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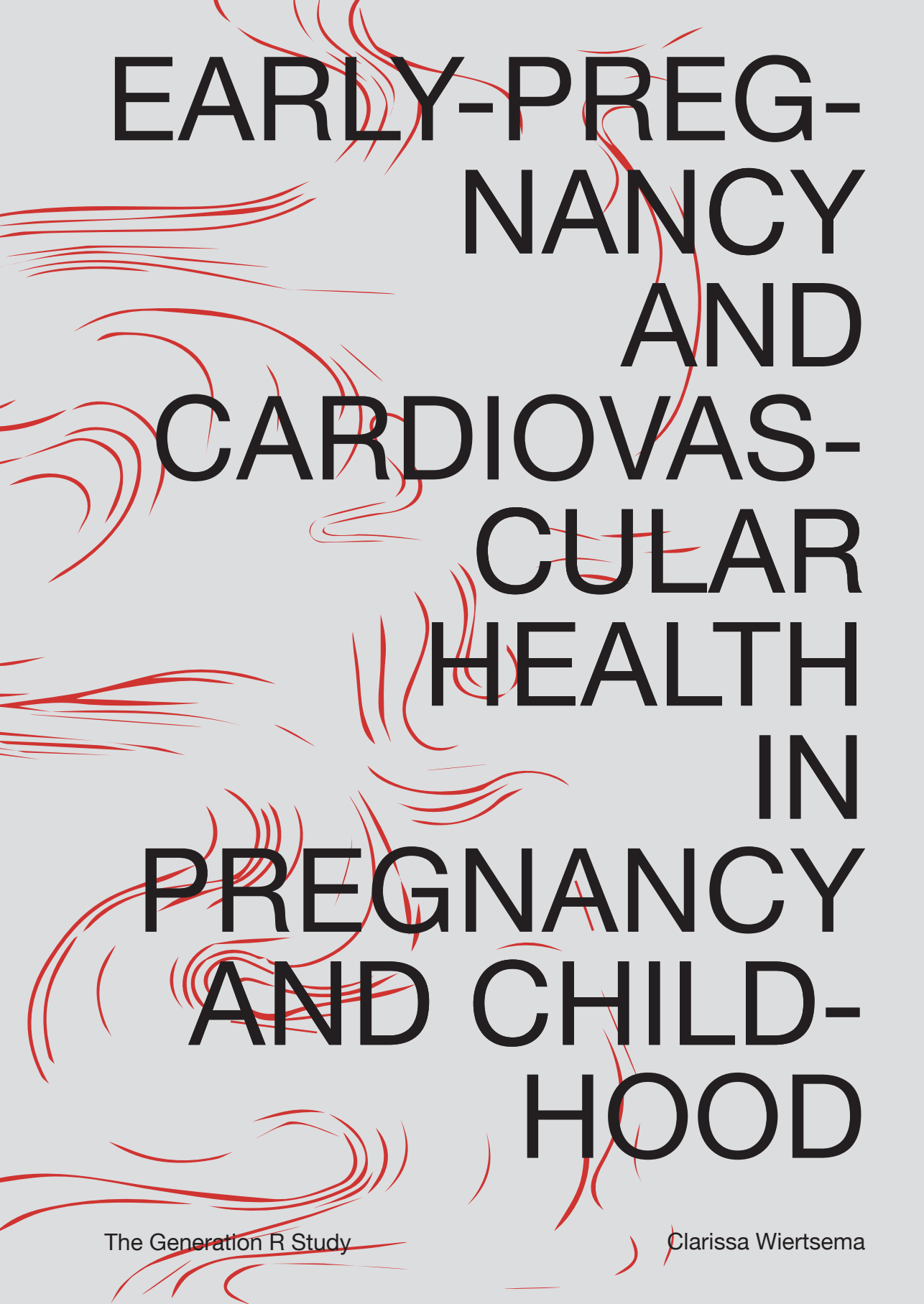
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The background of the cover is white, adorned with numerous red, fluid, and expressive brushstrokes that swirl and flow across the page, creating a sense of movement and energy.

EARLY-PREG- NANCY AND CARDIOVAS- CULAR HEALTH IN PREGNANCY AND CHILD- HOOD

The Generation R Study

Clarissa Wiertsema

EARLY-PREG- NANCY AND CARDIOVAS- CULAR HEALTH IN PREGNANCY AND CHILD- HOOD

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Early-pregnancy and Cardiovascular Health in Pregnancy and Childhood

The Generation R Study

De vroege zwangerschap en cardiovasculaire gezondheid
in de zwangerschap en de kindertijd

De Generation R Studie

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus

Prof. dr. A.L. Bredenoord

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

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CONTENTS



CHAPTER 1.	GENERAL INTRODUCTION	11
CHAPTER 2.	MATERNAL DIET IN EARLY-PREGNANCY AND PREGNANCY OUTCOMES	
2.1	Maternal DASH diet, gestational hemodynamic adaptations and hypertensive disorders	27
2.2	Maternal dietary glycemic index and load, gestational hemodynamic adaptations and hypertensive disorders	67
2.3	Maternal iron status, gestational hypertensive disorders and hemodynamic adaptations	107
CHAPTER 3.	CARDIOVASCULAR OUTCOMES IN CHILDHOOD	
3.1	Gestational hypertensive disorders and blood pressure, and cardiac structure and function in childhood	151
3.2	Gestational hypertensive disorders and blood pressure, and childhood markers for arterial health	179
3.3	Fetal and infant growth patterns, and childhood markers for arterial health	213
CHAPTER 4.	NOVEL PARAMETERS OF FIRST-TRIMESTER FETAL DEVELOPMENT	
4.1	First-trimester fetal proportion volumetric measurements using Virtual Reality	213
4.2	First-trimester organ volumetric measurements using Virtual Reality	265
CHAPTER 5.	GENERAL DISCUSSION	291
CHAPTER 6.	SUMMARY	323
CHAPTER 7.	ADDENDUM	
	Author's affiliations	336
	List of publications	337
	About the author	338
	PhD portfolio	338
	Dankwoord	339

MANUSCRIPTS BASED ON THIS THESIS

Chapter 2.1: Wiertsema CJ, Mensink-Bout SM, Duijts L, Mulders AGMGJ, Jaddoe VWV, Gaillard R. Associations of DASH diet in early-pregnancy with blood pressure patterns, placental hemodynamics and gestational hypertensive disorders. *Journal of the American Heart Association*, 2021 Jan 5;10(1):e017503. Doi: 10.1161/JAHA.120.017503.

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Chapter 2.3: Taeubert MJ, Wiertsema CJ, Vermeulen MJ, Quezada-Pinedo H, Reiss IK, Muckenthaler MU, Gaillard R. Maternal iron status in early-pregnancy and blood pressure throughout pregnancy, placental hemodynamics and the risk of gestational hypertensive disorders. *The Journal of Nutrition*, 2022 Feb 8;152(2):525-534. Doi: 10.1093/jn/nxab368.

Chapter 3.1: Wiertsema CJ, Bongers-Karmaoui MN, Mulders AGMGJ, Helbing WA, Hirsch A, Roest AAW, Jaddoe VWV, Gaillard R. Gestational hypertensive disorders and blood pressure throughout pregnancy, and alterations in cardiac structure and function in childhood. Submitted.

Chapter 3.2: Wiertsema CJ, Jaddoe VWV, Mulders AGMGJ, Gaillard R. Childhood blood pressure, carotid intima media thickness, and distensibility after in utero exposure to gestational hypertensive disorders. *Journal of the American Heart Association*, 2022 Feb;11(3):e023163. Doi: 10.1161/JAHA.121.023163.

Chapter 3.3: Gonzalves R, Wiertsema CJ, Silva CCV, Gaillard R, Steegers EAP, Santos S, Jaddoe VWV. Associations of fetal and infant growth pattern with early markers of arterial health in school-age children. Submitted.

Chapter 4.1: Wiertsema CJ, Erkamp JS, Mulders AGMGJ, Steegers EAP, Duijts L, Koning AHJ, Gaillard R, Jaddoe VWV. First-trimester fetal proportion volumetric measurements using a Virtual Reality approach. *Prenatal Diagnosis*, 2021 Jun;41(7):868-876. Doi: 10.1002/pd.5947.

Chapter 4.2: Wiertsema CJ, Sol CM, Mulders AGMGJ, Steegers EAP, Duijts L, Gaillard R, Koning AHJ, Jaddoe VWV. Innovative approach for first-trimester fetal organ volume measurements using a Virtual Reality system: The Generation R Next Study. *The Journal of Obstetrics and Gynaecology Research*, 2022 Mar;48(3):599-609. Doi: 10.1111/jog.15151.

CHAPTER



GENERAL INTRODUCTION



GENERAL INTRODUCTION

Cardiovascular diseases are the leading cause of morbidity and mortality worldwide¹. Annually around 37,000 deaths are caused by cardiovascular diseases in the Netherlands, which accounts for 25% of the total mortality². Cardiovascular disease burden can be lowered through preventive strategies that aim to modify risk factors such as obesity, hypertension, insulin resistance and dyslipidemia. Among others, dietary interventions have been identified as an important strategy to improve cardiovascular health. Research on preventive strategies primarily focus on traditional cardiovascular risk factors during late adulthood, but pregnancy-related risk factors that influence cardiovascular health are often overlooked.

From early-pregnancy onwards, maternal cardiovascular health seems to influence placental development and the ability to adapt to the increased metabolic demand of both mother and fetus. Hemodynamic adaptations require an initial decrease in systemic vascular resistance and an increase in plasma volume and cardiac output, followed by a decrease in blood pressure levels with a nadir in mid-pregnancy³. Further metabolic adaptations during pregnancy involve changes in the glucose and lipid homeostasis. Optimal hemodynamic and metabolic adaptations from early-pregnancy onwards, are essential to guarantee sufficient uteroplacental circulation and fetal nutrition supply³. Impaired maternal cardiovascular health may be related to suboptimal placental development and an inability to adequately adapt to pregnancy. Which in turn may result in impaired placental vascular development, elevated blood pressure levels and ultimately the development of gestational hypertensive disorders. Gestational hypertensive disorders, such as gestational hypertension or preeclampsia, occur in 5 to 10% of pregnancies and are a major cause of maternal and neonatal morbidity. Although gestational hypertensive disorders clinically manifest in later stages of pregnancy, the origin of this spectrum of disorders is likely to be found in early-pregnancy⁴. Maternal diet prior to and during early-pregnancy has been recognized to improve maternal cardiovascular health, and might also facilitate adequate hemodynamic responses to pregnancy and lead to a lower risk of gestational hypertensive disorders.

Accumulating evidence has shown that women who suffered from any gestational hypertensive disorder are at increased risk of developing cardiovascular disease far beyond pregnancy^{5,6}. Likewise, the offspring of pregnancies affected by gestational hypertensive disorders may have long-term cardiovascular health consequences. The Developmental Origins of Health and Disease (DOHaD) hypothesis proposes that adverse exposures during different stages of fetal and early-postnatal development initiate developmental adaptations which may lead to permanent alterations in the structure and function of

various organ systems^{7,8}. Fetal exposure to an adverse intrauterine environment related to preeclampsia or gestational hypertension, may have a direct effect on offspring cardiovascular development with possible implications for later life health^{9,10}. Gestational hypertension and preeclampsia represent the extremes of the gestational hypertensive disorder spectrum, but already poorer maternal cardiovascular health below the diagnostic threshold for gestational hypertensive disorders seems to influence offspring outcomes¹¹⁻¹³.

Identifying pregnant women and children from early-pregnancy onwards at risk of adverse cardiovascular consequences, may help to develop strategies at earlier stages in life to prevent adverse cardiovascular health in later life. Novel markers on first-trimester fetal development may help to elucidate mechanisms of fetal developmental adaptations. Specific focus to identify critical periods for adverse exposures and potential underlying mechanisms for adverse outcomes, might further aid in appropriate timing and specific targets for these preventive strategies.

Maternal diet in early-pregnancy and pregnancy outcomes

Recently, the Dutch Health Council came forth with new recommendations regarding a healthy diet during pregnancy¹⁴. This underlines the ongoing discussion and importance of maternal diet during pregnancy as a modifiable factor that can influence the health of mother and child. However, evidence to provide specific dietary recommendations to lower the risk of gestational hypertensive disorders remains limited. Diet is defined as the sum of food components, macro- and micronutrients consumed. A dietary pattern takes into account the combination of different nutrients, foods and beverages which are habitually consumed. The variety of foods and nutrients within a diet are likely to have interactive and synergistic effects, therefore it is important to consider diet as a dietary pattern to comprehend complex diet-disease relationships^{15, 16}. However, pathophysiological mechanisms involved in the development of gestational hypertensive disorders can also be modified by specific properties of food components or nutrients¹⁷.

In non-pregnant populations, the Dietary Approaches to Stop Hypertension (DASH) diet and the low-glycemic index diet have gained specific attention for their ability to improve cardiovascular health. The DASH dietary pattern is a diet high in fruits, vegetables, total grains, nuts, seeds, legumes and non-full-fat dairy products and low in animal protein, sugar and sodium¹⁸. Numerous observational and intervention studies have shown that adherence to the DASH diet leads to lower blood pressure levels, and improved lipid profile and fasting glucose concentrations¹⁹⁻²³. The glycemic index and load are commonly used dietary measures to qualify carbohydrate intake, and provide information on the postprandial glycemic response to carbohydrate containing food products^{24, 25}.

Adherence to a low-glycemic index diet can be achieved by consuming carbohydrate containing food products that are less likely to increase blood sugar levels referred to as low-glycemic index products, while avoiding products with a high-glycemic index. A low-glycemic index diet has also been associated with a lower risk of cardiovascular disease and mortality, when compared to diets with a high glycemic index²⁶.

Thus, maternal diet prior to and during pregnancy, has been recognized to improve maternal cardiovascular health. However, little is known about the influence of maternal diet on gestational hemodynamic adaptations during pregnancy and the risk of gestational hypertensive disorders. Identifying specific dietary patterns, food components or nutrients that reduce the risk of gestational hypertensive disorders, might improve future preventive strategies that can be translated into public health recommendations.

Cardiovascular outcomes in childhood

Common cardiovascular risk factors include higher blood pressure, impaired lipid profile, increased glucose levels and adiposity. Already during infancy, childhood and adolescence these cardiovascular risk factors can be identified and tend to track through adulthood, increasing the risk of evident cardiovascular disease in later life²⁷⁻³¹.

Adverse exposures during fetal and postnatal life may have a direct effect on cardiovascular health. Studies have shown that children small or large for gestational age and subsequent high infant growth rates seem to be at risk for cardiovascular disease in later life^{32, 33}. Offspring of pregnancies affected by gestational hypertensive disorders seem to have increased blood pressure levels and nearly a twofold increased risk of stroke in adulthood³⁴⁻³⁷. However, the underlying pathophysiological mechanisms for these associations remain unclear. Experimental studies indicate that features that are present in pregnancies affected by gestational hypertensive disorders, such as impaired uterine perfusion with altered pressure loads, intrauterine hypoxia and increased antiangiogenic factors, may negatively affect fetal cardiovascular development¹⁰. It is plausible that the effects of these adverse exposures are strongest during the first-trimester of pregnancy, which is a critical period for the initial development of the fetal cardiovascular system. For example, cardiomyocytes are predominantly formed during the first-trimester pregnancy and are directly responsible for a considerable part of the myocardial performance during an individual's life^{38, 39}. However, alterations in offspring cardiovascular structure and function, could also reflect genetic predisposition or shared lifestyle factors within a family as an underlying pathophysiological mechanism.

During adulthood, acquired cardiac structural and functional changes are associated with an increased risk of cardiovascular disease. Increased carotid intima media

thickness and decreased distensibility, signs of subclinical atherosclerosis and increased arterial stiffness, often coincide with increased blood pressure levels and are associated with increased risk of cardiovascular events. Early development of hypertension, atherosclerosis, arterial stiffness and cardiac dysfunction in children, might predispose them to evident cardiovascular disease in later life¹⁰. Therefore it is important to obtain a better understanding of underlying pathophysiologic mechanisms for the development of an adverse cardiovascular phenotype and to identify critical periods for exposure during fetal and postnatal life. This knowledge is crucial for the development of preventive strategies, with early life offering the best chance for primary prevention for cardiovascular disease in later life.

Novel parameters of first-trimester fetal development

The DOHaD hypothesis proposes that adverse exposures before and during pregnancy may initiate fetal developmental adaptations^{7, 8}. The first-trimester of pregnancy is a crucial period for organ development, with each organ having specific critical periods of development and growth. Already in the first-trimester of pregnancy adverse exposures may permanently reduce the number of cells in specific organs^{40, 41}. This may lead to alterations in the structure and function of various organ systems, which might predispose the offspring to poorer health on the long-term^{7, 8}. At first, this hypothesis was examined using birthweight as a proxy for fetal development⁷. More recently, studies have shown that suboptimal first-trimester development as measured by crown rump length, is associated with increased risks of adverse fetal, birth and child outcomes⁴²⁻⁴⁶.

To gain further insights in these potential fetal developmental adaptation mechanisms, novel parameters of first-trimester fetal development are essential⁷. With recent improvements in conventional two-dimensional and novel three-dimensional ultrasound, more advanced parameters of early fetal development than the traditional crown to rump length can be measured. Virtual Reality techniques additionally enable visualization of three-dimensional ultrasound datasets as a hologram, which offers optimal depth perception and opportunities for volumetric measurements of complex early-pregnancy fetal structures^{47, 48}. Novel volumetric measurements of fetal body parts and organs may be useful for detailed assessment of first-trimester growth and organ development. Studies on these parameters might improve understanding of mechanisms underlying fetal developmental adaptations that may lead to adverse health outcomes in later life. Before the value of these novel measurements for research in the field of DOHaD can be determined, the reproducibility of the measurements needs to be assessed.

GENERAL AIM OF THIS THESIS

The general aim of this thesis was to assess the critical role of early-pregnancy in maternal cardiovascular health during pregnancy and offspring cardiovascular development. Our specific objectives were (1) to assess the associations of early-pregnancy modifiable dietary factors with hemodynamic adaptations in pregnancy and the risk of gestational hypertensive disorders; (2) to assess the associations of gestational hypertensive disorders and perinatal growth with childhood cardiovascular outcomes; (3) to develop and assess the reproducibility of novel parameters of first-trimester fetal development. **Figure 1** shows an overview of the pathophysiological mechanisms and hypotheses studied within this thesis.

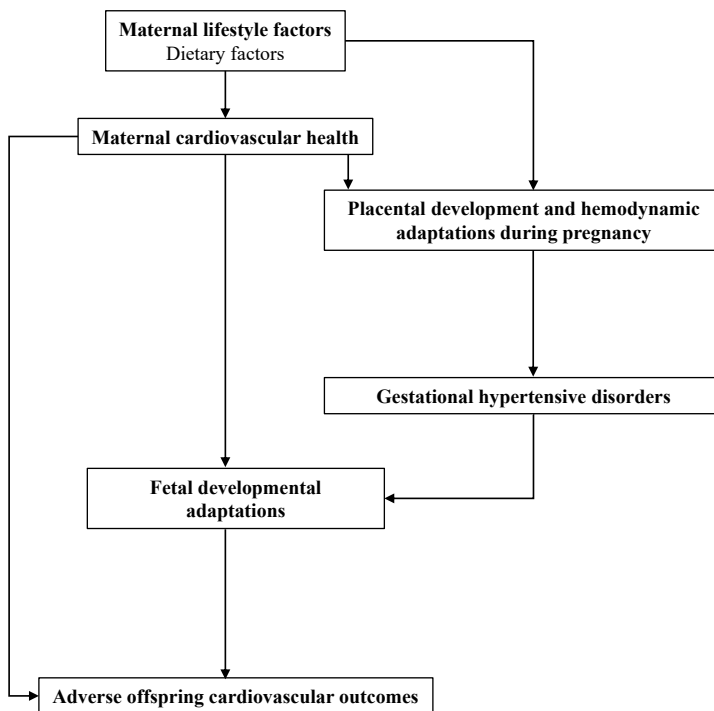


Figure 1. Overview of the pathophysiological mechanisms and hypotheses studied within this thesis.

GENERAL DESIGN

The studies within this thesis were embedded in the Generation R Study and the Generation R *Next* Study. The Generation R Study is a population-based prospective cohort study from early-pregnancy onwards in Rotterdam, The Netherlands⁴⁹. Women with an expected

delivery date between April 2002 and January 2006 were eligible for enrolment. In total 8,880 women were enrolled during pregnancy. Research visits were planned in early (<18 weeks gestations), mid (18-25 weeks gestation) and late-pregnancy (≥ 25 weeks gestation), and included parental anthropometric measurements, maternal blood and urine sample collections and ultrasound examinations. Further data collection was performed through self-administered questionnaires and medical records. From birth, data collection on the children was performed through questionnaires, physical examinations and body sample collections during visits to the Erasmus MC – Sophia Children’s Hospital research centre, and using data that was collected at the municipality health centre visits. At the age of 10 years children were invited for a follow-up research visit, which included a detailed cardiovascular follow-up using carotid ultrasound and Cardiovascular Magnetic Resonance in a specific subgroup.

The Generation R *Next* Study is a population-based prospective cohort study starting from preconception and embryonic life onwards in Rotterdam, the Netherlands. The Generation R *Next* Study was specifically designed to identify periconceptional environmental and health determinants that influence embryonic and early fetal development, and the consequences early fetal adaptations for the child’s later life health. Women with the wish to conceive or those who were pregnant at the time, were eligible for enrollment. From August 2017 to January 2021, 3,442 women were enrolled. Data collection was performed through self-administered questionnaires, medical records, anthropometric measurements and body sample collections. During visits to one of three dedicated research centers, two-dimensional and three-dimensional ultrasounds were obtained in the preconception phase, and at 7, 10, 12 and 30 weeks gestation. Using three-dimensional ultrasound datasets obtained in the first-trimester of pregnancy, in combination with Virtual Reality technology, detailed measurements of embryonic and early fetal development can be performed. From birth, data collection on the children was performed through questionnaires, physical examinations and body sample collections during visits to the Erasmus MC – Sophia Children’s Hospital research centre, and using data that was collected at the municipality health centre visits.

OUTLINE OF THIS THESIS

The objectives of this thesis are addressed in several studies. In **Chapter 2** studies on the associations of early-pregnancy dietary factors with placental vascular resistance, gestational blood pressure and hypertensive disorders are described. We studied whether adherence to a Dietary Approaches to Stop Hypertension diet or a low-Glycemic index

diet positively influences hemodynamic adaptations during pregnancy, and lowers the risk of gestational hypertensive disorders in **Chapter 2.1** and **Chapter 2.2**, respectively. In **Chapter 2.3** we examined the associations of early-pregnancy iron status with hemodynamic adaptations during pregnancy and the risk of gestational hypertensive disorders.

In **Chapter 3** we studied the associations of gestational blood pressure, hypertensive disorders and perinatal growth patterns with childhood cardiovascular outcomes. The associations of gestational blood pressure and hypertensive disorders on offspring cardiac structure and function are studied in **Chapter 3.1** and on offspring carotid intima media thickness and arterial stiffness in **Chapter 3.2**. In **Chapter 3.3** we examined the associations of fetal and infant growth with offspring carotid intima media thickness and arterial stiffness.

In **Chapter 4** we present reproducibility studies of novel volumetric parameters for the assessment of first-trimester fetal development using a Virtual Reality approach.

Finally, in **Chapter 5** we provide a general discussion in which we place our research findings in a broader perspective, with clinical implications and suggestions for future research.

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CHAPTER



MATERNAL DIET IN EARLY- PREGNANCY AND PREGNANCY OUTCOMES

2

CHAPTER

2.1

Associations of DASH diet in early-pregnancy with blood pressure patterns, placental hemodynamics and gestational hypertensive disorders

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BACKGROUND The Dietary Approaches to Stop Hypertension (DASH) diet improves blood pressure in non-pregnant populations. We hypothesized that adherence to the DASH diet during pregnancy improves hemodynamic adaptations, leading to a lower risk of gestational hypertensive disorders.

METHODS We examined whether the DASH diet score was associated with blood pressure, placental hemodynamics and gestational hypertensive disorders in a population-based cohort study among 3,414 Dutch women. We assessed DASH score using food-frequency-questionnaires. We measured blood pressure in early, mid and late-pregnancy (medians, 95% range: 12.9 (9.8-17.9), 20.4 (16.6-23.2), 30.2 (28.6-32.6) weeks gestation, respectively), and placental hemodynamics in mid and late-pregnancy (medians, 95% range: 20.5 (18.7-23.1), 30.4 (28.5-32.8) weeks gestation, respectively). Information on gestational hypertensive disorders was obtained from medical records.

RESULTS Lower DASH score quartiles were associated with a higher mid-pregnancy diastolic blood pressure, compared with the highest quartile (p -values <0.05). No associations were present for early and late-pregnancy diastolic blood pressure and systolic blood pressure throughout pregnancy. Compared to the highest DASH score quartile, the lower DASH score quartiles were associated with a higher mid and late-pregnancy umbilical artery pulsatility index (p -values ≤ 0.05), but not with uterine artery resistance index. No associations with gestational hypertensive disorders were present.

CONCLUSIONS A higher DASH diet score is associated with lower mid-pregnancy diastolic blood pressure and mid and late-pregnancy fetoplacental vascular resistance, but not with uteroplacental vascular resistance or gestational hypertensive disorders within a low-risk population. Further studies need to assess whether the effects of the DASH diet on gestational hemodynamic adaptations are more pronounced among higher-risk populations.

INTRODUCTION

Gestational hypertensive disorders affects up to 10% of pregnancies and are a major risk factor for maternal and neonatal morbidity and mortality¹. In non-pregnant populations, dietary interventions have been identified as an important strategy to reduce hypertension. The Dietary Approaches to Stop Hypertension (DASH) diet is a diet high in fruits, vegetables, total grains, nuts, seeds, legumes and non-full-fat dairy products and low in animal protein, sugar and sodium². Multiple observation and intervention studies have shown that adherence to the DASH diet leads to lower blood pressure levels and improves lipid profile and fasting glucose concentrations in non-pregnant adult populations³⁻⁷.

Not much is known about the influence of maternal adherence to the DASH diet during pregnancy on gestational hemodynamic adaptations or the risk of gestational hypertensive disorders. Recently, a study among 511 pregnant women from Ireland showed that higher adherence to the DASH diet was associated with a lower diastolic blood pressure and mean arterial pressure in early and late-pregnancy⁸. An intervention study in China among 85 pregnant women diagnosed with preexistent hypertension or gestational hypertension (i.e. developed <28 weeks of gestation) showed a lower incidence of preeclampsia in the group adhering to the DASH diet⁹. In contrast, two observational studies among 1,760 American and 66,651 Danish women showed no associations of maternal adherence to the DASH diet with the risks of gestational hypertension or preeclampsia^{10, 11}.

We hypothesized that maternal adherence to the DASH diet may improve maternal hemodynamic adaptations in pregnancy, leading to lower risks of gestational hypertensive disorders¹²⁻¹⁵. Therefore, we examined within a population-based cohort study among 3,414 low-risk pregnant women, the associations of maternal DASH diet score with systolic and diastolic blood pressure and placental vascular resistance throughout pregnancy and the risks of gestational hypertensive disorders.

METHODS

Study design and study sample

The study was embedded in the Generation R study, a population-based prospective cohort from early-pregnancy onwards in Rotterdam, The Netherlands¹⁶. Written informed consent was obtained of participating women. The study was approved by the Medical Ethical Committee of the Erasmus Medical Centre in Rotterdam, The Netherlands (MEC 198.782/2001/31). In total, 4,096 women of Dutch ethnicity were enrolled during

pregnancy. We excluded women with missing data on dietary intake (n=538), with missing data on all outcome measures (n=1) and with pre-existent hypertension (n=63). Finally, we excluded loss to follow up (n=3), multiple gestations (n=53) and pregnancies leading to fetal death (n=16) or induced abortions (n=8), leading to a cohort for analysis of 3,414 pregnant women (**Supplementary Figure S1**).

Maternal DASH score

Semi-quantitative self-administrated food frequency questionnaires (FFQ) of 293 food items were obtained at study enrolment (median 13.5 weeks of gestation, 95% range 10.2, 23.1) and assessed dietary intake in the three months prior. Previously, the FFQ was validated in 82 pregnant women with Dutch ethnic background^{17, 18}. As described previously, 136 of the 293 food items available from the FFQ were used to generate a DASH score². This score composed of eight food components, based mainly on the Fung method with a scoring system based on quintile rankings^{2, 19}. For intakes of total grains, vegetables, fruits, non-full-fat dairy products and nuts/seeds/legumes, participating women received a score from 1 (lowest quintile) to 5 (highest quintile). At the opposite, for intakes of red and processed meats, sugar-sweetened beverages/sweets/added sugars and sodium, participants were scored on a reverse scale. The food component scores were summed to calculate an overall DASH score for each participant. A lower DASH score characterizes a lower dietary quality². In line with previous studies, we constructed quartiles of the maternal DASH score to assess whether associations were restricted to a low DASH score only and constructed a maternal DASH Standard Deviation Score (SDS) to assess associations across the full range (range=10-37)¹⁰⁻¹².

Blood pressure in pregnancy

Systolic and diastolic blood pressure measurements were performed in early (median=12.9 weeks of gestation, 95% range 9.8-17.9), mid (median=20.4 weeks of gestation, 95% range 16.6-23.2) and late-pregnancy (median=30.2 weeks of gestation, 95% range 28.6-32.6) using a validated Omron 907 automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe BV, Hoofddorp, The Netherlands)²⁰. Blood pressure measurements were performed with the participant in upright seated position after a minimum waiting time of 5 minutes at rest. The cuff was placed around the upper arm at the level of the heart. The mean of two blood pressure measurements with a 60 seconds interval was used for further analysis²¹.

Placental hemodynamic parameters

Ultrasound examinations for placental hemodynamic parameters were carried out in two dedicated research centers during mid- (median=20.5 weeks of gestation, 95% range 18.7-23.1) and late-pregnancy (median=30.4 weeks of gestation, 95% range 28.5-32.8). Umbilical artery pulsatility index (UmPI), uterine artery resistance index (UtRI) and bilateral third trimester uterine artery notching were assessed as primary placental hemodynamic parameters, as these measures are most commonly used in clinical practice and strongly associated with the risks of gestational hypertensive disorders^{22, 23}. As a secondary outcome, we also assessed uterine artery pulsatility index (UtPI). The umbilical artery was assessed in a free floating part of the umbilical cord²³. The uterine arteries were identified at the crossover with the external iliac artery. For each Doppler measurement three consecutive flow velocity wave forms were recorded. The mean of three Doppler measurements was used. Bilateral notching resulting from increased uterine artery resistance was defined as an increase of the waveform at the start of diastole in both uterine arteries^{23, 24}.

Gestational hypertensive disorders

Information on gestational hypertensive disorders was obtained from medical records. Women suspected of gestational hypertensive disorders based on these records were crosschecked with the original hospital charts, as described previously^{25, 26}. Briefly, the following criteria were used to identify women with gestational hypertension: development of systolic blood pressure of at least 140 mmHg and/or diastolic blood pressure of at least 90 mmHg after 20 weeks of gestation in previously normotensive women²⁵⁻²⁷. These criteria and the presence of proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater or a 24-h urine collection containing at least 300 mg of protein) were used to identify women with preeclampsia²⁵⁻²⁷.

Covariates

Data on maternal age, education level, parity, prepregnancy weight, folic acid supplement use, alcohol use during pregnancy, smoking during pregnancy and total energy intake were collected by questionnaires. Height was measured at enrolment and used to calculate the prepregnancy BMI.

Statistical power

Power calculations within the Generation R study were performed based on 7,000 subjects during the design of the study²⁸. For a normally distributed continuous outcome it is possible to detect a difference of 0.08 SD with a type I error of 5% and a type II error of 20% (power 80%) if 25% of the cohort has the exposure, which corresponds to a mean difference of approximately 0.90 mmHg for systolic blood pressure and 0.70 mmHg for diastolic blood pressure. For gestational hypertensive disorders with a prevalence of approximately 7%, an odds ratio of 1.26 to 1.38 can be detected if 25% of the cohort has the relevant exposure²⁸.

Statistical analyses

First, we performed a non-response analysis comparing characteristics of women with information on dietary intake (**Supplementary Figure S1**) to women without information on dietary intake (**Supplementary Figure S2**). Second, one-way analysis of variance (ANOVA) and chi-square tests were used to compare population characteristics across the maternal DASH score quartiles. Third, we analyzed the associations of maternal DASH score quartiles with longitudinal systolic and diastolic blood pressure patterns in absolute values using linear mixed models, which take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome²⁹. We assumed a compound symmetry covariance structure and used REML estimation method. The DASH score quartiles were included in the models as intercept and as an interaction term with gestational age, to examine gestational age-independent (intercept) and gestational age-dependent differences (interaction DASH score quartiles and gestational age). We used these models as descriptive analyses that present the absolute values for systolic and diastolic blood pressure across the DASH quartiles to reflect clinical practice. Similar methods were used to examine the associations of maternal DASH score quartiles with longitudinal UmPI and UtRI patterns from second trimester onwards. Furthermore, we examined the associations of maternal DASH score quartiles and SDS with differences in systolic and diastolic blood pressure in each pregnancy period using linear regression models to further enable assessment of small differences in blood pressure levels in each pregnancy period, which are relevant from an etiological perspective and on a population level. Fourth, we examined the associations of maternal DASH score quartiles and SDS with differences in UmPI, UtRI and UtPI in mid and late-pregnancy using linear regression models and the risk of bilateral uterine artery notching using logistic regression models. Finally, we assessed the associations of maternal DASH score quartiles and SDS with the risk of gestational hypertensive disorders using logistic regression analyses. As maternal

dietary intake is known to be strongly related to other socio-demographic and lifestyle characteristics, analyses were first only adjusted for gestational age at intake in the basic model and subsequently additionally adjusted for maternal socio-demographic and lifestyle factors in the confounder model. To select potential confounders we used a directed acyclic graph and assessed whether covariates were associated with the exposure and outcome, or led to a >10% change in effect estimate when added to the univariate model³⁰. Using these criteria, maternal age, educational level, parity, prepregnancy body mass index (BMI), folic acid supplement use, smoking habits, alcohol use, total energy intake and gestational age at time of the measurements were included in the confounder model for the main analyses focused on the continuous outcomes systolic and diastolic blood pressure, UmPI and UtRI. As the number of cases for the adverse binary outcomes bilateral uterine artery notching, gestational hypertensive disorders, gestational hypertension and preeclampsia was relatively low, we only selected those confounders which led to a >10% change in effect estimate when added to the univariate model for these specific outcomes. These confounders models included parity, prepregnancy BMI, folic acid supplement use and gestational age at the time of intake. To assess whether associations were different according to maternal prepregnancy BMI or parity, we tested statistical interaction terms but none were significant (p -values>0.05)^{24, 26, 31}. We performed multiple sensitivity analyses: 1) We repeated the analyses excluding women with pre-existent or gestational diabetes, hypercholesterolemia or pre-existent heart diseases, as these women represent higher risk populations; 2) We repeated the analyses restricting to women who enrolled in early-pregnancy (i.e. <14 weeks of gestation) as adherence to the DASH diet from preconception and early-pregnancy onwards may have stronger effects on gestational hemodynamic adaptations; 3) We repeated the analyses for binary outcomes with adjustment for a propensity score, to enable correction for a larger number of maternal socio-demographic and lifestyle-related characteristics, considering the relatively low number of cases of adverse outcomes. We constructed a propensity score using a logistic regression model to estimate the probability of women having a dietary intake within DASH quartile 1 as compared to DASH quartile 4. The propensity score included maternal age, educational level, parity, prepregnancy BMI, folic acid supplement use, smoking habits, alcohol use, total energy intake and gestational age at time of intake. The propensity score was then included as a covariate in the regression models^{32, 33}. Missing data of covariates was imputed using multiple imputation. The amount of missing values was <8% for all covariates, except for prepregnancy BMI (13.7%) and folic acid supplementation (18%). Analysis were performed using IBM Statistical Package of Social Sciences version 25. The analysis for repeated measurements was performed using Statistical Analysis System version 9.4.

RESULTS

Participant characteristics

Population characteristics according to maternal DASH score quartiles are shown in **Table 1**. The mean DASH score was 24.6 (SD 4.6). Early-pregnancy mean systolic and diastolic blood pressure did not differ significantly across the maternal DASH score quartiles. Mid-pregnancy and late-pregnancy mean systolic and diastolic blood pressure were highest in the lowest maternal DASH score quartile, decreased over the higher maternal DASH score quartiles and were lowest in the highest maternal DASH score quartile (all p-values for univariate comparison across quartiles <0.05). Mid-pregnancy and late-pregnancy mean UtRI were highest in the lowest maternal DASH score quartile, decreased over the higher maternal DASH score quartiles and were lowest in the highest maternal DASH score quartile (all p-values for univariate comparison across quartiles <0.05). Mid and late-pregnancy mean UmPI did not differ significantly by maternal DASH score quartiles.

The composition of the DASH score and intake of food components according to DASH diet quartiles are shown in **Supplementary Table S1**. Non-response analysis showed that no differences in blood pressure or gestational hypertensive disorders were present among women with data on dietary intake compared to women without data on dietary intake (**Supplementary Table S2**).

Maternal DASH score and blood pressure throughout pregnancy

Figure 1 shows the systolic and diastolic blood pressure development during pregnancy in absolute values per maternal DASH score quartile. Women in the lowest DASH score quartile tended to have the highest overall systolic blood pressure and diastolic blood pressure throughout pregnancy, whereas women in the highest DASH score quartile tended to have the lowest overall systolic blood pressure and diastolic blood pressure throughout pregnancy. No consistent differences in the increase in blood pressure per week were present for the different maternal DASH score quartiles (p-values for interaction with gestational age >0.05). The regression coefficients for gestational age-independent (intercept) and gestational age-dependent differences (interaction of DASH score quartile and gestational age) are given in **Supplementary Table S3**.

The associations of maternal DASH score quartiles and SDS with differences in systolic and diastolic blood pressure in early, mid and late-pregnancy are given in **Table 2**. After adjustment for maternal socio-demographic and lifestyle factors, lower maternal DASH score quartiles, as compared to the highest maternal DASH score quartile, were

Table 1. Characteristics of the study population by DASH score quartile (n=3,414)

	Total group n=3,414	DASH quartile 1 score 10-21 n=860	DASH quartile 2 score 22-24 n=798	DASH quartile 3 score 25-27 n=836	DASH quartile 4 score 28-37 n=920	p-value [*]
Maternal age at enrollment, years	31.4 (4.4)	29.7 (5.0)	31.2 (4.2)	32.0 (3.9)	32.5 (3.8)	<0.001
Parity, n nulliparous	2,039 (59.9)	478 (55.7)	494 (62.1)	481 (57.6)	586 (63.8)	0.001
Prepregnancy BMI, kg/m ²	23.1 (3.8)	23.8 (4.4)	23.3 (3.9)	23.1 (3.8)	22.4 (2.9)	<0.001
Prepregnancy BMI ≥25	655 (22.2)	217 (29.0)	151 (22.4)	159 (21.9)	128 (16.1)	<0.001
Gestational weight gain, kg	10.8 (4.4)	10.8 (5.1)	10.8 (4.3)	10.7 (4.3)	10.8 (4.0)	1.00
Gestational age at intake, weeks	14.7(10.2, 23.1)	14.7 (9.6, 23.7)	14.6 (9.9, 23.4)	14.7 (9.9, 24.0)	14.8 (10.5, 22.5)	0.88
Education, n high	2,000 (59.3)	285 (33.7)	456 (58.0)	560 (67.9)	699 (76.5)	<0.001
Smoking, n continued during pregnancy	538 (17.0)	259 (32.2)	128 (17.6)	74 (9.5)	77 (9.0)	<0.001
Alcohol, n continued during pregnancy	1,570 (50.0)	304 (38.3)	358 (49.4)	425 (54.9)	483 (56.9)	<0.001
Folic acid supplement use, n yes	2,493 (89.1)	551 (80.8)	575 (88.9)	646 (92.7)	721 (93.3)	<0.001
Total energy intake, kcal/d	2,146.9 (511.5)	2,078.1 (548.1)	2,135.2 (535.6)	2,162.8 (491.9)	2206.8 (462.3)	<0.001
Systolic blood pressure, mmHg						
Early-pregnancy	117.3 (11.9)	117.8 (11.9)	117.4 (12.6)	117.3 (12.3)	116.6 (11.0)	0.29
Mid-pregnancy	118.5 (11.7)	119.5 (12.0)	118.9 (12.2)	118.0 (11.7)	117.5 (10.9)	0.002
Late-pregnancy	120.4(11.4)	121.3 (12.2)	121.1 (11.8)	119.7 (10.9)	119.5 (10.8)	0.001
Diastolic blood pressure, mmHg						
Early-pregnancy	68.5(9.2)	68.9 (9.2)	68.6 (10.1)	68.4 (9.0)	68.1 (8.5)	0.47
Mid-pregnancy	67.2(9.3)	68.3 (9.7)	67.7 (9.7)	66.9 (8.9)	66.1 (8.5)	<0.001
Late-pregnancy	69.4(9.2)	70.0 (9.6)	69.6 (9.3)	69.0 (8.7)	68.8 (9.0)	0.05
Umbilical artery pulsatility index						
Mid-pregnancy	1.19 (0.18)	1.20 (0.18)	1.20 (0.18)	1.18 (0.17)	1.17 (0.18)	0.01
Late-pregnancy	0.98 (0.17)	1.00 (0.18)	0.97 (0.16)	0.98 (0.16)	0.96 (0.16)	<0.001
Uterine artery resistance index						
Mid-pregnancy	0.535 (0.089)	0.535 (0.091)	0.535 (0.090)	0.535 (0.089)	0.535 (0.088)	1.00
Late-pregnancy	0.483 (0.078)	0.490 (0.076)	0.484 (0.076)	0.480 (0.081)	0.479 (0.08)	0.11
Third trimester bilateral uterine artery notching	48 (2.2)	13 (2.5)	11 (2.2)	10 (1.8)	14 (2.3)	0.91
Gestational hypertensive disorders						
Gestational hypertension	173 (5.3)	51 (6.3)	42 (5.4)	34 (4.2)	46 (5.2)	0.34
Preeclampsia	59 (1.9)	19 (2.4)	7 (1.0)	20 (2.5)	13 (1.5)	0.07

DASH, Dietary Approaches to Stop Hypertension. Sd, standard deviation. BMI, Body Mass Index. Kg, kilogram. Kcal/d, daily amount in kcal per day. Values are means (SD), median (95% range), or number (valid %). *P-values were obtained by ANOVA for continuous variables and by χ^2 for categorical variables.

