Vascular health in pregnancy; maternal and child outcomes

Nienke Bergen

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The Generation R Study

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Chapter 1

General introduction,
aims and outline of the thesis

GENERAL INTRODUCTION

Cardiometabolic health encompasses cardiovascular and metabolic diseases, including hypertension and the metabolic syndrome. These conditions are leading causes of preventable death worldwide. They share similar risk factors which can be modified by diet, lifestyle choices or targeted medical treatment. Recent attention has focused on pregnancy as having a unique role in the pathogenesis of cardiometabolic diseases in later life in women and their offspring.[1, 2]
During pregnancy important adaptations occur in the maternal circulation and metabolism to meet the increased metabolic demands of the mother and fetus. These adaptations include an initial fall in systemic vascular tone, an increase in cardiac output and expansion of plasma volume. This leads to a gradual lowering of the systolic and diastolic blood pressure until mid-pregnancy and thereafter a rise in blood pressure from mid-pregnancy to delivery. Pregnancy also leads to adaptations in maternal glucose metabolism, hemostasis and lipid metabolism. Normally, these adaptations result in an adequate placental perfusion and nutrient supply to the fetus. However, suboptimal adaptations may lead to increased risks of pregnancy complications of both the fetus and the mother.[3]
Early placental development is of great importance for normal fetal growth and development.[4] Placental development comprises both vasculogenesis and angiogenesis.[5, 6] Within these processes, the vascular endothelial growth factor (VEGF) system is essential.[5] Angiogenesis is not only essential for early placental development, but also crucial for organ growth and cardiovascular development in the embryo.[7] Evidence is accumulating that embryonic and fetal growth is important for the health of the child and an important predictor of one's futures' health.[8-10] This insight has led to the Development Origins of Health and Disease (DOHaD) paradigm, which states that prenatal insults and especially a suboptimal intrauterine environment can result in endocrine and metabolic adaptations in the fetus. Although these adaptations seem beneficial to the fetus at first, this eventually may lead to increased risks of non-communicable diseases in adulthood for these children.[11, 12]
Adequate embryonic and fetal growth and placentation depend on an optimal intrauterine environment, which is determined amongst others by environmental maternal conditions and exposures. Maternal nutrition has been recognized as one of the most important environmental factors influencing the development of the embryo, fetus, placenta, as well as maternal health.[13-15] In this respect, evidence indicates a role for micronutrients in the pathophysiology of child and maternal pregnancy outcomes.[16] Folate, being the most investigated nutrient in reproductive medicine is of interest. It is an essential substrate for intermediates of cell multiplication and cell differentiation. Folate together with vitamin B_{12} play an important role in the homocysteine metabolism.[17, 18] Experimental studies revealed that elevated homocysteine concentrations may induce cytotoxic and oxidative stress leading to endothelial cell impairment, cellular apoptosis and inhibited trophoblastic function.[19, 20] Elevated homocysteine concentrations can be treated by synthetic folic acid, food folate and other B-vitamins.[17, 18, 21]

Also for the mother, pregnancy course and outcome are of importance for future health. Recent attention focuses on maternal adaptation to pregnancy in relation to future cardiovascular disease. Increasing evidence has shown new cardiovascular risk factors that are related to pregnancy. These risk factors encompass the pregnancy complications gestational hypertension (GH) and preeclampsia (PE).[22 23-27] Women with these pregnancy complications have a higher weight and blood pressure after pregnancy, compared to women with a previous normotensive pregnancy.[24, 28-30] Other cardiovascular risk factors, such as insulin resistance, visceral adiposity and the metabolic syndrome, are also more often seen in these women after the pregannncy.[24, 29, 31, 32] Women with a previous gestational hypertensive disorder seem to be more susceptible to exhibit an atherogenic lipid profile after pregnancy compared to women with a previous normotensive pregnancy. Causal pathways relating hypertensive pregnancy disorders to chronic hypertension and cardiovascular disease later in life are unclear. Women with GH or PE might exhibit the phenotype of metabolic syndrome or impaired endothelial function during, but also directly after, pregnancy persisting throughout life.[22] It might be that this phenotype exists already prior to pregnancy. Exposure of women with this constitutional predisposition to the cardiovascular challenges of pregnancy may induce transient clinical disease that subsides after pregnancy (GH or PE) but is likely to re-emerge later in life as CVD.[33, 34] On the other hand it is also plausible that products of the dysfunctional placenta in hypertensive pregnancy disorders permanently compromise maternal cardiovasculature with long-lasting effects on cardiovascular health.

AIMS OF THIS THESIS

The overall aim of this thesis was to investigate the role of angiogenic factors, micronutrients involved in the homocysteine metabolism, and maternal blood pressure, in relation to maternal and child health during and after pregnancy. The questions to be addressed in this thesis are:

Part I: Maternal health

1) Do homocysteine, folate and vitamin B_{12} concentrations affect placental development and subsequently maternal and child health during pregnancy?
2) To what extent do maternal gestational blood pressure and hypertensive pregnancy disorders influence maternal cardiometabolic outcomes six years after delivery?

Part II: Child health

3) How do early pregnancy and umbilical cord blood markers of the homocysteine pathway and angiogenic markers relate to fetal and childhood growth?
4) Is maternal blood pressure associated with childhood blood pressure?

The studies presented in this theses were embedded in the Generation R Study, a population based prospective cohort study from fetal life until young adulthood in Rotterdam, the Netherlands.[35] The Generation R Study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life and childhood. All women living in the study area with a delivery date between April 2002 and January 2006 were eligible for enrolment. Enrolment was aimed at early pregnancy, but was possible until birth of the child. In total 8880 women enrolled prenatally of whom 80% during the early pregnancy period. Assessments were planned in early, mid- and late pregnancy. These included physical examinations, maternal blood collection, fetal ultrasound examinations and self-administered questionnaires. Several overlapping sources including obstetric care givers and Municipal health services provided information about perinatal and maternal outcomes. At the age of six years children and mothers were invited to visit the Generation R Research Centre to participate in a detailed body composition and cardiovascular follow-up assessment using innovative and detailed tools. Currently, the study encompasses approximately 6,500 actively participating children aged 12-16 years together with their parents. The Generation R Study has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre Rotterdam and the Medical Ethical Review Board of all participating hospitals. All participants provided written informed consent. The Generation R study follows the STROBE guidelines.

OUTLINE OF THE THESIS

The general aim of this thesis is to identify placental, maternal and fetal factors associated with (adverse) maternal and child health during and after pregnancy.

Maternal health - The first part of this thesis is focused on maternal health during and after pregnancy with emphasis on maternal cardiometabolic adaptation in relation to gestational hypertensive disorders. In **Chapter 2** we investigate the associations between early pregnancy homocysteine, folate and vitamin B_{12} concentrations and placentation and adverse pregnancy outcomes. In **Chapter 3** we examine the association between blood pressure in pregnancy, GH and PE with cardiovascular status six years after pregnancy. In **Chapter 4** we determine if women with previous GH and PE have a more atherogenic lipid profile six years after pregnancy compared to women with a previous normotensive pregnancy.

Child health - The second part of this thesis focuses on child health during its fetal life and during the first years of childhood. In **Chapter 5** we investigate associations of early pregnancy as well as umbilical cord homocysteine, folate and vitamin B_{12} concentrations with fetal growth. In **Chapter 6** we examine associations of both maternal and fetal sFlt-1 and PlGF with fetal and childhood growth. In **Chapter 7** we examine the associations of maternal but also paternal blood pressure throughout pregnancy and hypertensive disorders in pregnancy

with childhood blood pressure, and the identification of critical periods and the role of birth outcomes and childhood body mass index in these associations. Finally, in **Chapter 8**, the general discussion of this thesis, we reflect on the main findings in our studies in view of implications for general medical practice.

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Part I

Maternal health

Chapter 2

Homocysteine and folate concentrations in early pregnancy and the risk of adverse pregnancy outcomes

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ABSTRACT

Objective: To investigate associations between early pregnancy homocysteine, folate and vitamin B_{12} concentrations and placental weight, birth weight and adverse pregnancy outcomes.

Methods: This study was embedded in the Generation R Study, a population-based birth cohort study in Rotterdam, the Netherlands. In total 5805 pregnant women, were included. To analyse homocysteine, folate and vitamin B_{12} concentrations, blood was drawn in early pregnancy. These concentrations were divided into quintiles. Information on birth outcomes was retrieved from medical records. Multivariable regression analyses were used. Main outcome measures were placental weight, birth weight, small for gestational age at birth (SGA) ($<5^{th}$ percentile), prematurity and preeclampsia.

Results: High homocysteine concentrations (highest quintile) were associated with lower placental (difference 30g; *P*-value <0.001) and birth weight (difference 110g; *P*-value <0.001), and increased risk of SGA (odds ratio (OR) 1.7; *P*-value 0.006) compared with the lowest quintile (reference). Low folate concentrations (lowest quintile) were associated with lower placental weight (difference 26g; *P*-value 0.001) and birth weight (difference 125g; *P*-value <0.001), and increased risks of SGA (OR 1.9; *P*-value 0.002), prematurity (OR 2.2; *P*-value 0.002) and preeclampsia (OR 2.1; *P*-value 0.04) compared with the highest quintile (reference). The risk of developing SGA and preeclampsia was substantially higher in women who had higher homocysteine and lower folate concentrations. No associations were found with vitamin B_{12}.

Conclusions: Higher homocysteine and lower folate concentrations in early pregnancy are associated with lower placental weight and birth weight, and higher risk of adverse pregnancy outcomes. These findings suggest that high homocysteine and low folate concentrations in early pregnancy may adversely influence placentation and subsequently affect the success of pregnancy and birth outcomes.

INTRODUCTION

Vascular-related pregnancy complications are a major cause of maternal and fetal morbidity and mortality. The origin is thought to be related to early placentation, a process which involves trophoblast invasion and angiogenesis, but is also dependent on vascular and endothelial function.[1] Placental development in early pregnancy may be negatively influenced by increased maternal homocysteine concentrations.[2] Experimental studies revealed that moderately elevated homocysteine concentrations (16-24 μmol/L) may induce cytotoxic and oxidative stress consequently leading to endothelial cell impairment.[3] Additionally, exposure of trophoblast cells to homocysteine (20 μmol/L) may increase cellular apoptosis and lead to inhibition of trophoblastic function.[4] Homocysteine is thought to be related to early placentation, so it may therefore affect subsequent fetal growth. Birth weight as proxy for fetal growth is an important determinant of later health and morbidity.[5, 6] Placental vasculopathy might be associated with preterm birth[7, 8] which may also be the case for high homocysteine and low folate concentrations.[9] Other studies have confirmed that mild hyperhomocysteinemia is associated with vascular-related pregnancy complications, such as preeclampsia, recurrent miscarriages and intra-uterine growth restriction.[9, 10] However, most of these studies measured homocysteine concentrations at the end of pregnancy or after delivery, whereas it has been suggested that its role is during pregnancy, when placentation occurs.[2]

Homocysteine metabolism is influenced by multiple factors, including folate and vitamin B_{12} status.[11, 12] Elevated homocysteine concentrations can be treated by synthetic folic acid, food folate and other B vitamins.[11, 13] In addition, it has been shown that folic acid use has the potential to improve endothelial function independently of homocysteine.[14, 15] From this perspective folate and vitamin B_{12} are also of interest.

There is conflicting evidence as to what extend elevated maternal homocysteine is a risk factor for pregnancy complications, prospective, sufficiently powered studies from early pregnancy onwards are required.[16] We therefore have examined in this prospective cohort study whether homocysteine, folate and vitamin B_{12} concentrations affect placental development and subsequently fetal growth. We focused on placental parameters (placental weight and placental vascular resistance) as well as vascular-related pregnancy complications, such as spontaneous prematurity, small for gestational age (SGA) infants and preeclampsia, which are of great clinical relevance.

METHODS

Design and study population

This study was embedded in the Generation R Study, an ongoing population-based prospective cohort study.[17, 18] The Generation R study was designed to identify early environmental

and genetic determinants of growth, development and health from fetal life until young adulthood. It is conducted in Rotterdam, the second largest city in the Netherlands, and eligible women were those who were resident in the study area and delivered between April 2002 and January 2006. The study aimed to enrol women in early pregnancy (gestational age <18 weeks), but enrolment was possible until birth of the child. All midwifery practices and three hospitals located in the study area participated during the prenatal phase. The overall response rate was about 61%, and was based on the number of children born to eligible mothers during the inclusion period. Assessments in pregnancy, including physical examinations, fetal ultrasound examinations and questionnaires, were planned in each trimester.[18] Approval for the study was obtained from the Medical Ethical Committees of all participating hospitals. All participants provided written informed consent.[18]

For this study we restricted our analyses to women who enrolled during pregnancy in the Generation R Study (N = 8880). Blood samples were collected in 6230 mothers in early pregnancy. Women without data on homocysteine concentrations were excluded from the analyses (6%; n = 348). Women with twin pregnancies (n = 69) and women who delivered before 24 weeks of gestation (n = 6) were also excluded from the analyses. In the remaining cohort, 375 women were having two or more subsequent pregnancies. As exclusion of these women did not substantially change our results, they were included in the analyses. Finally, 5805 women with complete data on homocysteine concentrations and a singleton live born pregnancy were eligible for the present study (**Figure 1**).

Biomarkers

In early pregnancy (median 13.2 weeks of gestation, 90% range 11.4-16.2) venous blood samples were drawn and stored at room temperature before being transported to the regional laboratory for processing and storage for future studies. Processing was planned to finish within a maximum of 3 hours after venous puncture. The samples were centrifuged and thereafter stored at -80 °C.[17] To analyse homocysteine, folate and vitamin B_{12} concentrations, serum samples (vitamin B_{12}) and EDTA plasma samples (folate, homocysteine) were picked and transported to the Department of Clinical Chemistry at the Erasmus University Medical Centre, Rotterdam in 2008. After thawing, homocysteine, folate and vitamin B_{12} concentrations were analysed using an immunoelectrochemoluminence assay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, the Netherlands). The between-run coefficients of variation for plasma homocysteine were 3.1% at 7.2 μmol/L, 3.1% at 12.9 μmol/L, and 2.1% at 26.1 μmol/L, with an analytic range of 1-50 μmol/L. The same coefficient of variation for plasma folate was 8.9% at 5.6 nmol/L, 2.5% at 16.6 nmol/L, and 1.5% at 33.6 nmol/L, with an analytic range of 1.8-45.3 nmol/L. This coefficient of variation for serum vitamin B_{12} was 3.6% at 142 pmol/L, 7.5% at 308 pmol/L, and 3.1% at 633 pmol/L, with an analytic range of 44-1476 pmol/L.

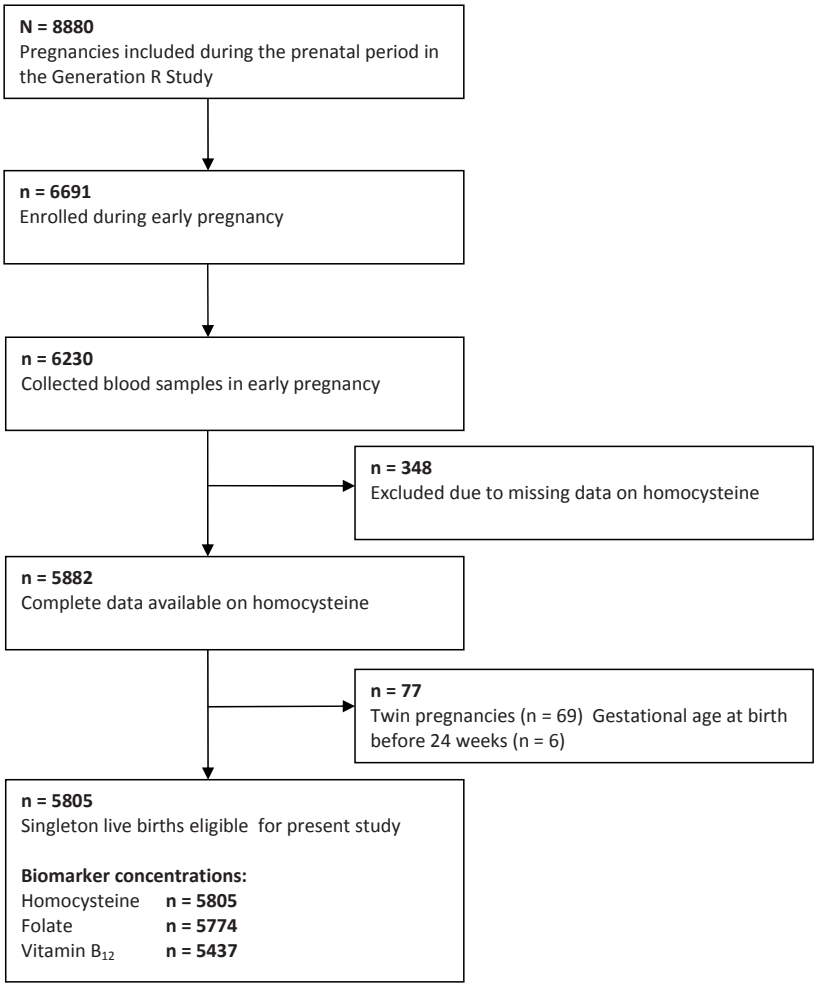

Figure 1 Flowchart.

Birth outcomes

Information concerning gestational age at birth (weeks), offspring sex, placental weight (grams) and birth weight (grams) were obtained from community midwives and hospital registries.[18] The definition of SGA was a gestational age-adjusted and sex-adjusted birth weight below the 5th percentile in this study cohort (less than -1.79 SD), according to the methodology of Niklasson et al.[19] Prematurity was defined as a spontaneous vaginal birth of an infant before 37.0 weeks of gestation (caesarean section, induction of labour not included).[20] The occurrence of hypertension and hypertension-related pregnancy complications in this study were cross-validated by a trained medical record abstractor using original blood pressure and proteinuria measurements noted in hospital medical records.[21] Preeclampsia was defined

according to criteria described by the International Society for the Study of Hypertension in Pregnancy (ISSHP).[22]

Placental vascular resistance

To determine placental vascular resistance, colour Doppler ultrasound examinations were performed in mid-pregnancy (median: 20.5 weeks; 90% range: 19.4, 22.0) and late pregnancy (median: 30.3 weeks; 90% range: 29.4, 31.6). Utero-placental vascular resistance was determined by uterine artery pulsatility index (UtA-PI). Fetal-placental vascular resistance was determined by umbilical artery pulsatility index (UmA-PI). For each measurement three consecutive uniform waveforms were recorded by pulsed Doppler ultrasound and the mean was used for further analyses.[23]

Covariates

Information on maternal age, educational level, geographical origin, maternal comorbidity (defined as the occurrence of chronic hypertension and/or heart disease and/or diabetes and/or high cholesterol and/r thyroid disease and/or systemic lupus erythematosus), parity and folic acid supplement use were obtained from the questionnaire at enrolment in the study. Maternal smoking habits, and alcohol and caffeine consumption were subsequently assessed by questionnaires in early, mid- and late pregnancy. As fetal growth is known to vary between ethnicities,[24] participating mothers gave details regarding information on their country of birth and that of their parents. This information was used to classify participants' ethnic background according to Statistics Netherlands, which previously has been described in detail.[24] Educational level was assessed by the highest completed education of the mother and classified into three categories: 1) primary school; 2) secondary school; and 3) university or college.[18] Folic acid supplement use was categorised in three groups: 1) started before conception; 2) start within 8 weeks of pregnancy; and 3) no use.[25] Weight and height were measured when the women were not wearing shoes or heavy clothing, and body mass index was calculated (weight in kilograms divided by height in metres squared). Information on fertility treatment was obtained from midwives and obstetricians.

Statistical analysis

First, we performed a nonresponse analysis by comparing characteristics of the women included in the analyses with those of women who were excluded from the analyses because of missing blood samples or missing data with regard to homocysteine concentrations. Differences were tested by using Student's T-test, Mann-Whitney's U-tests and Chi-square test.
Second, we created a standard deviation score (SDS) for each of the biomarkers, after logarithmic transformation, because the biomarkers were not normally distributed. We used linear regression models to assess the associations of the covariates (risk factors) with the biomarker

concentrations separately. To enable comparison of the effect estimates between risk factors, we present our results as change per SDS for continuous risk factors.

Third, the associations of biomarker concentrations with placental weight, birth weight and placental vascular resistance were analysed using multivariable linear regression models. Absolute differences in pulsatility indices were minor so SDS were used, which represent the deviation from the average based on the study population. Biomarkers were divided into quintiles and subsequently used as a categorical measure. This approach was chosen to explore the potential non-linearity of the association. The theoretically metabolically most favourable quintile (lowest quintile for homocysteine and highest quintiles for folate and vitamin B_{12}) was used as reference. This analysis allowed us to examine the effect of the association across the quintiles and whether the associations were apparent over the full exposure distribution or at the extremes only.

Fourth, the associations of biomarker concentrations with birth and pregnancy complications (prematurity, SGA infant and preeclampsia) were assessed using multivariable logistic regression models.

Lastly, we examined the association of women with both homocysteine concentrations in the highest quintile (≥8.3 μmol/L) and folate concentrations in the lowest quintile (≤9.2 nmol/L) with the risk of SGA infants and preeclampsia. The reference group was determined as women with simultaneously homocysteine concentrations in the lowest quintile (≤5.8 μmol/L) and folate concentrations in the highest quintile (≥25.9 nmol/L).

To further explore the effects of the chosen selection of women with available blood samples up to 18 weeks of gestation, we assessed a sensitivity analysis, repeating the regression analysis for the continuous outcome variables (placental weight and birth weight) and bi-variate outcome variables (prematurity, SGA infant and preeclampsia) in women in whom blood samples were collected before 13 weeks of gestation (n = 2968). When multiple comparisons were performed, the significance level was adjusted using Bonferroni correction.

We included potential confounders and effect modifiers in the models which were determined a priori and based on previously identified associations with birth outcomes and homocysteine or folate concentrations, namely maternal age, smoking, alcohol and caffeine consumption.[26] Next, we included offspring sex, parity, comorbidity, maternal height and weight, and geographical origin (as proxy for ethnicity) because these covariates had been shown to be associated with birth outcomes as well.[24] Educational level was also included as indicator for socio-economic status and is known to be associated with birth outcomes.[27, 28] We considered calorie intake as general estimate of nutrition intake and confounder in the model, but it did not change the effect estimate and therefore was not included in the final analysis. The same was true for mode of conception. Percentages of missing values in the covariates were provided in **Table 1** and ranged from 0% (maternal age) to 23.7% (folic acid use). For all analyses, missing values were imputed using the multiple imputation procedure.[29] Five imputed datasets were created using a fully conditional specified model to handle missing values. Imputations were

based on the relations between the covariates in the study, which were used to select the most likely value for a missing response. Data were analysed in each imputed dataset separately to obtain the effect estimates and standard errors. Pooled estimates were generated from these five imputed datasets and used to report estimates and their corresponding 95% confidence intervals. The pooled beta and odds ratio (OR) were calculated by taking the average of the beta's and OR's of the five imputed datasets. The pooled standard error (SE) to calculate the 95% confidence interval was then assessed using Rubin's rule[29]: $\sqrt{(W+(1+1/m)\times B)}$ with W the mean variance of the effect size within the imputed datasets; B the variance of the effect sizes between the imputed datasets; and m the number of imputed datasets (n = 5). Additional information about the imputation model is given in **Supplemental table 1**. Associations were considered significant at *P*-value <0.05. We performed statistical analyses using the Statistical Package of Social Sciences release 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Characteristics of the total study population were presented in **Table 1**. The mean age of the women in the whole cohort was 29.8 years and ranged from 15.3 to 46.3 years. Of all women, 56.9% were nulliparous, 59.3% were of White-European geographical origin, 46.9% finished higher education and 33.1% of the women started folic acid use before conception.

Nonresponse analysis (**Supplemental table 2**) showed that compared with infants born to women who did not provide blood samples, the infants included in the present study were less often born premature and had higher birth weights. The included women were older, taller, weighed less and were more often highly educated. They had a lower body mass index, more frequently used folic acid supplements, smoked less and were more likely to consume alcohol. They were more often nulliparous, of White-European origin and had more often conceived spontaneously.

Table 1 Baseline characteristics (n = 5805).

Maternal characteristics	
Age at intake (years), mean (SD)	29.8 (5.0)
Gestational age at intake (weeks), median (90% range)	13.4 (11.4, 16.5)
Height (cm), mean (SD)	167.6 (7.4)
Weight (kg), mean (SD)	68.8 (13.1)
BMI at intake (kg/m^2), median (90% range) (kg/m^2)†	23.5 (20.0, 30.4)
Nulliparous (%)	56.9
Missing	0.8
Geographical origin (%)	
White-European	59.3

Table 1 Baseline characteristics (n = 5805). (continued)

Maternal characteristics	
Surinamese	7.9
Turkish	7.5
Moroccan	5.2
Indonesian	2.8
Others	11.6
Missing	5.6
Education (%)	
Primary	4.3
Secondary	37.2
Higher	46.9
Missing	11.6
Comorbidity (%)	4.6
Missing	11.7
Spontaneous conception (%)	93.0
Missing	5.8
Folic acid supplement use (%)	
No use	18.8
Start before 8 weeks of pregnancy	24.5
Preconception start	33.1
Missing	23.7
Smoking (%)	
No smoking	64.2
Until pregnancy was known	8.1
Continued smoking	15.1
Missing	12.6
Alcohol consumption (%)	
No alcohol	40.2
Until pregnancy was known	13.2
Continued alcohol	34.4
Missing	12.2
Caffeine use in pregnancy (%)	89.5
Missing	6.1
Male gender of offspring (%)	50.4
Homocysteine concentration (μmol/L), median (90% range)	6.9 (5.3, 9.4)
Folate concentration (nmol/L), median (90% range)	15.8 (7.3, 30.6)
Vitamin B_{12} concentration (pmol/L), median (90% range)	169 (98, 298)

Abbreviation: Body mass index, BMI.
Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution.

Table 2 shows the effect of the independent risk factors on the biomarker concentrations. From this table it becomes clear that after multiple testing adjustments (8 independent risk factors) higher maternal age, being multiparous and preconceptional start of folic acid supplement use is negatively associated with homocysteine concentrations. Greater height and weight, secondary education only and continued smoking were positively associated with homocysteine concentrations.

Multiparity, greater weight, low education and smoking were negatively associated with folate concentrations. Greater height and higher maternal age, White-European geographical origin, fertility treatment, preconceptional start of folic acid supplement use and alcohol consumption were positively associated with folate concentrations.

Table 2 Maternal risk factors for early pregnancy biomarker concentrations (n = 5805).

Risk factors	**Homocysteine (SDS)**		**Folate (SDS)**		**Vitamin B_{12} (SDS)**	
	Beta (95% CI)	***P*-value**	Beta (95% CI)	***P*-value**	Beta (95% CI)	***P*-value**
Maternal age (years)						
Age (1 SD= 5.04)	-0.09 (-0.11, -0.06)	<0.001	0.29 (0.26, 0.31)	<0.001	0.08 (0.06, 0.11)	<0.001
<20	0.37 (0.22, 0.52)	<0.001	-0.95 (-1.1, -0.81)	<0.001	-0.11 (-0.26, 0.04)	0.15
20-24.9	0.26 (0.18, 0.34)	<0.001	-0.69 (-0.76, -0.62)	<0.001	-0.18 (-0.26, -0.10)	<0.001
25-29.9	0.05 (-0.01, 0.12)	0.11	-0.29 (-0.35, -0.22)	<0.001	-0.11 (-0.18, -0.04)	0.001
30-34.9	*Reference*		*Reference*		*Reference*	
35-39.9	0.04 (-0.04, 0.13)	0.32	0.07 (-0.01, 0.15)	0.09	0.06 (-0.03, 0.14)	0.21
>40	0.04 (-0.19, 0.28)	0.73	-0.11 (-0.33, 0.12)	0.35	0.17 (-0.07, 0.40)	0.16
BMI at intake (kg/m$^{2)}$						
BMI (1 SD= 4.41)	0.03 (0.001, 0.05)	0.04	-0.14 (-0.17, -0.12)	<0.001	-0.13 (-0.15, -0.10)	<0.001
<19.9	0.05 (-0.05, 0.14)	0.33	-0.05 (-0.14, 0.04)	0.27	0.05 (-0.04, 0.14)	0.27
20.24.9	*Reference*		*Reference*		*Reference*	
25-29.9	0.02 (-0.05, 0.08)	0.60	-0.21 (-0.27, -0.14)	<0.001	-0.12 (-0.18, -0.05)	<0.001
30-34.9	0.13 (0.03, 0.23)	0.01	-0.42 (-0.52, -0.32)	<0.001	-0.26 (-0.36, -0.16)	<0.001
>35	0.13 (-0.03, 0.28)	0.11	-0.47 (-0.62, -0.33)	<0.001	-0.49 (-0.64, -0.34)	<0.001
Maternal height (cm)						
Height (1 SD= 7.42)	0.07 (0.05, 0.10)	<0.001	0.15 (0.21, 0.17)	<0.001	0.07 (0.04, 0.09)	<0.001
Maternal weight at intake (kg)						
Weight (1 SD= 13.12)	0.06 (0.03, 0.09)	<0.001	-0.07 (-0.09, -0.04)	<0.001	-0.09 (-0.11, -0.06)	<0.001
Parity						
Nulliparous	*Reference*		*Reference*		*Reference*	
Multiparous	-0.09 (-0.14, -0.03)	0.001	-0.31 (-0.36, -0.26)	<0.001	-0.04 (-0.09, 0.02)	0.20
Geographical origin						
White-European	*Reference*		*Reference*		*Reference*	
Surinamese	0.05 (-0.05, 0.16)	0.29	-0.59 (-0.68, -0.50)	<0.001	-0.08 (-0.18, 0.02)	0.13
Turkish	0.05 (-0.06, 0.15)	0.38	-0.57 (-0.66, -0.47)	<0.001	-0.84 (-0.94, -0.74)	<0.001

Table 2 Maternal risk factors for early pregnancy biomarker concentrations (n = 5805). (continued)

Risk factors	Homocysteine (SDS)		Folate (SDS)		Vitamin B_{12} (SDS)	
	Beta (95% CI)	***P*-value**	Beta (95% CI)	***P*-value**	Beta (95% CI)	***P*-value**
Moroccan	-0.12 (-0.24, 0.01)	0.06	-0.80 (-0.91, -0.69)	<0.001	-0.00 (-0.12, 0.11)	0.95
Indonesian	-0.20 (-0.37, -0.03)	0.02	0.14 (-0.06, 0.34)	0.17	0.11 (-0.05, 0.27)	0.16
Others	-0.12 (-0.20, -0.03)	0.01	-0.53 (-0.61, -0.45)	<0.001	0.19 (0.11, 0.28)	<0.001
Education						
Primary	0.00 (-0.13, 0.13)	0.99	-0.92 (-1.05, -0.79)	<0.001	-0.30 (-0.43, -0.17)	<0.001
Secondary	0.28 (0.23, 0.34)	<0.001	-0.59 (-0.65, -0.54)	<0.001	-0.19 (-0.25, -0.13)	<0.001
Higher	*Reference*		*Reference*		*Reference*	
Comorbidity						
Yes	0.17 (0.04, 0.29)	0.01	0.08 (-0.05, 0.20)	0.23	-0.09 (-0.22, 0.04)	0.16
No	*Reference*		*Reference*		*Reference*	
Spontaneous conception						
Yes	*Reference*		*Reference*		*Reference*	
No	-0.13 (-0.41, 0.15)	0.35	0.37 (0.13, 0.62)	0.002	0.02 (-0.23, 0.26)	0.90
Folic acid supplement use						
No	0.52 (0.46, 0.59)	<0.001	-1.49 (-1.56, -1.42)	<0.001	-0.26 (-0.33, -0.19)	<0.001
Start before 8 weeks of pregnancy	0.16 (0.09, 0.22)	<0.001	-0.43 (-0.49, -0.37)	<0.001	-0.08 (-0.14, -0.01)	0.02
Preconception start	*Reference*		*Reference*		*Reference*	
Smoking						
No	*Reference*		*Reference*		*Reference*	
Until pregnancy was know	0.06 (-0.04, 0.16)	0.22	-0.08 (-0.18, 0.03)	0.17	0.02 (-0.08, 0.13)	0.67
Continued	0.35 (0.28, 0.42)	<0.001	-0.43 (-0.51, -0.36)	<0.001	-0.20 (-0.28, -0.12)	<0.001
Alcohol consumption						
No	*Reference*		*Reference*		*Reference*	
Until pregnancy was known	0.05 (-0.04, 0.13)	0.27	0.23 (0.14, 0.31)	<0.001	0.19 (0.10, 0.27)	<0.001
Continued	-0.07 (-0.14, -0.01)	0.03	0.34 (0.27, 0.40)	<0.001	0.30 (0.24, 0.35)	<0.001
Caffeine use						
No	*Reference*		*Reference*		*Reference*	
Yes	0.01 (-0.12, 0.14)	0.86	-0.10 (-0.23, 0.04)	0.16	0.09 (-0.04, 0.22)	0.19
Gender of offspring						
Male	*Reference*		*Reference*		*Reference*	
Female	-0.07 (-0.12, -0.02)	0.01	0.01 (-0.05, 0.06)	0.80	0.02 (-0.03, 0.08)	0.41

Abbreviations: Body mass index, BMI; Standard deviation score, SDS; Confidence interval, CI.
For continuous variables the effect estimates represent the change in biomarker SDS per increase of standard deviation of the risk factor. For categorical variables, the effect estimates represent the difference in biomarker concentration, given as SDS, compared to the reference group.
Values are adjusted for gestational age at blood sampling.

Turkish geographical origin, greater weight, low education and continued smoking were negatively associated with vitamin B_{12} concentrations. Greater height and higher maternal age, other geographical origin, preconceptional start of folic acid supplement use and alcohol consumption were positively associated with vitamin B_{12} concentrations.

Table 3 shows the multivariable analyses for placental weight and birth weight. Placental weight was approximately 15-30 grams lower in women in the two highest quintiles (homocysteine concentrations: >7.3 μmol/L) compared with women in the reference group (lowest quintile: ≤5.8 μmol/L). Infants born to women in the highest quintile had 110 grams lower birth weights compared with the reference group. Women with folate concentrations in the lowest quintile (folate concentration: ≤9.2 nmol/L) had a 26 grams lower placental weight compared with women in the reference group (folate concentration: ≥25.9 nmol/L). Compared with infants born to women in the reference group (highest quintile), infants born to women with folate concentrations below 19.0 nmol/L had 53-125 grams lower birth weights. In the partial R^2 model we estimated the R^2 change for placental weight and birth weight. It reveals that the contribution of homocysteine to the model after adjustment for all the covariates is 0.004 for placental weight and 0.005 for birth weight (both *P*-value <0.001). The R^2 change for the same outcomes when folate is added to the model after adjustment for all the covariates is 0.003 (*P*-value 0.006) and 0.004 (*P*-value <0.001), respectively.

In **Supplemental table 3** the results of the sensitivity analyses are given for the associations of first trimester homocysteine and folate concentrations with placental weight and birth weight. The effect estimates of these associations are stronger in these analyses compared to the analyses conducted in the complete population for analysis.

In **Figure 2**A-D the association is shown between homocysteine and folate concentrations and utero- and fetal-placental vascular resistance in mid- and late pregnancy. The categorical model showed that associations were only present at the extremes of the exposures. Women with folate concentrations in the lowest quintile had a significantly higher uterine artery pulsatility index (UtA-PI) in mid-pregnancy (difference SDS 0.23; 95% CI 0.10, 0.36; *P*-value 0.001) and a higher umbilical artery pulsatility index (UmA-PI) in late pregnancy (difference SDS 0.14; 95% CI 0.04, 0.24; *P*-value 0.006) compared with women in the reference group. Also, women with homocysteine concentrations in the highest quintile had a significantly higher UmA-PI in late pregnancy (difference SDS 0.11; 95% CI, 0.02, 0.20; *P*-value 0.02) compared with women in the reference group. However, this last association did not remain significant after multiple testing adjustments.

The associations between maternal biomarker concentrations and adverse pregnancy outcomes are shown in **Table 4**. An increasing risk of delivering a SGA infant was observed for women with homocysteine concentrations in the highest quintile (adjusted odds ratio (aOR), 1.68; *P*-value 0.006) compared with women in the reference group. Similar effects were seen for women with folate concentrations in the lowest quintile compared to the reference group (aOR, 1.91; *P*-value 0.002). Women with folate concentrations in the lowest quintile also had

Table 3 Associations between early pregnancy biomarker concentrations and placental weight and birth weight.

Biomarkers	Placental weight			Birth weight		
	grams†	Beta (95% CI)‡	***P*- value**	grams†	Beta (95% CI)‡	***P*-value**
Homocysteine µmol/L						
Q1 (<=5.8)	650 (151)	*Reference*		3464 (531)	*Reference*	
Q2 (5.8-6.6)	643 (148)	-8.2 (-21.0, 4.6)	0.21	3462 (554)	-5.3 (-38.3, 27.7)	0.75
Q3 (6.6-7.3)	636 (147)	-13.1 (-26.4, 0.2)	0.05	3422 (567)	-22.5 (-56.9, 11.9)	0.20
Q4 (7.3-8.3)	631 (149)	-14.7 (-28.0, -1.4)	0.03	3433 (580)	-6.4 (-40.9, 28.0)	0.72
Q5 (>=8.3)	617 (142)	-30.1 (-43.4, -16.7)	<0.001	3319 (575)	-110.1 (-144.5, -75.7)	<0.001
Folate nmol/L						
Q1 (<=9.2)	622 (146)	-26.0 (-40.6, -11.4)	<0.001	3314 (598)	-124.6 (-162.0, -87.2)	<0.001
Q2 (9.2-13.2)	638 (145)	-13.8 (-27.8, 0.24)	0.05	3425 (540)	-79.2 (-115.4, -43.0)	<0.001
Q3 (13.2-19.0)	642 (156)	-7.8 (-21.2, 5.6)	0.26	3458 (577)	-52.8 (-87.3, -18.2)	0.003
Q4 (19.0-25.9)	637 (150)	-4.6 (-17.8, 8.6)	0.49	3433 (553)	-41.8 (-75.9, -7.7)	0.02
Q5 (>=25.9)	641 (142)	*Reference*		3481 (531)	*Reference*	

Abbreviations: Confidence interval, CI; Quintile, Q.

Multivariable linear regression analysis with birth weight and placental weight as dependent variables and homocysteine and folate concentrations as independent variables. Q1 through Q5 represents the quintile distribution of the relative concentrations.

† All values in this column are means (SD).

‡ All values in this column are regression coefficients (95% CI) and their corresponding *P*-value. These values represent the difference between placental weight and birth weight in the specific quintile compared to the reference group.

Values are adjusted for gestational age at blood sampling, gestational age at birth, gender of offspring, maternal age at intake, parity, educational level, geographical origin, comorbidity, maternal height, maternal weight at intake, smoking, alcohol and caffeine use.

twice the risk of spontaneous prematurity (*P*-value 0.002) and of developing preeclampsia (*P*-value 0.04).

After multiple testing adjustment, the association of folate with preeclampsia was no longer significant. **Supplemental table 4** shows the results of the sensitivity analysis with regard to prematurity, SGA infants and preeclampsia. As a result of the reduced sample size, confidence intervals widened, and the associations between homocysteine and SGA infants and folate and preeclampsia were no longer significant, although the effect estimates were comparable to the results of the whole study cohort.

Lastly, women with both homocysteine concentrations in the highest quintile together with folate concentrations in the lowest quintile had a four times higher risk of a SGA infant (aOR, 4.04; 95% CI 1.97-8.28; *P*-value <0.001) and of developing preeclampsia (aOR, 4.27; 95% CI 1.2-15.03; *P*-value 0.02) than women with both homocysteine concentrations in the lowest quintile together with folate concentrations in the highest quintile.

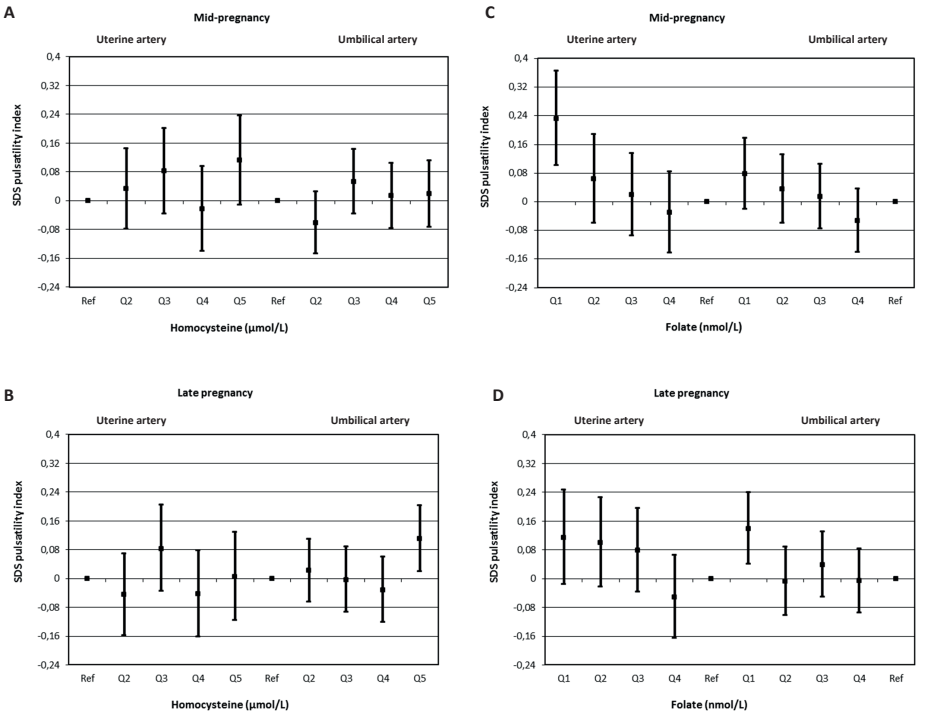

Figure 2 Associations between early pregnancy biomarker concentrations and placental vascular resistance.

Abbreviations: Reference, Ref; Quintile, Q; Standard deviation score, SDS.

Values are regression coefficients (error bars indicate 95% confidence intervals) and represent the difference in SDS of uterine artery and umbilical artery pulsatility index (UtA-PI and UmA-PI, respectively), compared to the reference group, in mid-(median 20.5 weeks) and late pregnancy (median 30.3 weeks). The analysis are based on respectively 2775 and 2730 measurements of UtA-PI in mid- and late pregnancy, and 4471 and 4656 measurements of UmA-PI in mid- and late pregnancy.

Values are adjusted for gestational age at measurement, gender of offspring, maternal age at intake, parity, educational level, geographical origin, comorbidity, maternal height, maternal weight at intake, smoking, alcohol and caffeine use.

DISCUSSION

In this prospective cohort study we demonstrated that higher homocysteine concentrations and lower folate concentrations were associated with lower placental weight, lower birth weight, and a higher risk of having a SGA infant. Furthermore, decreasing folate concentrations were also associated with an increasing risk of spontaneous prematurity and preeclampsia. Finally, women with both high homocysteine and low folate concentrations had a substantially increased risk of having a SGA infant or developing preeclampsia.

Table 4 Associations between early pregnancy biomarker concentrations and adverse birth and pregnancy outcomes.

Biomarkers	Prematurity			Small for gestational age at birth			Preeclampsia		
	n (%)	aOR (95% CI)	*P*-value	n (%)	aOR (95% CI)	*P*-value	n (%)	aOR (95% CI)	*P*-value
Homocysteine µmol/L									
Q1 (<=5.8)	34 (2.8)	*Reference*		54 (4.4)	*Reference*		20 (1.6)	*Reference*	
Q2 (5.8-6.6)	39 (3.1)	1.09 (0.68, 1.74)	0.73	68 (5.4)	1.26 (0.87, 1.84)	0.22	27 (2.2)	1.28 (0.71, 2.32)	0.41
Q3 (6.6-7.3)	39 (3.6)	1.24 (0.77, 1.99)	0.37	48 (4.4)	0.99 (0.66, 1.49)	0.96	19 (1.7)	1.08 (0.57, 2.05)	0.83
Q4 (7.3-8.3)	49 (4.5)	1.61 (1.02, 2.53)	0.04	49 (4.5)	1.07 (0.71, 1.60)	0.76	23 (2.1)	1.30 (0.70, 2.40)	0.41
Q5 (>=8.3)	46 (4.0)	1.36 (0.85, 2.17)	0.19	80 (7.0)	1.68 (1.16, 2.43)	0.006	29 (2.5)	1.60 (0.88, 2.90)	0.12
Folate nmol/L									
Q1 (<=9.2)	62 (5.3)	2.17 (1.34, 3.57)	0.002	88 (7.5)	1.91 (1.27, 2.87)	0.002	31 (2.6)	2.10 (1.05, 4.20)	0.04
Q2 (9.2-13.2)	30 (2.6)	1.04 (0.61, 1.77)	0.88	52 (4.5)	1.20 (0.78, 1.84)	0.41	27 (2.4)	1.84 (0.92, 3.65)	0.08
Q3 (13.2-19.0)	39 (3.4)	1.31 (0.80, 2.12)	0.28	60 (5.2)	1.39 (0.93, 2.08)	0.11	22 (1.9)	1.53 (0.77, 3.04)	0.23
Q4 (19.0-25.9)	44 (3.8)	1.41 (0.89, 2.25)	0.15	52 (4.5)	1.11 (0.74, 1.68)	0.61	24 (2.3)	1.70 (0.87, 3.32)	0.12
Q5 (>=25.9)	32 (2.8)	*Reference*		46 (4.0)	*Reference*		14 (1.3)	*Reference*	

Abbreviations: Adjusted odds ratio, aOR; Confidence interval, CI; Quintile, Q.
Multivariable logistic regression analysis with prematurity, small for gestational age at birth and preeclampsia as dependent variables and homocysteine and folate concentrations as independent variables. Q1 through Q5 represents the quintile distribution of the relative concentrations.
aOR (95% CI) and their corresponding *P*-value represents the risk of prematurity, having a small for gestational age infant or developing preeclampsia in the specific quintile compared to the reference group.
Values are adjusted for gestational age at blood sampling, gender of offspring, maternal age at intake, parity, educational level, geographical origin, comorbidity, maternal height, maternal weight at intake, smoking, alcohol and caffeine use.

Methodological considerations

To our knowledge, this is the largest study that examined the associations of homocysteine, folate and vitamin B_{12} concentrations in early pregnancy with several placental parameters and pregnancy outcomes. The large number of subjects studied significantly increases the accuracy of our effect estimate. In addition, detailed information was available for a large number of covariates. Some limitations of our study should be addressed. The response rate in the Generation R Study was approximately 61%.[18] Furthermore, from approximately 30% of the women a blood sample in early pregnancy was not obtained because they were enrolled later in pregnancy. Therefore, the underlying mechanism is most likely selective nonresponse (or delayed response). However, selective nonresponse only harms the validity of the study when the association between determinant and outcome differs between those included and excluded from the study. This is difficult to determine, since we do not know the associations between the determinants and outcomes of the women excluded from the study. Selection bias can therefore not be excluded.

Secondly, despite the large sample size, our results apply to a relatively healthy sample of pregnant women. Our estimates can therefore be too conservative and underestimate the true effect measures. Third, as we hypothesised that vascular-related pregnancy complications originate in the first-trimester of pregnancy we only measured biomarker concentrations in early pregnancy and not thereafter. Lastly, regression models were adjusted for several lifestyle (e.g. smoking, weight and height, caffeine and alcohol consumption) and socio-economic factors (e.g. educational level, geographical origin). The rationale is that these potential confounders, partially correlated with socio-economic factors, affect the folate-homocysteine pathway and thereby protein-, lipid- and DNA synthesis and DNA methylation important for embryonic growth and development.[26] However, since this is an observational study residual confounding cannot be excluded, even though we were able to adjust for a large number of potential confounders.

Interpretation

Our findings are consistent with series of investigations showing similar relations between higher homocysteine concentrations and a lower placental weight, lower birth weight and higher risk of SGA.[9, 30, 31] However, most previous studies were conducted at the end of late pregnancy or after delivery. As far as we know only two studies assessed the association between homocysteine concentrations in the first-trimester of pregnancy and intra-uterine growth.[30, 32] Murphy et al. observed that mothers with a homocysteine concentration above 8.44 μmol/L (which corresponds to our highest quintile of homocysteine) at eight weeks of pregnancy were three times more likely to give birth to an infant in the lowest birth weight tertile.[30] On the contrary, Dodds et al. did not observe a significant association between increased homocysteine concentrations in early pregnancy and SGA, but their homocysteine concentrations were relatively low (90th percentile at 5.71 μmol/L) which might be due to folic acid food fortification.[32]

Hyperhomocysteinemia during pregnancy is suggested to play a significant role in the pathogenesis of preeclampsia, in which endothelial cell dysfunction is a central theme.[1] This is also shown by studies performed in different stages of pregnancy.[9, 32, 33] We and others [34-36] were not able to find this association and suggest that an increased homocysteine concentration is more a consequence rather than a cause in the pathophysiology of preeclampsia.

In contrast to others, we did not find an association between homocysteine concentrations and prematurity.[9, 37] We did establish a significant association between a low folate concentration and spontaneous prematurity, which is in line with the findings of Scholl et al. and Siega-Riz and collegeous.[38, 39] It is important to realise that inconsistencies in these latter studies, due to differential definition of (spontaneous) preterm birth, timing of blood sampling and mandatory folic acid food fortification in some countries make it difficult to interpret the results.

