maternal and fetal pregnancy complications. We observed that maternal socio-demographic, lifestyle, and genetic factors were associated with the risks of prepregnancy overweight and obesity and excessive gestational weight gain. Maternal overweight and obesity were strongly associated with increased risks of gestational hypertensive disorders, gestational diabetes, caesarean delivery, large size for gestational age infants, and overweight and obesity in the offspring. Excessive gestational weight gain was associated with increased risks of gestational hypertension, caesarean delivery, large size for gestational age infants and overweight in the offspring. As compared to prepregnancy overweight and obesity, excessive gestational weight gain tended to have a limited influence on adverse pregnancy outcomes. In Chapter 2.5, we examined the associations of maternal prepregnancy body mass index with the risk of gestational hypertensive disorders in further detail. We observed that a higher maternal prepregnancy body mass index was associated with both higher systolic and diastolic blood pressure levels in all trimesters of pregnancy. The difference in blood pressure between body mass index categories was already present from first trimester onwards and remained stable throughout pregnancy.

In Chapter 2.6 and 2.7, the associations of maternal prepregnancy body mass index and gestational weight gain with childhood outcomes are described. We observed that higher maternal and paternal prepregnancy body mass index were associated with an adverse cardiovascular profile in the offspring, with stronger associations present for maternal prepregnancy body mass index. The associations of maternal prepregnancy body mass index with childhood outcomes were not explained by maternal pregnancy complications, maternal gestational weight gain, birth characteristics or infant growth. The associations of maternal prepregnancy body mass index with childhood fat mass measures and cardiovascular outcomes attenuated after adjustment for childhood current body mass index. These findings suggest that maternal prepregnancy body mass index may influence cardiovascular health of offspring partly through direct intrauterine mechanisms. In Chapter 2.7, we showed that, independent from maternal prepregnancy weight and weight gain in other periods, higher weight gain in early-pregnancy was associated with a higher childhood body mass index, a higher total fat mass percentage, an adverse body fat distribution, a higher systolic blood pressure, and higher insulin and C-peptide levels. Also, higher weight gain in early-pregnancy, but not in mid- or late-

pregnancy, was associated with increased risks of childhood overweight and clustering of cardiovascular risk factors. Thus, the effects of gestational weight gain on childhood outcomes may vary during pregnancy. Our results suggest that especially early pregnancy might be a specific and independent critical period for gestational weight gain.

In Chapter 3, we describe studies focused on the associations of placental hemodynamic function and fetal growth with maternal and childhood outcomes. In Chapter 3.1, we examined the influence of second and third trimester placental hemodynamic function on maternal and fetal pregnancy complications. We showed that placental resistance indices were influenced by maternal socio-demographic and lifestyle-related characteristics. Uterine artery resistance index tracked moderately from the second trimester to the third, whereas umbilical artery pulsatility index tracked poorly from the second trimester to the third. Higher placental resistance indices in the second and third trimesters and persistence in the highest tertile of uterine artery resistance index from the second trimester to the third were associated with increased risks of pre-eclampsia, preterm birth, and small size for gestational age at birth. We also explored whether impaired third trimester placental vascular function was associated with childhood outcomes (Chapter 3.2). We observed that higher third trimester umbilical and uterine artery vascular resistance were associated with lower fetal length and weight growth in third trimester, resulting in a smaller size at birth among boys and girls. These differences in length and weight growth became smaller from the age of 6 months onwards, but were still present at the age of 6 years. Higher third trimester umbilical artery vascular resistance, but not uterine artery vascular resistance, was associated with a higher childhood body mass index, higher total fat mass percentage, higher android/gynoid fat mass ratio, higher systolic blood pressure, and with a lower left ventricular mass. These associations were not explained by birth weight. Stronger associations tended to be present among girls as compared with boys.

In Chapter 3.3 we showed that fetal growth characteristics tracked moderately throughout gestation, with stronger tracking coefficients present in later pregnancy. Tracking coefficients were not materially influenced by maternal socio-demographic and lifestyle characteristics. First, second and third trimester fetal growth characteristics were associated with the risk of adverse birth outcomes. In Chapter 3.4, we observed that smaller first trimester fetal size was associated with an adverse body fat distribution, higher diastolic blood pressure, and an adverse blood cholesterol profile in childhood. First trimester fetal growth restriction was also associated with an increased risk of clustering of these cardiovascular risk factors in childhood. These associations were not explained by maternal, birth, and childhood characteristics. Thus, these findings suggest that the first trimester might be a critical period for cardiovascular and metabolic function in later life.