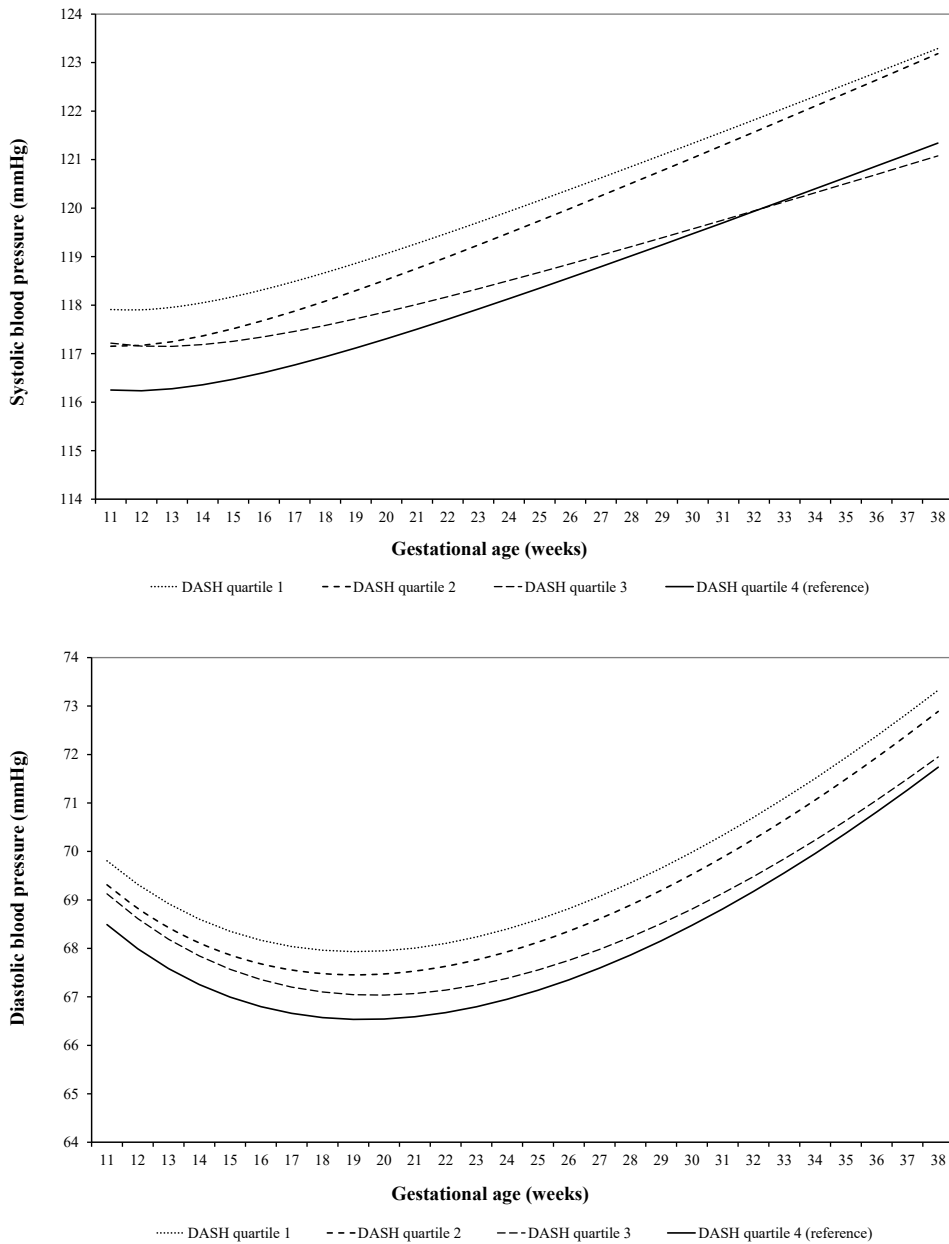


Figure 1. Blood pressure patterns in different DASH categories. Change in systolic blood pressure and diastolic blood pressure in mmHg for first quartile, second quartile, third quartile and fourth quartile. $SBP = \beta_0 + \beta_1 \times \text{DASH quartile} + \beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^{-2} + \beta_4 \times \text{DASH quartile} \times \text{gestational age}$. $DBP = \beta_0 + \beta_1 \times \text{DASH quartile} + \beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^{0.5} + \beta_4 \times \text{DASH quartile} \times \text{gestational age}$. In these models, ' $\beta_0 + \beta_1 \times \text{DASH}$ ' reflects the intercept and ' $\beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^{-2}$ ' reflects the slope of change in blood pressure per week for SBP, and ' $\beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^{0.5}$ ', reflects the slope of change in blood pressure per week for DBP. Our term of interest is β_4 , which reflects the difference in change in blood pressure per week per DASH category, as compared to women in the highest DASH score quartile (healthy diet). Estimates and p-values are given in [Supplementary Table S3](#).

Table 2. Associations of maternal DASH score with systolic and diastolic blood pressure in early, mid and late-pregnancy (n=3,414)*

DASH	Absolute values and differences in systolic blood pressure (mmHg)			
	Early-pregnancy n=2,831	Mid-pregnancy n=3,299	Late-pregnancy n=3,321	
Quartile 1	Absolute mean value (sd) [†] Confounder model [‡] 117.8 (11.9) -0.39 (-1.62, 0.84) n=702	119.5 (12.0) 0.05 (-1.09, 1.18) n=823	121.3 (12.2) -0.16 (-1.27, 0.96) n=825	
Quartile 2	Absolute mean value (sd) [†] Confounder model [‡] 117.4 (12.6) -0.35 (-1.53, 0.82) n=664	118.9 (12.2) 0.08 (-0.99, 1.15) n=773	121.1 (11.8) 0.45 (-0.60, 1.49) n=782	
Quartile 3	Absolute mean value (sd) [†] Confounder model [‡] 117.3 (12.3) -0.02 (-1.11, 1.17) n=704	118.0 (11.7) -0.13 (-1.18, 0.91) n=808	119.7 (10.9) -0.35 (-1.37, 0.68) n=815	
Quartile 4	Absolute mean value (sd) [†] Confounder model [‡] 116.6 (11.0) Reference n=761	117.5 (10.9) Reference n=895	119.5 (10.8) Reference n=899	
SDS [§]	0.19 (-0.26, 0.64)	-0.01 (-0.43, 0.40)	0.07 (-0.33, 0.48)	
DASH	Absolute values and differences in diastolic blood pressure (mmHg)			
	Early-pregnancy n=2,831	Mid-pregnancy n=3,298	Late-pregnancy n=3,320	
Quartile 1	Absolute mean value (sd) [†] Confounder model [‡] 68.9 (9.2) 0.18 (-0.77, 1.13) n=702	68.3 (9.7) 1.31* (0.42, 2.21) n=822	70.0 (9.6) 0.09 (-0.79, 0.97) n=825	
Quartile 2	Absolute mean value (sd) [†] Confounder model [‡] 68.6 (10.1) -0.18 (-1.09, 0.72) n=664	67.7 (9.7) 0.85* (0.01, 1.69) n=773	69.6 (9.3) -0.01 (-0.84, 0.81) n=781	
Quartile 3	Absolute mean value (sd) [†] Confounder model [‡] 68.4 (9.0) -0.19 (-1.07, 0.69) n=704	66.9 (8.9) 0.33 (-0.49, 1.15) n=808	69.0 (8.7) -0.21 (-1.02, 0.60) n=815	
Quartile 4	Absolute mean value (sd) [†] Confounder model [‡] 68.1 (8.5) Reference n=761	66.1 (8.5) Reference n=895	68.8 (9.0) Reference n=899	
SDS [§]	-0.05 (-0.39, 0.30)	-0.45* (-0.78, -0.12)	-0.06 (-0.38, 0.26)	

DASH, Dietary Approaches to Stop Hypertension. Sd, standard deviation. SBP, systolic blood pressure. DBP, diastolic blood pressure. *P-value <0.05. †Values are unadjusted mean blood pressure values (sd) and reflect the absolute value in SBP and DBP per DASH Quartile. ‡Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per maternal DASH score quartile. Groups are compared to women with the highest dietary quality according to the DASH score (quartile 4) as reference. Estimates are from multiple imputed data. Models are adjusted for maternal age, educational level, parity, prepregnancy BMI, smoking habits, alcohol use, folic acid use, total energy intake and gestational age at time of the measurements. §Estimates were based on multiple linear regression models with DASH as SDS.

associated with a higher mid-pregnancy diastolic blood pressure only (p-values <0.05). A higher maternal DASH score across the full range was also significantly associated with a lower mid-pregnancy diastolic blood pressure in the confounder model (difference -0.45 (95% CI: -0.78, -0.12) mmHg per SDS increase in maternal DASH score) but not with diastolic blood pressure in early or late-pregnancy or systolic blood pressure throughout pregnancy. In the basic models, lower maternal DASH score quartiles were associated with a higher systolic and diastolic blood pressure in mid and late-pregnancy as compared to the highest maternal DASH score quartile (all p-values <0.05) (**Supplementary Table S4**).

Maternal DASH score and placental vascular resistance

Supplementary Table S5 shows that in the basic models, compared to the highest maternal DASH score quartile, the lower maternal DASH score quartiles were associated with a higher UmPI in mid and late-pregnancy (p-values <0.05, p-values for trend <0.05), but not with UtRI or bilateral notching. We observed similar results when we used repeated measurement models to examine longitudinal placental vascular development from mid-pregnancy onwards (**Supplementary Table S6**). **Table 3** shows that as compared to the highest maternal DASH score quartile, the lowest maternal DASH score quartile was associated with a higher late-pregnancy UmPI (p-value <0.05) after adjustment for maternal socio-demographic and lifestyle factors. A higher maternal DASH score across the full range was also associated with a lower late-pregnancy UmPI (difference -0.008 (95% CI: -0.015, -0.002) per SDS increase in maternal DASH score). Similar tendencies were present for maternal DASH score quartiles and across the full range with mid-pregnancy UmPI, but these associations were not significant. No consistent associations of maternal DASH score quartiles and SDS with mid or late-pregnancy UtRI or bilateral notching were present after adjustment for maternal socio-demographic and lifestyle factors. Similarly, **Supplementary Table S7** shows that mid- and late-pregnancy mean UtPI did not differ significantly across maternal DASH score quartiles. No associations of maternal DASH score quartiles or SDS with UtPI were observed after adjustment for socio-demographic and lifestyle factors.

Maternal DASH score and risks of gestational hypertension and preeclampsia

Table 4 shows that maternal DASH score in quartiles and SDS were not significantly associated with the risks of any gestational hypertensive disorder, gestational hypertension or preeclampsia in the adjusted models. Comparable findings were present in the basic models (**Supplementary Table S8**).

Table 3. Associations of maternal DASH score with placental vascular resistance (n=3,414)

DASH		Absolute values and differences in UmpI			Absolute values and differences in UTRI			Bilateral notching [§]
		Mid-pregnancy n=2,527	Late-pregnancy n=2,776	Mid-pregnancy n=1,898	Late-pregnancy n=2,076	Mid-pregnancy n=1,898	Late-pregnancy n=2,076	
Quartile 1	Absolute mean value (sd) [†] Confounder model [‡]	1.20 (0.18) 0.012 (-0.009, 0.033) n=598	1.00 (0.18) 0.026 [*] (0.007, 0.044) n=672	0.535 (0.091) -0.002 (-0.014, 0.010) n=433	0.490 (0.076) 0.009 (-0.001, 0.019) n=496	n.a.	n.a.	n.a.
Quartile 2	Absolute mean value (sd) [†] Confounder model [‡]	1.20 (0.18) 0.019 (0.000, 0.039) n=600	0.97 (0.16) -0.002 (-0.016, 0.019) n=644	0.535 (0.090) 0.000 (-0.011, 0.011) n=448	0.484 (0.076) 0.005 (-0.004, 0.014) n=477	n.a.	n.a.	n.a.
Quartile 3	Absolute mean value (sd) [†] Confounder model [‡]	1.18 (0.17) 0.009 (-0.010, 0.028) n=630	0.98 (0.16) 0.015 (-0.003, 0.031) n=693	0.535 (0.089) -0.001 (-0.012, 0.010) n=468	0.480 (0.081) 0.000 (-0.009, 0.009) n=516	n.a.	n.a.	n.a.
Quartile 4	Absolute mean value (sd) [†] Confounder model [‡]	1.17 (0.18) Reference n=699	0.96 (0.16) Reference n=767	0.535 (0.088) Reference n=549	0.479 (0.08) Reference n=587	n.a.	n.a.	n.a.
SDS [§]		-0.007 (-0.015, 0.001)	-0.008 [*] (-0.015, -0.002)	0.001 (-0.003, 0.006)	-0.003 (-0.006, 0.001)	0.001 (-0.003, 0.006)	-0.003 (-0.006, 0.001)	1.02 (0.76, 1.36)

UmpI, umbilical artery pulsatility index. UTRI, uterine artery resistance index. DASH, Dietary Approaches to Stop Hypertension. Sd, standard deviation. *P-value <0.05. [†]Values are unadjusted mean values (sd) and reflect the absolute value in UmpI and UTRI per DASH Quartile. [‡]Values are regression coefficients (95% confidence interval) and reflect differences in umbilical artery pulsatility index and uterine artery resistance index per DASH quartile. Groups are compared to women with the highest dietary quality according to the DASH score (quartile 4) as reference. Models for UmpI and UTRI are adjusted for maternal age, educational level, parity, prepregnancy BMI, smoking habits, alcohol use, folic acid use, total energy intake and gestational age at time of the measurements. Models for bilateral notching are adjusted for parity, prepregnancy BMI, folic acid use and gestational age at time of measurement. [§]Values are odds ratios (95% confidence interval) that reflect difference in risks of third trimester notching per DASH quartile. Groups are compared to women with a healthy dietary pattern (quartile 4) as reference. Estimates are from multiple imputed data. ||Estimates were based on multiple linear regression models with DASH as SDS for uterine artery resistance index and umbilical artery resistance index, and on multiple logistic regression models with DASH as SDS for bilateral notching.

Table 4. Associations of maternal DASH score with the risks of gestational hypertensive disorders (n=3,414)

DASH	Gestational hypertensive disorders [†]	Gestational hypertension [†]	Preeclampsia [†]
	Odds ratio (95% CI) n _{cases} =232	Odds ratio (95% CI) n _{cases} =173	Odds ratio (95% CI) n _{cases} =59
Quartile 1	1.14 (0.78, 1.67) n _{cases} =70	1.04 (0.67, 1.60) n _{cases} =51	1.46 (0.70, 3.07) n _{cases} =19
Quartile 2	0.84 (0.56, 1.25) n _{cases} =49	0.91 (0.59, 1.42) n _{cases} =42	0.57 (0.23, 1.46) n _{cases} =7
Quartile 3	0.95 (0.64, 1.40) n _{cases} =54	0.73 (0.46, 1.16) n _{cases} =34	1.74 (0.85, 3.55) n _{cases} =20
Quartile 4	Reference n _{cases} =59	Reference n _{cases} =46	Reference n _{cases} =13
SDS [‡]	0.95 (0.83, 1.10)	0.96 (0.81, 1.12)	0.94 (0.72, 1.23)

DASH, Dietary Approaches to Stop Hypertension. CI, Confidence Interval. GHD, Gestational hypertensive disorders. GH, Gestational Hypertension. PE, Preeclampsia. [†]Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorders, gestational hypertension and preeclampsia per DASH quartile. Groups are compared to women with the highest dietary quality according to the DASH score (quartile 4) as reference. Estimates are from multiple imputed data. Models are adjusted for parity, prepregnancy BMI, folic acid use and gestational age at time of intake. [‡]Estimates were based on multiple logistic regression models with DASH as SDS.

Sensitivity analyses

Similar results were present when we excluded women with pre-existent and gestational diabetes (**Supplementary Table S9-S11**) and when we excluded women with hypercholesterolemia and/or a heart condition (**Supplementary Table S12-S14**). When we restricted to women who enrolled before 14 weeks of gestation, similar findings were present for systolic and diastolic blood pressure and gestational hypertensive disorders, but no associations of maternal DASH score quartiles or SDS with placental hemodynamic parameters were observed (**Supplementary Table S15-17**). When we used propensity scores to adjust for potential maternal socio-demographic and lifestyle-related confounding factors, we observed similar results for bilateral uterine artery notching and gestational hypertensive disorders as compared to conventional covariate adjustment in the multivariable regression models (**Supplementary Table S18**).

DISCUSSION

Within this low-risk population-based cohort study, we observed that a higher maternal DASH diet score was associated with a lower mid-pregnancy diastolic blood pressure, but not with diastolic blood pressure in early or late-pregnancy or systolic blood pressure throughout pregnancy. A higher maternal DASH diet score tended to be associated with a lower mid and late-pregnancy UmPI, but not with other placental hemodynamic parameters. No associations were present with the risks of gestational hypertensive disorders.

Interpretation of main findings

The DASH diet is a diet high in fruits, vegetables, total grains, nuts, seeds, legumes and non-full-fat dairy products, and low in animal protein, sugar and sodium². This dietary approach has gained substantial attention for its blood pressure lowering properties in non-pregnant populations. In the original clinical trial among 459 participants with systolic blood pressure of less than 160 mmHg and diastolic blood pressure of 80 to 95 mmHg, the DASH diet led to a significant reduction of systolic and diastolic blood pressure by 5.5 and 3.0 mmHg, with even stronger effects in hypertensive individuals³. These results have been reproduced in numerous other intervention and observational studies that suggest beneficial effects on cardiovascular risk factors and long-term cardiovascular outcomes^{4-7, 12}. The DASH diet is accordingly recommended by the American Heart Association to manage blood pressure, improve lipid profile and reduce the risks of heart attack and stroke³⁴. We hypothesized that maternal adherence to the DASH diet during pregnancy may also reduce the risks of gestational hypertensive disorders through its potential positive effects on blood pressure and vascular function.

Not much is known about the influence of maternal adherence to the DASH diet during pregnancy on blood pressure development or placental vascular resistance in pregnancy. The DASH diet has some resemblance in dietary properties when compared to the Mediterranean diet. Maternal adherence to a Mediterranean dietary pattern has been associated with lower blood pressure in early and mid-pregnancy and lower placental vascular resistance in low-risk and higher-risk populations³⁵⁻³⁸. In line with these findings, an observational study in Ireland among 511 women with a large-for-gestational-age infant in their previous pregnancy, showed that higher maternal adherence to the DASH diet in their second pregnancy was associated with a lower diastolic blood pressure and mean arterial pressure in early and late-pregnancy, but not in mid-pregnancy⁸. Within this study dietary intake was recorded in each trimester of pregnancy using a 3-day food diary, but no extensive adjustment for other lifestyle factors was performed⁸. A small intervention study among 34 Iranian women with gestational diabetes also described a favorable influence on third trimester systolic blood pressure after adhering to the DASH diet for 4 weeks compared to a control diet³⁵. Contrary, an observational study among 1,760 pregnant women in the United States showed no associations of DASH diet score with third trimester blood pressure in a low-risk multi-ethnic population¹⁰.

Only partly in line with the previous studies focused on adherence to a Mediterranean diet and the DASH diet, we did not find consistent associations of a higher maternal DASH score with systolic and diastolic blood pressure development throughout pregnancy after adjustment for socio-demographic and lifestyle factors in a low-risk population. A

higher maternal DASH diet score was only associated with a small reduction in mid-pregnancy diastolic blood pressure. A higher maternal DASH diet score also tended to be associated with lower umbilical artery vascular resistance in mid and late-pregnancy, but not with uteroplacental vascular resistance. The umbilical artery reflects the development of the fetoplacental vascular tree. Already small increases in mid and late-pregnancy fetoplacental vascular resistance are associated with increased risks of gestational hypertension and preeclampsia^{22, 23}. These observed associations with mid-pregnancy diastolic blood pressure and fetoplacental vascular resistance may be explained by improved endothelial cell function and reduction of oxidative stress through the DASH diet and potential positive effects on the renin-angiotensin-aldosterone system via sodium reduction^{12, 13, 15}. Through these mechanisms, the DASH diet may positively affect physiological hemodynamic adaptations in pregnancy, which could explain the strongest effect on mid-pregnancy diastolic blood pressure, when the physiological diastolic blood pressure dip in pregnancy occurs^{10, 40}. The vasomotor tone of the fetoplacental vasculature is fully regulated by endothelial derived vasoactive mediators, whereas the uteroplacental vascular bed is also influenced by autonomic regulation⁴¹⁻⁴³. Thus, the beneficial effects on endothelial function may be more apparent on the fetoplacental vasculature than the uteroplacental vasculature. The potential beneficial effects of the DASH diet on gestational hemodynamic adaptations may be more pronounced among higher risk populations^{8, 39}.

Three studies explored the effects of the DASH diet on the risks of gestational hypertensive disorders. A prospective cohort among 1,760 pregnant women in the United States did not observe any associations of first-trimester DASH diet score with gestational hypertension or preeclampsia¹⁰. A cohort among 66,651 women with singleton pregnancies in Denmark showed no association of maternal DASH diet score at 25 weeks gestation with the risk of gestational hypertensive disorders¹¹. In line with these previous studies, we observed no significant associations of maternal DASH score diet with the risk of gestational hypertensive disorders. We observed a tendency for an association of a higher maternal DASH score with a lower risk of preeclampsia, but this association was not significant. This might indicate a type II error due to a relatively small number of preeclampsia cases within our low-risk population. Contrary to our findings, a beneficial effect of the DASH diet was found in a randomized controlled trial in China among 85 high-risk pregnant women diagnosed with pre-existent hypertension or gestational hypertension. They found a lower incidence of preeclampsia when women adhered to the DASH diet compared to a control diet during a 12 week intervention period⁹. Thus, our findings suggest that in a low-risk pregnant population, higher maternal DASH diet score is not associated with a lower risk of gestational hypertensive disorders. Stimulating

maternal adherence to the DASH diet might be more clinically relevant in pregnant populations with a high a priori risk of gestational hypertensive disorders.

Within our low-risk Dutch population, we did not observe consistent and strong positive associations of higher maternal DASH diet score with systolic and diastolic blood pressure development throughout pregnancy, uteroplacental vascular resistance or the risks of gestational hypertensive disorders after considering maternal socio-demographic and lifestyle factors. There remained only a relatively small association of higher maternal DASH diet score with a lower mid-pregnancy diastolic blood pressure and a tendency to lower fetoplacental vascular resistance from mid-pregnancy onwards, after adjustment for maternal sociodemographic and lifestyle factors. These observed associations were small and within the normal range of maternal blood pressure and umbilical artery vascular resistance. However, we do consider these findings important from an etiological perspective and on a population level. Overall, we observed that participating women already adhered to components of the DASH diet and subsequently the range of DASH score within our study population was moderate. Possibly, among pregnant populations with a larger variability in dietary intake, the influence of higher maternal adherence to the DASH diet on gestational hemodynamic adaptations is more apparent. Within our study population, also blood pressure was mainly within the normotensive range. We excluded women with preexistent hypertension. Among pregnant women with an already increased baseline blood pressure, the beneficial effects of the DASH diet on gestational hemodynamic adaptations could be more apparent as was demonstrated in earlier research in non-pregnant populations³. Further studies are needed to explore the effects of adherence to the DASH diet in higher-risk multi-ethnic pregnant populations on gestational hemodynamic adaptations and the risks of gestational hypertensive disorders to assess whether recommending the DASH diet for these higher-risk pregnant women may improve their pregnancy outcomes.

Strengths and limitations

We had a prospective data collection from early-pregnancy onwards and a large sample size. The response rate for participation in the Generation R cohort was 61% at baseline, which reflects the number of participating pregnant women in the study as a percentage of the total number of pregnant women who fulfilled the eligibility criteria in the study area¹⁶. We restricted to women of Dutch ethnicity, which may have affected the generalizability of our findings. Information on gestational hypertensive disorders was obtained from medical records, using definitions of gestational hypertensive disorders used in clinical practice at the time²⁷. The definition of preeclampsia has been updated,

which might affect the generalizability of our findings to current clinical practice⁴⁴. Within our study population, we had a relatively small number of gestational hypertensive disorders and bilateral uterine artery notching cases, which might indicate a selection towards a relatively healthy low-risk population. Additionally, it may have led to lack of statistical power for these specific analyses and the possibility of a type II error. Further studies within larger populations with more cases of placental insufficiency and gestational hypertensive disorders are needed using the most up-to-date classification for gestational hypertensive disorders to examine these associations in further detail with increased statistical power. Women with preexistent hypertension or other cardiovascular diseases may be at increased risk of impaired gestational hemodynamic adaptations and developing gestational hypertensive disorders⁴⁵. Importantly, women with preexistent hypertension were excluded from our study population and we observed similar findings when we additionally excluded women with hypercholesterolemia and a heart condition from the analyses. Given the relatively young age of participating women, we consider it unlikely that a high percentage of women already had other pre-existent cardiovascular diseases, but we did not have more detailed information available. Further studies with detailed assessments of maternal cardiovascular health before and during pregnancy are needed to assess whether adherence to the DASH diet has a different effect on gestational hemodynamic adaptations in low-risk and higher-risk populations. Although the FFQs were validated previously and are a commonly used method to assess dietary intake, reporting bias may be an issue as the FFQ was self-administered and components of the DASH diet are food items that are generally known for their healthy or less healthy properties. We assessed maternal dietary intake by FFQ at enrolment in the study. Due to the design of our study, the timing of the FFQ administration is relatively broad²⁸. As the FFQ reflects maternal dietary intake in the three months prior, this approach allowed us to assess maternal dietary intake just before pregnancy and in the first half of pregnancy and reduces the risk of recall bias. Importantly, some women may have changed their diet already at an earlier stage in the preconception period in order to improve their own health and fertility, or may have changed their diet when they became pregnant. Further studies from preconception onwards are needed with detailed dietary assessments in the preconception period and during pregnancy to identify critical periods for maternal dietary intake on gestational hemodynamic adaptations and the risk of gestational hypertensive disorders. Information on a large number of covariates was available within our study to adjust for potential confounding within our main analyses. We could only adjust for a relatively small set of confounders for bilateral uterine artery notching and gestational hypertensive disorders due to number of cases. However, we observed similar results when

we used a propensity score to adjust for a larger number of maternal socio-demographic and lifestyle-related characteristics. As in any observational study residual confounding might still be an issue.

Conclusion

In a low-risk pregnant population, higher maternal adherence to DASH diet was associated with a lower mid-pregnancy diastolic blood pressure and tended to be associated with a lower mid and late-pregnancy umbilical artery vascular resistance, but not with systolic blood pressure, uteroplacental vascular resistance or the risk of gestational hypertensive disorders. Further studies are needed to assess whether maternal adherence to the DASH diet has more pronounced positive effects on gestational hemodynamic adaptations and the risks of gestational hypertensive disorders in higher-risk populations.

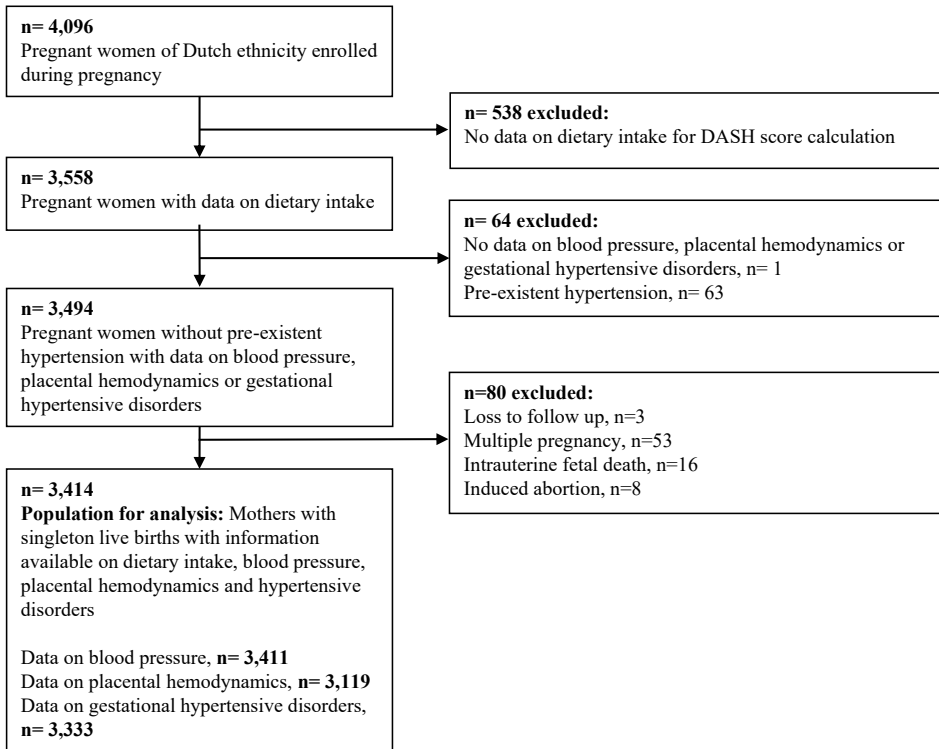
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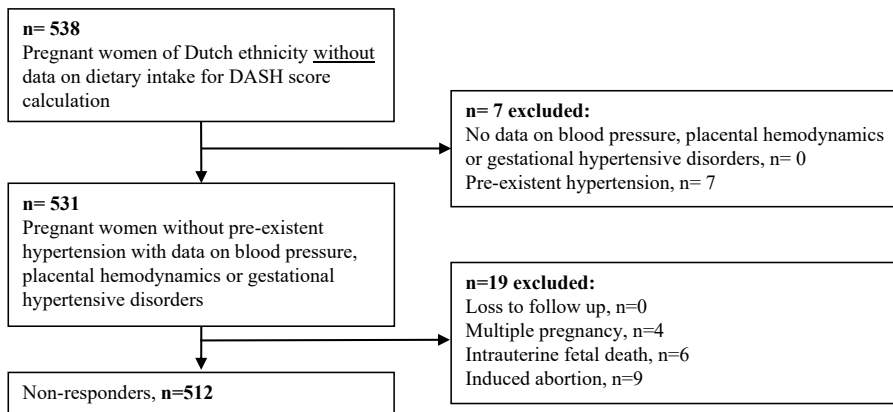
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SUPPLEMENTAL MATERIAL



Supplementary Figure S1. Flowchart of the study population.



Supplementary Figure S2. Flowchart of the non-responders.

Supplementary Table S1. Dietary intake of DASH food components by DASH score quartile (n=3,414)*

	Total group n=3,414	DASH quartile 1 score 10-21 n=860	DASH quartile 2 score 22-24 n=798	DASH quartile 3 score 25-27 n=836	DASH quartile 4 score 28-37 n=920	p-value
Total grains, g/d	174 (122, 218)	128 (88, 177)	168 (117, 208)	183 (139, 222)	203 (162, 246)	<0.001
Vegetables (excluding potatoes and condiments), g/d	143 (105, 186)	104 (76, 136)	129 (101, 160)	151 (121, 190)	188 (151, 231)	<0.001
Fruits, g/d	296 (192, 441)	202 (121, 299)	275 (187, 414)	319 (214, 454)	389 (281, 525)	<0.001
Non-full-fat dairy products, g/d	310 (171, 462)	191 (94, 386)	273 (154, 445)	326 (204, 473)	409 (266, 549)	<0.001
Nuts, seeds, legumes, g/d	13 (6, 23)	7 (2, 13)	11 (5, 18)	14 (7, 24)	22 (13, 35)	<0.001
Red and processed meats, g/d	53 (35, 75)	74 (54, 93)	60 (44, 78)	49 (34, 65)	36 (21, 52)	<0.001
Sugar-sweetened beverages, sweets and added sugars, g/d	65 (33, 140)	156 (83, 262)	76 (38, 148)	57 (31, 99)	39 (21, 63)	<0.001
Sodium, mg/d	3,317 (937)	3,464 (982)	3,337 (973)	3,311 (957)	3,168 (814)	<0.001

DASH, Dietary Approaches to Stop Hypertension. g/d, daily amount in grams and/or milliliters per day. mg/d, daily amount in milligrams per day. *Values are median (inter quartile range) or mean (sd).

Supplementary Table S2. Non-response analysis: characteristics of participating women with and without data on dietary intake*

	Participants with data on dietary intake [†]	Participants without data on dietary intake [‡]	p-value
	n=3,414	n=512	
Maternal age at enrolment, mean (sd), years	31.4 (4.4)	30.3 (5.3)	<0.001
Parity, n nulliparous (%)	2,039 (59.9)	291 (57.3)	0.27
Prepregnancy BMI, mean (sd)	23.1 (3.8)	23.1 (4.1)	0.80
Prepregnancy BMI ≥25	655 (22.2)	98 (23.1)	0.68
Gestational weight gain, mean (sd), kg	10.8 (4.4)	11.3 (4.8)	0.05
Gestational age at intake (weeks) [§]	14.7 (10.2, 23.1)	14.1 (10.3, 30.4)	<0.001
Higher education, n (%)	2,000 (59.3)	232 (46.6)	<0.001
Smoking, n continued (%)	538 (17.0)	116 (25.3)	<0.001
Alcohol consumption, n continued (%)	1,570 (50.0)	202 (44.4)	0.025
Folic acid supplement use, n (%)	2,493 (89.1)	332 (82.0)	<0.001
Systolic blood pressure, mean (sd), mmHg			
Early-pregnancy	117.3 (11.9)	117.6 (12.3)	0.60
Mid-pregnancy	118.5 (11.7)	118.5 (10.9)	0.92
Late-pregnancy	120.4 (11.4)	119.7 (11.4)	0.20
Diastolic blood pressure, mean (sd), mmHg			
Early-pregnancy	68.5 (9.2)	68.1 (9.5)	0.48
Mid-pregnancy	67.2 (9.3)	67.0 (9.5)	0.61
Late-pregnancy	69.4 (9.2)	69.5 (9.3)	0.76
Umbilical artery pulsatility index, mean (sd)			
Mid-pregnancy	1.19 (0.18)	1.22 (0.18)	0.008
Late-pregnancy	0.98 (0.17)	0.98 (0.18)	0.37
Uterine artery resistance index, mean (sd)			
Mid-pregnancy	0.535 (0.089)	0.545 (0.090)	0.08
Late-pregnancy	0.483 (0.078)	0.481 (0.077)	0.62
Late-pregnancy notching, n (%)	48 (2.2)	2 (0.6)	0.07
Gestational hypertensive disorders, n (%)			
Gestational hypertension	173 (5.3)	24 (4.9)	0.74
Preeclampsia	59 (1.9)	8 (1.7)	0.80

*Values are means (sd) or percentages. [†]Women with data on dietary intake as described in **Supplementary Figure S1**. [‡]Women without data on dietary intake as described in **Supplementary Figure S2**. [§]Median (95% range).

[§]Median (95% range).

Supplementary Table S3. Longitudinal associations between DASH score and systolic and diastolic blood pressure*

DASH	Difference in systolic blood pressure (mmHg)			
	Intercept	p-value [†]	Slope (mmHg (95%CI))	p-value [†]
Quartile 1	113.5	0.08	0.01 (-0.06, 0.08)	0.75
Quartile 2	112.5	0.56	0.04 (-0.03, 0.10)	0.31
Quartile 3	113.4	0.10	-0.05 (-0.11, 0.02)	0.18
Quartile 4	111.9	Reference	Reference	Reference

DASH	Difference in diastolic blood pressure (mmHg)			
	Intercept	p-value [†]	Slope (mmHg (95%CI))	p-value [†]
Quartile 1	100.1	0.08	0.01 (-0.04, 0.06)	0.70
Quartile 2	99.6	0.32	0.01 (-0.03, 0.06)	0.64
Quartile 3	99.7	0.25	-0.02 (-0.07, 0.04)	0.54
Quartile 4	98.9	Reference	Reference	Reference

DASH, Dietary Approaches to Stop Hypertension. *Values are based on repeated non-linear regression models and reflect the change in blood pressure in mmHg per DASH quartile compared to women with the highest dietary quality (quartile 4) as reference. Models are adjusted for gestational age at the time of measurements. [†]P-value reflects the significance level of the estimate.

Supplementary Table S4. Basic models: associations of maternal DASH score with systolic and diastolic blood pressure in early, mid and late-pregnancy (n=3,414)

DASH	Difference in systolic blood pressure (mmHg)		
	Early-pregnancy n=2,831	Mid-pregnancy n=3,299	Late-pregnancy n=3,321
Quartile 1 [†]	1.14 (-0.09, 2.36) n=702	1.97 (0.87, 3.08) [*] n=823	1.77 (0.69, 2.85) [*] n=825
Quartile 2 [†]	0.70 (-0.54, 1.94) n=664	1.28 (0.16, 2.41) [*] n=773	1.54 (0.45, 2.64) [*] n=782
Quartile 3 [†]	0.62 (-0.60, 1.84) n=704	0.49 (-0.62, 1.60) n=808	0.17 (-0.91, 1.25) n=815
Quartile 4 [†]	Reference n=761	Reference n=895	Reference n=899
SDS [‡]	-0.40 (-0.83, -0.04)	-0.77 (1.16, -0.37) [*]	-0.69 (-1.08, -0.30) [*]

DASH	Difference in diastolic blood pressure (mmHg)		
	Early-pregnancy n=2,831	Mid-pregnancy n=3,298	Late-pregnancy n=3,320
Quartile 1 [†]	0.69 (-0.25, 1.64) n=702	2.19 (1.32, 3.06) [*] n=822	1.11 (0.24, 1.97) [*] n=825
Quartile 2 [†]	0.38 (-0.58, 1.33) n=664	1.57 (0.68, 2.45) [*] n=773	0.75 (-0.13, 1.63) n=781
Quartile 3 [†]	0.23 (-0.71, 1.17) n=704	0.76 (-0.12, 1.64) n=808	0.20 (-0.67, 1.07) n=815
Quartile 4 [†]	Reference n=761	Reference n=895	Reference n=899
SDS [‡]	-0.28 (-0.58, 0.09)	-0.79 (-1.10, -0.48) [*]	-0.46 (-0.77, -0.15) [*]

DASH, Dietary Approaches to Stop Hypertension. *P-value <0.05. [†]Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per DASH quartile. Groups are compared to women with the highest dietary quality (quartile 4) as reference. Models are adjusted for gestational age at the time of intake. Estimates are from multiple imputed data. [‡]Estimates were based on multiple linear regression models with DASH as SDS. Models are adjusted for gestational age at the time of intake. Estimates are from multiple imputed data.