The effect of folate on the placental vasculature is not only suggested by the significant increase in placental weight, birth weight and decreased risk of SGA in the women with higher

folate concentrations, but also supported by our observed results that decreasing folate concentrations are associated with an increase in placental vascular resistance. These findings are sustained by several other studies.[25, 38, 40]

The overall clinical relevance of our findings is that placental development in early pregnancy is essential for optimal fetal growth and birth weight thereafter, in which the latter outcome is often used as end-point of different fetal growth patterns.[41] In this study, we show that women with high homocysteine and low folate concentrations have smaller placentas and a lower birth weight of 110 and 124 grams, respectively. These effect estimates induced by homocysteine and folate are of similar magnitude to, for example smoking which is a well-established risk factor for impaired fetal growth.[42] The relevance of our findings should also be considered against the background that newborns with impaired growth and compensatory accelerated postnatal growth are at risk for metabolic and cardiovascular disease in later life.[43, 44]

We also observed that low folate concentrations were associated with an increased risk of preeclampsia in contrast to the study of Guven and colleagues.[33]

Interestingly, our results on folate were more consistent than our findings on homocysteine. Folate does not only establish its effects through optimisation of the folate dependent homocysteine pathway. It also provides methyl groups for the synthesis of methionine and its derivate S-adenosyl –methionine. The latter is the most important methyl donor in the human body for genome programming by DNA methylation and represents one of the best known epigenetic mechanisms. Therefore, these folate dependent reactions are essential for placental and fetal growth and development.[45-47] Moreover, folate has been suggested to influence antioxidant defences through its role as a superoxide scavenger.[14] This may affect placental implantation and vascular remodelling independent of homocysteine status.[15, 48] These independent effects of folate together with the effect of homocysteine might also explain the substantial increased risk of adverse pregnancy outcomes in women with both low folate and high homocysteine concentrations.

We did not find any associations of vitamin B_{12} concentrations with pregnancy outcomes. This might be explained by the fact that folate is a substrate and vitamin B_{12} serves as a cofactor in the homocysteine metabolism, and thereby is not often a limiting factor.[49]

CONCLUSIONS

Our results showed that higher homocysteine concentrations and lower folate concentrations in early pregnancy were associated with lower fetal and placental size. Low folate concentrations in particular were associated with vascular-related pregnancy complications. The findings of the sensitivity analysis are in line with our hypothesis that both folate and homocysteine may establish their effects especially in early pregnancy when placentation occurs and these adverse pregnancy outcomes originate.

Several maternal lifestyle factors, such as smoking, alcohol consumption and folic acid supplement use, and weight as proxy of nutrition and lifestyle, affect the homocysteine, folate and vitamin B_{12} status. Therefore, our results, although observational and only indicative of causal relations, emphasize the importance of optimizing these preconceptional nutrition and lifestyle behaviour.

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SUPPLEMENTAL MATERIAL

Supplemental table 1 Details of the multiple imputation modelling.

Sofware used	SPSS 17.0 for windows (SPSS Inc, Chicago, IL, USA)
Imputation method and keysettings	Fully conditional specification (Markov chain Monte Carlo method); Maximum iterations: 10;
No of imputed data sets created	5
Variable included to be imputed and used as predictor	maternal folic acid supplementation, maternal age, maternal BMI, parity, gender of the child, maternal educational level, maternal smoking, maternal ethnicity, maternal alcohol use during pregnancy, maternal caffeine use during pregnancy, maternal comorbidity, maternal height, maternal weight, maternal fertilitity treatment, maternal calorie intake during pregnancy.
Variables included and only used as predictor	maternal folate concentration, maternal homocysteine concentration, maternal vitamin B_{12} concentration, pulsatility index umbilical artery mid-pregnancy, pulsatility index umbilical artery late pregnancy, pulsatility index uterine artery mid-pregnancy, pulsatility index late pregnancy, placental weight, birth weight, gestational age at birth, standard deviation score birth weight
Treatment of none normally distributed variables	Not applicable
Treatment of binary/categorical variables	Logistic regression and multinomial models
Statistical interaction included in the imputation models	None

Supplemental table 2 Characteristics of women included and excluded from the study.

	Total	**Included**	**Excluded**	***P*-value**[†]
	N = 8880	n = 5805	n = 3075	
Pregnancy outcomes				
Age at intake (years), mean (SD)	29.7 (5.3)	29.8 (5.0)	29.4 (5.8)	0.005
Gestational age at intake, median (90% range) (weeks)**	14.4 (11.6, 21.2)	13.4 (11.4, 16.5)	19.8 (12.6, 4.4)	<0.001
Height (cm), mean (SD)	167.1 (7.4)	167.6 (7.4)	166.3 (7.4)	<0.001
Weight (kg), mean (SD)	69.5 (13.3)	68.8 (13.1)	71.0 (13.6)	<0.001
BMI at intake (kg/m^2), median (90% range)**	23.9 (20.1, 30.9)	23.5 (20.0, 30.4)	24.8 (20.7, 32.0)	<0.001
Nulliparous (%)	54.8	56.9	50.7	<0.001
Missing	1.4	0.8	2.4	
Geographical origin (%)				
White-European	53.7	59.3	43.1	<0.001
Surinamese	8.3	7.9	9.1	
Turkish	8.4	7.5	10.1	
Moroccan	6.1	5.2	7.9	
Indonesian	2.7	2.8	2.6	
Others	13.0	11.6	15.7	
Missing	7.6	5.6	11.5	

Supplemental table 2 Characteristics of women included and excluded from the study. (continued)

	Total	**Included**	**Excluded**	***P*-value**[†]
	N = 8880	n = 5805	n = 3075	
Education (%)				
Primary	5.4	4.3	7.7	<0.001
Secondary	37.6	37.2	38.3	
Higher	43.0	46.9	35.7	
Missing	13.9	11.6	18.3	
Comorbidity (%)	4.7	4.6	4.9	0.26
Missing	13.5	11.7	16.9	
Spontaneous conception (%)	91.6	93.0	88.9	0.005
Missing	7.0	5.8	9.2	
Folic acid supplement use (%)				
No use	21.7	18.8	27.2	<0.001
Start before 8 weeks of pregnancy	23.0	24.5	20.2	
Preconception start	29.2	33.1	21.9	
Missing	26.1	23.7	30.7	
Smoking (%)				
No smoking	63.7	64.2	62.8	<0.001
Until pregnancy was known	7.1	8.1	5.4	
Continued smoking	14.7	15.1	13.8	
Missing	14.5	12.6	18.0	
Alcohol consumption (%)				
No alcohol	42.9	40.2	48.0	<0.001
Until pregnancy was known	11.8	13.2	9.0	
Continued alcohol	31.4	34.4	25.7	
Missing	14.0	12.2	17.4	
Caffeine use in pregnancy (%)	86.9	89.5	82.0	0.53
Missing	8.8	6.1	13.9	
Birth outcomes				
Male gender of offspring (%)	50.5	50.4	50.6	0.86
Placental weight (g), mean (SD)	635.8 (148.3)	635.9 (147.7)	635.7 (149.5)	0.95
Birthweight (g), mean (SD)	3407.8 (567.3)	3421.2 (563.1)	3380.2 (575.1)	0.002
Gestational age at birth (weeks), median (90% range)	40.1 (37.7, 41.7)	40.1 (38.0, 41.7)	40.0 (37.4, 41.7)	<0.001
Pregnancy complications				
Prematurity (%)	4.3	3.6	5.7	<0.001
Small for gestational age at birth (%)	4.9	5.2	4.4	0.49
Preeclampsia (%)	2.1	2.0	2.1	0.64

Abbreviation: Body mass index, BMI.

† Differences between women included versus excluded from the study were tested by using Student's t-test for continuous variables with a normal distribution and Mann-Whitney U-test for continuous variables with a skewed distribution. Chi-square test were used for categorical variables.

Supplemental table 3 Associations between **first trimester** biomarker concentrations and placental weight and birth weight (n = 2968).

Biomarkers	Placental weight			Birth weight		
	grams†	Beta (95% CI)‡	***P*-value**	grams†	Beta (95% CI)‡	***P*-value**
Homocysteine µmol/L						
Q1 (<=5.9)	654 (156)	*Reference*		3486 (553)	*Reference*	
Q2 (5.9-6.7)	642 (147)	-11.6 (-29.6, 6.5)	0.21	3480 (547)	-9.9 (-57.2, 37.3)	0.68
Q3 (6.7-7.4)	635 (139)	-16.3 (-35.0, 2.4)	0.09	3431 (553)	-30.8 (-79.1, 17.5)	0.21
Q4 (7.4-8.5)	630 (148)	-19.2 (-37.5, -0.8)	0.04	3452 (578)	-12.0 (-59.8, 35.8)	0.62
Q5 (>=8.5)	612 (138)	-37.8 (-56.5, -19.1)	<0.001	3324 (559)	-124.3 (-173.1, -75.5)	<0.001
Folate nmol/L						
Q1 (<=10.1)	625 (145)	-29.6 (-49.7, -9.5)	0.004	3347 (600)	-114.4 (-166.8, -61.9)	<0.001
Q2 (10.1-15.4)	631 (148)	-22.8 (-41.8, -3.8)	0.02	3409 (543)	-102.2 (-151.6, -52.8)	<0.001
Q3 (15.4-21.3)	640 (150)	-13.0 (-31.5, 5.5)	0.17	3475 (574)	-47.5 (-95.4, 0.5)	0.05
Q4 (21.3-27.5)	630 (147)	-16.3 (-34.7, 2.0)	0.08	3441 (531)	-46.0 (-93.8, 1.8)	0.06
Q5 (>=27.5)	647 (141)	*Reference*		3503 (542)	*Reference*	

Abbreviations : Quintile, Q; Confidence interval, CI.

Multivariable linear regression analysis with birth weight and placental weight as dependent variables and homocysteine and folate concentrations as independent variables. Q1 through Q5 represents the quintile distribution of the relative concentrations.

† All values in this column are means (SD).

‡ All values in this column are regression coefficients (95% CI) and their corresponding *P*-value. These values represent the difference between placental weight and birth weight in the specific quintile compared with the reference group.

Values are adjusted for gestational age at blood sampling, gestational age at birth, gender of offspring, maternal age at intake, parity, educational level, geographical origin, comorbidity, maternal height, maternal weight at intake, smoking, and alcohol and caffeine use.

Supplemental table 4 Associations between **first trimester** biomarker concentrations and adverse birth and pregnancy outcomes (n = 2968).

Biomarkers	**Prematurity**			**Small for gestational age at birth**			**Preeclampsia**		
	n (%)	aOR (95% CI)	***P*-value**	n (%)	aOR (95% CI)	***P*-value**	n (%)	aOR (95% CI)	***P*-value**
Homocysteine µmol/L									
Q1 (<=5.9)	16 (2.7)	*Reference*		24 (4.0)	*Reference*		9 (1.5)	*Reference*	
Q2 (5.9-6.7)	21 (3.4)	1.21 (0.62-1.36)	0.58	29 (4.7)	1.10 (0.62-1.95)	0.74	11 (1.8)	1.00 (0.40-2.47)	0.99
Q3 (6.7-7.4)	19 (3.3)	1.16 (0.59-2.30)	0.67	26 (4.6)	1.06 (0.59-1.90)	0.85	8 (1.4)	0.86 (0.32-2.26)	0.75
Q4 (7.4-8.5)	24 (4.0)	1.38 (0.72-2.67)	0.33	22 (3.7)	0.88 (0.48-1.61)	0.67	11 (1.8)	1.06 (0.43-2.63)	0.90
Q5 (>=8.5)	23 (3.9)	1.36 (0.70-2.66)	0.37	39 (6.6)	1.54 (0.88-2.67)	0.13	13 (2.2)	1.22 (0.50-2.97)	0.66
Folate nmol/L									
Q1 (<=10.1)	33 (5.5)	2.00 (1.02-3.94)	0.04	40 (6.7)	1.98 (1.10-3.56)	0.02	15 (2.5)	2.10 (0.77-5.75)	0.15
Q2 (10.1-15.4)	13 (2.2)	0.78 (0.37-1.67)	0.53	30 (5.0)	1.41 (0.78-2.54)	0.25	11 (1.8)	1.54 (0.57-4.15)	0.40
Q3 (15.4-21.3)	22 (3.8)	1.33 (0.69-2.56)	0.39	22 (3.8)	0.97 (0.53-1.79)	0.93	6 (1.0)	0.90 (0.30-2.73)	0.85
Q4 (21.3-27.5)	18 (3.1)	1.02 (0.51-2.01)	0.96	23 (3.9)	0.91 (0.50-1.65)	0.74	13 (2.2)	1.65 (0.64-4.23)	0.30
Q5 (>=27.5)	17 (2.9)	*Reference*		24 (4.1)	*Reference*		7 (1.2)	*Reference*	

Abbreviations: Adjusted odds ratio, aOR; Quintile, Q.

Multivariable logistic regression analysis with prematurity, small for gestational age at birth and preeclampsia as dependent variables and homocysteine and folate concentrations as independent variables. Q1 through Q5 represents the quintile distribution of the relative concentrations.

aOR (95% CI) and their corresponding *P*-value represents the risk of prematurity, having a small for gestational age infant or developing preeclampsia in the specific quintile compared to the reference group.

Values are adjusted for gestational age at blood sampling, gender of offspring, maternal age at intake, parity, educational level, geographical origin, comorbidity, maternal height, maternal weight at intake, smoking, and alcohol and caffeine use.

Chapter 3

Hypertensive disorders of pregnancy and subsequent maternal cardiovascular health

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ABSTRACT

Objective: To examine associations between hypertensive pregnancy disorders and maternal cardiovascular disease (CVD) in later life.

Methods: We examined the associations between blood pressure (BP) in pregnancy, gestational hypertension (GH) and preeclampsia (PE) with cardiovascular measurements 6 years after index pregnancy among 4912 women participating in the Generation R Study, the Netherlands. Main outcome measures were BP, left ventricular mass (LV mass), aortic root diameter (AOD), left atrial diameter (LAD), fractional shortening (FS), and carotid-femoral pulse wave velocity (PWV).

Results: Early pregnancy systolic and diastolic BP were associated with more adverse maternal cardiovascular measurements and a higher incidence of chronic hypertension 6 years after pregnancy. GH was associated with a higher BP, a higher PWV, a larger AOD and an increased LV mass 6 years after index pregnancy. Compared to previous normotensive pregnancies these women had a six-fold increased risk to develop chronic hypertension after pregnancy (OR 6.6, 95% CI 4.6-9.5). Compared to women with a normotensive pregnancy, women with PE had a higher BP and a higher risk for chronic hypertension (OR 4.5, 95% CI 2.6-7.8) at follow up. After adjustment for pre-pregnancy BMI in all the analyses on GH, PE and cardiovascular measurements, effect estimates attenuated up to 65%, but remained significant.

Conclusions: Both GH and PE are associated with markers of adverse maternal cardiovascular health after pregnancy with an increased risk for chronic hypertension. Women with GH and PE may be offered long-term cardiovascular follow-up incorporated in CVD risk management guidelines.

INTRODUCTION

Differences between men and women exist regarding age-dependent onset, severity, symptoms and outcomes of cardiovascular disease (CVD).[1] Increasing evidence has shown new cardiovascular risk factors exclusive to women related to pregnancy. These include gestational hypertension (GH) and preeclampsia (PE).[2] The exact mechanisms by which these risk factors contribute to long term CVD risk have not been clarified. Women with GH or PE may exhibit the phenotype of metabolic syndrome or impaired endothelial function during, but also directly after, pregnancy which is also seen in later life.[2, 3] These include family history of diabetes mellitus, pregravid diabetes mellitus, a high total cholesterol/high-density lipoprotein cholesterol ratio (>5), overweight and obesity, and elevated blood pressure status.[3] Exposure of women with this constitutional predisposition to the cardiovascular challenges of pregnancy may induce transient clinical disease that subsides after pregnancy (GH or PE) but is likely to re-emerge later in life as CVD.[4, 5] On the other hand it is also plausible that products of the dysfunctional placenta in hypertensive pregnancy disorders permanently compromise maternal cardiovasculature with long-lasting effects on cardiovascular health.[6, 7] In this respect pathophysiological studies may help to identify individuals after pregnancy with subclinical CVD as they might compose a target population for possible interventions before clinical signs and symptoms are evident. The aim of this study was to assess associations of maternal gestational blood pressure (BP) and hypertensive pregnancy disorders with cardiovascular outcomes 6 years after delivery.

METHODS

Design and study population

This study is embedded in the Generation R Study, a population-based cohort study.[8] The study protocol conforms to the ethical guidelines of the 1964 Declaration of Helsinki and its later amendments. The Medical Ethical Committee of the Erasmus Medical Centre Rotterdam approved the study and written consent was obtained from all participants. 5439 women with live born infants provided consent for postnatal analysis. We excluded women with missing or incomplete information on hypertensive pregnancy disorders (n = 110) or with known chronic hypertension before initial enrolment during pregnancy (n = 90). Also, women with cardiac abnormalities (n = 21), twin pregnancies (n = 34) and women being pregnant during their follow up visit at the research centre (n = 257) or with missing data on medication use at follow-up (n = 15) were excluded, leaving 4912 participants for the analyses (**Supplemental figure 1**).

Blood pressure, hypertensive disorders of pregnancy and chronic hypertension

BP was measured in early pregnancy (median 13.2 weeks gestation, 90% range [10.6-16.9]), mid- pregnancy (median 20.5 weeks gestation, 90% range [19.1-22.4]) and late pregnancy (median 30.2 weeks gestation, 90% range [29.1-31.9]) and 6 years after delivery (90% range 5.7-7.2 years) after with a validated automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe B.V., Hoofddorp, the Netherlands).[9] BP was measured in a research setting by trained research assistants wearing normal clothing (no white coats). Subsequently, mean arterial pressure (MAP) was derived. The presence of doctor diagnosed GH or PE was retrieved from hospital charts and was determined on the basis of the former 2001 criteria described by the International Society for the Study of Hypertension in Pregnancy.[10, 11] Information on chronic hypertension before onset of pregnancy was obtained through a questionnaire during pregnancy which was cross-checked with information from the original medical records and the Dutch obstetric database. Chronic hypertension at follow-up 6 years after pregnancy was defined as women using anti-hypertensive medication and/or having, in two subsequent readings, but at one single visit, a systolic BP above 140 mmHg or a diastolic BP above 90mmHg. The value of two BP readings over a 5 minute interval were documented for each participant. If women were having a systolic BP >140 mmHg or diastolic BP >90 mmHg in the first reading and also had a systolic BP >140 mmHg or diastolic BP >90 mmHg in the second reading we included them in the hypertension group. We are aware that international guidelines recommend ambulatory BP monitoring to define or confirm clinical diagnosis of hypertension. However ambulatory BP monitoring was not available. Instead we also performed a sensitivity analyses in which we labelled women as chronic hypertensive, only if they used BP medication at follow-up.

Cardiovascular measurements six years after pregnancy

Data on cardiovascular outcomes were collected 6 years after index pregnancy at our research centre (range, 4.9-7.0 years). Two-dimensional M-mode echocardiographic measurements were performed using the ATL-Philips Model HDI 5000 (Seattle, WA, USA) or the Logiq E9 (GE Medical Systems, Wauwatosa, WI, USA) devices. Aortic root diameter (AOD[sinus of Valsalva]) and fractional shortening (FS) were measured. Left ventricular mass (LV mass) was computed according to Devereux et al.[12] Arterial stiffness was assessed by carotid-femoral pulse wave velocity (PWV) using an automatic non-invasive, validated device (Complior®; Artech Medical, Pantin, France). The distance between the recording sites at the carotid (proximal) and femoral (distal) artery was measured.

Maternal anthropometrics

During pregnancy maternal height (cm) and weight (kg) were measured and body mass index (BMI) (kg/m^2) was calculated. Identical measurements were obtained 6 years after index pregnancy. Pre-pregnancy BMI was established at enrolment through a questionnaire. Pre-pregnancy weight highly correlated with the measured early pregnancy weight.

Covariates

Information on maternal age, educational level, ethnicity, gravidity, self-reported pre-pregnancy weight, folic acid supplementation, smoking, pre-pregnancy history of chronic hypertension was available from questionnaires administered during index pregnancy. Information about gestational age at birth, birth weight and placental weight was obtained from medical records. Six years after index pregnancy we obtained information on subsequent pregnancies in between the index pregnancy and follow-up, anti-hypertensive medication use and educational level through questionnaires. Regarding anti-hypertensive medication use at follow-up, information on ATC-codes was not available.

Statistical analysis

First, we performed a non-response analysis (**Supplemental table 1**). Second, missing values were imputed using the multiple imputations procedure with five imputations and these datasets were analysed together. Third, differences in maternal characteristics were compared between women with hypertensive pregnancy disorders and those with normotensive pregnancies using Student's t-test , Mann-Whitney U test and Chi-square test. Fourth, we used regression models to explore the combined effects of maternal BP in early and late pregnancy on vascular and cardiac outcomes, and chronic hypertension. In these analyses we divided maternal BP in early and late pregnancy into equal tertiles. We also performed conditional regression analyses to identify the independent associations of early, mid- and late pregnancy maternal BP, taking into account for their correlations, with vascular and cardiac outcomes and chronic hypertension. We constructed new systolic and diastolic BP variables, which are statistically independent from each other, by using standardised residuals obtained from linear regression models of maternal systolic and diastolic BP regressed on prior corresponding BP measurements (see for more details **Supplemental Information**).[13]

Fifth, using linear regression models, associations between women with GH or PE and women with a normotensive pregnancy and vascular and cardiac outcomes were assessed. These included; (1) basic model, adjusted for maternal age and visit interval; (2) confounder model, which in addition to model (1) included ethnicity, educational level, smoking, gravidity at follow-up, child's sex; (3) BMI model, which included BMI at follow-up in addition to model (2). In the BMI we observed whether changes in the effect estimates occurred after additional adjustment for BMI at follow-up. The difference between the effect estimates from model (2) and the effect estimates after adjustment for BMI was expressed as percentage change. The percentage change was calculated by the formula: 100 x (effect estimate$_{BMI}$ - effect estimate$_{confounder}$)/ (effect estimate$_{confounder}$ - 1). A 95% confidence interval (CI) for the percentage change of the effect estimate was calculated using a bootstrap method with 1000 resampling's.[14, 15] Using a similar approach multiple logistic regression models were used to examine the associations between hypertensive pregnancy disorders and normotensive pregnancies, and chronic hypertension at follow-up. Lastly, we carried out a sensitivity analysis by repeating the logistic

regression analysis and defining chronic hypertension only on the basis of anti-hypertensive medication use at follow-up. Statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA) and with R version 3.0.0 (libraries rmeta and metafor; The R foundation for Statistical Computing).

RESULTS

Table 1 shows maternal characteristics during index pregnancy and at follow-up. Women with a hypertensive pregnancy disorder had a higher BMI before and after the index pregnancy. They were also more often pregnant with their first child during the index pregnancy.

Table 1 Baseline characteristics by hypertensive pregnancy disorder (n = 4912).

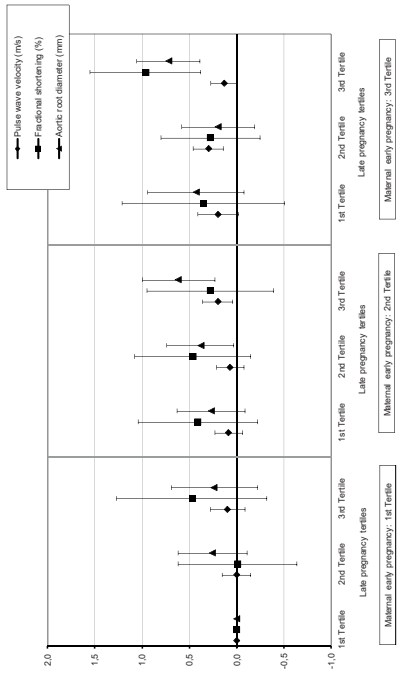
	Normotensive pregnancies	**GH**	**PE**
	n = 4612	n = 205	n = 95
Maternal characteristics (pregnancy)			
Age at intake (years), mean (SD)	30.3 (5.1)	30.7 (4.9)	29.6 (5.3)
Height (cm), mean (SD)	167 (7.5)	168 (7.3)[1]	166 (7.5)[3]
Pre-pregnancy BMI (kg/m^2), median (90% range)	22.7 (18.8, 31.6)	25.2 (19.9, 38.1)[1]	24.2 (19.2-38.9)[2]
Education, Higher (%)	42.5	44.9	32.6[2]
Ethnicity, European (%)	58.3	74.6[1]	55.8[3]
Gravidity at intake, Primigravida (%)	46.5	64.4[1]	69.5[2]
Smoking during pregnancy (%)	23.7	28.3	20.0[3]
Early pregnancy blood pressure (mmHg)			
Systolic (mmHg), mean (SD)	115 (12)	125 (13)[1]	121 (14)[2,3]
Diastolic (mmHg), mean (SD)	68 (9)	76 (11)[1]	74 (10)[2]
Mid-pregnancy blood pressure (mmHg)			
Systolic (mmHg), mean (SD)	116 (12)	127 (13)[1]	122 (14)[2,3]
Diastolic (mmHg), mean (SD)	67 (9)	76 (10)[1]	75 (90)[2]
Late pregnancy blood pressure (mmHg)			
Systolic (mmHg), mean (SD)	118 (12)	130 (13)[1]	128 (12)[2]
Diastolic (mmHg), mean (SD)	69 (9)	79 (9)[1]	79 (10)[2]
Birth characteristics			
Gestational age birth (weeks), median (90% range)	40.1 (37.1, 42.1)	40.0 (37.1, 42.0)	38.3 (31.2, 41.1)[2,3]
Birth weight (g), mean (SD)	3438.6 (532.3)	3375.4 (593.6)	2823.2 (833.2)[2,3]
Male sex (%)	50.2	47.8	45.3
Maternal characteristics (follow-up)			
No subsequent pregnancies (%)	6.9	12.2[1]	16.8[2]
BMI (kg/m^2), median (90% range)	24.6 (19.7, 35.2)	27.6 (21.2, 43.4)[1]	27.5 (20.0, 43.8)[2]

Table 1 Baseline characteristics by hypertensive pregnancy disorder (n = 4912) (continued)

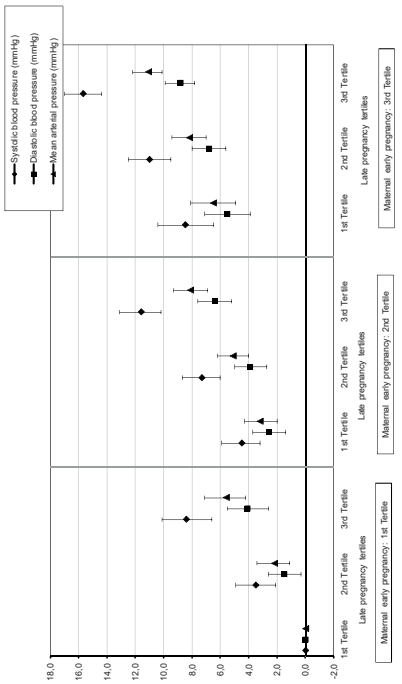
	Normotensive pregnancies	**GH**	**PE**
	n = 4612	n = 205	n = 95
Systolic blood pressure (mmHg), mean (SD)	119 (12)	130 (18)[1]	126 (15)[2]
Diastolic blood pressure (mmHg), mean (SD)	70 (10)	79 (13)[1]	78 (12)[2]
Mean arterial pressure (mmHg), median (90% range)	85 (73, 104)	94 (78, 125)[1]	92 (74, 118)[2]
Pulse wave velocity (m/s), mean (SD)	7.6 (1.1)	7.8 (1.2)[1]	7.6 (1.1)
Fractional shortening (%), mean (SD)	36.9 (4.9)	37.5 (4.7)	37.3 (5.4)
Aortic root diameter (mm), mean (SD)	27.7 (2.8)	28.7 (2.9)[1]	27.9 (3.0)[3]
Left ventricular mass (g), mean (SD)	130.0 (30.9)	143.1 (34.6)[1]	133.0 (33.3)[3]
End diastolic left ventricular diameter (mm), mean (SD)	48.3 (4.0)	49.5 (4.1)[1]	48.7 (4.4)[3]
End diastolic left ventricular posterior wall thickness (mm), mean (SD)	8.0 (1.0)	8.4 (1.4)[1]	8.1 (1.4)
End diastolic interventricular septum thickness (mm), mean (SD)	8.1 (1.3)	8.4 (1.4)[1]	8.0 (1.4)
Anti-hypertensive medication (%)	1.2	6.3[1]	6.3[2]
Hypertension (%)[†]	4.9	23.4[1]	17.9[2]

Abbreviations: Body mass index, BMI; Gestational hypertension, GH; Preeclampsia, PE.
Measurements were performed in early pregnancy (median 13.2 weeks gestation, 90% range [10.6-16.9]), mid-pregnancy (median 20.5 weeks gestation, 90% range [19.1-22.4]) and late pregnancy (median 30.2 weeks gestation, 90% range [29.1-31.9]) and six years after delivery (90% range 5.7-7.2 years).
Differences in subject characteristics between groups were assessed using Student's t-test for continuous variables with a normal distribution and Mann-Whitney U-test for continuous variables with a skewed distribution. Chi-square test were used for categorical variables.
† Defined as women using anti-hypertensive medication and/or having, in two subsequent readings, a systolic or diastolic blood pressure above 140 mmHg or 90 mmHg, respectively.
[1] Normotensive pregnancies versus GH with *P*-value < 0.05.
[2] Normotensive pregnancies versus PE with *P*-value < 0.05.
[3] GH versus PE with *P*-value < 0.05.

In **Figure 1** we presented the combined associations of maternal systolic and diastolic blood BP with cardiovascular outcomes and the risk of hypertension 6 years after pregnancy. As compared to women with a systolic or diastolic BP in the lowest tertiles during early and late pregnancy, those with a BP in the highest tertiles in early and late pregnancy had a higher systolic and diastolic BP, a higher MAP, a higher FS, a higher AOD, a higher LV mass and a higher risk of chronic hypertension 6 years after index pregnancy (all *P*-values <0.05 in confounder model).
Additionally, in the conditional analysis, which are presented in **Supplemental figure 2 and 3,** the independent associations of early, mid- and late pregnancy systolic and diastolic BP with cardiovascular outcomes and hypertension 6 years after pregnancy are shown. Early pregnancy systolic and diastolic BP were associated with BP, MAP, PWV, FS, AOD, LV mass and chronic hypertension 6 years after index pregnancy (all *P*-values <0.05 in confounder model).


A
Systolic blood pressure (mmHg)
Diastolic blood pressure (mmHg)
Mean arterial pressure (mmHg)
Difference in blood pressure at follow-up (beta) (95% CI)
Late pregnancy tertiles
Maternal early pregnancy: 1st Tertile
Maternal early pregnancy: 2nd Tertile
Maternal early pregnancy: 3rd Tertile

B
Pulse wave velocity (m/s)
Fractional shortening (%)
Aortic root diameter (mm)
Difference in cardio-vascular outcomes (beta) (95% CI)
Late pregnancy tertiles
Maternal early pregnancy: 1st Tertile
Maternal early pregnancy: 2nd Tertile
Maternal early pregnancy: 3rd Tertile


C
Left ventricular mass (g)
Difference in Left ventricular mass (beta) (95% CI)
Late pregnancy tertiles
Maternal early pregnancy: 1st Tertile
Maternal early pregnancy: 2nd Tertile
Maternal early pregnancy: 3rd Tertile

D
Hypertension (OR) (95% CI)
Late pregnancy tertiles
Maternal early pregnancy: 1st Tertile
Maternal early pregnancy: 2nd Tertile
Maternal early pregnancy: 3rd Tertile

Figure 1 Combined associations of maternal early and late pregnancy blood pressure measures with cardiovascular outcomes (A-C) and the risk of hypertension (D) six years after pregnancy (n =3551).
Abbreviation: Confidence interval, CI.
Effect estimates or odds ratios (95% Confidence Interval) are from multivariable linear or logistic regression models, respectively. Results are from multiple imputed data. Women using anti-hypertensive medication at follow-up are excluded from regression analysis with cardiovascular outcomes (**A-C**) (n = 52). Hypertension (**D**) is defined as women using anti-hypertensive medication at follow-up and/or having, in two subsequent blood pressure readings, a systolic or diastolic blood pressure above 140 mmHg or 90 mmHg, respectively.
Values are adjusted for maternal age at intake, visit interval, ethnicity, educational level, smoking, subsequent pregnancies between index and follow-up, and child's sex.

The associations of early pregnancy BP appeared more strongly related to cardiovascular outcomes at follow-up than associations of mid- and late pregnancy BP.

The associations between hypertensive pregnancy disorders and vascular, cardiac outcomes and the risk of chronic hypertension at follow-up are presented in **Table 2 and 3**. Compared to women with a previous normotensive pregnancy, GH was strongly associated with both vascular and cardiac outcomes and the risk of chronic hypertension. Women with a history of GH had a 10.8 mmHg higher systolic BP (95% CI 9.1-12.6), a 8.7 mmHg higher diastolic BP (95% CI 7.3-10.1), 0.22m/s higher PWV (95% CI 0.03-0.40), a 0.90 mm larger AOD (95% CI 0.49-1.32) and a 12.03 grams larger LV mass (95% CI 7.36-16.71) at follow-up. These women also had a six-fold higher risk to develop chronic hypertension 6 years after index pregnancy (OR 6.6, 95% CI 4.6-9.5). Additional adjustment for BMI at follow-up showed a large attenuation of the effect estimates, especially in AOD and LV mass (total percentage change 54.9 and 65.18, respectively). However, these effect estimates remained significant. Sensitivity analyses, in which chronic hypertension was defined only on the basis of anti-hypertensive medication use at follow up, showed similar results (**Supplemental table 2**). Compared to women with a previous normotensive pregnancy, women with a history of PE had higher systolic and diastolic BP at follow-up (6.3 mmHg; 95% CI, 3.9-8.8, and 6.8 mmHg; 95% CI 4.8-8.8, respectively). A history of PE was associated with a higher risk of chronic hypertension at follow-up (OR 4.5, 95% CI 2.6-7.8). Additional adjustment for BMI at follow-up showed that these associations attenuated by approximately 25%. Sensitivity analyses, in which chronic hypertension was defined only on the basis of anti-hypertensive medication use at follow up, showed similar results (**Supplemental table 2**). Cardiac outcomes were not associated with PE.

DISCUSSION

Women with a history of hypertensive pregnancy disorders exhibit a more unfavourable cardiovascular health after pregnancy compared to women with a previous normotensive pregnancy. Already 6 years after index pregnancy the prevalence of chronic hypertension shows an

Table 2 Associations of hypertensive pregnancy disorders with cardiovascular outcomes measured six years after pregnancy (n = 4837).

Outcome	Normotensive pregnancy	GH		PE	
	n = 4556	n = 192		n = 89	
		Beta (95% CI)	% Change (95% CI)	Beta (95% CI)	% Change (95% CI)
Systolic blood pressure (mmHg)					
Basic model	*Reference*	10.9 (9.1, 12.7)**		6.4 (3.9, 8.9)**	
Confounder model	*Reference*	10.8 (9.1, 12.6)**		6.3 (3.9, 8.8)**	
BMI model	*Reference*	8.6 (6.8, 10.3)**	-21.22 (-24.57, -18.29)**	4.4 (2.0, 6.8)**	-28.90 (-38.89, -21.30)**
Diastolic blood pressure (mmHg)					
Basic model	*Reference*	8.4 (7.0, 9.8)**		6.9 (4.9, 8.9)**	
Confounder model	*Reference*	8.7 (7.3, 10.1)**		6.8 (4.8, 8.8)**	
BMI model	*Reference*	6.7 (5.4, 8.1)**	-22.48 (-25.40, -19.44)**	5.1 (3.3, 7.0)**‡	-23.08 (-29.78, -17.59)**
Mean arterial pressure (mmHg)					
Basic model	*Reference*	9.2 (7.8, 10.6)**		6.7 (4.7, 8.7)**	
Confounder model	*Reference*	9.4 (8.0, 10.8)**		6.6 (4.6, 8.6)**	
BMI model	*Reference*	7.3 (6.0, 8.7)**	-21.99 (-24.96, -19.18)**	4.9 (3.0, 6.8)**	-24.94 (-31.88, -18.97)**
Pulse wave velocity (m/s)					
Basic model	*Reference*	0.20 (0.01, 0.39)*		0.04 (-0.22, 0.30)	
Confounder model	*Reference*	0.22 (0.03, 0.40)*		0.04 (-0.23, 0.30)	
BMI model	*Reference*	0.20 (0.01, 0.38)*	-7.98 (-19.19, -1.04)**	0.02 (-0.24, 0.29)	NA
Fractional shortening (%)					
Basic model	*Reference*	0.36 (-0.38, 1.09)		0.22 (-0.85, 1.28)	
Confounder model	*Reference*	0.37 (-0.37, 1.11)		0.19 (-0.87, 1.26)	
BMI model	*Reference*	0.25 (-0.49, 0.99)	NA	0.07 (-1.00, 1.14)	NA

Table 2 Associations of hypertensive pregnancy disorders with cardiovascular outcomes measured six years after pregnancy (n = 4837). (continued)

Outcome	Normotensive pregnancy	GH		PE	
	n = 4556	n = 192		n = 89	
		Beta (95% CI)	% Change (95% CI)	Beta (95% CI)	% Change (95% CI)
Aortic root diameter (mm)					
Basic model	*Reference*	1.01 (0.59, 1.43)**		0.24 (-0.37, 0.85)	
Confounder model	*Reference*	0.90 (0.49, 1.32)**		0.26 (-0.34, 0.87)	
BMI model	*Reference*	0.46 (0.05, 0.87)*	-54.9 (-70.75, -44.29)**	-0.10 (-0.69, 0.50)	NA
Left ventricular mass (g)					
Basic model	*Reference*	13.29 (8.59, 17.98)**		3.46 (-3.39, 10.31)	
Confounder model	*Reference*	12.03 (7.36, 16.71)‡		3.93 (-2.89, 10.75)	
BMI model	*Reference*	4.77 (0.45, 9.10)*	-65.18 (-78.08, -55.65)‡	-3.15 (-9.43, 3.13)	NA

Abbreviations: Gestational hypertension, GH; Preeclampsia, PE; Body mass index, BMI; Confidence interval, CI; Not applicable, NA.

Values are regression coefficients or % change (95% CI) and are based on linear regression models. % change represents the change in effect estimates after adjustment for BMI as potential mediator with corresponding 95% CI. Estimates are from multiple imputed data. Women using anti-hypertensive medication at follow-up were excluded from these analyses (n = 75).

Basic model: Adjusted for maternal age at intake and visit interval.

Confounder model: Basic model and additionally adjusted for ethnicity, educational level, smoking, subsequent pregnancies between index and follow-up, and child's sex.

BMI model: Confounder model and additionally adjusted for BMI at follow-up.

* *P*-value < 0.05

** *P*-value < 0.01

Table 3 Associations of hypertensive pregnancy disorders with the risk of hypertension six years after pregnancy (n = 4912).

Outcome			**Normotensive pregnancy**			**GH**				**PE**	
			n = 4612			n = 205				n = 95	
	n	%		n	%	Odds ratio (95% CI)	% Change (95% CI)	n	%	Odds ratio (95% CI)	% Change (95% CI)
Hypertension	228	4.9		48	23.4			17	17.9		
Basic model			*Reference*			5.8 (4.1-8.3)*				4.4 (2.6-7.6)*	
Confounder model			*Reference*			6.6 (4.6-9.5)*				4.5 (2.6-7.8)*	
BMI model			*Reference*			4.7 (3.2-6.9)*	-34.15 (-38.57, -29.83)*			3.5 (1.9-6.2)*	-28.66 (-37.18, -20.48)*

Abbreviations: Gestational hypertension, GH; Preeclampsia, PE; Confidence interval, CI, Body mass index, BMI.

Values are odds ratios or % change in odds ratio (95% CI) and are based on logistic regression models. % change represents the changes in odds ratio after adjustment for BMI at follow-up as potential mediator with corresponding 95% CI. Estimates are from multiple imputed data. Hypertension is defined as women using anti-hypertensive medication at follow-up and/or having, in two subsequent readings, a systolic or diastolic blood pressure above 140 mmHg or 90 mmHg, respectively.

Basic model: Adjusted for maternal age at intake and visit interval.

Confounder model: Basic model and additionally adjusted for ethnicity, educational level, smoking, subsequent pregnancies between index and follow-up, and child's sex.

BMI model: Confounder model and additionally adjusted for BMI at follow-up.

* *P*-value < 0.01

increased risk in both women with a history of GH or PE. The results further demonstrate that especially early pregnancy BP is strongly associated with the diagnosis chronic hypertension 6 years after pregnancy. Even though these results are strongly influenced by BMI at follow-up, effect estimates remained significant.

Methodological considerations

Strengths are the prospective data collection and large sample size. Complete information on pregnancies and pregnancy disorders that occurred in the years after the index pregnancy was not available. Instead we used gravidity at follow-up to account for pregnancies in between the index pregnancy and the follow-up measurement after 6 years. BP at study intake during pregnancy was higher in women with a history of GH than in women remaining normotensive throughout pregnancy. Pregnancy is associated with a physiologic decrease of BP which might suggest that non-random misclassification of the diagnosis chronic hypertension occurred in our study. Nevertheless, we think this is unlikely because information on chronic hypertension was cross-checked between multiple sources (maternal questionnaire in pregnancy and information from the original medical records and the Dutch obstetric database).

Finally, we are aware of the fact that our approach to define chronic hypertension at follow up might be suboptimal as 1) we defined chronic hypertension based upon BP measurements at one point in time at the 6 years follow-up visit (i.e. transient hypertension) and 2) international guidelines recommend that ambulatory BP monitoring should be used to define or confirm clinical diagnosis of hypertension because of prevalence of white coat hypertension. Transient hypertension is an established risk factor (i.e. early form) of chronic hypertension.[16, 17]

Unfortunately, ambulatory BP monitoring was not available. Besides, BP was measured in a research setting by trained research assistants wearing normal clothing (i.e. no white coats). We also performed a sensitivity analyses in which we labelled women as chronic hypertensive, only if they used BP medication at follow up. These analyses showed similar results (**Supplemental table 2**).

Interpretation

Studies show that hypertensive pregnancy disorders are associated with a woman's risk of CVD.[2] This provides opportunities to identify women at risk early in their lives when it may be possible to alter their risk trajectory. It has been shown that measures of arterial stiffness and left ventricular function are increased during pregnancy among women with hypertensive pregnancy disorders.[18, 19] Arterial stiffness and left ventricular function are thought to be independent predictors of chronic hypertension and CVD. Also aortic root dilatation may be secondary to hypertension.[18, 20, 21] Franz et al. showed differences in PWV after index pregnancy among former early, but not late, onset PE women.[22] Likewise, Ghossein-Doha et al.[23] showed increased left ventricular mass indices and decreased cardiac diastolic function among a population of mainly severe (early onset) PE postpartum as proxy for hypertension.

We observed increased PWV 6 years after index pregnancy among women with a history of GH. Similarly, women with a history of GH had an increased LV mass, a larger AOD, a higher BP and a higher risk of chronic hypertension. Interestingly, no differences were seen when comparing these cardiovascular measurements between women with PE and normotensive women, with the exception of an increased risk of chronic hypertension. Regrettably, GH has only rarely been included in postpartum research and despite evidence that differences in CVD risk exist between women with severe early-onset PE, mild late-onset PE and GH, most studies do not differentiate between the subgroups of hypertensive pregnancy disorders. One large longitudinal study which differentiated between (mild) PE and GH found that both GH and PE were associated with greater CVD risk factors. However after controlling for various confounders, results for PE were not significant anymore.[24] In a study by Wikström et al.[25] the risk of developing ischaemic heart disease was higher in women with severe PE compared with GH and mild PE.[25, 26] This was similar to results reported by Lykke et al.[27] Women with mild PE had a four-fold higher risk of chronic hypertension. However, this risk increased up to six-fold in women with a history of GH and severe PE, respectively.[25-27] Similar patterns in mean BP, usage of anti-hypertensive medication and chronic hypertension were reported by Verbeek et al.[26] with the highest incidence of chronic hypertension in severe PE followed by GH and finally mild PE. In our study the majority of preeclamptic women had mild PE (92%) with only eight women suffering from severe PE. These results strengthen our findings on GH showing that not only severe PE women exhibit an increased risk of CVD but also that women with GH are at increased of an adverse cardiovascular health profile after pregnancy. However, the results could also indicate that, for women with GH, this phenotype already existed prior to the index pregnancy. Women with GH should in either case not be excluded from (secondary) preventive interventions. A meta-analysis found that lifestyle interventions may alter cardiovascular risk after a history of PE up to 13% with relatively simple intervention measures.[28] These may include exercise, dietary counselling and support for smoking cessation assistance.

Causal pathways relating hypertensive pregnancy disorders to chronic hypertension and CVD are unclear. One hypothesis focuses on common risk factors including among others obesity, chronic hypertension and genetic constitution.[29] Both PE and atherosclerosis arise from vascular inflammation with endothelial dysfunction. It has been also been hypothesised that hypertensive pregnancy disorders worsen pre-existing subclinical CVD risk factors already present before index pregnancy or even induce de novo risk.[29] A large population-based study showed that most CVD risk factors remain higher after PE following adjustment for pre-pregnancy values.[30] It is possible that products of the dysfunctional placenta in PE could permanently compromise maternal cardiovasculature.[28] A study demonstrated that increased sensitivity to infused Angiotensin II exists in the postpartum state in women with a history of new-onset hypertension in pregnancy and that this increased sensitivity to Angiotensin II is present in the vasculature and in the adrenal glands, with a suggestion of sFlt-1 responsiveness.[31] This

study also showed that women with a history of new-onset hypertension in pregnancy were unable to modulate a response to infused Angiotensin II on the basis of salt intake. They suggested a dysregulation of the renin-angiotensin system.[31] Another study suggested persistence of left ventricular geometrical changes that herald the development of chronic hypertension.[23] In our study we observed significant associations regarding early pregnancy BP and all cardiac and vascular outcomes and the risk of chronic hypertension. Also the combined associations of maternal BP during early and late pregnancy were consistently associated with vascular, cardiac and hypertensive outcomes at follow-up. These findings may corroborate those of prior research supporting the theory that hypertensive pregnancy disorders share pathophysiology already programmed before the challenge of pregnancy that ultimately leads to CVD. However, they also cannot reject the hypothesis that hypertensive pregnancy disorders may cause permanent vascular damage thereby contributing to CVD risk.

CONCLUSIONS

Hypertensive pregnancy disorders are associated with an adverse cardiovascular health profile and an increased risk of chronic hypertension 6 years after the index pregnancy. It is important to assess both GH and PE when assessing chronic hypertension and CVD risks. Women with GH and PE may be offered long-term cardiovascular follow-up incorporated in CVD risk management guidelines. BP profiles measured from early pregnancy onwards might help to further distinguish women at risk of future chronic hypertension and CVD.

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SUPPLEMENTAL MATERIAL

Supplemental Information

Conditional regression models

Conditional regression models were used to examine the independent associations of maternal blood pressure (BP) development in each period of pregnancy with BP, cardiovascular outcomes and the risk of hypertension six years after index pregnancy, taking into account the correlation between maternal BP in early, mid- and late pregnancy.[1] For these analyses, we constructed new systolic and diastolic BP variables, which are statistically independent from each other, by using standardized residuals obtained from linear regression models of maternal systolic and diastolic BP regressed on all prior corresponding BP measurements: systolic BP is used as an example.[1] Systolic BP in early pregnancy is the starting point. Conditional change in systolic BP from early to mid-pregnancy is equivalent to the standardized residuals resulting from the linear regression model of mid-pregnancy BP on early pregnancy BP. Accordingly, the conditional change in mid-pregnancy BP to late pregnancy BP is given as the standardized residuals obtained from regressing late pregnancy BP on both mid-pregnancy BP and early pregnancy BP simultaneously.[2] By this approach, strong correlations are removed between systolic or diastolic BP measures in different periods in pregnancy which allows simultaneous inclusion of these related variables into one regression model. Subsequently, the influence of systolic or diastolic BP in specific periods in pregnancy can then be assessed in comparison with, and adjusted for, systolic or diastolic BP in the other pregnancy intervals.

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Supplemental table 1 Non-response analysis for maternal follow-up data six years after pregnancy.

	Follow-up at six years	**Loss to follow-up at six years**	***P*-value**
	n = 5439	n = 2759	
Maternal characteristics			
Age at intake (years), mean (SD)	30.2 (5.1)	28.2 (5.5)	<0.001
Gestational age at intake (weeks), median (90% range)	13.9 (10.8, 22.6)	14.5 (10.8, 23.8)	<0.001
Height (cm), mean (SD)	166.6 (7.5)	166.1 (7.3)	0.01
Pre-pregnancy weight (kg), mean (SD)	66.5 (13.3)	65.6 (13.3)	0.01
Weight at intake (kg), mean (SD)	69.6 (13.1)	69.3 (14.0)	0.43
Pre-pregnancy BMI (kg/m^2), median (90% range)	22.9 (18.9, 32.6)	22.6 (18.2, 33.1)	0.09
BMI at intake (kg/m^2), median (90% range)	24.1 (19.6, 33.7)	24.1 (19.1, 34.4)	0.59
Systolic blood pressure at intake (mmHg), mean (SD)	115.7 (12.3)	114.7 (12.4)	0.001
Diastolic blood pressure at intake (mmHg), mean (SD)	68.2 (9.5)	67.5 (9.8)	0.002
Gravidity, n (%)†			
1	263 (48.0)	1165 (42.2)	<0.001
≥2	2792 (51.3)	1543 (55.9)	
Educational level, n (%)†			
None/Primary	500 (9.8)	386 (16.5)	<0.001
Secondary	2301 (45.3)	1224 (52.4)	
Higher	2279 (44.9)	728 (31.1)	
Ethnicity, n (%)†			
Dutch/European	3172 (59.7)	1142 (47.3)	<0.001
Non-European	2144 (40.3)	1271 (52.7)	
Smoking, n (%)†			
No	3563 (73.4)	1612 (69.2)	<0.001
Yes	1291 (26.6)	716 (30.8)	
Pregnancy complications			
Gestational hypertension, n (%)†	226 (4.4)	79 (3.1)	0.01
Preeclampsia, n (%)†	106 (2.1)	73 (2.9)	0.04
Birth and infant characteristics			
Gestational age (weeks), median (90% range)	40.1 (36.9, 42.0)	40.0 (36.1, 42.1)	<0.001
Birth weight (g), mean (SD)	3410.4 (553.9)	3346.1 (599.3)	<0.001
Male sex, n (%)†	2714 (49.9)	1424 (51.7)	0.14

Abbreviation: Body mass index, BMI.