In Chapter 4 we provide a general discussion in which the studies described in this thesis are described in broader context, and implications and suggestions for future research are discussed.

In conclusion, findings from this thesis suggest that maternal, placental and fetal influences are associated with maternal and childhood cardiovascular health outcomes.

Although the observed associations were relatively small to moderate, they may be important for cardiovascular disease on a population level. Based on our findings, early pregnancy seems to be a critical period for health outcomes in pregnant women and their children. Preventive strategies should focus on improving maternal health status in the preconception period and in early pregnancy to improve maternal pregnancy outcomes and cardiovascular health status of the offspring. Health of the mother in early pregnancy may have important cardiovascular health consequences for mother and child.

Samenvatting

Hoofdstuk 1 beschrijft de achtergrond en hypothese voor de studies beschreven in dit proefschrift. Hart- en vaatziekten vormen een groot probleem voor de volksgezondheid. Vanwege de klinische impact van cardiovasculaire ziekten op oudere leeftijd, is onderzoek naar risicofactoren van cardiovasculaire ziekten voornamelijk gericht op volwassenen. Echter, in de laatste decennia, heeft een groot aantal studies aangetoond dat de cardiovasculaire gezondheid in jongere leeftijdsgroepen ook grote gevolgen heeft op lange termijn voor de volksgezondheid. Bij vrouwen van reproductieve leeftijd is de cardiovasculaire gezondheidsstatus van belang omdat dit de zwangerschap kan compliceren. Suboptimale maternale fysiologische aanpassingen tijdens de zwangerschap kunnen leiden tot zwangerschapscomplicaties, die op de lange termijn cardiovasculaire consequenties kunnen hebben voor zowel moeder als kind. Grootschalige epidemiologische studies hebben aangetoond dat kinderen, geboren met een laag en hoog geboortegewicht, een hoger risico hebben op het ontwikkelen van hart- en vaatziekten en diabetes mellitus type 2 op volwassen leeftijd. Gebaseerd op deze bevindingen is de hypothese ontwikkeld dat ongunstige factoren, tijdens verschillende stadia van de foetale en vroege postnatale ontwikkeling, leiden tot blijvende aanpassingen in de structuur, fysiologie en functie van verschillende orgaansystemen. Deze vroege programmering draagt bij aan de overleving op de korte termijn, maar verhoogt het risico op cardiovasculaire aandoeningen op latere leeftijd. Cardiovasculaire gezondheidsstatus van zwangere vrouwen en hun kinderen is dus belangrijk voor klinisch relevante, korte- en lange-termijn gezondheidsuitkomsten. Het identificeren van factoren, die van invloed zijn op de cardiovasculaire gezondheid van zwangere vrouwen en hun kinderen, kan bijdragen aan het ontwikkelen van strategieën om de cardiovasculaire gezondheid gedurende het hele leven en in toekomstige generaties te verbeteren. Daarom was het doel van de studies, gepresenteerd in dit proefschrift, om maternale, placentale en foetale factoren en kritieke ontwikkelingsperioden tijdens de zwangerschap, geassocieerd met cardiovasculaire gezondheidsuitkomsten bij moeders en kinderen, te identificeren.

De studies, beschreven in dit proefschrift, zijn onderdeel van het Generation R onderzoek, een populatie-gebaseerd prospectief cohort onderzoek vanaf het foetale leven tot in de jongvolwassenheid in Rotterdam, de tweede grootste stad van Nederland. Het Generation R onderzoek heeft tot doel factoren van invloed op de groei, ontwikkeling en gezondheid in het foetale leven en de kindertijd te identificeren.

In hoofdstuk 2 worden studies over maternale invloeden op moeder en kinduitkomsten beschreven. In hoofdstuk 2.1 tonen we aan dat maternale systolische en diastolische bloeddruk matig tracken tijdens de zwangerschap. Het tracken van bloeddruk was verminderd bij jongere, kortere, en niet-Europese vrouwen en bij vrouwen met een hogere gewichtstoename tijdens de zwangerschap. Verhoging van de systolische en diastolische bloeddruk van het tweede naar het derde trimester was

geassocieerd met een verhoogd risico op hypertensieve aandoeningen tijdens de zwangerschap.