Supplementary Table S5. Basic models: associations of DASH score with placental vascular resistance (n=3,414)

DASH	Umbilical artery pulsatility index ^{†‡}		Uterine artery resistance index ^{†‡}		Bilateral notching ^{†§}	
	Mid-pregnancy n=2,527	Late-pregnancy n=2,776	Mid-pregnancy n=1,898	Late-pregnancy n=2,076	Late-pregnancy n _{cases} =48	
Quartile 1	0.027 (0.007, 0.047) [*] n=598	0.038 (0.021, 0.055) [*] n=672	0.000 (-0.011, 0.012) n=433	0.010 (0.001, 0.020) [*] n=496	1.09 (0.51, 2.34) n _{cases} =13	
Quartile 2	0.024 (0.004, 0.044) [*] n=600	0.007 (-0.011, 0.024) n=644	0.000 (-0.011, 0.011) n=448	0.005 (-0.005, 0.014) n=477	0.95 (0.43, 2.11) n _{cases} =11	
Quartile 3	0.006 (-0.013, 0.026) n=630	0.015 (-0.002, 0.032) n=693	0.000 (-0.011, 0.011) n=468	0.001 (-0.009, 0.010) n=516	0.80 (0.35, 1.82) n _{cases} =10	
Quartile 4	Reference n=699	Reference n=767	Reference n=549	Reference n=587	Reference n _{cases} =14	
SDS	-0.013 (-0.020, -0.005) [*]	-0.013 (-0.019, -0.007) [*]	0.000 (-0.004, 0.005)	-0.003 (-0.007, 0.000)	1.02 (0.76, 1.36)	

DASH, Dietary Approaches to Stop Hypertension. UmPI, umbilical artery pulsatility index. UTRI, uterine artery resistance index. *P-value<0.05. †Values are regression coefficients (95% confidence interval) and reflect differences in UmPI and UTRI per DASH quartile. Groups are compared to women with the highest dietary quality according to the DASH score (quartile 4) as reference. Estimates are from multiple imputed data. ‡Models are adjusted for gestational age at the time of intake. §Values are odds ratios (95% confidence interval) that reflect difference in risks of late-pregnancy notching per DASH quartile. Groups are compared to women with a healthy dietary pattern (quartile 4) as reference. Estimates are from multiple imputed data. ||Estimates were based on multiple linear regression models with DASH as SDS for UmPI and UTRI, and on multiple logistic regression models with DASH as SDS for bilateral notching.

Supplementary Table S6. Longitudinal associations between DASH score and umbilical artery pulsatility index and uterine artery resistance index*

DASH	Difference in umbilical artery pulsatility index			
	Intercept	p-value [†]	Slope (95% CI)	p-value [†]
Quartile 1	1.642	0.39	0.0002 (-0.002, 0.002)	0.86
Quartile 2	1.690	0.01	-0.002 (-0.004, -0.000)	0.04
Quartile 3	1.628	0.66	-0.0001 (-0.002, 0.002)	0.96
Quartile 4	1.615	Reference	Reference	Reference
DASH	Difference in uterine artery resistance index			
	Intercept	p-value [†]	Slope (95% CI)	p-value [†]
Quartile 1	0.637	0.23	0.001 (-0.000, 0.002)	0.70
Quartile 2	0.636	0.21	0.001 (-0.000, 0.002)	0.64
Quartile 3	0.651	0.77	0.0001 (-0.001, 0.001)	0.54
Quartile 4	0.656	Reference	Reference	Reference

DASH, Dietary Approaches to Stop Hypertension. CI, Confidence interval. *Values are based on repeated non-linear regression models and reflect the change in umbilical artery pulsatility index and uterine artery resistance index per DASH quartile compared to women with the highest dietary quality (quartile 4) as reference. Models are adjusted for gestational age at the time of measurement. [†]P-value reflects the significance level of the estimate.

Supplementary Table S7. Secondary outcome: associations of DASH score with uterine artery pulsatility index (n=3,414)

DASH		Absolute values and differences in UtPI	
		Mid-pregnancy n=1,530	Late-pregnancy n=1,747
Quartile 1	Absolute mean value (sd) [*]	0.895 (0.275)	0.751 (0.199)
	Basic model ^{†,‡}	0.020 (-0.017, 0.057)	0.022 (-0.004, 0.048)
	Confounder model ^{†,§}	0.013 (-0.026, 0.053) n=342	0.013 (-0.015, 0.041) n=417
Quartile 2	Absolute mean value (sd) [*]	0.883 (0.261)	0.736 (0.189)
	Basic model ^{†,‡}	0.009 (-0.028, 0.045)	0.007 (-0.019, 0.032)
	Confounder model ^{†,§}	0.010 (-0.027, 0.046) n=354	0.006 (-0.020, 0.033) n=408
Quartile 3	Absolute mean value (sd) [*]	0.875 (0.252)	0.735 (0.206)
	Basic model ^{†,‡}	0.000 (-0.35, 0.036)	0.006 (-0.019, 0.031)
	Confounder model ^{†,§}	0.000 (-0.036, 0.035) n=394	0.004 (-0.021, 0.029) n=438
Quartile 4	Absolute mean value (sd) [*]	0.875 (0.256)	0.729 (0.187)
	Basic model ^{†,‡}	Reference	Reference
	Confounder model ^{†,§}	Reference n=440	Reference n=484
SDS	Basic model ^{†,}	-0.004 (-0.017)	-0.006 (-0.015, 0.003)
	Confounder model ^{†,}	-0.001 (-0.015, 0.014)	-0.003 (-0.013, 0.007)

UtPI, Uterine artery pulsatility index. DASH, Dietary Approaches to Stop Hypertension. Sd, standard deviation. CI, Confidence Interval. *Values are mean UtPI values (sd) and reflect the absolute value in uterine artery pulsatility index per DASH Quartile. P-values for comparison of absolute values among the four DASH quartiles were obtained by ANOVA (mid-pregnancy UtPI, p-value=0.693; late-pregnancy UtPI, p-value 0.387). [†]Values are regression coefficients (95% confidence interval) and reflect differences in UtPI per DASH Quartile. Groups are compared to women with the highest dietary quality according to the DASH score (Quartile 4) as reference. Estimates are from multiple imputed data. [§]Models are adjusted for maternal age, educational level, parity, prepregnancy BMI, smoking habits, alcohol use, folic acid use, total energy intake and gestational age at time of the measurements. ^{||}Estimates were based on multiple linear regression models with DASH dietary score as SDS.

Supplementary Table S8. Basic models: associations of maternal DASH score the risks of gestational hypertensive disorder (3,414)*

	Gestational hypertensive disorders	Gestational hypertension	Preeclampsia
DASH	Odds ratio (95% CI) n _{cases} =232	Odds ratio (95% CI) n _{cases} =173	Odds ratio (95% CI) n _{cases} =59
Quartile 1	1.31 (0.91, 1.88) n _{cases} =70	1.22 (0.81, 1.84) n _{cases} =51	1.62 (0.79, 3.30) n _{cases} =19
Quartile 2	0.96 (0.65, 1.42) n _{cases} =49	1.05 (0.68, 1.61) n _{cases} =42	0.62 (0.25, 1.57) n _{cases} =7
Quartile 3	1.00 (0.69, 1.47) n _{cases} =54	0.81 (0.51, 1.27) n _{cases} =34	1.70 (0.84, 3.43) n _{cases} =20
Quartile 4	Reference n _{cases} =59	Reference n _{cases} =46	Reference n _{cases} =13
SDS†	0.90 (0.79, 1.03)	0.90 (0.77, 1.05)	0.90 (0.70, 1.16)

DASH, Dietary Approaches to Stop Hypertension. CI, Confidence Interval. *Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorders, gestational hypertension and preeclampsia per DASH quartile. Groups are compared to women with the highest dietary quality according to the DASH score (quartile 4) as reference. Estimates are from multiple imputed data. Models are adjusted for gestational age at the time of intake. †Estimates were based on multiple logistic regression models with DASH as SDS.

Supplementary Table S9. Sensitivity analysis: associations of maternal DASH score with systolic and diastolic blood pressure in early, mid and late-pregnancy in participants without pre-existent diabetes or gestational diabetes (n=3,378)

		Absolute values and differences in systolic blood pressure (mmHg)		
DASH		Early-pregnancy n=2,802	Mid-pregnancy n=3,263	Late-pregnancy n=3,286
Quartile 1	Absolute mean value (sd) [†]	117.65 (11.79)	119.43 (12.02)	121.14 (12.01)
	Basic model ^{‡,§}	0.98 (-0.24, 2.20)	1.91 [*] (0.80, 3.02)	1.66 [*] (0.58, 2.73)
	Confounder model ^{‡,}	-0.53 (-1.77, 0.70) n=692	0.01 (-1.14, 1.15) n=809	-0.26 (-1.38, 0.86) n=811
Quartile 2	Absolute mean value (sd) [†]	117.17 (12.38)	118.77 (12.23)	120.93 (11.68)
	Basic model ^{‡,§}	0.48 (-0.76, 1.72)	1.22 [*] (0.09, 2.35)	1.44 [*] (0.35, 2.54)
	Confounder model ^{‡,}	-0.51 (-1.68, 0.67) n=657	0.06 (-1.02, 1.13) n=765	0.38 (-0.67, 1.43) n=774
Quartile 3	Absolute mean value (sd) [†]	117.17 (12.27)	117.94 (11.71)	119.64 (10.92)
	Basic model ^{‡,§}	0.53 (-0.69, 1.75)	0.43 (-0.69, 1.54)	0.16 (-0.92, 1.24)
	Confounder model ^{‡,}	-0.06 (-1.20, 1.08) n=696	-0.18 (-1.22, 0.87) n=799	-0.34 (-1.37, 0.68) n=806
Quartile 4	Absolute mean value (sd) [†]	116.63 (10.97)	117.48 (10.89)	119.48 (10.81)
	Basic model ^{‡,§}	Reference	Reference	Reference
	Confounder model ^{‡,}	Reference n=757	Reference n=890	Reference n=895
SDS [‡]	Basic model [‡]	-0.33 (-0.76, 0.11)	-0.75 [*] (-1.15, -0.35)	-0.66 [*] (-1.05, -0.27)
	Confounder model ^{‡,§}	0.26 (-0.19, 0.71)	-0.01 (-0.43, 0.41)	0.10 (-0.31, 0.51)
		Absolute values and differences in diastolic blood pressure (mmHg)		
DASH		Early-pregnancy [†] n=2,802	Mid-pregnancy [†] n=3,262	Late-pregnancy [†] n=3,285
Quartile 1	Absolute mean value (sd) [†]	68.80 (9.14)	68.22 (9.74)	69.86 (9.59)
	Basic model ^{‡,§}	0.65 (-0.29, 1.59)	2.16 [*] (1.28, 3.03)	1.03 [*] (0.16, 1.90)
	Confounder model ^{‡,}	0.15 (-0.81, 1.10) n=692	1.34 [*] (0.44, 2.24) n=808	0.04 (-0.84, 0.93) n=811
Quartile 2	Absolute [†]	68.43 (10.00)	67.56 (9.70)	69.47 (9.25)
	Basic model ^{‡,§}	0.25 (-0.70, 1.21)	1.49 [*] (0.60, 2.37)	0.63 (-0.25, 1.51)
	Confounder model ^{‡,}	-0.25 (-1.16, 0.66) n=657	0.82 (-0.02, 1.66) n=765	-0.08 (-0.91, 0.75) n=773
Quartile 3	Absolute mean value (sd) [†]	68.28 (8.97)	66.77 (8.87)	68.97 (8.73)
	Basic model ^{‡,§}	0.16 (-0.78, 1.10)	0.71 (-0.17, 1.59)	0.13 (-0.74, 1.00)
	Confounder model ^{‡,}	-0.25 (-1.13, 0.63) n=696	0.30 (-0.52, 1.12) n=799	-0.26 (-1.07, 0.55) n=806
Quartile 4	Absolute mean value (sd) [†]	68.10 (8.54)	66.05 (8.50)	68.83 (8.96)
	Basic model ^{‡,§}	Reference	Reference	Reference
	Confounder model ^{‡,}	Reference n=757	Reference n=890	Reference n=895
SDS [‡]	Basic model [‡]	-0.23 (-0.57, 0.11)	-0.79 [*] (-1.10, -0.47)	-0.43 [*] (-0.74, -0.12)
	Confounder model ^{‡,}	-0.03 (-0.37, 0.32)	-0.47 [*] (-0.80, -0.14)	-0.04 (-0.36, 0.28)

DASH, Dietary Approaches to Stop Hypertension. Sd, standard deviation. SBP, systolic blood pressure. DBP, diastolic blood pressure. *P-value<0.05. [†]Values are mean blood pressure values (sd) and reflect the absolute value in SBP and DBP per DASH Quartile. P-values for comparison of absolute values among the four DASH quartiles were obtained by ANOVA (early-pregnancy SBP, p-value=0.433; mid-pregnancy SBP, p-value=0.003; late-pregnancy SBP, p-value=0.003; early-pregnancy DBP, p-value=0.522; mid-pregnancy DBP, p-value<0.001; late-pregnancy DBP, p-value=0.081). [‡]Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per DASH Quartile. Groups are compared to women with the lowest DASH dietary score (Quartile 4) as reference. Estimates are from multiple imputed data. [§]Models are adjusted for gestational age at intake. ^{||}Models are adjusted for maternal age, educational level, parity, prepregnancy BMI, smoking habits, alcohol use, folic acid use, total energy intake and gestational age at time of the measurements. ^{*}Estimates were based on multiple linear regression models with DASH dietary score as SDS.

Supplementary Table S10. Sensitivity analysis: associations of DASH score with placental vascular resistance in participants without pre-existent or gestational diabetes (n=3,378)

DASH	Absolute values and differences in UmPI ^{††}			Absolute values and differences in URRI ^{††}			Bilateral notching ^{†††}
	Mid-pregnancy n=2,060	Late-pregnancy n=2,751	Mid-pregnancy n=1,884	Late-pregnancy n=2,505	Mid-pregnancy n=1,884	Late-pregnancy n=2,505	
Quartile 1	Absolute mean value (sd) [†]	1.201 (0.181)	0.999 (0.177)	0.536 (0.091)	0.490 (0.076)	0.490 (0.076)	n.a.
	Basic model ^{†§}	0.026 [†] (0.006, 0.046)	0.036 [†] (0.019, 0.053)	0.000 (-0.11, 0.012)	0.010 [†] (0.001, 0.020)	0.010 [†] (0.001, 0.020)	1.05 (0.52, 2.37)
	Confounder model [†]	0.011 (-0.010, 0.032)	0.024 [†] (0.006, 0.043)	-0.003 (-0.015, 0.010)	0.009 (-0.001, 0.019)	0.009 (-0.001, 0.019)	1.12 (0.51, 2.45)
Quartile 2	Absolute mean value (sd) [†]	1.199 (0.184)	0.969 (0.160)	0.535 (0.090)	0.484 (0.076)	0.484 (0.076)	n.a.
	Basic model ^{†§}	0.024 [†] (0.004, 0.044)	0.007 (-0.010, 0.024)	0.000 (-0.011, 0.012)	0.005 (-0.005, 0.014)	0.005 (-0.005, 0.014)	0.96 (0.43, 2.13)
	Confounder model [†]	0.019 (-0.001, 0.039)	0.002 (-0.016, 0.019)	0.000 (-0.011, 0.011)	0.005 (-0.004, 0.014)	0.005 (-0.004, 0.014)	0.95 (0.43, 2.11)
Quartile 3	Absolute mean value (sd) [†]	1.180 (0.186)	0.978 (0.163)	0.535 (0.089)	0.480 (0.081)	0.480 (0.081)	n.a.
	Basic model ^{†§}	0.005 (-0.014, 0.025)	0.015 (-0.002, 0.032)	0.000 (-0.011, 0.012)	0.000 (-0.009, 0.010)	0.000 (-0.009, 0.010)	0.81 (0.36, 1.84)
	Confounder model [†]	0.008 (-0.011, 0.27)	0.015 (-0.002, 0.032)	0.000 (-0.012, 0.011)	0.000 (-0.010, 0.009)	0.000 (-0.010, 0.009)	0.83 (0.37, 1.89)
Quartile 4	Absolute mean value (sd) [†]	1.174 (0.182)	0.962 (0.162)	0.535 (0.088)	0.479 (0.077)	0.479 (0.077)	n.a.
	Basic model ^{†§}	Reference	Reference	Reference	Reference	Reference	Reference
	Confounder model [†]	Reference	Reference	Reference	Reference	Reference	Reference
SDS [*]	Basic model [†]	-0.012 [†] (-0.019, -0.005)	-0.013 [†] (-0.019, -0.006)	0.001 (-0.004, 0.005)	-0.003 (-0.007, 0.000)	-0.003 (-0.007, 0.000)	1.01 (0.76, 1.35)
	Confounder model ^{†§}	-0.006 (-0.014, 0.001)	-0.008 [†] (-0.015, -0.001)	0.002 (-0.003, 0.006)	-0.003 (-0.006, 0.001)	-0.003 (-0.006, 0.001)	1.01 (0.76, 1.36)

UmPI, umbilical artery pulsatility index. URRI, umbilical artery resistance index. DASH, Dietary Approaches to Stop Hypertension. [†]P-value<0.05. ^{††}Values are mean values (sd) and reflect the absolute value in UmPI and URRI per DASH Quartile. P-values for comparison of absolute values among the four DASH quartiles were obtained by ANOVA (mid-pregnancy UmPI, p-value=0.016; late-pregnancy UmPI, p-value<0.001; mid-pregnancy URRI, p-value=1.000; late-pregnancy URRI, p-value=0.108). ^{†††}Values for UmPI and URRI are regression coefficients (95% confidence interval) and reflect the difference in UmPI and URRI per DASH Quartile. Values for bilateral notching are odds ratios (95% confidence interval) that reflect difference in risks of bilateral notching per DASH quartile. Groups are compared to women with the lowest DASH dietary score (Quartile 4) as reference. Estimates are from multiple imputed data. [§]Models are adjusted for gestational age at intake. ^{||}Models for UmPI and URRI are adjusted for maternal age, educational level, parity, prepregnancy BMI, smoking habits, alcohol use, folic acid use, total energy intake and gestational age at time of the measurements. Models for bilateral notching are adjusted for parity, prepregnancy BMI, folic acid use and gestational age at time of measurement. ^{*}Estimates were based on multiple linear regression models with DASH dietary score as SDS for UmPI and URRI; and on multiple logistic regression models with DASH dietary score as SDS for bilateral notching.

Supplementary Table S11. Sensitivity analysis: associations of maternal DASH score the risks of gestational hypertensive disorder in participants without pre-existent or gestational diabetes (n=3,378)*

		Gestational hypertensive disorders	Gestational hypertension	Preeclampsia	
		Odds ratio (95% CI) n _{cases} =224	Odds ratio (95% CI) n _{cases} =166	Odds ratio (95% CI) n _{cases} =224	
DASH	Quartile 1	Basic model [†]	1.31 (0.91, 1.88)	1.21 (0.80, 1.84)	1.63 (0.80, 3.33)
		Confounder model [‡] n _{cases} =68	1.15 (0.79, 1.69) n _{cases} =49	1.04 (0.67, 1.61) n _{cases} =19	1.50 (0.72, 3.16) n _{cases} =19
Quartile 2	Basic model [†]	0.89 (0.60, 1.34)	1.00 (0.64, 1.55)	0.54 (0.20, 1.41)	
	Confounder model [‡] n _{cases} =45	0.79 (0.53, 1.20) n _{cases} =39	0.87 (0.55, 1.37) n _{cases} =6	0.50 (0.19, 1.33) n _{cases} =6	
Quartile 3	Basic model [†]	1.01 (0.69, 1.48)	0.81 (0.51, 1.28)	1.71 (0.84, 3.45)	
	Confounder model [‡] n _{cases} =53	0.96 (0.65, 1.43) n _{cases} =33	0.74 (0.46, 1.18) n _{cases} =20	1.78 (0.87, 3.62) n _{cases} =20	
Quartile 4	Basic model [†]	Reference	Reference	Reference	
	Confounder model [‡] n _{cases} =58	Reference n _{cases} =45	Reference n _{cases} =13	Reference n _{cases} =13	
SDS [§]	Basic model [†]	0.91 (0.79, 1.04)	0.91 (0.78, 1.06)	0.90 (0.69, 1.16)	
	Confounder model [‡]	0.96 (0.83, 1.11)	0.96 (0.82, 1.14)	0.93 (0.71, 1.22)	

DASH, Dietary Approaches to Stop Hypertension. CI, Confidence Interval. GHD, Gestational hypertensive disorders. GH, Gestational Hypertension. PE, Preeclampsia. *Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorders, gestational hypertension and preeclampsia per DASH Quartile. Groups are compared to women with the lowest DASH dietary score (Quartile 1) as reference. Estimates are from multiple imputed data. [†]Models are adjusted for gestational age at intake. [‡]Models are adjusted for parity, prepregnancy BMI, folic acid use and gestational age at time of intake. [§]Estimates were based on multiple logistic regression models with DASH dietary score as SDS.

Supplementary Table S12. Sensitivity analysis: associations of maternal DASH score with systolic and diastolic blood pressure in early, mid and late-pregnancy in participants without heart condition or hypercholesterolemia (n=3,356)*

		Absolute values and differences in systolic blood pressure (mmHg)		
DASH		Early-pregnancy [†] n=2,789	Mid-pregnancy [†] n=3,246	Late-pregnancy [†] n=3,265
Quartile 1	Absolute mean value (sd) [†]	117.77 (11.84)	119.56 (12.05)	121.25 (12.17)
	Basic model ^{‡,§}	1.09 (-0.14, 2.33)	2.07 [*] (0.95, 3.18)	1.73 [*] (0.64, 2.82)
	Confounder model ^{‡,}	-0.44 (-1.68, 0.81) n=688	0.12 (-1.03, 1.26) n=809	-0.20 (-1.33, 0.93) n=808
Quartile 2	Absolute mean value (sd) [†]	117.44 (12.61)	118.82 (12.21)	121.04 (11.75)
	Basic model ^{‡,§}	0.75 (-0.50, 2.00)	1.30 [*] (0.17, 2.43)	1.51 [*] (0.41, 2.61)
	Confounder model ^{‡,}	-0.35 (-1.54, 0.83) n=655	0.06 (-1.02, 1.14) n=762	0.39 (-0.67, 1.45) n=771
Quartile 3	Absolute mean value (sd) [†]	117.26 (12.29)	118.02 (11.68)	119.68 (10.95)
	Basic model ^{‡,§}	0.61 (-0.62, 1.84)	0.52 (-0.60, 1.64)	0.15 (-0.94, 1.25)
	Confounder model ^{‡,}	-0.01 (-1.16, 1.15) n=696	-0.11 (-1.17, 0.94) n=795	-0.36 (1.40, 0.68) n=802
Quartile 4	Absolute mean value (sd) [†]	116.63 (11.02)	117.45 (10.93)	119.51 (10.87)
	Basic model ^{‡,§}	Reference	Reference	Reference
	Confounder model ^{‡,}	Reference n=750	Reference n=880	Reference n=884
SDS [*]	Basic model [‡]	-0.38 (-0.82, 0.06)	-0.79 [*] (-1.19, -0.39)	-0.67 [*] (-1.07, -0.28)
	Confounder model [‡]	0.23 (-0.23, 0.68)	-0.03 (-0.45, 0.39)	0.10 (-0.31, 0.51)
		Absolute values and differences in diastolic blood pressure (mmHg)		
DASH		Early-pregnancy [†] n=2,789	Mid-pregnancy [†] n=3,245	Late-pregnancy [†] n=3,264
Quartile 1	Absolute mean value (sd) [†]	68.70 (9.01)	68.27 (9.80)	69.88 (9.62)
	Basic model ^{‡,§}	0.56 (-0.39, 1.50)	2.20 [*] (1.32, 3.09)	1.03 [*] (0.15, 1.90)
	Confounder model ^{‡,}	0.02 (-0.94, 0.97) n=688	1.28 [*] (0.37, 2.18) n=808	0.01 (-0.88, 0.90) n=808
Quartile 2	Absolute mean value (sd) [†]	68.61 (10.08)	67.67 (9.76)	69.53 (9.26)
	Basic model ^{‡,§}	0.45 (-0.51, 1.41)	1.60 [*] (0.70, 2.50)	0.67 (-0.21, 1.56)
	Confounder model ^{‡,}	-0.15 (-1.06, 0.76) n=655	0.84 [*] (-0.01, 1.69) n=762	-0.10 (-0.94, 0.73) n=770
Quartile 3	Absolute mean value (sd) [†]	68.32 (9.03)	66.80 (8.90)	69.03 (8.75)
	Basic model ^{‡,§}	0.20 (-0.74, 1.15)	0.73 (-0.16, 1.62)	1.18 (-0.70, 1.05)
	Confounder model ^{‡,}	-0.23 (-1.11, 0.66) n=696	0.29 (-0.54, 1.11) n=795	-0.22 (-1.03, 0.59) n=802
Quartile 4	Absolute mean value (sd) [†]	68.10 (8.57)	66.06 (8.55)	68.85 (9.01)
	Basic model ^{‡,§}	Reference	Reference	Reference
	Confounder model ^{‡,}	Reference n=750	Reference n=880	Reference n=884
SDS [*]	Basic model [‡]	-0.21 (-0.54, 0.13)	-0.80 [*] (-1.11, -0.48)	-0.43 [*] (-0.74, -0.12)
	Confounder model [‡]	0.01 (-0.34, 0.36)	-0.43 [*] (-0.77, -0.10)	-0.02 (-0.35, 0.30)

DASH, Dietary Approaches to Stop Hypertension. Sd, standard deviation. SBP, systolic blood pressure. DBP, diastolic blood pressure. *P-value<0.05. [†]Values are mean blood pressure values (sd) and reflect the absolute value in SBP and DBP per DASH Quartile. P-values for comparison of absolute values among the four DASH quartiles were obtained by ANOVA (early-pregnancy SBP, p-value=0.324; mid-pregnancy SBP, p-value=0.001; late-pregnancy SBP, p-value=0.002; early-pregnancy DBP, p-value=0.324; mid-pregnancy DBP, p-value=0.001; late-pregnancy DBP, p-value=0.002). [‡]Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per DASH Quartile. Groups are compared to women with the lowest DASH dietary score (Quartile 4) as reference. Estimates are from multiple imputed data. [§]Models are adjusted for gestational age at intake. ^{||}Models are adjusted for maternal age, educational level, parity, prepregnancy BMI, smoking habits, alcohol use, folic acid use, total energy intake and gestational age at time of the measurements. *Estimates were based on multiple linear regression models with DASH dietary score as SDS.

Supplementary Table S13. Sensitivity analysis: associations of DASH score with placental vascular resistance in participants heart condition or hypercholesterolemia (n=3,356)

DASH	Absolute values and differences in UmPI ^{†,‡}				Absolute values and differences in UHR ^{†,‡}			
	Mid-pregnancy n=2,482	Late-pregnancy n=2,729	Mid-pregnancy n=1,864	Late-pregnancy n=2,042	Mid-pregnancy n=1,864	Late-pregnancy n=2,042	Mid-pregnancy n=1,864	Late-pregnancy n=2,042
Quartile 1	Absolute mean value (sd) [†]	1.201 (0.182)	1.000 (0.178)	0.535 (0.091)	0.490 (0.076)	n.a.		
	Basic model ^{‡,§}	0.026 [*] (0.006, 0.047)	0.037 [*] (0.020, 0.054)	0.001 (-0.011, 0.012)	0.010 [*] (0.001, 0.020)	1.17 (0.54, 2.56)		
	Confounder model ^{‡,}	0.011 (-0.010, 0.033)	0.025 [*] (0.007, 0.044)	-0.002 (-0.014, 0.010)	0.010 (0.000, 0.020)	1.2 (0.454, 2.67)	n _{cases} =13	
Quartile 2	Absolute mean value (sd) [†]	1.198 (0.184)	0.970 (0.160)	0.535 (0.089)	0.484 (0.076)	n.a.		
	Basic model ^{‡,§}	0.024 [*] (0.004, 0.044)	0.006 (-0.011, 0.024)	0.001 (-0.011, 0.012)	0.005 (-0.005, 0.014)	1.01 (0.45, 2.28)		
	Confounder model ^{‡,}	0.019 (-0.001, 0.039)	0.002 (-0.016, 0.019)	0.001 (-0.011, 0.012)	0.006 (-0.004, 0.015)	0.88 (0.39, 2.03)	n _{cases} =11	
Quartile 3	Absolute mean value (sd) [†]	1.180 (0.186)	0.975 (0.163)	0.534 (0.089)	0.480 (0.081)	n.a.		
	Basic model ^{‡,§}	0.006 (-0.014, 0.026)	0.012 (-0.005, 0.029)	0.000 (-0.011, 0.011)	0.000 (-0.009, 0.010)	0.86 (0.37, 1.98)		
	Confounder model ^{‡,}	0.009 (-0.011, 0.028)	0.012 (-0.005, 0.029)	-0.001 (-0.012, 0.010)	0.000 (-0.009, 0.009)	0.87 (0.38, 2.01)	n _{cases} =10	
Quartile 4	Absolute mean value (sd) [†]	1.174 (0.182)	0.963 (0.162)	0.535 (0.088)	0.479 (0.077)	n.a.		
	Basic model ^{‡,§}	Reference	Reference	Reference	Reference	Reference		
	Confounder model ^{‡,}	Reference	Reference	Reference	Reference	Reference		
SDS [*]	Basic model [‡]	-0.012 [*] (-0.019, -0.005)	-0.013 [*] (-0.019, -0.007)	0.000 (-0.004, 0.004)	-0.003 (-0.007, 0.000)	0.97 (0.74, 1.33)	n _{cases} =13	
	Confounder model [‡]	-0.007 (-0.014, 0.001)	-0.008 [*] (-0.015, -0.001)	0.001 (-0.003, 0.006)	-0.003 (-0.007, 0.001)	0.99 (0.74, 1.33)		

UmPI, umbilical artery pulsatility index. UHR, umbilical artery resistance index. DASH, Dietary Approaches to Stop Hypertension. Sd, standard deviation. *P-value<0.05. †Values are mean values (sd) and reflect the absolute value in UmPI and UHR per DASH Quartile. P-values for comparison of absolute values among the four DASH quartiles were obtained by ANOVA (mid-pregnancy UmPI, p-value=0.019; late-pregnancy UmPI, p-value<0.001; mid-pregnancy UHR, p-value=0.998; late-pregnancy UHR, p-value=0.101). ‡Values for UmPI and UHR are regression coefficients (95% confidence interval) and reflect the difference in UmPI and UHR per DASH Quartile. Values for bilateral notching are odds ratios (95% confidence interval) that reflect difference in risks of bilateral notching per DASH Quartile. Groups are compared to women with the lowest DASH dietary score (Quartile 4) as reference. Estimates are from multiple imputed data. §Models are adjusted for gestational age at intake. ||Models for UmPI and UHR are adjusted for maternal age, educational level, parity, prepregnancy BMI, smoking habits, alcohol use, folic acid use, total energy intake and gestational age at time of measurement. Models for bilateral notching are adjusted for parity, prepregnancy BMI, folic acid use and gestational age at time of measurement. Estimates were based on multiple linear regression models with DASH dietary score as SDS for UmPI and UHR; and on multiple logistic regression models with DASH dietary score as SDS for bilateral notching.

Supplementary Table S14. Sensitivity analysis: associations of maternal DASH score the risks of gestational hypertensive disorder in participants without heart condition or hypercholesterolemia (n=3,356)*

		Gestational hypertensive disorders	Gestational hypertension	Preeclampsia
DASH		Odds ratio (95% CI) n _{cases} =227	Odds ratio (95% CI) n _{cases} =167	Odds ratio (95% CI) n _{cases} =59
Quartile 1	Basic model [†]	1.32 (0.92, 1.90)	1.23 (0.81, 1.86)	1.63 (0.80, 3.32)
	Confounder model [‡]	1.17 (0.80, 1.71) n _{cases} =69	1.06 (0.69, 1.64) n _{cases} =50	1.49 (0.71, 3.12) n _{cases} =19
Quartile 2	Basic model [†]	0.95 (0.64, 1.41)	1.04 (0.68, 1.61)	0.62 (0.25, 1.57)
	Confounder model [‡]	0.83 (0.55, 1.25) n _{cases} =48	0.90 (0.58, 1.41) n _{cases} =41	0.57 (0.22, 1.44) n _{cases} =7
Quartile 3	Basic model [†]	0.98 (0.67, 1.45)	0.78 (0.49, 1.24)	1.70 (0.84, 3.44)
	Confounder model [‡]	0.93 (0.63, 1.38) n _{cases} =52	0.70 (0.44, 1.13) n _{cases} =32	1.74 (0.85, 3.55) n _{cases} =20
Quartile 4	Basic model [†]	Reference	Reference	Reference
	Confounder model [‡]	Reference n _{cases} =58	Reference n _{cases} =45	Reference n _{cases} =13
SDS [§]	Basic model [†]	0.89 (0.78, 1.02)	0.89 (0.77, 1.04)	0.90 (0.69, 1.15)
	Confounder model [‡]	0.94 (0.82, 1.09)	0.94 (0.80, 1.11)	0.93 (0.71, 1.22)

DASH, Dietary Approaches to Stop Hypertension. GHD, Gestational hypertensive disorders. GH, Gestational Hypertension. PE, Preeclampsia. *Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorders, gestational hypertension and preeclampsia per DASH Quartile. Groups are compared to women with the lowest DASH dietary score (Quartile 1) as reference. Estimates are from multiple imputed data. [†]Models are adjusted for gestational age at intake. [‡]Models are adjusted for parity, prepregnancy BMI, folic acid use and gestational age at time of intake. [§]Estimates were based on multiple logistic regression models with DASH dietary score as SDS.

Supplementary Table S15. Sensitivity analysis: associations of maternal DASH score with systolic and diastolic blood pressure in early, mid and late-pregnancy in participants enrolled in the first-trimester of pregnancy (n=1,888)

		Absolute values and differences in systolic blood pressure (mmHg)		
DASH		Early-pregnancy [†] n=1,869	Mid-pregnancy [†] n=1,854	Late-pregnancy [†] n=1,842
Quartile 1	Absolute mean value (sd) [†]	118.03 (11.97)	120.14 (11.80)	120.96 (12.16)
	Basic model ^{‡,§}	1.03 (-0.48, 2.55)	2.02 [*] (0.52, 3.51)	0.73 (-0.74, 2.19)
	Confounder model ^{‡,}	-0.10 (-1.64, 1.43) n=464	0.81 (-0.71, 2.32) n=462	-0.52 (-2.02, 0.97) n=457
Quartile 2	Absolute mean value (sd) [†]	117.66 (12.59)	119.47 (12.10)	120.71 (11.95)
	Basic model ^{‡,§}	0.67 (-0.86, 2.19)	1.35 (-0.16, 2.85)	0.47 (-1.00, 1.95)
	Confounder model ^{‡,}	-0.12 (-1.56, 1.32) n=450	0.48 (-0.95, 1.90) n=447	-0.28 (-1.68, 1.13) n=445
Quartile 3	Absolute mean value (sd) [†]	117.67 (12.10)	118.89 (12.12)	119.82 (11.06)
	Basic model ^{‡,§}	0.70 (-0.81, 2.21)	0.75 (-0.74, 2.25)	-0.40 (-1.86, 1.06)
	Confounder model ^{‡,}	-0.07 (-1.45, 1.39) n=462	0.01 (-1.39, 1.42) n=457	-1.06 (-2.44, 0.32) n=456
Quartile 4	Absolute mean value (sd) [†]	116.95 (11.03)	118.15 (10.83)	120.21 (10.56)
	Basic model ^{‡,§}	Reference	Reference	Reference
	Confounder model ^{‡,}	Reference n=493	Reference n=488	Reference n=484
SDS [*]	Basic model [‡]	-0.36 (-0.90, 0.18)	-0.78 [*] (-1.32, -0.25)	-0.35 (-0.87, 0.18)
	Confounder model ^{‡,§}	0.04 (-0.52, 0.60)	-0.35 (-0.90, 0.21)	0.11 (-0.44, 0.65)
		Absolute values and differences in diastolic blood pressure (mmHg)		
DASH		Early-pregnancy [†] n=1,869	Mid-pregnancy [†] n=1,853	Late-pregnancy [†] n=1,841
Quartile 1	Absolute mean value (sd) [†]	69.43 (9.31)	68.60 (9.75)	69.89 (9.53)
	Basic model ^{‡,§}	1.03 (-0.15, 2.21)	2.36 [*] (1.17, 3.54)	0.44 (-0.73, 1.60)
	Confounder model ^{‡,}	0.80 (-0.38, 1.97) n=464	1.87 [*] (0.68, 3.07) n=461	-0.20 (-1.35, 0.95) n=457
Quartile 2	Absolute mean value (sd) [†]	69.15 (10.06)	68.06 (9.90)	69.59 (9.27)
	Basic model ^{‡,§}	0.76 (-0.43, 1.94)	1.81 [*] (0.62, 3.01)	0.14 (-1.04, 1.31)
	Confounder model ^{‡,}	0.35 (-0.76, 1.45) n=450	1.28 [*] (0.16, 2.40) n=447	-0.47 (-1.55, 0.62) n=444
Quartile 3	Absolute mean value (sd) [†]	68.88 (9.23)	67.15 (9.17)	69.20 (8.98)
	Basic model ^{‡,§}	0.48 (-0.69, 1.66)	0.88 (-0.31, 2.07)	-0.24 (-1.41, 0.93)
	Confounder model ^{‡,}	-0.08 (-1.17, 1.01) n=462	0.30 (-0.81, 1.40) n=457	-0.86 (-1.92, 0.21) n=456
Quartile 4	Absolute mean value (sd) [†]	68.40 (8.42)	66.29 (8.39)	69.43 (8.74)
	Basic model ^{‡,§}	Reference	Reference	Reference
	Confounder model ^{‡,}	Reference n=493	Reference n=488	Reference n=484
SDS [*]	Basic model [‡]	-0.39 (-0.81, 0.03)	-0.80 [*] (-1.23, -0.38)	-0.24 (-0.66, 0.18)
	Confounder model ^{‡,§}	-0.32 (-0.75, 0.11)	-0.63 [*] (-1.07, -0.19)	-0.01 (-0.43, 0.41)

DASH, Dietary Approaches to Stop Hypertension. Sd, standard deviation. SBP, systolic blood pressure. DBP, diastolic blood pressure. *P-value<0.05. [†]Values are mean blood pressure values (sd) and reflect the absolute value in SBP and DBP per DASH Quartile. P-values for comparison of absolute values among the four DASH quartiles were obtained by ANOVA (early-pregnancy SBP, p-value=0.553; mid-pregnancy SBP, p-value=0.059; late-pregnancy SBP, p-value=0.435; early-pregnancy DBP, p-value=0.359; mid-pregnancy DBP, p-value=0.001; late-pregnancy DBP, p-value=0.716). [‡]Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per DASH Quartile. Groups are compared to women with the lowest DASH dietary score (Quartile 4) as reference. Estimates are from multiple imputed data. [§]Models are adjusted for gestational age at intake. ^{||}Models are adjusted for maternal age, educational level, parity, prepregnancy BMI, smoking habits, alcohol use, folic acid use, total energy intake and gestational age at time of the measurements. ^{*}Estimates were based on multiple linear regression models with DASH dietary score as SDS.

Supplementary Table S17. Sensitivity analysis: associations of maternal DASH score the risks of gestational hypertensive disorder in participants enrolled in the first-trimester of pregnancy (n=1,888)*

DASH		Gestational hypertensive disorders	Gestational hypertension	Preeclampsia
		Odds ratio (95% CI) n _{cases} =124	Odds ratio (95% CI) n _{cases} =96	Odds ratio (95% CI) n _{cases} =28
Quartile 1	Basic model [†]	1.22 (0.75, 1.99)	1.03 (0.60, 1.78)	2.29 (0.79, 6.66)
	Confounder model [‡]	1.10 (0.66, 1.82) n _{cases} =39	0.91 (0.51, 1.61) n _{cases} =28	2.04 (0.68, 6.11) n _{cases} =11
Quartile 2	Basic model [†]	0.75 (0.44, 1.30)	0.85 (0.48, 1.50)	0.21 (0.02, 1.80)
	Confounder model [‡]	0.65 (0.37, 1.14) n _{cases} =24	0.72 (0.40, 1.30) n _{cases} =23	0.19 (0.02, 1.65) n _{cases} =1
Quartile 3	Basic model [†]	0.88 (0.52, 1.48)	0.63 (0.34, 1.16)	2.28 (0.78, 6.61)
	Confounder model [‡]	0.76 (0.44, 1.29) n _{cases} =28	0.51 (0.27, 0.98)* n _{cases} =17	2.16 (0.74, 6.30) n _{cases} =11
Quartile 4	Basic model [†]	Reference	Reference	Reference
	Confounder model [‡]	Reference n _{cases} =33	Reference n _{cases} =28	Reference n _{cases} =5
SDS [§]	Basic model [†]	0.93 (0.77, 1.12)	0.96 (0.78, 1.18)	0.82 (0.57, 1.20)
	Confounder model [‡]	0.96 (0.79, 1.17)	1.00 (0.80, 1.25)	0.86 (0.58, 1.27)

DASH, Dietary Approaches to Stop Hypertension. CI, Confidence Interval. GHD, Gestational hypertensive disorders. GH, Gestational Hypertension. PE, Preeclampsia. *Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorders, gestational hypertension and preeclampsia per DASH Quartile. Groups are compared to women with the lowest DASH dietary score (Quartile 1) as reference. Estimates are from multiple imputed data. [†]Models are adjusted for gestational age at intake. [‡]Models are adjusted for parity, prepregnancy BMI, folic acid use, and gestational age at time of intake. [§]Estimates were based on multiple logistic regression models with DASH dietary score as SDS.