Differences in subject characteristics between groups were assessed using Student's t-test for continuous variables with a normal distribution and Mann-Whitney U-test for continuous variables with a skewed distribution. Chi-square test were used for categorical variables.

† Values are observed numbers and valid percentages.

Supplemental table 2 Associations of hypertensive pregnancy disorders with the risk of hypertension six years after pregnancy (n = 4912).

Outcome	**Normotensive pregnancies**	**GH**		**PE**	
	n =4612	n = 205		n = 95	
		Odds ratio (95% CI)[†]	Odds ratio (95% CI)[‡]	Odds ratio (95% CI)[†]	Odds ratio (95% CI)[‡]
Hypertension					
Basic model	*Reference*	5.8 (4.1-8.3)**	5.4 (2.9-10.0)**	4.4 (2.6-7.6)**	5.6 (2.4-13.5)**
Confounder model	*Reference*	6.6 (4.6-9.5)**	7.2 (3.7-13.8)**	4.5 (2.6-7.8)**	5.8 (2.4-14.3)**
BMI model	*Reference*	4.7 (3.2-6.9)**	5.5 (2.8-11.0)**	3.5 (1.9-6.2)**	4.9 (2.0-12.3)**

Abbreviations: Gestational hypertension, GH; Preeclampsia, PE; Confidence interval, CI, Body mass index, BMI.

Values are odds ratios and are based on logistic regression models. Estimates are from multiple imputed data.

Basic model: Adjusted for maternal age at intake and visit interval.

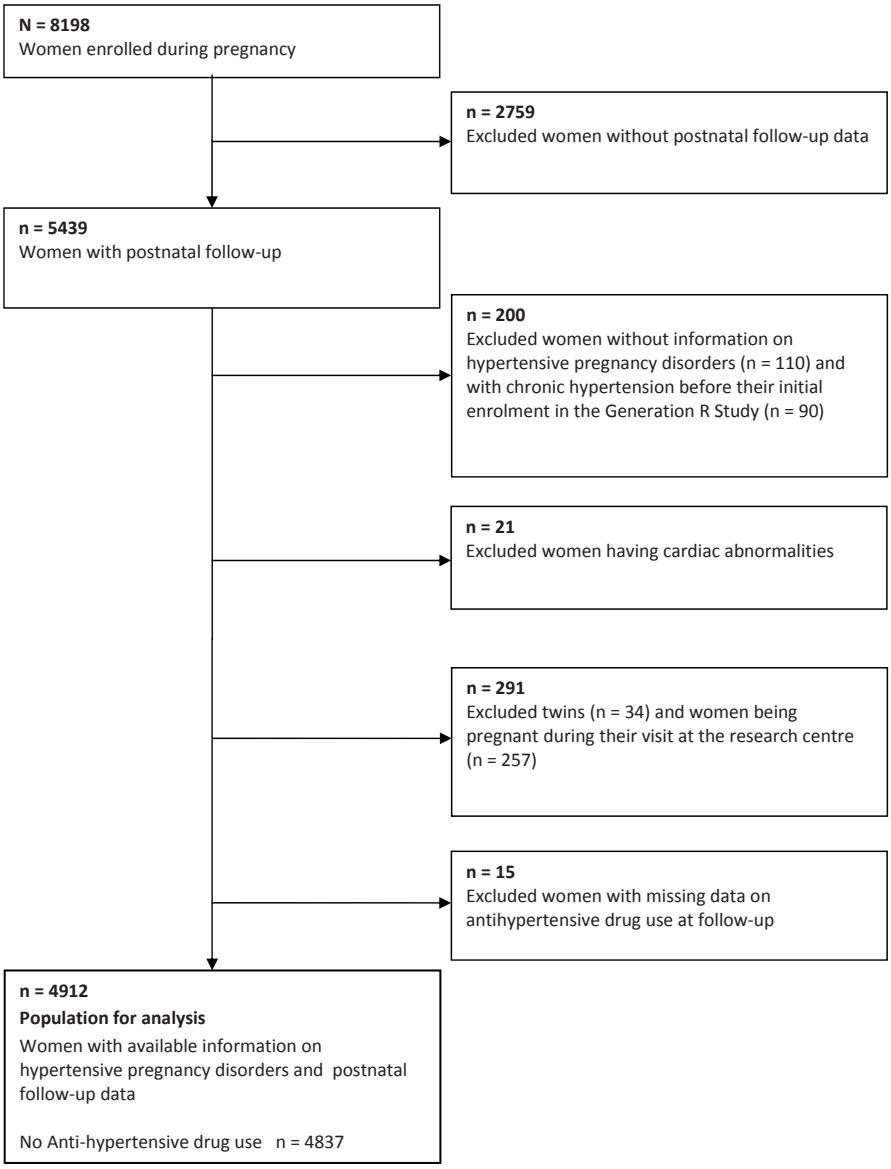
Confounder model: basic model and additionally adjusted for ethnicity, educational level, smoking, subsequent pregnancies between index and follow-up, and child's sex.

BMI model: confounder model and additionally adjusted for BMI at follow-up.

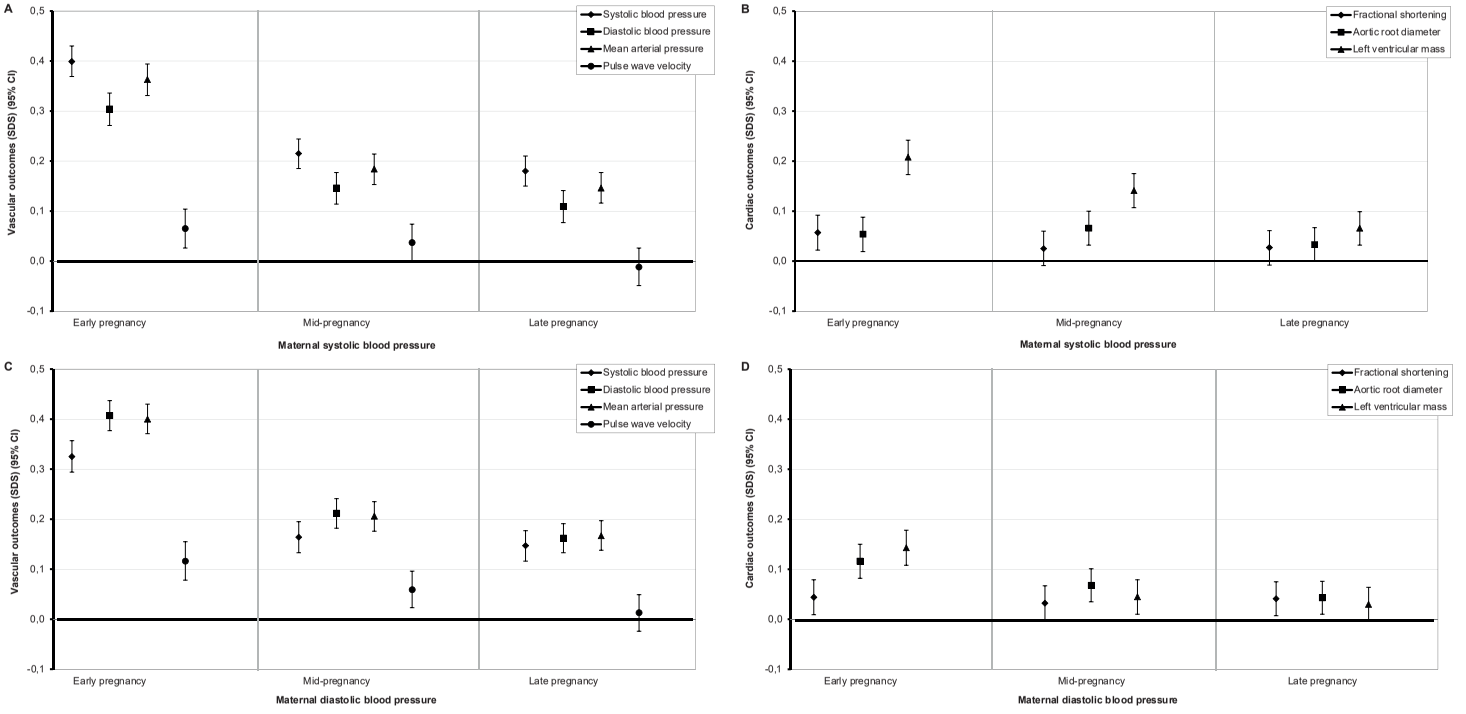
† Hypertension defined as women using anti-hypertensive medication and/or having, in two subsequent readings, a systolic or diastolic blood pressure above 140 mmHg or 90 mmHg, respectively.

‡ Hypertension defined as women using anti-hypertensive medication.

** *P*-value < 0.01



Supplemental figure 1 Flowchart

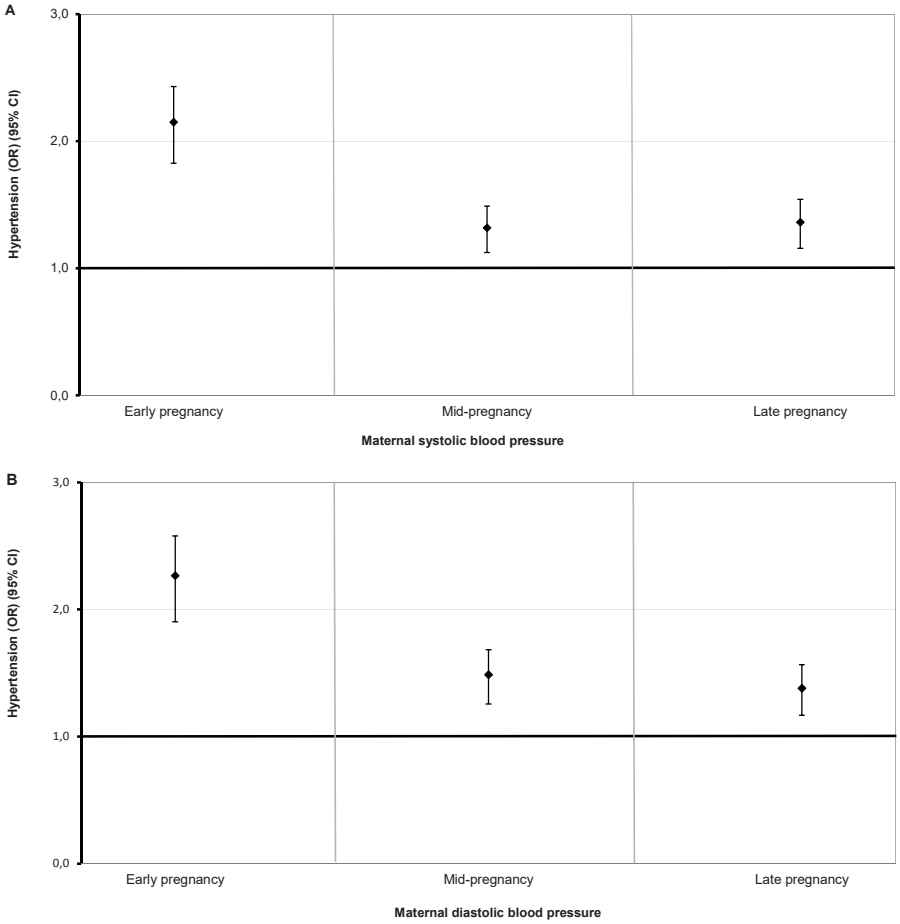


Supplemental figure 2 Associations of systolic (A,B) and diastolic (C,D) blood pressure measures in pregnancy with cardiovascular outcomes six years after pregnancy from conditional analyses (n = 3551).

Abbreviations: Standard deviation score, SDS; Cofidence interval, CI.

Values are regression coefficients (95% CI) from multivariable linear regression models and reflect the difference in cardiovascular outcomes per SDS change in early pregnancy systolic and diastolic blood pressure and per SDS change in standardised residual change in systolic and diastolic blood pressure in mid- and late pregnancy from conditional regression analyses (see for details of conditional regression models **Supplemental Information**). Women using anti-hypertensive medication are excluded from these analysis (n = 52).

Values are adjusted for maternal age, visit interval, ethnicity, educational level, smoking, subsequent pregnancies between index and follow-up and child's sex.



Supplemental figure 3 Associations of systolic (A) and diastolic (B) blood pressure measures in pregnancy with hypertension six years after pregnancy from conditional analyses (n = 3551).
Abbreviations: Odds ratio, OR; Confidence interval, CI.
Values are regression coefficients (95% CI) from multivariable logistic regression models and reflect the difference in the risk of hypertension at follow-up per SDS change in early pregnancy systolic and diastolic blood pressure and per SDS change in standardised residual change in systolic and diastolic blood pressure in mid- and late pregnancy from conditional regression models (see for details of conditional regression models **Supplemental Information**).
Values are adjusted for maternal age, visit interval, ethnicity, educational level, smoking, subsequent pregnancies between index and follow-up and child's sex.

Chapter 4

Maternal lipid profile six years after a gestational hypertensive disorder

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ABSTRACT

Objective: To assess if women with a previous gestational hypertensive disorder (GHD), including gestational hypertension and preeclampsia, have a more atherogenic lipid profile six years after pregnancy compared to women with a previous normotensive pregnancy.

Methods: In a population-based prospective cohort study, we included 4933 women during pregnancy, including 302 women with a GHD. Six years after pregnancy, we determined maternal lipid profile (total-cholesterol, triglycerides, HDL-c, LDL-c, lipoprotein[a] and apolipoprotein B) and glucose levels.

Results: Women with a previous GHD had a more atherogenic lipid profile six years after pregnancy compared to women with a previous normotensive pregnancy. These atherogenic lipid profiles were a result of higher levels of triglycerides, LDL-c and apolipoprotein B and lower levels of HDL-c. Differences in lipid profile between women with a previous GHD and women with a previous normotensive pregnancy were attenuated after adjustment for pre-pregnancy body mass index (BMI). Between women from both groups, no differences were observed in total-cholesterol, lipoprotein[a] and glucose levels.

Conclusions: Women with a previous GHD show a more atherogenic lipid profile six years after pregnancy than women with a previous normotensive pregnancy. The increased risk of cardiovascular disease after a GHD might result from an atherogenic lipid profile after pregnancy, primarily driven by pre-pregnancy BMI.

INTRODUCTION

An atherogenic lipid profile, consisting of high levels of total-cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-c), lipoprotein(a) (Lp[a]) or apolipoprotein B (apoB), or low levels of high-density lipoprotein cholesterol (HDL-c), increases the risk of future CVD, stroke and transient ischemic attack.[1, 2] High levels of these individual lipids can stimulate lipid absorption by macrophages in the arterial vessel wall. This process will induce local vascular inflammation and the formation of atherosclerotic plaques.[3] Women with previous gestational hypertensive disorders (GHD) such as gestational hypertension (GH) and pre-eclampsia (PE) are also more likely to develop cardiovascular disease (CVD) in later life.[4-7] Both conditions are characterized by hypertension in pregnancy but only PE is a placenta mediated syndrome with abnormal placentation leading to proteinuria, systemic inflammation and organ dysfunction.[6, 8] Multiple factors may contribute to the process of CVD after GH and PE, such as accelerative development of classical cardiovascular risk factors after pregnancy.[9-13] Some of these cardiovascular risk factors, such as weight and blood pressure after pregnancy, are consistently higher in women with a previous GHD compared to women with a previous normotensive pregnancy.[10, 14-16] Other cardiovascular risk factors, such as insulin resistance, visceral adiposity and the metabolic syndrome, are also more prevalent in these women.[10, 15, 17, 18] As a result, women with a previous GHD seem to be more susceptible to exhibit an atherogenic lipid profile after pregnancy compared to women with a previous normotensive pregnancy. Nevertheless, studies on their lipid profiles after pregnancy show contradictory results[10, 14-17, 19-22] and current clinical cardiovascular guidelines do not provide uniform recommendations on lipid profile assessment after a GHD.[23-25]

The aim of this study was to assess if women with a previous GHD have a more atherogenic lipid profile six years after pregnancy compared to women with a previous normotensive pregnancy. As GH and PE do share similar risk factors (e.g. obesity, advanced maternal age, nulliparity and diabetes) but differ in their pathophysiological pathways we also assess the individual association between GH and PE, and maternal lipid profile after pregnancy.

METHODS

Design and study population

This study was embedded in the Generation R Study, a multi-ethnic and population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[26, 27] Approval has been obtained by the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam, the Netherlands.[28] For the present study we included women with: a live born singleton (exclusion of twin pregnancies), available information regarding postnatal development and information on the occurrence of GHD. Women were excluded when they had a

history of chronic hypertension prior to enrolment in the Generation R Study, if they were pregnant during the follow-up visit or in case information on cholesterol or glucose mediating medication at follow-up was missing. The final population for analysis comprised 4933 women (**Figure 1**). All women included in this study gave informed consent (MEC 198.782/2001/31).

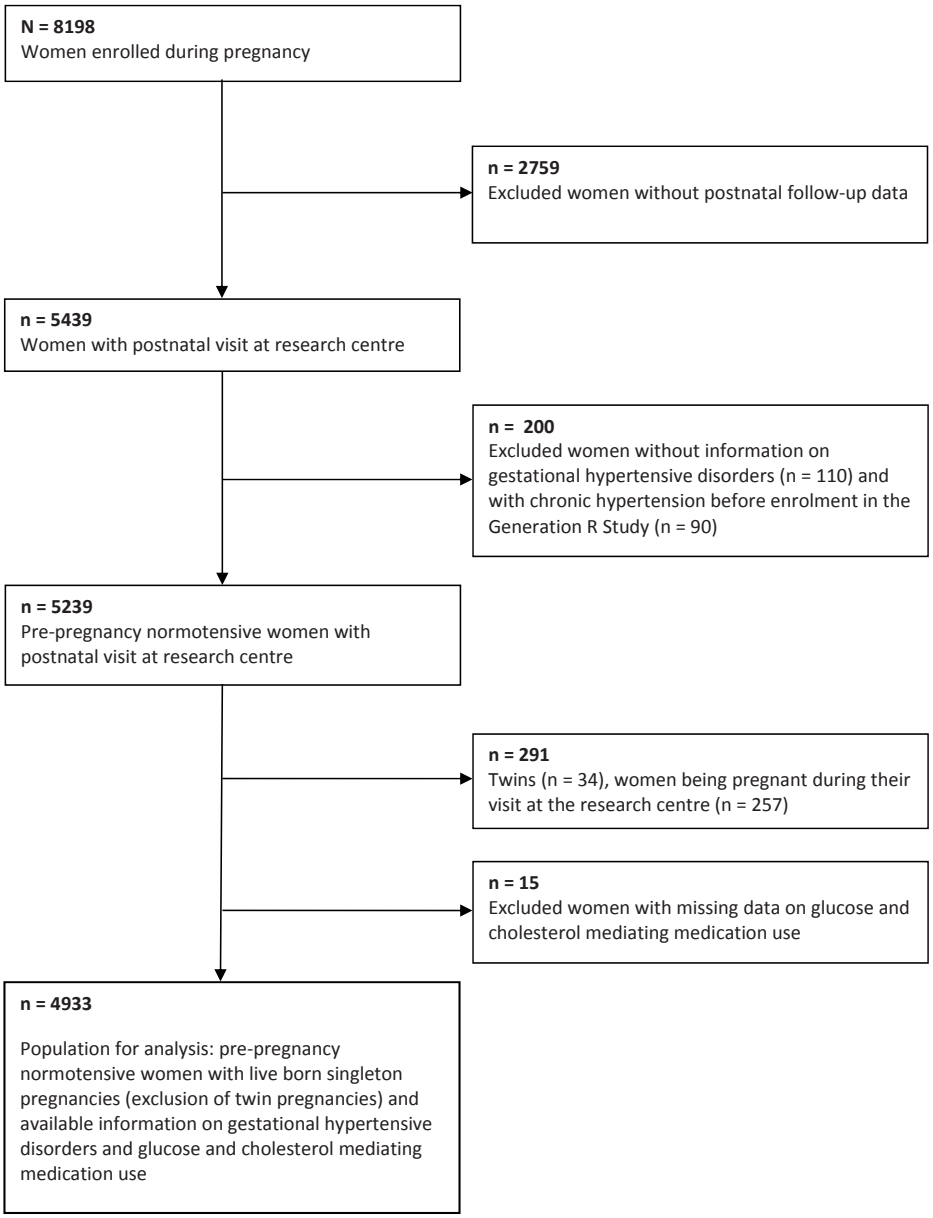

Figure 1 Flowchart

Blood pressure, hypertensive disorders of pregnancy and chronic hypertension

Trained research assistants wearing usual clothing (i.e. no white coats) measured blood pressure in early pregnancy (median 13.9 weeks of gestation [90% range 10.8, 22.5]) and six years after index pregnancy (median 6.0 years [90% range, 5.7 to 7.3]), with the validated Omron 907 automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe B.V., Hoofddorp, the Netherlands).[29] To prevent differences due to position, women sat in a standardised supine position with the cuff placed around the right upper arm. In case of an upper arm exceeding 33 centimeters a larger cuff (32 – 42 cm) was used. The mean value of two blood pressure readings over a five-minute interval was documented for each participant.

Women with a GHD were affected by GH or PE during the index pregnancy. Information on doctor diagnosed GH or PE was retrieved from hospital charts.[30] The diagnosis was determined on the basis of the former criteria of 2001 described by the International Society for the Study of Hypertension in Pregnancy.[31, 32] GH was defined by a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg after 20 weeks of gestation in previously normotensive women. PE was defined as de novo GH with concurrent new onset proteinuria in a random urine sample with no evidence of urinary tract infection.[31] We obtained information on chronic hypertension before pregnancy from three sources: a questionnaire during pregnancy, information from the original medical records and the Dutch obstetric database.[30, 33]

Maternal anthropometrics

Maternal height (cm) and weight (kg) without shoes were measured in early pregnancy and body mass index (BMI) (kg/m^2) was calculated. Identical measurements were obtained during follow-up six years after index pregnancy. Pre-pregnancy BMI was established at study enrolment by questionnaire and was highly correlated with BMI measured in early pregnancy (Pearson's correlation coefficient r 0.95 (*P*-value <0.001)) indicating good intra-individual agreement.[34] Previous research has shown that self-reported BMI of women around the reproductive age is accurate.[35]

Maternal glucose and plasma lipid levels at follow-up six years after index pregnancy

Non-fasting blood samples were obtained six years after index pregnancy by trained research nurses and were temporarily stored at our research centre at room temperature. Twice a day, these blood samples were transported to a dedicated laboratory facility of the regional laboratory in Rotterdam, the Netherlands (STAR-MDC) for further processing and storage at -80 °C. All collected EDTA plasma samples were processed within four hours after venous puncture.[27] Between 2013 and 2014 the samples were transferred from the STAR MC laboratory to the laboratory of Vascular Medicine of the Erasmus Medical Centre Rotterdam. After thawing, the following lipids could be analysed in the EDTA plasma samples: total-cholesterol (mmol/L),

triglycerides (mmol/L), HDL-c (mmol/L), LDL-c (mmol/L), apoB (g/L) and Lp(a) (g/L). Also, plasma glucose (mmol/L) was determined. Samples were analysed using the Vital Scientific (Merck) Selectra E Chemistry Analyzer (Vital Scientific N.V., Dieren, the Netherlands). Details of the mean range of the intra-assay and inter-assay precision with the coefficient of variation (CV) per lipid are provided in **Supplemental table 1**. Remnant cholesterol was calculated as (total-cholesterol – LDL-c) – HDL-c and non-HDL-c level as total-cholesterol – HDL-c.

Covariates

Information on maternal characteristics during pregnancy including maternal age, self-reported pre-pregnancy weight, gravidity, parity, ethnicity, educational level and smoking was available from questionnaires repeatedly applied during pregnancy. We obtained information on gestational age at birth and birth weight from midwifery and obstetric medical records.[36, 37] Six years after index pregnancy, questionnaires were used to obtain information on cholesterol and glucose mediating medication, smoking as well as gravidity and parity at follow-up. The time interval between delivery at index pregnancy and the follow-up visit was calculated and referred to as time interval. The time of blood sampling during the follow-up visit after pregnancy was also documented and used as a covariate.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA) and R version 3.0.0 (R foundation for Statistical Computing, Vienna, Austria, packages rmeta and metafor).[38] Maternal characteristics during pregnancy and follow-up were compared between women with a previous gestational hypertensive disorder and women with a previous normotensive index pregnancy. We used Student's t-test to analyse continuous variables with a normal distribution (presented as means with a standard deviation) and the Kruskal-Wallis test for variables with a skewed distribution (presented as medians with a 90% confidence interval [CI]). Categorical variables were analysed with Chi-square tests. A *P*-value <0.05 was considered statistically significant. The association between a previous GHD and lipid and glucose levels after pregnancy was assessed through linear and logistic regression analyses. Triglyceride levels were log transformed to obtain a normal distribution. Lp(a) could not obtain a normal distribution and was therefore analysed with logistic regression analyses using a cut-off value above the 80th percentile. We also created cut-off values for lipid and glucose levels to define dyslipidemia and examine whether differences between women with a previous normotensive pregnancy and women with a previous GHD were driven by extreme values. Dyslipidemia was defined as exceeding the cut-off value of at least one of the lipids. The 90th percentile was used as the cut-off value for total-cholesterol, triglycerides, LDL-c, apoB and glucose. For Lp(a) we applied a cut-off value above the 80th percentile and for HDL-c a cut-off value below the 10th percentile. Differences were tested using chi-square tests. We repeated all linear and logistic regression

analyses to test whether results differed between women with previous GH and PE (**Supplemental tables 5 and 6**). To perform regression analyses we selected potential confounders depending on their association with the outcome of interest and/or based on previous studies and/or based on a change in effect estimate of more than 10%. The final regression models included the following confounders; (1) basic model, adjusted for maternal age at intake, visit interval and time of blood sampling; (2) confounder model, which in addition to model (1) included ethnicity, educational level, smoking and gravidity at follow-up); (3) BMI model, which in addition to model (2) included pre-pregnancy BMI. Results of linear and logistic regression analyses are presented as regression coefficients (β) and odds ratios (OR) with a 95% CI. A *P*-value <0.05 (*) or <0.001 (**) was considered statistically significant.

For women included in our analyses, missing values of confounders that were used in the three regression models were imputed through multiple imputation procedures.[39] This procedure was carried out by taking five draws for each missing value which provided five complete data sets. Each dataset was analysed separately and results were integrated into one final result by computing the mean over the five repeated analysis, including the variance, confidence interval and *P*-value. The following confounders had missing values (for women with a previous normotensive pregnancy and women with a previous GHD respectively): pre-pregnancy BMI

Table 1 Subject characteristics by gestational hypertensive disorder (n = 4933).

	Normotensive Pregnancy	**GHD**	***P*-value**
	n = 4631	n = 302	
Maternal characteristics (pregnancy)			
Age at intake (years), mean (SD)	30.1 (5.1)	30.4 (5.1)	0.74
Gestational age at intake (weeks), median (90% range)	13.9 (10.9, 22.2)	13.6 (10.5, 22.9)	0.23
Pre-pregnancy BMI (kg/m2), median (90% range)	22.7 (18.7, 31.5)	24.9 (19.8, 38.5)	<0.001
Normal BMI (≥18.5 and < 25.0), n (%)	2790 (73.0)	133 (51.0)	
High BMI (≥25.0), n (%)	1030 (27.0)	128 (49.0)	
BMI in first trimester (kg/m2), median (90% range)	23.9 (19.6, 32.6)	26.1 (20.3, 39.0)	<0.001
Systolic blood pressure at intake (mmHg), mean (SD)	114.9 (11.7)	123.4 (13.2)	<0.001
Diastolic blood pressure at intake (mmHg), mean (SD)	67.4 (8.9)	75.6 (10.3)	<0.001
Primigravida, n (%)	2152 (46.7)	198 (65.6)	<0.001
Non-European ethnicity, n (%)	1917 (41.4)	94 (31.1)	0.001
Lower educational level, n (%)	555 (12.0)	24 (7.9)	0.06
Smoking, n (%)	1222 (26.4)	83 (27.5)	0.77

Abbreviations: Body mass index, BMI; Gestational hypertensive disorder, GHD.

Values are numbers with valid percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution. Presented values are not imputed. Statistical testing was carried out through Student's t-test for continuous variables with a normal distribution and Kruskal-Wallis test for continuous variables with a skewed distribution. Chi-square tests were used for categorical variables.

Table 2 Subject characteristics by gestational hypertensive disorder six years after index pregnancy (n = 4933).

	Normotensive Pregnancy	**GHD**	***P*-value**
	n = 4631	n = 302	
Maternal characteristics (follow-up)			
Visit interval (years), median (90% range)	6.0 (5.7, 7.3)	6.1 (5.7, 7.5)	0.14
Medication use, n (%)			0.19
Cholesterol mediating medication	18 (0.4)	1 (0.4)	
Glucose mediating medication	22 (0.5)	4 (1.3)	
Smoking, n (%)	958 (20.7)	57 (18.9)	0.70
BMI (kg/m^2), median (90% range)	24.6 (19.7, 35.2)	27.6 (20.6, 43.5)	<0.001
Systolic blood pressure (mmHg), mean (SD)	118.5 (12.1)	128.2 (17.5)	<0.001
Diastolic blood pressure (mmHg), mean (SD)	70.4 (9.5)	78.5 (12.2)	<0.001
Lipids at follow-up			
Total-cholesterol (mmol/L), mean (SD)	4.85 (0.89)	4.95 (0.92)	0.08
Triglycerides (mmol/L), median (90% range)	1.12 (0.61, 2.48)	1.21 (0.67, 2.48)	<0.001
HDL-c (mmol/L), mean (SD)	1.37 (0.34)	1.32 (0.33)	0.02
LDL-c (mmol/L), mean (SD)	2.64 (0.58)	2.73 (0.61)	0.02
Non-HDL-c (mmol/L), mean (SD)	3.48 (0.90)	3.63 (0.95)	0.009
Remnant cholesterol (mmol/L), mean (SD)	0.84 (0.49)	0.90 (0.50)	0.06
Lp(a) (mmol/L), median (90% range)	0.16 (0.0, 2.85)	0.17 (0.0, 1.26)	0.26
ApoB (g/L), mean (SD)	0.80 (0.19)	0.83 (0.19)	0.005
Glucose (mmol/L), mean (SD)	5.48 (0.98)	5.47 (0.83)	0.82

Abbreviations: Apolipoprotein B, ApoB; Body mass index, BMI; Gestational hypertensive disorder, GHD; Lipoprotein(a), Lp(a).
Values are numbers with valid percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution. Presented values are not imputed. Statistical testing was carried out through Student's t-test for continuous variables with a normal distribution and Kruskal-Wallis test for continuous variables with a skewed distribution. Chi-square tests were used for categorical variables.

(17.5% and 13.6%), ethnicity (2.2% and 0.7%), educational level (15.2% and 12.3%), smoking in pregnancy (10.8% and 6.6%), gravidity at follow-up (0.6% and 0%) and blood sampling (13.3% and 14.9%). A sensitivity analysis was performed to observe differences in observed and expected values of confounders before and after imputation (**Supplemental table 4**). Lastly, to test for non-response bias we compared subject characteristics between women with and without available follow-up data six years after index pregnancy (**Supplemental table 2**).

Table 3 Association of gestational hypertensive disorders with lipid profile and glucose six years after index pregnancy (n = 4933).

	Normotensive Pregnancy	**GHD**
	n = 4631	n = 302
		Beta (95% CI)
Total-cholesterol (mmol/L)		
Basic model	*Reference*	0.09 (-0.02, 0.20)
Confounder model	*Reference*	0.11 (-0.01, 0.23)
BMI model	*Reference*	0.08 (-0.03, 0.20)
Triglycerides (log, mmol/L)		
Basic model	*Reference*	0.09 (0.04, 0.14)**
Confounder model	*Reference*	0.10 (0.04, 0.15)**
BMI model	*Reference*	0.04 (-0.01, 0.10)
HDL-c (mmol/L)		
Basic model	*Reference*	-0.05 (-0.10, -0.01)*
Confounder model	*Reference*	-0.06 (-0.10, -0.02)**
BMI model	*Reference*	-0.01 (-0.05, 0.04)
LDL-c (mmol/L)		
Basic model	*Reference*	0.08 (0.01, 0.16)*
Confounder model	*Reference*	0.09 (0.01, 0.17)*
BMI model	*Reference*	0.06 (-0.02, 0.14)
Non-HDL-c (mmol/L)		
Basic model	*Reference*	0.14 (0.03, 0.26)*
Confounder model	*Reference*	0.17 (0.05, 0.29)**
BMI model	*Reference*	0.09 (-0.03, 0.21)
Remnant cholesterol (mmol/L)		
Basic model	*Reference*	0.06 (-0.01, 0.12)
Confounder model	*Reference*	0.08 (0.01, 0.14)*
BMI model	*Reference*	0.02 (-0.04, 0.09)
ApoB (g/L)		
Basic model	*Reference*	0.03 (0.01, 0.06)**
Confounder model	*Reference*	0.04 (0.02, 0.07)**
BMI model	*Reference*	0.02 (-0.00, 0.05)
Lp(a) (OR, >p80, >0.84g/L)†		
Basic model	*Reference*	1.2 (0.88, 1.6)
Confounder model	*Reference*	1.3 (0.91, 1.7)
BMI model	*Reference*	1.2 (0.86, 1.6)
Glucose (mmol/L)		
Basic model	*Reference*	-0.02 (-0.14, 0.10)
Confounder model	*Reference*	-0.02 (-0.15, 0.11)
BMI model	*Reference*	-0.02 (-0.16, 0.11)

Abbreviations: Body mass index, BMI; Confidence interval, CI; Gestational hypertensive disorder, GHD; Odds ratio, OR.
Values are regression coefficients (β with 95% confidence interval) and are based on linear regression models except for Lp(a)† which was based on a logistic regression model (OR with 95% confidence interval).
Estimates are from multiple imputed data.
Basic model: Adjusted for maternal age at intake, visit interval and time of blood sampling.
Confounder model: basic model and additionally adjusted for ethnicity, educational level, smoking and gravidity at follow-up.
BMI model: Confounder model and additionally adjusted for pre-pregnancy BMI.
*P-value < 0.05
**P-value < 0.01

RESULTS

Tables 1 and 2 show maternal characteristics before, during and six years after index pregnancy. Compared to women with a previous normotensive pregnancy, women with a GHD showed a higher BMI both before and after pregnancy and a higher systolic and diastolic blood pressure both at study enrolment and after pregnancy. In total, 0.4% and 0.5% of all women used cholesterol and glucose mediating medication after pregnancy respectively. The use of cholesterol and glucose regulating medication was similar between women with a previous normotensive pregnancy and women with a previous GHD.
The association between a GHD, and lipid and glucose levels at follow-up is presented in **Table 3** and **Supplemental table 3**. Six years after index pregnancy, women with a previous GHD had higher levels of triglycerides, LDL-c, non-HDL-c, remnant cholesterol and apoB, and lower levels of HDL-c than women with a previous normotensive pregnancy. After additional adjustment for pre-pregnancy BMI these results attenuated to non-significant levels. No differences were observed in total-cholesterol, Lp(a) and glucose levels. Sensitivity analyses showed that especially women with previous GH had higher lipid levels after pregnancy than women with a previous normotensive pregnancy ([higher levels of triglycerides, LDL-c, non-HDL-c, remnant cholesterol and apoB, and lower levels of HDL-c] **Supplemental table 5**). Women with previous PE had higher triglyceride levels than women with a previous normotensive pregnancy. Results attenuated to non-significant levels after adjustment for pre-pregnancy BMI. The prevalence of dyslipidemia, defined as LDL-c levels > 90th percentile, was higher among women with previous GHD than women with a previous normotensive pregnancy (**Supplemental table 3**). This result remained significant after adjusting for confounders, including pre-pregnancy BMI (OR 1.6; 95% CI 1.1-2.4, P-value 0.015 [data not shown]) and was most profound in women with previous GH (**Supplemental table 6**). Women with previous PE were more often affected by dyslipidemia resulting from triglyceride levels > 90th percentile (**Supplemental table 6**).
An additional sensitivity analysis was performed to examine whether values of confounders used for linear and logistic regression analyses differed before and after multiple imputation

(**Supplemental table 4**). No major differences were observed. Linear and logistic regression analyses showed similar results when confounders were not imputed (data not shown).

DISCUSSION

The results of this study suggest that women with a previous GHD have a more atherogenic lipid profile six years after pregnancy than women with a previous normotensive pregnancy. The association between GHD and lipid profile after pregnancy is largely explained by pre-pregnancy BMI.

Methodological considerations

The main strengths of our study are the prospective data collection from early pregnancy onwards until six years after index pregnancy, the large sample size of 4933 participants, the detailed analysis of lipid profiles and the wide variety of included ethnicities, which gives a good representation of the general population. Given the prospective nature of this study, information on microalbuminuria or other markers of kidney injury might have been interesting to examine. Unfortunately, this data was not available within our study. An important issue that might have affected the generalizability of our results is the selection of a relatively healthy population (**Supplemental table 2**). Women without postnatal follow-up information were on average younger and more often of non-European descent. These women tended to have a lower pre-pregnancy BMI and lower education, they smoked more often and suffered more often from PE. A second is that, due to unavailability of pre-pregnancy data on glucose and lipid levels (as is also the case in most other studies focusing on pregnant women and lipid profile after pregnancy), we cannot exclude the possibility that changes in glucose levels and lipid profile preceded the onset of the GHD. The third issue is that we did not obtain lipid and glucose levels in the fasting state, which might have introduced a random measurement error. Finally, the observational nature of this study does not allow for inference of causality.

Interpretation

The results of this large prospective cohort study show that women with previous GHD have higher levels of triglycerides, LDL-c, non-HDL-c, remnant cholesterol and apoB and lower levels of HDL-c after pregnancy compared to women with a previous normotensive pregnancy. Pre-pregnancy BMI largely explained these results as most lipid levels attenuated after adjustment for this factor. However, when pre-pregnancy BMI was taken into account, women with previous GHD still had a higher risk of having LDL-c levels above the 90th percentile compared to women with a previous normotensive pregnancy. A smaller study, comparing lipid levels 7.8 years after pregnancy between women with a previous GHD (GH [n = 105] or PE [n = 63]) and women with a previous normotensive pregnancy, showed results similar to ours without

adjusting for pre-pregnancy BMI.[15] Women with previous GH showed higher levels of triglycerides, apoB and glucose, and lower levels of HDL-c. Women with previous PE showed higher levels of LDL-c and apoB than women with a previous normotensive pregnancy. In the study concerned, pre-pregnancy BMI was not taken in to consideration and might therefore have overestimated their results.[15] Other studies comparing lipid profile and glucose levels between women with only previous PE and women with a previous normotensive pregnancy show no differences within the first 10 years after pregnancy (1.5 to 9.1 years).[19, 20, 22] However, after a longer time period (up to 16 years after pregnancy), women with previous PE do seem to have a more atherogenic lipid profile, but this is largely driven by BMI.[14, 21] These results are in line with our findings, suggesting pre-pregnancy BMI is an important factor contributing to an atherogenic lipid profile after a pregnancy affected by GHD.

We observed no differences in total-cholesterol, Lp(a) and glucose levels between women with a previous GHD and women with a previous normotensive pregnancy. Lp(a) is a plasma lipoprotein consisting of an LDL-like particle, apolipoprotein B100 and apolipoprotein(a).[40] The structure of apolipoprotein(a) resembles plasminogen and plasmin which can stimulate a prothrombotic effect. The LDL-like particle of Lp(a) contains a high concentration of cholesterol which can induce atherogenesis through deposition of cholesterol in the arterial vessel wall.[41] Both characteristics increase the risk of CVD substantially.[42] In our study, no differences were observed in Lp(a) levels between women with a previous GHD and women with a previous normotensive pregnancy. As Lp(a) is to a large extent genetically determined, one could hypothesise that lipid levels after pregnancy in women with previous GHD might be less genetically determined and more through environmental factors such as BMI. This hypothesis supports our findings of pre-pregnancy BMI attenuating most lipid levels after pregnancy to non-significant levels.

Additional analyses showed that our results were stronger in women with previous GH than in women with previous PE. This might result from a greater constitutional cardiovascular burden at the start of pregnancy (higher pre-pregnancy BMI and higher blood pressure at study enrolment) in women with GH than in women with PE (**Table 1**). These constitutional risk factors are likely to remain after pregnancy which increases the risk of developing an atherogenic lipid profile. Women with PE also exhibit a greater constitutional cardiovascular burden at the start of pregnancy but less than women with GH. This might explain why women with previous GH have a more atherogenic lipid profile after pregnancy than women with previous PE.

There is an established association between GHD and the risk for CVD in later life.[6, 43] Depending on the type and the severity of the GHD, the occurrence of CVD can be seven times larger compared to women with a previous normotensive pregnancy.[43] Currently, there are several guidelines in practice for the prevention of CVD after a GHD.[23-25] Nevertheless, it remains controversial whether to assess a woman's lipid profile after a GHD and a uniform recommendation of these clinical guidelines is not available. The guideline of the American

Heart Association for the prevention of stroke in women, as well as the National Institute for Health and Care Excellence and the American College of Obstetricians and Gynecologists guidelines on the management of hypertensive disorders during pregnancy advise to perform annual measurement of a lipid profile and glucose level after a GHD. [23-25] Contrarily, the guidelines of the European Society of Cardiology on CVD prevention and of the American Heart Association on the prevention of CVD in women do not address lipid profile assessment after a GHD, although the former does recommend periodic screening for hypertension and diabetes in these women.[44, 45]

The association between an atherogenic lipid profile and the risk of future CVD is evident within the general population.[46, 47] Nevertheless, results from similar studies on women with previous GHD remain inconsistent, possibly due to a variety in study methodology. The most important methodological differences are: the time interval between pregnancy and lipid profile assessment (varying between 0.5 and 16 years),[10, 14, 15, 17, 19-21] incomplete lipid profile assessment,[10, 14, 17, 19-21] differences in statistical models to adjust for confounding[14, 20] and differences in the definition of GHD.[10, 21]

Interestingly, our study shows that differences in lipid profile between women with a previous normotensive pregnancy and women with previous GHD are most likely the result of a higher pre-pregnancy BMI in the latter. BMI and blood pressure are important constitutional risk factors for developing a GHD as well as developing future CVD.[48]

Lipid profile assessment after a GHD, especially in overweight women, might help to identify those women at risk for CVD. Based on our results we foremost suggest to encourage women to achieve a healthy weight status before pregnancy as pre-pregnancy weight is strongly associated with an atherogenic lipid profile after pregnancy and with an increased risk of developing a GHD.[48]

Conclusions

Women with a previous GHD have a more atherogenic lipid profile six years after pregnancy than women with a previous normotensive pregnancy. This is more likely resulting from a higher pre-pregnancy BMI than from the GHD itself. Weight counseling before the onset of pregnancy might therefore be beneficial in reducing the risk of developing an atherogenic lipid profile after pregnancy and possibly CVD in later life.[49]

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SUPPLEMENTAL MATERIAL

Supplemental table 1 Mean range of the intra-assay precision with the coefficient of variation(CV) per lipid concentration.

	Mean range of the intra-assay precision	**Coefficient of variation**
Triglycerides	55-448	< 0.80%
Apolipoprotein B	24.2-156	< 2.63%
Total-cholesterol	108-254	< 1.62%
Glucose	4.4-188	< 3.78%
HDL-c	0.204-1.25	< 0.82%
LDL-c	101-164	< 0.67%
Lipoprotein(a)	26.9-52.3	< 2%
	Mean range of the inter-assay precision	**Coefficient of variation**
Triglycerides	90-238	< 1.48%
Apolipoprotein B	25.4-158	< 2.89%
Total-cholesterol	104-245	< 1.22%
Glucose	86.6-248	< 1.88%
HDL-c	0.44	< 1.88%
LDL-c	108-135	< 1.45%
Lipoprotein(a)	26.2-52.2	< 3.06%

Supplemental table 2 Subject characteristics between women with follow-up visit six years after pregnancy and women without follow-up visit (n = 8198).

Maternal characteristics	**Follow-up six years after index pregnancy**	**Loss to follow-up**	***P*-value**
	n = 5439	n = 2759	
Age at intake (years), mean (SD)	30.2 (5.1)	28.2 (5.5)	<0.001
Gestational age at intake (weeks), median (90% range)	13.9 (10.8, 22.6)	14.5 (10.8, 23.8)	<0.001
Pre-pregnancy BMI (kg/m^2), median (90% range)	22.9 (18.9, 32.6)	22.6 (18.2, 33.1)	0.02
SBP at intake (mmHg), mean (SD)	115.7 (12.3)	114.7 (12.4)	0.001
DBP at intake (mmHg), mean (SD)	68.2 (9.5)	67.5 (9.8)	0.002
Primigravida, n (%)	2338 (43.0)	1332 (48.3)	<0.001
Non-European ethnicity, n (%)	2192 (40.3)	1454 (52.7)	<0.001
Lower educational level, n (%)	533 (9.8)	455 (16.5)	<0.001
Smoking, n (%)	1446 (26.6)	849 (30.8)	<0.001
Gestational hypertension, n (%)	239 (4.4)	85 (3.1)	0.009
Preeclampsia, n (%)	114 (2.1)	80 (2.9)	0.04

Abbreviations: Body mass index, BMI; Systolic blood pressure, SBP; Diastolic blood pressure, DBP.
Values are numbers with valid percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution.
Statistical testing was carried out through Student's t-test for continuous variables with a normal distribution and Kruskal-Wallis test for continuous variables with a skewed distribution. Chi-square tests were used for categorical variables.

Supplemental table 3 Maternal glucose and lipid profile six years after pregnancy (n = 4933).

Outcomes	Normotensive Pregnancy	GHD	*P*-value
	n = 4631	n = 302	
Total-cholesterol > 90th percentile, n (%)	397 (9.9)	29 (11.3)	0.47
Triglycerides > 90th percentile, n (%)	397 (9.9)	29 (11.3)	0.47
HDL-c < 10th percentile, n (%)	399 (10.0)	27 (10.5)	0.76
LDL-c > 90th percentile, n (%)	387 (9.7)	39 (15.2)	0.004
Non-HDL-c > 90th percentile, n (%)	391 (9.8)	34 (13.3)	0.07
Remnant cholesterol > 90th percentile, n (%)	394 (9.8)	31 (12.1)	0.24
Lp(a) > 80th percentile, n (%)	837 (21.2)	61 (24.3)	0.24
ApoB > 90th percentile, n (%)	394 (9.8)	32 (12.5)	0.17
Glucose > 90th percentile, n (%)	404 (10.1)	22 (8.6)	0.43

Abbreviations: Apolipoprotein B, ApoB; lipoprotein(a), Lp(a); Gestational hypertensive disorder, GHD.
Values are numbers with percentages and are not imputed. Statistical testing was carried out through chi-square tests.

Supplemental table 4 Observed and expected values for confounders.

	Normotensive Pregnancy		GHD	
	n = 4631		n = 302	
	Observed	**Expected**	**Observed**	**Expected**
Maternal characteristics (pregnancy)				
Age at intake (years), mean (SD)	30.1 (5.1)	30.1 (5.1)	30.4 (5.1)	30.4 (5.1)
Pre-pregnancy BMI (kg/m^2), median (90% range)	22.7 (18.8, 31.6)	22.8 (18.8, 31.7)	24.9 (19.8, 38.5)	24.8 (19.8, 38.1)
Normal BMI (≥18.5 and < 25.0), n (%)	2790 (60.2)	3331 (71.9)	133(44.0)	154 (51.0)
High BMI (≥25.0), n (%)	1030 (22.2)	1300 (28.1)	128 (42.4)	148 (49.0)
Non-European ethnicity, n (%)	1828 (39.5)	1919 (41.4)	92 (30.5)	94 (31.1)
Lower educational level, n (%)	417 (9.0)	555 (12.0)	19 (6.3)	24 (7.9)
Smoking, n (%)	1095 (23.6)	1213 (26.2)	77 (25.5)	85 (28.1)
Primigravid, n (%)	318 (6.9)	406 (8.8)	41 (17.6)	48 (15.9)
Maternal characteristics (follow-up)				
Visit interval (years), median (90% range)	6.0 (5.7, 7.3)	6.0 (5.7, 7.3)	6.1 (5.7, 7.5)	6.1 (5.7, 7.5)
Blood sampling before 10:00 h	1129 (24.4)	1272 (27.5)	71 (23.5)	81 (26.8)

Abbreviations: Body mass index, BMI; Gestational hypertensive disorder, GHD.
Values are numbers with percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution.

Supplemental table 5 Association of gestational hypertensive disorders with lipid profile and glucose six years after index pregnancy (n = 4933).