In hoofdstuk 2.2 onderzochten we de associaties van maternale pariteit met maternale zwangerschaps-gerelateerde hemodynamische aanpassingen, bloedstroomsnelheidsprofielen van de arteria uterina en hypertensieve zwangerschapscomplicaties. We vonden dat nullipare vrouwen een hogere systolische en diastolische bloeddruk in elk trimester van de zwangerschap hadden, en een hoger risico op een vroegdiastolische indeuking, ook wel notch genoemd, in het bloedstroomsnelheidsprofiel van de arteria uterina in het derde trimester. Nullipare vrouwen hadden ook een hoger risico op hypertensieve zwangerschapscomplicaties. De eerste zwangerschap is dus mogelijk een belangrijke risicofactor voor maternale hemodynamische maladaptaties en vasculaire complicaties tijdens de zwangerschap. Vervolgens hebben we de associaties van maternale pariteit met kinduitkomsten onderzocht (hoofdstuk 2.3). We lieten zien dat de kinderen van nullipare moeders een verminderde foetale groei hebben vanaf het derde trimester. Ook hebben zij een hoger risico op vroeggeboorte en een te laag geboortegewicht voor de zwangerschapsduur. Deze kinderen hebben echter een lager risico op een te hoog geboortegewicht voor de zwangerschapsduur. Kinderen van nullipare moeders hebben een versnelde groei in de eerste 24 maanden van het leven, een hoger risico op overgewicht en een ongunstig metabool profiel op de kinderleeftijd. Deze bevindingen suggereren dat maternale nulliparitеit langdurige cardiovasculaire gevolgen kan hebben voor kinderen.

In hoofdstuk 2.4 beschrijven we de associaties van maternale body mass index voor de zwangerschap en gewichtstoename tijdens de zwangerschap met maternale en foetale zwangerschapscomplicaties. We zagen dat maternale sociaal-demografische factoren, leefstijlfactoren en genetische factoren geassocieerd waren met het risico op overgewicht en obesitas voor de zwangerschap en een overmatige gewichtstoename tijdens de zwangerschap. Maternaal overgewicht en obesitas voor de zwangerschap waren sterk geassocieerd met een verhoogd risico op zwangerschapshypertensie, preeclampsie, zwangerschapsdiabetes, het ondergaan van een keizersnede, het krijgen van een kind met een hoog geboortegewicht, en een hoger risico op overgewicht en obesitas bij hun kinderen. Overmatige gewichtstoename tijdens de zwangerschap was ook geassocieerd met een verhoogd risico op zwangerschapshypertensie, het ondergaan van een keizersnede, het krijgen van een kind met een hoog geboortegewicht en een hoger risico op overgewicht op de kinderleeftijd. In vergelijking met overgewicht en obesitas voor de zwangerschap, waren de associaties van overmatige gewichtstoename tijdens de zwangerschap met nadelige zwangerschapsuitkomsten minder sterk. In hoofdstuk 2.5 werden de associaties van maternale body mass index voor de zwangerschap met het risico op hypertensieve aandoeningen tijdens de zwangerschap meer in detail onderzocht. We vonden dat een hogere maternale body mass index voor de zwangerschap geassocieerd was met zowel hogere systolische als diastolische bloeddruk in alle trimesters van de zwangerschap. Het verschil in bloeddruk tussen body mass index groepen was al aanwezig vanaf het eerste trimester en bleef stabiel gedurende de zwangerschap.