Supplementary Table S18. Associations of maternal DASH score with the risks of gestational hypertensive disorder with adjustment for propensity score (1,780) *

DASH	Bilateral notching	Gestational hypertensive disorders	Gestational hypertension	Preeclampsia
	Late-pregnancy n _{cases} =48	Odds ratio (95% CI) n _{cases} =232	Odds ratio (95% CI) n _{cases} =173	Odds ratio (95% CI) n _{cases} =59
Quartile 1	1.18 (0.49, 2.83) n _{cases} =13	1.15 (0.75, 1.75) n _{cases} =70	0.96 (0.59, 1.57) n _{cases} =51	1.89 (0.84, 4.23) n _{cases} =19
Quartile 4	Reference n _{cases} =14	Reference n _{cases} =59	Reference n _{cases} =46	Reference n _{cases} =13
Propensity score	1.46 (0.25, 8.49)	0.61 (0.27, 1.36)	0.42 (0.17, 1.05)	1.85 (0.39, 8.68)

DASH, Dietary Approaches to Stop Hypertension. CI, Confidence Interval. GHD, Gestational hypertensive disorders. GH, Gestational Hypertension. PE, Preeclampsia. *Values are odds ratios (95% confidence interval) that reflect difference in risks of bilateral uterine artery notching, gestational hypertensive disorders, gestational hypertension and preeclampsia per DASH quartile. DASH score quartile 1 is compared to DASH score quartile 4 as a reference category. Estimates are from multiple imputed data. Models are adjusted for propensity scores that were calculated using a logistic regression model to predict the likelihood of having a DASH score in quartile 1 rather than quartile 4.

CHAPTER

2.2

Associations of dietary glycemic index and load with blood pressure, placental hemodynamic parameters and gestational hypertensive disorders

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PURPOSE The aim of this study was to examine the associations of dietary glycemic index and load with maternal blood pressure, placental hemodynamic parameters and the risk of gestational hypertensive disorders.

METHODS In a population-based cohort among 3,378 pregnant Dutch women, dietary glycemic index and load were assessed from food frequency questionnaires at median 13.4 (95% range 9.9-22.9) weeks gestation. Blood pressure was measured in early, mid and late-pregnancy. Placental hemodynamic parameters were measured in mid and late-pregnancy by ultrasound. Data on gestational hypertensive disorders was acquired from medical records.

RESULTS Mean dietary glycemic index (SD) was 58 (3) and mean dietary glycemic load (SD) was 155 (47). Dietary glycemic index was not associated with blood pressure, placental hemodynamic parameters and the risk of gestational hypertensive disorders. Higher dietary glycemic load SDS was associated with a higher diastolic blood pressure in early-pregnancy, remaining after adjustment for socio-demographic and lifestyle factors ((0.98 (95% CI 0.35-1.61) mmHg per SDS increase in glycemic load). No significant associations of dietary glycemic index and load quartiles with longitudinal blood pressure patterns from early to late-pregnancy were present. No associations of glycemic load with blood pressure or placental hemodynamic parameters and the risk of gestational hypertensive disorders were present.

CONCLUSIONS Within this low-risk pregnant population, we did not find consistent associations of dietary glycemic index and load with maternal blood pressure, placental hemodynamic parameters and the risk of gestational hypertensive disorders. Further studies need to assess whether the effects on gestational hemodynamic adaptations are more pronounced among high-risk women with an impaired glucose metabolism.

INTRODUCTION

Gestational hypertensive disorders affect up to 10% of pregnancies and are a major risk factor for maternal and neonatal morbidity and mortality¹. Women with a medical history of gestational hypertensive disorders are at increased risk of chronic hypertension and cardiovascular disease in later life². In non-pregnant populations, the quality and quantity of carbohydrate intake seem to influence blood pressure and other cardiovascular risk factors, including body weight, impaired lipid metabolism and insulin resistance³⁻⁵. The glycemic index and load are commonly used dietary measures to qualify carbohydrate intake, and provide information on the postprandial glycemic response to carbohydrate containing food products^{6, 7}. A low-glycemic index diet can be achieved by consuming carbohydrate containing food products that are less likely to increase blood sugar levels referred to as low-glycemic index products, while avoiding products with a high-glycemic index. For a low-glycemic load diet the daily quantity of carbohydrates is additionally taken into account. A meta-analysis consisting of 14 intervention studies comprising 1,097 healthy non-pregnant individuals with a mean age ranging from 28-54 years, showed that a daily glycemic index reduction of 10 units lowered systolic and diastolic blood pressure by 1.1 mmHg and 1.3 mmHg, respectively³. This meta-analysis also showed that a daily glycemic load reduction of 28 units lowered systolic and diastolic blood pressure by 2.0 mmHg³.

During pregnancy, replacing high-glycemic index products by lower glycemic index products may also have favorable effects on pregnancy outcomes, especially among women at increased risk of an impaired glucose metabolism⁸. A low-glycemic index diet during pregnancy is suggested to have beneficial effects on glucose metabolism, lipid profile, gestational weight gain and the risk of delivering a large-for-gestational-age-infant⁸⁻¹⁶. Dietary glycemic index and load have a direct effect on postprandial glucose levels. Higher glucose levels during pregnancy can impair endothelial function through oxidative stress and vascular inflammation, with elevated blood pressure and impaired placental function as a possible result predisposing to an increased risk of gestational hypertensive disorders¹⁷⁻¹⁹. It has already been shown that higher glucose levels are associated with a higher risk of gestational hypertensive disorders²⁰. However, not much is known about the effects of low-glycemic index and load diets on gestational hemodynamic adaptations and the risk of gestational hypertensive disorders. A case-control study in Iran among 202 pregnant women, showed that a daily dietary glycemic load above the median was associated with an increased risk of gestational hypertension²¹. Likewise, an intervention study in Italy among 370 overweight pregnant women found a lower incidence

of gestational hypertension among women who were prescribed a low-glycemic index diet²². No previous studies have examined the influence of low-glycemic index and load diets on gestational blood pressure and placental hemodynamic adaptations, which are major determinants for the development of gestational hypertensive disorders.

We hypothesized that a lower dietary glycemic index and load during pregnancy positively influence hemodynamic adaptations during pregnancy, leading to a lower risk of gestational hypertensive disorders. Therefore, we examined the associations of dietary glycemic index and load with blood pressure and placental vascular resistance throughout pregnancy and the risks of gestational hypertensive disorders within a population-based cohort study among 3,378 pregnant women.

METHODS

Study design and study sample

The study was embedded in the Generation R study, a population-based prospective cohort from early-pregnancy onwards in Rotterdam, The Netherlands^{23, 24}. In total, 4,096 Dutch women were enrolled during pregnancy. Information on dietary intake was available for 3,558 women. We excluded women with pre-existent hypertension and diabetes, with missing outcome data, and non-singleton live-births ($n=180$). The population for analysis consisted of 3,378 pregnant women (**Figure 1**). This study was performed in accordance with the ethical standard laid down in the Declaration of Helsinki and was approved by the Medical Ethical Committee of the Erasmus Medical Centre in Rotterdam, The Netherlands (MEC 198.782/2001/31). All participating women gave written informed consent prior to their inclusion in the study.

Maternal dietary glycemic index and glycemic load

Semi-quantitative food frequency questionnaires (FFQ) consisting of 293 food items were obtained at study enrollment (median=13.4 weeks of gestation, 95% range 9.9-22.9). The FFQ considered dietary intake of the three months prior and was validated in a subgroup of 83 Dutch women against three non-consecutive 24h dietary recalls, with further confirmation using nutritional biomarkers²⁵. Intraclass correlation coefficients between nutrient intake estimates from the FFQ and from the 24h dietary recalls ranged from 0.47 to 0.77 for macronutrients, and was 0.60 for total carbohydrate intake. We calculated mean dietary glycemic index and load per day as described previously²⁶. We used the dietary glycemic index as primary exposure, as this is most commonly used in

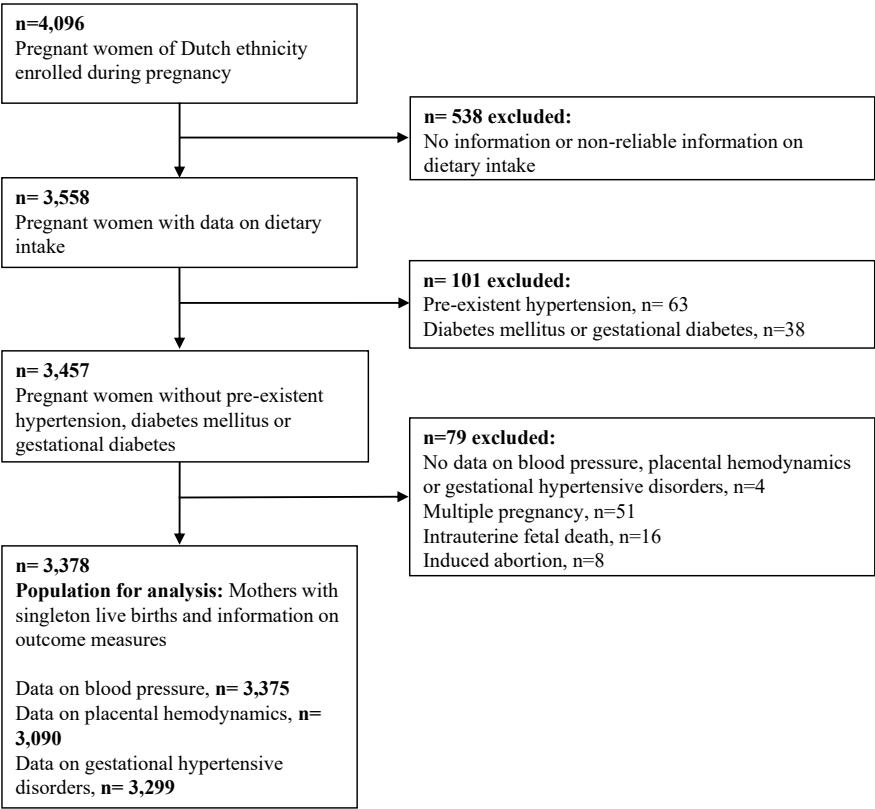


Figure 1. Flowchart of the study population.

clinical and research settings. As the dietary glycemic load additionally takes into account the daily quantity of carbohydrates consumed, it provides additional information on postprandial glucose levels but might also be more sensitive for measurement error^{6, 7, 26}. Glycemic index values were obtained from the glycemic index database on the Dutch diet by the Medical Research Council Human Nutrition Research, and assigned to each individual food item in the FFQ²⁷. This database was developed using a standardized approach of calculating dietary glycemic index and load to facilitate research into the health effect of dietary glycemic index and load²⁷. This approach is used in comparable observational studies that investigated the associations of dietary glycemic index and load with adverse birth and child outcomes^{28, 29}. Mean dietary glycemic index per day was calculated by summing the product of the carbohydrate intake of each food item with its glycemic index, which was then divided by the total amount of carbohydrates consumed per day. The mean dietary glycemic load per day was calculated by summing the product

of the carbohydrate intake of each food item with its glycemic index^{6, 7, 26}. To examine whether associations were restricted to women with a relatively high dietary glycemic index or load within our study population, and to explore whether a linear tendency was present, we constructed quartiles of dietary glycemic index and load for our study population. Since a linear tendency was present, we also constructed standard deviation scores (SDS) of dietary glycemic index and load to assess the continuous associations of dietary glycemic index and load per 1-SDS increase with gestational hemodynamic developmental outcomes. Finally, to increase clinical interpretability, dietary glycemic index per day was categorized into categories using similar cut-offs as used for individual food product: low-glycemic index diet (≤ 55), a normal-glycemic index diet (56-69) and a high-glycemic index diet (≥ 70) as a secondary analysis^{16, 26}. We consider this method in line with studies that recommend a low-glycemic index diet by replacing high-glycemic index food products with low-glycemic index food products as dietary intervention²⁶.

Blood pressure in pregnancy

Systolic blood pressure and diastolic blood pressure were measured in early, mid and late-pregnancy (medians, 95% range=12.9 (9.8-17.2), 20.4 (18.6-23.2), 30.2 (28.6-32.6) weeks gestation, respectively) using an Omron 907 automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe BV, Hoofddorp, The Netherlands)³⁰. The participant was seated in upright position with feet on the floor. The cuff was placed around the non-dominant arm supported at the level of the heart. Blood pressure measurement started after a minimum of 5 minutes at rest. The mean systolic and diastolic blood pressure was calculated of two readings with a 60 seconds interval³¹.

Placental hemodynamic parameters

Placental hemodynamic parameters were measured in mid and late-pregnancy (medians, 95% range=20.5 (18.8-22.9), 30.4 (28.5-32.6) weeks gestation, respectively) using a detailed ultrasonography protocol^{32, 33}. The umbilical artery pulsatility index (UmPI) was measured in a free-floating part of the umbilical cord and the uterine artery resistance index (UtRI) at the crossover with the external iliac artery. Three sequential flow velocity wave forms were recorded with the mean of three Doppler measurements being used for further analysis. Bilateral uterine artery notching was defined as an upturn of the velocity waveform at the beginning of diastole in both uterine arteries³³.

Gestational hypertensive disorders

Information on preeclampsia and gestational hypertension was obtained from medical records and cross checked with the original hospital charts, as described previously^{34, 35}. Gestational hypertension was defined as a systolic blood pressure of at least 140 mmHg and/or diastolic blood pressure of at least 90 mmHg after 20 weeks of gestation in previously normotensive women. These criteria including the manifestation of proteinuria were used to identify preeclampsia³⁶.

Covariates

Data on maternal age, education level, parity, prepregnancy weight, folic acid supplement use, alcohol consumption during pregnancy, smoking during pregnancy, and the diagnosis of pre-existent hypertension, pre-existent diabetes mellitus and gestational diabetes mellitus were collected by questionnaires during pregnancy. Information on dietary factors were obtained from the FFQ. Height was measured at enrolment to calculate the prepregnancy body mass index (BMI).

Statistical power

As previously described, statistical power was calculated based on 7,000 subjects within the Generation R Study²³. For a normally distributed continuous outcome a difference of 0.08 SD is detectable with type I error of 5% and a type 2 error of 20% (power of 80%), if 25% of the study population is exposed. This corresponds with an approximate difference of 0.90 mmHg for systolic and 0.70 mmHg for diastolic blood pressure. For gestational hypertensive disorders an odds ratio of 1.26 is detectable if 25% is exposed.

Statistical analyses

First, we performed a non-response analysis to compare women with information on dietary glycemic index and load to those without to explore whether differences in characteristics between those women are present. Second, we examined the associations of glycemic index and load quartiles with longitudinal blood pressure patterns throughout pregnancy using unbalanced repeated measurement models. These models take into account the correlation of measurements within the same participant and allow for incomplete outcome data³⁷. We constructed the best fitting model using fractional polynomials³⁸. These models can be written as follows: Systolic blood pressure: $\beta_0 + \beta_1 \times \text{GI/GL quartile} + \beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^2 + \beta_4 \times \text{GI/GL quartile} \times \text{gestational age}$. Diastolic blood pressure: $\beta_0 + \beta_1 \times \text{GI/GL quartile} + \beta_2 \times \text{gestational age} +$

$\beta_3 \times \text{gestational age}^{0.5} + \beta_4 \times \text{GI/GL quartile} \times \text{gestational age}$. In these models, ' $\beta_0 + \beta_1 \times \text{GI/GL quartile}$ ' reflects the intercept. The intercept reflects the mean systolic and diastolic blood pressure value for the glycemic index and load categories. ' $\beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^{-2}$ ' reflects the slope of change in systolic blood pressure per week, and ' $\beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^{0.5}$ ', reflects the slope of change in diastolic blood pressure per week. Our term of interest is ' $\beta_4 \times \text{GI/GL quartile} \times \text{gestational age}$ ', which reflects the difference in blood pressure change per week per glycemic index or load quartile, as compared to women in the lowest glycemic index or load quartile. As a second step, we examined the associations of dietary glycemic index and load SDS and quartiles with differences in early, mid and late-pregnancy blood pressure separately using linear regression models to identify potential critical periods in gestational hemodynamic adaptations important from an etiological perspective. Third, we examined the associations of dietary glycemic index and load in SDS and quartiles with differences in umbilical artery pulsatility index and uterine artery resistance index in mid and late-pregnancy using linear regression models and the risk of bilateral uterine artery notching using logistic regression models. Finally, we examined the risk on gestational hypertensive disorders using logistic regression models.

Potential confounding by maternal socio-demographic and lifestyle factors needs to be taken into account as it is well-known that dietary intake is strongly related to these other maternal characteristics. Potential confounders were selected beforehand using a directed acyclic graph (**Supplementary figure S1**). We constructed four different adjustment models as it well-known that dietary exposures are strongly related to socio-demographic, lifestyle and other dietary factors, which may explain potential associations. 1) Basic model, in which we adjusted for gestational age at intake; 2) Socio-demographic model, in which we additionally adjusted for maternal age, educational level and parity; 3) Lifestyle model in which we additionally adjusted for prepregnancy BMI, folic acid use, smoking habits and alcohol use, and total energy intake; 4) Dietary model: in which we additionally adjusted for dietary fiber intake, salt intake and gestational weight gain if we found significant associations in the lifestyle model. These dietary factors are closely linked to dietary glycemic index and load, and may also influence the development of gestational hypertensive disorders. Covariates were included in the models if they were associated with both outcome and exposure ($p\text{-value} < 0.05$ and $> 10\%$ change in effect estimate when added to the univariate model)³⁹.

We conducted four sensitivity analyses: 1) We repeated the analyses for dietary glycemic index using a cut-off to classify diets into a low, normal or high-glycemic index diet; 2) We repeated the analyses restricted to women with a prepregnancy $\text{BMI} \geq 25$, as

they represent a population at higher risk of impaired glucose metabolism who may be more prone to adverse effects of a higher dietary glycemic index and load diet; 3) We repeated the analyses restricted to participants who were enrolled in early-pregnancy (i.e. <14 weeks of gestation) as adherence to a lower dietary glycemic index and load already during preconception and early-pregnancy may have stronger effects on gestational hemodynamic adaptations. 4) We repeated the main analyses among participants with complete data on all covariates (non-imputed data). P-values <0.05 were considered as statistical significant. We used data from multiple imputations to reduce potential bias due to missing values of covariates. We used the Fully Conditional Specifications (FCS) method. In the imputation model all covariates and outcomes were included as predictor variables, and maternal weight and height at enrolment, paternal age and BMI, family income status, gestational age at birth and birth weight were included as additional predictor variables. We created five independent datasets, that were analyzed together and presented the pooled effect estimates. Analysis were performed using IBM Statistical Package of Social Sciences version 25. The analysis for repeated measurements was performed using Statistical Analysis System version 9.4.

RESULTS

Participant characteristics

Table 1 shows that the mean dietary glycemic index (SD) was 57.7 (3.3) and the mean dietary glycemic load (SD) was 154.7 (46.9). A low-glycemic index diet according to the individual food product classification was consumed by 1,059 (31%) pregnant women, whereas only 1 woman consumed a high-glycemic index diet according to the individual food product classification. No consistent differences were present in characteristics between women with information on dietary glycemic index and load to those without this information (**Supplementary Table S1**).

Dietary glycemic index and load with blood pressure throughout pregnancy

Figure 2 shows the longitudinal systolic and diastolic blood pressure patterns throughout pregnancy per dietary glycemic index quartile. Women in the lowest dietary glycemic index quartile had the lowest systolic and diastolic blood pressure throughout pregnancy when compared to the other quartiles, although there were no significant differences in the increase of blood pressure per week present between quartiles (p-values for interaction of dietary glycemic index quartile with gestational age ≥ 0.05). Similarly,

Table 1. Characteristics of the study population by glycemic index quartile (n=3,378)

	Total group n=3,378	Glycemic index Quartile 1 n=844	Glycemic index Quartile 2 n=845	Glycemic index Quartile 3 n=845	Glycemic index Quartile 4 n=844	p-value*
Maternal age at enrollment, years	31.4 (4.4)	32.4 (4.0)	31.6 (4.1)	31.1 (4.4)	30.4 (4.8)	<0.001
Gestational age at intake, weeks	13.5 (5.4, 38.1)	13.8 (10.5, 23.2)	13.6 (10.0, 24.4)	13.5 (9.8, 23.1)	13.5 (9.8, 22.6)	0.19
Parity, n nulliparous	2,019 (59.9)	523 (62.1)	524 (62.0)	500 (59.3)	472 (56.2)	0.04
Prepregnancy BMI, kg/m ²	23.1 (3.8)	22.8 (3.5)	23.0 (3.7)	23.2 (4.0)	23.3 (3.9)	0.04
Prepregnancy BMI ≥25, n	636 (21.8)	134 (18.2)	147 (20.3)	173 (23.7)	182 (25.1)	0.005
Gestational weight gain, kg	10.8 (4.4)	10.6 (4.1)	10.8 (4.5)	10.9 (4.4)	10.7 (4.7)	0.59
Education, n high	1,985 (59.5)	593 (70.8)	527 (62.9)	481 (58.0)	384 (46.3)	<0.001
Glycemic index, per day	57.7 (3.3)	53.8 (1.4)	56.5 (0.6)	58.6 (0.7)	62.1 (1.9)	<0.001
Glycemic load, per day	154.7 (46.9)	132.2 (32.8)	147.7 (38.7)	159.5 (43.5)	179.5 (55.9)	<0.001
Carbohydrate intake, g/d	267.0 (75.1)	245.7 (60.4)	261.5 (68.5)	272.1 (74.1)	288.9 (88.3)	<0.001
Protein intake, g/d	79.1 (19.1)	82.1 (18.1)	80.6 (18.9)	78.8 (19.1)	74.9 (19.6)	<0.001
Fat intake, g/d	86.4 (24.3)	85.5 (23.7)	87.4 (24.0)	87.9 (24.6)	84.8 (24.8)	0.02
Fiber intake, g/d	23.4 (6.9)	25.1 (7.0)	24.1 (6.7)	23.1 (6.6)	21.4 (6.8)	<0.001
Total energy intake, kcal/d	2,146.3 (511.0)	2,061.4 (451.8)	2,137.6 (495.6)	2,180.6 (516.1)	2,205.4 (563.3)	<0.001
Smoking, n continued during pregnancy	531 (17.0)	88 (11.3)	109 (13.9)	135 (17.2)	199 (25.4)	<0.001
Alcohol, n continued during pregnancy	1,559 (50.2)	442 (56.8)	404 (52.1)	369 (47.4)	344 (44.4)	<0.001
Early-pregnancy, ≥1 glass per week	830 (26.9)	256 (30.8)	207 (24.9)	192 (23.1)	175 (21.1)	<0.001
Mid-pregnancy, ≥1 glass per week	378 (12.3)	119 (31.5)	95 (25.1)	96 (25.4)	68 (18.0)	<0.001
Late-pregnancy, ≥1 glass per week	444 (14.7)	142 (32.0)	118 (26.6)	104 (23.4)	80 (18.0)	<0.001
Folic acid supplement use, n/yes	467 (89.1)	642 (90.9)	626 (89.7)	627 (91.3)	572 (84.5)	<0.001
Systolic blood pressure, mmHg						
Early-pregnancy	117.1 (11.8)	116.3 (11.1)	116.9 (11.7)	118.0 (12.1)	117.4 (12.3)	0.05
Mid-pregnancy	118.4 (11.7)	117.0 (11.3)	118.5 (11.2)	119.1 (11.6)	118.9 (12.6)	0.001
Late-pregnancy	120.3 (11.4)	119.1 (10.7)	120.3 (11.2)	120.7 (11.4)	121.0 (12.1)	0.005
Diastolic blood pressure, mmHg						
Early-pregnancy	68.4 (9.2)	68.0 (8.9)	68.2 (8.9)	68.6 (9.7)	68.8 (9.0)	0.30
Mid-pregnancy	67.1 (9.2)	66.4 (8.7)	66.7 (8.6)	67.7 (9.9)	67.7 (9.7)	0.004
Late-pregnancy	69.3 (9.1)	68.7 (9.0)	69.4 (8.7)	69.3 (9.5)	69.7 (9.4)	0.181
Uterine artery resistance index						
Mid-pregnancy	0.535 (0.089)	0.540 (0.093)	0.533 (0.087)	0.533 (0.087)	0.534 (0.088)	0.49
Late-pregnancy	0.483 (0.078)	0.486 (0.082)	0.482 (0.075)	0.481 (0.073)	0.482 (0.080)	0.76
Umbilical artery pulsatility index						
Mid-pregnancy	1.188 (0.183)	1.178 (0.173)	1.194 (0.187)	1.198 (0.184)	1.182 (0.189)	0.16
Late-pregnancy	0.977 (0.166)	0.970 (0.171)	0.971 (0.159)	0.981 (0.161)	0.985 (0.174)	0.22
Late-pregnancy uterine artery notching	48 (2.2)	15 (2.7)	11 (2.0)	6 (1.1)	16 (3.0)	0.147
Gestational hypertensive disorders						
Gestational hypertension	166 (5.1)	38 (4.7)	45 (5.5)	39 (4.8)	44 (5.5)	0.84
Preeclampsia	58 (1.9)	16 (2.0)	11 (1.4)	18 (2.3)	13 (1.7)	0.57

BMI, Body Mass Index. Kg, kilogram. Kcal/d, daily amount in kcal per day. Values are means (SD), median (95% range), or number (valid %). *P-values were obtained by chi-square tests for categorical variables, one-way ANOVA for continuous variables.

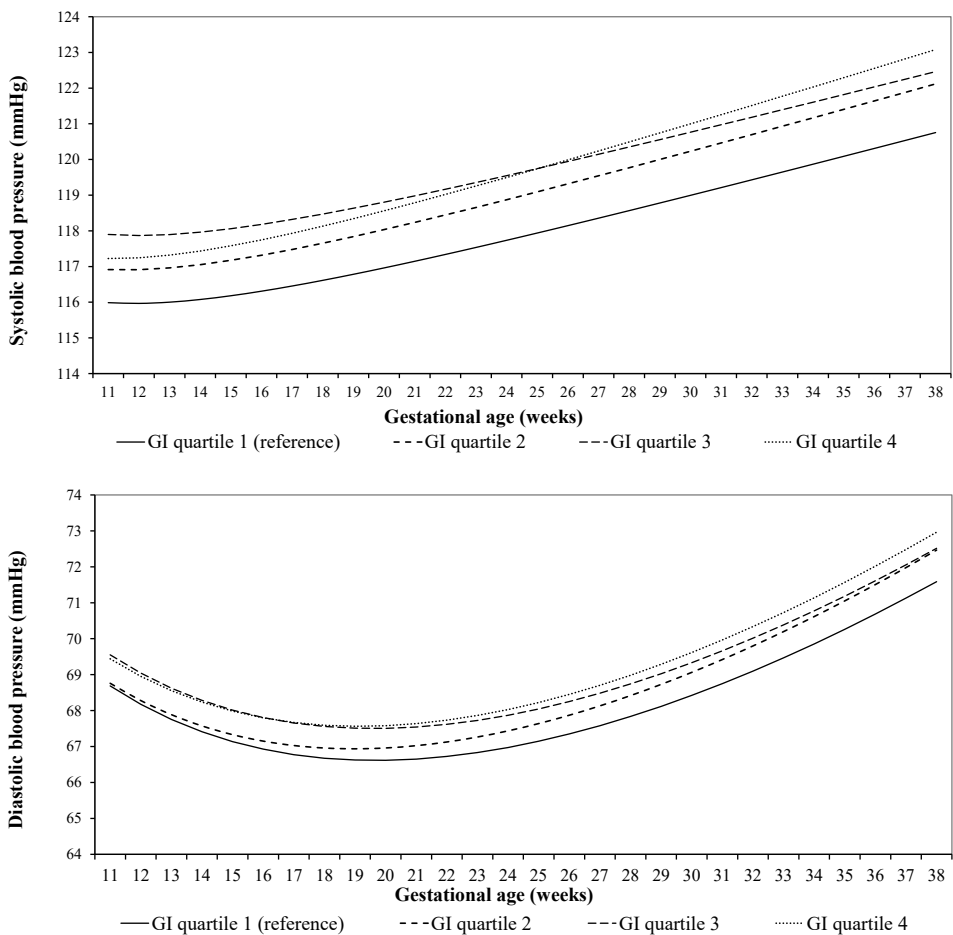


Figure 2. Blood pressure patterns in different glycemic index (GI) quartiles from repeated measurement models. Change in SBP and DBP in mmHg for first quartile, second quartile, third quartile and fourth quartile. $SBP = \beta_0 + \beta_1 \times GI \text{ quartile} + \beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^2 + \beta_4 \times GI \text{ quartile} \times \text{gestational age}$. $DBP = \beta_0 + \beta_1 \times GI \text{ quartile} + \beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^{0.5} + \beta_4 \times GI \text{ quartile} \times \text{gestational age}$. In these models, ' $\beta_0 + \beta_1 \times GI$ ' reflects the intercept and ' $\beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^{0.5}$ ' reflects the slope of change in blood pressure per week for SBP, and ' $\beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^{0.5}$ ' reflects the slope of change in blood pressure per week for DBP. Our term of interest is β_4 , which reflects the difference in change in blood pressure per week per GI category, as compared to women in the lowest GI score quartile. Estimates and p-values from repeated measurement models are given in **Supplementary Table S2**.

no significant associations of dietary glycemic load quartiles with longitudinal blood pressure development throughout pregnancy were present (p-values for interaction of dietary glycemic index quartile with gestational age ≥ 0.05) (**Figure 3**). The regression coefficients for a gestational age-dependent and a gestational age-independent effect for dietary glycemic index and load quartiles are shown in **Supplementary Table S2**.

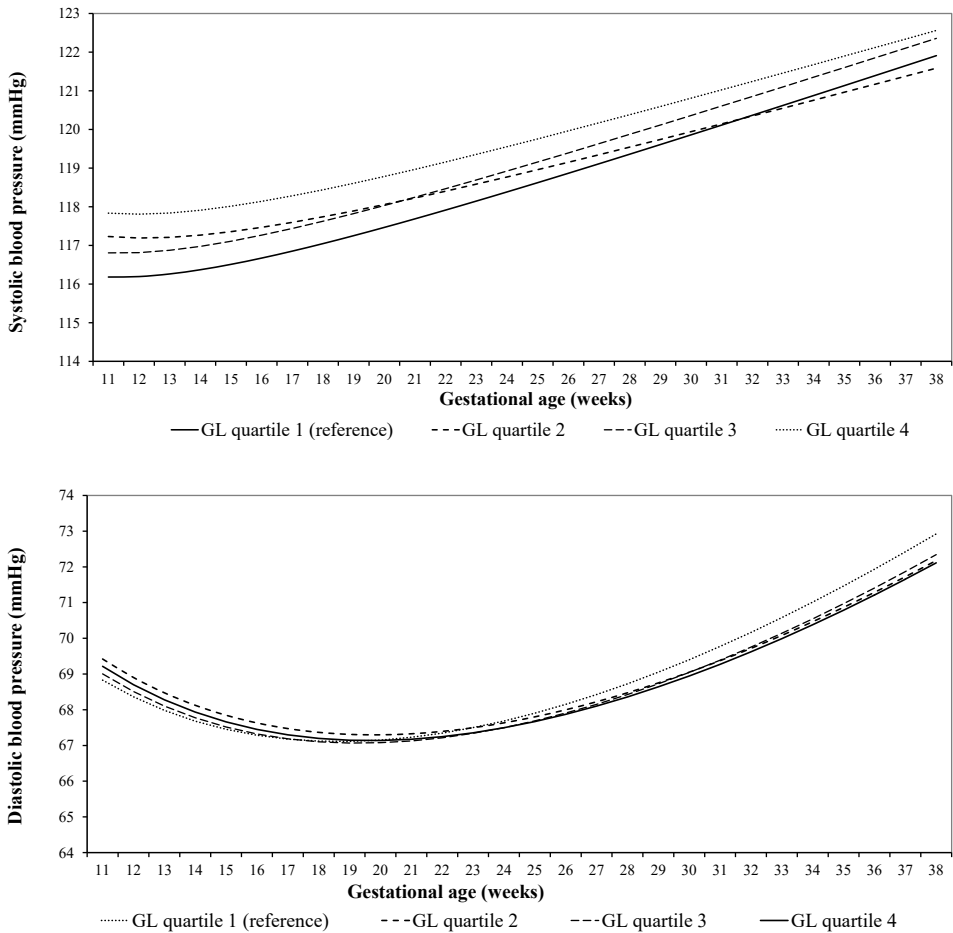


Figure 3. Blood pressure patterns in different glycemic load (GL) quartiles from repeated measurement models. Change in SBP and DBP in mmHg for first quartile, second quartile, third quartile and fourth quartile. $SBP = \beta_0 + \beta_1 \times GL \text{ quartile} + \beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^2 + \beta_4 \times GL \text{ quartile} \times \text{gestational age}$. $DBP = \beta_0 + \beta_1 \times GL \text{ quartile} + \beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^{0.5} + \beta_4 \times GL \text{ quartile} \times \text{gestational age}$. In these models, ' $\beta_0 + \beta_1 \times GL$ ' reflects the intercept and ' $\beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^2$ ' reflects the slope of change in blood pressure per week for SBP, and ' $\beta_2 \times \text{gestational age} + \beta_3 \times \text{gestational age}^{0.5}$ ', reflects the slope of change in blood pressure per week for DBP. Our term of interest is β_4 , which reflects the difference in change in blood pressure per week per GL category, as compared to women in the lowest GL score quartile. Estimates and p-values from repeated measurement models are given in **Supplementary Table S2**.

Table 2 shows that a higher dietary glycemic index and load across the full range were associated with a higher early, mid and late-pregnancy systolic blood pressure in the basic model, but these associations disappeared after adjustment for socio-demographic factors. A higher dietary glycemic load across the full range was associated with a higher early-pregnancy diastolic blood pressure, which persisted after full adjustment for socio-demographic and lifestyle factors (0.98 (95% CI 0.35-1.61) mmHg per SDS increase in

glycemic load). The effect estimate only partly attenuated but remained significant after additional adjustment for gestational weight gain, salt intake and dietary fiber intake (0.84 (95% CI 0.20, 1.50) mmHg per SDS increase in glycemic load). No other associations of dietary glycemic index and load across the full range with diastolic blood pressure were present. Dietary glycemic index and load quartiles were not associated with systolic and diastolic blood pressure in the full models (**Supplementary Table S3A-B**).

Dietary glycemic index and load with placental vascular resistance

Table 3 shows that no consistent associations of dietary glycemic index and load across the full range with UmPI, UtRI and bilateral uterine artery notching were present after considering other maternal socio-demographic and lifestyle characteristics. A higher glycemic load was only associated with a lower uterine artery resistance (p-value<0.05). This association remained present after additional adjustment for dietary factors. No associations of dietary glycemic index and load quartiles with placental hemodynamic parameters were present (**Supplementary Table S4A-B**).

Maternal glycemic index and load and risks of gestational hypertensive disorders

Table 4 shows that dietary glycemic index and load across the full range were not associated with the risk of any gestational hypertensive disorder in the basic or adjusted models. No associations of dietary glycemic index and load quartiles with gestational hypertensive disorders were present (**Supplementary Table S5A-B**).

Sensitivity analyses

No associations were present with blood pressure, placental vascular resistance and gestational hypertensive disorders when we repeated the analyses using clinical cut-offs to classify glycemic index diets (**Supplementary Table 6A-C**). When we restricted our analyses to women with a BMI ≥ 25 , a higher dietary glycemic index across the full range was only associated with a higher late-pregnancy UmPI in all models (p-value<0.05) (**Supplementary Table S7A-C**). When we restricted to women who enrolled in the study before 14 weeks of gestation, no consistent associations with blood pressure, placental hemodynamic parameters and risk of gestational hypertensive disorders were present (**Supplementary Table S8A-C**). When we restricted to women with complete data on all covariates, we observed similar findings as in the main analyses (**Supplementary Table S9A-C**).

Table 2. Associations of dietary glycemic index and glycemic load with systolic and diastolic blood pressure during pregnancy in total population (n=3,375)^a

Glycemic index (SDS)	Differences in systolic blood pressure in mmHg (CI 95%)		
	Early-pregnancy (n=2,802)	Mid-pregnancy (n=3,263)	Late-pregnancy (n=3,286)
Basic models [†]	0.38 (-0.06, 0.82)	0.51 (0.11, 0.91)*	0.58 (0.19, 0.97)*
Socio-demographic models [‡]	0.14 (-0.31, 0.58)	0.20 (-0.20, 0.61)	0.30 (-0.09, 0.70)
Lifestyle models [§]	-0.04 (-0.46, 0.38)	0.03 (-0.36, 0.41)	0.15 (-0.24, 0.53)
Glycemic load (SDS)	Differences in diastolic blood pressure in mmHg (CI 95%)		
	Early-pregnancy (n=2,802)	Mid-pregnancy (n=3,262)	Late-pregnancy (n=3,285)
Basic models [†]	0.31 (-0.03, 0.65)	0.41 (0.09, 0.72)*	0.26 (-0.05, 0.58)
Socio-demographic models [‡]	0.25 (-0.10, 0.60)	0.29 (-0.3, 0.61)	0.18 (-0.14, 0.49)
Lifestyle models [§]	0.21 (-0.12, 0.54)	0.21 (-0.09, 0.52)	0.13 (-0.17, 0.43)
Glycemic load (SDS)	Differences in systolic blood pressure in mmHg (CI 95%)		
	Early-pregnancy (n=2,802)	Mid-pregnancy (n=3,263)	Late-pregnancy (n=3,286)
Basic model [†]	0.81 (0.37, 1.25)*	0.40 (-0.01, 0.80)	0.47 (0.08, 0.86)*
Socio-demographic models [‡]	0.77 (0.33, 1.20)*	0.33 (-0.07, 0.73)	0.42 (0.03, 0.80)*
Lifestyle models [§]	0.23 (-0.59, 1.05)	-0.10 (-0.84, 0.64)	0.05 (-0.68, 0.78)
Glycemic load (SDS)	Differences in diastolic blood pressure in mmHg (CI 95%)		
	Early-pregnancy (n=2,802)	Mid-pregnancy (n=3,262)	Late-pregnancy (n=3,285)
Basic models [†]	0.35 (0.02, 0.69)*	0.06 (-0.26, 0.38)	-0.03 (-0.35, 0.28)
Socio-demographic models [‡]	0.35 (0.01, 0.69)*	0.05 (-0.27, 0.36)	-0.04 (-0.35, 0.27)
Lifestyle models [§]	0.98 (0.35, 1.61)*	0.30 (-0.28, 0.88)	0.27 (-0.31, 0.84)

SDS, standard deviation score. CI, Confidence Interval. Values are regression coefficients (95% confidence interval) from multiple linear regression models and reflect the differences in mmHg blood pressure per one increase in standard deviation score of maternal glycemic index and glycemic load. Estimates are from multiple imputed data. [†]Basic models are adjusted for gestational age at time of intake. [‡]Socio-demographic models are adjusted for maternal age, educational level, parity and gestational age at time of measurements. [§]Lifestyle models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of the measurements. *P-value <0.05.