	Normotensive pregnancy	**GH**	**PE**
	n = 4631	n = 207	n = 95
		Beta (95% CI)	Beta (95% CI)
Total-cholesterol (mmol/L)			
Basic model	*Reference*	0.11 (-0.02, 0.24)	0.04 (-0.16, 0.24)
Confounder model	*Reference*	0.15 (0.01, 0.28)*	0.03 (-0.18, 0.24)
BMI model	*Reference*	0.11 (-0.03, 0.25)	0.02 (-0.20, 0.23)
Triglycerides (log, mmol/L)			
Basic model	*Reference*	0.08 (0.02, 0.14)*	0.12 (0.02, 0.21)*
Confounder model	*Reference*	0.09 (0.02, 0.16)**	0.11 (0.01, 0.22)*
BMI model	*Reference*	0.03 (-0.04, 0.09)	0.08 (-0.02, 0.18)
HDL-c (mmol/L)			
Basic model	*Reference*	-0.05 (-0.10, -0.004)*	-0.05 (-0.13, 0.03)
Confounder model	*Reference*	-0.07 (-0.12, -0.01)*	-0.05 (-0.13, 0.03)
BMI model	*Reference*	-0.00 (-0.05, 0.05)	-0.02 (-0.09, 0.06)
LDL-c (mmol/L)			
Basic model	*Reference*	0.11 (0.02, 0.19)*	0.04 (-0.09, 0.17)
Confounder model	*Reference*	0.12 (0.03, 0.21)*	0.02 (-0.12, 0.16)
BMI model	*Reference*	0.09 (-0.01. 0.18)	0.00 (-0.14, 0.14)
Non-HDL-c (mmol/L)			
Basic model	*Reference*	0.17 (0.03, 0.30)*	0.09 (-0.11, 0.29)
Confounder model	*Reference*	0.21 (0.07, 0.35)**	0.08 (-0.14, 0.29)
BMI model	*Reference*	0.11 (-0.03, 0.25)	0.03 (-0.18, 0.24)
Remnant cholesterol (mmol/L)			
Basic model	*Reference*	0.06 (-0.02, 0.13)	0.05 (-0.06, 0.16)
Confounder model	*Reference*	0.08 (0.01, 0.16)*	0.06 (-0.06, 0.18)
BMI model	*Reference*	0.02 (-0.05, 0.10)	0.03 (-0.09, 0.15)
ApoB (g/L)			
Basic model	*Reference*	0.04 (0.01, 0.07)**	0.02 (-0.02, 0.06)
Confounder model	*Reference*	0.05 (0.02, 0.08)**	0.02 (-0.03, 0.06)
BMI model	*Reference*	0.03 (0.00, 0.06)	0.01 (-0.04, 0.05)
Lp(a) (OR, >p80, >0.84g/L)†			
Basic model	*Reference*	1.2 (0.84, 1.7)	1.2 (0.68, 2.0)
Confounder model	*Reference*	1.3 (0.91, 1.9)	1.1 (0.59, 2.0)
BMI model	*Reference*	1.2 (0.86, 1.8)	1.1 (0.57, 1.9)
Glucose (mmol/L)			
Basic model	*Reference*	-0.06 (-0.21, 0.08)	0.07 (-0.15, 0.29)
Confounder model	*Reference*	-0.07 (-0.23, 0.09)	0.10 (-0.15, 0.34)
BMI model	*Reference*	-0.07 (-0.23, 0.09)	0.09 (-0.15, 0.33)

Abbreviations: Body Mass Index, BMI; Confidence Interval, CI; Gestational hypertension, GH; Odds Ratio, OR; Preeclampsia, PE.

Values are regression coefficients (β with 95% confidence interval) and are based on linear regression models except for Lp(a)† which was based on a logistic regression model (OR with 95% confidence interval). Estimates are from multiple imputed data.
Basic model: Adjusted for maternal age at intake, visit interval and time of blood sampling.
Confounder model: basic model and additionally adjusted for ethnicity, educational level, smoking and gravidity at follow-up.
BMI model: Confounder model and additionally adjusted for pre-pregnancy BMI.
**P*-value < 0.05
***P*-value < 0.01

Supplemental table 6 Maternal glucose and lipid profile six years after pregnancy (n = 4933).

	Normotensive Pregnancy	**GH**	**PE**	***P*-value**
	n = 4631	n = 207	n = 95	
Total-cholesterol > 90th percentile, n (%)	397 (9.9)	22 (12.2)	7 (9.1)	0.58
Triglycerides > 90th percentile, n (%)	397 (9.9)[B]	15 (8.3)	14 (18.2)	0.04
HDL-c < 10th percentile, n (%)	399 (10.0)	18 (10.1)	9 (11.7)	0.88
LDL-c > 90th percentile, n (%)	387 (9.7)[A]	29 (16.1)	10 (13.0)	0.01
Non-HDL-c > 90th percentile, n (%)	391 (9.8)	27 (15.1)	7 (9.1)	0.07
Remnant cholesterol > 90th percentile, n (%)	394 (9.8)	20 (11.2)	11 (14.3)	0.38
Lp(a) > 80th percentile, n (%)	837 (21.2)	43 (24.4)	18 (24.0)	0.50
ApoB > 90th percentile, n (%)	394 (9.8)	24 (13.4)	8 (10.4)	0.30
Glucose > 90th percentile, n (%)	404 (10.1)	14 (7.8)	8 (10.4)	0.60

Abbreviations: Apolipoprotein B, ApoB; Lipoprotein(a), Lp(a); Gestational hypertension, GH; Preeclampsia, PE.
Values are numbers with percentages. Statistical testing was carried out through chi-square tests.
[A]Significant (*P*-value < 0.05) differences in distribution between women with GH and women with a normotensive pregnancy.
[B]Significant (*P*-value < 0.05) differences in distribution between women with PE and women with a normotensive pregnancy.

Part II

Child health

Chapter 5

Maternal and neonatal markers of the homocysteine pathway and fetal growth

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ABSTRACT

Objective: Suboptimal dietary intake during pregnancy may have long-term health implications in children. These effects may be mediated by fetal growth. We investigated associations of early pregnancy and umbilical cord total homocysteine (tHcy), folate, total and active vitamin B_{12} concentrations with fetal growth parameters repeatedly measured in pregnancy and at birth.

Methods: This study was performed in 5890 pregnant women, participating in a population-based prospective cohort study. Blood samples were obtained from women in early pregnancy and from the umbilical vein at delivery. Fetal size parameters were repeatedly measured by ultrasound. Information about birth anthropometrics was retrieved from medical records.

Results: High early pregnancy maternal tHcy (≥8.31 μmol/L), as compared with low maternal tHcy (≤5.80 μmol/L), and low early pregnancy maternal folate (≤9.10 nmol/L), as compared with high maternal folate (≥25.81 nmol/L) concentrations, were associated with reduced weight growth patterns throughout pregnancy, resulting in birth weight differences of -102.3 g (95% CI -139.6, -65.0) and -113.0 g (95% CI -159.6, -66.3), respectively. Low umbilical cord folate concentrations (≤15.20 nmol/L) as compared with high umbilical cord folate concentrations (≥28.41 nmol/L) were also associated with a lower birth weight and birth length (*P*-value <0.001). Interestingly, compared with higher umbilical cord vitamin B_{12}, lower umblical cord vitamin B_{12} concentrations were associated with a higher weight, length and head circumference at birth (*P*-value <0.01).

Conclusions: Early pregnancy maternal and umbilical cord markers of the homocysteine pathway are significantly associated with fetal growth patterns. These differences arise from mid-pregnancy onwards**.**

INTRODUCTION

Poor nutrition and other environmental exposures, acting in different stages of fetal development, have been associated with adverse birth outcomes, including fetal growth restriction.[1, 2] Increased maternal total homocysteine (tHcy) concentrations may be a marker of an adverse environmental status and have been associated with higher risks of small for gestational age children.[3] A proposed mechanisms underlying this association is that high tHcy generates reactive oxygen species inducing oxidative stress and ultimately leading to endothelial cell dysfunction, especially within the placental vasculature.[4] The homocysteine metabolism is regulated by gene-nutrient interactions and is largely dependent on the intake of B vitamins, including folate and vitamin $B_{12.}$ Suboptimal status or deficiency in these micronutrients can lead to disturbances in the homocysteine metabolism. In addition to their function in the homocysteine metabolism, folate and vitamin B_{12} are also important for DNA synthesis and cell proliferation.[5] During pregnancy, the cell proliferation necessary for uterine enlargement and placental and fetal growth and development increases the requirement of folate and vitamin B_{12} not only in the mother but also in the fetus. To our knowledge, only a few small studies examined the effects of neonatal tHcy, folate and vitamin B_{12} on birth weight with inconsistent results.[6-9] Furthermore, these studies focused on birth weight as proxy for fetal growth. Similar birth weights may be the result of different fetal exposures and growth patterns. Assessing fetal growth parameters in different stages of pregnancy may give more insight in identifying specific critical periods.

We therefore examined the association of early pregnancy maternal tHcy, folate, total and active B_{12} concentrations with fetal growth parameters repeatedly measured in different stages of pregnancy and the effect of umbilical cord tHcy, folate, total and active B_{12} concentrations on birth anthropometrics.

METHODS

Design and study population

This study was embedded in the Generation R Study, an ongoing population-based prospective cohort study from early fetal life onward. The study was approved by the Medical Ethics Committee at Erasmus University Medical Centre Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from all participants. All pregnant women were enrolled between 2001 and 2005.[10]

In total, 8880 mothers were enrolled during pregnancy. Blood samples were collected in 6362 mothers in early pregnancy. Mothers without data on homocysteine concentrations were excluded from the analyses (n = 392). We also excluded twin pregnancies (n = 69), loss to follow-up (n = 1), pregnancies that ended before a gestational age of 24 weeks (n = 7) and

mothers without any fetal growth measurements (n = 3). Of the remaining 5890 mothers, 391 were included twice or more within the study. Exclusion of these subjects did not substantially change our results and were therefore included in the analyses presented.

Biomarkers

Maternal venous samples were collected in early pregnancy (median 13.5 weeks of gestation, 90% range 10.5-17.2). Directly after delivery (median 40.1 weeks of gestation, 90% range 37.0- 42.0), midwives or obstetricians collected samples from the umbilical cord vein. After collection, blood samples were stored at room temperature for a maximum of 3h before being transported to the regional laboratory for processing and storage for future studies. The samples were centrifuged and thereafter stored at –80 °C. To analyse tHcy, folate, total and active vitamin B_{12} concentrations, serum samples (total and active vitamin B_{12}) and EDTA plasma samples (folate, tHcy) were picked and transported to the Department of Clinical Chemistry of the Erasmus Medical Centre, Rotterdam in 2008. After thawing tHcy, folate, total and active vitamin B_{12} concentrations were analysed using an immunoelectrochemoluminence assay on the Architect System (Abott Diagnostics B.V., Hoofddorp, the Netherlands). This system automatically neutralises potential native intrinsic factor antibodies, even in case of high load.[11] The between run coefficients of variation and analytic ranges for tHcy, folate, total and active vitamin B_{12} concentrations are presented in **Supplemental table 1**.

Fetal growth parameters

Fetal ultrasound examinations were performed in early (median 13.2 weeks of gestation, 90% range 11.1-17.0), mid- (median 20.5 weeks of gestation, 90% range 19.1-22.6) and late pregnancy (median 30.4 weeks of gestation, 90% range 29.0-32.2) by using the Aloka® model SSD-1700 (Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Seatle, WA, USA). Gestational age was established by using data from the first fetal ultrasound examination.[12] Early pregnancy measurements were therefore not included in the growth analyses. Fetal growth parameters included head circumference, biparietal diameter, abdominal circumference, and femur length and were measured trans abdominally using standardised ultrasound procedures in mid- and late pregnancy. Estimated fetal weight was calculated using the formula by Hadlock et al.[13] All ultrasound examinations were carried out by experienced sonographers who also underwent additional training according to guidelines from the Fetal Medicine Foundation to achieve optimal reproducibility. More details regarding ultrasound procedures have been described previously.[12] Longitudinal growth curves and gestational-age-adjusted standard deviation (SD) scores were constructed for all fetal growth parameters. These gestational-age-adjusted SD scores were based on reference growth curves from the whole study population and represent the equivalent of z-scores.

Birth outcomes

Date of birth, birth anthropometrics (weight, length and head circumference), and fetal gender were obtained from medical records and hospital registries. The regression models with head circumference at birth an birth length as outcome were additionally adjusted for postconceptional age (gestational age at birth). Since head circumference and length at birth were not routinely measured at birth, missing birth measures were completed with data from the first month routine visit at the child health centre. Of all measurements, 31% and 20% were based on the first month visit for head circumference and birth length, respectively. To account for this, the regression models with head circumference at birth and birth length as outcome were, if it concerned a measure that was completed using data from the first month routine visit at the child health centre, additionally adjusted for postconceptional age (gestational age + postnatal age of measurement from the child health centre) and also for the moment, that is, the time of measurement at the birth or child health centre.

Covariates

Information on maternal age, educational level, ethnicity, parity and folic acid supplement use was available from questionnaires at enrolment in the study. Maternal smoking and alcohol consumption were assessed by questionnaires applied in early, mid- and late pregnancy.[10] Maternal weight and height were measured at enrolment in the study. Subsequently body mass index was calculated. Information on fertility treatment was obtained from midwives and obstetricians. Blood pressure was measured with the validated Omron 907® automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe B.V. Hoofddorp, the Netherlands). The presence of preeclampsia was retrieved from medical records after delivery.[14]

Statistical analysis

First, Spearman's correlation coefficients were determined to assess associations between early pregnancy maternal and umbilical cord tHcy, folate, total and active vitamin B_{12} concentrations. Second, the associations of umbilical cord tHcy, folate, total and active vitamin B_{12} concentrations with weight, length and head circumference at birth were analysed using multivariable linear regression models. Third, we assessed the associations between early pregnancy maternal tHcy, folate, total and active vitamin B_{12} concentrations and repeatedly measured fetal growth parameters ((estimated fetal) weight, head circumference and (femur) length) using unbalanced repeated measurement regression models with a unstructured covariance structure. These regression models take the correlation of multiple measurements within one subject into account and assess both the time-independent and time-dependent effect of tHcy, folate, total and active vitamin B_{12} on fetal growth. Moreover, they have an optimal use of available measurements by allowing for incomplete outcome data.[15] For all regression analyses maternal and umbilical cord tHcy, folate, total and active vitamin B_{12} concentrations were categorised as quintiles and subsequently used as a categorical measure. If available clinical

cut-off values were also used to define sufficient versus deficient. These two approaches were chosen to explore the potential nonlinearity of the association. Trend tests were alos included in the analyses. Confounding were selected as a result of exploratory analyses and included in the analyses if the effect estimates of the fetal growth parameters changed more than 5%. The multivariable models focused on early pregnancy tHcy concentrations were additionally adjusted for early pregnancy maternal folate and vitamin B_{12} concentrations. Likewise, the models focused on early pregnancy folate concentrations were additionally adjusted for maternal tHcy and total vitamin B_{12} concentrations, and the models focused on early pregnancy active and total B_{12} concentrations were additionally adjusted for maternal tHcy and folate concentrations. Umbilical cord tHcy concentrations were additionally adjusted for maternal tHcy, umbilical cord folate concentrations, and vitamin B_{12} concentrations. Umbilical cord folate concentrations were additionally adjusted for maternal folate, umbilical cord tHcy, and vitamin B_{12} concentrations. Umbilical cord active and total vitamin B_{12} concentrations were additionally adjusted for maternal vitamin B_{12}, umbilical cord tHcy, and folate concentrations. Mediation analyses were performed by calculating the percentage change of the effect estimate using the formula: $100 \times (\text{effect estimate}_{\text{mediator}} - \text{effect estimate}_{\text{confounder}})/(\text{effect estimate}_{\text{confounder}} - 1)$. A 95% confidence interval (CI) for the percentage change of the effect estimate was calculated using a bootstrap method with 1000 resamplings.[16-18] Finally, we tested the effect modification between the biomarkers by multiplying these with each other. If *P*-value <0.10 was fulfilled, additional linear regression analyses were performed.

For all analyses, missing values in covariates, that is, potential confounders, were imputed using the multiple imputation procedure. This did not apply to the biomarkers or fetal growth characteristics.[19] Details regarding the multiple imputation modelling are given in **Supplemental table 2**. Associations were considered significant at *P*-value <0.05. We performed statistical analyses using the Statistical Package of Social Sciences release 21.0 for Windows (SPSS Inc, Chicago, IL, USA). The unbalanced repeated measurements analysis, including the PROX MIXED module, was performed with the Statistical Analysis System version 9.2 (SAS, Institute Ic. Gary NC, USA) and with R version 3.0.0 (libraries rmeta and metafor; The R foundation for Statistical Computing).

RESULTS

Maternal characteristics of the study population are presented in **Table 1**. Gestational age at birth ranged from 24.9 to 43.4 with a median of 40.1 weeks. Fetal growth parameters were available in 5624 (96.8%) and 5615 (96.7%) children in mid- and late pregnancy, respectively. Early pregnancy maternal tHcy, folate, total and active vitamin B_{12} concentrations were all significantly positively correlated with the concentrations of their biomarkers in umbilical cord blood (**Supplemental table 3**).

Table 1 Baseline characteristics (n = 5890).

Maternal characteristics	
Maternal age at intake (years)	29.8 (5.1)
Gestational age at intake (weeks)	13.2 (10.5, 17.2)
Height (cm)	167.0 (7.4)
Weight (kg)	68.8 (13.1)
BMI at intake (kg/m^2)	23.7 (19.4, 33.4)
Calorie intake (kcal)	2033.2 (561.1)
Parity, nulliparous (%)	56.8
Educational level, primary (%)	9.1
Race/Ethnicity, Non-European (%)	37.3
Smoking during pregnancy, yes (%)	25.0
Folic acid supplement use, no (%)	19.0
Placental weight (g)	634.1 (145.4)
Maternal biomarker concentrations	
Gestational age at blood sampling (weeks)	13.2 (10.5, 17.2)
Maternal total homocysteine (µmol/L)	6.9 (4.9, 10.5)
Maternal folate (nmol/L)	15.7 (6.2, 34.3)
Maternal total vitamin B_{12} (pmol/L)	169.0 (83.0, 351.0)
Maternal active vitamin B_{12} (pmol/L)	42.0 (20.0, 83.0)
Fetal and newborn characteristics	
Mid-pregnancy measurements	96.9
Gestational age (weeks)	20.5 (19.1, 22.6)
Estimated fetal weight (g)	377.4 (84.4)
Femur length (mm)	33.4 (3.3)
Head circumference (mm)	178.9 (13.4)
Late pregnancy measurements	96.7
Gestational age (weeks)	30.3 (29.0, 32.2)
Estimated fetal weight (g)	1612.3 (250.5)
Femur length (mm)	57.4 (3.0)
Head circumference (mm)	284.8 (12.2)
Birth measurements	99.5
Gestational age (weeks)	40.1 (37.0, 42.0)
Weight (g)	3420.6 (564.3)
Length (mm)	509.9 (28.6)
Head circumference (mm)	349.8 (23.5)
Male sex (%)	50.6
Neonatal biomarker concentrations (%)	59.8
Umbilical cord total homocysteine (µmol/L)	9.0 (5.7, 14.8)
Umbilical cord folate (nmol/L)	20.7 (11.7, 36.2)
Umbilical cord total vitamin B_{12} (pmol/Ll)	300.5 (139.0, 731.0)
Umbilical cord active vitamin B_{12} (pmol/L)	87.0 (41.0, 128.0)

Abbreviation: Body mass index, BMI.
Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution.

Table 2 Associations of maternal total homocysteine, folate, total and active vitamin B_{12} concentrations with different birth anthropometrics.

Biomarker	Birth weight (g)	Birth length (mm)†	Head circumference (mm)†
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
tHcy (µmol/L)			
Q1 (≤5.80)	*Reference*	*Reference*	*Reference*
Q2 (5.81-6.60)	-6.2 (-39.8, 27.4)	1.0 (-0.8, 2.8)	0.9 (-0.4, 2.2)
Q3 (6.61-7.30)	-25.4 (-61.0, 10.1)	-0.9 (-2.8, 1.0)	-0.5 (-1.9, 0.9)
Q4 (7.31-8.30)	1.3 (-34.8, 37.4)	1.4 (-0.5, 3.4)	0.0 (-1.4, 1.4)
Q5 (≥8.31)	-102.3 (-139.6, -65.0)	-1.5 (-3.6, 0.5)	-1.6 (-3.1, -0.1)
P-value for trend	<0.001	0.60	0.06
Folate (nmol/L)			
Q1 (≤9.10)	-113.0 (-159.6, -66.3)	-3.3 (-5.8, -0.8)	-2.0 (-4.0, -0.1)
Q2 (9.11-13.10)	-78.5 (-117.8, -39.3)	-2.4 (-4.5, -0.3)	-1.2 (-2.7, 0.4)
Q3 (13.11-18.90)	-51.1 (-86.7, -15.5)	-1.3 (-3.1, 0.6)	-0.2 (-1.6, 1.2)
Q4 (18.91-25.80)	-36.4 (-70.5, -0.3)	-1.9 (-3.8, -0.1)	-1.3 (-2.7, 0.0)
Q5 (≥25.81)	*Reference*	*Reference*	*Reference*
P-value for trend	<0.001	0.04	0.14
Total vitamin B_{12} (pmol/L)			
Q1 (≤119.0)	42.5 (6.9, 78.0)	0.5 (-1.4, 2.5)	-0.6 (-2.0, 0.8)
Q2 (119.01-153.0)	25.8 (-9.1, 60.7)	0.0 (-1.9, 1.9)	-0.4 (-1.8, 1.0)
Q3 (153.01-189.0)	53.8 (18.8, 88.7)	1.3 (-0.6, 3.2)	0.5 (-0.9, 1.8)
Q4 (189.01-244.0)	6.7 (-28.2, 41.6)	0.2 (-1.7, 2.1)	-0.3 (-1.7, 1.0)
Q5 (≥244.01)	*Reference*	*Reference*	*Reference*
P-value for trend	0.008	0.83	0.28
Active vitamin B_{12} (pmol/L)			
Q1 (≤30)	15.6 (-25.2, 56.4)	-1.1 (-3.3, 1.1)	-0.7 (-2.3, 0.8)
Q2 (31-38)	4.8 (-36.7, 46.2)	-1.0 (-3.2, 1.2)	0.1 (-1.5, 1.6)
Q3 (39-46)	26.6 (-14.8, 68.0)	-0.9 (-3.1, 1.3)	-0.4 (-1.9, 1.2)
Q4 (47-59)	27.5 (-13.2, 68.2)	0.0 (-2.1, 2.2)	0.4 (-1.2, 1.9)
Q5 (≥60)	*Reference*	*Reference*	*Reference*
P-value for trend	0.35	0.31	0.30

Abbreviations: Total homocysteine, tHcy; Confidence interval, CI; Quintile, Q.

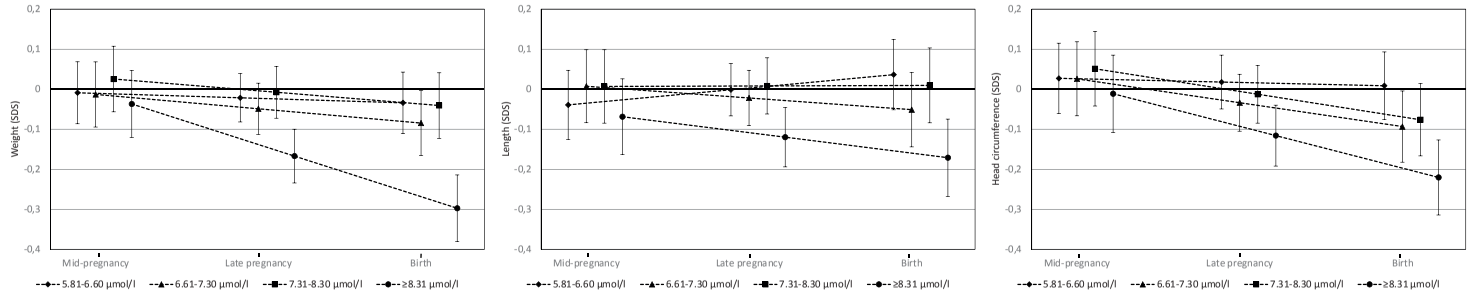
Multivariable linear regression analysis with weight, length and head circumference as dependent variables and maternal tHcy, folate, total and active B_{12} concentrations as independent variables. Q1 through Q5 represents the quintile distribution of the relative concentrations. Values are regression coefficients (95% CI) and represent the difference in weight, length or head circumference in offspring in the specific quintile compared with the reference quintile. Estimates are from multiple imputed data.

Values are adjusted for gestational age at measurement, maternal age, maternal height and body mass index, gender offspring, educational level, ethnicity, parity, smoking, calorie intake and folic acid supplement use.

Maternal tHcy concentrations are additionally adjusted for maternal folate and vitamin B_{12} concentrations. Maternal folate concentrations are additionally adjusted for maternal tHcy and vitamin B_{12} concentrations. Maternal total and active B_{12} concentrations are additionally adjusted maternal tHcy and folate concentrations.

† Combined measurements of length and head circumference at birth are additionally adjusted for post-conceptional age and origin of the data (birth or the first child health centre visit).

A. Homocysteine



B. Folate

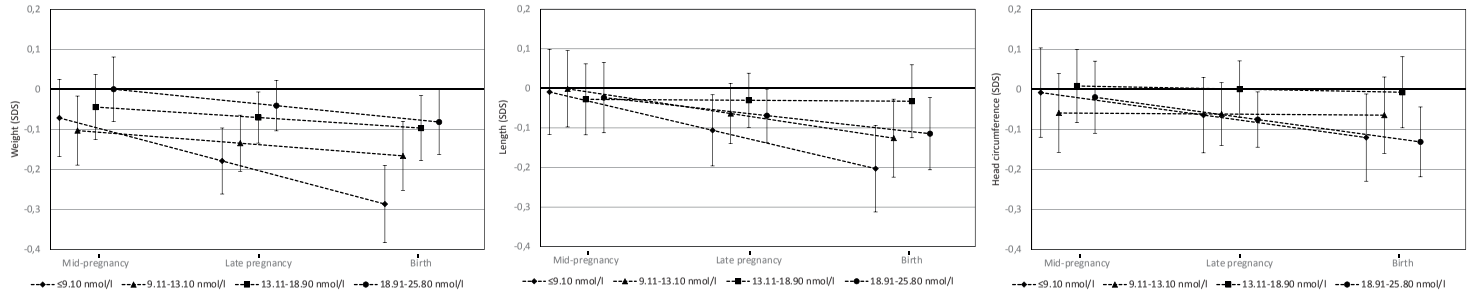


Figure 1 Associations of early pregnancy maternal total homocysteine (A) and folate (B) concentrations with fetal growth characteristics.
Abbreviations: Standard deviation score, SDS.
Results are based on repeated measurement regression models. Effect estimates (95% confidence interval represented by error bars) reflect the difference in gestational-age-adjusted SD scores of fetal weight, length and head circumference in mid- and late pregnancy and at birth among early pregnancy maternal total homocysteine (tHcy) (**A**) and folate (**B**) categories. The reference category, tHcy concentrations below 5.81 µmol/L and folate concentrations above 25.80 nmol/L, is represented by the zero-line in the graphs. Estimates are from multiple imputed data. Values are adjusted for gestational age at measurement, maternal age, maternal height and body mass index, gender offspring, educational level, ethnicity, parity, smoking, calorie intake and folic acid supplement use. Maternal tHcy concentrations are additionally adjusted for maternal folate and vitamin B_{12} concentrations. Maternal folate concentrations are additionally adjusted for maternal tHcy and vitamin B_{12} concentrations. Models with combined measures of length at birth or head circumference are additionally adjusted for the origin of data (measured at birth or at the first child health centre visit).

Compared with tHcy concentrations in the lower quintile (<5.81 μmol/L), early pregnancy maternal tHcy concentrations in the higher quintile (≥8.31 μmol/L) were associated with reduced fetal head circumference, length and weight growth patterns from late pregnancy onwards (**Figure 1A** and **Table 2**), with a smaller size at birth (difference in birth weight, length and head circumference at birth: -103.2 g (95% Confidence Interval (CI): -139.6, -65.0), -1.5 mm (95% CI: -3.6, -0.0) and -1.6 mm (95% CI: -3.1, -0.1), respectively). As compared with folate concentrations in the higher quintile (>25.80 nmol/L), early pregnancy maternal folate concentrations in the lower two quintiles (ie. ≤9.10 nmol/L and 9.11-13.10 nmol/L) were also associated with reduced fetal weight growth patterns from mid-pregnancy onwards (**Figure 1B** and **Table 2**), with a -113.0 g (95% CI: -159.6, -66.3) lower weight at birth in mothers with folate concentrations ≤9.10 nmol/L and a -78.5 g (95% CI: -117.8, -39.3) lower weight at birth

Table 3 Associations of maternal total homocysteine, folate, total and active vitamin B_{12} concentrations with birth weight.

	Birth weight	
	Beta (95% CI)	% change (95% CI)
tHcy (μmol/L)		
Model 1	-12.8 (-18.1, -7.4)	
Model 2	-6.1 (-10.6, -1.6)	-50.9 (-60.4, -42.9)
Folate (nmol/L)		
Model 1	3.9 (2.3, 5.5)	
Model 2	2.6 (1.1, 4.0)	-34.5 (-43.3, -26.0)
Total vitamin B_{12} (pmol/L)		
Model 1	-0.2 (-0.3, -0.0)	
Model 2	-0.2 (-0.3, -0.01)	-3.6 (-19.9, 16.8)
Active vitamin B_{12} (pmol/L)		
Model 1	-0.3 (-1.0, 0.3)	
Model 2	-0.5 (-1.0, 0.1)	NA

Abbreviations: Total homocysteine, tHcy; Confidence interval, CI.
Multivariable linear regression analysis with birth weight as dependent variables and maternal tHcy, folate, total and active B_{12} concentrations as independent variables. Estimates are from multiple imputed data.
Model 1: adjusted for gestational age at measurement, maternal age, maternal height and body mass index, gender offspring, educational level, ethnicity, parity, smoking, calorie intake and folic acid supplement use.
Maternal tHcy concentrations additionally adjusted for maternal folate and vitamin B_{12} concentrations.
Maternal folate concentrations additionally adjusted for maternal tHcy and vitamin B_{12} concentrations.
Maternal total and active vitamin B_{12} concentrations additionally adjusted for maternal tHcy and folate concentrations.
Model 2: model 1 and additionally adjusted for placental weight.
In the intermediate model, the percentage change of the effect estimate was calculated by the formula: $100 \times (\text{effect estimate}_{\text{mediator}} - \text{effect estimate}_{\text{confounder}})/(\text{effect estimate}_{\text{confounder}} - 1)$. A 95% confidence interval (CI) for the percentage change of the effect estimate was calculated using a bootstrap method with 1000 resamplings.

in mothers with folate concentrations 9.11-13.10 nmol/L. Similar trends regarding maternal folate concentrations were observed for the other folate quintile categories and for fetal head circumference and length growth patterns.

When looking at clinical cut-off values, deficient early pregnancy folate concentrations (i.e. <8nmol/L) were also associated with lower birth weight with a 57.1 g difference (95% CI: -95.5, -18.8) (**Supplemental table 4**). No associations were found between total and active vitamin B_{12} concentrations and fetal growth (**Supplemental figure 1 and 2**).

Table 3 shows that the associations between maternal tHcy and folate concentrations and fetal growth were modified through placental weight. Almost 50% of the associations between maternal early pregnancy folate concentrations and birth weight were explained by the intermediate effect of folate on placental weight.

The results presented in **Table 4** show the associations of umbilical cord tHcy, folate, total and active vitamin B_{12} concentrations with weight, length and head circumference at birth. Compared with newborns with tHcy concentrations in the lower quintile (≤7.20 μmol/L), tHcy concentrations in the quintiles above (9.61 μmol/L) were associated with a significantly smaller head circumference. Folate concentrations in the lower quintile (≤15.20 nmol/L) were associated with a lower birth weight and birth length (-122.0 g, 95% CI: -168.1, -75.8 and -3.1 mm, 95% CI: -5.5, -0.7) as compared with newborns with folate concentrations in the highest quintile (28.41 nmol/L). In contrast to what would be expected total and active vitamin B_{12} concentrations in newborns were inversely associated with all birth anthropometrics. Finally, we did not find a significant interaction between folate concentrations and total and active vitamin B_{12} concentrations.

DISCUSSION

Our findings show that higher early pregnancy maternal tHcy and lower folate concentrations are adversely associated with fetal growth patterns. These differences are mediated through placenta weight. Additionally, newborns with higher tHcy concentrations have a lower head circumference and newborns with lower folate concentrations have a lower birth weight and length. Remarkably, newborns with lower total or active vitamin B_{12} concentrations have a higher weight, length and head circumference at birth.

Methodological considerations

Biomarker studies are limited by biological interactions, collinearity, and residual confounding. We did not have information on the role of intrinsic factor in vitamin B_{12} absorption in the body for our study population. Nor did we have the availability of the prevalence of intrinsic factor antibodies in the assay, known to influence vitamin B_{12} concentrations. However, the assay that we used automatically neutralises potential native intrinsic factor antibodies.[11]

Table 4 Associations of umbilical cord total homocysteine, folate, total and active vitamin B_{12} concentrations with different birth anthropometrics.

Biomarker	Birth weight (g)	Birth length (mm)[†]	Head circumference (mm)[†]
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
tHcy (µmol/L)			
Q1 (≤7.2)	*Reference*	*Reference*	*Reference*
Q2 (7.21-8.40)	-7.1 (-49.3, 35.1)	-1.4 (-3.7, 0.9)	-1.7 (-3.4, 0.0)
Q3 (8.41-9.60)	-16.3 (-58.6, 26.0)	-0.8 (-3.1, 1.4)	-0.9 (-2.6, 0.8)
Q4 (9.61-11.40)	13.3 (-30.2, 56.7)	0.2 (-2.2, 2.5)	-2.5 (-4.2, -0.7)
Q5 (≥11.41)	-6.6 (-52.3, 39.1)	-1.1 (-3.6, 1.4)	-3.0 (-4.9, -1.2)
P-value for trend	0.75	0.87	0.02
Folate (nmol/L)			
Q1 (≤15.20)	-122.0 (-168.1, -75.8)	-3.1 (-5.5, -0.7)	-0.8 (-2.6, 1.0)
Q2 (15.21-18.80)	-81.1 (-126.4, -35.9)	-2.6 (-5.1, -0.2)	-0.4 (-2.1, 1.4)
Q3 (18.81-22.80)	-82.5 (-125.6, -38.4)	-1.7 (-4.1, 0.6)	-1.0 (-2.7, 0.8)
Q4 (22.81-28.40)	-45.5 (-88.3, -2.8)	-1.2 (-3.5, 1.1)	-0.1 (-1.7, 1.6)
Q5 (≥28.41)	*Reference*	*Reference*	*Reference*
P-value for trend	<0.001	0.001	0.09
Total vitamin B_{12} (pmol/L)			
Q1 (≤201)	258.1 (209.1, 307.2)	6.1 (3.5, 8.7)	4.8 (2.9, 6.7)
Q2 (202-266)	177.7 (131.6, 223.9)	4.0 (1.5, 6.5)	2.8 (1.0, 4.6)
Q3 (267-339)	165.1 (120.2, 210.0)	3.6 (1.2, 6.0)	2.4 (0.6, 4.2)
Q4 (340-459)	127.7 (83.5, 171.8)	1.8 (-0.5, 4.2)	1.9 (0.1, 3.6)
Q5 (≥460)	*Reference*	*Reference*	*Reference*
P-value for trend	<0.001	<0.001	0.001
Active vitamin B_{12} (pmol/L)			
Q1 (≤59)	224.9 (163.0, 286.8)	7.1 (3.8, 10.5)	4.7 (2.3, 7.1)
Q2 (60-78)	130.4 (71.5, 189.3)	3.9 (0.7, 7.0)	2.8 (0.5, 5.1)
Q3 (79-96)	146.4 (90.7, 202.0)	3.7 (0.7, 6.6	1.9 (-0.3, 4.1)
Q4 (97-124)	122.4 (69.2, 175.6)	2.6 (-0.2, 5.4)	2.6 (0.6, 4.7)
Q5 (≥125)	*Reference*	*Reference*	*Reference*
P-value for trend	<0.001	<0.001	<0.001

Abbreviations: Total homocysteine, tHcy; Quintile, Q; Confidence interval, CI.

Multivariable linear regression analysis with weight, length and head circumference as dependent variables and umbilical cord tHcy, folate, total and active B_{12} concentrations as independent variables. Q1 through Q5 represents the quintile distribution of the relative concentrations. Values are regression coefficients (95% CI) and represent the difference in weight, length or head circumference in offspring in the specific quintile compared with the reference quintile. Estimates are from multiple imputed data.

Values are adjusted for gestational age at measurement, maternal age, maternal height and body mass index, gender offspring, educational level, ethnicity, parity, smoking, calorie intake and folic acid supplement use.

Umbilical cord tHcy concentrations are additionally adjusted for maternal tHcy, umbilical cord folate and vitamin B_{12} concentrations.

Umbilical cord folate concentrations are additionally adjusted for maternal folate, umbilical cord tHcy and vitamin B_{12} concentrations.
Umbilical cord total B_{12} concentrations are additionally adjusted for maternal total B_{12}, umbilical cord tHcy and folate concentrations.
Umbilical cord active B_{12} concentrations are additionally adjusted for maternal active B_{12}, umbilical cord tHcy and folate concentrations.
† Combined measurements of length and head circumference at birth are additionally adjusted for post-conceptional age and origin of the data (birth or the first child health centre visit).

Furthermore, by testing effect modification between folate and vitamin B_{12} concentrations and by adjusting biomarkers for each other and for calorie intake, we tried to account for potential biological interactions of residual confounding. Nevertheless, we cannot rule out that the results were caused by other unmeasured factors.

Strengths of this study were the prospective design, the significant number of measurements and the large number of detailed information available. However, from approximately 30% of the mothers, an early pregnancy blood sample was not obtained as they were enrolled later in pregnancy. Also data were more complete in higher educated mothers. The underlying mechanism is most likely selective nonresponse which only harms the validity of the study if the association between determinant and outcome differs between those included and excluded from the study. Two birth cohorts from Scandinavia were able to compare well-established associations between participants included and excluded from the study, and similar associations were found.[20, 21] Therefore, selection bias seems unlikely.[20]

Interpretation

We observed a negative association between early pregnancy maternal tHcy concentrations and fetal weight, length and head circumference in pregnancy. Previous studies mainly focused on the association between maternal tHcy concentrations and birth weight and showed inconsistent associations.[3, 7, 22-25] Sample size, different cut-offs for birth weight, confounders, and timing of maternal blood sampling may have contributed to these conflicting results. Also, birth weight is the end point of different fetal growth patterns and cannot provide information regarding growth across different time periods in pregnancy. Neonates can reach the same birth weight by different fetal growth trajectories.[26]

Total homocysteine concentrations were significantly higher in umbilical cord blood as compared to the maternal circulation. This finding is in contrast to earlier studies who reported lower or similar tHcy concentrations in umbilical cord blood. Maternal concentrations tend to decline (up to 50% compared to non-pregnant women) in the first and mid-pregnancy as a result of physiological responses to pregnancy, such as hemodilution, and a period of increased remethylation of tHcy due to increased demands for methionine by the fetus.[27] This, however, is a transitory situation, with tHcy returning to initial values at the end of pregnancy.[7] This may, in part, explain these conflicting results. We suggest that maternal tHcy is an important predictor of tHcy concentrations in the developing fetus which is supported by the

results of Malinow *et al*.[28] They found a descending concentration gradient from maternal to umbilical venous to umbilical arterial circulations and proposed that tHcy in the fetus arises from maternal blood. We also observed that newborns with high circulating tHcy concentrations had a lower head circumference and, although not significant, tended to have a lower birth weight and birth length. Different explanations may apply towards the effects of high tHcy concentrations in both the maternal and neonatal circulation and fetal growth. Elevated tHcy concentrations are associated with cytotoxic and oxidative stress consequently leading to endothelial cell impairment in possibly both the placental vasculature and endothelial cells in the developing fetus.[29] Also, growing evidence suggests that excessive tHcy may increase cellular apoptosis leading to the inhibition of trophoblastic function.[30] It is plausible that these metabolic events affect fetal growth directly and also indirectly through placental vascular dysfunction since these processes both involve rapid cell division.

Humans do not have the ability to synthesize folate. The demand for folate is therefore entirely reliant on dietary intake. This explains the significant correlation between maternal and umbilical cord folate concentrations in our study since the fetus is completely dependent on the maternal folate supply. In addition, folate demand increases during pregnancy, partially because of the critical importance of folate in the tHcy metabolism, but also as a consequence of increased DNA synthesis, cellular division and proliferation, and the growth and development of both the placenta and the fetus. It is, therefore, not surprising that folate concentrations are higher in umbilical cord blood, which is attributable to an active transport of folate across the placenta mediated by three different placental folate receptors.[31]

Previous studies found inconsistent results with regard to the association between maternal vitamin B_{12} status and birth weight.[32, 33] Maternal vitamin B_{12} concentrations tended to be positively related to weight and length growth. We observed a highly significant inverse relation between total and active vitamin B_{12} concentrations in the neonatal circulation and weight, length and head circumference at birth. The same results were found by a number of earlier studies.[8, 9, 34] They were unable to provide an explanation regarding possible underlying mechanism for this remarkable phenomenon. Likewise, we can also only speculate about the potential underlying mechanism. Perhaps, reverse causality could have palyed a role. In this concept, it is not the higher amount of vitamin B_{12} that is associated with larger fetal growth parameters but rather the idea that larger babies require larger amounts of vitamin B_{12} , that is, they have utilized more vitamin B_{12} in their tissues. This would then be presented by the observed inverse association. To test this, a Mendelian randomisation model could be applied. However to do so, larger numbers are required. Further studies are needed to disentangle possible underlying mechanisms.

Finally, the early pregnancy period is critical for placental development, embryogenesis, and embryonic programming. Relatively little is known about the implications of maternal nutrition during this particular period on the developing fetus. Previously, others from our study group reported on positive associations between maternal folic acid supplementation and

folate levels in early pregnancy and increased early fetal size. This emphasizes the importance of the early pregnancy period on fetal growth throughout pregnancy.[35, 36]

CONCLUSIONS

Our study showed that higher early pregnancy maternal tHcy and lower folate concentrations are associated with impaired fetal growth patterns and correlated with tHcy and folate concentrations in umbilical cord blood. Especially, low umbilical cord folate concentrations were associated with a lower birth weight in offspring. The relevance of our findings should also be considered against the background that newborns with impaired growth and compensatory accelerated postnatal growth are at risk for metabolic and cardiovascular disease in later life.[37] Therefore, long-term prospective studies from pregnancy throughout adulthood are required to determine possible consequences in later life.

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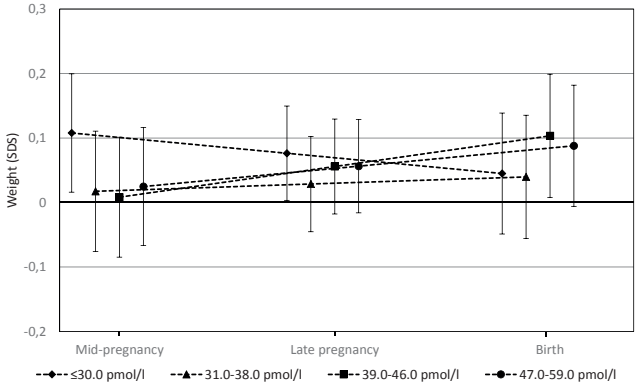
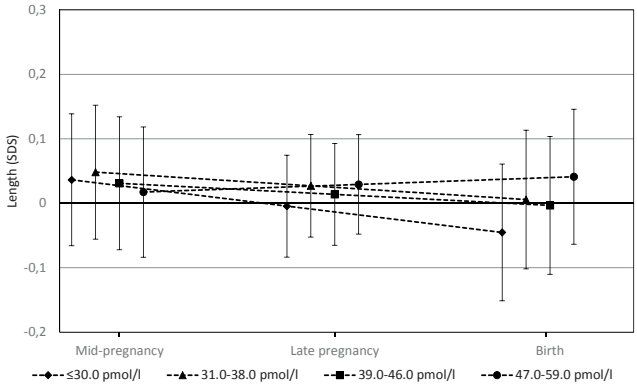
SUPPLEMENTAL MATERIAL

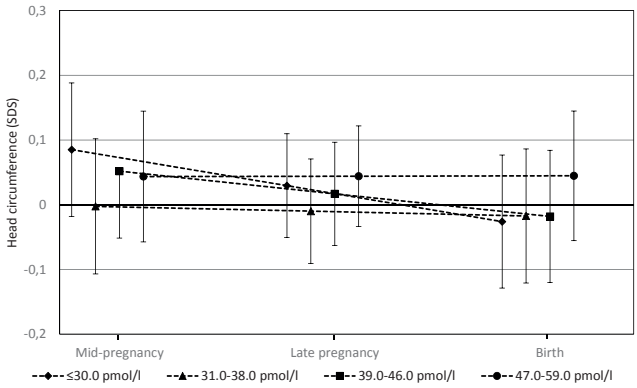
A. Weight



B. Length



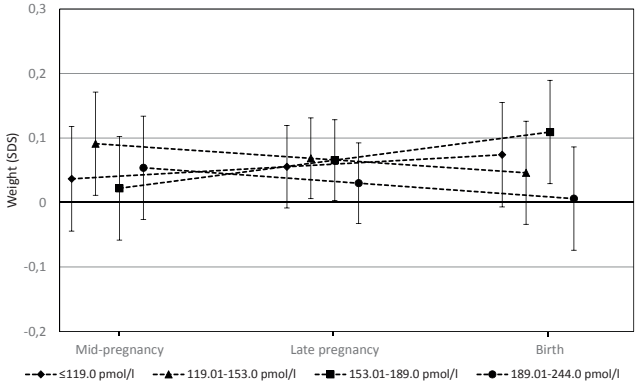
C. Head circumference



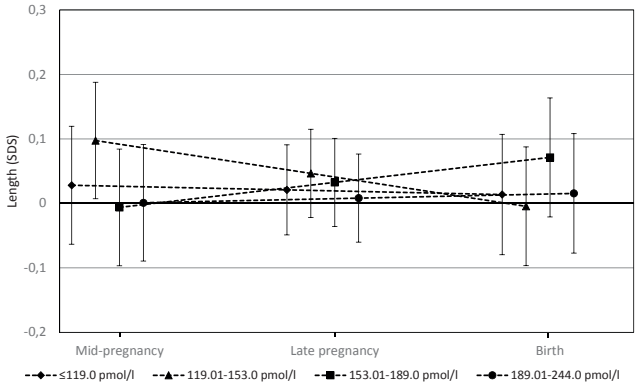
Supplemental figure 1 Associations of early pregnancy maternal total vitamin B_{12} concentrations with fetal growth characteristics.
Abbreviations: Standard deviation score, SDS.

Results are based on repeated measurement regression models. Effect estimates (95% confidence interval represented by error bars) reflect the difference in gestational-age-adjusted SD scores of fetal weight, length and head circumference in mid- and late pregnancy and at birth among early pregnancy maternal total vitamin B_{12} categories. The reference category, total vitamin B_{12} concentrations above 244.0 pmol/L, is represented by the zero-line in the graphs. Estimates are from multiple imputed data.
Values are adjusted for gestational age at measurement, maternal age, maternal height and body mass index, gender offspring, educational level, ethnicity, parity, smoking, calorie intake and folic acid supplement use. Total vitamin B_{12} concentrations are additionally adjusted for maternal total homocysteine and folate concentrations.
Models with combined measures of length at birth or head circumference are additionally adjusted for the origin of data (measured at birth or at the first child health centre visit).

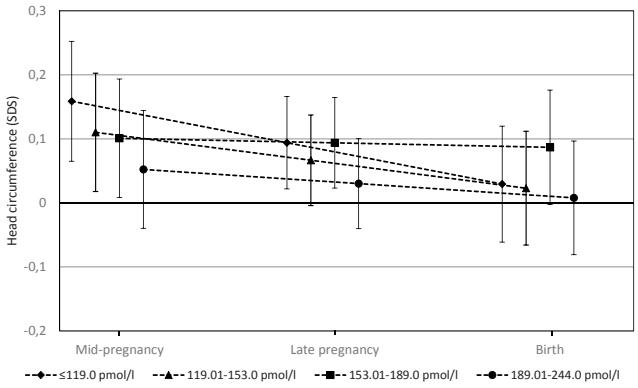
A. Weight



B. Length



C. Head circumference



Supplemental figure 2 Associations of early pregnancy maternal active vitamin B_{12} concentrations with fetal growth characteristics.
Abbreviations: Standard deviation score, SDS.

Results are based on repeated measurement regression models. Effect estimates (95% confidence interval represented by error bars) reflect the difference in gestational-age-adjusted SD scores of fetal weight, length and head circumference in mid- and late pregnancy and at birth among early pregnancy maternal active vitamin B_{12} categories. The reference category, active vitamin B_{12} concentrations above 60.0 pmol/L, is represented by the zero-line in the graphs. Estimates are from multiple imputed data.
Values are adjusted for gestational age at measurement, maternal age, maternal height and body mass index, gender offspring, educational level, ethnicity, parity, smoking, calorie intake and folic acid supplement use. Active vitamin B_{12} concentrations are additionally adjusted for maternal total homocysteine and folate concentrations.
Models with combined measures of length at birth or head circumference are additionally adjusted for the origin of data (measured at birth or at the first child health centre visit).

Supplemental table 1 The between-run coefficients of variation for total homocysteine, folate, total vitamin B_{12} and active vitamin B_{12} concentrations.

	Between run coefficient		**Between run coefficient**		**Between run coefficient**		**Analytic range**
	First		Second		Third		
	%		%		%		
Plasma tHcy	3.1	7.2 µmol/L	3.1	12.9 µmol/L	2.1	26.1 µmol/L	1-50 µmol/L
Plasma folate	8.9	5.6 nmol/L	2.5	16.6 nmol/L	1.5	33.6 nmol/L	1.8-45.3 nmol/L
Serum total vitamin B_{12}	3.6	142 pmol/L	7.5	308 pmol/L	3.1	633 pmol/L	44-1476 pmol/L
Serum active vitamin B_{12}	8.2	38.2 pmol/L	9.8	109.9 pmol/L		-	5-128 pmol/L

Abbreviation: Total homocysteine, tHcy.