In **hoofdstuk 2.6** en **2.7** beschrijven we de associaties van maternale body mass index voor de zwangerschap en gewichtstoename tijdens de zwangerschap met kinduitkomsten. We zagen dat hogere body mass index van zowel de moeder als de vader geassocieerd was met een ongunstig cardiovasculair profiel bij de kinderen. Body mass index van de moeder was sterker geassocieerd met nadelige kinduitkomsten dan body mass index van de vader. De associaties van maternale body mass index voor de zwangerschap met de cardiovasculaire gezondheid van het kind werden niet verklaard door maternale zwangerschapscomplicaties, maternale gewichtstoename tijdens de zwangerschap, geboortefactoren of groei van het kind in de eerste 24 maanden. De associaties van maternale body mass index voor de zwangerschap met gedetailleerde vetuitkomsten en cardiovasculaire uitkomsten bij het kind zwakten wel af na correctie voor huidig body mass index van het kind. Deze bevindingen suggereren dat maternale body mass index voor de zwangerschap van invloed is op de cardiovasculaire gezondheid van hun kinderen, mogelijk voor een deel via directe intra-uteriene mechanismen. In **hoofdstuk 2.7** hebben we aangetoond dat een hogere maternale gewichtstoename aan het begin van de zwangerschap was geassocieerd met een hogere body mass index en totale vetmassa, een ongunstigere androïde/gynoïde vet-ratio, meer subcutaan en preperitoneaal buikvet, een hogere systolische bloeddruk en hogere insuline- en C-peptidelevels van het kind op de leeftijd van 6 jaar. Deze bevindingen waren onafhankelijk van het gewicht van de moeder voor de zwangerschap en van gewichtstoename later in de zwangerschap. Ook was hogere gewichtstoename in het begin van de zwangerschap, maar niet later in de zwangerschap, geassocieerd met een verhoogd risico op overgewicht en clustering van cardiovasculaire risicofactoren bij kinderen. De effecten van gewichtstoename tijdens de zwangerschap op cardiovasculaire gezondheid van het kind, kunnen dus afhankelijk zijn van de periode waarin de gewichtstoename plaatsvindt. Onze resultaten suggereren dat specifiek de vroege zwangerschap een kritieke periode is voor gewichtstoename tijdens de zwangerschap.

In **hoofdstuk 3** beschrijven we studies gericht op de hemodynamische functie van de placenta en groei van de foetus, in relatie tot moeder- en kinduitkomsten. In **hoofdstuk 3.1** onderzochten we de invloed van tweede en derde trimester bloedstroomsnelheidsprofielen van de arteria uterina en arteria umbilicalis op maternale en foetale zwangerschapscomplicaties. We toonden aan dat de bloedstroomsnelheidsprofielen werden beïnvloed door sociaal-demografische en leefstijl-gerelateerde factoren van de moeder. Tracking van de resistance index van arteria uterina van tweede naar derde trimester was matig, en tracking van de resistance index van de arteria umbilicalis van tweede naar derde trimester was laag. Hogere placentale resistance indices in het tweede en derde trimester van de zwangerschap waren geassocieerd met een hoger risico op preeclampsie, vroeggeboorte en een te laag geboortegewicht voor de zwangerschapsduur. We zagen ook dat een hogere vaatweerstand in de arteria umbilicalis en arteria uterina in het derde trimester van de zwangerschap geassocieerd was met een verminderde foetale lengtegroei en gewichtstoename in het derde trimester, wat resulteert in een kleiner kind bij de geboorte bij zowel jongens als meisjes. Deze verschillen in lengte en gewicht werden kleiner vanaf de leeftijd van 6 maanden, maar waren nog steeds

aanwezig op de leeftijd van 6 jaar. Een hogere vaatweerstand in de arteria umbilicalis in het derde trimester, maar niet een verhoogde vaatweerstand in de arteria uterina, was geassocieerd met een hogere body mass index, een hogere totale vetmassa, een ongunstigere androïde/gynoïde vet-ratio, een hogere systolische bloeddruk, en met een lagere linker ventrikel massa op de kinderleeftijd. Deze associaties werden niet verklaard door geboortegewicht. Sterkere associaties leken aanwezig te zijn bij meisjes dan bij jongens (**hoofdstuk 3.2**).

In **hoofdstuk 3.3** hebben we laten zien dat de foetale groeimetingen matig tracken gedurende de zwangerschap, waarbij tracking sterker aanwezig was later in de zwangerschap. Tracking coëfficiënten werden niet sterk beïnvloed door maternale sociaaldemografische factoren en leefstijlfactoren. Eerste, tweede en derde trimester foetale groeimetingen waren geassocieerd met het risico op ongunstige geboorte-uitkomsten. In **hoofdstuk 3.4** hebben we aangetoond dat een kleinere foetale grootte in het eerste trimester van de zwangerschap geassocieerd was met een ongunstige verdeling van lichaamsvet, hogere diastolische bloeddruk en een ongunstig cholesterolprofiel op de kinderleeftijd. Eerste trimester foetale groeivertraging was ook geassocieerd met een verhoogd risico op clustering van deze cardiovasculaire risicofactoren op de kinderleeftijd. Deze associaties werden niet verklaard door maternale factoren, geboortefactoren of kindfactoren. Deze bevindingen suggereren dat het eerste trimester een kritieke periode zou kunnen zijn voor cardiovasculaire en metabolische functie op latere leeftijd.