Table 3. Associations of dietary glycemic index and glycemic load with uterine artery resistance index, umbilical artery pulsatility index and bilateral uterine artery notching in total population (n=3,090)

	Differences in UmPI (95% CI) [*]		Differences in UfRI (95% CI) [*]		Bilateral notching (95% CI) [†] n _{Cases} =48
	Mid-pregnancy n=2,505	Late-pregnancy n=2,751	Mid-pregnancy n=1,884	Late-pregnancy n=2,060	
<u>Glycemic index (SDS)</u>					
Basic models [‡]	-0.001 (-0.008, 0.007)	0.007 (0.000, 0.013)	-0.004 (-0.008, 0.001)	-0.001 (-0.004, 0.003)	1.12 (0.84, 1.49)
Socio-demographic models [§]	-0.002 (-0.009, 0.005)	0.005 (-0.001, 0.011)	-0.004 (-0.008, 0.001)	-0.001 (-0.004, 0.003)	1.10 (0.95, 1.28)
Lifestyle models	-0.003 (-0.011, 0.004)	0.004 (-0.002, 0.011)	-0.004 (-0.008, 0.000)	-0.001 (-0.005, 0.002)	1.10 (0.82, 1.48)
<u>Glycemic load (SDS)</u>					
Basic models [‡]	-0.003 (-0.010, 0.004)	0.002 (-0.004, 0.008)	0.000 (-0.004, 0.005)	0.000 (0.003, 0.004)	0.99 (0.74, 1.33)
Socio-demographic models [§]	-0.003 (-0.010, 0.004)	0.002 (-0.004, 0.008)	0.000 (-0.004, 0.005)	0.001 (-0.001, 0.002)	0.98 (0.84, 1.13)
Lifestyle models	-0.011 (-0.025, 0.003)	0.007 (-0.005, 0.020)	-0.004 (-0.012, 0.004)	-0.009 (-0.015, -0.002)*	1.05 (0.59, 1.84)

UmPI, umbilical artery pulsatility index. UfRI, uterine artery pulsatility index. SDS, standard deviation score. CI, Confidence Interval. *Values are regression coefficients (95% confidence interval) from multiple linear regression models and reflect the differences in umbilical artery pulsatility index and uterine artery resistance index per one increase in standard deviation score of maternal glycemic index and glycemic load. †Estimates are from multiple imputed data. ‡Values are odds ratios (95% confidence interval) from multiple logistic regression models and reflect the difference in risks of bilateral uterine artery notching per one increase in standard deviation score of maternal glycemic index and load. §Estimates are from multiple imputed data. †Basic models are adjusted for gestational age at time of intake. ‡Socio-demographic models are adjusted for maternal age, educational level, parity and gestational age at time of measurements. §Lifestyle models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of the measurements. *P-value <0.05.

Table 4. Associations of dietary glycemic index and glycemic load with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia in total population (n=3,299)^a

	Gestational hypertensive disorders	Gestational hypertension	Preeclampsia
	OR (95% CI) n _{cases} =224	OR (95% CI) n _{cases} =166	OR (95% CI) n _{cases} =58
<u>Glycemic index (SDS)</u>			
Basic models [†]	1.00 (0.87, 1.14)	1.02 (0.87, 1.19)	0.92 (0.71, 1.20)
Socio-demographic models [‡]	0.99 (0.86, 1.14)	1.01 (0.86, 1.19)	0.94 (0.72, 1.23)
Lifestyle models [§]	0.98 (0.85, 1.13)	1.00 (0.85, 1.19)	0.92 (0.70, 1.21)
<u>Glycemic load (SDS)</u>			
Basic model [†]	1.04 (0.91, 1.19)	1.03 (0.88, 1.20)	1.06 (0.83, 1.37)
Socio-demographic models [‡]	1.05 (0.92, 1.21)	1.04 (0.89, 1.22)	1.10 (0.85, 1.42)
Lifestyle models [§]	0.98 (0.75, 1.30)	1.02 (0.75, 1.41)	0.89 (0.53, 1.49)

SDS, standard deviation score; CI, Confidence Interval. ^aValues are odds ratios (95% confidence interval) from multiple logistic regression models and reflect the difference in risks of gestational hypertensive disorders, gestational hypertension and preeclampsia per one increase in standard deviation score of maternal glycemic index and glycemic load. Estimates are from multiple imputed data. [†]Basic models are adjusted for gestational age at time of intake.

[‡]Socio-demographic models are adjusted for maternal age, educational level, parity and gestational age at time of intake. [§]Lifestyle models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of intake. *P-value <0.05.

DISCUSSION

In this prospective cohort study we observed that dietary glycemic index and load during pregnancy were not consistently associated with blood pressure throughout pregnancy, placental vascular resistance or the risk of gestational hypertensive disorders after considering other maternal socio-demographic and lifestyle characteristics. Higher dietary glycemic load across the full range was only associated with a higher diastolic blood pressure in early-pregnancy.

Interpretation of main findings

There is an increasing interest in low-glycemic index and load diets as a lifestyle intervention during pregnancy to improve birth outcomes²⁶. In this low-risk pregnant population we observed that dietary glycemic index and load during pregnancy were not consistently associated with blood pressure and placental vascular resistance throughout pregnancy when also considering other socio-demographic and lifestyle factors. We only observed that a higher dietary glycemic load was associated with a higher early-pregnancy diastolic blood pressure after adjustment for socio-demographic, lifestyle and other dietary factors, but the effect estimate was only small. To our knowledge, we are the first study to investigate the associations of dietary glycemic index and load with blood pressure and placental vascular resistance during pregnancy. A meta-analysis of

randomized controlled trials among 1,097 healthy non-pregnant individuals indicated that a lower glycemic index or load diet is associated with a lower systolic and diastolic blood pressure³. The observed differences between this meta-analysis and our study may be explained by the overrepresentation of participants at high-risk of impaired glucose metabolism due to adiposity in the trials included in the meta-analysis, and a greater magnitude of change in dietary glycemic index and load in the included intervention trials. As many of the studies also aimed to achieve weight reduction, it is hard to isolate the effect on blood pressure alone and to make the comparison with a pregnant population³. Finally, physiological changes related to pregnancy may further complicate the comparison of our results among a pregnant population to this meta-analyses among non-pregnant populations. During pregnancy a physiological decrease in systemic vascular resistance results in an initial decrease in blood pressure levels and physiologic metabolic adaptations during pregnancy lead to increased insulin resistance⁴⁰. In our study, we observed no associations of dietary glycemic index and load with blood pressure in overweight or obese pregnant women, but a higher dietary glycemic index was associated with a higher umbilical artery pulsatility index in late-pregnancy only. Possibly, different effects of dietary glycemic index and load on vascular function might be present among pregnant women, as pregnancy related adaptations in the cardiovascular system occur. It could be hypothesized that the effects on endothelial function are most apparent in the fetoplacental vasculature as the vasomotor tone is completely driven by endothelial derived mediators⁴¹⁻⁴³. Pregnancy related insulin resistance and subsequent effect on the endothelium will be more apparent in late-pregnancy, especially in overweight women. Although we did not observe consistent associations of maternal dietary glycemic index and load with gestational hemodynamic adaptations in our low-risk population, possible effects of the dietary glycemic index and load on gestational hemodynamic adaptations may be more pronounced among higher risk populations.

Only two studies examined the effects of carbohydrate quality on the risk of gestational hypertension and preeclampsia. A case-control study in Iran among 202 pregnant women showed a lower incidence of gestational hypertension when women consumed a below average daily glycemic load, but no associations were found for the glycemic index²¹. Within this Iranian study, recall and observer bias could be an issue as dietary intake of the previous year was assessed by a dietitian after the 20th week of pregnancy once gestational hypertension was already diagnosed and only prepregnancy BMI, age and education were considered as confounding factors²¹. Second, an intervention study in Italy among 370 overweight pregnant women found a lower incidence of gestational hypertension among women prescribed a customized low-glycemic index diet with physical activity counseling

according to the ACOG and ACSM recommendations^{22,44}. We observed no effects of dietary glycemic index and load on the risk of gestational hypertensive disorders. The different findings can be explained as our study population reflects a low-risk population and we were able to correct for more confounding factors in our statistical analysis.

Within our low-risk Dutch population, we observed no consistent associations of dietary glycemic index and load with hemodynamic adaptations and the risk of gestational hypertensive disorders. Our study population reflects a relatively healthy pregnant population at low-risk for impaired glucose metabolism and at low-risk for gestational hypertensive disorders as we excluded women with diabetes and preexistent hypertension. Also among overweight and obese women, who are at higher risk for impaired glucose metabolism, we did not find consistent associations. Possibly, the beneficial effects of a lower dietary glycemic index and load on gestational hemodynamic adaptations are only apparent in diabetic women with profound impaired glucose metabolism who are at high risk of developing gestational hypertensive disorders. As we only had a small number of women with diabetes and gestational diabetes, we were not able to assess these associations. Furthermore, the dietary glycemic index and load within our study population were within a normal range, when compared to classification used for individual food products. Effects on gestational hemodynamic adaptations might only be present when larger differences from a higher dietary glycemic index and load to a lower dietary-glycemic index and load are achieved. The FFQ assessment in our study mainly reflected dietary intake in preconception period and the first-trimester of pregnancy, which allowed us to investigate the association of dietary glycemic index and load on hemodynamic adaptations from early-pregnancy onwards. Importantly, pregnancy related insulin resistance increases from mid-pregnancy onwards and effects of dietary glycemic index and load may be more pronounced in the second half of pregnancy.

Strengths and limitations

The prospective data collection from early-pregnancy onwards with repeatedly measured blood pressure and placental hemodynamic parameters within a large study sample are major strengths of our study. The overall response rate for participation in the Generation R study was 61% and the participation in the self-administrated FFQs was 78%²⁴. As we restricted to a Dutch population, this may have affected the generalizability of our findings. Furthermore, we had a relatively small number of gestational hypertensive disorder cases which indicates a possible selection towards a relatively healthy population. This relatively low number of cases might have caused a decreased statistical power for the gestational hypertensive disorder analyses. Studies in higher-risk population with

more cases of preeclampsia and gestational hypertension are needed to examine these associations further. The FFQ is a widely used method to assess dietary intake in large observational studies, but relies on self-reported data which may be prone to over- or underreporting of dietary intake. Although the FFQ was not directly validated for the estimation of dietary glycemic index and load, the FFQ was shown to be a reliable tool for the estimation of total carbohydrate intake in a validation study conducted in the same area as the study area²⁵. Within this validation study using 24h dietary recalls and nutritional biomarkers, intake of carbohydrate was only slightly underestimated with the use of the FFQ²⁵. When compared to the general Dutch population, we observed only a slightly lower mean maternal early-pregnancy dietary glycemic index²⁷. This might be explained by slight underreporting of carbohydrate containing food products or may reflect our relatively healthier study population. The mean dietary glycemic index within our study was in line with the mean dietary glycemic index in other observational studies during pregnancy which are comparable in demographic and other lifestyle characteristics^{28, 29}. We examined the associations of maternal dietary glycemic index and load with multiple outcomes, which might increase the risk of chance findings due to multiple testing. We did not perform correction for multiple testing as the evaluated outcome measures are strongly correlated. The observed associations of dietary glycemic load with early-pregnancy diastolic blood pressure among the total study population and dietary glycemic index with late-pregnancy umbilical artery pulsatility index among overweight and obese women, should be considered hypothesis generating and need further replication. Lastly, it is well-known that dietary intake is strongly related to socio-demographic and lifestyle factors. Detailed information about a large number of maternal sociodemographic and lifestyle factors was available within our study. Residual confounding might still be an issue because of the observational study design, for example by physical activity.

Conclusion

Within a low-risk pregnant population, we did not find consistent associations of dietary glycemic index and load with blood pressure throughout pregnancy, placental vascular resistance and the risk of gestational hypertensive disorders. Further studies should focus on the effects of dietary glycemic index and load on gestational hemodynamic adaptations and the risk of gestational hypertensive disorders within pregnant populations at higher risk of impaired glucose metabolism.

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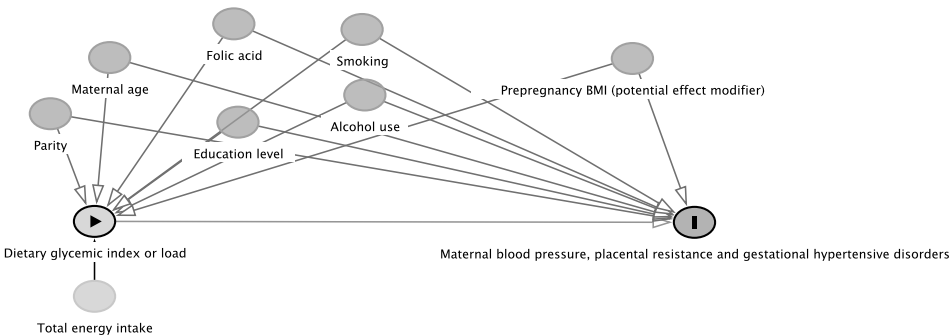
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SUPPLEMENTARY MATERIAL

Supplementary Table S1. Non-response analysis: characteristics of the total study population versus participating women without data on dietary intake

	Total group	Participants without data on dietary intake ^a
	n=3,378	n=506
Maternal age at enrolment, years	31.4 (4.4)	30.2 (5.3)
Gestational age at intake, weeks	13.5 (5.4-38.1)	14.1 (10.3-30.4)
Parity, n nulliparous	2,019 (59.9)	290 (57.8)
Prepregnancy BMI, kg/m ²	23.1 (3.8)	23.1 (4.1)
Prepregnancy BMI ≥25, n	636 (21.8)	96 (22.9)
Gestational weight gain, kg	10.8 (4.4)	11.2 (4.9)
Education, n high	1,985 (59.5)	229 (46.5)
Smoking, n continued during pregnancy	531 (17.0)	116 (25.7)
Alcohol, n continued during pregnancy	1,559 (50.2)	200 (44.5)
Early-pregnancy, ≥1 glass per week	830 (26.9)	98 (21.9)
Mid-pregnancy, ≥1 glass per week	378 (12.3)	34 (8.9)
Late-pregnancy ≥1 glass per week	444 (14.7)	45 (11.4)
Folic acid supplement use, n yes	2,467 (89.1)	328 (82.2)
Systolic blood pressure, mmHg		
Early-pregnancy	117.1 (11.8)	117.7 (12.2)
Mid-pregnancy	118.4 (11.7)	118.6 (10.9)
Late-pregnancy	120.3 (11.4)	119.7 (11.4)
Diastolic blood pressure, mmHg		
Early-pregnancy	68.4 (9.2)	68.0 (11.4)
Mid-pregnancy	67.1 (9.2)	66.0 (9.4)
Late-pregnancy	69.3 (9.1)	68.0 (9.3)
Uterine artery resistance index		
Mid-pregnancy	0.535 (0.089)	0.545 (0.090)
Late-pregnancy	0.483 (0.078)	0.480 (0.077)
Umbilical artery pulsatility index		
Mid-pregnancy	1.188 (0.183)	1.216 (0.180)
Late-pregnancy	0.977 (0.166)	0.985 (0.180)
Late-pregnancy uterine artery notching	48 (2.2)	2 (0.7)
Gestational hypertensive disorders		
Gestational hypertension	166 (5.1)	24 (5.0)
Preeclampsia	58 (1.9)	8 (1.7)

Values are means (SD), median (95% range) or n (valid %). ^aWomen of Dutch ethnicity enrolled during pregnancy with singleton live births without data on dietary intake.



Supplementary Figure S1. Directed Acyclic Graph for confounder selection.

Supplementary Table S2. Longitudinal associations of glycemic index and load with systolic and diastolic blood pressure from repeated measurement models[†]

	Systolic blood pressure in mmHg			
	Intercept	p-value [†]	Slope (mmHg (95%CI))	p-value [†]
GI quartile 1	111.89	Reference	Reference	Reference
GI quartile 2	112.64	0.40	0.02	0.64
GI quartile 3	113.88	0.03	-0.01	0.83
GI quartile 4	112.68	0.38	0.04	0.25
GL quartile 1	111.66	<0.001	Reference	
GL quartile 2	113.27	0.07	-0.05	0.14
GL quartile 3	112.36	0.43	-0.01	0.85
GL quartile 4	113.72	0.02	-0.04	0.29

	Diastolic blood pressure in mmHg			
	Intercept	p-value [†]	Slope (mmHg (95%CI))	p-value [†]
GI quartile 1	99.24	Reference	Reference	Reference
GI quartile 2	98.99	0.72	0.03	0.26
GI quartile 3	100.08	0.23	0.002	0.93
GI quartile 4	99.75	0.47	0.02	0.34
GL quartile 1	98.96	Reference	Reference	Reference
GL quartile 2	100.10	0.10	-0.04	0.06
GL quartile 3	99.45	0.48	-0.03	0.29
GL quartile 4	99.83	0.21	-0.05	0.10

CI, Confidence interval. Values are based on repeated non-linear regression models and reflect the change in blood pressure in mmHg per glycemic index and glycemic load quartile compared to women with the highest dietary quality (quartile 1) as reference. Models are adjusted for gestational age at the time of intake. [†]P-value reflects the significance level of the estimate.

Supplementary Table S3a. Basic model: Associations of dietary glycemic index and load quartiles with systolic and diastolic blood pressure during pregnancy in total population (n=3,375)*

Differences in systolic blood pressure in mmHg (95% CI)			
	Early-pregnancy n=2,802	Mid-pregnancy n=3,263	Late-pregnancy n=3,286
GI quartile 1	reference	reference	reference
GI quartile 2	0.55 (-0.69, 1.80)	1.47 (0.34, 2.60)*	1.20 (0.10, 2.30)*
GI quartile 3	1.66 (0.42, 2.90)*	2.00 (0.87, 3.13)*	1.63 (0.53, 2.72)*
GI quartile 4	1.03 (-0.21, 2.26)	1.85 (0.72, 2.98)*	1.83 (0.73, 2.94)*
GL quartile 1	reference	reference	Reference
GL quartile 2	1.09 (-0.14, 2.36)	0.62 (-0.52, 1.75)	0.03 (-1.07, 1.13)
GL quartile 3	0.98 (-0.25, 2.20)	0.39 (-0.75, 1.52)	0.57 (-0.53, 1.67)
GL quartile 4	2.07 (0.84, 3.31)*	0.98 (-0.16, 2.11)	1.12 (0.02, 2.22)*
Differences in diastolic blood pressure in mmHg (95% CI)			
	Early-pregnancy n=2,802	Mid-pregnancy n=3,262	Late-pregnancy n=3,285
GI quartile 1	reference	reference	reference
GI quartile 2	0.17 (-0.79, 1.14)	0.38 (-0.52, 1.27)	0.67 (-0.23, 1.54)
GI quartile 3	0.58 (-0.38, 1.54)	1.36 (0.46, 2.25)*	0.63 (-0.25, 1.52)
GI quartile 4	0.83 (-0.13, 1.78)	1.29 (0.39, 2.18)*	0.96 (0.07, 1.84)*
GL quartile 1	reference	reference	reference
GL quartile 2	0.55 (-0.41, 1.50)	0.33 (-0.56, 1.23)	-0.47 (-1.36, 0.42)
GL quartile 3	0.27 (-0.68, 1.22)	0.10 (-0.80, 1.00)	-0.44 (-1.33, 0.44)
GL quartile 4	0.63 (-0.33, 1.58)	-0.08 (-0.98, 0.81)	-0.39 (-1.28, 0.49)

CI, Confidence Interval. GI, glycemic index. GL, glycemic load. Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per glycemic index or glycemic load quartile. Groups are compared to women with the highest dietary quality (quartile 1) as reference. Estimates are from multiple imputed data. Models are adjusted for gestational age at time of intake. *P-value <0.05.

Supplementary Table S3b. Confounder model: Associations of dietary glycemic index and load quartiles with systolic and diastolic blood pressure during pregnancy in total population (n=3,375)*

Differences in systolic blood pressure in mmHg (95% CI)			
	Early-pregnancy n=2,802	Mid-pregnancy n=3,263	Late-pregnancy n=3,286
GI quartile 1	reference	reference	reference
GI quartile 2	-0.03 (-1.20, 1.14)	0.86 (-0.20, 1.92)	0.61 (-0.43, 1.66)
GI quartile 3	0.75 (-0.42, 1.92)	1.00 (-0.07, 2.07)	0.79 (-0.26, 1.84)
GI quartile 4	-0.22 (-1.41, 0.97)	0.47 (-0.63, 1.56)	0.56 (-0.52, 1.64)
GL quartile 1	Reference	reference	reference
GL quartile 2	0.67 (-0.63, 1.96)	0.39 (-0.79, 1.58)	0.11 (-1.05, 1.27)
GL quartile 3	-0.10 (-1.63, 1.43)	-0.50 (-1.89, 0.90)	0.07 (-1.30, 1.44)
GL quartile 4	0.30 (-1.70, 2.29)	-0.51 (-2.32, 1.31)	0.14 (-1.65, 1.92)
Differences in diastolic blood pressure in mmHg (95% CI)			
	Early-pregnancy n=2,802	Mid-pregnancy n=3,262	Late-pregnancy n=3,285
GI quartile 1	reference	reference	reference
GI quartile 2	-0.08 (-0.99, 0.82)	0.03 (-0.80, 0.86)	0.33 (-0.49, 1.15)
GI quartile 3	0.24 (-0.67, 1.14)	0.80 (-0.04, 1.64)	0.27 (-0.56, 1.10)
GI quartile 4	0.48 (-0.44, 1.40)	0.69 (-0.17, 1.54)	0.49 (-0.37, 1.34)
GL quartile 1	reference	Reference	reference
GL quartile 2	0.68 (-0.32, 1.68)	0.40 (-0.53, 1.33)	-0.15 (-1.07, 0.77)
GL quartile 3	0.48 (-0.71, 1.66)	-0.01 (-1.10, 1.09)	-0.25 (-1.33, 0.84)
GL quartile 4	0.99 (-0.55, 2.54)	-0.17 (-1.60, 1.25)	-0.15 (-1.56, 1.26)

CI, Confidence Interval. GI, glycemic index. GL, glycemic load. Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per glycemic index and glycemic load quartile. Groups are compared to women with the highest dietary quality (quartile 1) as reference. Estimates are from multiple imputed data. Models are adjusted for maternal age, ethnicity, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use and gestational age at time of the measurements. *P-value <0.05.

Supplementary Table S4a. Basic model: Associations of dietary glycemic index and load quartiles with umbilical artery pulsatility index, uterine artery resistance index and bilateral notching in total population (n=3,090)

	Differences in UmPI (95% CI) ^a			Differences in UTRI (95% CI) ^b			Bilateral notching (95% CI) ^c
	Mid-pregnancy n=2,505	Late-pregnancy n=2,751	Mid-pregnancy n=1,884	Late-pregnancy n=2,060	Late-pregnancy n _{Cases} =48		
GI quartile 1	reference	reference	reference	reference	Reference		
GI quartile 2	0.015 (-0.005, 0.035)	0.001 (-0.016, 0.019)	-0.008 (-0.019, 0.003)	-0.004 (-0.013, 0.006)	0.72 (0.33, 1.58)		
GI quartile 3	0.018 (-0.002, 0.039)	0.011 (-0.006, 0.029)	-0.007 (-0.018, 0.004)	-0.005 (-0.014, 0.005)	0.41 (0.16, 1.06)		
GI quartile 4	0.003 (-0.017, 0.023)	0.016 (-0.002, 0.033)	-0.007 (-0.018, 0.005)	-0.003 (-0.013, 0.006)	1.12 (0.55, 2.29)		
GL quartile 1	reference	reference	reference	reference	reference		
GL quartile 2	-0.002 (-0.022, 0.018)	-0.023 (-0.041, -0.006)*	-0.005 (-0.016, 0.006)	-0.003 (-0.012, 0.007)	0.99 (0.44, 2.23)		
GL quartile 3	-0.001 (-0.021, 0.019)	-0.015 (-0.033, 0.002)	0.005 (-0.006, 0.016)	0.005 (-0.005, 0.014)	1.23 (0.57, 2.69)		
GL quartile 4	-0.006 (-0.026, 0.015)	0.000 (-0.018, 0.018)	-0.003 (-0.015, 0.009)	-0.001 (-0.011, 0.008)	0.90 (0.39, 2.10)		

UmPI, umbilical artery pulsatility index. UTRI, uterine artery resistance index. CI, Confidence Interval. GI, glycemic index. GL, glycemic load. ^aValues are regression coefficients (95% confidence interval) and reflect differences in umbilical artery pulsatility index and uterine artery resistance index per glycemic index and glycemic load quartile. Groups are compared to women with the highest dietary quality (quartile 1) as reference. ^bValues are odds ratios (95% confidence interval) that reflect difference in risks of bilateral notching per glycemic index a glycemic load quartile. Groups are compared to women with the highest dietary quality (quartile 1) as reference. Estimates are from multiple imputed data. ^cModels are adjusted for gestational age at time of intake. *P-value <0.05.

Supplementary Table S4b. Confounder models: Associations of dietary glycemic index and load quartiles with umbilical artery pulsatility index, uterine artery resistance index and bilateral notching in total population (n=3,090)

	Differences in UmPI (95% CI): ‡			Differences in UtRI (95% CI): ‡			Bilateral notching (95% CI) †, ‡ n _{cases} =48
	Mid-pregnancy n=2,505	Late-pregnancy n=2,751	reference	Mid-pregnancy n=1,884	Late-pregnancy n=2,060	reference	
GI quartile 1	reference	reference	reference	reference	reference	reference	
GI quartile 2	0.011 (-0.009, 0.030)	0.000 (-0.017, 0.018)	-0.008 (-0.019, 0.003)	-0.003 (-0.013, 0.006)	0.71 (0.32, 1.53)		
GI quartile 3	0.012 (-0.008, 0.032)	0.007 (-0.011, 0.024)	-0.007 (-0.018, 0.004)	-0.006 (-0.015, 0.004)	0.39 (0.15, 1.04)		
GI quartile 4	-0.005 (-0.025, 0.015)	0.010 (-0.008, 0.028)	-0.008 (-0.019, 0.004)	-0.005 (-0.015, 0.005)	1.06 (0.53, 2.10)		
GL quartile 1	reference	reference	reference	reference	reference	reference	
GL quartile 2	-0.001 (-0.023, 0.021)	-0.017 (-0.037, 0.002)	-0.008 (-0.020, 0.005)	-0.009 (-0.019, 0.001)	1.05 (0.42, 2.59)		
GL quartile 3	-0.006 (-0.032, 0.020)	-0.012 (-0.035, 0.011)	0.000 (-0.015, 0.015)	-0.007 (-0.020, 0.005)	1.33 (0.46, 3.87)		
GL quartile 4	-0.014 (-0.047, 0.020)	0.003 (-0.027, 0.032)	-0.011 (-0.030, 0.008)	-0.019 (-0.036, -0.003)*	0.98 (0.24, 4.04)		

UmPI, umbilical artery pulsatility index. UtRI, uterine artery resistance index. CI, Confidence Interval. GI, glycemic index. GL, glycemic load. †Values are regression coefficients (95% confidence interval) and reflect differences in umbilical artery pulsatility index and uterine artery resistance index per glycemic index and glycemic load quartile. Groups are compared to women with the highest dietary quality (quartile 1) as reference. ‡Values are odds ratios (95% confidence interval) that reflect difference in risks of bilateral notching per glycemic index a glycemic load quartile. Groups are compared to women with the highest dietary quality (quartile 1) as reference. Estimates are from multiple imputed data. †Models are adjusted for maternal age, ethnicity, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use and gestational age at time of the measurements. *P-value <0.05.

Supplementary Table S5a. Basic models: Associations of dietary glycemic index and load quartiles with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia in total population (n=3,299)*

	Gestational hypertensive disorders	Gestational hypertension	Preeclampsia
	Odds ratio (95% CI) n _{cases} =224	Odds ratio (95% CI) n _{cases} =166	Odds ratio (95% CI) n _{cases} =58
GI quartile 1	reference	reference	reference
GI quartile 2	1.03 (0.70, 1.52)	1.18 (0.76, 1.84)	0.69 (0.32, 1.49)
GI quartile 3	1.06 (0.72, 1.56)	1.03 (0.65, 1.63)	1.14 (0.58, 2.25)
GI quartile 4	1.07 (0.72, 1.57)	1.16 (0.75, 1.82)	0.83 (0.40, 1.74)
GL quartile 1	reference	reference	reference
GL quartile 2	1.06 (0.72, 1.56)	1.35 (0.86, 2.12)	0.50 (0.22, 1.11)
GL quartile 3	1.04 (0.71, 1.54)	1.18 (0.74, 1.87)	0.78 (0.39, 1.58)
GL quartile 4	1.15 (0.78, 1.68)	1.25 (0.79, 1.98)	0.95 (0.48, 1.85)

CI, Confidence Interval. GI, glycemic index. GL, glycemic load. Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorders, gestational hypertension and preeclampsia per glycemic index and glycemic load quartile. Groups are compared to women with the highest dietary quality (quartile 1) as reference. Estimates are from multiple imputed data. Models are adjusted for gestational age at time of intake. *P-value <0.05.

Supplementary Table S5b. Confounder models: Associations of dietary glycemic index and load quartiles with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia in total population (n=3,299)*

	Gestational hypertensive disorders	Gestational hypertension	Preeclampsia
	Odds ratio (95% CI) n _{cases} =224	Odds ratio (95% CI) n _{cases} =166	Odds ratio (95% CI) n _{cases} =58
GI quartile 1	reference	reference	reference
GI quartile 2	1.00 (0.67, 1.48)	1.12 (0.71, 1.76)	0.68 (0.31, 1.50)
GI quartile 3	0.97 (0.65, 1.45)	0.91 (0.57, 1.47)	1.09 (0.54, 2.21)
GI quartile 4	1.03 (0.68, 1.54)	1.10 (0.69, 1.77)	0.84 (0.39, 1.81)
GL quartile 1	reference	reference	reference
GL quartile 2	1.10 (0.70, 1.71)	1.50 (0.90, 2.51)	0.41 (0.17, 0.98)*
GL quartile 3	1.05 (0.63, 1.77)	1.35 (0.73, 2.48)	0.55 (0.21, 1.40)
GL quartile 4	1.14 (0.57, 2.24)	1.54 (0.70, 3.41)	0.51 (0.15, 1.78)

CI, Confidence Interval. GI, glycemic index. GL, glycemic load. Values are odds ratios (95% confidence interval) that reflect difference in risks of Gestational hypertensive disorders, gestational hypertension and preeclampsia per glycemic index and glycemic load quartile. Groups are compared to women with the highest dietary quality (quartile 1) as reference. Estimates are from multiple imputed data. Models are adjusted for maternal age, ethnicity, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use and gestational age at time of the measurements. *P-value <0.05.

Supplementary Table S6a. Sensitivity analysis: Associations of dietary glycemic index with clinical cut-off for low- and normal-glycemic diet with systolic and diastolic blood pressure during pregnancy (n=3,374)^{*}

		Differences in systolic blood pressure in mmHg (CI 95%)		
		Early-pregnancy	Mid-pregnancy	Late-pregnancy
Normal-GI		reference n=1,938	reference n=2,238	reference n=2,262
Low-GI	Basic model [†]	-0.92 (-1.87, 0.03)	-1.58 (-2.45, -0.72)*	-1.18 (-2.02, -0.34)*
	Lifestyle model [‡]	-0.01 (-0.91, 0.90) n=863	-0.63 (1.45, -1.19) n=1,024	-0.36 (-1.17, 0.45) n=1,023
		Differences in diastolic blood pressure in mmHg (CI 95%)		
		Early-pregnancy	Mid-pregnancy	Late-pregnancy
Normal-GI		reference n=1,938	reference n=2,237	reference n=2,261
Low-GI	Basic model [†]	-0.61 (-1.35, 0.12)	-1.03 (0.34, 1.71)*	-0.64 (-1.32, 0.03)
	Lifestyle model [‡]	-0.27 (-0.97, 0.43) n=863	-0.53 (-1.17, 0.12) n=1,024	-0.32 (-0.96, 0.32) n=1,023

GI, glycemic index. ^{*}Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure of a low-glycemic index diet compared to women with a normal-glycemic index diet as reference. Estimates are from multiple imputed data. [†]Basic models are adjusted for gestational age at time of intake. [‡]Confounder models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of the measurements. *P-value <0.05.

Supplementary Table S6b. Sensitivity analysis: Associations of dietary glycemic index with clinical cut-off for low- and normal-glycemic diet with umbilical artery pulsatility index, uterine artery resistance index and bilateral notching (n=3,089)

	Differences in UmPI (95% CI) ^a		Differences in UTRI (95% CI) ^b		Bilateral notching (95% CI) ^c	
	Mid-pregnancy	Late-pregnancy	Mid-pregnancy	Late-pregnancy	Late-pregnancy	Late-pregnancy
Normal-GI	reference n=1,706	reference n=1,897	reference n=1,255	reference n=1,409	reference n _{cases} =30	
Low-GI	Basic model ^d	-0.006 (-0.021, 0.009)	-0.006 (-0.007, 0.020)	0.006 (-0.003, 0.014)	0.005 (-0.002, 0.012)	1.32 (0.73, 2.39)
	Lifestyle model ^e	-0.001 (-0.017, 0.014)	-0.001 (-0.014, 0.013)	0.006 (-0.003, 0.014)	0.006 (-0.001, 0.013)	1.41 (0.77, 2.60)
	n=799	n=853	n=629	n=651	n _{cases} =18	

UmPI, umbilical artery pulsatility index. UTRI, uterine artery resistance index. CI, Confidence Interval. GI, glycemic index. Values are regression coefficients (95% confidence interval) and reflect the differences in umbilical artery pulsatility index and uterine artery resistance index of women with a low-glycemic index diet compared to women with a normal-glycemic index diet as reference. Estimates are from multiple imputed data. ^aValues are odds ratios (95% confidence interval) from multiple logistic regression models and reflect the differences in risks of bilateral notching of women with a normal-glycemic index diet compared to women with a low-glycemic index diet as reference. Estimates are from multiple imputed data. ^bBasic models are adjusted for gestational age at time of intake. ^cConfounder models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, and folic acid use and gestational age at time of the measurements. ^dP-value <0.05.

Supplementary Table S6c. Sensitivity analysis: Associations of dietary glycemic index with clinical cut-off for low and normal glycemic diet with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia (n=3,298)[†]

		Gestational hypertensive disorders	Gestational hypertension	Preeclampsia
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Normal-GI		reference	reference	reference
n=2,262		n _{cases} =155	n _{cases} =115	n _{cases} =40
Low-GI	Basic model [†]	0.97 (0.73, 1.30)	0.97 (0.69, 1.36)	0.97 (0.56, 1.71)
n=1,036	Lifestyle model [‡]	1.03 (0.75, 1.40)	1.05 (0.74, 1.50)	0.98 (0.55, 1.74)
		n _{cases} =69	n _{cases} =51	n _{cases} =18

OR, odds ratio. CI, Confidence Interval. GI, glycemic index. [†]Values are odds ratios (95% confidence interval) from multiple logistic regression models and reflect the difference in risks of gestational hypertensive disorders, gestational hypertension and preeclampsia of women with a low-glycemic index diet compared to women with a normal-glycemic index diet as reference. Estimates are from multiple imputed data. [‡]Basic models are adjusted for gestational age at time of intake. [§]Confounder models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of the measurements. *P-value <0.05.

Supplementary Table S7a. Sensitivity analysis: Associations of dietary glycemic index and load with systolic and diastolic blood pressure during pregnancy in population with BMI_≥25 (n=766)[†]

<u>Glycemic index (SDS)</u>		Differences in systolic blood pressure in mmHg (CI 95%)		
		Early-pregnancy (n=623)	Mid-pregnancy (n=728)	Late-pregnancy (n=741)
Basic model [†]		-0.22 (-1.23, 0.80)	-0.16 (-1.09, 0.76)	0.23 (-0.69, 1.16)
Socio-demographic model [‡]		-0.39 (-1.36, 0.68)	-0.46 (-1.39, 0.47)	-0.08 (-1.01, 0.86)
Lifestyle model [§]		-0.32 (-1.32, 0.68)	-0.45 (-1.36, 0.46)	-0.18 (-1.09, 0.74)
		Differences in diastolic blood pressure in mmHg (CI 95%)		
		Early-pregnancy (n=623)	Mid-pregnancy (n=728)	Late-pregnancy (n=741)
Basic model [†]		-0.08 (-0.85, 0.69)	-0.27 (-0.99, 0.44)	-0.11 (-0.78, 0.57)
Socio-demographic model [‡]		-0.09 (-0.87, 0.69)	-0.27 (-1.00, 0.47)	-0.06 (-0.75, 0.62)
Lifestyle model [§]		0.16 (-0.59, 0.92)	-0.12 (-0.81, 0.56)	0.09 (-0.57, 0.76)
<u>Glycemic load (SDS)</u>		Differences in systolic blood pressure in mmHg (CI 95%)		
		Early-pregnancy (n=623)	Mid-pregnancy (n=728)	Late-pregnancy (n=741)
Basic model [†]		1.11 (0.13, 2.08)*	0.25 (-0.66, 1.15)	0.79 (-0.06, 1.64)
Socio-demographic model [‡]		0.98* (0.005, 1.96)*	0.07 (-0.82, 0.96)	0.64 (-0.19, 1.47)
Lifestyle model [§]		-0.10 (-2.04, 1.84)	-1.29 (-3.00, 0.42)	-0.90 (-2.53, 0.74)
		Differences in diastolic blood pressure in mmHg (CI 95%)		
		Early-pregnancy (n=623)	Mid-pregnancy (n=728)	Late-pregnancy (n=741)
Basic model [†]		0.01 (-0.75, 0.77)	-0.32 (-1.02, 0.38)	-0.27 (-0.91, 0.37)
Socio-demographic model [‡]		-0.05 (-0.82, 0.71)	-0.29 (-0.99, 0.41)	-0.29 (-0.93, 0.35)
Lifestyle model [§]		1.31 (-0.20, 2.82)	-0.28 (-1.56, 1.01)	0.43 (-0.82, 1.68)

SDS, standard deviation score. CI, Confidence Interval. [†]Values are regression coefficients (95% confidence interval) from multiple linear regression models and reflect the differences in mmHg blood pressure per one increase in standard deviation score of maternal glycemic index and glycemic load. Estimates are from multiple imputed data. [‡]Basic models are adjusted for gestational age at time of intake. [§]Socio-demographic models are adjusted for maternal age, educational level, parity and gestational age at time of measurements. [§]Lifestyle models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of the measurements. *P-value <0.05.

Supplementary Table S7b. Sensitivity analysis: Associations of dietary glycemic index and load with umbilical artery pulsatility index, uterine artery resistance index and bilateral notching in population with BMI \geq 25 (n=766)

	Differences in UmPI (95% CI) ^a		Differences in UTRI (95% CI) ^a		Bilateral notching (95% CI) ^a
	Mid-pregnancy n=547	Late-pregnancy n=609	Mid-pregnancy n=380	Late-pregnancy n=459	
<u>Glycemic index (SDS)</u>					
Basic model [†]	-0.010 (-0.027, 0.007)	0.019 (0.005, 0.033)*	0.003 (-0.007, 0.013)	0.005 (-0.003, 0.012)	Late-pregnancy n _{cases} =11 0.92 (0.44, 1.90)
Socio-demographic model [§]	-0.009 (-0.025, 0.008)	0.018 (0.004, 0.032)*	0.004 (-0.007, 0.014)	0.004 (-0.003, 0.012)	0.94 (0.46, 1.91)
Lifestyle model	-0.012 (-0.029, 0.005)	0.016 (0.002, 0.030)*	0.003 (-0.008, 0.013)	0.004 (-0.004, 0.012)	0.86 (0.43, 1.83)
<u>Glycemic load (SDS)</u>					
Basic model [†]	-0.010 (-0.026, 0.007)	0.011 (-0.003, 0.025)	-0.001 (-0.011, 0.010)	0.005 (-0.002, 0.013)	Late-pregnancy n _{cases} =11 0.81 (0.39, 1.66)
Socio-demographic model [§]	-0.007 (-0.022, 0.009)	0.011 (-0.003, 0.025)	0.001 (-0.010, 0.011)	0.006 (-0.002, 0.014)	0.80 (0.38, 1.68)
Lifestyle model	-0.031 (-0.064, 0.002)	0.016 (-0.013, 0.044)	0.007 (-0.015, 0.029)	0.004 (-0.012, 0.020)	0.86 (0.17, 4.25)

SDS, standard deviation score. CI, Confidence Interval. UmPI, umbilical artery pulsatility index. UTRI, uterine artery resistance index. Values are regression coefficients (95% confidence interval) from multiple linear regression models and reflect the differences in umbilical artery pulsatility index and uterine artery resistance index per one increase in standard deviation score of maternal glycemic index and glycemic load. Estimates are from multiple imputed data. Values are odds ratios (95% confidence interval) from multiple logistic regression models and reflect the difference in risks of bilateral notching per one increase in standard deviation score of maternal glycemic index and load. Estimates are from multiple imputed data. [†]Basic models are adjusted for gestational age at time of intake. [§]Socio-demographic models are adjusted for maternal age, educational level, parity and gestational age at time of measurements. ^{||}Lifestyle models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of the measurements. *P-value <0.05.