Supplemental table 2 Details of the multiple imputation modelling.

Sofware used	SPSS 21.0 for windows (SPSS Inc, Chicago, IL, USA)
Imputation method and keysettings	Fully conditional specification (Markov chain Monte Carlo method); Maximum iterations: 10;
Number of imputed data sets created	5
Variable included to be imputed and used as predictor	maternal weight before pregnancy, maternal weight at intake, maternal height, systolic blood pressure, diastolic blood pressure, haemoglobin, calorie intake, maternal educational level, maternal ethnicity, ethnicity of the child, parity, breastfeeding, maternal smoking, maternal alcohol use and caffeine intake, folic acid supplement use, maternal comorbidity, preeclampsia, gestational diabetes, pregnancy induced hypertension, maternal fertility treatment, maternal marital status, netto income household, placental weight.
Variables included and only used as predictor	Maternal age, gravidity, gender of the child, head circumference, abdominal circumference, femur length, estimated fetal weight and gestational age at ultrasound measurement in mid- and late pregnancy; birth weight, head circumference, length and gestational age at birth; gestational age at blood sampling; maternal early pregnancy folate, homocysteine, sFlt-1, PlGF, PAI-2, total and active vitamin B_{12} concentrations; maternal mid-pregnancy sFlt-1 and PlGF concentrations; neonatal homocysteine, folate, sFlt-1, PlGF and total vitamin B_{12} concentrations.
Treatment of none normally distributed variables	Log-transformation: maternal early pregnancy folate, homocysteine, sFlt-1, PlGF, PAI-2, total and active vitamin B_{12} concentrations; maternal mid-pregnancy sFlt-1 and PlGF concentrations; neonatal homocysteine, folate, sFlt-1, PlGF and total vitamin B_{12} concentrations. Quadratic-transformation: gestational age at birth
Treatment of binary/categorical variables	Logistic regression and multinomial models
Statistical interaction included in the imputation models	None

Abbreviations: soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF; Plasminogen activator inhibitor type 2, PAI-2.

Supplemental table 3 Spearman's rank correlation coefficients between maternal and umbilical cord total homocysteine, folate, total and active B_{12} concentrations.

	Maternal concentrations				Umbilical cord concentrations			
	tHcy (µmol/L)	Folate (nmol/L)	Total vitamin B_{12} (pmol/L)	Active vitamin B_{12} (pmol/L)	tHcy (µmol/L)	Folate (nmol/L)	Total vitamin B_{12} (pmol/L)	Active vitamin B_{12} (pmol/L)
Maternal concentrations								
tHcy (µmol/L)	1							
Folate (nmol/L)	-0.29**	1						
Total vitamin B_{12} (pmol/L)	-0.23**	0.16**	1					
Active vitamin B_{12} (pmol/L)	-0.28**	0.18**	0.65**	1				
Umbilical cord concentrations								
tHcy (µmol/L)	0.39**	-0.21**	-0.26**	-0.31**	1			
Folate (nmol/L)	-0.17**	0.40**	0.12**	0.14**	-0.30**	1		
Total vitamin B_{12} (pmol/L)	-0.20**	0.09**	0.49**	0.51**	0.33**	0.15**	1	
Active vitamin B_{12} (pmol/L)	-0.20**	0.08**	0.38**	0.61**	-0.38**	0.19**	0.70**	1

Abbreviations: Total homocysteine, tHcy.

** *P*-value < 0.001

Supplemental table 4 Associations of **maternal** total homocysteine, folate, total and active vitamin B_{12} clinical cut-off values with birth weight.

Biomarkers	**Birth weight**
	n = 5890
	Beta (95% CI)
tHcy	
≤22 µmol/L (n = 5878)	*Reference*
>22 µmol/L (n = 12, 0.2%)	-49.7 (-284.8, 185.5)
Folate	
<8 nmol/L (n = 807, 13.7%)	-57.1 (-95.5, -18.8)
≥8 nmol/L (n = 5052)	*Reference*
Total vitamin B_{12}	
<145 pmol/L (n = 1935, 32.9%)	4.5 (-19.1, 28.1)
≥145 pmol/L (n = 3581)	*Reference*
Active vitamin B_{12}	
<21 pmol/L (n = 207, 3.5%)	-20.3 (-80.1, 39.6)
≥21 pmol/L (n =3873)	*Reference*

Abbreviations: Total homocysteine, tHcy.
Multivariable linear regression analysis with birth weight as dependent variables and maternal tHcy, folate, total and active B_{12} concentrations as independent variables. Estimates are from multiple imputed data.
Values are adjusted for gestational age at measurement, maternal age, maternal height and body mass index, gender offspring, educational level, ethnicity, parity, smoking, calorie intake and folic acid supplement use.
Maternal tHcy concentrations are additionally adjusted for maternal folate and vitamin B_{12} concentrations.
Maternal folate concentrations are additionally adjusted for maternal tHcy and vitamin B_{12} concentrations.
Maternal total and active vitamin B_{12} concentrations are additionally adjusted for maternal tHcy and folate concentrations.

Chapter 6

Early pregnancy maternal and fetal angiogenic factors and fetal and childhood growth

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Adapted from Human Reprod. 2015;30:1302-13

ABSTRACT

Objective: An imbalance in maternal and fetal soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) concentrations has been suggested to affect pregnancy outcomes. However, their effects on longitudinal fetal and childhood growth remain largely unknown. In this study we investigated associations of maternal and fetal sFlt-1 and PlGF concentrations with repeatedly measured fetal and childhood growth parameters.

Methods: This study was performed in 5980 mothers and 4108 of their children, participating in the Generation R Study; a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands (2001-2005). Blood samples were obtained from mothers in early and mid-pregnancy and from the umbilical vein at delivery. Fetal and childhood growth characteristics (weight and length) were measured repeatedly by ultrasound and physical examinations until the age of 6 years. We assessed the associations of maternal and fetal angiogenic factors with fetal and childhood growth using repeated measurement regression models. Logistic regression models were used to determine associations between angiogenic factors and small for gestational age at birth (SGA).

Results: Compared with early pregnancy maternal sFlt-1 concentrations in the lowest quintile, early pregnancy maternal sFlt-1 concentrations in the highest quintile were associated with a higher fetal weight growth resulting in a higher birth weight (difference in birth weight 0.33 standard deviation score (SDS); (95% Confidence Interval (CI) 0.25-0.41), a lower risk of SGA (Odds Ratio (OR) 0.36; 95% CI 0.27-0.48) and a subsequent higher weight growth until the age of 6 years. Early pregnancy maternal PlGF concentrations in the lowest quintile were associated with a reduced weight growth pattern resulting in a smaller birth weight (difference in birth weight -0.34 SDS; 95% CI -0.44, -0.25), an increased risk of SGA (OR 3.48; 95% CI 2.39-5.08) and a lower weight growth throughout childhood. An early pregnancy maternal sFlt-1:PlGF ratio in the highest quintile was associated with a higher fetal weight growth pattern from 30 weeks onwards, resulting in a higher weight at birth (difference in birth weight 0.09 SDS; *P*-value <0.05), which remained present until the age of two years. Newborns with higher umbilical cord sFlt-1 concentrations, lower PlGF concentrations or a higher sFlt-1:PlGF ratio showed a lower fetal and childhood weight growth from 30 weeks gestation onwards until the age of 6 years (*P*-value <0.05). Similar patterns were observed in relation to fetal and childhood length growth.

Conclusions: An angiogenic profile that is characterised by both low early pregnancy maternal sFlt-1 and PlGF concentrations and higher sFlt-1 concentrations, lower PlGF concentrations or a higher sFlt-1:PlGF ratio in umbilical cord blood is associated with a reduced fetal and childhood growth. Both a maternal and fetal angiogenic imbalance may affect fetal and

childhood growth. Changes in angiogenic profiles may be involved in the pathways linking fetal growth restriction with the long-term risk of vascular disease in adulthood.

INTRODUCTION

Impaired fetal growth is associated with neonatal morbidity and mortality[1] and a subsequent risk of developing cardiovascular disease in later life.[2, 3] Early placental development is of great importance for normal fetal growth and development.[4] Placental development comprises both vasculogenesis and angiogenesis.[5, 6] Within these processes, the vascular endothelial growth factor (VEGF) system is essential.[5] Placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) are included in the VEGF-system. PlGF is produced by endothelial cells, cyto- and syncytiotrophoblasts and binds to VEGF receptor 1 (VEGFR-1 or Flt-1), thereby promoting proliferation, migration and activation of endothelial cells. sFlt-1 is the soluble form of Flt-1 and is synthesised by the trophoblast of the placenta,[7] but also made in cells outside the placenta such as endothelial cells and monocytes.[8, 9] sFlt-1 binds PlGF, thereby reducing the free circulating concentrations of PlGF.[10, 11] Low maternal PlGF concentrations have been related to adverse maternal and fetal outcomes.[12, 13] Results regarding the associations of sFlt-1 with fetal growth seem not consistent.[11] One of our previous studies focused on the maternal circulation and pregnancy outcome.[13] Only a few small studies evaluated the associations between maternal and fetal PlGF and sFlt-1 concentrations and intrauterine growth restriction.[14, 15] Angiogenesis is not only essential for early placental development, but also crucial for organ growth and cardiovascular development in the embryo.[16] However, little is known about the impact of sFlt-1 and PlGF in the fetal circulation on the development of the cardiovascular system and subsequent fetal and childhood growth.

In the present study, we therefore examined associations of both maternal and umbilical cord PlGF and sFlt-1 concentrations with small for gestational age at birth (SGA) and repeatedly measured fetal and childhood size measurements to estimate growth.

METHODS

Design and study population

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[17] In total, 8880 mothers were enrolled during their pregnancy. We excluded pregnancies not leading to singleton live births (n = 197), loss to follow-up (n = 45), pregnancies that ended before a gestational age of 24 weeks (n = 10) and mothers without any fetal growth measurements (n = 3). For the growth analysis with the maternal sFlt-1 and PlGF concentrations we additionally excluded mothers without sFLt-1 or PlGF concentrations available in early pregnancy (n = 2645), leading to 5980 mothers available for analysis (**Supplemental figure 1**). For the growth analysis with umbilical cord data we excluded children without sFlt-1 or PlGF concentrations available from umbilical cord blood (n = 4517), leading to 4108 children available for analysis

(**Supplemental figure 1**). The study has been approved by the Medical Ethics Committee of the Erasmus MC, University Medical Centre, Rotterdam, the Netherlands. Written informed consent was obtained from all mothers for both maternal and child data.

Agiogenic factors

In early (median 13.2 weeks of gestation, 90% range 10.5-17.2) and mid-pregnancy (median 20.4 weeks of gestation, 90% range 19.1-22.4) maternal venous blood plasma samples were drawn. Immediately following delivery (median 40.1 weeks of gestation, 90% range 37.4-42.0), 30 mL cord blood from the umbilical vein was collected. The samples were temporally stored at room temperature before being transported to the regional laboratory for processing and storage for future studies. Processing was planned to finish within a maximum of 3 hours after venous puncture. Details of processing procedures have been described previously.[18] Blood samples were centrifuged and thereafter stored at -80°C. To analyse sFlt-1 and PlGF, ethylenediaminetetraacetic acid (EDTA) plasma samples were picked and transported to the Department of Clinical Chemistry of the Erasmus Medical Centre, Rotterdam. After thawing sFlt-1 and PlGF concentrations were analysed using a prototype of a microparticle-enhanced immunoassay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, the Netherlands). The between-run coefficients of variation and analytical ranges are listed in **Supplemental table 1**.

Fetal and childhood growth measurements

Fetal ultrasound examinations were carried out in two dedicated research centres in early, mid- and late pregnancy. We established gestational age by using data from the first ultrasound examination.[19] In mid- and late pregnancy, fetal head circumference, abdominal circumference, and femur length (FL) were measured to the nearest millimetre using standardised ultrasound procedures. Estimated fetal weight (EFW) was subsequently calculated by using formula of Hadlock.[20] Gestational-age-adjusted standard deviation scores (SDS) of all fetal growth characteristics were constructed based on data from the study group.[19] Information about date of birth, fetal gender, birth weight and length was obtained from medical records and hospital registries. Gestational-age-adjusted SDS for birth weight and length were constructed using North European growth Standards.[21] SGA at birth was defined as a sex and gestational age adjusted birth weight below the 10th percentile (<-1.39 SDS).

Well-trained staff in the Community Health Centres obtained postnatal growth characteristics according to standard schedule and procedures at the ages of 3 months, 6 months, 12 months, 24 months, 36 months, 48 months and 72 months. SDS for childhood growth characteristics were obtained with Dutch growth reference Charts (Growth Analyser 3.0; Dutch Growth Research Foundation, Rotterdam, Netherlands). Catch-up growth was defined as a gain in SDS for weight greater than 0.67 SDS between birth and two years.[22]

Covariates

Information on maternal age, ethnicity, education, maternal comorbidity (defined as the occurrence of chronic hypertension and/or heart disease and/or diabetes and/or high cholesterol and/or thyroid disease and/or Systemic Lupus Erythematosus and/or arthritis) parity and folic acid supplement use was obtained from self-administrated questionnaires at enrolment. Education was assessed by the highest completed education of the mother. Information on maternal smoking habits was obtained by questionnaires.[17] Maternal weight and height were measured at intake to calculate body mass index (BMI, kg/m^2). Information on fertility treatment was obtained from community midwives and obstetricians. Maternal blood pressure was measured at enrolment. The mean value of two blood pressure readings over a 60-second interval was documented.[23] Information about the presence of preeclampsia, pregnancy induced hypertension and gestational diabetes was retrieved from medical records after delivery.[24] Information about breastfeeding was obtained by questionnaires in infancy.

Statistical analysis

First, we performed nonresponse analyses for the analysis in the mothers and children separately. Differences were tested using Student's *t* test, Mann-Whitney's U-test and chi-square test. Second, correlations between sFlt-1 and PlGF in maternal and umbilical cord blood were determined with the use of Spearman's rank correlation coefficients.

Third, early to mid-pregnancy changes in sFlt-1 and PlGF (difference in concentrations / gestational weeks interval) was referred to as delta sFlt-1 and delta PlGF, respectively. Fourth, maternal and umbilical cord sFlt-1:PlGF ratio were calculated ((sFlt-1×1000):PlGF) in early and mid-pregnancy and at birth.

Subsequently, we explored the associations of maternal and umbilical cord sFlt-1 and PlGF concentrations with repeatedly measured fetal and childhood growth characteristics ((estimated fetal) weight and (femur) length) using unbalanced repeated measurement regression models with an unstructured covariance structure. These regression models take the correlation of multiple measurements within one subject into account and assess both the time-independent and time-dependent effect of sFlt-1 and PlGF on fetal and childhood growth. Moreover, they have an optimal use of available measurements by allowing for incomplete outcome data.[25]

Finally, we examined the associations of maternal and umbilical cord sFlt-1 and PlGF concentrations with SGA by using logistic regression models. Similar analyses were performed for the associations between maternal and umbilical cord sFlt-1 and PlGF concentrations and catch-up growth. For all regression analyses, maternal and umbilical cord sFlt-1 and PlGF concentrations and sFlt-1:PlGF ratio, as well as maternal delta sFlt-1 and PlGF concentrations were categorised as quintiles and subsequently used as a categorical measure. Based on previous studies suggesting that lower sFlt-1 and higher PlGF concentrations are associated with a lower risk of adverse pregnancy outcomes, we used as reference groups the lowest quintiles

for early pregnancy maternal sFlt-1; delta sFlt-1; early and mid-pregnancy sFlt-1:PlGF ratio; umbilical cord sFlt-1; umbilical cord sFlt-1:PlGF ratio and the highest quintiles for early pregnancy maternal PlGF; delta PlGF; umbilical cord PlGF.

Confounding variables were determined a priori and based on previous literature.[13, 26] Potential confounders were then selected as a result of exploratory analysis and included in the analysis if the effect estimates changed >10%. By using this approach maternal age, folic acid supplement use, maternal height and BMI, systolic blood pressure, education, ethnicity, parity, smoking and fetal gender were included in the final analysis. Models focused on childhood growth outcomes were additionally adjusted for age at visit and breastfeeding. Missing data of the covariates were completed using the Markov Chain Monte Carlo multiple imputation technique.[27] The percentages of missing values within the population for analysis were lower than 12 %, except for folic acid supplement use (24.3%). To explore the effect of preeclampsia, pregnancy induced hypertension, gestational diabetes and maternal comorbidity on the association between angiogenic factors and fetal and childhood growth, we excluded these mothers and subsequently assessed the repeated measurements regression models. Since exclusion of these mothers did not materially change our results, they were included in the analysis. Associations were considered significant at *P*-value <0.05. The unbalanced repeated measurements regression analyses were performed with the Statistical Analysis System version 9.3 (SAS, Institute Inc. Cary NC, USA). The remaining analyses were performed using the Statistical Package of Social Sciences release 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Maternal and child characteristics are presented in **Table 1**. Fetal and childhood growth characteristics are presented in **Table 2**. **Supplemental table 2** presents the correlations between maternal sFlt-1 and PlGF concentrations in early and mid-pregnancy and also the correlation between umbilical cord sFlt-1 and PlGF concentrations as well as the correlations between these biomarkers in the maternal and fetal (umbilical cord) circulation. In **Supplemental table 3 and 4** nonresponse analysis are presented. Mothers included in the analysis were on average taller, had a lower BMI, were more often nulliparous and of western ethnicity, were higher educated and gave birth to larger babies.

Figure 1A shows that mothers with higher sFlt-1 concentrations in early pregnancy had a higher fetal weight growth from 20 weeks onwards, resulting in a significantly higher birth weight when compared with mothers in the reference group. Children of these mothers remained heavier until the age of 6 years, although this difference decreased with increasing age. **Figure 1C** shows that an increase in maternal sFlt-1 concentrations from early to mid-pregnancy resulted in lower fetal weight growth from 30 weeks onwards. However, in contrast to children of mothers in the fourth and third quintile, children of mothers with the highest

Table 1 Baseline characteristics.

	Mothers with available blood samples	**Children with available blood samples**
	n = 5980	n = 4108
Maternal characteristics		
Age at intake (years)	29.8 (5.1)	29.5 (5.2)
Gestational age at intake (weeks)	13.4 (10.6, 17.4)	14.4 (10.9, 22.8)
Height (cm)	167.5 (7.4)	167.7 (7.4)
Weight at intake (kg)	68.8 (13.1)	69.4 (13.1)
BMI at intake (kg/m^2)	23.5 (19.3, 33.3)	23.8 (19.3, 33.2)
Blood pressure		
Systolic	115.6 (12.3)	115.4 (12.3)
Diastolic	68.2 (9.6)	67.9 (9.5)
Parity at intake, %		
Nulliparous	43.2	44.0
Multipara	56.8	56.0
Education, %		
Primary/secondary school	41.7	43.8
Higher education	46.6	44.9
Missing	11.6	11.3
Race/Ethnicity, %		
Western	58.9	57.8
Non-Western	35.4	36.7
Missing	5.6	5.5
Smoking, %		
No	64.1	64.5
Yes, until pregnancy was known	8.4	7.8
Yes, continued	16.6	17.6
Missing	10.9	10.1
Folic acid supplement use, %		
No use	19.1	21.5
Start before eight weeks	24.3	23.9
Preconception start	33.0	30.3
Missing	23.7	24.3
Comorbidity, %		
No	94.6	95.4
Yes	5.1	4.6
Missing	0.3	0.1
Hypertensive pregnancy disorder, %		
No	90.5	93.7
Preeclampsia	2.1	1.2

Table 1 Baseline characteristics. (continued)

	Mothers with available blood samples	**Children with available blood samples**
	n = 5980	n = 4108
Pregnancy induced hypertension	3.9	3.5
Missing	3.5	1.6
Gestational diabetes, %		
No	95.1	97.9
Yes	1.0	0.8
Missing	3.9	1.3
Small for gestational age at birth, %		
No	89.3	91.3
Yes	10.1	8.5
Missing	0.6	0.2
Early pregnancy biomarker concentrations		
Gestational age blood sampling (< 18 weeks)	13.2 (10.5, 17.2)	
sFlt-1 (ng/mL)	5.1 (2.2, 11.9)	
PlGF (pg/mL)	43.1 (17.6, 156.1)	
sFlt-1:PlGF ratio	116.7 (26.1, 355.6)	
Mid-pregnancy biomarker concentrations		
Gestational age blood sampling (18-25 weeks)	20.4 (19.1, 22.4)	
sFlt-1 (ng/mL)	4.9 (1.8, 14.2)	
PlGF (pg/mL)	199.7 (90.2, 502.1)	
sFlt-1:PlGF ratio	24.6 (7.1, 85.0)	
Infant characteristics		
Umbilical cord blood biomarker concentrations		
Gestational age at blood sampling		40.1 (37.4, 42.0)
sFlt-1 (ng/mL)		0.5 (0.2, 2.2)
PlGF (pg/mL)		8.6 (2.0, 15.7)
sFlt-1:PlGF ratio		53.5 (18.0, 276.3)
Gender, %		
Boy	50.6	51.3
Girl	49.4	48.7

Abbreviations: Body mass index, BMI; soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF. Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution.

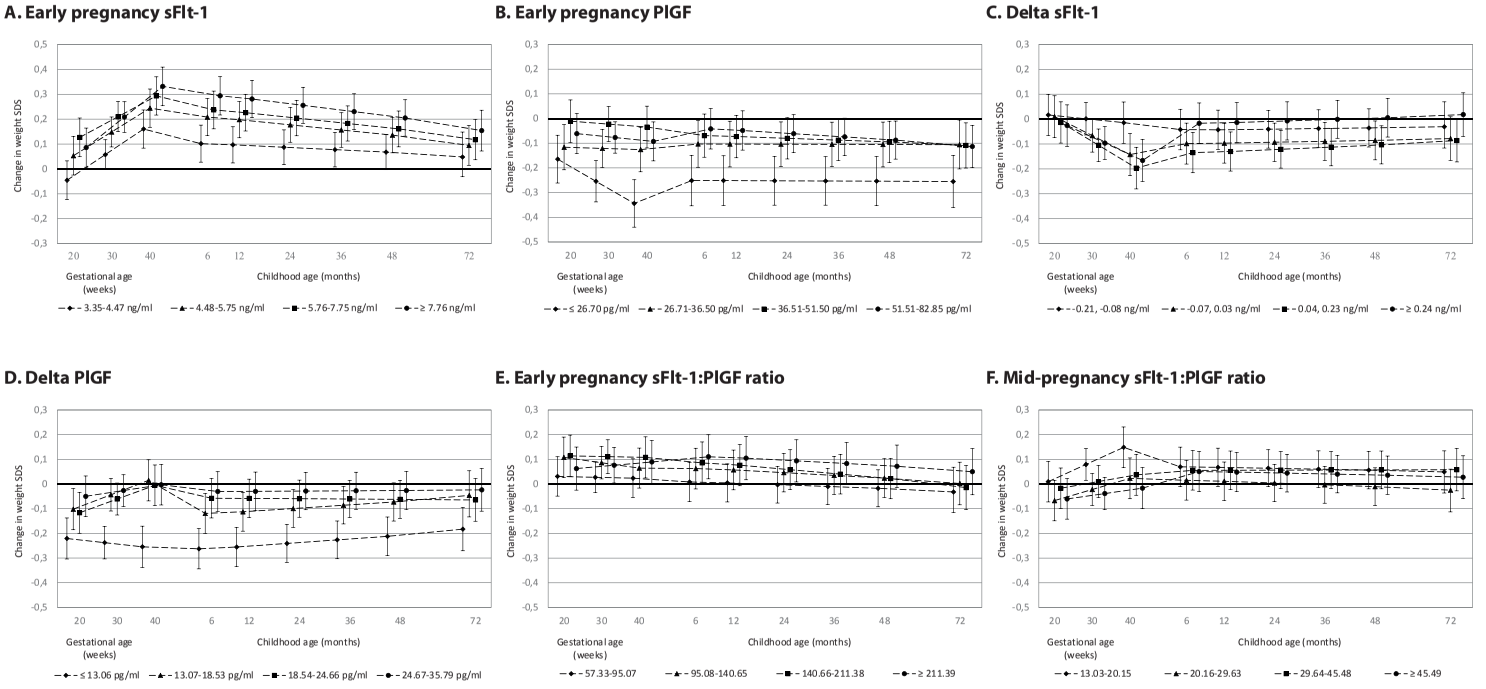


Figure 1 Associations between maternal sFlt-1 and PlGF concentrations and repeatedly measured fetal and childhood weight growth (n = 5980).
Abbreviations: Soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF; Standard deviation score, SDS.
Results are based on repeated measurement regressions models. Effect estimates (95% confidence interval represented by error bars) reflect the differences in gestational age adjusted SD scores of fetal and childhood weight at 20, 30 and 40 weeks gestation and at 6, 12, 24, 36, 48 and 72 months postnatally among maternal sFlt-1 and PlGF categories. The reference categories (**A**. Early pregnancy sFlt-1 ≤ 3.34 ng/mL; **B**. Early pregnancy PlGF ≥ 82.86 pg/mL; **C**. Delta sFlt-1 ≤ -0.22 ng/mL; **D**. Delta PlGF ≥ 35.80 pg/mL; **E**. Early pregnancy sFlt-1:PlGF ratio ≤ 57.32; **F**. Mid-pregnancy maternal sFlt-1:PlGF ratio ≤ 13.02) are represented by the zero-line in the graphs.
Values are adjusted for gestational age (blood sampling), maternal age, height, body mass index, parity, education, ethnicity, smoking, folic acid supplement use, systolic blood pressure and fetal gender. Childhood analyses are additionally adjusted for breastfeeding.

increase in sFlt-1 during pregnancy (fifth quintile) did not have a significantly lower weight growth from 6 months onwards when compared with the reference group. **Figure 1B** and **1D** show that mothers with lower PlGF concentrations in early pregnancy and the smallest increase in PlGF concentrations from early to mid-pregnancy had a lower fetal weight growth from 20 weeks onwards, resulting in a smaller newborn. Children of these mothers remained smaller until the age of 6 years; also this difference decreased with increasing age. Mothers with an early pregnancy maternal sFlt-1:PlGF ratio in the highest quintile showed a higher fetal weight growth from 30 weeks onwards resulting in a higher weight at birth (difference in birth weight 0.09 SDS; *P*-value <0.05), which remained present until the age of two years (difference in weight at two years 0.09 SDS; 95% Confidence Interval (CI) 0.01, 0.18) (**Figure 1E**). A mid-pregnancy maternal sFlt-1:PlGF ratio in the second quintile was associated with a higher fetal weight growth from 30 weeks onwards, but did not track into childhood (**Figure 1F**). Similar tendencies were observed for the association between maternal sFlt-1 and PlGF concentrations and fetal and childhood length growth (**Supplemental figure 2**).

The individual data points and confidence intervals for the different quintiles regarding these associations of the repeated measurement analyses are given in **Supplemental tables 5** and **6**. **Figure 2A** shows that children with the highest sFlt-1 concentrations in umbilical cord blood had a lower weight growth from 30 weeks onwards, resulting in a smaller birth weight. These children remained smaller until the age of 6 years. **Figure 2B** shows that the lowest PlGF concentrations in umbilical cord blood were associated a lower weight growth from 30 weeks gestation onwards, resulting in a lower weight at birth and a lower weight at the age of 6 years. Compared with umbilical cord sFlt-1:PlGF ratio in the reference group (lowest quintile), an umbilical cord sFlt-1:PlGF ratio in the highest quintile was associated with a lower fetal weight growth which resulted in a lower birth weight (difference in birth weight -0.51 SDS; 95% CI -0.61, -0.42). These children remained smaller until the age of 6 years (difference in weight at 6 years -0.19 SDS; 95% CI -0.30, -0.08) (**Figure 2C**). Similar tendencies were observed for the association between umbilical cord sFlt-1 and PlGF concentrations and fetal and childhood length growth (**Supplemental figure 3**).

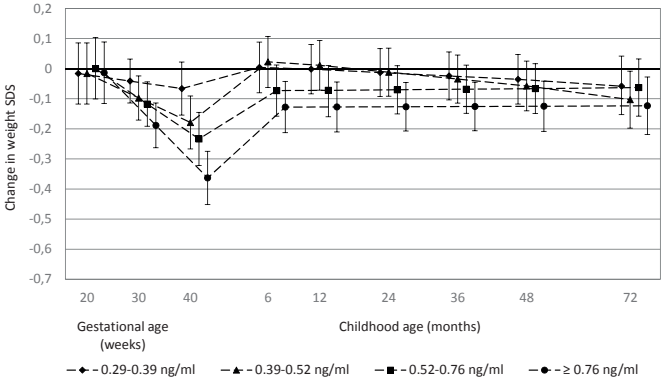
The individual data points and confidence intervals for the different quintiles regarding these associations of the repeated measurement analyses are given in **Supplemental tables 7** and **8**. **Table 3** shows that as compared with the reference group, mothers with early pregnancy maternal sFlt-1 concentrations in the highest quintile had a significantly lower risk of having a SGA child (Odds Ratio (OR) 0.36; 95% CI 0.27-0.48) and a lower risk of postnatal catch-up growth (**Supplemental table 9**). Early pregnancy maternal PlGF concentrations in the lowest quintile were associated with a higher risk of SGA (OR 3.48; 95% CI 2.39-5.08). Mothers with the smallest increase in maternal PlGF from early to mid-pregnancy had an increased risk of having a SGA child (OR 2.32; *P*-value <0.001). Subsequently, these children had a 1.5 times increased risk of postnatal catch-up growth. Newborns with umbilical cord sFlt-1 concentrations in the highest quintile and PlGF concentrations in the lowest quintile were more often

Table 2 Fetal and childhood growth measurements.

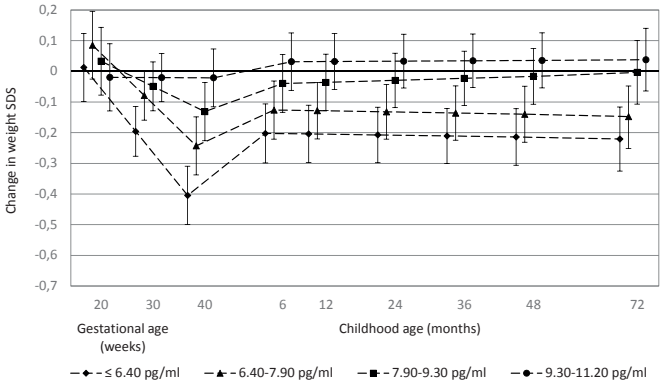
	Mothers with available blood samples	**Children with available blood samples**
	n = 5980	n = 4108
Fetal		
Mid-pregnancy measurements, %	96.8	95.7
Gestational age (weeks)	20.5 (19.1, 22.6)	20.5 (18.9, 22.8)
Estimated fetal weight (g)	377.3 (84.2)	382.9 (94.5)
Femur length (mm)	33.4 (3.3)	33.5 (3.6)
Late pregnancy measurements, %	96.6	98.7
Gestational age (weeks)	30.4 (29.0, 32.2)	30.3 (28.8, 32.3)
Estimated fetal weight (g)	1611.9 (250.6)	1613.6 (251.4)
Femur length (mm)	57.4 (3.0)	57.4 (52.9, 62.4)
Birth measurements, %	99.5	99.9
Weight (g)	3420.1 (564.2)	3461.1 (503.6)
Length (cm)	50.2 (2.4)	50.2 (2.3)
Childhood		
Visit 5-10 months, %	71.6	70
Age (months)	6.2 (5.4, 7.5)	6.2 (5.4, 7.7)
Weight (kg)	7.9 (0.9)	7.9 (0.9)
Length (cm)	67.7 (2.6)	67.7 (2.7)
Visit 10-13 months, %	64.7	63.4
Age (months)	11.1 (10.2, 12.3)	11.1 (10.2, 12.4)
Weight (kg)	9.7 (1.1)	9.7 (1.1)
Length (cm)	74.4 (2.7)	74.5 (2.6)
Visit 23-35 months, %	62.5	63.4
Age (months)	25.0 (23.6, 30.5)	25.1 (23.5, 30.7)
Weight (kg)	13.1 (1.6)	13.1 (1.6)
Length (cm)	88.7 (3.7)	88.9 (3.7)
Visit 35-44 months, %	54.3	56.2
Age (months)	36.7 (35.6, 39.8)	36.8 (35.6, 39.8)
Weight (kg)	15.2 (1.9)	15.3 (1.9)
Length (cm)	97.4 (3.8)	97.4 (3.8)
Visit 72 months, %	70.4	71.6
Age (months)	72.5 (68.9, 87.7)	73.2 (69.2, 90.6)
Weight (kg)	23.2 (4.1)	23.6 (4.3)
Length (cm)	119.4 (5.9)	120.1 (6.1)

Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution.

A. Umbilical cord sFlt-1



B. Umbilical cord PlGF



C. Umbilical cord sFlt-1:PlGF ratio

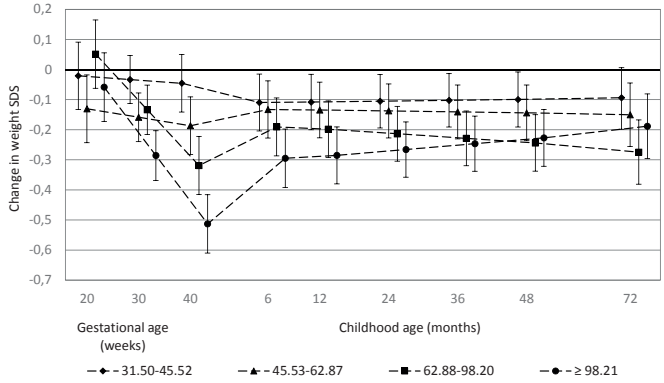


Figure 2 Associations between umbilical cord sFlt-1 and PlGF concentrations and repeatedly measured fetal and childhood weight growth (n = 4108).

Abbreviations: Soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF; Standard deviation score, SDS.

Results are based on repeated measurement regressions models. Effect estimates (95% confidence interval represented by error bars) reflect the differences in gestational age adjusted SD scores of fetal and childhood weight at 20, 30 and 40 weeks gestation and at 6, 12, 24, 36, 48 and 72 months postnatally among umbilical cord sFlt-1:PlGF ratio, sFlt-1 and PlGF categories. The reference categories (**A**. Umbilical cord sFlt-1 concentrations <0.29 ng/mL; **B**. Umbilical cord PlGF concentrations >11.20 pg/mL; **C**. Umbilical cord sFlt-1:PlGF ratio ≤ 31.49) are represented by the zero-line in the graphs.
Values are adjusted for gestational age (blood sampling), maternal age, height, body mass index, parity, education, ethnicity, smoking, folic acid supplement use, systolic blood pressure and fetal gender. Childhood analyses are additionally adjusted for breastfeeding.

Table 3 Associations of maternal and umbilical cord sFlt-1 and PlGF concentrations with small for gestational age children.

	Small for gestational age at birth		**Small for gestational age at birth**	
	n = 5980		n = 4108	
	OR (95% CI)	***P*-value**	OR (95% CI)	***P*-value**
Maternal				
Early pregnancy sFlt-1 (ng/mL)				
Q1 (≤ 3.34)	*Reference*			
Q2 (3.35-4.47)	0.61 (0.47-0.79)	<0.001		
Q3 (4.48-5.75)	0.44 (0.33-0.58)	<0.001		
Q4 (5.76-7.75)	0.47 (0.36-0.61)	<0.001		
Q5 (≥ 7.76)	0.36 (0.27-0.48)	<0.001		
Early pregnancy PlGF (pg/mL)				
Q1 (≤ 26.70)	3.48 (2.39-5.08)	<0.001		
Q2 (26.71-36.50)	2.21 (1.54-3.17)	<0.001		
Q3 (36.51-51.50)	1.69 (1.21-2.36)	0.002		
Q4 (51.51-82.85)	1.57 (1.18-2.11)	0.002		
Q5 (≥ 82.86)	*Reference*			
Delta sFlt-1 (ng/mL)				
Q1 (≤ -0.22)	*Reference*			
Q2 (-0.21, -0.08)	0.83 (0.60-1.16)	0.27		
Q3 (-0.07, 0.03)	1.41 (1.41-1.04)	0.03		
Q4 (0.04, 0.23)	1.49 (1.11-2.00)	0.007		
Q5 (≥ 0.24)	1.08 (0.80-1.47)	0.61		
Delta PlGF (pg/mL)				
Q1 (≤ 13.06)	2.32 (1.75-3.07)	<0.001		
Q2 (13.07-18.53)	1.03 (0.77-1.46)	0.71		
Q3 (18.54-24.66)	1.17 (0.87-1.58)	0.30		
Q4 (24.67-35.79)	0.94 (0.69-1.26)	0.66		
Q5 (≥ 35.80)	*Reference*			

Table 3 Associations of maternal and umbilical cord sFlt-1 and PlGF concentrations with small for gestational age children. (continued)

	Small for gestational age at birth		**Small for gestational age at birth**	
	n = 5980		n = 4108	
	OR (95% CI)	***P*-value**	OR (95% CI)	***P*-value**
Early pregnancy sFlt-1:PlGF ratio				
Q1 (≤ 57.32)	*Reference*			
Q2 (57.33-95.07)	0.94 (0.72-1.23)	0.66		
Q3 (95.08-140.65)	0.71 (0.53-0.96)	0.03		
Q4 (140.66-211.38)	0.74 (0.54-1.01)	0.06		
Q5 (≥ 211.39)	0.82 (0.60-1.12)	0.22		
Mid-pregnancy sFlt-1:PlGF ratio				
Q1 (≤ 13.02)	*Reference*			
Q2 (13.03-20.15)	0.72 (0.54-0.98)	0.03		
Q3 (20.16-29.63)	0.82 (0.61-1.11)	0.20		
Q4 (29.64-45.48)	0.84 (0.63-1.13)	0.26		
Q5 (≥ 45.49)	1.08 (0.82-1.44)	0.58		
Fetal				
Umbilical cord sFlt-1 (ng/mL)				
Q1 (≤ 0.29)			*Reference*	
Q2 (0.29-0.39)			1.16 (0.72-1.88)	0.54
Q3 (0.39-0.52)			2.51 (1.65-3.83)	<0.001
Q4 (0.52-0.76)			2.20 (1.44-3.38)	<0.001
Q5 (≥ 0.76)			3.48 (2.31-5.25)	<0.001
Umbilical cord PlGF (pg/mL)				
Q1 (≤ 6.40)			4.29 (2.81-6.55)	<0.001
Q2 (6.40-7.90)			2.23 (1.43-3.48)	<0.001
Q3 (7.90-9.30)			1.76 (1.11-2.79)	0.02
Q4 (9.30-11.20)			1.06 (0.64-1.76)	0.81
Q5 (≥ 11.20)			*Reference*	
Umbilical cord sFlt-1:PlGF ratio				
Q1 (≤ 31.49)			*Reference*	
Q2 (31.50-45.52)			1.69 (0.97-2.94)	0.07
Q3 (45.53-62.87)			2.26 (1.33-3.83)	0.003
Q4 (62.88-98.20)			3.68 (2.22-6.10)	<0.001
Q5 (≥ 98.21)			6.23 (3.82-10.16)	<0.001

Abbreviations: soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF; Odds ratio, OR; Confidence interval, CI; Quintile, Q.

Multivariable logistic regression analysis with small for gestational age at birth as dependent variable and maternal and umbilical cord sFlt-1 and PlGF as independent variables.

Analysis are adjusted for gestational age at blood sampling, maternal age, height, body mass index, parity, education, ethnicity, smoking, folic acid supplement use, systolic blood pressure and fetal gender.

born SGA (OR 3.48; 95% CI 2.31-5.25 and OR 4.29; 95% CI 2.81-6.55, respectively) and were having two times more often postnatal catch-up growth. Also, an umbilical cord sFlt-1:PlGF ratio in the highest quintile was associated with a higher risk of being SGA at birth (OR 6.23; 95% CI 3.82-10.16) followed by an increased risk of postnatal catch-up growth.

DISCUSSION

Findings from this large population-based prospective cohort study suggest that higher maternal sFlt-1 concentrations in early pregnancy resulted in a higher fetal weight growth and a subsequent heavier child at the age of 6 years. An increase in maternal sFlt-1 concentrations from early to mid-pregnancy, however, resulted in a lower fetal weight growth. We observed a lower fetal growth in mothers with lower PlGF concentrations in early pregnancy as well as in mothers with a relatively small increase in PlGF concentrations from early to mid-pregnancy, followed by smaller children at the age of 6 years. A higher early pregnancy maternal sFlt-1:PlGF ratio was associated with an increased fetal growth, followed by larger children at the age of two years. High umbilical cord sFlt-1 concentrations, low umbilical cord PlGF concentrations and a high umbilical cord sFlt-1:PlGF ratio were associated with a lower weight growth from 30 weeks gestation onwards until the age of 6 years.

Methodological considerations

This study was conducted within a large birth cohort with the availability of a large number of prospectively collected data. Fetal growth was assessed by actually measuring fetal growth characteristics in mid- and late pregnancy instead of using only birth outcomes as proxy for fetal growth. To our knowledge, this is the first study that has investigated the associations of sFlt-1 and PlGF in umbilical cord blood with repeatedly measured fetal and childhood growth characteristics. However, some limitations should be considered. The Generation R Study is characterised by a relatively highly educated and healthy study population compared to the population in the study area.[28] Our estimates can therefore be too conservative and underestimate the true effect measures. Moreover, 67% of the mothers had early pregnancy blood samples available and 46% of the newborns had umbilical cord blood samples available. Nonresponse analyses for both populations for analysis showed a similar pattern. Characteristics of those included in the analysis showed that mothers were on average taller, had a lower BMI, were more often nulliparous and of western ethnicity, were higher educated and gave birth to larger babies when compared with those not included in the study. This nonresponse would lead to biased effect estimates if associations would differ between those included and not included in the analyses. This is difficult to ascertain, but seems unlikely.[29] The nonresponse at baseline and at follow-up might have led to a selection towards a more healthy population, which might affect the generalizability of our results. We used a multiple

imputation model in this study for missing values in covariates. This reduces selection bias due to random missing in the covariates. Last, we were able to control for a large number of potential confounders. However, residual confounding cannot be ruled out completely due to the observational design of the study.

Interpretation

The associations between maternal angiogenic factors and birth weight have frequently been studied.[12, 13, 30-32] However, most studies have focused on pregnancy outcomes and complications. In line with our results, low maternal PlGF concentrations have consistently been associated with adverse birth outcomes.[12, 13, 32, 33] However, studies on the association between maternal sFlt-1 concentrations and fetal growth have shown inconsistent results. A number of factors, including differences in study population and design, confounders, biological differences and differences in gestational age at blood sampling may have contributed to these conflicting results. In the same cohort as our current study, Coolman et al.[13] showed that higher maternal sFlt-1 and PlGF concentrations in early pregnancy were associated with a higher birth weight. However, in our previous study, we did not find an association between early pregnancy maternal sFlt-1 concentrations and SGA. This may be explained by a stricter definition of SGA, different cut-off points for sFlt-1 and other exclusion criteria for the population of analysis in this previous study. Similar results were found by Smith et al.[30] They observed a reduced risk of delivering a small for gestational age infant in mothers with higher sFlt-1 and PlGF concentrations at 10-14 weeks of gestation (odds ratio (OR) 0.92; *P*-value <0.05 and OR 0.93; *P*-value <0.05, respectively). Furthermore, Åsvold et al.[32] found a 6-fold increased risk on having a small for gestational age newborn in women with low sFlt-1 in the first trimester and high sFlt-1 concentrations in the second trimester.

Others, however, have shown a negative association between increased sFlt-1 and birth weight, but not earlier than the second trimester.[11] Hypoxia has been shown to increase sFlt-1.[34] Recent studies have therefore proposed that the positive relation between early pregnancy sFlt-1 concentrations and fetal weight reflects the low oxygen environment needed for early placental and embryonic development,[13, 26] whereas in subsequent development high sFlt-1 concentrations may reflect the response to fetal-placental hypoxia which is associated with placental impairment.[4] This is in accordance with the adverse effect of an increase in sFlt-1 between early and mid-pregnancy on fetal growth as shown by our data and is substantiated by others.[32, 35] Additionally we observed a positive association of early pregnancy maternal sFlt-1:PlGF ratio with fetal growth. Since we found high maternal sFlt-1 concentrations to be positively associated with fetal growth our results concerning the sFlt-1:PlGF ratio may be a reasonable consequence. Åsvold et al.[32] found a higher risk of SGA in women with an angiogenic profile characterised by a low early pregnancy maternal sFlt-1:PlGF ratio and a subsequent high maternal sFlt-1:PlGF ratio in mid-pregnancy suggesting an adverse effect of low early pregnancy sFlt:PlGF ratio. In contrast to earlier studies, we did not find a clear

association between mid-pregnancy sFlt-1:PlGF ratio's and fetal and postnatal growth.[36, 37] However, Stepan et al.[36] examined the predictive value of these markers in a high-risk population with pregnancies characterised by abnormal uterine perfusion. Herraiz et al.[37] obtained blood samples later in pregnancy (gestational age > 24 weeks) and used a different definition to diagnose SGA.

Suboptimal placental growth and function due to an angiogenic imbalance is unable to secure optimal oxygen and nutritional supply to the fetus resulting in developmental adaptations with a permanent influence on growth and development in later life.[3, 38] In line with this hypothesis we observed that differences in length and weight growth starting in pregnancy as a result of an unfavourable angiogenic prolife led to smaller children until the age of 6 years. Moreover, these children showed more often postnatal catch-up growth which is known to be associated with an increased risk of disease in childhood.[22] We therefore propose that alterations in maternal sFlt-1 and PlGF may have long-term effects on childhood growth and development.

To our knowledge, only a few small studies examined the association between fetal sFlt-1 and PlGF concentrations and intrauterine growth restriction.[14, 15] These studies did not take into account postnatal growth patterns. In line with these previous studies, we observed that low PlGF and high sFlt-1 concentrations in umbilical cord blood were associated with slower fetal growth rates resulting in children with a lower birth weight. Moreover, we also showed that these children remained smaller until the age of 6 years. Unlike our results with regard to maternal sFlt-1:PlGF ratio and fetal and childhood growth, newborns with a higher umbilical cord sFlt-1:PlGF ratio had lower fetal growth rates and remained smaller throughout childhood. These results may suggest a significant role for umbilical cord sFlt-1:PlGF ratio particularly in the fetus.

We were not able to find a correlation between maternal and fetal sFlt-1 and PlGF concentrations. Also, maternal concentrations were much higher than the concentrations present in umbilical cord blood. This could be explained by the enhanced secretion of sFlt-1 and PlGF from the placenta into the maternal circulation rather than into the fetal circulation as both villous and extravillous trophoblasts contain very high levels of sFlt-1 and are in direct contact with the maternal circulation.[39] This supports the idea that angiogenic factors in the fetal circulation are mainly produced by the fetus itself rather than having a placental origin. Additionally, previous studies have shown that sFlt-1 and PlGF concentrations are also detectable in males and non-pregnant females as sFlt-1 and PlGF are both also expressed on cells different from those of the placenta[40] and after birth still contribute to organ growth and repair.[41] An angiogenic imbalance may result in adverse vascular effects by affecting endothelial function which is possibly adversely associated with fetal and childhood growth. Such a pathophysiologic mechanism may therefore be involved in the well-established association of fetal growth restriction with the long-term risk of vascular disease in adulthood.[42] However, future studies are needed to gain a further understanding of the effects of angiogenic factors

on early and late growth patterns in children and possible cardiovascular consequences in later life.

CONCLUSIONS

In conclusion, an angiogenic profile that is characterised by both low maternal sFlt-1 and PlGF concentrations in early pregnancy, as well as a subsequent relatively small increase in PlGF towards mid-pregnancy is associated with a reduced fetal and childhood growth. Higher sFlt-1 concentrations, lower PlGF concentrations or a higher sFlt-1:PlGF ratio in umbilical cord blood also seem to impair fetal and childhood growth. These results remained present even after exclusion of pregnancy-related and pre-existing maternal diseases which may influence fetal growth.