In **hoofdstuk 4** worden de in dit proefschrift beschreven studies in een bredere context beschouwd, en implicaties en suggesties voor toekomstig onderzoek besproken.

Concluderend suggereren de bevindingen van dit proefschrift dat maternale, placentale en foetale factoren geassocieerd zijn met cardiovasculaire gezondheidsuitkomsten in moeder en kind. Hoewel de waargenomen associaties relatief van kleine grootte zijn, zijn ze mogelijk belangrijk voor het verklaren van hart- en vaatziekten op populatieniveau. Op basis van onze bevindingen, blijkt de vroege zwangerschap een kritieke periode voor gezondheidsuitkomsten van zwangere vrouwen en hun kinderen te zijn. Preventieve strategieën moeten zich daarom ook richten op het verbeteren van de maternale gezondheidsstatus in de preconceptionele periode en in het begin van de zwangerschap om maternale zwangerschapsuitkomsten en de cardiovasculaire gezondheid van kinderen te verbeteren.

Chapter 6

Authors' affiliations
Publication list
About the author
PhD portfolio
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Publication list

First author

1. **Gaillard R**, Steegers EA, Hofman A, Jaddoe VW. Associations of maternal obesity with blood pressure and the risks of gestational hypertensive disorders. The Generation R Study. *J Hypertens*. 2011;29(5):937-44.
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9. **Gaillard R**, Eilers PH, Yassine S, Hofman A, Steegers EA, Jaddoe VW. Risk factors and consequences of maternal anaemia and elevated haemoglobin levels during pregnancy: a population-based prospective cohort study. *Paediatr Perinat Epidemiol.* 2014;28(3):213-26.
10. **Gaillard R**, Steegers EA, De Jongste JC, Hofman A, Jaddoe VW. Tracking of fetal growth characteristics during different trimesters and the risks of adverse birth outcomes. *Int J Epidemiol.* 2014. doi: 10.1093/ije/dyu036.
11. **Gaillard R**, Jaddoe VW. Assessment of fetal growth by customised growth charts. *Ann Nutr Metab.* In press.
12. **Gaillard R**, Rurangirwa AA, Williams MA, Hofman A, Mackenbach JP, Franco OH, Steegers EA, Jaddoe VW. Associations of maternal parity with fetal and childhood growth, and cardiometabolic risk factors in childhood. The Generation R Study. *Hypertension.* In press.
13. **Gaillard R**, Steegers EA, Hofman A, Franco OH, Jaddoe VW. Maternal weight gain in different periods of pregnancy and childhood cardio-metabolic outcomes. The Generation R Study. *Submitted.*

Co-author

14. Bouthoorn SH, **Gaillard R**, Steegers EA, Hofman A, Jaddoe VW, van Lenthe FJ, Raat H. Ethnic differences in blood pressure and hypertensive complications during pregnancy: the Generation R study. *Hypertension*. 2012;60(1):198-205.
15. Rurangirwa AA, **Gaillard R**, Steegers EA, Hofman A, Jaddoe VW. Hemodynamic adaptations in different trimesters among nulliparous and multiparous pregnant women; the Generation R study. *Am J Hypertens*. 2012;25(8):892-9.
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22. Durmuş B, Heppe DH, Gishti O, Manniesing R, Abrahamse-Berkeveld M, van der Beek EM, Hofman A, Duijts L, **Gaillard R**, Jaddoe VW. Total and abdominal fat outcomes in school-age children associated with infant breastfeeding patterns. *Am J Clin Nutr.* 2014. doi: 10.3945/ajcn.113.075937.
23. Gishti O, **Gaillard R**, Manniesing R, Abrahamse-Berkeveld M, van der Beek EM, Heppe DH, Steegers EA, Hofman A, Duijts L, Durmuş B, Jaddoe VW. Fetal and infant growth patterns associated with total and abdominal fat distribution in school-age children. *J Clin Endocrinol Metab.* 2014:jc20134345.
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25. Bakker H, **Gaillard R**, Franco OH, Hofman A, van der Heiden AJ, Steegers EA, Taal HR, Jaddoe VW. Fetal and infant growth patterns and kidney function at school age. *J Am Soc Nephrol.* In press.
26. Gishti O, **Gaillard R**, Durmuş B, Hofman A, Duijts L, Franco OH, Jaddoe VW. Infant diet and metabolic outcomes in school-age children.The Generation R Study. *Eur J Clin Nutr.* In press.