Supplementary Table S7c. Sensitivity analysis: Associations of dietary glycemic index and load with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia in population with BMI \geq 25 (n=766)^{*}

	Gestational hypertensive disorders	Gestational hypertension	Preeclampsia
<u>Glycemic index (SDS)</u>	Odds ratio (95% CI) n _{cases} =89	Odds ratio (95% CI) n _{cases} =74	Odds ratio (95% CI) n _{cases} =15
Basic model [†]	0.81 (0.63, 1.03)	0.71 (0.58, 0.98)*	1.08 (0.60, 1.94)
Socio-demographic model [‡]	0.85 (0.66, 1.10)	0.80 (0.61, 1.06)	1.14 (0.61, 2.14)
Lifestyle model [§]	0.84 (0.64, 1.10)	0.80 (0.60, 1.07)	1.07 (0.53, 2.16)
<u>Glycemic load (SDS)</u>	Odds ratio (95% CI) n _{cases} =89	Odds ratio (95% CI) n _{cases} =74	Odds ratio (95% CI) n _{cases} =15
Basic model [†]	1.00 (0.80, 1.26)	0.95 (0.74, 1.22)	1.27 (0.77, 2.10)
Socio-demographic model [‡]	1.04 (0.82, 1.32)	0.98 (0.76, 1.27)	1.34 (0.80, 2.24)
Lifestyle model [§]	0.95 (0.57, 1.59)	0.86 (0.50, 1.48)	1.38 (0.38, 5.03)

SDS, standard deviation score. CI, Confidence Interval. ^{*}Values are odds ratios (95% confidence interval) from multiple logistic regression models and reflect the difference in risks of gestational hypertensive disorders, gestational hypertension and preeclampsia per one increase in standard deviation score of maternal glycemic index and glycemic load. Estimates are from multiple imputed data. [†]Basic models are adjusted for gestational age at time of intake. [‡]Socio-demographic models are adjusted for maternal age, educational level, parity and gestational age at time of intake. [§]Lifestyle models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of intake. ^{*}P-value <0.05.

Supplementary Table S8a. Sensitivity analysis: Associations of dietary glycemic index and load with systolic and diastolic blood pressure during pregnancy in population with study enrollment <14 weeks of gestation (n=1,867)^{*}

<u>Glycemic index (SDS)</u>	Differences in systolic blood pressure in mmHg (CI 95%)		
	Early-pregnancy (n=1,848)	Mid-pregnancy (n=1,833)	Late-pregnancy (n=1,821)
Basic model [†]	0.12 (-0.43, 0.67)	0.34 (-0.21, 0.89)	0.32 (-0.22, 0.85)
Socio-demographic model [‡]	-0.06 (-0.61, 0.50)	0.10 (-0.45, 0.65)	0.12 (-0.42, 0.65)
Lifestyle model [§]	-0.17 (-0.70, 0.36)	-0.02 (-0.54, 0.51)	0.06 (-0.46, 0.58)
	Differences in diastolic blood pressure in mmHg (CI 95%)		
	Early-pregnancy (n=1,848)	Mid-pregnancy (n=1,832)	Late-pregnancy (n=1,820)
Basic model [†]	0.32 (-0.11, 0.75)	0.51 (0.07, 0.95)*	0.30 (-0.13, 0.72)
Socio-demographic model [‡]	0.29 (-0.14, 0.73)	0.41 (-0.02, 0.83)	0.24 (-0.19, 0.67)
Lifestyle model [§]	0.31 (-0.09, 0.72)	0.42 (0.001, 0.83)	0.27 (-0.13, 0.67)
<u>Glycemic load (SDS)</u>	Differences in systolic blood pressure in mmHg (CI 95%)		
	Early-pregnancy (n=1,848)	Mid-pregnancy (n=1,833)	Late-pregnancy (n=1,820)
Basic model [†]	0.57 (0.03, 1.12)*	0.50 (-0.05, 1.04)	0.35 (-0.17, 0.88)
Socio-demographic model [‡]	0.59 (0.05, 1.13)*	0.50 (-0.04, 1.03)	0.36 (-0.16, 0.88)
Lifestyle model [§]	-0.11 (-1.14, 0.92)	-0.09 (-1.11, 0.92)	-0.02 (-1.02, 0.97)
	Differences in diastolic blood pressure in mmHg (CI 95%)		
	Early-pregnancy (n=1,848)	Mid-pregnancy (n=1,832)	Late-pregnancy (n=1,820)
Basic model [†]	0.24 (-0.19, 0.66)	0.16 (-0.27, 0.59)	-0.02 (-0.45, 0.40)
Socio-demographic model [‡]	0.25 (-0.17, 0.67)	0.17 (-0.26, 0.60)	0.01 (-0.41, 0.42)
Lifestyle model [§]	0.96 (0.17, 1.75)*	0.87 (0.07, 1.67)*	0.53 (-0.24, 1.31)

SDS, standard deviation score. CI, Confidence Interval. ^{*}Values are regression coefficients (95% confidence interval) from multiple linear regression models and reflect the differences in mmHg blood pressure per one increase in standard deviation score of maternal glycemic index and glycemic load during early-pregnancy. Estimates are from multiple imputed data. [†]Basic models are adjusted for gestational age at time of intake. [‡]Socio-demographic models are adjusted for maternal age, educational level, parity and gestational age at time of measurements. [§]Lifestyle models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of the measurements. ^{*}P-value <0.05.

Supplementary Table S8b. Sensitivity analysis: Associations of dietary glycemic index and load with uterine artery resistance index, umbilical artery pulsatility index and bilateral notching in population with study enrollment <14 weeks of gestation (n=1,867)

	Differences in UmPI (95% CI) [†]		Differences in UTRI (95% CI) [†]		Bilateral notching (95% CI) [†]
	Mid-pregnancy n=1,504	Late-pregnancy n=1,602	Mid-pregnancy n=1,156	Late-pregnancy n=1,222	
<u>Glycemic index (SDS)</u>					
Basic model [†]	0.000 (-0.010, 0.009)	0.003 (-0.005, 0.011)	0.003 (-0.008, 0.003)	0.001 (-0.006, 0.003)	Late-pregnancy n _{cases} =30 0.92 (0.63, 1.34)
Socio-demographic model [‡]	-0.002 (-0.011, 0.008)	0.001 (-0.007, 0.009)	0.001 (-0.005, 0.004)	-0.001 (-0.005, 0.004)	0.95 (0.65, 1.40)
Lifestyle model [§]	-0.003 (-0.013, 0.006)	0.000 (-0.009, 0.008)	-0.003 (-0.008, 0.003)	-0.001 (-0.006, 0.003)	0.95 (0.78, 1.15)
<u>Glycemic load (SDS)</u>					
Basic model [†]	-0.003 (-0.012, 0.007)	-0.005 (-0.014, 0.003)	0.002 (-0.004, 0.007)	0.001 (-0.004, 0.005)	Late-pregnancy n _{cases} =30 1.02 (0.71, 1.48)
Socio-demographic model [‡]	-0.002 (-0.011, 0.007)	-0.005 (-0.013, 0.003)	0.002 (-0.003, 0.008)	0.002 (-0.001, 0.004)	0.89 (0.74, 1.42)
Lifestyle model [§]	-0.008 (-0.026, 0.010)	-0.004 (-0.020, 0.012)	-0.008 (-0.019, 0.002)	-0.011 (-0.019, -0.002)*	1.08 (0.52, 2.25)

SDS, standard deviation score. CI, Confidence Interval. UmPI, umbilical artery pulsatility index. UTRI, uterine artery resistance index. Values are regression coefficients (95% confidence interval) from multiple linear regression models and reflect the differences in umbilical artery pulsatility index and uterine artery resistance index per one increase in standard deviation score of maternal glycemic index and glycemic load during early-pregnancy. Estimates are from multiple imputed data. [†]Values are odds ratios (95% confidence interval) from multiple logistic regression models and reflect the difference in risks of bilateral notching per one increase in standard deviation score of maternal glycemic index and glycemic load during early-pregnancy. Estimates are from multiple imputed data. [‡]Basic models are adjusted for gestational age at time of intake. [§]Socio-demographic models are adjusted for maternal age, educational level, parity and gestational age at time of measurements. ^{||}Lifestyle models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of the measurements. *P-value <0.05.

Supplementary Table S8c. Sensitivity analysis: Associations of dietary glycemic index and load with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia in population with study enrollment <14 weeks of gestation (n=1,867)

	Gestational hypertensive disorders	Gestational hypertension	Preeclampsia
<u>Glycemic index (SDS)</u>	Odds ratio (95% CI) n _{cases} =119	Odds ratio (95% CI) n _{cases} =91	Odds ratio (95% CI) n _{cases} =28
Basic model [†]	1.01 (0.84, 1.23)	1.06 (0.85, 1.31)	0.88 (0.60, 1.30)
Socio-demographic model [‡]	1.01 (0.83, 1.24)	1.07 (0.85, 1.35)	0.84 (0.56, 1.27)
Lifestyle model [§]	1.01 (0.84, 1.22)	1.06 (0.85, 1.32)	0.87 (0.60, 1.25)
<u>Glycemic load (SDS)</u>	Odds ratio (95% CI) n _{cases} =119	Odds ratio (95% CI) n _{cases} =91	Odds ratio (95% CI) n _{cases} =28
Basic model [†]	1.04 (0.86, 1.25)	1.04 (0.84, 1.28)	1.06 (0.73, 1.53)
Socio-demographic model [‡]	1.05 (0.87, 1.27)	1.05 (0.85, 1.29)	1.10 (0.76, 1.59)
Lifestyle model [§]	1.04 (0.71, 1.55)	1.25 (0.80, 1.94)	0.59 (0.26, 1.33)

SDS, standard deviation score. CI, Confidence Interval. *Values are odds ratios (95% confidence interval) from multiple logistic regression models and reflect the difference in risks of gestational hypertensive disorders, gestational hypertension and preeclampsia per one increase in standard deviation score of maternal glycemic index and glycemic load during early-pregnancy. Estimates are from multiple imputed data. [†]Basic models are adjusted for gestational age at time of intake. [‡]Socio-demographic models are adjusted for maternal age, educational level, parity and gestational age at time of measurements. [§]Lifestyle models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of intake. *P-value <0.05.

Supplementary Table S9a. Sensitivity analysis: Associations of dietary glycemic index and glycemic load with systolic and diastolic blood pressure during pregnancy in complete cases^{*}

<u>Glycemic index (SDS)</u>	Differences in systolic blood pressure in mmHg (CI 95%)		
	Early-pregnancy	Mid-pregnancy	Late-pregnancy
Basic model [†]	0.38 (-0.06, 0.82) n=2,802	0.51 (0.11, 0.91)* n=3,263	0.58 (0.19, 0.97)* n=3,286
Lifestyle model [‡]	-0.03 (-0.52, 0.46) n=2,052	-0.13 (-0.58, 0.32) n=2,357	0.10 (-0.35, 0.54) n=2,394
	Differences in diastolic blood pressure in mmHg (CI 95%)		
	Early-pregnancy	Mid-pregnancy	Late-pregnancy
Basic model [†]	0.31 (-0.03, 0.65) n=2,802	0.41 (0.09, 0.72)* n=3,262	0.26 (-0.05, 0.58) n=3,285
Lifestyle model [‡]	0.15 (-0.23, 0.52) n=2,052	0.25 (-0.10, 0.60) n=2,356	0.25 (-0.10, 0.60) n=2,393
<u>Glycemic load (SDS)</u>	Differences in systolic blood pressure in mmHg (CI 95%)		
	Early-pregnancy	Mid-pregnancy	Late-pregnancy
Basic model [†]	0.81 (0.37, 1.25)* n=2,802	0.40 (-0.01, 0.80) n=3,263	0.47 (0.08, 0.86)* n=3,286
Lifestyle model [‡]	-0.13 (-1.10, 0.84) n=2,052	-0.46 (-1.34, 0.41) n=2,357	-0.08 (-0.94, 0.77) n=2,394
	Differences in diastolic blood pressure in mmHg (CI 95%)		
	Early-pregnancy	Mid-pregnancy	Late-pregnancy
Basic model [†]	0.35 (0.02, 0.69)* n=2,802	0.06 (-0.26, 0.38) n=3,262	-0.03 (-0.35, 0.28) n=3,285
Lifestyle model [‡]	0.76 (0.03, 1.50)* n=2,052	-0.09 (-0.77, 0.59) n=2,356	0.03 (-0.64, 0.69) n=2,393

SDS, standard deviation score. CI, Confidence Interval. *Values are regression coefficients (95% confidence interval) from multiple linear regression models and reflect the differences in mmHg blood pressure per one increase in standard deviation score of maternal glycemic index and glycemic load. Estimates are from complete cases (non-imputed data). [†]Basic models are adjusted for gestational age at time of intake. [‡]Lifestyle models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of the measurements. *P-value <0.05.

Supplementary Table S9b. Sensitivity analysis: Associations of dietary glycemic index and glycemic load with uterine artery resistance index, umbilical artery pulsatility index and bilateral uterine artery notching in complete cases

	Differences in UmPI (95% CI) [†]			Differences in UTRI (95% CI) [†]		Bilateral notching (95% CI) [†]	
	Mid-pregnancy	Late-pregnancy		Mid-pregnancy	Late-pregnancy	Early-pregnancy	Late-pregnancy
<u>Glycemic index (SDS)</u>							
Basic model [‡]	-0.001 (-0.008, 0.007) n=2,505	0.007 (0.000, 0.013) n=2,751		-0.004 (-0.008, 0.001) n=1,884	-0.001 (-0.004, 0.003) n=2,060	1.12 (0.84, 1.49) n _{cases} =48	
Lifestyle model [§]	-0.005 (-0.014, 0.003) n=1,798	0.003 (-0.005, 0.010) n=2,016		-0.003 (-0.008, 0.002) n=1,348	0.000 (-0.004, 0.004) n=1,495	1.04 (0.73, 1.48) n _{cases} =34	
<u>Glycemic load (SDS)</u>							
Basic model [‡]	-0.003 (-0.010, 0.004) n=2,505	0.002 (-0.004, 0.008) n=2,751		0.000 (-0.004, 0.005) n=1,884	0.000 (0.003, 0.004) n=2,060	0.99 (0.74, 1.33) n _{cases} =48	
Lifestyle model [§]	-0.009 (-0.026, 0.007) n=1,798	0.005 (-0.009, 0.019) n=2,016		0.001 (-0.009, 0.010) n=1,348	-0.008 (-0.016, 0.000) n=1,495	0.95 (0.85, 1.07) n _{cases} =34	

UmPI, umbilical artery pulsatility index. UTRI, uterine artery resistance index. SDS, standard deviation score. CI, Confidence Interval. Values are regression coefficients (95% confidence interval) from multiple linear regression models and reflect the differences in umbilical artery pulsatility index and uterine artery resistance index per one increase in standard deviation score of maternal glycemic index and glycemic load. Estimates are from complete cases (non-imputed data). [†]Values are odds ratios (95% confidence interval) from multiple logistic regression models and reflect the difference in risks of bilateral uterine artery notching per one increase in standard deviation score of maternal glycemic index and load. Estimates are from multiple imputed data. [‡]Basic models are adjusted for gestational age at time of intake. [§]Lifestyle models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of the measurements. *P-value <0.05.

Supplementary Table S9c. Sensitivity analysis: Associations of dietary glycemic index and glycemic load with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia in complete cases*

	Gestational hypertensive disorders	Gestational hypertension	Preeclampsia
	OR (95% CI)	OR (95% CI)	OR (95% CI)
<u>Glycemic index (SDS)</u>			
Basic model [†]	1.00 (0.87, 1.14) n _{cases} =224	1.02 (0.87, 1.19) n _{cases} =166	0.92 (0.71, 1.20) n _{cases} =58
Lifestyle model [‡]	0.99 (0.94, 1.05) n _{cases} =154	0.97 (0.91, 1.04) n _{cases} =116	0.87 (0.63, 1.19) n _{cases} =42
<u>Glycemic load (SDS)</u>			
Basic model [†]	1.04 (0.91, 1.19) n _{cases} =224	1.03 (0.88, 1.20) n _{cases} =166	1.06 (0.83, 1.37) n _{cases} =58
Lifestyle model [‡]	0.98 (0.69, 1.38) n _{cases} =514	1.06 (0.72, 1.57) n _{cases} =116	0.78 (0.42, 1.46) n _{cases} =42

SDS, standard deviation score; CI, Confidence Interval. *Values are odds ratios (95% confidence interval) from multiple logistic regression models and reflect the difference in risks of gestational hypertensive disorders, gestational hypertension and preeclampsia per one increase in standard deviation score of maternal glycemic index and glycemic load. Estimates are from complete cases (non-imputed data). [†]Basic models are adjusted for gestational age at time of intake. [‡]Lifestyle models are adjusted for maternal age, educational level, parity, prepregnancy BMI, kcal, smoking habits, alcohol use, folic acid use and gestational age at time of intake. *P-value <0.05.

CHAPTER

2.3

Maternal iron status in early-pregnancy and blood pressure throughout pregnancy, placental hemodynamics and the risk of gestational hypertensive disorders

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BACKGROUND In non-pregnant populations, higher serum ferritin, which reflects high iron stores, is associated with an increased risk of hypertension. We hypothesized that a dysregulated maternal iron status in early-pregnancy may lead to impaired gestational hemodynamic adaptations, leading to an increased risk of gestational hypertensive disorders.

OBJECTIVE Examine the associations of maternal iron status with maternal blood pressure, placental hemodynamic parameters and the risks of gestational hypertensive disorders.

METHODS In a population-based prospective cohort study among 5,983 pregnant women, we measured maternal serum ferritin, transferrin saturation, serum iron and transferrin concentrations at median 13.2 weeks gestation (95% range 9.6, 17.6). Maternal blood pressure was measured in early, mid, and late-pregnancy, and placental hemodynamic parameters in mid and late-pregnancy by ultrasound. Information on gestational hypertensive disorders was collected from medical records. We examined the associations of maternal early-pregnancy iron status with maternal systolic and diastolic blood pressure, placental hemodynamic parameters and the risks of gestational hypertensive disorders using linear and logistic regression models.

RESULTS Higher maternal early-pregnancy serum ferritin concentrations were associated with higher systolic and diastolic blood pressure throughout pregnancy in the basic models (p -values <0.05). After adjustment for maternal inflammation, sociodemographic and lifestyle factors, higher maternal early-pregnancy serum ferritin concentrations were only associated with a higher early-pregnancy diastolic blood pressure (0.27 (95% CI 0.03, 0.51) mmHg per SDS increase in serum ferritin) and with a higher mid-pregnancy umbilical artery pulsatility index (p -value <0.05). No associations were present with the risk of gestational hypertensive disorders.

CONCLUSIONS No consistent associations of maternal iron status in early-pregnancy with gestational hemodynamic adaptations or the risks of gestational hypertensive disorders were present. Further studies are needed to examine the potential role of iron metabolism in the development of gestational hypertensive disorders within higher risk populations.

INTRODUCTION

Gestational hypertensive disorders, which include gestational hypertension and preeclampsia, are a leading cause of maternal and neonatal morbidity and mortality¹. Gestational hypertension is characterized by the late onset of hypertension in pregnancy in previously normotensive women, while preeclampsia is defined as gestational hypertension with the presence of high protein levels in the urine². Both high and low maternal hemoglobin concentrations in early-pregnancy have been associated with elevated blood pressure levels during pregnancy, impaired placental function and a higher risk of gestational hypertensive disorders³⁻⁶. The underlying pathophysiological mechanisms for these associations are unclear, but it has been hypothesized that a dysregulated iron status may play a role.

A dysregulated iron status can cause oxidative stress. Iron overload leads to more production of reactive oxygen species (ROS), whereas iron deficiency can cause leakage of ROS through mitochondrial damage^{7,8}. Oxidative stress leads to endothelial damage and impaired vasoreactivity, which may negatively affect placental development and gestational hemodynamic adaptations, predisposing to the development of gestational hypertensive disorders⁹⁻¹³. Already in non-pregnant populations, increased serum ferritin concentrations, which reflect high iron stores, have been associated with the risk of hypertension, increased arterial stiffness, and a higher risk of cardiovascular disease¹⁴⁻²⁴. In pregnant populations, far less is known about the influence of maternal iron status in early-pregnancy on gestational hemodynamic adaptations and the risk of gestational hypertensive disorders²⁵⁻²⁹. Two observational studies among 484 healthy Polish pregnant women and 57 healthy nulliparous American women reported that lower serum iron concentrations at 12 weeks gestation, were associated with a higher risk of gestational hypertensive disorders^{25, 26}. In contrast, an observational study among 47 pregnant women with diabetes mellitus reported no association of serum iron concentrations at 12 weeks gestation with the risk of preeclampsia²⁷. On the contrary, also iron supplementation in early-pregnancy has been associated with a higher risk of gestational hypertensive disorders^{28, 29}.

We hypothesized that both decreased and increased maternal iron store concentrations are associated with a higher maternal blood pressure and impaired placental vascular resistance throughout pregnancy, leading to a higher risk of gestational hypertensive disorders. Therefore, we examined the associations of maternal early-pregnancy iron status with maternal systolic and diastolic blood pressure during pregnancy, placental hemodynamic parameters and the risks of gestational hypertensive disorders within a population-based cohort of 5,983 multi-ethnic pregnant women.

METHODS

Study design and study sample

This study was embedded in the Generation R Study, a prospective population-based cohort study from early-pregnancy onwards in Rotterdam, the Netherlands³⁰. Written informed consent was obtained for all participants. The study was approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center Rotterdam (MEC 198.782/2001/31). In total, 7,069 women enrolled in early-pregnancy. Data on maternal iron markers in early-pregnancy was available for 6,159 women. We excluded women with pre-existent hypertension (n=107), multiple pregnancies (n=68) and absent exposure data (n=1). The total population for analysis consisted of 5,983 women (**Figure 1**).

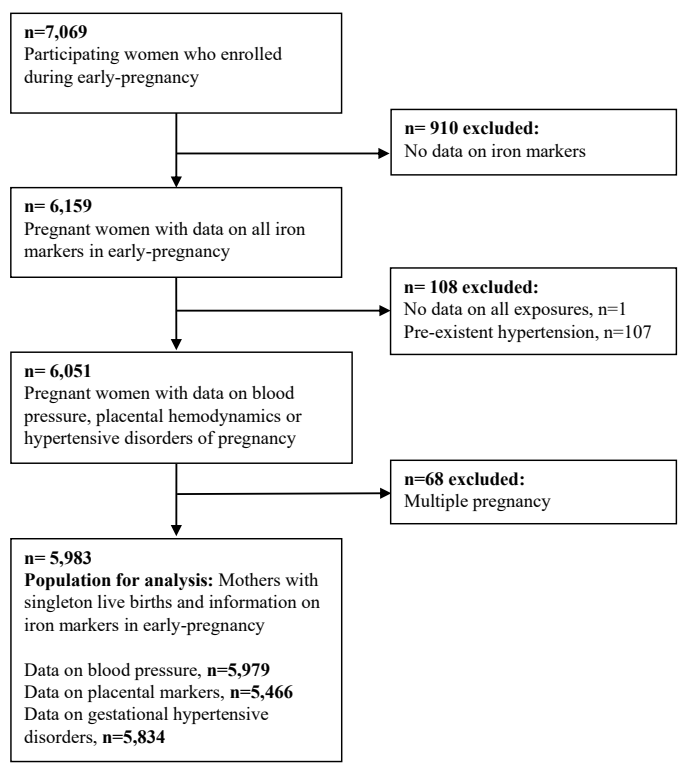


Figure 1. Flowchart of the study population.

Maternal iron status

Serum ferritin, serum iron and transferrin concentrations were measured from maternal non-fasting venous blood samples that were collected during early-pregnancy (median=13.2 weeks gestation (95% range 9.6, 17.6))³¹. Ferritin was measured by

electrochemiluminescence immunoassay on the Cobas e411 analyzer (Roche). Iron was measured by colorimetric assay and transferrin by immunoturbidimetric assay both by the C502 module on the Cobas 8000 (Roche)³². As serum ferritin is considered the gold standard to assess iron stores, we used serum ferritin concentrations as our primary exposure³³. To obtain further insight in maternal iron status, we additionally used transferrin saturation (TSAT), serum iron and transferrin as secondary exposures. These measures provide additional information on the bioavailability of iron in the body. TSAT was calculated using serum iron and transferrin concentrations ($TSAT = (\text{serum iron} * 100) / (\text{transferrin} * 25.1)$) to reflect the iron-bound part of the total iron binding capacity.

We constructed quintiles of all iron makers to assess whether associations were restricted to lower or higher iron stores, and to explore potential non-linear effects. We also constructed standard deviation scores (SDS) of all iron markers to assess the continuous associations across the full range. Serum ferritin was log-transformed prior to the construction of the SDS due to its skewed distribution.

Gestational hemodynamic adaptations

Systolic and diastolic blood pressure were measured in early- (median=13.2 weeks gestation, 95% range 9.6, 17.6), mid- (median=20.5 weeks gestation, 95% range 18.7, 23.1), and late-pregnancy (median=30.4 weeks gestation, 95% range 28.6, 32.8). An Omron 907 automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe BV, Hoofddorp, the Netherlands) was used to perform the blood pressure measurements, as previously described^{34, 35}. Two blood pressure measurements were performed at a 60-second interval, from which the mean blood pressure was calculated^{34, 35}.

Ultrasounds were performed in mid- (median=20.5 weeks gestation, 95% range 18.7, 23.1 weeks) and late-pregnancy (median=30.4 weeks gestation, 95% range 28.6, 32.8 weeks). Mid and late-pregnancy placental vascular resistance was evaluated with recorded flow velocity waveforms from the uterine and umbilical arteries³⁶. Umbilical artery pulsatility index (UmPI) was measured in a free-floating loop of the umbilical cord. Uterine artery resistance index (UtRI) was measured in the uterine arteries near the crossover with the external iliac artery. A higher uterine artery resistance index and umbilical artery pulsatility index indicate increased placental vascular resistance. The presence of uterine artery notching was assessed. The presence of uterine artery notching reflects an increase in resistance to blood flowing into the placenta and is used for identification of high-risk pregnancies.

Gestational hypertensive disorders

Gestational hypertensive disorder status was obtained from medical records, which were cross-checked with the original hospital charts³⁷. Gestational hypertension was defined as a diastolic blood pressure of at least 90 mmHg and/or systolic blood pressure of at least 140 mmHg after 20 weeks of gestation in women without previous hypertension. Preeclampsia was defined as gestational hypertension, including the presence of proteinuria (defined as a 24h urine sample containing at least 300 mg of protein, one catheter sample reading of at least 1+, or two or more dipstick readings of at least 2+)².

Covariates

Information on maternal age, ethnicity, pre-pregnancy weight, educational level, parity, folic acid supplementation, and maternal smoking was obtained from questionnaires. Ethnicity was categorized in European and non-European ethnicity. European ethnicity included Dutch and other European ethnicities. Non-European ethnicity included Indonesian, Cape Verdian, Moroccan, Antillean, Surinamese, Turkish, African, American (western and non-western), Asian (western and non-western), and Oceanian ethnicities. Height was measured at the intake visit and was used to calculate pre-pregnancy body mass index (BMI). Maternal hemoglobin and CRP concentrations were measured in the same non-fasting venous blood samples that were used for the measurement of the iron markers, as described previously³⁸.

Statistical analyses

First, we performed a non-response analysis comparing women with information on early-pregnancy maternal iron markers to those without. Second, we used one-way analyses of variance or chi-square tests to compare the participant characteristics across serum ferritin quartiles. Third, we examined the associations of maternal serum ferritin in categories (quintiles) and serum ferritin continuously (SDS) with systolic and diastolic blood pressure in early, mid and late-pregnancy, UmPI and UtRI in mid and late-pregnancy, and the risk of bilateral uterine artery notching using linear and logistic regression models, respectively. Fourth, we examined the associations of serum ferritin quintiles and SDS with the risk of gestational hypertensive disorders using logistic regression analyses. We constructed two different models: 1) basic model: adjusted for gestational age at the time of blood sampling and gestational age at the time of blood pressure measurement or ultrasound; 2) confounder model: additionally adjusted for maternal sociodemographic factors, lifestyle factors and inflammation, including maternal age, ethnicity, educational level,

parity, pre-pregnancy BMI, folic acid supplementation, smoking, and CRP concentrations. Confounders were based on the literature and selected if they were associated with serum ferritin and the outcomes of interest, or if they led to a change in effect estimate of >10% when the covariate was included into the univariate model. As the effects of an impaired maternal iron status may be more pronounced among pregnant women who also have abnormal hemoglobin levels, we calculated the interaction terms of maternal serum ferritin with maternal hemoglobin for each outcome. Only the interaction terms for maternal systolic and diastolic blood pressure were significant and we repeated these analyses stratified by maternal hemoglobin levels, defined as low (≤ 11 g/dl), normal (> 11 g/dl, < 13.2 g/dl), and high (≥ 13.2 g/dl) respectively³.

Next, we performed several sensitivity analyses to examine the robustness of our findings: 1) We repeated the analyses using maternal early-pregnancy TSAT, serum iron, and transferrin concentrations as secondary exposures that further reflect the bioavailability of maternal iron; 2) As higher serum ferritin concentrations may be explained by acute inflammation, we performed a sensitivity analysis excluding all women with CRP concentrations > 10 mg/ml who may suffer from acute inflammation, while still additionally adjusting for CRP concentrations across the full range to consider the impact of chronic low-grade inflammation; 3) We repeated the analyses with maternal serum ferritin concentrations, additionally adjusted for transferrin concentrations, as physiological changes in transferrin concentrations occur to regulate the bioavailability of the iron stores³⁹.

We imputed missing data of the covariates using multiple imputation. The percentage of missing values was $< 10\%$, with the exception for smoking (11%), pre-pregnancy BMI (18%) and folic acid supplementation (24%). P-values < 0.05 were considered significant. Analyses were performed using Statistical Package of Social Science (SPSS) version 25.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Participant characteristics

Table 1 shows the participant characteristics. Participating women were on average 30 years old, with a mean pre-pregnancy BMI of 23.5 kg/m^2 , 40% were higher educated, and 57% were nulliparous. The median serum ferritin concentration was $52.2 \text{ } \mu\text{g/L}$ (95% range 9.9, 203.7). Iron deficiency was observed in 7.2% (serum ferritin $< 15 \text{ } \mu\text{g/L}$) and iron overload was observed in 6.7% (serum ferritin $> 150 \text{ } \mu\text{g/L}$) of women⁴⁰. No differences in

Table 1. Characteristics of the study population by early-pregnancy serum ferritin quintiles (n=5,983)^a

	Total group n=5,983	Serum ferritin quintile 1 n=1,196	Serum ferritin quintile 2 n=1,197	Serum ferritin quintile 3 n=1,196	Serum ferritin quintile 4 n=1,198	Serum ferritin quintile 5 n=1,196	p-value ^f
Serum ferritin, range, µg/L	2 – 390	2 – 26	26 – 42	42 – 62	63 – 96	96 – 390	
Serum ferritin, µg/L	52.2 (9.9, 203.7)	17.3 (6.7, 25.9)	34.2 (26.8, 42.1)	52.2 (42.8, 62.4)	76.2 (63.3, 94.2)	128.4 (96.5, 295.7)	<0.001
TSAT, %	24.5 (10.5)	18.4 (8.9)	24.0 (8.9)	26.1 (9.9)	26.3 (10.4)	28.0 (11.7)	<0.001
Iron, µmol/L	17.1 (6.6)	14.7 (6.5)	17.5 (6.0)	17.9 (6.3)	17.6 (6.5)	18.0 (7.0)	<0.001
Transferrin, g/L	2.9 (0.5)	3.3 (0.5)	3.0 (0.4)	2.8 (0.4)	2.7 (0.4)	2.6 (0.3)	<0.001
Hemoglobin, g/dL	12.3 (0.9)	12.0 (1.0)	12.3 (0.9)	12.4 (0.9)	12.4 (0.9)	12.4 (0.9)	<0.001
CRP, mg/L	4.5 (0.6, 25.7)	4.8 (0.7, 23.6)	4.4 (0.6, 22.1)	4.4 (0.7, 24.4)	4.3 (0.6, 29.1)	4.4 (0.5, 32.8)	<0.001
Maternal age, years	29.7 ± 5.1	29.0 ± 5.6	29.4 ± 5.3	29.8 ± 5.0	30.1 ± 4.8	30.5 ± 4.4	<0.001
Parity, n nulliparous	3,399 (56.8)	481 (40.2)	602 (50.3)	682 (57.0)	764 (63.8)	870 (72.7)	<0.001
Pre-pregnancy BMI, kg/m ²	23.5 (4.2)	23.5 (4.1)	23.3 (4.2)	23.3 (4.0)	23.6 (4.2)	23.9 (4.4)	<0.001
Gestational age at blood sampling, weeks	13.2 (9.6, 17.6)	13.9 (7.6, 17.9)	13.5 (9.5, 17.7)	13.2 (9.6, 17.4)	12.9 (9.5, 17.4)	12.8 (9.5, 17.1)	<0.001
Education level, n higher	2,501 (41.8)	369 (30.9)	467 (39.0)	533 (44.6)	546 (45.6)	586 (49.0)	<0.001
Ethnicity, n European [†]	3,500 (61.0)	470 (39.3)	668 (55.8)	740 (61.9)	797 (66.5)	825 (69.0)	<0.001
Smoking, n continued during pregnancy	990 (16.5)	188 (15.7)	228 (19.0)	212 (17.7)	195 (16.3)	167 (14.0)	<0.05
Folic acid supplement use, n yes	3,413 (57.0)	532 (44.5)	634 (53.0)	703 (58.8)	755 (63.0)	789 (66.0)	<0.001
Systolic blood pressure, mmHg							
Early-pregnancy	115.3 (12.0)	113.5 (11.9)	114.8 (11.8)	115.1 (11.8)	115.9 (12.0)	116.9 (12.3)	<0.001
Mid-pregnancy	116.9 (11.8)	115.5 (11.6)	116.1 (11.6)	116.9 (11.5)	117.2 (12.1)	118.9 (11.9)	<0.001
Late-pregnancy	118.3 (11.8)	116.5 (11.6)	117.3 (11.4)	118.6 (12.2)	119.0 (12.0)	119.7 (11.8)	<0.001
Diastolic blood pressure, mmHg							
Early-pregnancy	67.9 (9.3)	66.8 (9.2)	67.2 (9.0)	67.7 (9.2)	68.6 (9.4)	69.4 (9.4)	<0.001
Mid-pregnancy	67.0 (9.2)	66.2 (9.3)	66.4 (8.6)	66.6 (9.1)	67.4 (9.5)	68.5 (9.3)	<0.001
Late-pregnancy	68.9 (9.2)	67.7 (9.5)	68.2 (8.7)	68.6 (9.2)	69.6 (9.2)	70.3 (9.0)	<0.001
Uterine artery resistance index							
Mid-pregnancy	0.54 (0.09)	0.54 (0.08)	0.54 (0.09)	0.54 (0.09)	0.55 (0.09)	0.53 (0.09)	0.08
Late-pregnancy	0.48 (0.08)	0.49 (0.08)	0.49 (0.08)	0.48 (0.08)	0.48 (0.08)	0.48 (0.08)	0.26
Umbilical artery pulsatility index							
Mid-pregnancy	1.20 (0.18)	1.18 (0.18)	1.20 (0.18)	1.20 (0.18)	1.21 (0.18)	1.22 (0.18)	<0.05
Late-pregnancy	0.98 (0.17)	0.98 (0.17)	0.98 (0.17)	0.98 (0.17)	0.98 (0.17)	0.98 (0.17)	0.94
Bilateral uterine artery notching	108 (1.8)	19 (1.6)	25 (2.1)	22 (1.8)	20 (1.7)	22 (1.8)	0.90
Gestational hypertensive disorders							
Preeclampsia	123 (2.1)	20 (1.7)	32 (2.7)	25 (2.1)	20 (1.7)	26 (2.2)	0.38
Gestational hypertension	228 (3.8)	26 (2.2)	35 (2.9)	44 (3.7)	47 (3.9)	76 (6.4)	<0.05

BMI, Body Mass Index; CRP, C-reactive protein; SD, standard deviation; TSAT, transferrin saturation. Values are mean (SD), median (95% range) or n (valid %). ^aDifferences between characteristics in serum ferritin quintiles were tested with one-way ANOVA for continuous variables, and χ^2 -test for categorical variables. ^bIn the full study population, European ethnicity consisted of 52.5% Dutch and 8.5% other European ethnicities. Non-European ethnicities, consisting of 8.5% Surinamese, 8.1% Turkish, 6.0% Moroccan, 4.2% Cape Verdean, 3.0% Indonesian, 2.9% Antillean, 2.7% Asian, 1.9% African and 0.1% Oceania.

maternal blood pressure, placental hemodynamic parameters or gestational hypertensive disorders were present for women with data on early-pregnancy iron markers compared to those without (**Supplementary Table S1**).

Early-pregnancy serum ferritin concentrations and blood pressure throughout pregnancy

In the basic models, higher serum ferritin concentrations across the full range were associated with higher systolic and diastolic blood pressure throughout pregnancy (all p-values <0.05) (**Supplementary Table S2**). After adjustment for maternal sociodemographic factors, lifestyle factors and inflammation, no associations of serum ferritin concentrations in quintiles or across the full range with systolic blood pressure throughout pregnancy were present (**Table 2**). Higher serum ferritin concentrations across the full range were only associated with higher early-pregnancy diastolic blood pressure (0.27 (95% CI 0.03, 0.51) mmHg per SDS increase in serum ferritin), but not with mid- or late diastolic blood pressure. No associations for the serum ferritin quintiles with diastolic blood pressure were present. Analyses stratified for maternal hemoglobin concentrations, showed that the strongest effect for serum ferritin concentrations across the full range with early-pregnancy diastolic blood pressure was present for women with high hemoglobin concentrations (p-value <0.05) (**Supplementary Table S3**).

Early-pregnancy serum ferritin concentrations and placental hemodynamic parameters

In the confounder model, as compared to the third serum ferritin quintile, the lowest serum ferritin quintile was associated with a lower mid-pregnancy UmPI, whereas the highest serum ferritin quintile was associated with a higher mid-pregnancy UmPI (all p-values <0.05) (**Table 3**). Higher serum ferritin concentrations across the full range were also associated with a higher mid-pregnancy UmPI (0.010 (95% CI 0.005, 0.016) per SDS increase in serum ferritin). No associations were present with the UtRI or the risk of bilateral uterine artery notching. In the basic models, similar findings were present (**Supplementary Table S4**).

Early-pregnancy serum ferritin concentrations and risks of gestational hypertensive disorders

Table 4 shows that serum ferritin quintiles and serum ferritin concentrations across the full range were not associated with the risks of any gestational hypertensive disorder in the

Table 2. Associations of early-pregnancy serum ferritin with systolic blood pressure and diastolic blood pressure during pregnancy (n=5,979)^a

Differences in systolic blood pressure (mmHg)						
Early-pregnancy		Mid-pregnancy		Late-pregnancy		
Early-pregnancy serum ferritin	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n
Quintile 1 [†]	-0.07 (-0.99, 0.85)	1,189	-0.09 (-1.02, 0.83)	1,121	-0.64 (-1.59, 0.31)	1,110
2–26 $\mu\text{g/L}$						
Quintile 2 [†]	0.33 (-0.57, 1.24)	1,190	-0.33 (-1.24, 0.57)	1,141	-0.75 (-1.67, 0.17)	1,146
26–42 $\mu\text{g/L}$						
Quintile 3	reference	1,187	reference	1,132	reference	1,127
42–63 $\mu\text{g/L}$						
Quintile 4 [†]	0.14 (-0.76, 1.04)	1,190	-0.36 (-1.26, 0.54)	1,156	-0.12 (-1.03, 0.80)	1,144
63–96 $\mu\text{g/L}$						
Quintile 5 [†]	0.35 (-0.56, 1.26)	1,184	0.64 (-0.26, 1.55)	1,159	-0.08 (-1.01, 0.84)	1,150
96–390 $\mu\text{g/L}$						
SDS [‡]	-0.01 (-0.31, 0.30)	5,940	0.20 (-0.10, 0.51)	5,709	0.18 (-0.13, 0.49)	5,709

Differences in diastolic blood pressure (mmHg)						
Early-pregnancy		Mid-pregnancy		Late-pregnancy		
Early-pregnancy serum ferritin	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n
Quintile 1 [†]	-0.23 (-0.94, 0.48)	1,189	0.16 (-0.56, 0.89)	1,120	-0.06 (-0.79, 0.67)	1,110
2–26 $\mu\text{g/L}$						
Quintile 2 [†]	-0.02 (-0.72, 0.68)	1,190	0.07 (-0.64, 0.78)	1,141	0.0 (-0.72, 0.72)	1,146
26–42 $\mu\text{g/L}$						
Quintile 3	reference	1,187	reference	1,132	reference	1,126
42–63 $\mu\text{g/L}$						
Quintile 4	0.53 (-0.16, 1.23)	1,190	0.38 (-0.33, 1.08)	1,155	0.56 (-0.15, 1.28)	1,144
63–96 $\mu\text{g/L}$						
Quintile 5 [†]	0.68 (-0.03, 1.38)	1,184	0.90 (0.18, 1.61)*	1,159	0.62 (-0.10, 1.34)	1,150
96–390 $\mu\text{g/L}$						
SDS [‡]	0.27 (0.03, 0.51)*	5,940	0.23 (-0.01, 0.47)	5,707	0.23 (-0.02, 0.47)	5,676

BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling, gestational age at time of blood pressure measurements and CRP levels. [†]Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 $\mu\text{g/L}$ – 63 $\mu\text{g/L}$) as reference. Estimates are from multiple imputed data. [‡]Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per log serum ferritin SDS. *P-value <0.05.