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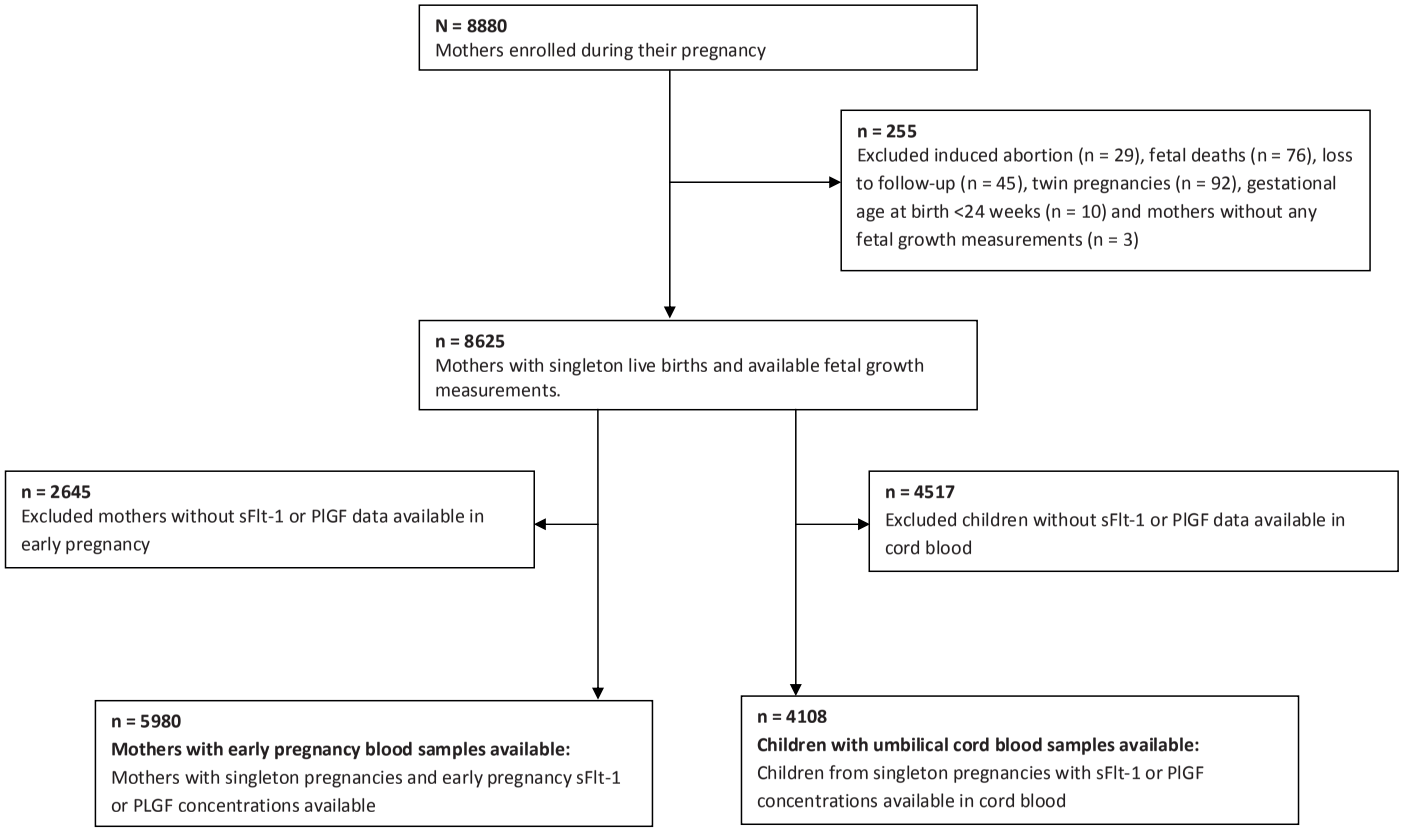
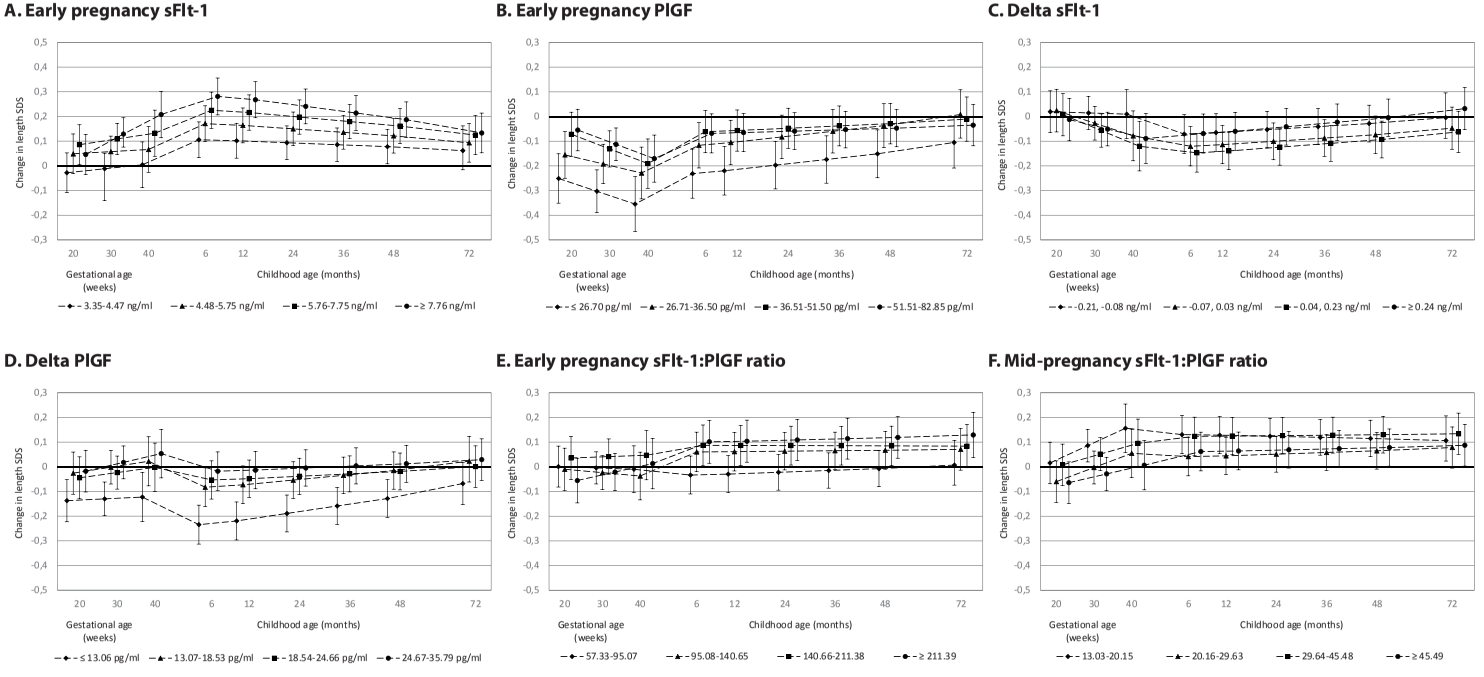
SUPPLEMENTAL MATERIAL

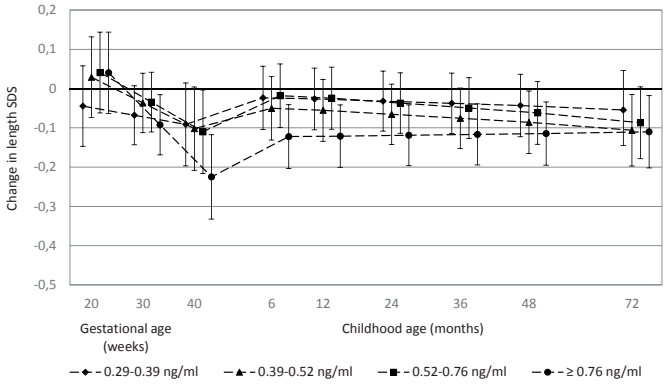


Supplemental figure 1 Flowchart.

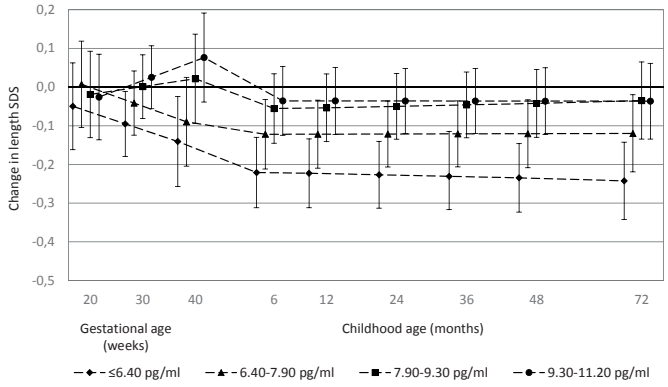


Supplemental figure 2 Associations between maternal sFlt-1 and PlGF concentrations and repeatedly measured fetal and childhood length growth (n = 5980).
Abbreviations: Soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF; Standard deviations score, SDS.
Results are based on repeated measurement regressions models. Effect estimates (95% confidence interval represented by error bars) reflect the differences in gestational age adjusted SD scores of fetal and childhood length at 20, 30 and 40 weeks gestation and at 6, 12, 24, 36, 48 and 72 months postnatally among maternal sFlt-1 and PlGF categories. The reference categories (**A.** Early pregnancy sFlt-1 ≤ 3.34 ng/mL; **B**. Early pregnancy PlGF ≥ 82.86 pg/mL; **C**. Delta sFlt-1 ≤ -0.22 ng/mL; **D**. Delta PlGF ≥ 35.80 pg/mL; **E**. Early pregnancy maternal sFlt-1:PlGF ratio ≤ 57.32; **F**. Mid-pregnancy maternal sFlt-1:PlGF ratio ≤ 13.02) are represented by the zero-line in the graphs. Values are adjusted for gestational age (blood sampling), maternal age, height, body mass index, parity, education, ethnicity, smoking, folic acid supplement use, systolic blood pressure and fetal gender. Childhood analyses are additionally adjusted for breastfeeding.

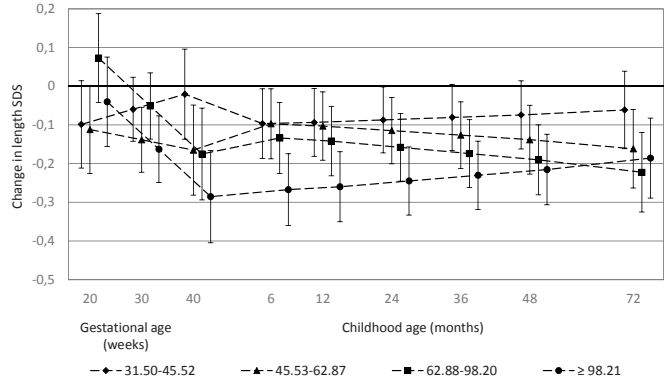
A. Umbilical cord sFlt-1



B. Umbilical cord PlGF



C. Umbilical cord sFlt-1:PlGF ratio



Supplemental figure 3 Associations between umbilical cord sFlt-1 and PlGF concentrations and repeatedly measured fetal and childhood length growth (n = 4108).
Abbreviations: Soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF; Standard deviations score, SDS.

Results are based on repeated measurement regressions models. Effect estimates (95% confidence interval represented by error bars) reflect the differences in gestational age adjusted SD scores of fetal and childhood length at 20, 30 and 40 weeks gestation and at 6, 12, 24, 36, 48 and 72 months postnatally among umbilical cord sFlt-1:PlGF ratio, sFlt-1 and PlGF categories. The reference categories (**A**. Umbilical cord sFlt-1 concentrations <0.29 ng/mL; **B**. Umbilical cord PlGF concentrations >11.20 pg/mL; **C**. Umbilical cord sFlt-1:PlGF ratio ≤ 31.49) are represented by the zero-line in the graphs.
Values are adjusted for gestational age (blood sampling), maternal age, height, body mass index, parity, education, ethnicity, smoking, folic acid supplement use, systolic blood pressure and fetal gender.
Childhood analyses are additionally adjusted for breastfeeding.

Supplemental table 1 Between-run coefficients of variation of angiogenic factors.

Angiogenic factors	**Between run coefficients**				**Analytic range**
	First		Second		
sFlt-1, plasma	2.8 %	5.5 ng/mL	2.3 %	34 ng/mL	0-150 ng/mL
PlGF, plasma	4.7 %	24 pg/mL	3.8 %	113 pg/mL	10-1500 pg/mL

Abbreviations: soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF.
The angiogenic factors were analysed using a microparticle-enhanced immunoassay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, the Netherlands). The assays used to analyse sFlt-1 and PlGF were performed with pre-launch assays.

Supplemental table 2 Spearman correlation coefficients between maternal and umbilical cord sFlt-1 and PlGF concentrations.

	Mother								**Child**			
	<18 weeks				**18-25 weeks**				**Birth**			
	sFlt-1 (ng/mL)		PlGF (pg/mL)		sFlt-1 (ng/mL)		PlGF (pg/mL)		sFlt-1 (ng/mL)		PlGF (pg/mL)	
	r	n	r	n	r	n	r	n	r	n	r	n
Mother												
<18 weeks												
sFlt-1 (ng/mL)	1											
PlGF (pg/mL)	0.127**	5918	1									
18-25 weeks												
sFlt-1 (ng, mL)	0.747**	5233	0.059**	5245	1							
PlGF (pg/mL)	0.139**	5235	0.447**	5247	0.130**	5267	1					
Child												
Birth												
sFlt-1 (nmg/mL)	0.023	2861	0.03	2874	0.034	2572	0.049*	2574	1			
PlGF(pg/mL)	-0.024	2579	0.002	2589	-0.005	2300	0.041	2302	0.001	2570	1	

Abbreviations: soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF; correlation coefficient, r; number, n.
* *P*-value < 0.05
** *P*-value < 0.01

Supplemental table 3 Nonresponse analysis of mothers included versus excluded from the study.

	Included	**Excluded**	***P*-value**
	n = 5980	n = 2900	
Maternal characteristics			
Age at intake (years)	29.4 (5.8)	29.8 (5.1)	0.006
Gestational age at intake (weeks)	13.4 (10.6, 17.4)	19.9 (11.9, 30.5)	<0.001
Height (cm)	167.5 (7.4)	166.3 (7.4)	<0.001
Weight at intake (kg)	68.8 (13.1)	71.1 (13.7)	<0.001
BMI at intake (kg/m^2)	23.5 (19.3, 33.3)	24.8 (19.7, 34.8)	<0.001
BMI before pregnancy (kg/m^2)	22.6 (18.7, 32.1)	22.8 (18.5, 33.2)	0.01
Blood pressure at intake (mmHg)			
Systolic	115.5 (12.3)	114.9 (12.0)	0.03
Diastolic	68.2 (9.6)	67.3 (9.6)	<0.001
Nulliparous, %	56.8	50.5	<0.001
Education, %			<0.001
Primary/secondary school	47.2	56.2	
Higher education	52.8	43.8	
Race/ethnicity, %			<0.001
Western	62.5	48.7	
Non-Western	37.5	51.3	
Smoking, %			0.002
No	72.0	74.4	
Yes, until pregnancy known	9.4	7.0	
Yes, continued	18.6	18.6	
Folic acid supplement use, %			<0.001
No use	25.0	39.5	
Start before eight weeks	31.9	29.4	
Preconception start	43.2	31.1	
Comorbidity, %			
No	94.9	94.9	0.96
Yes	5.1	5.1	
Hypertensive pregnancy disorder, %			
No	93.8	94.6	0.06
Preeclampsia	2.1	2.3	
Pregnancy induced hypertension	4.1	3.1	
Gestational diabetes, %			
No	99.0	98.8	0.50
Yes	1.0	1.2	
Small for gestational age at birth, %			
No	89.8	89.6	0.73
Yes	10.2	10.4	

Supplemental table 3 Nonresponse analysis of mothers included versus excluded from the study. (continued)

	Included	**Excluded**	***P*-value**
	n = 5980	n = 2900	
Male gender, %	50.6	50.1	0.65
Mid-pregnancy measurements			
Estimated fetal weight (g)	377.3 (84.2)	395.6 (119.9)	<0.001
Femur length (mm)	33.4 (3.3)	33.9 (4.5)	<0.001
Late pregnancy measurements			
Estimated fetal weight (g)	1611.9 (250.6)	1624.6 (299.7)	0.06
Femur length (mm)	57.4 (3.0)	57.6 (3.4)	0.002
Birth outcomes			
Weight (g)	3420.1 (564.2)	3357.8 (581.1)	<0.001
Length (cm)	50.2 (2.4)	50.1 (2.4)	0.03

Abbreviation: Body mass index, BMI.
Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution.
Differences between women included versus excluded from the study were tested by using Student's t-test for continuous variables and Mann-Whitney U-test for continuous variables with a skewed distribution. Chi-square tests were used for categorical variables.

Supplemental table 4 Nonresponse analysis of children included versus excluded from the study.

	Included	**Excluded**	***P*-value**
	n = 4108	n = 4772	
Maternal characteristics			
Age at intake (years)	29.5 (5.2)	29.8 (5.4)	0.06
Gestational age at intake (weeks)	14.4 (10.9, 22.8)	14.5 (10.9, 23.8)	0.001
Height (cm)	167.7 (7.4)	166.7 (7.4)	<0.001
Weight at intake (kg)	69.4 (13.1)	69.6 (13.6)	0.48
BMI at intake (kg/m^2)	23.8 (19.3, 33.2)	24.0 (19.5, 34.2)	0.001
BMI before pregnancy (kg/m^2)	22.6 (18.6, 32.2)	22.7 (18.7, 32.8)	0.03
Blood pressure at intake (mmHg)			
Systolic	115.4 (12.3)	115.3 (12.2)	0.98
Diastolic	67.9 (9.5)	67.9 (9.7)	0.88
Nulliparous, %	56.0	53.7	0.03
Education, %			0.002
Primary/secondary school	49.4	50.6	
Higher education	50.6	49.4	
Race/ethnicity, %			<0.001
Western	61.2	55.5	
Non-Western	38.8	44.5	
Smoking, %			0.11
No	71.7	73.6	
Yes, until pregnancy known	8.7	8.7	
Yes, continued	19.6	17.7	
Folic acid supplement use, %			0.27
No use	28.4	30.3	
Start before eight weeks	31.5	30.7	
Preconception start	40.0	39.0	
Comorbidity, %			
No	94.4	92.5	0.03
Yes	5.6	7.5	
Hypertensive pregnancy disorder, %			
No	95.2	93.0	<0.001
Preeclampsia	1.2	3.1	
Pregnancy induced hypertension	3.6	3.9	
Gestational diabetes, %			
No	99.2	98.7	0.05
Yes	0.8	1.3	
Small for gestational age at birth, %			
No	91.4	88.2	<0.001
Yes	8.6	11.8	

Supplemental table 4 Nonresponse analysis of children included versus excluded from the study. (continued)

	Included	**Excluded**	***P*-value**
	n = 4108	n = 4772	
Male gender, %	51.3	49.7	0.13
Mid-pregnancy measurements			
Estimated fetal weight (g)	382.9 (95.5)	382.4 (98.2)	0.81
Femur length (mm)	33.5 (3.6)	33.6 (3.7)	0.39
Late pregnancy measurements			
Estimated fetal weight (g)	1613.6 (251.4)	1617.8 (280.2)	0.47
Femur length (mm)	57.4 (3.0)	57.5 (3.2)	0.23
Birth outcomes			
Weight (g)	3461.1 (503.6)	3346.2 (619.1)	<0.001
Length (cm)	50.2 (2.3)	50.1 (2.5)	0.02

Abbreviation: Body mass index, BMI.
Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution.
Differences between women included versus excluded from the study were tested by using Student's t-test for continuous variables and Mann-Whitney U-test for continuous variables with a skewed distribution. Chi-square tests were used for categorical variables.

Supplemental table 5 Associations of **maternal** sFlt-1 and PlGF concentrations with fetal and childhood **weight** growth (n = 5980).

	Change in weight SDS								
	Fetal			Childhood					
	20 weeks gestation	30 weeks gestation	40 weeks gestation	6 months	12 months	24 months	36 months	48 months	72 months
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
Early pregnancy sFlt-1 (ng/mL)									
Q1 (≤ 3.34)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Q2 (3.35-4.47)	-0.05 (-0.12; 0.03)	0.06 (0.00; 0.12)	0.16 (0.08; 0.24)*	0.10 (0.03; 0.18)*	0.10 (0.02; 0.17)*	0.09 (0.02; 0.16)*	0.08 (0.01; 0.15)*	0.07 (0.00; 0.14)	0.05 (-0.03; 0.13)
Q3 (4.48-5.75)	0.05 (-0.02; 0.03)	0.15 (0.09; 0.21)*	0.24 (0.17; 0.32)*	0.21 (0.13; 0.28)*	0.20 (0.13; 0.27)*	0.18 (0.11; 0.25)*	0.16 (0.09; 0.23)*	0.14 (0.06; 0.21)*	0.09 (0.01; 0.17)*
Q4 (5.76-7.75)	0.13 (0.05; 0.20)*	0.21 (0.15; 0.27)*	0.29 (0.22; 0.37)*	0.24 (0.16; 0.31)*	0.23 (0.16; 0.31)*	0.20 (0.13; 0.28)*	0.18 (0.11; 0.25)*	0.16 (0.09; 0.23)*	0.12 (0.04; 0.20)*
Q5 (≥ 7.76)	0.09 (0.01; 0.16)*	0.21 (0.15; 0.27)*	0.33 (0.25; 0.41)*	0.29 (0.22; 0.37)*	0.28 (0.21; 0.36)*	0.26 (0.18; 0.33)*	0.23 (0.16; 0.30)*	0.20 (0.13; 0.28)*	0.15 (0.07; 0.24)*
Early pregnancy PlGF (pg/mL)									
Q1 (≤ 26.70)	-0.16 (-0.26; -0.07)*	-0.25 (-0.34; -0.17)*	-0.34 (-0.44; -0.25)*	-0.25 (-0.35; -0.15)*	-0.25 (-0.35; -0.15)*	-0.25 (-0.35; -0.15)*	-0.25 (-0.35; -0.15)*	-0.25 (-0.35; -0.15)*	-0.26 (-0.36; -0.15)*
Q2 (26.71-36.50)	-0.12 (-0.21; -0.03)*	-0.12 (-0.20; -0.04)*	-0.13 (-0.22; -0.04)*	-0.10 (-0.20; -0.01)*	-0.10 (-0.20; -0.01)*	-0.10 (-0.19; -0.01)*	-0.10 (-0.19; -0.01)*	-0.11 (-0.20; -0.01)*	-0.10 (-0.20; -0.01)*
Q3 (36.51-51.50)	-0.01 (-0.10; 0.08)	-0.02 (-0.16; 0.01)	-0.03 (-0.12; 0.05)	-0.07 (-0.16; 0.02)	-0.07 (-0.16; 0.01)	-0.08 (-0.16; 0.00)	-0.09 (-0.17; 0.00)*	-0.09 (-0.18; -0.01)*	-0.11 (-0.20; -0.02)*
Q4 (51.51-82.85)	-0.06 (-0.14; 0.02)	-0.08 (-0.14; -0.01)*	-0.09 (-0.17; -0.01)*	-0.04 (-0.12; 0.04)	-0.05 (-0.13; 0.03)	-0.06 (-0.14; 0.02)	-0.07 (-0.15; 0.00)	-0.09 (-0.16; -0.01)*	-0.11 (-0.20; -0.03)*
Q5 (≥ 82.90)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*

Supplemental table 5 Associations of **maternal** sFlt-1 and PlGF concentrations with fetal and childhood **weight** growth (n = 5980). (continued)

Delta sFlt-1 (ng/mL)									
Q1 (≤ -0.22)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Q2 (-0.21, -0.08)	0.02 (-0.07; 0.10)	0.00 (-0.06; 0.07)	-0.01 (-0.10; 0.07)	-0.04 (-0.12; 0.04)	-0.04 (-0.12; 0.04)	-0.04 (-0.12; 0.04)	-0.04 (-0.11; 0.04)	-0.04 (-0.11; 0.04)	-0.03 (-0.12; 0.06)
Q3 (-0.07, 0.03)	0.01 (-0.07; 0.09)	-0.07 (-0.13; 0.00)*	-0.14 (-0.23; -0.06)*	-0.10 (-0.18; -0.02) *	-0.10 (-0.18; -0.02) *	-0.09 (-0.17; -0.02) *	-0.09 (-0.17; -0.01) *	-0.09 (-0.16; -0.01) *	-0.08 (-0.17; 0.01)
Q4 (0.04, 0.23)	-0.01 (-0.10; 0.07)	-0.11 (-0.17; -0.04)*	-0.20 (-0.28; -0.11)*	-0.13 (-0.22; -0.05) *	-0.13 (-0.21; -0.05) *	-0.12 (-0.20; -0.05) *	-0.11 (-0.19; -0.04) *	-0.10 (-0.18; -0.03) *	-0.09 (-0.17; 0.00) *
Q5 (≥ 0.24)	-0.03 (-0.11; 0.06)	-0.10 (-0.16; -0.03)*	-0.17 (-0.25; -0.08)*	-0.02 (-0.10; 0.07)	-0.01 (-0.09; 0.07)	-0.01 (-0.08; 0.07)	0.00 (-0.08; 0.08)	0.01 (-0.07; 0.08)	0.02 (-0.07; 0.11)
Delta PlGF (pg/mL)									
Q1 (≤ 13.06)	-0.22 (-0.30; -0.14) *	-0.24 (-0.30; -0.17) *	-0.25 (-0.34; -0.17) *	-0.26 (-0.34; -0.18) *	-0.26 (-0.33; -0.18) *	-0.24 (-0.32; -0.16) *	-0.23 (-0.30; -0.15) *	-0.21 (-0.29; -0.13) *	-0.18 (-0.27; -0.10) *
Q2 (13.07-18.53)	-0.10 (-0.19; -0.02)*	-0.04 (-0.11; 0.02)	0.02 (-0.07; 0.10)	-0.12 (-0.20; -0.04) *	-0.11 (-0.19; -0.03) *	-0.10 (-0.18; -0.02) *	-0.09 (-0.16; -0.01) *	-0.07 (-0.15; 0.00)	-0.05 (-0.13; 0.04)
Q3 (18.54-24.66)	-0.12 (-0.20; -0.03) *	-0.06 (-0.13; 0.00)	0.00 (-0.09; 0.08)	-0.06 (-0.14; 0.02)	-0.06 (-0.14; 0.02)	-0.06 (-0.13; 0.02)	-0.06 (-0.14; 0.01)	-0.06 (-0.14; 0.01)	-0.06 (-0.15; 0.02)
Q4 (24.67-35.79)	-0.05 (-0.13; 0.03)	-0.03 (-0.09; 0.04)	0.00 (-0.08; 0.08)	-0.03 (-0.11; 0.05)	-0.03 (-0.11; 0.05)	-0.03 (-0.10; 0.05)	-0.03 (-0.10; 0.05)	-0.03 (-0.10; 0.05)	-0.02 (-0.11; 0.06)
Q5 (≥ 35.80)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*

Supplemental table 5 Associations of **maternal** sFlt-1 and PlGF concentrations with fetal and childhood **weight** growth (n = 5980). (continued)

Early pregnancy sFl-1:PlGF ratio									
Q1 (≤ 57.32)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Q2 (57.33-95.07)	0.03 (-0.05; 0.11)	0.03 (-0.03; 0.09)	0.02 (-0.05; 0.10)	0.01 (-0.07; 0.09)	0.00 (-0.07; 0.08)	-0.00 (-0.07; 0.07)	-0.01 (-0.08; 0.06)	-0.01 (-0.09; 0.06)	-0.03 (-0.12; 0.05)
Q3 (95.08-140.65)	0.11 (0.03; 0.19)*	0.09 (0.02; 0.15)*	0.06 (-0.02; 0.15)	0.06 (-0.02; 0.14)	0.06 (-0.02; 0.14)	0.05 (-0.03; 0.12)	0.04 (-0.04; 0.11)	0.02 (-0.05; 0.10)	0.00 (-0.08; 0.09)
Q4 (140.66-211.38)	0.11 (0.03; 0.20)*	0.11 (0.04; 0.18)*	0.11 (0.02; 0.19)*	0.09 (0.00; 0.17)*	0.08 (-0.01; 0.16)	0.06 (-0.02; 0.14)	0.04 (-0.04; 0.12)	0.02 (-0.06; 0.10)	-0.01 (-0.10; 0.07)
Q5 (≥ 211.39)	0.06 (-0.02; 0.15)	0.08 (0.00; 0.15)*	0.09 (0.00; 0.18)*	0.11 (0.02; 0.20)*	0.10 (0.02; 0.19)*	0.09 (0.01; 0.18)*	0.08 (-0.00; 0.17)	0.07 (-0.01; 0.16)	0.05 (-0.04; 0.14)
Mid-pregnancy sFlt-1:PlGF ratio									
Q1 (≤ 13.02)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Q2 (13.03-20.15)	0.01 (-0.07; 0.09)	0.08 (0.02; 0.14)*	0.15 (0.07; 0.23)*	0.07 (-0.01; 0.15)	0.07 (-0.01; 0.15)	0.06 (-0.01; 0.14)	0.06 (-0.01; 0.13)	0.06 (-0.02; 0.13)	0.05 (-0.04; 0.13)
Q3 (20.16-29.63)	-0.07 (-0.15; 0.01)	-0.02 (-0.09; 0.04)	0.02 (-0.06; 0.11)	0.01 (-0.07; 0.10)	0.01 (-0.07; 0.09)	0.00 (-0.07; 0.08)	-0.00 (-0.08; 0.07)	-0.01 (-0.09; 0.07)	-0.02 (-0.11; 0.06)
Q4 (29.64-45.48)	-0.02 (-0.10; 0.06)	0.01 (-0.05; 0.07)	0.04 (-0.04; 0.12)	0.06 (-0.02; 0.14)	0.06 (-0.02; 0.13)	0.06 (-0.02; 0.13)	0.06 (-0.02; 0.13)	0.06 (-0.02; 0.13)	0.06 (-0.03; 0.14)
Q5 (≥ 45.49)	-0.06 (-0.14; 0.02)	-0.04 (-0.10; 0.03)	-0.02 (-0.10; 0.07)	0.05 (-0.03; 0.13)	0.05 (-0.03; 0.13)	0.04 (-0.03; 0.12)	0.04 (-0.04; 0.12)	0.04 (-0.04; 0.11)	0.03 (-0.06; 0.11)

Abbreviations: soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF; Standard deviation score, SDS; Confidence interval, CI; Quintile, Q.
Fetal and childhood weight growth among maternal sFlt-1 and PlGF categories, based on repeated measurement regression models.
Values are adjusted for gestational age (blood sampling), maternal age, height, body mass index, parity, education, ethnicity, smoking, folic acid supplement use, systolic blood pressure and fetal gender. Childhood analysis are additionally adjusted for breastfeeding.
* *P*-value < 0.05

Supplemental table 6 Associations of **maternal** sFlt-1 and PlGF concentrations with fetal and childhood **length** growth (n = 5980).

	Change in length SDS								
	Fetal			Childhood					
	20 weeks gestation	**30 weeks gestation**	**40 weeks gestation**	**6 months**	**12 months**	**24 months**	**36 months**	**48 months**	**72 months**
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
Early pregnancy sFlt-1 (ng/mL)									
Q1 (≤ 3.34)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Q2 (3.35-4.47)	-0.03 (-0.11; 0.05)	-0.01 (-0.07; 0.12)	0.00 (-0.09; 0.10)	0.11 (0.03; 0.18)*	0.10 (0.03; 0.17)*	0.09 (0.03; 0.16)*	0.09 (0.02; 0.15)*	0.08 (0.01; 0.15)*	0.06 (-0.02; 0.14)
Q3 (4.48-5.75)	0.05 (-0.03; 0.13)	0.06 (-0.01; 0.12)	0.07 (-0.03; 0.16)	0.17 (0.10; 0.24)*	0.16 (0.09; 0.23)*	0.15 (0.08; 0.22)*	0.14 (0.07; 0.20)*	0.12 (0.05; 0.19)*	0.09 (0.01; 0.17)*
Q4 (5.76-7.75)	0.09 (0.01; 0.17)*	0.11 (0.05; 0.17)*	0.13 (0.04; 0.23)*	0.23 (0.15; 0.30)*	0.22 (0.14; 0.29)*	0.20 (0.13; 0.27)*	0.18 (0.11; 0.25)*	0.16 (0.09; 0.23)*	0.12 (0.05; 0.20)*
Q5 (≥ 7.76)	0.05 (-0.04; 0.13)	0.13 (0.06; 0.18)*	0.21 (0.11; 0.30)*	0.28 (0.21; 0.36)*	0.27 (0.19; 0.34)*	0.24 (0.17; 0.31)*	0.21 (0.14; 0.28)*	0.19 (0.12; 0.26)*	0.13 (0.05; 0.21)*
Early pregnancy PlGF (pg/mL)									
Q1 (≤ 26.70)	-0.25 (-0.35; -0.15)*	-0.30 (-0.39; -0.22)*	-0.35 (-0.47; -0.24)*	-0.23 (-0.33; -0.13)*	-0.22 (-0.32; -0.12)*	-0.20 (-0.29; -0.10)*	-0.17 (-0.27; -0.08)*	-0.15 (-0.25; -0.05)*	-0.11 (-0.21; 0.00)*
Q2 (26.71-36.50)	-0.16 (-0.25; -0.06)*	-0.19 (-0.27; -0.11)*	-0.23 (-0.33; -0.12)*	-0.12 (-0.21; -0.03)*	-0.11 (-0.20; -0.02)*	-0.08 (-0.17; 0.01)	-0.06 (-0.15; 0.03)	-0.04 (-0.13; 0.05)	0.01 (-0.09; 0.10)
Q3 (36.51-51.50)	-0.07 (-0.16; 0.02)	-0.13 (-0.20; -0.06)*	-0.19 (-0.29; -0.09)*	-0.06 (-0.15; 0.02)	-0.06 (-0.14; 0.03)	-0.05 (-0.13; 0.03)	-0.04 (-0.12; 0.04)	-0.03 (-0.11; 0.05)	-0.01 (-0.10; 0.08)
Q4 (51.51-82.85)	-0.05 (-0.14; 0.03)	-0.11 (-0.18; -0.05)*	-0.17 (-0.27; -0.08)*	-0.07 (-0.15; 0.01)	-0.07 (-0.14; 0.01)	-0.06 (-0.31; 0.02)	-0.05 (-0.13; 0.02)	-0.05 (-0.12; 0.03)	-0.04 (-0.12; 0.05)
Q5 (≥ 82.90)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*

Supplemental table 6 Associations of **maternal** sFlt-1 and PlGF concentrations with fetal and childhood **length** growth (n = 5980). (continued)

Delta sFlt-1 (ng/mL)									
Q1 (≤ -0.22)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Q2 (-0.21, -0.08)	0.02 (-0.07; 0.10)	0.01 (-0.05; 0.08)	0.01 (-0.09; 0.11)	-0.07 (-0.15; 0.01)	-0.07 (-0.14; 0.01)	-0.05 (-0.13; 0.02)	-0.04 (-0.11; 0.03)	-0.03 (-0.10; 0.05)	0.00 (-0.09; 0.08)
Q3 (-0.07, 0.03)	0.02 (-0.06; 0.11)	-0.03 (-0.10; 0.04)	-0.08 (-0.18; 0.02)	-0.12 (-0.20; -0.04)*	-0.11 (-0.19; -0.04)*	-0.10 (-0.18; -0.03)*	-0.09 (-0.16; -0.01)*	-0.07 (-0.15; 0.00)	-0.05 (-0.13; 0.04)
Q4 (0.04, 0.23)	0.01 (-0.08; 0.09)	-0.06 (-0.12; 0.01)	-0.12 (-0.22; -0.02)*	-0.15 (-0.23; -0.07)*	-0.14 (-0.22; -0.06)*	-0.12 (-0.20; -0.05)*	-0.11 (-0.18; -0.04)*	-0.09 (-0.17; -0.02)*	-0.06 (-0.15; 0.02)
Q5 (≥ 0.24)	-0.01 (-0.1; 0.07)	-0.05 (-0.1; 0.02)	-0.09 (-0.2; 0.01)	-0.07 (-0.15; 0.01)	-0.06 (-0.14; 0.02)	-0.04 (-0.12; 0.03)	-0.02 (-0.10; 0.05)	0.00 (-0.08; 0.07)	0.03 (-0.05; 0.12)
Delta PlGF (pg/mL)									
Q1 (≤ 13.06)	-0.14 (-0.22; -0.05)*	-0.13 (-0.20; -0.06)*	-0.12 (-0.22; -0.02)*	-0.23 (-0.31; -0.16)*	-0.22 (-0.30; -0.14)*	-0.19 (-0.26; -0.11)*	-0.16 (-0.23; -0.08)*	-0.13 (-0.20; -0.05)*	-0.07 (-0.15; 0.02)
Q2 (13.07-18.53)	-0.03 (-0.11; 0.06)	0.00 (-0.07; 0.07)	0.02 (-0.08; 0.12)	-0.08 (-0.16; 0.00)*	-0.07 (-0.15; 0.00)	-0.05 (-0.13; 0.02)	-0.03 (-0.11; 0.04)	-0.02 (-0.09; 0.06)	0.02 (-0.6; 0.11)
Q3 (18.54-24.66)	-0.04 (-0.13; 0.04)	-0.02 (-0.09; 0.04)	0.00 (-0.10; 0.10)	-0.05 (-0.13; 0.02)	-0.05 (-0.12; 0.03)	-0.04 (-0.11; 0.03)	-0.03 (-0.10; 0.04)	-0.02 (-0.09; 0.06)	0.00 (-0.08; 0.08)
Q4 (24.67-35.79)	-0.02 (-0.10; 0.07)	0.02 (-0.05; 0.08)	0.05 (-0.04; 0.15)	-0.02 (-0.10; 0.06)	-0.01 (-0.09; 0.06)	0.00 (-0.08; 0.07)	0.00 (-0.07; 0.08)	0.01 (-0.06; 0.09)	0.03 (-0.05; 0.11)
Q5 (≥ 35.80)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*

Supplemental table 6 Associations of **maternal** sFlt-1 and PlGF concentrations with fetal and childhood **length** growth (n = 5980). (continued)

Early pregnancy sFl-1:PlGF ratio									
Q1 (≤ 57.32)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Q2 (57.33-95.07)	0.00 (-0.08; 0.08)	-0.00 (-0.07; 0.06)	-0.01 (-0.10; 0.08)	-0.03 (-0.11; 0.04)	-0.03 (-0.10; 0.04)	-0.02 (-0.09; 0.05)	-0.01 (-0.09; 0.06)	-0.01 (-0.08; 0.07)	0.01 (-0.07; 0.09)
Q3 (95.08-140.65)	-0.01 (-0.10; 0.08)	-0.02 (-0.09; 0.04)	-0.04 (-0.13; 0.06)	0.06 (-0.02; 0.14)	0.06 (-0.02; 0.14)	0.06 (-0.01; 0.14)	0.07 (-0.01; 0.14)	0.07 (-0.01; 0.14)	0.07 (-0.01; 0.16)
Q4 (140.66-211.38)	0.04 (-0.05; 0.12)	0.04 (-0.03; 0.11)	0.05 (-0.05; 0.15)	0.09 (0.00; 0.17)	0.09 (-0.01; 0.17)*	0.09 (0.01; 0.16)*	0.09 (0.01; 0.16)*	0.08 (0.01; 0.16)*	0.08 (-0.00; 0.17)
Q5 (≥ 211.39)	-0.06 (-0.15; 0.03)	-0.02 (-0.10; 0.05)	0.01 (-0.09; 0.12)	0.10 (0.01; 0.19)*	0.10 (0.02; 0.19)*	0.11 (0.03; 0.19)*	0.11 (0.03; 0.20)*	0.12 (0.03; 0.20)*	0.13 (0.04; 0.22)*
Mid-pregnancy sFlt-1:PlGF ratio									
Q1 (≤ 13.02)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Q2 (13.03-20.15)	0.02 (-0.07; 0.10)	0.09 (0.02; 0.15)*	0.16 (0.06; 0.25)*	0.13 (0.05; 0.21)*	0.13 (0.05; 0.20)*	0.12 (0.05; 0.20)*	0.12 (0.05; 0.19)*	0.11 (0.04; 0.19)*	0.11 (0.02; 0.19)*
Q3 (20.16-29.63)	-0.06 (-0.14; 0.02)	-0.00 (-0.07; 0.06)	0.05 (-0.04; 0.15)	0.04 (-0.04; 0.12)	0.04 (-0.03; 0.12)	0.05 (-0.02; 0.12)	0.06 (-0.01; 0.13)	0.06 (-0.01; 0.14)	0.08 (-0.01; 0.16)
Q4 (29.64-45.48)	0.01 (-0.08; 0.09)	0.05 (-0.02; 0.12)	0.10 (-0.00; 0.19)	0.12 (0.05; 0.20)*	0.12 (0.05; 0.20)*	0.13 (0.05; 0.20)*	0.13 (0.06; 0.20)*	0.13 (0.06; 0.20)*	0.13 (0.05; 0.22)*
Q5 (≥ 45.49)	-0.06 (-0.15; 0.02)	-0.03 (-0.10; 0.04)	0.01 (-0.09; 0.11)	0.06 (-0.02; 0.14)	0.06 (-0.01; 0.14)	0.07 (-0.00; 0.15)	0.07 (-0.00; 0.15)	0.08 (0.00; 0.15)*	0.09 (0.00; 0.17)*

Abbreviations: soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF; Standard deviation score, SDS; Confidence interval, CI; Quintile, Q.

Fetal and childhood length growth among maternal sFlt-1 and PlGF categories, based on repeated measurement regression models.

Values are adjusted for gestational age (blood sampling), maternal age, height, body mass index, parity, education, ethnicity, smoking, folic acid supplement use, systolic blood pressure and fetal gender. Childhood analysis are additionally adjusted for breastfeeding.

* *P*-value < 0.05

Supplemental table 7 Associations of **umbilical cord** sFlt-1 and PlGF concentrations with fetal and childhood **weight** growth (n = 4108).

	Change in weight SDS								
	Fetal			Childhood					
	20 weeks gestation	30 weeks gestation	40 weeks gestation	6 months	12 months	24 months	36 months	48 months	72 months
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
sFlt-1 (ng/mL)									
Q1 (≤ 0.29)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Q2 (0.29-0.39)	-0.02 (-0.12; 0.09)	-0.04 (-0.11; 0.03)	-0.07 (-0.15; 0.02)	0.00 (-0.08; 0.09)	0.00 (-0.08; 0.08)	-0.01 -0.09; 0.07)	-0.02 (-0.10; 0.06)	-0.04 (-0.12; 0.05)	-0.06 (-0.15; 0.04)
Q3 (0.39-0.52)	-0.02 (-0.12; 0.09)	-0.10 (-0.17; -0.02)*	-0.18 (-0.27; -0.09)*	0.02 (-0.06; 0.11)	0.01 (-0.07; 0.09)	-0.01 (-0.09; 0.07)	-0.03 (-0.11; 0.05)	-0.06 (-0.14; 0.03)	-0.10 (-0.20; -0.01)*
Q4 (0.52-0.76)	0.00 (-0.10; 0.10)	-0.12 (-0.19; -0.04)*	-0.23 (-0.32; -0.15)*	-0.07 (-0.16; 0.01)	-0.07 (-0.15; 0.01)	-0.07 (-0.15; 0.01)	-0.07 (-0.15, 0.01)	-0.07 (-0.15; 0.02)	-0.06 (-0.16; 0.03)
Q5 (≥ 0.76)	-0.01 (-0.12; 0.09)	-0.19 (-0.26; -0.11)*	-0.36 (-0.45; -0.27)*	-0.13 (-0.21; -0.04)*	-0.13 (-0.21; -0.04)*	-0.13 (-0.21; -0.05)*	-0.13 (-0.21; -0.05)*	-0.12 (-0.21; -0.04)*	-0.12 (-0.22; -0.03)*
PlGF (pg/mL)									
Q1 (≤ 6.40)	0.01 (-0.10; 0.12)	-0.20 (-0.28; -0.11)*	-0.40 (-0.50; -0.31)*	-0.20 (-0.30; -0.11)*	-0.20 (-0.30; -0.11)*	-0.21 (-0.30; -0.12)*	-0.21 (-0.30; -0.12)*	-0.21 (-0.30; -0.12)*	-0.22 (-0.33; -0.12)*
Q2 (6.40-7.90)	0.09 (-0.03; 0.20)	-0.08 (-0.16; 0.00)	-0.24 (-0.34; -0.15)*	-0.13 (-0.22; -0.03)*	-0.13 (-0.22; -0.04)*	-0.13 (-0.22; -0.04)*	-0.14 (-0.22; -0.05)*	-0.14 (-0.23; -0.05)*	-0.15 (-0.25; -0.04)*
Q3 (7.90-9.30)	0.03 (-0.08; 0.14)	-0.05 (-0.13; 0.03)	-0.13 (-0.23; -0.04)*	-0.04 (-0.13; 0.06)	-0.04 (-0.13; 0.06)	-0.03 (-0.13; 0.06)	-0.02 (-0.11; 0.07)	-0.02 (-0.11; 0.07)	0.00 (-0.11; 0.10)
Q4 (9.30-11.20)	-0.02 (-0.13; 0.09)	-0.02 (-0.10; 0.06)	-0.02 (-0.12; 0.07)	0.03 (-0.06; 0.13)	0.03 (-0.06; 0.12)	0.03 (-0.05; 0.12)	0.03 (-0.05; 0.12)	0.04 (-0.05; 0.13)	0.04 (-0.06; 0.14)
Q5 (≥ 11.20)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*

Supplemental table 7 Associations of **umbilical cord** sFlt-1 and PlGF concentrations with fetal and childhood **weight** growth (n = 4108). (continued)

sFl-1:PlGF ratio									
Q1 (≤ 31.49)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Q2 (31.50-45.52)	-0.02 (-0.13; 0.09)	-0.03 (-0.11; 0.05)	-0.05 (-0.14; 0.05)	-0.11 (-0.20; -0.01)*	-0.11 (-0.20; -0.02)*	-0.11 (-0.19; -0.02)*	-0.10 (-0.19; -0.01)*	-0.10 (-0.19; -0.01)*	-0.09 (-0.20; .01)
Q3 (45.53-62.87)	-0.13 (-0.24; -0.02)	-0.16 (-0.24; -0.08)*	-0.19 (-0.28; -0.09)*	-0.13 (-0.23; -0.04)*	-0.13 (-0.23; -0.04)*	-0.14 (-0.23; -0.05)*	-0.14 (-0.23; -0.05)*	-0.14 (-0.24; -0.05)*	-0.15 (-0.26; -0.04)*
Q4 (62.88-98.20)	0.05 (-0.06; 0.16)	-0.13 (-0.22; -0.05)*	-0.32 (-0.42; -0.22)	-0.19 (-0.29; -0.09)*	-0.20 (-0.29; -0.10)*	-0.21 (-0.30; -0.12)*	-0.23 (-0.32; -0.14)*	-0.24 (-0.34; -0.15)*	-0.27 (-0.38; -0.17)*
Q5 (≥ 98.21)	-0.06 (-0.17; 0.06)	-0.29 (-0.37; -0.20)*	-0.51 (-0.61; -0.42)	-0.29 (-0.39; -0.20)*	-0.29 (-0.38; -0.19)*	-0.27 (-0.36; -0.17)*	-0.25 (-0.34; -0.15)*	-0.23 (-0.32; -0.13)*	-0.19 (-0.30; -0.08)*

Abbreviations: soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF; Standard deviation score, SDS; Confidence interval, CI; Quintile, Q.

Fetal and childhood weight growth among umbilical cord sFlt-1 and PlGF categories, based on repeated measurement regression models..

Values are adjusted for gestational age (blood sampling), maternal age, height, body mass index, parity, education, ethnicity, smoking, folic acid supplement use, systolic blood pressure and fetal gender. Childhood analysis are additionally adjusted for breastfeeding.

* *P*-value < 0.05

Supplemental table 8 Associations of **umbilical cord** sFlt-1 and PlGF concentrations with fetal and childhood **length** growth (n = 4108).

	Change in length SDS								
	Fetal			Childhood					
	20 weeks gestation	30 weeks gestation	40 weeks gestation	6 months	12 months	24 months	36 months	48 months	72 months
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
sFlt-1 (ng/mL)									
Q1 (≤ 0.29)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Q2 (0.29-0.39)	-0.04 (-0.15; 0.06)	-0.07 (-0.14; 0.01)	-0.09 (-0.20; 0.01)	-0.02 (-0.10; 0.06)	-0.03 (-0.10; 0.05)	-0.03 (-0.11; 0.04)	-0.04 (-0.11; 0.04)	-0.04 (-0.12; 0.04)	-0.05 (-0.15; 0.04)
Q3 (0.39-0.52)	0.03 (-0.07; 0.13)	-0.04 (-0.11; 0.04)	-0.10 (-0.21; 0.00)	-0.05 (-0.13; 0.03)	-0.06 (-0.13; 0.02)	-0.07 (-0.14; 0.01)	-0.08 (-0.15; 0.00)	-0.09 (-0.17; -0.01)*	-0.11 (-0.20; -0.02)*
Q4 (0.52-0.76)	0.04 (-0.06; 0.14)	-0.03 (-0.11; 0.04)	-0.11 (-0.22; 0.00)*	-0.02 (-0.10; 0.06)	-0.02 (-0.10; 0.05)	-0.04 (-0.11; 0.04)	-0.05 (-0.13; 0.03)	-0.06 (-0.14; 0.02)	-0.09 (-0.18; 0.00)
Q5 (≥ 0.76)	0.04 (-0.06; 0.14)	-0.09 (-0.17; -0.02)*	-0.22 (-0.33; -0.12)*	-0.12 (-0.20; -0.04)*	-0.12 (-0.20; -0.04)*	-0.12 (-0.20; -0.04)*	-0.12 (-0.19; -0.04)*	-0.11 (-0.20; -0.03)*	-0.11 (-0.20; -0.02)*
PlGF (pg/mL)									
Q1 (≤ 6.40)	-0.05 (-0.6; 0.06)	-0.10 (-0.18; -0.01)*	-0.14 (-0.26; -0.02)*	-0.22 (-0.31; -0.13)*	-0.22 (-0.31; -0.13)*	-0.23 (-0.31; -0.14)*	-0.23 (-0.35; -0.14)*	-0.23 (-0.32; -0.15)*	-0.24 (-0.34; -0.14)*
Q2 (6.40-7.90)	0.01 (-0.10; 0.12)	-0.04 (-0.12; 0.04)	-0.09 (-0.20; 0.02)	-0.12 (-0.21; -0.03)*	-0.12 (-0.21; -0.03)*	-0.12 (-0.21; -0.04)*	-0.12 (-0.21; -0.04)*	-0.12 (-0.21; -0.03)*	-0.12 (-0.22; -0.02)*
Q3 (7.90-9.30)	-0.02 (-0.13; 0.09)	0.00 (-0.08; 0.08)	0.02 (-0.09; 0.14)	-0.06 (-0.15; 0.03)	-0.05 (-0.14; 0.03)	-0.05 (-0.13; 0.04)	-0.05 (-0.13; 0.04)	-0.04 (-0.13; 0.05)	-0.03 (-0.13; 0.06)
Q4 (9.30-11.20)	-0.03 (-0.14; 0.08)	0.03 (-0.06; 0.11)	0.08 (-0.04; 0.19)	-0.04 (-0.12; 0.05)	-0.04 (-0.12; 0.05)	-0.04 (-0.12; 0.05)	-0.04 (-0.12; 0.05)	-0.04 (-0.12; 0.05)	-0.04 (-0.13; 0.06)
Q5 (≥ 11.20)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*

Supplemental table 8 Associations of **umbilical cord** sFlt-1 and PlGF concentrations with fetal and childhood **length** growth (n = 4108). (continued)

sFl-1:PlGF ratio									
Q1 (≤ 31.49)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Q2 (31.50-45.52)	-0.10 (-0.21; 0.01)	-0.06 (-0.14; 0.02)	-0.02 (-0.14; 0.10)	-0.10 (-0.19; -0.01)*	-0.09 (-0.18; -0.01)*	-0.09 (-0.17; -0.00)*	-0.08 (-0.17; 0.00)	-0.07 (-0.16; 0.01)	-0.06 (-0.16; 0.04)
Q3 (45.53-62.87)	-0.11 (-0.23; 0.00)	-0.14 (-0.22; -0.05)*	-0.16 (-0.28; -0.05)*	-0.10 (-0.19; -0.01)*	-0.10 (-0.19; -0.01)*	-0.11 (-0.20; -0.03)*	-0.13 (-0.21; -0.04)*	-0.14 (-0.23; -0.05)*	-0.16 (-0.26; -0.06)*
Q4 (62.88-98.20)	0.07 (-0.04; 0.19)	-0.05 (-0.14; 0.03)	-0.18 (-0.29; -0.06)*	-0.13 (-0.23; -0.04)*	-0.14 (-0.23; -0.05)*	-0.16 (-0.25; -0.07)*	-0.17 (-0.26; -0.09)*	-0.19 (-0.28; -0.10)*	-0.22 (-0.33; -0.12)*
Q5 (≥ 98.21)	-0.04 (-0.16; 0.08)	-0.16 (-0.25; -0.08)*	-0.29 (-0.40; -0.17)*	-0.27 (-0.36; -0.17)*	-0.26 (-0.35; -0.17)*	-0.24 (-0.33; -0.16)*	-0.23 (-0.32; -0.14)*	-0.22 (-0.31; -0.12)*	-0.19 (-0.29; -0.08)*

Abbreviations: soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF; Standard deviation score, SDS; Confidence interval, CI; Quintile, Q.