About the author

Romy Gaillard was born March 13th 1988 in Schiedam, The Netherlands. She graduated from secondary school (gymnasium) at the Groen van Prinsterer Lyceum in Vlaardingen in 2006. In the same year, she started her medical education at the Erasmus University, Rotterdam, The Netherlands. During the second year of her medical education, she was invited to participate in the Master of Science program Health Sciences, specialisation Clinical Epidemiology, at the Netherlands Institute for Health Sciences. As part of the Master of Science program, she participated in a summer course at the Harvard School of Public Health, Boston, MA, USA. In 2010, she obtained her 'doctoral' degree in medicine cum laude, and started her Master of Science research project at the department of Epidemiology, within the Generation R Study. In 2011, she received her Master of Science degree in Clinical Epidemiology and expanded her research project in her current PhD-project entitled 'Cardiovascular health in pregnant women and their children' under supervision of Prof. dr. V.W.V. Jaddoe (Departments of Epidemiology and Pediatrics) and Prof. dr. E.A.P. Steegers (Department of Obstetrics and Gynaecology). The results of this work are presented in this dissertation. During her PhD-project, she received a grant from the EarlyNutrition Brain Mobility program enabling her to work as a research fellow at the department of Epidemiology and Project Viva at the Harvard School of Public Health, Boston, MA, USA under supervision of Prof. M.A. Williams and Prof. dr. M.W. Gillman, respectively. From September 2014 onwards, she will work as a research fellow at the Raine Study, Perth, Australia (under supervision of dr. R.C. Huang) for a period of two months, after which she will start her medical internships. She expects to graduate as a medical doctor in 2016.

PhD Portfolio

Summary PhD training and teaching activities

Name PhD student:	Romy Gaillard
Erasmus MC Department:	Epidemiology
Research School:	Netherlands Institute for Health Sciences
PhD period:	May 2011 - June 2014
Promotors:	Prof. dr. V.W.V. Jaddoe, Prof. dr. E.A.P. Steegers

1. PhD training

	Year	Workload (ECTS)
General courses		
Master's degree Health Sciences, specialization Clinical Epidemiology, NIHES, Erasmus University Rotterdam, the Netherlands	2008-2011	
Principles of Research in Medicine and Epidemiology		0.7
Introduction to Data-analysis		1.0
Regression Analysis		1.9
Methods of Clinical Research		0.7
Clinical Trials		0.7
Topics in Meta-analysis		0.7
Survival Analysis		1.9
Case-control Studies		0.7
Study Design		4.3
Introduction to Public Health		0.7
Methods of Health Services Research		0.7
Introduction to Decision-making in Medicine		0.7
Topics in Health and Diseases in the Elderly		0.7
Advanced courses		
Missing values in Clinical Research		0.7
Courses for the Quantitative Researcher		1.4
Analysis of Growth Data		0.6
Introduction to Clinical Research		0.9
Advanced Topics in Decision-making in Medicine		1.9
Pharmaco-epidemiology and Drug Safety		1.9
Intervention Research and Clinical Trials		0.9
Diagnostic Research		1.9
Advanced Topics in Clinical Trials		1.9
Advanced Analysis of Prognosis Studies		0.9
Prognosis Research		0.9
Principles and Epidemiologic Data-analysis		0.9
Planning and Evaluation of Screening		1.4
Summer Programme at Harvard School of Public Health, Boston, USA	2010	4.0
General academic skills		
Scientific Writing in English for Publication, Erasmus MC, the Netherlands		2.0
Instellingsgebonden regelgeving en stralingshygiëne niveau 5R, Erasmus MC, the Netherlands	2011	0.7