Table 3. Associations of early-pregnancy serum ferritin with umbilical artery pulsatility index, uterine artery resistance index, and third trimester bilateral uterine artery notching (n=5,466)^a

Early-pregnancy serum ferritin	Umbilical artery pulsatility index			Uterine artery resistance index			Bilateral notching		
	Mid-pregnancy		Late-pregnancy		Mid-pregnancy		Late-pregnancy		n _{cases}
	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	
Quintile 1 [†] 2–26 μg/L	-0.019 (-0.036, -0.002)*	914	-0.005 (-0.020, 0.011)	925	0.003 (-0.007, 0.012)	673	0.002 (-0.007, 0.010)	593	19
Quintile 2 [†] 26–42 μg/L	-0.002 (-0.018, 0.015)	922	-0.006 (-0.021, 0.010)	953	0.005 (-0.004, 0.015)	677	0.003 (-0.005, 0.012)	638	25
Quintile 3 42–63 μg/L	reference	922	reference	957	reference	699	reference	655	22
Quintile 4 [†] 63–96 μg/L	0.009 (-0.007, 0.026)	912	-0.006 (-0.021, 0.009)	974	0.009 (0.000, 0.018)	682	0.000 (-0.008, 0.008)	682	20
Quintile 5 [†] 96–390 μg/L	0.017 (0.000, 0.033)*	925	-0.006 (-0.022, 0.009)	973	-0.001 (-0.010, 0.008)	699	-0.005 (-0.013, 0.004)	695	22
SDS ^b	0.010 (0.005, 0.016)*	4,595	-0.001 (-0.006, 0.005)	4,782	-0.000 (-0.004, 0.003)	3,430	-0.002 (-0.005, 0.001)	3,263	108

BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking habits, gestational age at time of blood sampling, gestational age at time of ultrasound measurements and CRP levels. ^aValues are regression coefficients (95% confidence interval) and reflect differences in umbilical artery pulsatility index and uterine artery resistance index per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 μg/L–63 μg/L) as reference. Estimates are from multiple imputed data. ^bValues are odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 μg/L – 63 μg/L) as reference. Estimates are from multiple imputed data. ^cValues are regression coefficients (95% confidence interval) that reflect the difference in umbilical artery pulsatility index and uterine artery resistance index per log serum ferritin SDS or odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per log serum ferritin SDS. ^dP-value < 0.05.

Table 4. Associations of early-pregnancy serum ferritin with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia (n=5,834)^a

Early-pregnancy serum ferritin	Gestational Hypertensive Disorder		Gestational hypertension		Preeclampsia	
	Odds ratio (95% CI)	n _{cases}	Odds ratio (95% CI)	n _{cases}	Odds ratio (95% CI)	n _{cases}
Quintile 1 [†] 2 – 26 µg/L	0.85 (0.57, 1.27)	46	0.86 (0.52, 1.44)	26	0.84 (0.45, 1.55)	20
Quintile 2 [†] 26 – 42 µg/L	1.10 (0.77, 1.57)	67	0.95 (0.60, 1.52)	35	1.36 (0.80, 2.34)	32
Quintile 3 42 – 63 µg/L	reference	69	reference	44	reference	25
Quintile 4 [†] 63 – 96 µg/L	0.85 (0.60, 1.21)	67	0.90 (0.59, 1.39)	47	0.75 (0.41, 1.37)	20
Quintile 5 [†] 96 – 390 µg/L	1.20 (0.86, 1.66)	102	1.35 (0.91, 2.01)	76	0.94 (0.54, 1.65)	26
SDS [‡]	1.08 (0.96, 1.22)	351	1.15 (0.99, 1.34)	228	0.98 (0.81, 1.18)	123

BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. [†]Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling and CRP levels. [‡]Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 µg/L – 63 µg/L) as reference. Estimates are from multiple imputed data. [§]Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per log serum ferritin as SDS.

confounder model. In the basic model, the higher serum ferritin quintiles as compared to the third quintile, and serum ferritin concentrations across the full range were associated with a higher risk of gestational hypertensive disorders and gestational hypertension, but not preeclampsia (all p-values <0.05) (**Supplementary Table S5**).

Sensitivity analyses

Higher TSAT was associated with a lower systolic and diastolic blood pressure in early-pregnancy (p-values <0.05), but not in mid- or late-pregnancy in the confounder model (**Supplementary Table S6**). Higher serum iron concentrations were associated with a lower systolic blood pressure in early-pregnancy (p-value <0.05), but not in mid- or late-pregnancy or with diastolic blood pressure throughout pregnancy in the confounder model (**Supplementary Table S7**). Higher transferrin concentrations were associated with higher systolic and diastolic blood pressure throughout pregnancy (p-values <0.05) in the confounder model (**Supplementary Table S8**). No consistent associations were found for TSAT, serum iron and transferrin concentrations with placental hemodynamic parameters or the risks of any gestational hypertensive disorder (**Supplementary Tables S9-S14**). When we excluded women with acute inflammation (CRP >10mg/L), we found stronger associations for serum ferritin concentrations across the full range with diastolic blood pressure throughout pregnancy as compared to the main analysis for serum ferritin (all p-values <0.05) (**Supplementary Tables S15-S17**). We observed that higher serum

ferritin concentrations across the full range were associated with higher systolic and diastolic blood pressure throughout pregnancy (all p -values <0.05) when we additionally adjusted the confounder model for transferrin concentrations, but no associations were present for placental hemodynamic parameters or the risks of gestational hypertensive disorders (**Supplementary Tables S18-S20**).

DISCUSSION

In this population-based prospective cohort study, we found no consistent associations of maternal early-pregnancy iron status with maternal blood pressure and placental vascular resistance throughout pregnancy or the risks of gestational hypertensive disorders after considering maternal inflammatory, sociodemographic and lifestyle factors.

Gestational hypertensive disorders are a leading cause of maternal and neonatal morbidity and mortality¹. Increased oxidative stress has been suggested to play a role in impaired placental development and the pathophysiology of gestational hypertensive disorders⁴¹. Both iron overload and iron deficiency can induce increased levels of oxidative stress^{7,8}. High levels of oxidative stress can lead to endothelial damage and impaired vasoreactivity, which may negatively affect placental development and gestational hemodynamic adaptations, predisposing to the development of gestational hypertensive disorders⁹⁻¹³. We hypothesized that both higher and lower serum ferritin concentrations in early-pregnancy might lead to higher maternal blood pressure, impaired placental hemodynamics and an increased risk of gestational hypertensive disorders.

Already in non-pregnant populations, it has been suggested that higher serum ferritin concentrations are associated with the risk of hypertension, increased arterial stiffness, and a higher risk of cardiovascular disease, but findings are inconsistent¹⁴⁻²⁴. Two large prospective observational studies showed that higher serum ferritin concentrations were associated with an increased risk of hypertension^{14,16}. However, two large prospective observational studies among 4,509 Chinese and 2,895 French adult men and women, showed no associations of serum ferritin concentrations with the risk of hypertension after a more thorough adjustment for potential confounding factors^{42,43}. In line with these studies in non-pregnant populations, we did not find consistent associations of maternal iron status in early-pregnancy with maternal blood pressure development or placental vascular resistance throughout pregnancy, after adjustment for maternal inflammation, sociodemographic and lifestyle related factors. In the basic models we observed consistent associations of higher early-pregnancy serum ferritin concentrations with a higher maternal systolic and diastolic pressure throughout pregnancy, a higher umbilical artery pulsatility

index in mid-pregnancy and a higher risk of gestational hypertensive disorders. After adjustment for maternal inflammation, sociodemographic and lifestyle factors, only the associations of higher maternal early-pregnancy serum ferritin concentrations with a higher diastolic blood pressure in early-pregnancy and a higher umbilical artery pulsatility index in mid-pregnancy remained. This indicates that the associations of serum ferritin with our outcomes are for a large part explained by sociodemographic and lifestyle related factors, such as maternal age, BMI and parity. These observed tendencies may be more pronounced in early-pregnancy since the iron measurements were conducted close to these time-points. A high iron status may lead to a higher blood pressure during pregnancy and impaired placental function due to increased levels of oxidative stress^{10, 11, 13}. A high iron status may also lead to higher hemoglobin levels, which has been previously associated with higher blood pressure throughout pregnancy³. In the current study, we indeed observed the strongest effects of high serum ferritin concentrations on early-pregnancy diastolic blood pressure among pregnant women who also had a high hemoglobin level. Iron availability is strongly influenced through physiological feedback, which may be even more pronounced in pregnancy, since iron stores are increasingly being mobilized during the course of pregnancy to facilitate placental and fetal development⁴⁴. Iron availability in the body is largely influenced by levels of transferrin, which is the main iron carrier in the blood. Transferrin concentrations decrease in the presence of iron overload, but they increase in the presence of iron deficiency to make it readily available for cells to use. We observed that the associations of serum ferritin concentrations with systolic and diastolic blood pressure during pregnancy were stronger when additionally adjusting for transferrin, which may further suggest that relatively higher serum ferritin concentrations negatively affect maternal blood pressure development in pregnancy. However, the observed effect is only small from a clinical perspective, but may be considered important on a population level and from an etiological perspective. We observed no consistent associations for other secondary measures of maternal iron status with blood pressure development in pregnancy. Higher early-pregnancy TSAT was associated with lower systolic and diastolic blood pressure in early-pregnancy, and higher early-pregnancy serum iron was associated with lower systolic blood pressure in early-pregnancy only. We cannot explain these findings. It may reflect a chance finding, or be related to the measurement of these iron markers. Both TSAT and serum iron are influenced by recent dietary intake and diurnal variation⁴⁵. Thus, special care is needed in the interpretation of these findings, since these iron markers were determined from non-fasting blood samples and might not reflect the overall iron status accurately. To summarize, suboptimal maternal iron status in early-pregnancy within the normal range

was not consistently associated with maternal gestational hemodynamic adaptations after adjustment for inflammatory, sociodemographic and lifestyle factors. Further studies are needed to explore whether more pronounced iron overload or iron deficiency affects gestational hemodynamic adaptations.

In women already suffering from preeclampsia during later stages of pregnancy, apparent differences in iron biomarkers are present when compared to healthy pregnant women⁴⁶. However only few previous studies investigated the association of serum iron in early-pregnancy with the risk of gestational hypertensive disorders²⁵⁻²⁷. A case-control study from Poland among 484 healthy pregnant women, and an observational study from the United States among 57 healthy nulliparous women found that lower serum iron concentrations from fasting samples at 12 weeks gestation, were associated with a higher risk of gestational hypertensive disorders^{25,26}. It should be mentioned that adjustment for confounders was limited in these studies. In contrast, an observational study among 47 pregnant women with pre-gestational type 1 diabetes mellitus found no association of serum iron concentrations at 12 weeks gestation with preeclampsia²⁷. As gestational age at the time of blood sampling was quite similar in our and previous studies, it seems unlikely that differences in gestational age at measurement of iron status explained any discrepancies between our and previous studies. With regards to iron overload, a retrospective study from Thailand among 400 pregnant women showed that iron supplementation in early-pregnancy was associated with an increased risk of gestational hypertensive disorders²⁸. Similarly, a randomized placebo controlled trial among 727 Iranian non-anemic pregnant women reported that iron supplementation in early-pregnancy was associated with an increased risk of gestational hypertensive disorders, but baseline iron status was not determined in either of these studies²⁹. We did not find consistent associations of maternal early-pregnancy iron status with the risk of gestational hypertensive disorders. Different findings in these previous studies and our study may be explained by our non-fasting blood samples that influence the measurement of serum iron and our relatively healthy population in comparison, since most of these previous studies had a higher percentage of gestational hypertensive disorders cases. Thus, our findings suggest that within our population, iron status in early-pregnancy is not associated with the risk of gestational hypertensive disorders.

The response rate for participating in the Generation R Study was 61% for all the eligible women in the study area at the time of enrollment. Biased estimates are unlikely since they are more commonly caused by loss to follow-up rather than from non-response at baseline⁴⁷. Moreover, selection on the availability of iron status is unlikely to have affected the generalizability of the results, since we observed no substantial differences

in the characteristics of women with data on early-pregnancy iron markers compared to those without. Due to the design of our study, gestational age at measurement of iron status in early-pregnancy was relatively broad. During early placental development the syncytiotrophoblast can adapt to small increases in ROS by producing antioxidants⁴⁸. A dysregulated iron status in early-pregnancy may increase the risk of gestational hypertensive disorders through small increases in oxidative stress that negatively influence placental development. In our study, iron markers were measured at a median of 13.2 weeks, partly after early-placentation. However, we still consider our markers as adequate proxies of iron status from early gestation onwards, since the relative trend of iron status among the participants is likely to remain similar from conception to early-pregnancy. For example, women with relatively low iron levels at conception are likely to continue having lower iron levels in early-pregnancy compared to the rest of the study population. As individual absolute iron levels might differ depending on gestational age at blood sampling, we adjusted all analyses for gestational age at the time of blood sampling. We were able to adjust our analyses for maternal sociodemographic and lifestyle factors, but due to the observational nature of this study, residual confounding might still be present due to unmeasured factors such as iron supplementation.

We did not observe consistent associations of maternal iron status in early-pregnancy with gestational hemodynamic adaptations or the risk of gestational hypertensive disorders. Our study population was mainly composed of relatively young, higher educated women with a pre-pregnancy BMI within the normal range without pre-existent hypertension. Pregnant populations with older women with a higher BMI would be at a higher risk for gestational hypertensive disorders. Furthermore, all markers of iron metabolism were mostly within the normal range. The prevalence of iron deficiency (serum ferritin <15 µg/L) in our study population was 7% and was slightly lower, compared to the general Dutch population⁴⁹. Together, these factors may reflect a selection towards a relatively healthy and lower-risk pregnant population. Effects on gestational hemodynamic adaptations might be more pronounced in women with evidently low or high iron stores or at higher risk for the development of gestational hypertensive disorders. In addition, the relatively low number of cases of iron deficiency or iron overload, and of gestational hypertensive disorders may also have led to reduced statistical power. Studies in higher risk populations could help to consolidate these initial findings and assess the potential associations of more extreme dysregulations in iron metabolism with hemodynamic adaptations in pregnancy. Moreover, the interpretation of iron status is particularly difficult in pregnancy due to iron stores being increasingly mobilized with gestational age progression in a reaction to higher iron requirements to facilitate placental and

fetal development⁴⁴. Repeated measurement of fasting blood samples within the same participant are needed to assess longitudinal changes of iron parameters in pregnancy and their effect on maternal gestational hemodynamic adaptations. Since serum ferritin concentrations can be influenced by an inflammatory state, other important factors involved in the regulation of iron status such as hepcidin, IL-6 and erythropoietin, in combination with other markers of iron metabolism that are less affected by inflammation like the soluble transferrin receptor, could have aided the interpretation of our results^{50, 51}. This is especially important in the context of this study, since inflammatory processes are suggested to be involved in the pathophysiology of gestational hypertensive disorders⁵². Unfortunately, these markers were not available in our cohort due to the considerable costs of these measurements. Further studies including these markers could provide a broader picture on the role of maternal iron metabolism in the development of gestational hypertensive disorders.

Conclusion

We found no consistent associations of early-pregnancy serum ferritin concentrations with maternal blood pressure, placental hemodynamic parameters, or the risks of gestational hypertensive disorders after considering maternal inflammation, sociodemographic and lifestyle related factors. Further studies are needed to investigate the potential role of iron metabolism on gestational hemodynamic adaptations and the development of gestational hypertensive disorders within higher risk populations.

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. Non-response analysis: characteristics of participating women with and without data on iron markers¹

	Participants with data on iron markers	Participants without data on iron markers
	n=5,983	n=2,478
CRP levels, median (95% range), mg/L	4.5 (0.6, 25.7)	3.9 (0.5, 31.5)
Maternal age at enrolment, mean (SD), years	29.7 (5.1)	29.3 (5.9)
Parity, nulliparous n (%)	3,399 (56.8)	1,251 (51.6)
Pre-pregnancy BMI, mean (SD), kg/m ²	23.5 (4.2)	23.7 (4.5)
Gestational age at intake, median (95% range), weeks	13.4 (9.9, 17.8)	20.4 (11.9, 31.8)
Higher education n (%)	2,501 (41.8)	729 (34.5)
European ethnicity n (%)	3,500 (58.5)	1,042 (46.4)
Continued smoking during pregnancy n (%)	990 (16.5)	384 (18.7)
Folic acid supplement use n (%)	3,413 (57.0)	999 (59.3)
Systolic blood pressure, mean (SD), mmHg		
Early-pregnancy	115.3 (12.0)	114.5 (11.8)
Mid-pregnancy	116.9 (11.8)	114.9 (12.0)
Late-pregnancy	118.3 (11.8)	117.3 (12.0)
Diastolic blood pressure, mean (SD), mmHg		
Early-pregnancy	67.9 (9.3)	68.0 (9.6)
Mid-pregnancy	67.0 (9.2)	66.7 (9.2)
Late-pregnancy	68.9 (9.2)	68.7 (9.2)
Uterine artery resistance index, mean (SD)		
Mid-pregnancy	0.54 (0.09)	0.55 (0.09)
Late-pregnancy	0.48 (0.08)	0.49 (0.08)
Umbilical artery pulsatility index, mean (SD)		
Mid-pregnancy	1.20 (0.18)	1.20 (0.19)
Late-pregnancy	0.98 (0.17)	0.99 (0.17)
Bilateral uterine artery notching, n (%)	108 (1.8)	35 (2.8)
Gestational hypertensive disorders, n (%)		
Preeclampsia	123 (2.1)	45 (1.9)
Gestational hypertension	228 (3.8)	76 (3.2)

¹ Abbreviations: BMI, body mass index; CRP, C-reactive protein; SD, standard deviation.

Supplementary Table S2. Associations of early-pregnancy serum ferritin with systolic blood pressure and diastolic blood pressure during pregnancy in basic models (n=5,979).²

	Differences in systolic blood pressure (mmHg)					
	Early-pregnancy		Mid-pregnancy		Late-pregnancy	
Early-pregnancy serum ferritin	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n
Quintile 1 ³ 2 – 26 $\mu\text{g/L}$	-1.28 (-2.24, -0.31)*	1,189	-1.31 (-2.29, -0.33)*	1,121	-2.02 (-3.00, -1.04)*	1,110
Quintile 2 ³ 26 – 42 $\mu\text{g/L}$	-0.11 (-1.07, 0.85)	1,190	-0.75 (-1.72, 0.22)	1,141	-1.26 (-2.23, -0.28)*	1,146
Quintile 3 42 – 63 $\mu\text{g/L}$	reference	1,187	reference	1,132	reference	1,127
Quintile 4 ³ 63 – 96 $\mu\text{g/L}$	0.75 (-0.21, 1.71)	1,190	0.22 (-0.75, 1.18)	1,156	0.44 (-0.53, 1.41)	1,144
Quintile 5 ³ 96 – 390 $\mu\text{g/L}$	1.60 (0.64, 2.57)*	1,184	1.89 (0.93, 2.86)*	1,159	1.08 (0.11, 2.05)*	1,150
SDS ⁴	0.85 (0.54, 1.16)*	5,940	1.04 (0.73, 1.35)*	5,709	1.06 (0.75, 1.38)*	5,677
	Differences in diastolic blood pressure (mmHg)					
	Early-pregnancy		Mid-pregnancy		Late-pregnancy	
Early-pregnancy serum ferritin	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n
Quintile 1 ³ 2 – 26 $\mu\text{g/L}$	-0.61 (-1.35, 0.14)	1,189	-0.29 (-1.05, 0.48)	1,120	-0.75 (-1.51, 0.01)	1,110
Quintile 2 ³ 26 – 42 $\mu\text{g/L}$	-0.27 (-1.01, 0.48)	1,190	-0.18 (-0.94, 0.57)	1,141	-0.37 (-1.12, 0.39)	1,146
Quintile 3 42 – 63 $\mu\text{g/L}$	reference	1,187	reference	1,132	reference	1,126
Quintile 4 ³ 63 – 96 $\mu\text{g/L}$	0.92 (0.17, 1.66)*	1,190	0.79 (0.04, 1.54)*	1,155	0.98 (0.23, 1.73)*	1,144
Quintile 5 ³ 96 – 390 $\mu\text{g/L}$	1.61 (0.86, 2.35)*	1,184	1.96 (1.21, 2.71)*	1,159	1.64 (0.89, 2.40)*	1,150
SDS ⁴	0.71 (0.47, 0.95)*	5,940	0.73 (0.49, 0.97)*	5,707	0.81 (0.57, 1.06)*	5,676

¹Abbreviations: CI, Confidence Interval; SDS, standard deviation score. ²Models are adjusted for gestational age at time of blood sampling and gestational age at time of blood pressure measurements. ³Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 $\mu\text{g/L}$ – 63 $\mu\text{g/L}$) as reference. Estimates are from multiple imputed data. ⁴Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per log serum ferritin SDS. *P-value <0.05.

Supplementary Table S3. Associations of early-pregnancy serum ferritin with systolic and diastolic blood pressure stratified by low, normal or high hemoglobin levels (n=5,979)^{1,2,3}

Low hemoglobin levels (≤ 11 g/dl)					
Differences in systolic blood pressure (mmHg)					
Early-pregnancy		Mid-pregnancy		Late-pregnancy	
Early-pregnancy serum ferritin	β (95% CI)	n	β (95% CI)	n	β (95% CI)
SDS	-0.84 (-1.71, 0.03)	542	-0.55 (-1.49, 0.39)	510	-0.47 (-1.41, 0.47)
Differences in diastolic blood pressure (mmHg)					
Early-pregnancy		Mid-pregnancy		Late-pregnancy	
Early-pregnancy serum ferritin	β (95% CI)	n	β (95% CI)	n	β (95% CI)
SDS	-0.43 (-1.12, 0.27)	542	0.04 (-0.67, 0.75)	510	0.25 (-0.45, 0.95)
Normal hemoglobin levels (> 11 g/dl, < 13.2 g/dl)					
Differences in systolic blood pressure (mmHg)					
Early-pregnancy		Mid-pregnancy		Late-pregnancy	
Early-pregnancy serum ferritin	β (95% CI)	n	β (95% CI)	n	β (95% CI)
SDS	0.11 (-0.26, 0.48)	4,400	0.39 (0.02, 0.77)*	4,232	0.33 (-0.05, 0.70)
Differences in diastolic blood pressure (mmHg)					
Early-pregnancy		Mid-pregnancy		Late-pregnancy	
Early-pregnancy serum ferritin	β (95% CI)	n	β (95% CI)	n	β (95% CI)
SDS	0.26 (-0.02, 0.54)	4,400	0.29 (0.01, 0.58)*	4,231	0.18 (-0.10, 0.47)
High hemoglobin levels (≥ 13.2 g/dl)					
Differences in systolic blood pressure (mmHg)					
Early-pregnancy		Mid-pregnancy		Late-pregnancy	
Early-pregnancy serum ferritin	β (95% CI)	n	β (95% CI)	n	β (95% CI)
SDS	0.06 (-0.79, 0.91)	998	-0.33 (-1.15, 0.48)	968	0.09 (-0.78, 0.95)
Differences in diastolic blood pressure (mmHg)					
Early-pregnancy		Mid-pregnancy		Late-pregnancy	
Early-pregnancy serum ferritin	β (95% CI)	n	β (95% CI)	n	β (95% CI)
SDS	0.66 (0.00, 1.33)*	998	-0.27 (-0.94, 0.39)	967	0.15 (-0.56, 0.87)

*Abbreviations: CI, Confidence Interval; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling, gestational age at time of blood pressure measurements, and CRP levels. ³Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per log serum ferritin SDS. *P-value < 0.05 .

Supplementary Table S4. Associations of early-pregnancy serum ferritin with umbilical artery pulsatility index, uterine artery resistance index, and third trimester bilateral uterine artery notching in basic models (n=5,466)^{1,2}

Early-pregnancy serum ferritin	Umbilical artery pulsatility index				Uterine artery resistance index				Bilateral uterine artery notching	
	Mid-pregnancy		Late-pregnancy		Mid-pregnancy		Late-pregnancy		Late-pregnancy	
	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	Odds ratio (95% CI) ⁴	n _{cases}
Quintile 1 ³	-0.014 (-0.031, 0.002)	914	-0.005 (-0.020, 0.010)	925	0.006 (-0.004, 0.015)	673	0.003 (-0.005, 0.012)	593	0.96 (0.51, 1.80)	19
2 – 26 $\mu\text{g/L}$										
Quintile 2 ³	0.000 (-0.017, 0.016)	922	-0.006 (-0.021, 0.009)	953	0.007 (-0.003, 0.016)	677	0.004 (-0.005, 0.012)	638	1.18 (0.66, 2.11)	25
26 – 42 $\mu\text{g/L}$										
Quintile 3	reference	922	reference	957	reference	699	reference	655	reference	22
42 – 63 $\mu\text{g/L}$										
Quintile 4 ³	0.009 (-0.008, 0.025)	912	-0.006 (-0.021, 0.009)	974	0.008 (-0.001, 0.017)	682	0.000 (-0.009, 0.008)	682	0.87 (0.47, 1.61)	20
63 – 96 $\mu\text{g/L}$										
Quintile 5 ³	0.016 (0.000, 0.033)	925	-0.005 (-0.021, 0.010)	973	-0.003 (-0.012, 0.006)	699	-0.005 (-0.013, 0.003)	695	0.94 (0.52, 1.72)	22
96 – 390 $\mu\text{g/L}$										
SDS ⁵	0.008 (0.003, 0.014) [*]	4,595	0.000 (-0.005, 0.005)	4,782	-0.002 (-0.005, 0.001)	3,430	-0.003 (-0.006, -0.000) [*]	3,263	0.98 (0.81, 1.19)	108

¹Abbreviations: CI, Confidence Interval; SDS, standard deviation score. ²Models are adjusted for gestational age at time of blood sampling and gestational age at time of ultrasound measurements. ³Values are regression coefficients (95% confidence interval) and reflect differences in umbilical artery pulsatility index and uterine artery resistance index per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 $\mu\text{g/L}$ – 63 $\mu\text{g/L}$) as reference. Estimates are from multiple imputed data. ⁴Values are odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 $\mu\text{g/L}$ – 63 $\mu\text{g/L}$) as reference. Estimates are from multiple imputed data. ⁵Values are regression coefficients (95% confidence interval) that reflect the difference in umbilical artery pulsatility index and uterine artery resistance index per log serum ferritin SDS or odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per log serum ferritin SDS. ^{*}P-value < 0.05.

Supplementary Table S5. Associations of early-pregnancy serum ferritin with gestational hypertensive disorder, gestational hypertension and preeclampsia in basic models (n=5,834)^{1,2}

Early-pregnancy serum ferritin	Gestational Hypertensive Disorder		Gestational hypertension		Preeclampsia	
	Odds ratio (95% CI)	n _{CASES}	Odds ratio (95% CI)	n _{CASES}	Odds ratio (95% CI)	n _{CASES}
Quintile 1 ³ 2 – 26 µg/L	0.66 (0.45, 0.98)*	46	0.61 (0.37, 0.99)*	26	0.75 (0.42, 1.37)	20
Quintile 2 ³ 26 – 42 µg/L	0.98 (0.69, 1.38)	67	0.81 (0.52, 1.28)	35	1.25 (0.74, 2.13)	32
Quintile 3 42 – 63 µg/L	reference	69	reference	44	reference	25
Quintile 4 ³ 63 – 96 µg/L	0.97 (0.68, 1.37)	67	1.06 (0.70, 1.61)	47	0.80 (0.44, 1.45)	20
Quintile 5 ³ 96 – 390 µg/L	1.50 (1.10, 2.07)*	102	1.74 (1.19, 2.55)*	76	1.08 (0.62, 1.88)	26
SDS ⁴	1.28 (1.14, 1.43)*	351	1.43 (1.24, 1.64)*	228	1.06 (0.89, 1.28)	123

¹Abbreviations: CI, Confidence Interval; SDS, standard deviation score. ²Models are adjusted for gestational age at time of blood sampling. ³Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 µg/L – 63 µg/L) as reference. Estimates are from multiple imputed data. ⁴Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per log serum ferritin as SDS. *P-value <0.05.

Supplementary Table S6. Associations of early-pregnancy TSAT with systolic blood pressure and diastolic blood pressure during pregnancy (n=5,979)^{1,2}

Differences in systolic blood pressure (mmHg)						
TSAT	Early-pregnancy		Mid-pregnancy		Late-pregnancy	
	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n
Quintile 1 ³ 1 – 15 %	1.20 (0.28, 2.11)*	1,188	0.72 (-0.21, 1.64)	1,128	0.36 (-0.58, 1.30)	1,117
Quintile 2 ³ 15 – 21 %	0.18 (-0.72, 1.08)	1,194	-0.15 (-1.05, 0.76)	1,141	0.12 (-0.81, 1.04)	1,121
Quintile 3 21 – 26 %	reference	1,184	reference	1,124	reference	1,136
Quintile 4 ³ 26 – 33 %	-1.33 (-2.22, -0.43)*	1,187	0.23 (-0.67, 1.13)	1,151	-0.34 (-1.26, 0.58)	1,150
Quintile 5 ³ 33 – 85 %	-1.73 (-2.63, -0.82)*	1,187	-0.70 (-1.61, 0.21)	1,165	-0.64 (-1.56, 0.29)	1,153
SDS ⁴	-0.86 (-1.17, -0.56)*	5,940	-0.32 (-0.62, -0.02)*	5,709	-0.33 (-0.64, -0.02)*	5,677

Differences in diastolic blood pressure (mmHg)						
TSAT	Early-pregnancy		Mid-pregnancy		Late-pregnancy	
	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n
Quintile 1 ³ 1 – 15 %	1.04 (0.33, 1.76)*	1,188	0.59 (-0.13, 1.32)	1,128	0.42 (-0.31, 1.15)	1,117
Quintile 2 ³ 15 – 21 %	0.39 (-0.30, 1.09)	1,194	-0.16 (-0.87, 0.54)	1,140	0.17 (-0.54, 0.89)	1,121
Quintile 3 21 – 26 %	reference	1,184	reference	1,124	reference	1,136
Quintile 4 ³ 26 – 33 %	-0.60 (-1.30, 0.10)	1,187	0.16 (-0.54, 0.87)	1,151	0.16 (-0.55, 0.87)	1,149
Quintile 5 ³ 33 – 85 %	-0.75 (-1.45, -0.04)*	1,187	-0.33 (-1.05, 0.38)	1,164	-0.04 (-0.76, 0.68)	1,153
SDS ⁴	-0.47 (-0.70, -0.23)*	5,940	-0.11 (-0.35, 0.13)	5,707	-0.04 (-0.28, 0.20)	5,676

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score; TSAT, transferrin saturation. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling, gestational age at time of blood pressure measurements and CRP levels. ³Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per TSAT quintile. Groups are compared to women in quintile 3 (TSAT: 21% – 26%) as reference. Estimates are from multiple imputed data. ⁴Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per TSAT SDS. *P-value <0.05.

Supplementary Table S7. Associations of early-pregnancy serum iron with systolic blood pressure and diastolic blood pressure during pregnancy (n=5,979)^{1,2}

Differences in systolic blood pressure (mmHg)						
Serum iron	Early-pregnancy		Mid-pregnancy		Late-pregnancy	
	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n
Quintile 1 ³ 1 – 11 $\mu\text{mol/L}$	0.28 (-0.64, 1.20)	1,189	-0.21 (-1.12, 0.71)	1,134	-0.01 (-0.94, 0.93)	1,118
Quintile 2 ³ 11 – 15 $\mu\text{mol/L}$	0.23 (-0.67, 1.14)	1,163	-0.30 (-1.21, 0.61)	1,104	-0.04 (-0.96, 0.89)	1,094
Quintile 3 15 – 18 $\mu\text{mol/L}$	reference	1,196	reference	1,142	reference	1,147
Quintile 4 ³ 18 – 23 $\mu\text{mol/L}$	-1.13 (-2.03, -0.23)*	1,192	0.06 (-0.84, 0.96)	1,156	-0.45 (-1.36, 0.46)	1,157
Quintile 5 ³ 23 – 47 $\mu\text{mol/L}$	-1.21 (-2.11, -0.30)*	1,200	-0.51 (-1.41, 0.39)	1,173	-0.15 (-1.06, 0.77)	1,161
SDS ⁴	-0.52 (-0.82, -0.22)*	5,940	-0.02 (-0.32, 0.28)	5,709	-0.11 (-0.42, 0.20)	5,677

Differences in diastolic blood pressure (mmHg)						
Serum iron	Early-pregnancy		Mid-pregnancy		Late-pregnancy	
	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n
Quintile 1 ³ 1 – 11 $\mu\text{mol/L}$	0.48 (-0.23, 1.19)	1,189	0.33 (-0.39, 1.05)	1,134	0.25 (-0.48, 0.98)	1,118
Quintile 2 ³ 11 – 15 $\mu\text{mol/L}$	0.67 (-0.03, 1.37)	1,163	0.41 (-0.30, 1.13)	1,103	-0.01 (-0.73, 0.71)	1,094
Quintile 3 15 – 18 $\mu\text{mol/L}$	reference	1,196	reference	1,142	reference	1,147
Quintile 4 ³ 18 – 23 $\mu\text{mol/L}$	0.03 (-0.69, 0.70)	1,192	0.76 (0.06, 1.46)*	1,156	-0.03 (-0.71, 0.71)	1,156
Quintile 5 ³ 23 – 47 $\mu\text{mol/L}$	-0.25 (-0.95, 0.45)	1,200	0.26 (-0.45, 0.96)	1,172	0.36 (-0.35, 1.08)	1,161
SDS ⁴	-0.16 (-0.40, 0.07)	5,940	0.11 (-0.12, 0.34)	5,707	0.09 (-0.15, 0.33)	5,676

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling, gestational age at time of blood pressure measurements and CRP levels. ³Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per serum iron quintile. Groups are compared to women in quintile 3 (serum iron: 15 $\mu\text{mol/L}$ – 18 $\mu\text{mol/L}$) as reference. Estimates are from multiple imputed data. ⁴Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per serum iron SDS. *P-value <0.05.

Supplementary Table S8. Associations of early-pregnancy transferrin with systolic blood pressure and diastolic blood pressure during pregnancy (n=5,979)^{1,2}

Differences in systolic blood pressure (mmHg)					
Early-pregnancy			Mid-pregnancy		
Transferrin	β (95% CI)	n	β (95% CI)	n	β (95% CI)
Quintile 1 ³ 1.3 – 2.5 g/L	-1.61 (-2.56, -0.67)*	1,029	-0.81 (-1.75, 0.14)	1,004	-1.37 (-2.33, -0.41)*
Quintile 2 ³ 2.5 – 2.7 g/L	-0.57 (-1.51, 0.37)	1,025	0.06 (-0.88, 1.00)	987	-0.59 (-1.55, 0.37)
Quintile 3 2.7 – 2.9 g/L	reference	1,142	reference	1,097	reference
Quintile 4 ³ 2.9 – 3.2 g/L	0.09 (-0.79, 0.97)	1,345	1.02 (0.14, 1.90)*	1,299	-0.08 (-0.98, 0.81)
Quintile 5 ³ 3.2 – 4.8 g/L	1.95 (1.07, 2.83)*	1,399	1.42 (0.54, 2.31)*	1,322	0.60 (-0.30, 1.50)
SDS ⁴	1.16 (0.86, 1.45)*	5,940	0.86 (0.57, 1.16)*	5,709	0.62 (0.32, 0.93)*
Differences in diastolic blood pressure (mmHg)					
Early-pregnancy			Mid-pregnancy		
Transferrin	β (95% CI)	n	β (95% CI)	n	β (95% CI)
Quintile 1 ³ 1.3 – 2.5 g/L	-0.84 (-1.58, -0.11)*	1,029	-0.33 (-1.07, 0.41)	1,004	-0.44 (-1.19, 0.31)
Quintile 2 ³ 2.5 – 2.7 g/L	0.01 (-0.72, 0.74)	1,025	0.23 (-0.51, 0.97)	986	0.12 (-0.63, 0.87)
Quintile 3 2.7 – 2.9 g/L	reference	1,142	reference	1,096	reference
Quintile 4 ³ 2.9 – 3.2 g/L	0.68 (0.00, 1.36)	1,345	0.72 (0.03, 1.41)*	1,299	-0.10 (-0.80, 0.60)
Quintile 5 ³ 3.2 – 4.8 g/L	1.76 (1.07, 2.45)*	1,399	1.57 (0.87, 2.26)*	1,322	0.63 (-0.08, 1.33)
SDS ⁴	0.92 (0.69, 1.14)*	5,940	0.63 (0.40, 0.86)*	5,707	0.33 (0.09, 0.57)*

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling, gestational age at time of blood pressure measurements and CRP levels. ³Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per transferrin quintile. Groups are compared to women in quintile 3 (transferrin: 2.7 g/L – 2.9 g/L) as reference. Estimates are from multiple imputed data. ⁴Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per transferrin SDS. *P-value <0.05.

Supplementary Table S9. Associations of early-pregnancy TSAT with umbilical artery pulsatility index, uterine artery resistance index, and third trimester bilateral uterine artery notching (n=5,466)^{1,2}

TSAT	Umbilical artery pulsatility index			Uterine artery resistance index			Bilateral uterine artery notching		
	Mid-pregnancy	Late-pregnancy		Mid-pregnancy	Late-pregnancy		Late-pregnancy		n _{cases}
	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	
Quintile 1 ³ 1 – 15 %	-0.004 (-0.021, 0.012)	904	0.004 (-0.011, 0.020)	929	0.001 (-0.009, 0.010)	654	0.002 (-0.006, 0.011)	606	1.06 (0.55, 2.05)
Quintile 2 ³ 15 – 21 %	-0.010 (-0.026, 0.007)	913	-0.015 (-0.031, 0.000)*	957	0.000 (-0.009, 0.010)	669	0.002 (-0.007, 0.010)	652	0.97 (0.51, 1.86)
Quintile 3 ³ 21 – 26 %	reference	882	reference	944	reference	674	reference	636	reference
Quintile 4 ³ 26 – 33 %	0.005 (-0.011, 0.022)	936	-0.008 (-0.023, 0.007)	966	0.006 (-0.004, 0.015)	696	-0.001 (-0.010, 0.007)	672	1.36 (0.74, 2.50)
Quintile 5 ³ 33 – 85 %	0.008 (-0.008, 0.025)	960	-0.008 (-0.023, 0.007)	986	-0.001 (-0.010, 0.008)	737	-0.005 (-0.013, 0.003)	697	1.31 (0.71, 2.43)
SDS ⁵	0.005 (-0.001, 0.010)	4,595	-0.003 (-0.008, 0.002)	4,782	-0.002 (-0.005, 0.001)	3,430	-0.003 (-0.006, -0.000)*	3,263	1.06 (0.86, 1.29)

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score; TSAT, transferrin saturation. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking habits, gestational age at time of blood sampling, gestational age at time of ultrasound measurements and CRP levels. ³Values are regression coefficients (95% confidence interval) and reflect differences in umbilical artery pulsatility index and uterine artery resistance index per TSAT quintile. Groups are compared to women in quintile 3 (TSAT: 21% – 26 %) as reference. Estimates are from multiple imputed data. ⁴Values are odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per TSAT quintile. Groups are compared to women in quintile 3 (TSAT: 21% – 26%) as reference. Estimates are from multiple imputed data. ⁵Values are regression coefficients (95% confidence interval) that reflect the difference in umbilical artery pulsatility index and uterine artery resistance index per TSAT SDS or odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per TSAT SDS. *P-value < 0.05.