Fetal and childhood length growth among umbilical cord sFlt-1 and PlGF categories, based on repeated measurement regression models.

Values are adjusted for gestational age (blood sampling), maternal age, height, body mass index, parity, education, ethnicity, smoking, folic acid supplement use, systolic blood pressure and fetal gender. Childhood analysis are additionally adjusted for breastfeeding.

* *P*-value < 0.05

Supplemental table 9 Associations of maternal and umbilical cord sFlt-1 and PlGF concentrations with postnatal catch-up growth.

	Catch-up growth		**Catch-up growth**	
	n = 5980		n = 4108	
	OR (95% CI)	***P*-value**	OR (95% CI)	***P*-value**
Maternal				
Early pregnancy sFlt-1 (ng/mL)				
Q1 (≤ 3.34)	*Reference*			
Q2 (3.35-4.47)	0.71 (0.56-0.90)	0.005		
Q3 (4.48-5.75)	0.72 (0.57-0.92)	0.008		
Q4 (5.76-7.75)	0.66 (0.51-0.84)	0.001		
Q5 (≥ 7.76)	0.67 (0.52-0.86)	<0.001		
Early pregnancy PlGF (pg/mL)				
Q1 (≤ 26.70)	1.31 (0.93-1.85)	0.09		
Q2 (26.71-36.50)	1.20 (0.87-1.65)	0.22		
Q3 (36.51-51.50)	1.05 (0.78-1.41)	0.70		
Q4 (51.51-82.85)	1.28 (0.98-1.68)	0.08		
Q5 (≥ 82.86)	*Reference*			
Delta sFlt-1 (ng/mL)				
Q1 (≤ -0.22)	*Reference*			
Q2 (-0.21, -0.08)	1.07 (0.81-1.41)	0.57		
Q3 (-0.07, 0.03)	1.12 (0.85-1.46)	0.40		
Q4 (0.04, 0.23)	1.09 (0.83-1.43)	0.46		
Q5 (≥ 0.24)	1.33 (1.02-1.74)	0.03		
Delta PlGF (pg/mL)				
Q1 (≤ 13.06)	1.58 (1.21-2.06)	<0.001		
Q2 (13.07-18.53)	1.12 (0.85-1.47)	0.39		
Q3 (18.54-24.66)	1.11 (0.85-1.46)	0.42		
Q4 (24.67-35.79)	1.16 (0.89-1.52)	0.26		
Q5 (≥ 35.80)	*Reference*			
Early pregnancy sFlt-1:PlGF ratio				
Q1 (≤ 57.32)	*Reference*			
Q2 (57.33-95.07)	0.88 (0.68-1.14)	0.33		
Q3 (95.08-140.65)	0.86 (0.66-1.13)	0.27		
Q4 (140.66-211.38)	0.77 (0.58-1.02)	0.07		
Q5 (≥ 211.39)	0.79(0.59-1.07)	0.13		
Mid-pregnancy sFlt-1:PlGF ratio				
Q1 (≤ 13.02)	*Reference*			
Q2 (13.03-20.15)	0.99 (0.76-1.29)	0.92		
Q3 (20.16-29.63)	0.99 (0.76-1.29)	0.92		
Q4 (29.64-45.48)	1.06 (0.82-1.38)	0.65		

Supplemental table 9 Associations of maternal and umbilical cord sFlt-1 and PlGF concentrations with postnatal catch-up growth. (continued)

	Catch-up growth		**Catch-up growth**	
	n = 5980		n = 4108	
	OR (95% CI)	***P*-value**	OR (95% CI)	***P*-value**
Q5 (≥ 45.49)	1.20 (0.92-1.57)	0.17		
Fetal				
Umbilical cord sFlt-1 (ng/mL)				
Q1 (≤ 0.29)			*Reference*	
Q2 (0.29-0.39)			1.66 (1.18-2.32)	0.004
Q3 (0.39-0.52)			2.13 (1.54-2.95)	<0.001
Q4 (0.52-0.76)			2.09 (1.51-2.89)	<0.001
Q5 (≥ 0.76)			2.50 (1.81-3.45)	<0.001
Umbilical cord PlGF (pg/mL)				
Q1 (≤ 6.40)			1.92 (1.36-2.71)	<0.001
Q2 (6.40-7.90)			1.94 (1.38-2.73)	<0.001
Q3 (7.90-9.30)			1.19 (0.83-1.70)	0.34
Q4 (9.30-11.20)			1.44 (1.02-2.05)	0.04
Q5 (≥ 11.20)			*Reference*	
Umbilical cord sFlt-1:PlGF ratio				
Q1 (≤ 31.49)			*Reference*	
Q2 (31.50-45.52)			1.18 (0.82-1.70)	0.38
Q3 (45.53-62.87)			1.36 (0.95-1.94)	0.09
Q4 (62.88-98.20)			1.80 (1.27-2.55)	0.001
Q5 (≥ 98.21)			2.52 (1.78-3.58)	<0.001

Abbreviations: soluble fms-like tyrosine kinase 1, sFlt-1; Placental growth factor, PlGF; Odds ratio, OR; Confidence interval, CI; Quintile, Q.

Multivariable logistic regression analysis with catch-up growth as dependent variable and maternal and umbilical cord sFlt-1 and PlGF as independent variables.

Values are adjusted for gestational age (blood sampling), maternal age, height, body mass index, parity, education, ethnicity, smoking, folic acid supplement use, systolic blood pressure and fetal gender.

Chapter 7

Associations of maternal and paternal blood pressure patterns and hypertensive disorders during pregnancy with childhood blood pressure

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ABSTRACT

Objective: Hypertensive disorders in pregnancy may affect offspring cardiovascular risk. We examined the associations of maternal blood pressure throughout pregnancy and hypertensive disorders in pregnancy with childhood blood pressure. Specific focus was on the comparison with paternal blood pressure effects, the identification of critical periods and the role of birth outcomes and childhood body mass index in the observed associations.

Methods: This study was embedded in a population-based prospective cohort study among 5,310 mothers, fathers and their children. We measured maternal blood pressure in each trimester of pregnancy and paternal blood pressure once. Information about hypertensive disorders in pregnancy was obtained from medical records. We measured childhood blood pressure at the median age of 6.0 years (95% range 5.7, 8.0).

Results: Both maternal and paternal blood pressure were positively associated with childhood blood pressure (all *P*-values <0.05), with similar effect estimates. Conditional regression analyses showed that early, mid- and late pregnancy maternal blood pressure were all independent and positively associated with childhood blood pressure, with the strongest effect estimates for early pregnancy. As compared to children from mothers without hypertensive disorders in pregnancy, those from mothers with hypertensive disorders in pregnancy had 0.13 SDS (95% Confidence Interval: 0.05 to 0.21) higher diastolic blood pressure. The observed associations were not materially affected by birth outcomes and childhood body mass index.

Conclusions: Both maternal and paternal blood pressure affect childhood blood pressure, independent of fetal and childhood growth measures, with the strongest effect for maternal blood pressure in early pregnancy.

INTRODUCTION

Gestational hypertension and preeclampsia affect up to 8% of all pregnant women worldwide and are associated with both maternal and offspring cardiovascular health and disease.[1-4] It has been suggested that these associations are explained by maternal vasculotoxic factors in pregnancies with hypertensive disorders, which affect vascular development.[5, 6] Also, early placental and fetal microvasculature maladaptations may lead to a higher blood pressure in both pregnant women and their offspring.[7] Next to hypertensive disorders in pregnancy, also higher blood pressure within the normal range during pregnancy may be associated with higher offspring blood pressure.[8-12] Thus far, it is not known whether the associations of maternal blood pressure with offspring blood pressure are explained by direct maternal or intra-uterine mechanisms or just reflect shared family-based lifestyle-related or genetic factors. Comparing maternal and paternal blood pressure effects may help to disentangle the direct maternal or intra-uterine mechanisms.[13] Also, it is not known which period of pregnancy is most critical for the effects of maternal blood pressure on their offspring blood pressure. Finally, the associations of hypertensive disorders in pregnancy, with childhood blood pressure may partly be explained by a lower offspring birth weight and a higher body mass index.[8] Therefore, we examined in a population-based prospective cohort study from early pregnancy onwards among 5310 mothers, fathers and children, the associations of maternal blood pressure in different periods of pregnancy and hypertensive disorders in pregnancy with blood pressure in their school-aged children. Specific focus was on the comparison with paternal blood pressure effects, the identification of critical periods and the role of birth outcomes and childhood body mass index in the observed associations.

METHODS

Design and study population

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[14, 15] The study has been approved by the local Medical Ethics Committee. Written informed consent was obtained from the parents. All pregnant women were enrolled between 2001 and 2005. Of all eligible children in the study area, 61% participated at birth in the study. In total 8713 initially normotensive mothers had information about blood pressure measurements available, of whom 8475 gave birth to singleton live-born children. In total, 5810 (69%) of these children participated in detailed follow-up studies at the age of 6 years. We excluded children with missing blood pressure measurements (n = 477) or with congenital cardiac abnormalities (n = 23) leading to a population for analysis of 5310 mothers and their children (Flow chart is given in **Supplemental figure 1**).

Maternal and paternal blood pressure

We measured maternal and paternal blood pressure using the Omron 907 automated digital oscillometric spygmanometer (OMRON Healthcare Europe, Hoofddorp, the Netherlands).[16] As described previously, all participants were seated in an upright position with back support and were asked to relax for 5 minutes.[17] 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. For participants with an upper arm circumference exceeding 33 cm, a larger cuff (32-42 cm) was used. We used the mean value of 2 blood pressure readings over a 60-second interval. Blood pressure was measured in 4098 mothers in early pregnancy (gestational age median 13.4 (95% range 9.8-17.5) weeks), 5006 mothers in mid-pregnancy (gestational age median 20.5 (95% range 18.5-23.5) weeks) and 5104 mothers in late pregnancy (gestational age median 30.2 (95% range, 28.5-32.9) weeks). Three, two, and one blood pressure measurements were available for 3842, 1214, and 254 mothers, respectively. Of the population for analysis, blood pressure was measured during mid-pregnancy in 3805 fathers.

Hypertensive disorders in pregnancy

Information on hypertensive disorders in pregnancy, including gestational hypertension and preeclampsia, was obtained through medical records.[18] Mothers suspected of any hypertensive disorder in pregnancy based on the records were crosschecked with original charts by a trained medical record abstractor.[18] The following criteria were used to identify women with gestational hypertension: development of systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg after 20 weeks of gestation in previously normotensive women. These criteria and the presence of proteinuria (defined as 2 or more dipstick readings of 2 or greater, or 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 preeclampsia.[19]

Childhood blood pressure

Childhood 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).[20] A cuff was selected with a cuff width approximately 40% of the arm circumference and long enough to cover 90% of the arm circumference.[20] We used the mean systolic and diastolic blood pressure values using the last three blood pressure readings. Using normative values from the "Fourth report on the diagnosis, evaluation and treatment of high blood pressure in children and adolescents" from the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents,[21,22] we calculated the standard deviation scores (SDS) for individual systolic and diastolic blood pressure values. Subsequently, we used these individual SDS to categorise children in blood pressure tertiles. Children whose average systolic and/or diastolic blood pressure based on three readings was ≥ 95th percentile for age, sex and height were classified as hypertensive.

Covariates

We assessed maternal and paternal age at enrolment in the study. Information on maternal and paternal ethnicity and educational level, maternal parity, folic acid supplement use, smoking and alcohol consumption was obtained by questionnaires.[14] At enrolment, we measured maternal and paternal height and weight without shoes and heavy clothing and calculated body mass index. Information on infant sex, gestational age at birth and birth weight was obtained from medical records. At the age of 6 years, we measured child height and weight and calculated body mass index.

Statistical Analysis

First, we performed a non-response analysis by comparing subject characteristics between children with and without follow-up blood pressure measurements by using T-tests, Chi-square tests and Mann-Whitney tests. Second, we examined maternal longitudinal blood pressure patterns during pregnancy for mothers in tertiles of childhood blood pressure. For these analyses, we used mixed effects regression models. These regression models enable analyses on repeatedly measured outcomes, accounting for the correlation between repeated measurements within the same participant, and allowing for incomplete outcome data.[23] Details of the mixed effects regression models are given in the **Supplemental methods**. We also examined the associations of maternal blood pressure in different periods of pregnancy and paternal blood pressure with childhood blood pressure in three linear regression models; (1) a confounder model, which included covariates selected based on their associations with the outcome of interest based on previous studies or a change in effect estimate of >10%; (2) a birth model, which included gestational age and weight at birth in addition to the confounder model; (3) a childhood model, which included child current body mass index in addition to the confounder model. We used similar multiple regression models to examine the associations of hypertensive disorders in pregnancy with childhood blood pressure. Third, we used similar linear and logistic regression models to explore the combined effects of maternal blood pressure in early and late pregnancy and the combined effects of maternal blood pressure and paternal blood pressure on childhood blood pressure and risk of hypertension. For these analyses, we created tertiles of both maternal and paternal blood pressure. Fourth, we performed conditional regression analyses to identify the independent associations of maternal blood pressure measurements in early, mid- and late pregnancy, taking into account their correlations, with childhood blood pressure and risk of hypertension.[24] We constructed blood pressure values for each trimester, which are statistically independent from blood pressure values for other trimesters, by using standardized residuals obtained from regression of blood pressure values at a specific time point (dependent variable) on blood pressure values obtained at a previous time point.[24-26] This approach enables identification of critical periods for maternal blood pressure during pregnancy that independently of other periods during pregnancy influence childhood blood pressure. Details of these conditional regression models

are given in the **Supplemental methods**. To reduce potential bias associated with missing data, missing values of covariates (maternal and paternal ethnicity, educational level and body mass index, paternal age, maternal parity, folic acid supplement use, smoking and alcohol consumption, infant birth weight and child body mass index), were multiple imputed (n = 5 imputations), according to the Fully Conditional Specification method (predictive mean matching), assuming no monotone missing pattern. We report the pooled effect estimates after the multiple imputation procedure [27] Subject characteristics before and after imputation and the percentages of missing values are given in **Supplemental table 1.** The multiple imputation procedure was performed using Statistical Package for the Social Sciences version 21.0. Statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp). The mixed effects regression analyses were performed with the Statistical Analysis System, Prox Mixed module (version 9.3; SAS Institute Inc, Cary NC).

RESULTS

Table 1 shows the participant characteristics. In our cohort, 410 children (7.7%) were classified as hypertensive. Results from the non-response analysis showed that as compared to children with blood pressure follow-up measurements, those without these measurements had a lower birth weight and gestational age. Mothers of children with blood pressure measurements were older, used less alcohol but smoked more frequently as compared to mothers of children who were lost to follow up. Moreover, maternal systolic blood pressure throughout pregnancy was lower in the children without follow up blood pressure measurements (**Supplemental table 2**).

Figure 1 shows that as compared to children in the lowest tertile of systolic blood pressure, those in the highest tertile had mothers with a higher systolic blood pressure throughout pregnancy. For each tertile of childhood blood pressure, maternal blood pressure increased with advanced gestational age. There was no significant difference in the slope of maternal systolic blood pressure between the tertiles of their children blood pressure. For all childhood diastolic blood pressure tertiles, maternal diastolic blood pressure had a mid-pregnancy dip, with an increase thereafter. Diastolic blood pressure was the highest throughout pregnancy for mothers from children in the highest tertile. The exact corresponding regression coefficients for gestational age-independent (intercept) and gestational age dependent differences (interaction childhood blood pressure and gestational age) are given in **Supplemental table 3**. Additional analyses showed that higher maternal blood pressure in early, mid- and late pregnancy and paternal blood pressure were all, separately, associated with higher childhood blood pressure (all *P*-values <0.05). The effect estimates for mother and father were similar and not affected by birth outcomes or childhood body mass index (**Supplemental table 4**).

Table 1 Subject characteristics (n = 5310).

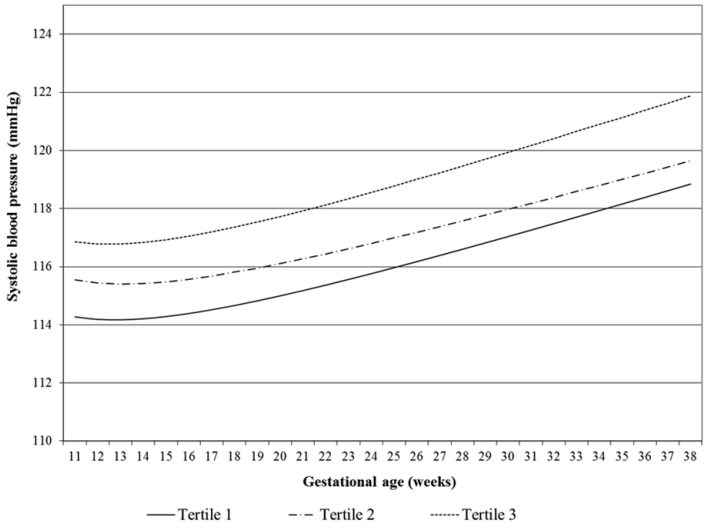
Maternal characteristics	
Age (years), median (95% range)	30.9 (19.7, 39.3)
Height (cm), mean (SD)	167.5 (7.5)
Weight (kg), mean (SD)	69.3 (12.7)
Body mass index (kg/m^2), mean (SD)	23.5 (3.8)
Parity, n (%)	
0	3008 (56.6)
≥1	2302 (43.4)
Educational level mother, n (%)	
Primary or secondary	2854 (53.7)
Higher	2456 (46.3)
Ethnicity, n (%)	
European	3167 (59.6)
Non-European	2143 (40.4)
Smoking during pregnancy, n (%)	
No	3792 (71.4)
Yes	1518 (28.6)
Alcohol using during pregnancy, n (%)	
No	2478 (46.7)
Yes	2832 (53.3)
Folic acid supplements during pregnancy, n (%)	
No	1695 (31.9)
Yes	3615 (68.1)
Blood pressure	
Early pregnancy	
Gestational age (weeks), median (95% range)	13.4 (9.8, 17.5)
Systolic blood pressure (mmHg), mean (SD)	115.5 (12.0)
Diastolic blood pressure (mmHg), mean (SD)	68.1 (9.3)
Mid-pregnancy	
Gestational age (weeks), median (95% range)	20.5 (18.5, 23.5)
Systolic blood pressure (mmHg), mean (SD)	116.8 (11.9)
Diastolic blood pressure (mmHg), mean (SD)	67.2 (9.3)
Late pregnancy	
Gestational age (weeks), median (95% range)	30.2 (28.5, 32.9)
Systolic blood pressure (mmHg), mean (SD)	118.4 (11.9)
Diastolic blood pressure (mmHg), mean (SD)	69.1 (9.2)
Hypertensive disorders in pregnancy, n (%)	
Any	308 (5.8)
Gestational hypertension	215 (4.0)
Preeclampsia	93 (1.8)

Paternal characteristics	
Age (years), median (95% range)	33.0 (21.7, 45.2)
Height (cm), mean (SD)	181.9 (7.7)
Weight (kg), mean (SD)	83.7 (11.6)
Body mass index (kg/m^2), mean (SD)	25.3 (3.2)
Ethnicity, n (%)	
European	3274 (61.7)
Non-European	2036 (38.3)
Educational level, n (%)	
Primary or secondary	2896 (54.5)
Higher	2414 (45.5)
Systolic blood pressure (mmHg), mean (SD)	130.2 (13.5)
Diastolic blood pressure (mmHg), mean (SD)	73.4 (10.6)
Birth characteristics	
Female, n (%)	2656 (50.0)
Gestational age (weeks), median (95% range)	40.1 (35.9, 42.3)
Birth weight (g), mean (SD)	3430 (548)
Childhood characteristics	
Age (years), median (95% range)	6.0 (5.7, 8.0)
Height (cm), mean (SD)	119.5 (6.1)
Weight (kg), mean (SD)	23.3 (4.3)
Body mass index (kg/m^2), mean (SD)	16.2 (1.9)
Systolic blood pressure (mmHg), mean (SD)	102.7 (8.2)
Diastolic blood pressure (mmHg), mean (SD)	60.7 (6.9)
†Z score Systolic blood pressure, mean (SD)	0.53 (0.7)
†Z score Diastolic blood pressure, mean (SD)	0.34 (0.6)
‡Blood pressure ≥ 95th percentile, n (%)	410 (7.7)

Values represent means (SD), medians (95% range), or numbers (%).
† Z scores of systolic and diastolic blood pressure are calculated using normative values from the "Fourth report on the diagnosis, evaluation and treatment of high blood pressure in children and adolescents" from the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents.
‡ Blood pressure ≥ 95th percentile (systolic and/or diastolic blood pressure ≥ 95th percentile) for age, sex and height on three measurements.
Subject characteristics before and after imputation are shown **Supplemental table 1**.

Figure 2A shows the combined associations of maternal blood pressure during early and late pregnancy. As compared to children from mothers with a blood pressure in the lowest tertiles during both early and late pregnancy, those with a blood pressure in the highest tertiles during both early and late pregnancy had 0.24 SDS (95% Confidence Interval (CI) 0.16, 0.31) and 0.18 SDS (95% CI 0.11, 0.24) higher systolic and diastolic blood pressure, respectively.

A. Systolic blood pressure



B. Diastolic blood pressure

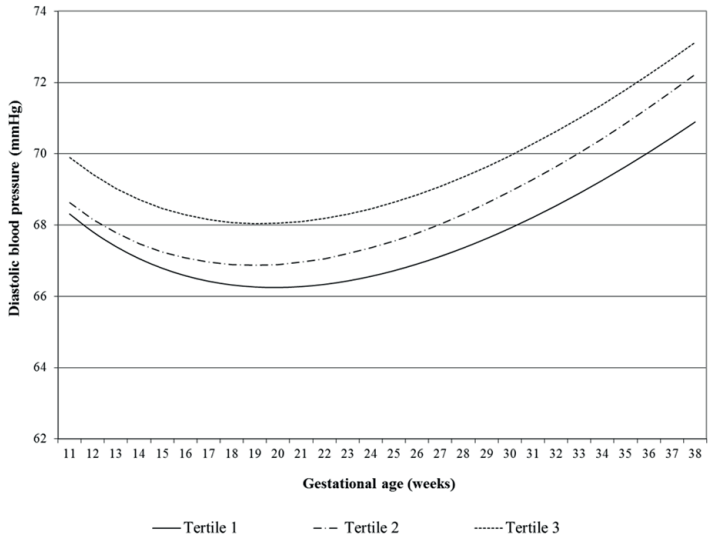


Figure 1 Maternal blood pressure patterns from children in different blood pressure tertiles (n = 5310). Maternal blood pressure pattern per childhood blood pressure tertile. (**A**) Systolic blood pressure. Difference in maternal systolic blood pressure (mmHg) between childhood systolic blood pressure tertiles based on mixed effects regression models. Model: Maternal systolic blood pressure = $\beta_0 + \beta_1$* child systolic blood pressure tertile + β_2 * gestational age + β_3 * gestational age^{-2} + β_4 * child systolic blood pressure tertile* gestational age. (**B**) Diastolic blood pressure. Difference in maternal diastolic blood pressure (mmHg) for childhood diastolic blood pressure tertiles based on repeated measurement analysis. Model: Maternal diastolic blood pressure = $\beta_0 + \beta_1$* child diastolic blood pressure tertile + β_2 * gestational age + β_3 * gestational age$^{0.5}$ + β_4 * child diastolic blood pressure tertile * gestational age. Effect estimates (95% confidence intervals) are given in **Supplemental table 3**.

Also, within each tertile of maternal early pregnancy blood pressure, maternal late pregnancy blood pressure was associated with a higher childhood blood pressure with the strongest effect estimates in early pregnancy. **Figure 2B** shows the combined associations of maternal early pregnancy and paternal blood pressure. As compared to children from mothers and fathers with a blood pressure in the lowest tertiles, those from mothers and fathers with a blood pressure in the highest tertiles had 0.26 SDS (95% CI 0.17, 0.37) and 0.19 SDS (95% CI 0.11, 0.28) higher systolic and diastolic blood pressure, respectively. Results from the confounder and birth models for these stratified analyses are given in **Supplemental figure 2(A-D)**. None of the statistical interactions terms were significant.

Figure 3A shows the results of the combined associations of maternal blood pressure during early and late pregnancy with the risk of childhood hypertension. Children of mothers with a systolic and diastolic blood pressure in the highest tertiles during both early and late pregnancy had a higher risk of hypertension: Odds Ratio (OR) 2.66 (95% CI 1.71, 4.13) and 1.63 (95% CI 1.09, 2.46), respectively, as compared to children from mothers with a systolic and diastolic blood pressure in the lowest tertiles during both early and late pregnancy.

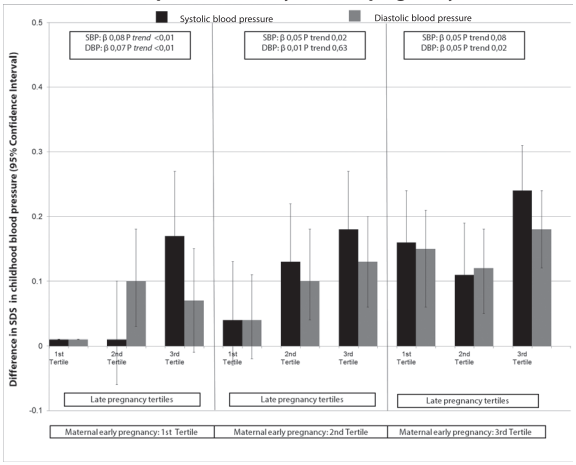
Figure 3B shows the combined associations of maternal early pregnancy and paternal blood pressure. Children of mothers and fathers with blood pressure in the highest tertiles had a higher risk of having hypertension OR 2.18 (95% CI 1.25, 3.79) and 2.20 (95% CI 1.25, 3.93), respectively for systolic and diastolic blood pressure, as compared to children from mothers and fathers with a systolic and diastolic blood pressure in the lowest tertiles.

Figure 4A shows that maternal blood pressure in early, mid- and late pregnancy were all independently associated with childhood blood pressure (all *P*-values <0.05). The strongest effect estimates were observed for early pregnancy maternal blood pressure (differences in childhood systolic and diastolic blood pressure 0.08 SDS (95% CI 0.05, 0.10) and 0.05 SDS (95% CI 0.03, 0.07) per standardised residual increase in maternal systolic and diastolic blood pressure).

Figure 4B shows that maternal systolic blood pressure, but not diastolic, in early, mid- and late pregnancy were all independently associated with the risk of childhood hypertension (all *P*-values <0.05). The strongest effect estimates were observed for early pregnancy maternal blood pressure (OR in childhood risk of hypertension 1.25 (95% CI 1.11, 1.42) per standardised residual increase in maternal systolic blood pressure).

Table 2 shows that as compared to children from mothers without hypertensive disorders in pregnancy, those from mothers with hypertensive disorders in pregnancy had a higher diastolic, but not systolic, blood pressure. These associations were mainly driven by gestational hypertension (difference in diastolic blood pressure 0.13 SDS (95% CI 0.05, 0.21) between children from mothers with and without gestational hypertension). Preeclampsia was not associated with childhood blood pressure.

A. Maternal blood pressure in early and late pregnancy



B. Maternal and paternal blood pressure

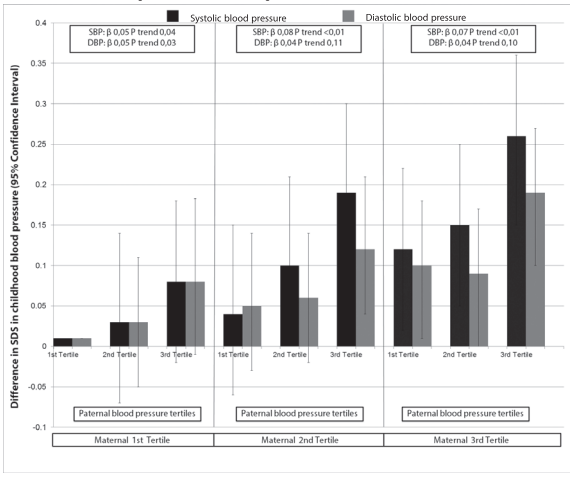


Figure 2 Combined associations of maternal and paternal blood pressure with childhood blood pressure (n = 5310).

Abbreviations: Body mass index, BMI; Diastolic blood pressure, DBP; Systolic blood pressure, SBP; Standard deviation score, SDS.

Values are regression coefficients (95% confidence interval) from multiple linear regression models. Estimates are based on multiple imputed data.

Values are adjusted for maternal age, gestational age at measurement, pre-pregnancy body mass index, parity, ethnicity, educational level, smoking and alcohol consumption during pregnancy, folic acid supplement intake and childhood body mass index.

Estimates regarding childhood systolic blood pressure are assessed by combining parental systolic blood pressure tertiles.

Estimates regarding childhood diastolic blood pressure are assessed by combining parental diastolic blood pressure tertiles. The interaction term of maternal late and early pregnancy blood pressure and for the interaction term of maternal and paternal blood pressure were not statistically significant.

Table 2 Associations of hypertensive disorders in pregnancy with childhood blood pressure (n = 5310).

	Childhood blood pressure (SDS)		
	Confounder model	Birth model	Childhood model
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
None (n = 4888)	*Reference*	*Reference*	*Reference*
Any complications (n = 308)			
Childhood systolic blood pressure	0.07 (-0.02, 0.15)	0.03 (-0.05, 0.12)	0.06 (-0.02, 0.14)
Childhood diastolic blood pressure	0.10 (0.02, 0.17)**	0.08 (0.01, 0.15)*	0.10 (0.02, 0.17)**
Gestational hypertension (n = 215)			
Childhood systolic blood pressure	0.06 (-0.04, 0.15)	0.04 (-0.06, 0.13)	0.06 (-0.04, 0.15)
Childhood diastolic blood pressure	0.13 (0.05, 0.21)**	0.11 (0.03, 0.19)**	0.13 (0.05, 0.21)**
Preeclampsia (n = 93)			
Childhood systolic blood pressure	0.14 (-0.01, 0.28)	0.06 (-0.08, 0.21)	0.14 (-0.01, 0.28)
Childhood diastolic blood pressure	0.03 (-0.09, 0.15)	-0.01 (-0.13, 0.11)	0.03 (-0.09, 0.15)

Abbreviations: Standard deviation score; SDS, Confidence interval, CI.
Values are regression coefficients (95% confidence interval) based on multiple linear regression models. Estimates are from multiple imputed data.
Pregnancies without gestational hypertension or preeclampsia were taken as reference category.
Confounder model: Adjusted for maternal age, pre-pregnancy body mass index, ethnicity, parity, educational level, smoking during pregnancy, alcohol consumption, and folic acid supplement intake.
Birth model: confounder model and additionally adjusted for gestational age at birth and birth weight.
Childhood model: confounder model and additionally adjusted for childhood current body mass index.
*P<0.05 **P<0.01

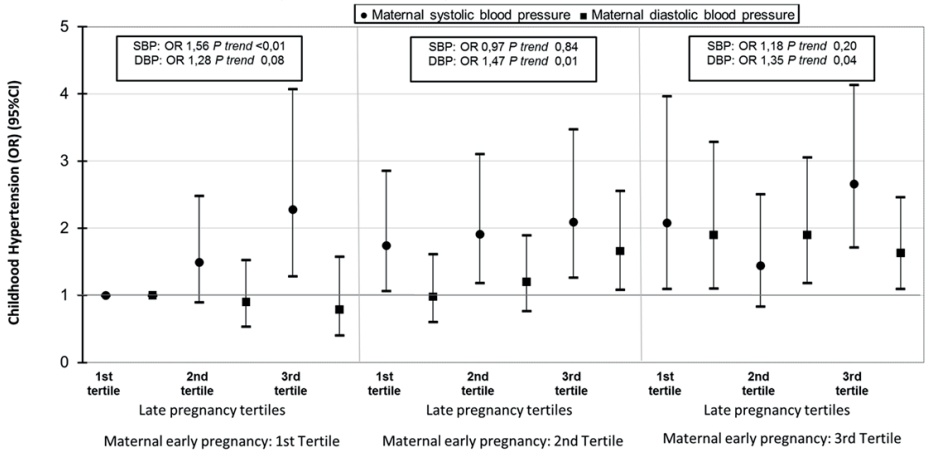
DISCUSSION

In this population-based prospective cohort study, we observed that both higher maternal blood pressure throughout pregnancy and paternal blood pressure are associated with higher childhood blood pressure. Early, mid- and late pregnancy maternal blood pressure were all independently associated with childhood blood pressure, with the strongest effect estimates for early pregnancy. Gestational hypertension was associated with higher childhood diastolic blood pressure. The observed associations were largely independent from fetal and childhood growth measures.

Methodological considerations

A major strength of our study is the prospective design from early pregnancy onwards within a large population-based cohort. Furthermore, we measured maternal blood pressure in different pregnancy periods. Not all mothers had blood pressure measurements in each trimester of pregnancy. Restricting our analyses to mothers who had blood pressure measurements in all three trimesters (n = 3842), revealed similar results as in the full group. Of all children from mothers with information about blood pressure and pregnancy complications 65%

A. Maternal blood pressure in early and late pregnancy



B. Maternal and paternal blood pressure

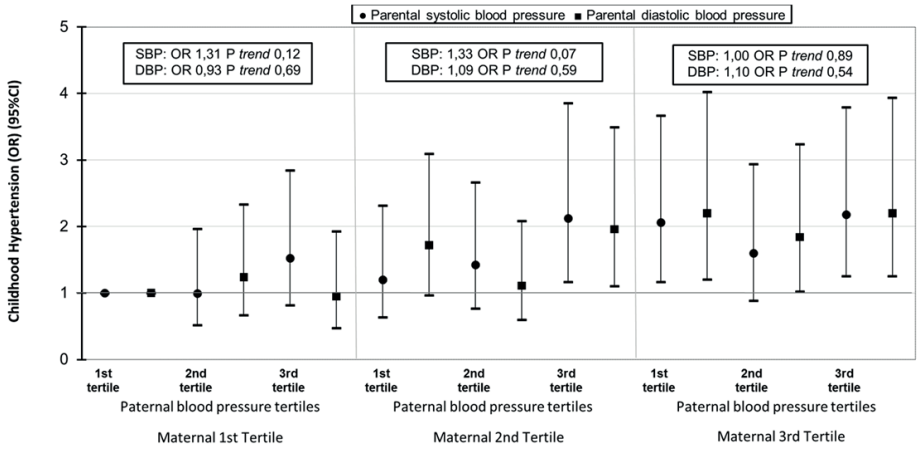


Figure 3 combined associations of maternal and paternal blood pressure with childhood hypertension (n = 5310).

Abbreviations: Body mass index, BMI; Diastolic blood pressure, DBP; Odds ratio, OR; Systolic blood pressure, SBP; Standard deviation score, SDS.

Values are regression coefficients (95% confidence interval) from logistic regression models. Estimates are based on multiple imputed data.

Values are adjusted for maternal age, gestational age at measurement, pre-pregnancy body mass index, parity, ethnicity, educational level, smoking and alcohol consumption during pregnancy, folic acid supplement intake and childhood body mass index.

Estimates regarding childhood hypertension are assessed by combining parental systolic and diastolic blood pressure tertiles, respectively.

participated in the follow-up measurements at the age of 6 years and had blood pressure information available. As compared to children with blood pressure follow-up measurements, those without follow-up measurements had mothers with lower systolic blood pressure throughout pregnancy and at birth a lower weight and younger gestational age. A selective loss to follow-up may have reduced variation in blood pressure development and therefore reduced the power to detect differences. Also, loss to follow-up would lead to selection bias if the associations of maternal blood pressure with childhood blood pressure would be different between those included and those not included in the final analyses. Although we do not expect this likely, selection bias cannot be excluded. Blood pressure has a large within subject-variation and is also liable to measurement error. This measurement error may have led to an underestimation of the observed effect estimates.[17] Furthermore, the number of cases with hypertensive disorders in pregnancy was relatively small, which might have led to lack of power for the associations of hypertensive disorders in pregnancy with childhood blood pressure. Family history of hypertension may also influence childhood blood pressure. Unfortunately, information about family history of hypertension, was only available in a small subset of our cohort. Finally, although we performed adjustment for a large number of potential maternal and paternal confounders, residual confounding by other socio-economic or lifestyle related factors might still be present, as in any observational study.

Interpretation

We hypothesised that higher maternal blood pressure within the normal range during pregnancy and hypertensive disorders in pregnancy influence blood pressure development in childhood. This hypothesis is based on previous studies suggesting that hypertensive disorders in pregnancy are associated with higher offspring blood pressure.[2, 8, 9, 28] A study among 6343 mother-children pairs in the United Kingdom, showed that gestational hypertension, but not preeclampsia, was associated with a higher blood pressure in children at the age of 9 years.[8] A systemic review and meta-analyses, with data from 18 studies, showed that children of mothers with preeclampsia had a higher blood pressure in childhood and early adulthood.[28] A recent study from the United Kingdom suggested that children of mothers with hypertensive disorders in pregnancy had higher blood pressure at the age of 7 to 18 years.[12] In line with the results of these previous studies, we observed that higher maternal blood pressure during pregnancy was associated with a higher blood pressure in children aged 6 years. Children from mothers with hypertensive disorders in pregnancy had a higher diastolic blood pressure, compared to children of mothers without hypertensive disorders in pregnancy. These associations were mainly driven by gestational hypertension, and not present for
preeclampsia. Thus, results from both previous and our study suggest that maternal blood pressure during pregnancy affect childhood blood pressure. Yet, not much is known about the specific maternal and paternal effects, critical periods, and role of fetal and childhood growth in the associations.

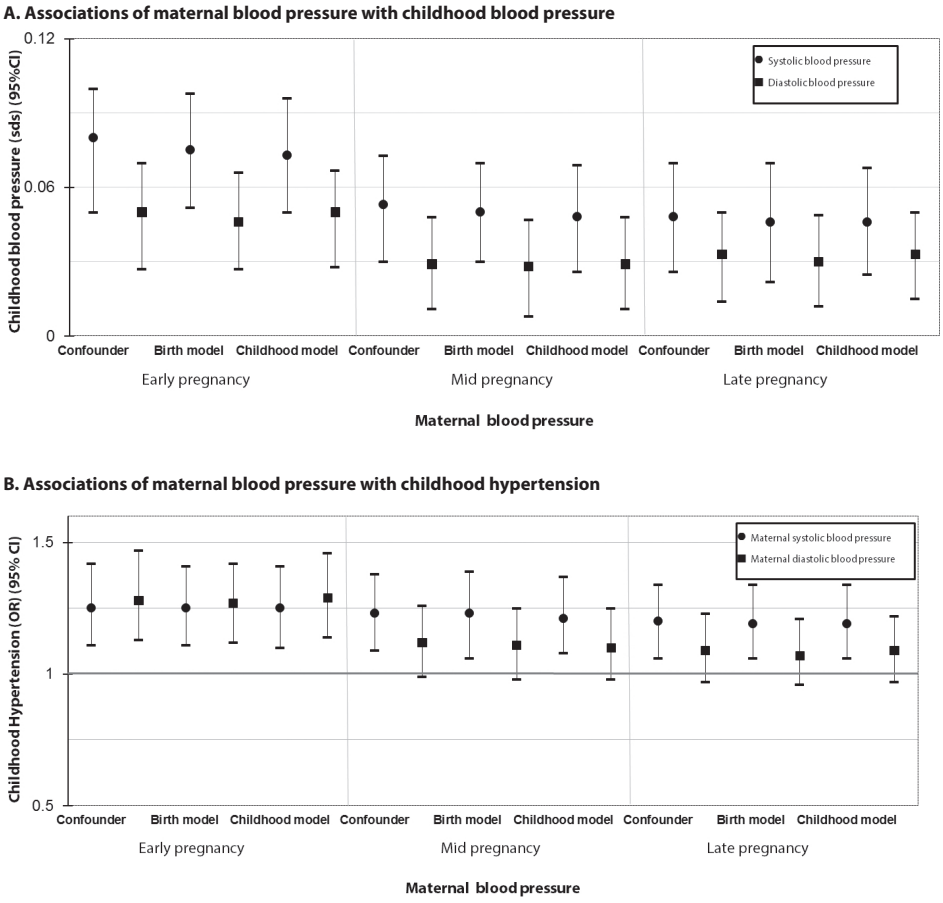


Figure 4 Associations of maternal blood pressure with childhood blood pressure and hypertension from conditional regression models (n = 5310).
Abbreviations: Odds ratio, OR; Standard deviation score, SDS.
Values are linear (**A**) and logistic (**B**) regression coefficients (95% Confidence Interval) that reflect the difference in childhood systolic and diastolic blood pressure per standardised residual for maternal blood pressure during each trimester of pregnancy independent from previous trimesters blood pressure measurements.
Confounder model: Adjusted for maternal age, pre-pregnancy body mass index, ethnicity, parity, educational level, smoking during pregnancy, alcohol consumption and folic acid supplement intake.
Birth model: confounder model and additionally adjusted for birth weight and gestational age.
Childhood model: confounder model and additionally adjusted for child current body mass index.

In the current study, we observed that both, maternal and paternal blood pressure were associated with childhood blood pressure and the risk of hypertension. Also, within each tertile of maternal blood pressure, higher paternal blood pressure was associated with childhood blood pressure. Only a few previous studies have explored the effect of maternal and paternal blood

pressure on childhood blood pressure and the risk of hypertension.[29-31] These studies suggest that both higher maternal and paternal blood pressure are associated with an increased risk for higher childhood blood pressure.[29, 31] The presence of hypertension in both parents has an additive effects on childhood blood pressure levels.[29, 32] A recent study suggested that children from hypertensive parents had a higher risk of hypertension.[32] Similar associations for maternal and paternal blood pressure suggest that genetic or shared family based factors, rather than direct intra-uterine programming may explain the associations of maternal blood pressure with childhood blood pressure.[13] Thus, our results suggest that both maternal and paternal blood pressure are important for childhood blood pressure, in a similar magnitude.

We aimed to identify critical periods during pregnancy that impact childhood blood pressure. Our results suggest that early, mid- and late pregnancy are all independently associated with childhood blood pressure. Differences between early, mid- and late pregnancy were small, but slightly stronger effect estimates were observed for early pregnancy. A recent study from a prospective cohort in the United Kingdom, also showed that early pregnancy appears to be the most contributable period during pregnancy on childhood blood pressure.[12] Some mechanisms have been hypothesised to underlie the association of maternal blood pressure levels during early pregnancy with blood pressure levels in offspring.[7] A higher maternal blood pressure in early pregnancy may be a marker of maternal and placental vascular maladaptations,[33] leading to fetal growth restriction and abnormal fetal vascular development,[34] which may subsequently affect childhood blood pressure.[35] Also, higher maternal blood pressure levels in early pregnancy may be predictors of hypertensive disorders in pregnancy that are in turn predictors of maternal and offspring cardiovascular diseases later in life. Thus although blood pressure in each period of pregnancy seems to be independently associated with childhood blood pressure, especially early pregnancy may be critical for childhood blood pressure.

Consistent evidence suggests that preterm birth and low birth weight are associated with childhood blood pressure, although the effects seem to be small.[36, 37] Also, body mass index is one of the strongest predictors of blood pressure in childhood.[26] Therefore, associations of maternal blood pressure with childhood pressure may be partly explained by preterm birth, low birth weight and high body mass index. However, we observed that the effect estimates of parental blood pressure or hypertensive disorders in pregnancy with childhood blood pressure did not materially change after additional adjustment for birth outcomes or childhood body mass index. We also explored whether including size at birth for gestational age, instead of birth weight, would affect the results, but this was not the case. These findings are in line with the large study from the United Kingdom, showing that the effects of hypertensive disorders in pregnancy on childhood blood pressure were largely independent from maternal and childhood obesity.[2] Current results suggest that the associations of parental blood pressure and hypertensive disorders in pregnancy with childhood blood pressure are not explained by fetal and childhood growth measures.

The prevalence of hypertension in children and adolescents in the Western countries has been reported at 1% to 5%.[38] Except the already known childhood risk factors, such as body mass index in developing primary hypertension, other parental factors should be considered in screening guidelines.[38] Young offspring from mothers, who had high blood pressure in early pregnancy or gestational hypertension may be specific groups at risk for having a high blood pressure from childhood onwards. Whether these findings can be translated to primary prevention strategies for primary hypertension in children and adolescents should be further studied.

CONCLUSIONS

In summary, our results suggest that both higher maternal blood pressure throughout pregnancy and paternal blood pressure influence childhood blood pressure. Early, mid- and late pregnancy maternal blood pressure were all independently associated with childhood blood pressure, with the strongest effect estimates for early pregnancy. The observed associations were largely independent from fetal and childhood growth measures. Further follow-up studies are needed to investigate whether parental blood pressure and hypertensive disorders in pregnancy affect cardiovascular risk at older ages.

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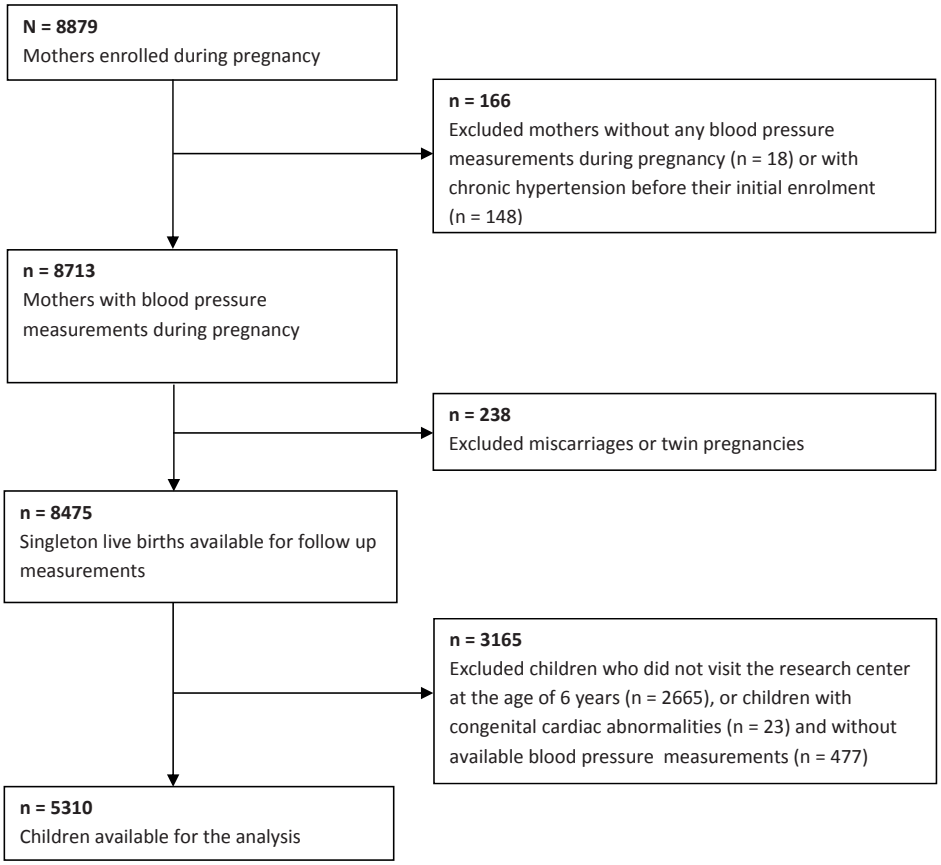
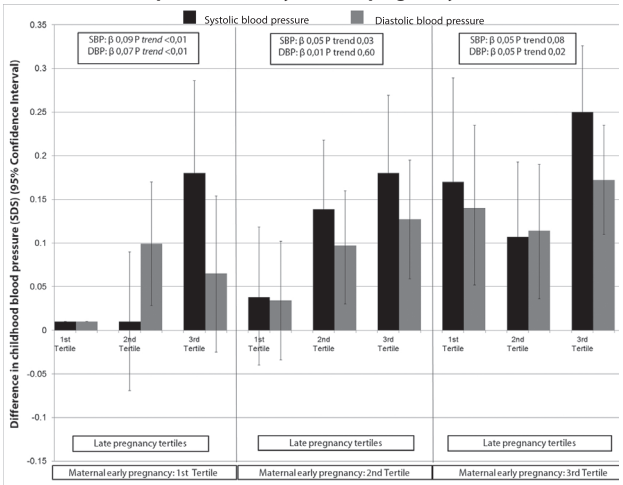
SUPPLEMENTAL MATERIAL

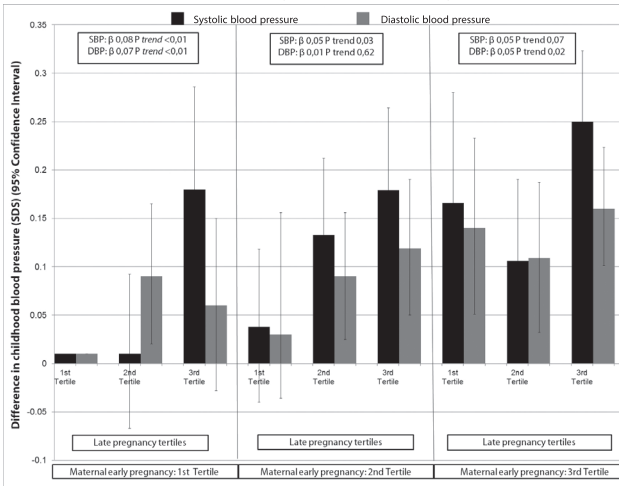


Supplemental figure 1 Flowchart

A. Maternal blood pressure in early and late pregnancy, confounder model



B. Maternal blood pressure in early and late pregnancy, birth model



Supplemental figure 2 Combined associations of maternal and paternal blood pressure with childhood blood pressure, confounder and birth models (n = 5310).