	Year	Workload (ECTS)
Seminars and workshops		
Dag voor de jonge onderzoeker, NVK, Veldhoven, the Netherlands	2011	0.5
Invitational Conference 'Opsporing van foetale groeivertraging' KNOV, Utrecht, the Netherlands	2012	0.5
Generation R research meetings, Erasmus MC, The Netherlands	2011-2012	1.0
Seminars at the department of Epidemiology, Erasmus MC, The Netherlands	2011-2012	1.0
Seminars at the department of Epidemiology, Harvard School of Public Health, Boston, USA	2013-2014	1.0
Stress and Health Disparities Symposium, Harvard Catalists, Harvard School of Public Health, Boston, USA	2013	0.5
1st Asia Working Group on Growth, Danone, Singapore	2013	0.2
(Inter)national congresses and presentations		
Invited speaker		
Child Growth Trajectory Workshop, Munich, Germany. *Oral presentation*	2013	1.4
19e Nederlands/Vlaams Doelencongres Infertiliteit, Gynaecologie en Obstetrie, Rotterdam, The Netherlands. *Oral presentation*	2013	1.4
Power of programming, Munich, Germany. *Oral presentation*	2014	1.4
Other		
Conference of Epidemiological Studies in Europe, Paphos, Cyprus. *Oral presentation*	2010	1.4
Early Genetics Consortium Meeting, London, United Kingdom. *Oral presentation*	2012	0.7
Early Nutrition Project. Research Workshop on Assessing Early Growth and Adiposity, Obergurgl, Austria. *Oral presentation*	2012	1.4
Werkgroep Epidemiologie Nederland, Rotterdam, the Netherlands. *Oral presentation*	2012	1.4
XVIII ISSHP World Congress, Geneva, Switzerland. *Oral and poster presentation*	2012	1.4
Early Nutrition Project. Placenta Research Workshop, Seggau, Austria. *Oral presentation*	2012	1.4
Generation R Research Meeting, Erasmus MC, the Netherlands.	2012	1.0
Developmental Origins of Health and Disease (DOHaD), Rotterdam, the Netherlands. *Oral presentation*	2012	1.4
3rd Biannual Early Nutrition meeting, Copenhagen, Denmark. *Poster presentation*	2013	0.7
Sophia onderzoekersdag, Rotterdam, the Netherlands. *Poster presentation*	2013	0.7
Developmental Origins of Health and Disease (DOHaD), Singapore. *Poster presentation*	2013	0.7
Early Nutrition Project. Anthropometry and Body Composition Workshop, Amsterdam, the Netherlands. *Oral presentation*	2013	1.4
Nutrition and Growth Conference, Barcelona, Spain. *Oral and poster presentation*	2014	1.4

	Year	Workload (ECTS)
International research projects		
International research project at Harvard School of Public Health, Boston, USA *Manuscript: Maternal inflammation during pregnancy and childhood adiposity & Maternal parity and birth outcomes. A sibling-comparison study.*	2013-2014	
Scholarships and grants		
Early Nutrition Brain Mobility Grant, €42.000	2013	
Vereniging Trustfonds Erasmus Universiteit Rotterdam, several travel grants	2010-2013	
Other		
Reviewed articles for Plos One, Am J Hypert, Eur J Epidemiol, Hypertension		
METC application for 'Generation R Focus at 9 visit'		
Participation in organizing DOHaD conference, Rotterdam, the Netherlands		
2. Teaching		
Supervising Master's and Bachelor's theses		
Akashi A. Rurangirwa, Clinical Epidemiology, Nihes, the Netherlands. Project title: *Hemodynamic adaptations in different trimesters among nulliparous and multiparous pregnant women & Associations of maternal parity with fetal and childhood growth, and cardio-metabolic risk factors*	2011-2012	4.0
Siham Yassine, Medical student, Erasmus MC, the Netherlands. Project title: *Risk factors and consequences of maternal anaemia and elevated haemoglobin levels during pregnancy*	2012	2.0
Guilherme A.F. Godoy, Medical student, Federal University of Minas Gerais, Brazil. Project title: *Maternal thyroid hormones during pregnancy, childhood adiposity and cardiovascular risk factors*	2013	2.0
Olta Gishti, Clinical Epidemiology, Nihes, the Netherlands. Project title: *Body mass index, total and abdominal body fat distribution and cardiovascular risk factors in school-age children*	2013	2.0
Fernanda M. Collares, Medical student, Federal University of Rio Grande do Sul, Brazil. Project title: *Maternal thyroid hormones, obesity and weight gain during pregnancy*	2014	2.0
Aleksandra Jelena, Medical student, University of Belgrade, Serbia. Project title: *Maternal fatty acids during pregnancy and childhood blood pressure*	2014	2.0
Sunayna Bahadoer, Student Health Sciences, VU, The Netherlands. Project title: *Ethnic differences in maternal obesity during pregnancy*	2014	2.0

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The Road goes ever on and on
Down from the door where it began.
Now far ahead the Road has gone,
And I must follow, if I can,
Pursuing it with eager feet,
Until it joins some larger way
Where many paths and errands meet.
And whither then? I cannot say.

~

J.R.R. Tolkien