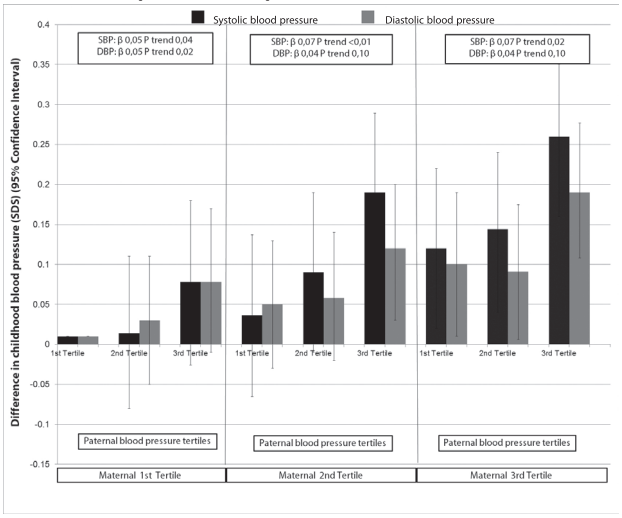
Values are regression coefficients (95% confidence intervals) from multiple linear regression models. Estimates are based on multiple imputed data.

Supplemental figure A-B: Estimates regarding childhood systolic blood pressure are assessed by combining maternal early pregnancy with late pregnancy systolic blood pressure tertiles. Estimates regarding childhood diastolic blood pressure are assessed by combining maternal early with late pregnancy diastolic blood pressure tertiles.

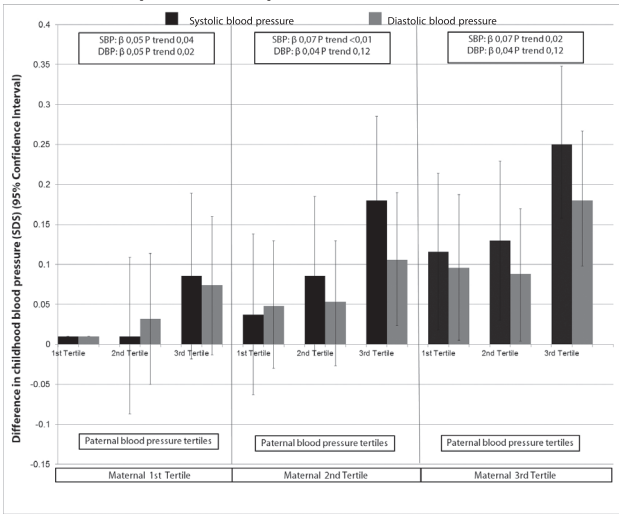
Confounder model (**A**): Adjusted for maternal age, gestational age at measurement, pre-pregnancy body mass index, parity, ethnicity, educational level, smoking and alcohol consumption during pregnancy, folic acid supplement intake

Birth model (**B**): confounder model and additionally adjusted for birth weight and gestational age at birth.

C. Maternal and paternal blood pressure, confounder model



D. Maternal and paternal blood pressure, birth model



Supplemental figure 2 Combined associations of maternal and paternal blood pressure with childhood blood pressure, confounder and birth models (n = 5310).

Values are regression coefficients (95% confidence intervals) from multiple linear regression models. Estimates are based on multiple imputed data.

Supplemental figure C-D: Estimates regarding childhood systolic blood pressure are assessed by combining maternal early pregnancy systolic blood pressure tertiles with paternal systolic blood pressure tertiles. Estimates regarding childhood diastolic blood pressure are assessed by combining maternal early pregnancy diastolic blood pressure tertiles with paternal diastolic blood pressure tertiles.

Confounder model (**C**): Adjusted for maternal and paternal age, gestational age at measurement, (pre-pregnancy) body mass index, ethnicity, educational level, maternal smoking and alcohol consumption during pregnancy, folic acid supplement intake.

Birth model (**D**): confounder model and additionally adjusted for birth weight and gestational age at birth.

Supplemental methods

Mixed effects regression models

We used unbalanced repeated measurement regression models to examine maternal longitudinal blood pressure patterns in tertiles of childhood blood pressure. These models take the correlation between repeated measurements within the same subject into account by modelling the correlated errors of these measurements.[1] Both gestational age-independent (difference constant over time) and gestational age-dependent (difference not-constant over time) effects were assessed. We constructed best-fitting models for maternal blood pressure patterns. 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 goodness of fit. Since adding fractional polynomials of gestational age to the model improved the model fit, we included these fractional polynomials in the final models. We used a compound symmetry covariance structure. Childhood blood pressure in tertiles were included in these models as intercept and as an interaction term with gestational age. The final models can be written as:

Maternal systolic blood pressure:

Difference in maternal systolic blood pressure (mmHg) between childhood systolic blood pressure tertiles based on repeated measurement regression analysis = $\beta_0 + \beta_1$* child systolic blood pressure tertile + β_2 * gestational age + β_3 * gestational age^{-2} + β_4 * child systolic blood pressure tertile* gestational age.

Maternal diastolic blood pressure:

Difference in maternal diastolic blood pressure (mmHg) for childhood diastolic blood pressure tertiles based on repeated measurement analysis = $\beta_0 + \beta_1$* child diastolic blood pressure tertile + β_2 * gestational age + β_3 * gestational age$^{0.5}$ + β_4 * child diastolic blood pressure tertile * gestational age.

Conditional regression analyses

We performed conditional regression analyses to identify the independent associations of first, second and third trimester maternal blood pressure, taking into account their correlations, with childhood blood pressure.[2] We constructed blood pressure values for each trimester, which are statistically independent from blood pressure values for other trimesters, by using standardised residuals obtained from regression of blood pressure values at a specific time point (dependent variable) on blood pressure values obtained at a previous time point.[3,4] These standardised residuals, which are assumed to be independent of the estimated regression line (and thus from the previous blood pressure), were taken forward to the regression models as independent variable with childhood blood pressure (dependent variable). As conditional blood pressure measurements are statistically independent of each other, this approach allows inclusion of blood pressure values from different trimesters simultaneously in one linear regression model when continuous childhood blood pressure was the outcome, or in one logistic regression model when childhood hypertension was the outcome. For the conditional analyses, we imputed maternal blood pressure measures. Results from these datasets were pooled and presented in the conditional results.

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Supplemental table 1 Subject characteristics in the original and imputed dataset (n = 5310).

	Observed	Imputed
Maternal characteristics		
Age (years), median (95% range)	30.9 (19.7, 39.3)	30.9 (19.7, 39.3)
Height (cm), mean (SD)	167.5 (7.4)	167.5 (7.5)
Missing, n (%)	19 (3.6)	
Weight (kg), mean (SD)	69.3 (12.8)	69.3 (12.7)
Missing, n (%)	18 (3.4)	
Body mass index (kg/m^2), mean (SD)	24.7 (4.3)	24.7 (4.3)
Missing, n (%)	36 (6.8)	
Parity, n (%)		
0	2990 (56.3)	3008 (56.6)
≥1	2280 (42.9)	2302 (43.4)
Missing	40 (0.8)	
Educational level mother, n (%)		
Primary or secondary	2673 (50.3)	2854 (53.7)
Higher	2295 (43.2)	2456 (46.3)
Missing	342 (6.5)	
Ethnicity, n (%)		
European	3135 (59.0)	3167 (59.6)
Non-European	2059 (38.8)	2143 (40.4)
Missing	116 (2.2)	
Smoking during pregnancy, n (%)		
No	3476 (65.4)	3792 (71.4)
Yes	1224 (23.1)	1518 (28.6)
Missing	610 (11.5)	
Alcohol using during pregnancy, n (%)		
No	2138 (40.3)	2478 (46.7)
Yes	2512 (47.3)	2832 (53.3)
Missing	660 (12.4)	
Folic acid supplements during pregnancy, n (%)		
No	1017 (19.2)	1695 (31.9)
Yes	3023 (56.9)	3615 (68.1)
Missing	1270 (23.9)	
Blood pressure		
Early pregnancy		
Gestational age (weeks), median (95% range)	13.2 (9.8, 17.4)	13.4 (9.8, 17.5)
Systolic blood pressure (mmHg), mean (SD)	115.5 (12.0)	115.5 (12.0)
Diastolic blood pressure (mmHg), mean (SD)	68.1 (9.3)	68.1 (9.3)
Mid-pregnancy		
Gestational age (weeks), median (95% range)	20.5 (18.5, 23.5)	20.5 (18.5, 23.5)

Supplemental table 1 Subject characteristics in the original and imputed dataset (n = 5310). (continued)

	Observed	Imputed
Systolic blood pressure (mmHg), mean (SD)	116.8 (11.9)	116.8 (11.9)
Diastolic blood pressure (mmHg), mean (SD)	67.2 (9.3)	67.2 (9.3)
Late pregnancy		
Gestational age (weeks), median (95% range)	30.2 (28.5, 32.9)	30.2 (28.5, 32.9)
Systolic blood pressure (mmHg), mean (SD)	118.4 (11.9)	118.4 (11.9)
Diastolic blood pressure (mmHg), mean (SD)	69.1 (9.2)	69.1 (9.2)
Hypertensive disorders in pregnancy, n (%)		
Any	308 (5.8)	308 (5.8)
Gestational hypertension	215 (4.0)	215 (4.0)
Preeclampsia	93 (1.8)	93 (1.8)
Paternal characteristics		
Age (years), median (95% range)	33.0 (22.3, 45.8)	33.0 (21.7, 45.2)
Missing, n (%)	1284 (24.2)	
Height (cm), mean (SD)	182.1 (7.9)	181.9 (7.7)
Missing, n (%)	1,289 (24.3)	
Weight (kg), mean (SD)	83.9 (13.0)	83.7 (11.6)
Missing, n (%)	1286 (24.2)	
Body mass index (m/kg^2), mean (SD)	25.3 (3.4)	25.3 (3.2)
Missing, n (%)	1293 (24.4)	
Ethnicity, n (%)		
European	2736 (51.5)	3274 (61.7)
Non-European	1155 (21.8)	2036 (38.3)
Missing	1419 (26.7)	
Educational level father, n (%)		
Primary or secondary	1661 (31.3)	2896 (54.5)
Higher	1841 (34.7)	2414 (45.5)
Missing	1808 (34.0)	
Systolic blood pressure (mmHg), mean (SD)	130.2 (13.5)	130.2 (13.5)
Diastolic blood pressure (mmHg), mean (SD)	73.4 (10.6)	73.4 (10.6)
Birth characteristics		
Female, n (%)	2656 (50.0)	2656 (50.0)
Gestational age (weeks), median (95% range)	40.1 (35.9, 42.3)	40.1 (35.9, 42.3)
Birth weight (g), mean (SD)	3431 (548)	3430 (548)
Missing, n (%)	8 (0.1)	
Small size for gestational age, n (%)	267 (5.0)	267 (5.0)
Appropriate size for gestational age, n (%)	4777 (89.9)	4777 (89.9)
Childhood characteristics		
Age (years), median (95% range)	6.0 (5.7, 8.0)	6.0 (5.7, 8.0)
Height (cm), mean (SD)	119.5 (6.0)	119.5 (6.1)

Supplemental table 1 Subject characteristics in the original and imputed dataset (n = 5310). (continued)

	Observed	Imputed
Missing, n (%)	7 (0.1)	
Weight (kg), mean (SD)	23.3 (4.3)	23.3 (4.3)
Missing, n (%)	7 (0.1)	
Body mass index (kg/m^2), mean (SD)	16.2 (1.9)	16.2 (1.9)
Missing, n (%)	7 (0.1)	
Systolic blood pressure (mmHg), mean (SD)	102.7 (8.2)	102.7 (8.2)
Diastolic blood pressure (mmHg), mean (SD)	60.7 (6.9)	60.7 (6.9)
†Z score Systolic blood pressure, mean (SD)	0.53 (0.7)	0.53 (0.7)
†Z score Diastolic blood pressure, mean (SD)	0.34 (0.6)	0.34 (0.6)
‡Blood pressure ≥ 95th percentile, n (%)	410 (7.7)	410 (7.7)

Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (95% range) for continuous variables with a skewed distribution.

† Z scores of systolic and diastolic blood pressure are calculated using normative values from the "Fourth report on the diagnosis, evaluation and treatment of high blood pressure in children and adolescents" from the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents.

‡ Blood pressure ≥ 95th percentile (systolic and/or diastolic blood pressure ≥ 95th percentile) for age, height and gender on repeated measurement.

Supplemental table 2 Subject characteristics in children with and without follow-up blood pressure measurements (n = 8452)

	Children with follow-up blood pressure measurements	**Loss to follow-up**	***P*-value**
	n = 5310	n = 3142	
Maternal characteristics			
Age (years), median (95% range)	30.9 (19.7, 39.3)	28.9 (18.5, 39.0)	< 0.01
Height (cm), mean (SD)	167.5 (7.4)	166.4 (7.4)	< 0.01
Weight (kg), mean (SD)	69.3 (12.8)	69.1 (13.6)	0.55
Body mass index (kg/m^2), mean (SD)	23.5 (4.1)	23.6 (4.5)	0.55
Parity (%)			
0	2,990 (56.3)	1,663 (54)	0.02
≥1	2,280 (42.9)	1,414 (46)	
Educational level mother, n (%)			< 0.01
Primary or secondary	2,673 (50.3)	1,781 (56.7)	
Higher	2,295 (43.2)	936 (39.8)	
Ethnicity, n (%)			< 0.01
European	3,135 (59.0)	1,407 (44.8)	
Non-European	2,059 (38.8)	1,386 (44.1)	
Smoking during pregnancy, n (%)			< 0.01
No	3,476 (65.4)	1,887 (60.1)	
Yes	1,224 (23.1)	783 (24.9)	
Alcohol using during pregnancy, n (%)			< 0.01
No	2,138 (40.3)	1,471 (46.8)	
Yes	2,512 (47.3)	1,152 (36.7)	
Folic acid supplements during pregnancy, n (%)			< 0.01
No	1,017 (19.2)	823 (26.2)	
Yes	3,023 (56.9)	1,393 (44.3)	
Blood pressure			
Early pregnancy			
Gestational age (weeks), median (95% range)	13.2 (9.8, 17.4)	13.4 (9.5, 17.6)	< 0.01
Systolic blood pressure (mmHg), mean (SD)	115.5 (12.0)	114.7 (12.1)	< 0.01
Diastolic blood pressure (mmHg), mean (SD)	68.1 (9.3)	67.7 (9.3)	0.08
Mid-pregnancy			
Gestational age (weeks), median (95% range)	20.5 (18.5, 23.5)	20.4 (18.5, 23.9)	0.07
Systolic blood pressure (mmHg), mean (SD)	116.8 (11.9)	115.8 (11.8)	< 0.01
Diastolic blood pressure (mmHg), mean (SD)	67.2 (9.3)	66.6 (9.0)	0.01
Late pregnancy			
Gestational age (weeks), median (95% range)	30.2 (28.5, 32.9)	30.3 (28.4, 32.9)	0.02
Systolic blood pressure (mmHg), mean (SD)	118.4 (11.9)	117.4 (12.0)	< 0.01

Supplemental table 2 Subject characteristics in children with and without follow-up blood pressure measurements (n = 8452) (continued)

	Children with follow-up blood pressure measurements	**Loss to follow-up**	***P*-value**
	n = 5310	n = 3142	
Diastolic blood pressure (mmHg), mean (SD)	69.1 (9.2)	68.5 (9.2)	< 0.01
Hypertensive disorders in pregnancy, n (%)			
Any	305 (5.8)	160 (5.1)	0.58
Gestational hypertension	215 (4.0)	95 (3.0)	0.03
Preeclampsia	93 (1.8)	65 (2.1)	0.02
Paternal characteristics			
Age (years), median (95% range)	33.0 (22.3, 45.8)	31.8 (21.0, 44.5)	< 0.01
Height (cm), mean (SD)	182.1 (7.9)	181.8 (7.7)	< 0.01
Weight (kg), mean (SD)	83.9 (13.0)	83.6 (11.6)	< 0.01
Body mass index (kg/m^2), mean (SD)	25.3 (3.4)	25.3 (3.6)	0.59
Ethnicity, n (%)			< 0.01
European	2,736 (51.5)	1,150 (36.6)	
Non-European	1,155 (21.8)	697 (22.2)	
Educational level father, n (%)			< 0.01
Primary or secondary	1,661 (31.3)	818 (26.0)	
Higher	1,841 (34.7)	722 (23.0)	
Systolic blood pressure (mmHg), mean (SD)	130.2 (13.5)	129.8 (13.6)	0.25
Diastolic blood pressure (mmHg), mean (SD)	73.4 (10.6)	72.9 (10.9)	0.12
Birth characteristics			
Female, n (%)	2,656 (50.0)	1,534 (48.9)	0.30
Gestational age (weeks), median (95% range)	40.1 (35.9, 42.3)	40.0 (35.0, 42.4)	< 0.01
Birth weight (g), mean (SD)	3,431 (548)	3,387 (576)	< 0.01

Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (95% range) for continuous variables with a skewed distribution.
Differences in subject characteristics comparing the groups with and without blood pressure measurements were evaluated using Student's t-test for continuous variables with a normal distribution and Mann-Whitney U-test for continuous variables with a skewed distribution. Chi-square tests were used for categorical variables.

Supplemental table 3 Effect estimates from the longitudinally measured maternal blood pressure and childhood blood pressure.

		Difference in systolic blood pressure		
Childhood blood pressure	**Intercept** (mmHg)	***P*-value**	**Slope** (mmHg/week of gestation)	***P*-value**
Tertile 1	109.6	< 0.001	-0.02	0.51
Tertile 2	111.1	0.15	-0.03	0.17
Tertile 3	112.0	< 0.001	*Reference*	
		Difference in diastolic blood pressure		
	Intercept (mmHg)	***P*-value**	**Slope** (mmHg/week of gestation)	***P*-value**
Tertile 1	97.7	< 0.001	-0.02	0.22
Tertile 2	97.8	< 0.001	0.01	0.48
Tertile 3	99.1	< 0.001	*Reference*	

Values are based on mixed effects regression models and reflect the change in maternal blood pressure during pregnancy in mmHg per tertile of childhood blood pressure compared to the reference group of children in the highest tertile.

Supplemental table 4 associations of maternal and paternal blood pressure during pregnancy with childhood blood pressure (n = 5310).

	Childhood blood pressure (SDS)		
	Confounder Model	Birth Model	Childhood Model
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
Maternal blood pressure (SDS)			
Early pregnancy (n = 4098)			
Systolic blood pressure	0.07 (0.05, 0.09)**	0.07 (0.05, 0.09)**	0.07 (0.04, 0.09)**
Diastolic blood pressure	0.04 (0.02, 0.06)**	0.04 (0.02, 0.06)**	0.04 (0.02, 0.06)**
Mid-pregnancy (n = 5006)			
Systolic blood pressure	0.08 (0.06, 0.10)**	0.08 (0.06, 0.10)**	0.07 (0.05, 0.09)**
Diastolic blood pressure	0.05 (0.04, 0.07)**	0.05 (0.03, 0.07)**	0.05 (0.04, 0.07)**
Late pregnancy (n = 5104)			
Systolic blood pressure	0.08 (0.06, 0.10)**	0.08 (0.06, 0.10)**	0.08 (0.06, 0.10)**
Diastolic blood pressure	0.06 (0.04, 0.08)**	0.06 (0.04, 0.08)**	0.06 (0.04, 0.08)**
Paternal blood pressure (n = 3805)			
Systolic blood pressure	0.05 (0.03, 0.08)**	0.05 (0.03, 0.08)**	0.06 (0.04, 0.08)**
Diastolic blood pressure	0.06 (0.04, 0.08)**	0.05 (0.03, 0.07)**	0.06 (0.04, 0.08)**

Abbreviation: Standard deviation score, SDS; Confidence interval, CI.
Values are regression coefficients (95% confidence interval) based from multiple linear regression models. Estimates are based on multiple imputed data.
Confounder model: Adjusted for maternal age, gestational age at measurement, pre-pregnancy body mass index, parity, ethnicity, educational level, smoking and alcohol consumption during pregnancy, and folic acid intake; Models focused on paternal blood pressure are adjusted for paternal age, body mass index, ethnicity and educational level.
Birth model: confounder model and additionally adjusted for gestational age at birth and birth weight.
Childhood model: confounder model and additionally adjusted for childhood current body mass index.
* *P*-value < 0.05
** *P*-value < 0.01

Chapter 8

General discussion

INTRODUCTION

The overall aim of this thesis was to investigate the role of angiogenic factors, micronutrients involved in the homocysteine metabolism, and maternal blood pressure, in relation to maternal and child health during and after pregnancy. We focused on maternal health during and after pregnancy with emphasis on maternal cardiometabolic adaptation in relation to gestational hypertensive disorders (**Part I**), and on offspring health during fetal life and throughout the first years of childhood (**Part II**). Higher homocysteine and lower folate concentrations were associated with lower placental weight, fetal and child growth and a higher risk for preeclampsia (**Chapter 2**, **Chapter 5**). Newborns with impaired growth and women with preeclampsia (PE) are at risk of a higher blood pressure in later life. Thereby, in these children and women improvement in their cardiovascular status can be achieved since these markers of the homocysteine pathway can be modified by diet, lifestyle choices or targeted medical treatment (i.e. folic acid). We also demonstrated that both higher maternal blood pressure throughout pregnancy and paternal blood pressure are associated with childhood blood pressure and that these associations were largely independent from fetal and childhood growth measures (**Chapter 7**). As maternal blood pressure is also strongly associated with maternal cardiometabolic status after pregnancy (**Chapter 3**, **Chapter 4**), this highlights the importance of adequate follow-up and treatment of not only women, but also their children after pregnancy.

From my position as in-training resident general medicine I would like to discuss clinical implications resulting from my thesis with respect to general medicine, in particular the role of the general practitioner in relation to preconception counselling (care and uptake) as well as follow-up of women and their children after pregnancy.

Clinical implications in general medicine

Assessing causality in observational research is not possible. However, the results from this thesis, combined with those from other studies, do emphasize the importance of a healthy lifestyle during pregnancy as well as the importance of early follow-up after pregnancy complicated by a hypertensive disorder with regard to women but also their children. Women planning pregnancy as well as pregnant women are more receptive to health messages.[1] Therefore, the periconception period offers a great opportunity to implement health behaviour changes. In 2004 the Peristat Study showed that perinatal mortality rates in the Netherlands were high and declined slower compared to other European countries.[2-4] These results created awareness on the changes that had to be made in preconception care, antenatal care, care during delivery, but also the care after pregnancy. Thereafter the concept of achieving "healthy pregnancy" gained high priority in the Netherlands. In response to the Peristat Study, several studies were initiated to take a closer look at cause and effect concerning unfavourable perinatal health outcomes. These studies aimed:

1) To gain knowledge about mechanisms and lifestyle habits involved in achieving a healthy pregnancy.
2) To study how care-uptake works.
3) To detect how preventive strategies can be incorporated in general practice.

One of these initiatives was the Healthy Pregnancy 4 All Study, which was designed to evaluate the effectiveness of the interventions in the preconception and antenatal period.[5] This study was one of the first studies to combine the medical and social aspects of care. General practitioners actively participated and provided preconception care consultations in order 1) to evaluate risk factors, and 2) to educate women how to *achieve* a healthy pregnancy.

Care before pregnancy

Preconception care includes any intervention to optimize a woman's health before pregnancy with the aim to improve maternal, newborn and child health outcomes. Preconception care has proven to be effective in improving pregnancy outcomes.[10] The timing of preconception counselling, namely before conception, is crucial to maximize the benefit. As many couples are unaware of their risk status and of the fact that the first period of pregnancy is crucial, they do not seek information before pregnancy occurs.[6] For this reason the health care worker has to actively approach couples in their reproductive ages and spread the message of the importance of preconception counseling.[7] In the Netherlands primary care has become the cornerstone of the health services system. The task of a primary care physician is to manage and coordinate a patient's care, as well as to be their adviser when it comes to the pro's and cone's with regard to diagnostic tools and treatment, but also making decisions about the best and most appropriate use of medical services. The majority of pregnant women have regular contact with their general practitioner.[8] Therefore general practitioners have been identified as the perfect health professional to identify, but perhaps most importantly also to coordinate, the kind of care a couple needs before, during and after pregnancy.[7, 9]

Several tools have been developed to assist the general practitioner regarding preconception care.

1) The Dutch College of General Practitioners' (NHG) practice guideline 'Preconception care'. This guideline was published in 2011. It provides the general practitioner with recommendations to inform couples how to achieve the best possible health before conception.[10]
2) Thuisarts.nl. In 2012 Thuisarts.nl was launched by the Dutch Minister of Health. This website was developed for the general public and supports the general practitioner-patient interaction and communication before, during and after visiting the general practitioner.[11]
3) Zwangerwijzer.nl. This interactive preconception tool was launched in 2010 to be used by parents to be as well as health care providers. It is publicly and freely available and designed to identify and raise awareness of preconception risks.[12]

Unfortunately, despite these initiatives utilization of preconception care is still limited because of low awareness of the availability and the benefits of the service for patients but also for health care providers.[6, 13-16] This should be an important target point for campaigns and recruitment strategies.[17] Committed health care providers are needed to recruit women for preconception care and it should be delivered in a comprehensive approach.[18] Besides the fact that most general practitioners are unaware of the delivery of comprehensive preconception care it requires also training of the general practitioner to feel capable to do so.[19-22] To inform but also train general practitioner continuing vocational training programs can be used. Uptake can be maximized by rewarding participation by accreditation points.

The workload of general practitioners has increased recent years. This makes it difficult to find the time to deliver preconception care.[23] A possible solution can be the use of a general practice-based nurse specialist trained to provide preconception care consultations. General practice-based nurse specialists have proven to be an indispensable part of the team in general practice, which is shown in diabetic and psychiatric care, pulmonary disease and special care for the elderly. Reaching patients could be achieved by sending personal invitation letters to women for preconception care. For this a similar infrastructure as used for the annual flu vaccination and diabetic care, can be applied. Also, handing out leaflets, hanging posters in waiting rooms and posting a link on the website of the general practice has shown to be a valuable tool to reach patients.[24]

Care after pregnancy

Not only preconception care is important for the health of mothers and their children. Also, the follow-up of mothers, fathers and their children after pregnancy is essential.

The results of this thesis showed that hypertensive pregnancy disorders already influence measures of cardiometabolic health of the mother, but also the child, after a period of 6 years. Therefore CVD risk assessment in these women but also their children is of great importance. Concerning the mothers, in 2014, the Dutch multidisciplinary guideline 'Cardiovascular risk management after a reproductive disorder' was launched.[25] This guideline was an initiative by the Dutch Society of Obstetrics and Gynaecology (NVOG), together with the Dutch Societies for Internal medicine, Cardiology, Neurology and Radiology launched. The guideline mainly focused on (severe) PE as risk factor for adverse future cardiovascular outcomes including ischemic heart disease and stroke but not dyslipidaemia. However, our results show that also metabolic health matters and that gestational hypertension (GH) is also associated with a greater CVD risk. Women with a history of GH should therefore not be neglected when it comes to cardiometabolic follow-up after pregnancy. This group of patients will too benefit from preventive interventions.[26]

A few problems arise when trying to incorporate the Dutch guideline into general practice.

1) "Timing" of follow-up.
2) Which system should be used for follow-up?

The optimal timing of regular CVD risk assessment after a hypertensive pregnancy disorder is widely debated since the *absolute* CVD risk in this young population of mothers as assessed by CVD risk models is actually low.[27] It is advised to actively follow and treat women after pregnancy at least until their blood pressure has normalized and proteinuria has resolved.[25] However, other studies do not support this but recommend follow up not sooner than two years postpartum as spontaneous recovery from the hypertensive pregnancy may still occur.[28] In this respect the Dutch guideline even advises to start CVD risk assessment after the age of 50 years.[25] However, in women after a hypertensive pregnancy disorder the CVD risk is increased at all ages and at an earlier stage. Results from two recent studies by Benschop et al.[29, 30] strengthen this. They demonstrate that that already one year after pregnancy 41.5% of women with previously severe PE have some form of hypertension including sustained hypertension, masked hypertension, and white-coat hypertension, diagnosed with ambulatory blood pressure monitoring. With sheer office blood pressure measurement only 24% of women would have been diagnosed as hypertensive. For this reason Benschop et al. advise ambulatory blood pressure measurements in all women at high risk of developing hypertension and possibly future cardiovascular disease, starting one year after the index pregnancy.[29] They also showed that women with previous preeclampsia have more modifiable cardiovascular risk factors and develop coronary artery calcification approximately five years earlier than women with normotensive pregnancies. Their excessive cardiovascular risk becomes apparent by the age of 45 years.Women might therefore benefit from regular cardiovascular screening and intervention before this age.[30]

Nowadays young mothers have little available time to actually visit the clinic because of their expanding family. For this reason they are difficult to reach and to motivate. The general practitioner might act as the ideal health professional to coordinate the care for these women after pregnancy. They are in regular contact with these women and have ongoing contact with them and their families. General practitioners can inform women about their risk profile in order to make them more aware of their future cardiometabolic health risk. In this respect we would like to advocate annual screening, preferably with ambulatory blood pressure measurements, combined with cardiometabolic five-yearly extensive screening.[31]

Alternatively, the cardiovascular health (CVH) score as developed by the American Heart Association (AHA) can be used.[32] The CVH score was designed to improve CVH while simultaneously reducing the risk of CVD related death. The score includes three health factors (blood pressure as well as total-cholesterol and glucose concentrations) and four health behaviours (body mass index [BMI], smoking habit, diet and physical activity).[32] Rather than emphasizing risk factors, the score underlines the importance of a healthy behaviour.

GENERAL CONCLUSION

The present thesis demonstrates the importance of healthy lifestyle behaviours before and during pregnancy, as well as the follow-up of women with a pregnancy complicated by a hypertensive pregnancy disorder as well as their children. Homocysteine, folate, vitamin B_{12} concentrations and the (anti) angiogenic factors soluble fms-like tyrosine kinase 1 and placental growth factor are important biomarkers for placentation, fetal growth and the development of adverse pregnancy outcomes. Gestational hypertension and preeclampsia are associated with an altered cardiovascular status and more atherogenic lipid profile after pregnancy with potential lifelong consequences. Moreover, both maternal and paternal blood pressure are associated with childhood blood pressure. With these results this thesis emphasizes the importance of appropriate care before, during, but also after pregnancy. The general practitioner represents the ideal health professional to coordinate this with the assistance of general practice-based nurse specialists.

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Chapter 9

Summary / Samenvatting

SUMMARY

Cardiometabolic diseases are the leading cause of preventable death worldwide. Recent attention has focused on pregnancy as having an important role in the pathogenesis of cardiometabolic diseases in both women and their children. During pregnancy important adaptations occur in the maternal circulation and metabolism to meet the increased metabolic demands of the mother and fetus. Suboptimal adaptations may lead to increased risk of pregnancy complications with potential long term effects for both the mother and her child. The exact mechanisms by which pregnancy course and outcome contribute to long term cardiometabolic health have not been clarified. The general aim of this thesis is to identify placental, maternal and fetal factors associated with (adverse) maternal and child health during and after pregnancy.

All studies were embedded in the Generation R Study. This is a large prospective cohort study in Rotterdam, the Netherlands. Between 2001 and 2006, 8880 pregnant women were included. The Generation R Study was designed to identify environmental determinants of health in the mother, and growth, development and health in fetal life and childhood. Currently these children have reached the age of fourteen years.

The first part of this thesis was focused on maternal health during and after pregnancy with emphasis on maternal cardiometabolic adaptation in relation to gestational hypertensive disorders. In **Chapter 2** we investigated the associations between early pregnancy homocysteine, folate and vitamin B_{12} concentrations and placentation and adverse pregnancy outcomes including preeclampsia (PE). We showed that high homocysteine concentrations were associated with a lower placental weight with an increased risk of a small for gestational age (SGA) child at birth. Low folate concentrations were associated with a lower placental weight, an increased risk of SGA and pre-term birth. The risk for PE was also higher in these women. In **Chapter 3** we examined the association between blood pressure in pregnancy, gestational hypertension (GH) and PE with cardiovascular status six years after pregnancy. Early pregnancy systolic and diastolic blood pressure were associated with more adverse maternal cardiovascular measurements and a higher incidence of chronic hypertension six years after pregnancy. GH was associated with a higher blood pressure, a higher risk for chronic hypertension and also more adverse maternal cardiovascular measurements six years after pregnancy. Women with PE had a higher blood pressure and higher risk of developing chronic hypertension at follow-up. In **Chapter 4** we determined if women with previous GH and PE had a more atherogenic lipid profile six years after pregnancy compared to women with a previous normotensive pregnancy. Especially women with previous GH, and to a lesser extend women with previous PE, showed a more atherogenic lipid profile six years after pregnancy compared to women with a previous normotensive pregnancy.

The second part of this thesis focused on child health during its fetal life and during the first years of childhood. In **Chapter 5** we investigated associations of early pregnancy as well as

umbilical cord homocysteine, folate and vitamin B_{12} concentrations with fetal growth. Higher early pregnancy maternal homocysteine and lower early pregnancy maternal folate concentrations were associated with reduced fetal growth. Neonatal folate concentrations were associated with a lower birth weight and length. In **Chapter 6** we examined associations of both maternal and fetal soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) with fetal and childhood growth. Children of mothers with an angiogenic profile that is characterized by both low maternal sFlt-1 and low PlGF in early pregnancy, as well as a subsequent relatively small increase in PlGF towards mid-pregnancy were persistently smaller throughout pregnancy. This difference remained present until the age of 6 years. Higher sFlt-1 concentrations, lower PlGF concentrations or a higher sFlt-1:PlGF ratio in umbilical cord blood also seem to impair fetal and childhood growth. In **Chapter 7** we examined the associations of maternal blood pressure throughout pregnancy and hypertensive disorders in pregnancy with childhood blood pressure. Our specific focus was on comparison with paternal blood pressure effects, the identification of critical periods and the role of birth outcomes and childhood body mass index in these associations. We observed that both maternal and paternal blood pressure were associated with childhood blood pressure with similar effect estimates. Early, mid- and late pregnancy maternal blood pressure were independent and positively associated with childhood blood pressure, with the strongest effect estimates for early pregnancy. All the observed associations were independent of fetal and childhood growth measurements.

In the last part of this thesis, encompassing **Chapter 8**, we reflected on the main findings in our studies in view of implications for general medical practice. We highlight on the importance of healthy nutrition and lifestyle behaviours, preconception care as well as the significance of early follow-up with regard to women but also their children with hypertensive pregnancy disorders.

SAMENVATTING

Hart- en vaatziekten zijn wereldwijd de belangrijkste oorzaak van vermijdbare sterfte. Bekende risicofactoren voor hart- en vaatziekten zijn een hoog cholesterol, hoge bloeddruk of suikerziekte. Er komt echter steeds meer aandacht voor vrouw-, maar ook kind-specifieke risicofactoren zoals een ernstige verhoogde bloeddruk tijdens de zwangerschap. Tijdens de zwangerschap treden belangrijke aanpassingen op in de bloedsomloop van de moeder. Dit gebeurt onder meer om de groeiende baby van voldoende voedingsstoffen en zuurstof te voorzien en de foetale afvalstoffen af te voeren. Suboptimale aanpassingen kunnen leiden tot een verhoogd risico op zwangerschapscomplicaties met mogelijk langdurige nadelige effecten voor zowel de moeder als haar kind. De exacte mechanismen waardoor het verloop en de uitkomst van de zwangerschap bijdragen tot het ontstaan van hart- en vaatziekten bij zowel moeder als kind, zijn nog niet opgehelderd. Het doel van dit proefschrift is om factoren bij zowel moeder als kind te identificeren die geassocieerd zijn met (nadelige) gezondheid van hart- en vaatstelsel van moeders en kinderen tijdens maar ook na de zwangerschap.

Dit alles is onderzocht binnen het Generation R Onderzoek. Generation R is een grootschalig populatie-gebaseerd prospectief cohort onderzoek waarin bijna 10.000 kinderen vanaf de vroege zwangerschap tot de jonge volwassenheid gevolgd worden. In dit geboorte cohort worden de groei, ontwikkeling en gezondheid van Rotterdamse kinderen bestudeerd. In relatie tot groei en ziekte worden zowel factoren die te maken hebben met voeding, leefstijl, omgeving als biologische factoren bekeken. Alle kinderen zijn tussen 2001 en 2006 geboren. Gegevens zijn verzameld met vragenlijsten en medische dossiers. Tijdens de zwangerschap werden de aanstaande moeders onderzocht bij een zwangerschapsduur van ongeveer 12, 20 en 30 weken. Op die drie meetmomenten werden de groei en ontwikkeling van de baby en daarnaast de gezondheid van de moeder, waaronder de bloeddruk, gevolgd aan de hand van echo's en lichamelijk onderzoek. Daarnaast zijn van alle zwangerschappen gegevens over de geboorte, inclusief mogelijke complicaties, bekend. Zes en negen jaar na de zwangerschap kwamen de moeders en hun kinderen terug in ons onderzoekscentrum. Bij dat bezoek hebben we de bloeddruk gemeten, bloed afgenomen en echometingen verricht van bijvoorbeeld het hart. Dit werd gedaan om het risico op hart- en vaatziekten, ook wel de cardiovasculaire status, te meten. Het onderzoek loopt nog steeds en momenteel hebben de kinderen de leeftijd van veertien jaar bereikt. Dit meetmoment is niet in dit proefschrift meegenomen.

Het eerste deel van dit proefschrift was gericht op de gezondheid van de moeder tijdens en na de zwangerschap, met de nadruk op hoge bloeddruk in de zwangerschap. In **Hoofdstuk 2** onderzochten we de relaties tussen enkele factoren in het bloed (biomarkers) zoals homocysteïne, foliumzuur en vitamine B_{12} concentraties en ongunstige zwangerschapsuitkomsten, waaronder groeivertraging bij het kind en zwangerschapsvergiftiging (pre-eclampsie). De biomarkers werden in het eerste trimester in het bloed van de moeder gemeten. We toonden aan dat hoge homocysteïne concentraties geassocieerd waren met een kleinere moederkoek

(placenta) en met een verhoogd risico op een kind dat groei vertraagd was. Lage foliumzuur concentraties waren ook geassocieerd met een lager placenta gewicht, een verhoogde kans op een groei vertraagd kind en daarnaast ook een verhoogd risico op vroeggeboorte. Het risico op pre-eclampsie was ook hoger bij deze vrouwen. In **Hoofdstuk 3** hebben we het verband onderzocht tussen bloeddruk tijdens de zwangerschap, zwangerschapshypertensie en pre-eclampsie met de cardiovasculaire status zes jaar na de zwangerschap. Zwangerschapshypertensie was geassocieerd met een hogere bloeddruk, een hoger risico op chronische hypertensie en ook meer negatieve maternale cardiovasculaire metingen zes jaar na de zwangerschap. Vrouwen met pre-eclampsie hadden ook een hogere bloeddruk en een hoger risico op het ontwikkelen van chronische hypertensie bij de follow-up. In **Hoofdstuk 4** stelden we daarnaast vast dat zes jaar na de zwangerschap zowel vrouwen met zwangerschapshypertensie als vrouwen met pre-eclampsie ook een ongunstiger lipidenprofiel hadden, gemeten in het bloed, in vergelijking met vrouwen met een normale zwangerschap. Vooral vrouwen met zwangerschapshypertensie, en in mindere mate vrouwen met pre-eclampsie, vertoonden zes jaar na de zwangerschap een meer atherogeen lipidenprofiel in vergelijking met vrouwen met een normale zwangerschap.

Het tweede deel van dit proefschrift richtte zich op de gezondheid van kinderen tijdens de zwangerschap en tijdens de eerste jaren van hun kindertijd. In **Hoofdstuk 5** onderzochten we associaties van de eerder genoemde biomarkers homocysteïne, foliumzuur en vitamine B_{12} met de groei van het ongeboren kind tijdens de zwangerschap. Dit wordt ook wel foetale groei genoemd. De biomarkers werden in het bloed van de moeder in het eerste trimester gemeten maar ook in het navelstreng bloed. Hogere maternale homocysteïne concentraties en lagere foliumzuur concentraties in het eerste trimester waren geassocieerd met verminderde foetale groei. Neonatale foliumzuurconcentraties waren geassocieerd met een lager geboortegewicht en kleinere geboortelengte. In **Hoofdstuk 6** onderzochten we de invloed van maternale en neonatale biomarkers die zijn betrokken bij de ontwikkeling en functie van de placenta. We zagen dat een bepaald profiel dat gekenmerkt werd door lage waarden van de placenta biomarker sFlt-1 in combinatie met lage waarden van de placenta biomarker PlGF van invloed was op de groei van het kind zowel tijdens als na de zwangerschap. Dit effect bleef aanwezig tot op de leeftijd van zes jaar. Dit laat zien dat de functie van de placenta niet alleen tijdens maar ook tot ver na de zwangerschap belangrijk is voor de groei en ontwikkeling van het kind. In **Hoofdstuk 7** hebben we het verband onderzocht tussen het bloeddruk verloop tijdens de zwangerschap en de bloeddruk bij het kind gemeten op de leeftijd van zes jaar. Ook keken we naar de bloeddruk van de vader in relatie tot de kind bloeddruk. We zagen dat zowel de maternale als de paternale bloeddruk geassocieerd was met de bloeddruk van het kind. De maternale bloeddruk in alle trimesters was positief en onafhankelijk geassocieerd met de bloeddruk van kinderen. Al deze associaties waren onafhankelijk van foetale groei en groei tijdens de kindertijd. In het laatste deel van dit proefschrift, dat **Hoofdstuk 8** omvat, hebben we de belangrijkste bevindingen in onze studies besproken met het oog op de implicaties voor

de algemene medische praktijk. We benadrukken het belang van een gezonde levensstijl en voedingspatroon, counseling over risico's (preconceptiezorg) en de betekenis van goede maar ook tijdige follow-up na de zwangerschap voor zowel de vrouwen, maar ook hun kinderen, die een zwangerschap hebben doorgemaakt die gecompliceerd is door zwangerschapshypertensie of pre-eclampsie.

Chapter 10

About the author
List of publications
PhD portfolio
Dankwoord

LIST OF PUBLICATIONS

1. **Bergen NE**, Schalekamp-Timmermans S, Roos-Hesselink J, Roeters-van Lennep JE, Jaddoe VWV, Steegers EAP. Hypertensive disorders of pregnancy and subsequent maternal cardiovascular health. *Eur J Epidemiol*. 2018 Aug;33(8):763-771.

2. Benschop L, **Bergen NE**, Schalekamp-Timmermans S, Jaddoe VWV, Mulder MT, Steegers EAP, Roeters-van Lennep JE. Maternal lipid profile 6 years after a gestational hypertensive disorder. *J Clin Lipidol*. 2018 Mar-Apr;12(2):428-436.

3. Miliku K, **Bergen NE**, Bakker H, Hofman A, Steegers EA, Gaillard, R, Jaddoe VW. Associations of Maternal and Paternal Blood Pressure Patterns and Hypertensive Disorders during Pregnancy with Childhood Blood Pressure. *J Am Heart Assoc*. 2016 Oct 14;5(10).

4. **Bergen NE**, Schalekamp-Timmermans S, Jaddoe VW, Hofman A, Lindemans J, Russcher H, Tiemeier H, Steegers-Theunissen RP, Steegers EA. Maternal and Neonatal Markers of the Homocysteine Pathway and Fetal Growth: The Generation R Study. *Paediatr Perinat Epidemiol*. 2016 Jul;30(4):386-96.

5. **Bergen NE**, Bouwland-Both MI, Steegers-Theunissen RP, Hofman A, Russcher H, Lindemans J, Jaddoe VW, Steegers EA. Early pregnancy maternal and fetal angiogenic factors and fetal and childhood growth: the Generation R Study. *Hum Reprod*. 2015 Jun;30(6):1302-13.

6. **Bergen NE**, Jaddoe VW, Timmermans S, Hofman A, Lindemans J, Russcher H, Raat H, Steegers-Theunissen RP, Steegers EA. Homocysteine and folate concentrations in early pregnancy and the risk of adverse pregnancy outcomes: the Generation R Study. *BJOG*. 2012 May;119(6):739-51.

7. van Oppenraaij RH, **Bergen NE**, Duvekot JJ, de Krijger RR, Hop Ir WC, Steegers EA, Exalto N. Placental vascularization in early onset small for gestational age and preeclampsia. *Reprod Sci*. 2011 Jun;18(6):586-93.

PHD PORTFOLIO

Name PhD student:	Nienke Bergen
Erasmus Department:	Obstetrics and Gynaecology
Research School:	Netherlands Institute for Health Sciences
PhD period:	2009 – 2019
Promotors:	Prof. dr. E.A.P. Steegers, Prof. dr. V.W.V. Jaddoe
Co-promotor:	Dr. S Schalekamp-Timmermans

	Year	Workload (ECTS)
1. PhD Training		
- Master of Health Sciences, specialization in Genetic Epidemiology, NIHES	2010-2012	
General courses		
Principles of Research in Medicine		0.7
Clinical Decision Analysis		0.7
Topics in Meta-analysis		0.7
Health Economics		0.7
Genome Wide Association Analysis		1.4
Conceptional Foundation of Epidemiologic Study Design		0.7
Cohort Studies		0.7
Case-control Studies		0.7
Principles of Genetic Epidemiology		0.7
Genomics in Molecular Medicine		1.4
Study Design		4.3
Biostatistical Methods I: Basic Principles		5.7
Biostatistical Methods II: Popular Regression Models		4.3
Genetic-epidemiologic Research Methods		5.7
SNP's and Human Diseases		1.4
Advanced courses		
Introduction to Clinical and Public Health Genomics		1.4
Advances in Population-based studies of Complex Genetic Disorders		1.4
Family-based Genetic Analysis		1.4
Advances in Genomics Research		0.4
General academic skills courses		
Basic introduction Course on SPSS (MOLMED)	2009	1.0
CPO minicursus: Methodologie van patiëntgebonden Onderzoek en Voorbereiding Subsidieaanvragen (Het Congresbureau)	2010	0.3

Biomedical English Writing and Communication	2011	4.0
ErasmusAge Workshop on basic principles of Nutritional Epidemiology	2012	0.5

Seminars and workshops

NEDWEP symposium, Groningen, The Netherlands	2009	0.3
Annual PhD-day Sophia's Children's Hospital, Rotterdam, The Netherlands	2010	0.3
Eagle and Birth Cohort Consortium Workshop, Oslo, Norway	2010	0.6
Attending seminars of the department of Obstetrics and Gynaecology, Rotterdam, The Netherlands	2009-2013	1.0
Attending the Generation R research meetings, Rotterdam, The Netherlands	2009-2013	1.0
Attending seminars at the department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands	2009-2013	1.0

(Inter)national conferences - presentations

ISSHP Congress, Melbourne, Australia – oral presentation	2010	1.4
SGI Congress, Miami Beach, Florida, USA – oral presentation	2011	1.4
SGI Congress, San Diego, California, USA – poster presentation	2012	1.4
DoHad Satellite Meeting, Rotterdam, The Netherlands – poster presentation	2012	1.4

Other

Reviewed articles for PlosOne, British Journal of Nutrition, Chemistry and Laboratory Medicine

2. Teaching activities

Supervising Bachelor's thesis: Maaike van Son, student Nutriton and Dietetics, The Hague University, The Netherlands.	2010	2.0
Supervising Master's thesis: Elke Jacobs, Medical Student, Erasmus University, Rotterdam, The Netherlands.	2011	2.0
Supervising Master's thesis: Stephanie Hoogerwerf, Pharmacology student, Utrecht, The Netherlands.	2012-2013	2.0