Supplementary Table S10. Associations of early-pregnancy serum iron with umbilical artery pulsatility index, uterine artery resistance index, and third trimester bilateral uterine artery notching (n=5,466)^{1,2}

	Umbilical artery pulsatility index				Uterine artery resistance index				Bilateral uterine artery notching	
	Mid-pregnancy		Late-pregnancy		Mid-pregnancy		Late-pregnancy		Late-pregnancy	
Serum iron	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	Odds ratio (95% CI) ⁴	n _{cases}
Quintile 1 ³ 1 – 11 $\mu\text{mol/L}$	0.006 (-0.010, 0.023)	923	0.016 (0.001, 0.032)*	943	-0.001 (-0.011, 0.008)	682	0.000 (-0.008, 0.009)	638	0.97 (0.52, 1.78)	23
Quintile 2 ³ 11 – 15 $\mu\text{mol/L}$	0.008 (-0.008, 0.025)	875	0.002 (-0.013, 0.017)	927	0.000 (-0.009, 0.010)	641	-0.003 (-0.012, 0.005)	616	0.82 (0.43, 1.53)	18
Quintile 3 ³ 15 – 18 $\mu\text{mol/L}$	reference	918	reference	964	reference	695	reference	645	reference	23
Quintile 4 ³ 18 – 23 $\mu\text{mol/L}$	0.008 (-0.009, 0.024)	922	0.010 (-0.005, 0.025)	953	-0.002 (-0.011, 0.008)	679	-0.005 (-0.014, 0.003)	661	1.01 (0.56, 1.83)	23
Quintile 5 ³ 23 – 47 $\mu\text{mol/L}$	0.020 (0.004, 0.037)*	957	0.002 (-0.013, 0.017)	995	0.001 (-0.008, 0.010)	733	-0.010 (-0.019, -0.002)*	703	0.88 (0.48, 1.62)	21
SDS ⁵	0.004 (-0.002, 0.009)	4,595	-0.004 (-0.009, 0.001)	4,782	-0.001 (-0.004, 0.002)	3,430	-0.003 (-0.006, -0.001)*	3,263	1.03 (0.84, 1.26)	108

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking habits, gestational age at time of blood sampling, gestational age at time of ultrasound measurements and CRP levels. ³Values are regression coefficients (95% confidence interval) and reflect differences in umbilical artery pulsatility index and uterine artery resistance index per serum iron quintile. Groups are compared to women in quintile 3 (serum iron: 15 $\mu\text{mol/L}$ – 18 $\mu\text{mol/L}$) as reference. Estimates are from multiple imputed data. ⁴Values are odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per serum iron quintile. Groups are compared to women in quintile 3 (serum iron: 15 $\mu\text{mol/L}$ – 18 $\mu\text{mol/L}$) as reference. Estimates are from multiple imputed data. ⁵Values are regression coefficients (95% confidence interval) that reflect the difference in umbilical artery pulsatility index and uterine artery resistance index per serum iron SDS or odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per serum iron SDS. *P-value < 0.05.

Supplementary Table S11. Associations of early-pregnancy transferrin with umbilical artery pulsatility index, uterine artery resistance index, and third trimester bilateral uterine artery notching (n=5,466)^{1,2}

	Umbilical artery pulsatility index			Uterine artery resistance index			Bilateral uterine artery notching	
	Mid-pregnancy	Late-pregnancy	Mid-pregnancy	Late-pregnancy	Mid-pregnancy	Late-pregnancy	Odds ratio (95% CI) ⁴	n _{cases}
Transferrin								
Quintile 1 ³								23
1.3 – 2.5 g/L								
Quintile 2 ³								19
2.5 – 2.7 g/L								
Quintile 3								27
2.7 – 2.9 g/L								
Quintile 4 ³								18
2.9 – 3.2 g/L								
Quintile 5 ³								21
3.2 – 4.8 g/L								
SDS ⁵								108

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking habits, gestational age at time of blood sampling, gestational age at time of ultrasound measurements and CRP levels. ³Values are regression coefficients (95% confidence interval) and reflect differences in umbilical artery pulsatility index and uterine artery resistance index per transferrin quintile. Groups are compared to women in quintile 3 (transferrin: 2.7 g/L – 2.9 g/L) as reference. Estimates are from multiple imputed data. ⁴Values are odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per transferrin quintile. Groups are compared to women in quintile 3 (transferrin: 2.7 g/L – 2.9 g/L) as reference. Estimates are from multiple imputed data. ⁵Values are regression coefficients (95% confidence interval) that reflect the difference in umbilical artery pulsatility index and uterine artery resistance index per transferrin SDS or odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per transferrin SDS. *P-value < 0.05.

Supplementary Table S12. Associations of early-pregnancy TSAT with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia (n=5,834)^{1,2}

TSAT	Gestational Hypertensive Disorder			Gestational hypertension			Preeclampsia		
	Odds ratio (95% CI)	n _{cases}		Odds ratio (95% CI)	n _{cases}		Odds ratio (95% CI)	n _{cases}	
Quintile 1 ³ 1 – 15 %	0.99 (0.69, 1.40)	82		0.98 (0.64, 1.50)	56		0.96 (0.54, 1.72)	26	
Quintile 2 ³ 15 – 21 %	0.69 (0.47, 1.00)	55		0.53 (0.33, 0.86)*	29		0.99 (0.56, 1.75)	26	
Quintile 3 21 – 26 %	reference	71		reference	47		reference	24	
Quintile 4 ³ 26 – 33 %	1.03 (0.73, 1.45)	75		1.02 (0.67, 1.55)	51		1.03 (0.58, 1.84)	24	
Quintile 5 ³ 33 – 85 %	0.94 (0.66, 1.34)	68		0.90 (0.59, 1.39)	45		1.02 (0.57, 1.84)	23	
SDS ⁴	1.05 (0.93, 1.18)	351		1.06 (0.91, 1.22)	228		1.04 (0.86, 1.26)	123	

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score; TSAT, transferrin saturation. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling and CRP levels. ³Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per TSAT quintile. Groups are compared to women in quintile 3 (TSAT: 21% – 26%) as reference. Estimates are from multiple imputed data. ⁴Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per TSAT as SDS. *P-value <0.05.

Supplementary Table S13. Associations of early-pregnancy serum iron with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia (n=5,834)^{1,2}

Serum iron	Gestational Hypertensive Disorder			Gestational hypertension			Preeclampsia		
	Odds ratio (95% CI)	n _{CASES}		Odds ratio (95% CI)	n _{CASES}		Odds ratio (95% CI)	n _{CASES}	
Quintile 1 ³	1.00 (0.70, 1.42)	81		1.05 (0.68, 1.64)	53		0.91 (0.52, 1.57)	28	
1 – 11 µmol/L									
Quintile 2 ³	0.79 (0.55, 1.14)	60		0.89 (0.57, 1.40)	41		0.63 (0.35, 1.15)	19	
11 – 15 µmol/L									
Quintile 3	reference	70		reference	42		reference	28	
15 – 18 µmol/L									
Quintile 4 ³	0.94 (0.66, 1.33)	66		1.07 (0.69, 1.65)	46		0.73 (0.41, 1.30)	20	
18 – 23 µmol/L									
Quintile 5 ³	1.10 (0.77, 1.55)	74		1.09 (0.70, 1.69)	46		1.13 (0.66, 1.94)	28	
23 – 47 µmol/L									
SDS ⁴	1.03 (0.92, 1.16)	351		1.03 (0.89, 1.19)	228		1.05 (0.86, 1.27)	123	

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling and CRP levels. ³Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per serum iron quintile. Groups are compared to women in quintile 3 (serum iron: 15 µmol/L – 18 µmol/L) as reference. Estimates are from multiple imputed data. ⁴Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per serum iron as SDS.

Supplementary Table S14. Associations of early-pregnancy transferrin with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia (n=5,834)^{1,2}

Transferrin	Gestational Hypertensive Disorder			Gestational hypertension			Preeclampsia		
	Odds ratio (95% CI)	n _{cases}		Odds ratio (95% CI)	n _{cases}		Odds ratio (95% CI)	n _{cases}	
Quintile 1 ³ 1.3 – 2.5 g/L	0.82 (0.57, 1.17)	61		0.88 (0.57, 1.36)	42		0.71 (0.39, 1.29)	19	
Quintile 2 ³ 2.5 – 2.7 g/L	0.88 (0.62, 1.24)	67		0.88 (0.57, 1.35)	43		0.91 (0.52, 1.59)	24	
Quintile 3 2.7 – 2.9 g/L	reference	78		reference	49		reference	29	
Quintile 4 ³ 2.9 – 3.2 g/L	0.82 (0.59, 1.14)	79		0.88 (0.58, 1.32)	54		0.72 (0.42, 1.24)	25	
Quintile 5 ³ 3.2 – 4.8 g/L	0.70 (0.50, 1.00)*	66		0.73 (0.47, 1.13)	40		0.70 (0.40, 1.20)	26	
SDS ⁴	0.94 (0.84, 1.06)	351		0.91 (0.78, 1.06)	228		1.00 (0.83, 1.21)	123	

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling and CRP levels. ³Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per transferrin quintile. Groups are compared to women in quintile 3 (transferrin: 2.7 g/L – 2.9 g/L) as reference. Estimates are from multiple imputed data. ⁴Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per transferrin as SDS. *P-value <0.05.

Supplementary Table S15. Associations of early-pregnancy serum ferritin with systolic blood pressure and diastolic blood pressure during pregnancy restricting to women with CRP <10 mg/L (n=4,892)^{1,2}

Differences in systolic blood pressure (mmHg)						
Early-pregnancy serum ferritin	Early-pregnancy		Mid-pregnancy		Late-pregnancy	
	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n
Quintile 1 ³	0.07 (-0.95, 1.09)	935	-0.06 (-1.09, 0.97)	881	-0.53 (-1.57, 0.51)	873
2 – 26 $\mu\text{g/L}$						
Quintile 2 ³	0.41 (-0.56, 1.39)	1,001	-0.44 (-1.41, 0.54)	957	-0.52 (-1.52, 0.47)	969
26 – 42 $\mu\text{g/L}$						
Quintile 3	reference	975	reference	935	reference	935
42 – 63 $\mu\text{g/L}$						
Quintile 4 ³	0.09 (-0.89, 1.06)	1,001	-0.50 (-1.50, 0.49)	972	-0.27 (-1.26, 0.72)	963
63 – 96 $\mu\text{g/L}$						
Quintile 5 ³	0.69 (-0.31, 1.68)	949	0.72 (-0.28, 1.72)	926	-0.40 (-1.41, 0.61)	921
96 – 390 $\mu\text{g/L}$						
SDS ⁴	0.07 (-0.28, 0.41)	4,861	0.22 (-0.12, 0.55)	4,671	0.02 (-0.33, 0.36)	4,661
Differences in diastolic blood pressure (mmHg)						
Early-pregnancy serum ferritin	Early-pregnancy		Mid-pregnancy		Late-pregnancy	
	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n
Quintile 1 ³	-0.17 (-0.96, 0.63)	935	0.02 (-0.80, 0.82)	881	-0.24 (-1.06, 0.57)	873
2 – 26 $\mu\text{g/L}$						
Quintile 2 ³	0.07 (-0.69, 0.83)	1,001	-0.02 (-0.80, 0.75)	957	0.07 (-0.71, 0.85)	969
26 – 42 $\mu\text{g/L}$						
Quintile 3	reference	975	reference	935	reference	934
42 – 63 $\mu\text{g/L}$						
Quintile 4 ³	0.66 (-0.10, 1.42)	1,001	0.27 (-0.50, 1.04)	971	0.57 (-0.21, 1.34)	963
63 – 96 $\mu\text{g/L}$						
Quintile 5 ³	0.99 (0.22, 1.77)*	949	1.14 (0.35, 1.92)*	926	0.61 (-0.19, 1.40)	921
96 – 390 $\mu\text{g/L}$						
SDS ⁴	0.33 (0.06, 0.60)*	4,861	0.32 (0.05, 0.59)*	4,670	0.27 (0.00, 0.54)*	4,660

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling, gestational age at time of blood pressure measurements and CRP levels. ³Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 $\mu\text{g/L}$ – 63 $\mu\text{g/L}$) as reference. Estimates are from multiple imputed data. ⁴Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per log serum ferritin SDS. *P-value <0.05.

Supplementary Table S16. Associations of early-pregnancy serum ferritin with umbilical artery pulsatility index, uterine artery resistance index, and third trimester bilateral uterine artery notching restricting to women with CRP <10 mg/L (n=4,892).^{1,2}

Early-pregnancy serum ferritin	Umbilical artery pulsatility index				Uterine artery resistance index				Bilateral uterine artery notching	
	Mid-pregnancy		Late-pregnancy		Mid-pregnancy		Late-pregnancy		Late-pregnancy	
	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	Odds ratio (95% CI) ⁴	n _{cases}
Quintile 1 ³ 2 – 26 $\mu\text{g/L}$	-0.031 (-0.050, -0.013)*	719	-0.007 (-0.025, 0.010)	723	0.001 (-0.011, 0.010)	529	0.002 (-0.008, 0.011)	471	0.88 (0.44, 1.78)	16
Quintile 2 ³ 26 – 42 $\mu\text{g/L}$	-0.010 (-0.028, 0.008)	773	-0.008 (-0.025, 0.008)	815	0.003 (-0.007, 0.013)	573	0.003 (-0.005, 0.012)	550	0.95 (0.50, 1.82)	20
Quintile 3 ³ 42 – 63 $\mu\text{g/L}$	reference	757	reference	799	reference	579	reference	555	reference	20
Quintile 4 ³ 63 – 96 $\mu\text{g/L}$	-0.001 (-0.017, 0.019)	770	-0.013 (-0.029, 0.004)	823	0.007 (-0.003, 0.017)	585	0.001 (-0.008, 0.010)	577	0.85 (0.45, 1.64)	18
Quintile 5 ³ 96 – 390 $\mu\text{g/L}$	0.005 (-0.014, 0.023)	741	-0.021 (-0.038, -0.004)*	788	-0.004 (-0.014, 0.006)	574	-0.006 (-0.015, 0.003)	560	0.60 (0.29, 1.23)	13
SDS ⁵	0.010 (0.004, 0.016)*	3,760	-0.004 (-0.010, 0.002)	3,948	0.000 (-0.003, 0.004)	2,840	-0.003 (-0.006, 0.000)	2,713	0.90 (0.71, 1.14)	87

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking habits, gestational age at time of blood sampling, gestational age at time of ultrasound measurements and CRP levels. ³Values are regression coefficients (95% confidence interval) and reflect differences in umbilical artery pulsatility index and uterine artery resistance index per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 $\mu\text{g/L}$ – 63 $\mu\text{g/L}$) as reference. Estimates are from multiple imputed data. ⁴Values are odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 $\mu\text{g/L}$ – 63 $\mu\text{g/L}$) as reference. Estimates are from multiple imputed data. *P-value <0.05.

Supplementary Table S17. Associations of early-pregnancy serum ferritin with gestational hypertensive disorder, gestational hypertension and preeclampsia restricting to women with CRP <10 mg/L (n=4,892)

Early-pregnancy serum ferritin	Gestational Hypertensive Disorder		Gestational hypertension		Preeclampsia	
	Odds ratio (95% CI)	n _{cases}	Odds ratio (95% CI)	n _{cases}	Odds ratio (95% CI)	n _{cases}
Quintile 1 ³ 2 – 26 µg/L	0.70 (0.45, 1.11)	32	0.86 (0.49, 1.51)	21	0.50 (0.24, 1.07)	11
Quintile 2 ³ 26 – 42 µg/L	0.98 (0.66, 1.45)	53	0.87 (0.52, 1.46)	28	1.11 (0.61, 2.01)	25
Quintile 3 ³ 42 – 63 µg/L	reference	59	reference	37	reference	22
Quintile 4 ³ 63 – 96 µg/L	0.81 (0.55, 1.19)	54	0.86 (0.53, 1.38)	37	0.72 (0.38, 1.37)	17
Quintile 5 ³ 96 – 390 µg/L	1.03 (0.71, 1.48)	71	1.13 (0.73, 1.77)	52	0.83 (0.44, 1.56)	19
SDS ⁴	1.09 (0.95, 1.25)	269	1.10 (0.92, 1.30)	175	1.07 (0.86, 1.34)	94

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling and CRP levels. ³Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 µg/L – 63 µg/L) as reference. Estimates are from multiple imputed data. ⁴Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per log serum ferritin as SDS.

Supplementary Table S18. Associations of early-pregnancy serum ferritin with systolic blood pressure and diastolic blood pressure during pregnancy additionally adjusting for transferrin levels (n=5,979)^{1,2}

Differences in systolic blood pressure (mmHg)							
Early-pregnancy serum ferritin		Early-pregnancy		Mid-pregnancy		Late-pregnancy	
		β (95% CI)	n	β (95% CI)	n	β (95% CI)	n
Quintile 1 ³		-1.74 (-2.72, -0.76)*	1,189	-1.39 (-2.37, -0.40)*	1,121	-1.66 (-2.67, -0.65)*	1,110
2 – 26 μg/L							
Quintile 2 ³		-0.18 (-1.08, 0.72)	1,190	-0.72 (-1.63, 0.19)	1,141	-1.07 (-2.00, -0.15)*	1,146
26 – 42 μg/L							
Quintile 3	reference	reference	1,187	reference	1,132	reference	1,127
42 – 63 μg/L							
Quintile 4 ³		0.40 (-0.49, 1.30)	1,190	-0.16 (-1.05, 0.74)	1,156	0.04 (-0.87, 0.96)	1,144
63 – 96 μg/L							
Quintile 5 ³		0.96 (0.05, 1.87)*	1,184	1.11 (0.20, 2.02)*	1,159	0.29 (-0.65, 1.22)	1,150
96 – 390 μg/L							
SDS ⁴		0.83 (0.47, 1.18)*	5,940	0.90 (0.54, 1.25)*	5,709	0.69 (0.33, 1.06)*	5,677

Differences in diastolic blood pressure (mmHg)							
Early-pregnancy serum ferritin		Early-pregnancy		Mid-pregnancy		Late-pregnancy	
		β (95% CI)	n	β (95% CI)	n	β (95% CI)	n
Quintile 1 ³		-1.70 (-2.46, -0.95)*	1,189	-0.84 (-1.61, -0.06)*	1,120	-0.67 (-1.46, 0.12)	1,110
2 – 26 μg/L							
Quintile 2 ³		-0.47 (-1.17, 0.23)	1,190	-0.23 (-0.94, 0.48)	1,141	-0.19 (-0.91, 0.53)	1,146
26 – 42 μg/L							
Quintile 3	reference	reference	1,187	reference	1,132	reference	1,126
42 – 63 μg/L							
Quintile 4 ³		0.77 (0.07, 1.46)*	1,190	0.53 (-0.17, 1.23)	1,155	0.66 (-0.06, 1.37)	1,144
63 – 96 μg/L							
Quintile 5 ³		1.21 (0.51, 1.92)*	1,184	1.26 (0.54, 1.97)*	1,159	0.84 (0.11, 1.57)*	1,150
96 – 390 μg/L							
SDS ⁴		1.03 (0.75, 1.31)*	5,940	0.77 (0.49, 1.05)*	5,707	0.54 (0.26, 0.82)*	5,676

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling, gestational age at time of blood pressure measurements, CRP levels and transferrin levels. ³Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 $\mu\text{g/L}$ – 63 $\mu\text{g/L}$) as reference. Estimates are from multiple imputed data. ⁴Values are regression coefficients (95% confidence interval) and reflect the difference in mmHg blood pressure per log serum ferritin SDS. *P-value <0.05.

Supplementary Table S19. Associations of early-pregnancy serum ferritin with umbilical artery pulsatility index, uterine artery resistance index, and third trimester bilateral uterine artery notching additionally adjusting for transferrin levels (n=5,466)^{1,2}

Early-pregnancy serum ferritin	Umbilical artery pulsatility index				Uterine artery resistance index				Bilateral uterine artery notching	
	Mid-pregnancy		Late-pregnancy		Mid-pregnancy		Late-pregnancy		Late-pregnancy	
	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	β (95% CI)	n	Odds ratio (95% CI) ⁴	n _{cases}
Quintile 1 ³ 2 – 26 $\mu\text{g/L}$	-0.019 (-0.037, -0.001)*	914	-0.002 (-0.019, 0.014)	925	-0.001 (-0.011, 0.010)	673	0.002 (-0.007, 0.012)	593	1.01 (0.51, 2.02)	19
Quintile 2 ³ 26 – 42 $\mu\text{g/L}$	-0.002 (-0.018, 0.015)	922	-0.005 (-0.020, 0.010)	953	0.004 (-0.005, 0.014)	677	0.004 (-0.005, 0.012)	638	1.18 (0.65, 2.15)	25
Quintile 3 ³ 42 – 63 $\mu\text{g/L}$	reference	922	reference	957	reference	699	reference	655	reference	22
Quintile 4 ³ 63 – 96 $\mu\text{g/L}$	0.009 (-0.007, 0.026)	912	-0.006 (-0.021, 0.009)	974	0.009 (0.000, 0.019)*	682	0.000 (-0.008, 0.008)	682	0.86 (0.46, 1.60)	20
Quintile 5 ³ 96 – 390 $\mu\text{g/L}$	0.016 (0.000, 0.033)	925	-0.007 (-0.023, 0.008)	973	0.000 (-0.010, 0.009)	699	-0.005 (-0.013, 0.004)	695	0.89 (0.48, 1.65)	22
SDS ⁵	0.010 (0.004, 0.017)*	4,595	-0.002 (-0.008, 0.004)	4,782	0.001 (-0.003, 0.005)	3,430	-0.003 (-0.006, 0.000)	3,263	0.94 (0.74, 1.21)	108

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking habits, gestational age at time of blood sampling, gestational age at time of ultrasound measurements, CRP levels and transferrin levels. ³Values are regression coefficients (95% confidence interval) and reflect differences in umbilical artery pulsatility index and uterine artery resistance index per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 $\mu\text{g/L}$ – 63 $\mu\text{g/L}$) as reference. Estimates are from multiple imputed data. ⁴Values are odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 $\mu\text{g/L}$ – 63 $\mu\text{g/L}$) as reference. Estimates are from multiple imputed data. ⁵Values are regression coefficients (95% confidence interval) that reflect the difference in umbilical artery pulsatility index and uterine artery resistance index per log serum ferritin SDS or odds ratios (95% confidence interval) that reflect difference in risks of third trimester bilateral uterine artery notching per log serum ferritin SDS. *P-value < 0.05.

Supplementary Table S20. Associations of early-pregnancy serum ferritin with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia additionally adjusting for transferrin levels (n=5,834)^{1,2}

Early-pregnancy serum ferritin	Gestational Hypertensive Disorder			Gestational hypertension			Preeclampsia		
	Odds ratio (95% CI)	n _{CASES}		Odds ratio (95% CI)	n _{CASES}		Odds ratio (95% CI)	n _{CASES}	
Quintile 1 ³ 2 – 26 µg/L	0.88 (0.58, 1.34)	46		0.90 (0.53, 1.54)	26		0.84 (0.44, 1.62)	20	
Quintile 2 ³ 26 – 42 µg/L	1.11 (0.78, 1.59)	67		0.97 (0.61, 1.54)	35		1.37 (0.79, 2.35)	32	
Quintile 3 42 – 63 µg/L	reference	69		reference	44		reference	25	
Quintile 4 ³ 63 – 96 µg/L	0.85 (0.59, 1.21)	67		0.90 (0.58, 1.38)	47		0.75 (0.41, 1.37)	20	
Quintile 5 ³ 96 – 390 µg/L	1.18 (0.85, 1.65)	102		1.33 (0.89, 1.98)	76		0.94 (0.53, 1.66)	26	
SDS ⁴	1.07 (0.93, 1.22)	351		1.13 (0.95, 1.34)	228		0.97 (0.78, 1.21)	123	

¹Abbreviations: BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; SDS, standard deviation score. ²Models are adjusted for maternal age, ethnicity, educational level, parity, pre-pregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling, CRP levels and transferrin levels. ³Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 µg/L – 63 µg/L) as reference. Estimates are from multiple imputed data. ⁴Values are odds ratios (95% confidence interval) that reflect difference in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per log serum ferritin as SDS.

CHAPTER



CARDIOVASCULAR- LAF OUTCOMES IN CHILDHOOD



CHAPTER

3.1

Gestational hypertensive disorders and blood pressure throughout pregnancy, and alterations in cardiac structure and function in childhood

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AIM To assess whether gestational hypertensive disorders and higher maternal blood pressure during pregnancy were associated with subclinical changes in offspring cardiac structure and function during childhood.

METHODS In a population-based prospective study among 2,502 mother-offspring pairs, maternal blood pressure was measured in early, mid, and late-pregnancy and information on gestational disorders were obtained from medical records. Offspring left and right ventricular end-diastolic volumes and ejection fractions, and left ventricular mass were assessed by Cardiovascular Magnetic Resonance at the median age of 10 years.

RESULTS Offspring exposed to preeclampsia, but not gestational hypertension, had a lower right ventricular ejection fraction (difference: -0.31 SDS (95% CI -0.60 , -0.02)), however no associations with other cardiac outcomes were present. Higher maternal diastolic blood pressure in early and late-pregnancy were associated with lower left and right ventricular end-diastolic volumes (p -values <0.05), with the strongest effect in early-pregnancy. No associations of systolic blood pressure with offspring outcomes were present. These associations persisted after additional adjustment for birth and child factors. Paternal systolic and diastolic blood pressure were not associated with offspring cardiac outcomes.

CONCLUSIONS No consistent associations of gestational hypertensive disorder status with childhood cardiac outcomes were present. Higher maternal diastolic blood pressure throughout pregnancy, but not systolic blood pressure, was associated with lower childhood left and right ventricular end-diastolic volumes. Stronger maternal-offspring than paternal-offspring associations were present, which may suggest that suboptimal maternal gestational hemodynamic adaptations affects offspring cardiac structure through direct intrauterine effects. Further studies need to replicate these findings and examine underlying mechanisms.

INTRODUCTION

Multiple large-scale observational studies have shown that maternal preeclampsia is associated with offspring congenital cardiac defects, ranging from minor septal defects to more severe cardiac anomalies¹⁻³. These findings suggest a potential pathophysiological link between the development of gestational hypertensive disorders and abnormal fetal cardiogenesis, but only few studies investigated whether gestational hypertensive disorders are also associated with subclinical alterations in cardiac structure and function in the offspring. Two previous observational studies suggest alterations in cardiac structure during infancy and adolescence in offspring exposed to gestational hypertensive disorders, but no studies have been performed during childhood^{4,5}. Gestational hypertension and preeclampsia represent the extremes of the gestational hypertensive disorder spectrum. These pregnancy complications seem to reflect an extreme inability of the maternal cardiovascular system to adequately adapt to pregnancy. A higher maternal blood pressure during pregnancy, already below the clinical definition of gestational hypertensive disorders, has been associated with several adverse cardiovascular outcomes in the offspring⁶⁻⁸. It is unknown whether a higher maternal blood pressure during the course of pregnancy is also associated with alterations in cardiac structure and function during childhood.

Cardiomyocytes are predominantly formed during the first trimester of pregnancy and are directly responsible for a considerable part of the myocardial performance during an individual's life^{9,10}. Impaired maternal gestational hemodynamic adaptations may lead to reduced uteroplacental perfusion, a relative state of hypoxia and increased placental vascular resistance, which may affect fetal cardiac development through a direct intra-uterine effect¹¹⁻¹³. However, observed differences in offspring cardiac structure and function could also reflect risk factors or genetic predisposition shared between a mother and child, especially since women with a history of gestational hypertension or preeclampsia also have alterations in cardiac structure and function^{3,14,15}.

We hypothesized that offspring exposed to gestational hypertensive disorders, and already a higher gestational blood pressure across the full range, display subclinical alterations in cardiac structure and function during childhood. The right ventricle is predominant in the fetal circulation and increased placental resistance associated with gestational hypertensive disorders primarily influences right ventricular pressures during intrauterine life¹⁶. Therefore, we expected stronger effects on right ventricular measures. We first examined the associations of gestational hypertension and preeclampsia, and maternal blood pressure throughout pregnancy with offspring cardiac structure and

function measured by Cardiovascular Magnetic Resonance (CMR) at the age of 10 years among 2,502 mother-offspring pairs. Second, we explored whether critical periods for the associations of maternal gestational blood pressure with these offspring outcomes were present as we expected the strongest effect in early-pregnancy. Lastly, we explored potential underlying mechanisms by a maternal-offspring and paternal-offspring association comparison. Stronger maternal-offspring associations would support a direct intra-uterine effect on fetal cardiac development.

METHODS

Design and study population

This study was embedded in the Generation R study, a population-based prospective cohort from fetal life onwards in Rotterdam, The Netherlands¹⁷. All participants gave written informed consent. The study was approved by the local Medical Ethical Committee (MEC 198.782/2001/31). Of 8,879 women that were enrolled during pregnancy, of which 5 women did not have blood pressure measurements during pregnancy or information on gestational hypertensive disorders. Women with pre-existent hypertension (n=141) and non-singleton non-live births (n=201) were excluded. A random subgroup of 2,978 children were invited for CMR measurements at the age of 10 years, of which good quality CMR scans were available for 2,519 children. We excluded 17 children with a cardiac anomaly. The total population for analysis consisted of 2,502 mother-offspring pairs, of which 2,454 mother-offspring pairs had data on gestational hypertensive disorders and 2,497 mother-offspring pairs had data on gestational blood pressure (**Figure 1**).

Parental blood pressure and gestational hypertensive disorders

Maternal blood pressure was measured in early, mid and late-pregnancy (medians, IQR 13.1 (12.1, 14.5), 20.4 (19.9, 20.9), 30.2 (29.9, 30.8) weeks gestation, respectively), as described previously¹⁸. Paternal blood pressure was measured at study enrollment. An Omron 907 automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe BV, Hoofddorp, The Netherlands) was used for the maternal and paternal blood pressure measurements¹⁹. The mean of two measurements with a 60 second interval was used for further analysis.

Information on gestational hypertensive disorders was obtained from medical records that were cross-checked with the original hospital charts²⁰. In short, gestational hypertension was defined as a systolic blood pressure of at least 140 mmHg and/or

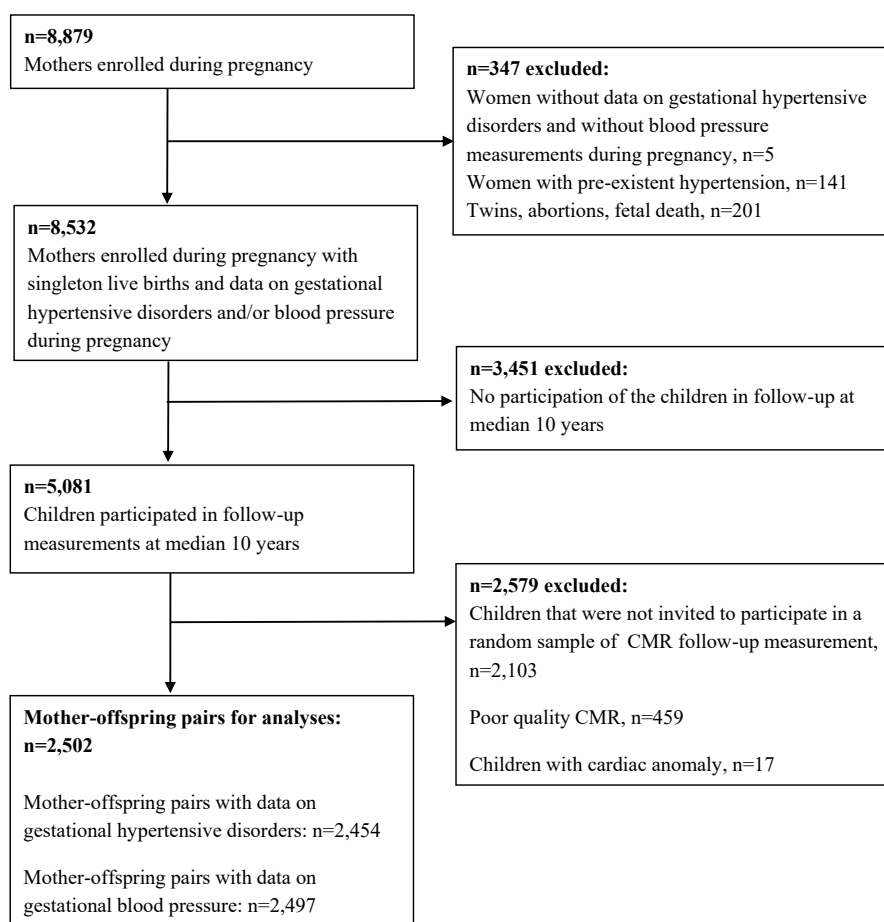


Figure 1. Flowchart of the study population.

diastolic blood pressure of at least 90 mmHg after 20 weeks of gestation in previously normotensive women. These criteria including the manifestation of proteinuria were used to identify preeclampsia²¹.

Offspring cardiac measurements in childhood

At median 9.9 years (IQR 9.8, 10.4), Cardiovascular Magnetic Resonance (CMR) was performed as described previously using a clinical wide-bore Discovery MR 750 3T scanner (GE Healthcare, Milwaukee, MI)²². Localizer images followed by ECG gated breath-held scans were acquired. A short-axis steady-state free precession cine stack was then obtained with basal slice alignment and covering the ventricles and part of the

atria with continuous 8mm thick slices over several end expiration breath-holds. Post-processing analysis was performed according to the guidelines of the Society for CMR by a commercial party (Precision Image Analysis, Kirkland, WA, USA) using Medis QMASS software (Medis Medical Imaging, Leiden, the Netherlands). Briefly, the right and left ventricular short-axis endocardial and left ventricular epicardial borders were semi-automatically contoured. Structural cardiac outcomes included SD scores of left ventricular mass (LVM), left and right ventricular end-diastolic volume (LVEDV and RVEDV, respectively) and left ventricular mass to volume ratio (LVMVR). LVMR was calculated as LVM/LVEDV. Functional cardiac outcomes included SD scores of left and right ventricular ejection fraction (LVEF and RVEF, respectively). Because cardiac outcomes are strongly dependent of child size, the cardiac outcomes were adjusted for body surface area (BSA) using Generalized Additive Models for Location, Size and Shape using R (based on full cohort, $n=3,018$)²².

Covariates

At enrollment, we collected information on education level, ethnicity, maternal prepregnancy weight, folic acid supplementation, smoking and alcohol consumption during pregnancy by prenatal questionnaires. Prepregnancy body mass index (BMI) was calculated using height measured at the intake appointment. Information on gestational age at birth, birth weight and child sex were obtained from medical records^{23,24}. Breastfeeding status was collected by postnatal questionnaires. Child blood pressure, height and weight (to calculate BMI) were measured during the research visit.

Statistical analysis

Differences in population characteristics between maternal gestational hypertensive disorder categories were examined with ANOVA for continuous variables and by χ^2 for categorical variables. We performed a non-response analysis to compare population characteristics of participants with offspring CMR measurements to those without. We constructed standard deviation scores (SDS) for continuous exposures and outcomes to enable comparison of effect estimates for all the analyses. First, we examined the associations of gestational hypertensive disorders and maternal systolic and diastolic blood pressure in early, mid and late-pregnancy with offspring cardiac outcomes using linear regression models. Potential confounders and mediators were identified and pictured in a directed acyclic graph (**Supplementary Figure S1**). The confounders were selected based on their association with exposure and outcome or a change in effect of $>10\%$.

To explore the effect of confounders and mediators we constructed different adjustment models. In the *basic model* we adjusted for child's age and sex, time difference with BSA measurement and CMR (as these measures were conducted at different visits to the research centre). In the *confounder model*, we additionally adjusted for maternal prepregnancy BMI, educational level, ethnicity, folic acid supplementation, smoking and alcohol consumption during pregnancy. If we found significant associations the *confounder model* was additionally adjusted for child's gestational age and weight at birth, breastfeeding status, child body mass index, and child systolic blood pressure (*mediator models*).

Second, we used conditional linear regression analyses to investigate independent associations of maternal blood pressure in early, mid and late-pregnancy with offspring outcomes to explore critical periods²⁵⁻²⁷. These models take the correlation between maternal blood pressure measurements at different time-points throughout pregnancy into account. Using standardized residuals from linear regression models of maternal blood pressure regressed on all the previous blood pressure measurements, maternal systolic and diastolic blood pressure variables were constructed that are statistically independent of each other. This approach allows inclusion of all maternal blood pressure measures simultaneously in one regression model. Thus, associations of maternal systolic and diastolic blood pressure in each period with childhood outcomes can be assessed adjusted for, and compared with, maternal systolic and diastolic blood pressure in other periods of pregnancy.

Third, we examined the associations of paternal early-pregnancy systolic and diastolic blood pressure with offspring cardiac outcomes to compare the strength of these paternal-offspring associations with the strength of the maternal-offspring associations. Stronger maternal-offspring associations would support a potential direct intrauterine effect of maternal blood during pregnancy on offspring cardiac outcomes, while similar or stronger associations for paternal blood pressure with offspring outcomes would suggest that these associations are more likely driven by genetic predisposition or shared lifestyle risk factors²⁸. We explored whether effect modification was present by testing interaction terms for gestational hypertensive disorder status and maternal blood pressure with child sex, gestational age at birth and gestational-age-and-sex-adjusted-birthweight. No consistent interactions were present (p -values >0.05), and therefore we did not perform stratified analysis. We performed multiple imputations for missing data on covariates ($<15\%$ missing values, except for folic acid supplementation (23%) and prepregnancy BMI (18%)). Analyses were performed using IBM SPSS version 25 (SPSS Inc., Chicago, Illinois).

Table 1. Characteristics of the total study population (n=2,502) and by gestational hypertensive disorder status (n=2,454)*

	Total population n=2,502	Normotensive pregnancy n=2,322	Gestational hypertension n=87	Preeclampsia n=45	p-value*
<u>Maternal characteristics</u>					
Maternal age, mean (sd), years	31.0 (4.8)	31.0 (4.8)	31.4 (4.6)	30.1 (5.1)	0.34
Prepregnancy BMI, median (IQR), kg/m ²	22.5 (20.8, 25.0)	22.4 (20.8, 24.8)	25.0 (22.7, 30.9)	23.5 (21.6, 27.7)	<0.01
Parity, n nulliparous (%)	1,466 (58.9)	1,345 (58.2)	59 (67.8)	38 (84.4)	<0.01
Education level, n higher (%)	1,262 (53.1)	1,175 (53.2)	44 (52.4)	19 (43.2)	0.42
Ethnicity, n European (%)	1,600 (65.1)	1,478 (64.7)	63 (72.4)	28 (62.2)	0.31
Folic acid supplement use, n yes (%)	1,540 (80.0)	1,422 (79.8)	58 (86.6)	32 (78.0)	0.38
Smoking during pregnancy, n yes (%)	316 (14.2)	291 (14.1)	15 (18.8)	3 (7.3)	0.23
Alcohol consumption during pregnancy, n yes (%)	946 (43.0)	883 (43.1)	33 (42.3)	18 (42.9)	0.99
Systolic blood pressure, mean (sd), mmHg					
Early-pregnancy	115.7 (11.8)	115.3 (11.5)	124.6 (13.1)	120.3 (12.8)	<0.01
Mid-pregnancy	116.6 (11.8)	116.1 (11.5)	126.3 (13.8)	121.5 (12.4)	<0.01
Late-pregnancy	118.1 (11.3)	117.4 (11.0)	128.2 (12.1)	128.0 (12.0)	<0.01
Diastolic blood pressure, mean (sd), mmHg					
Early-pregnancy	68.0 (9.2)	67.6 (8.9)	76.3 (11.4)	72.6 (10.1)	<0.01
Mid-pregnancy	67.0 (9.2)	66.4 (8.9)	77.4 (10.6)	73.3 (9.7)	<0.01
Late-pregnancy	68.9 (9.0)	68.2 (8.5)	79.1 (9.9)	80.6 (9.8)	<0.01
<u>Paternal characteristics</u>					
Age, mean (sd), years	33.5 (5.3)	33.5 (5.3)	33.5 (5.5)	32.4 (4.1)	0.47
BMI, median (IQR), kg/m ²	24.9 (23.0, 27.2)	24.9 (23.0, 27.2)	26.0 (23.6, 28.4)	25.4 (21.8, 27.2)	0.05
Education level, n higher (%)	994 (56.2)	926 (56.4)	38 (55.1)	14 (45.2)	0.45
Ethnicity, n European (%)	1,568 (65.0)	1,450 (64.7)	62 (72.9)	30 (68.2)	0.27
Systolic blood pressure, mean (sd), mmHg	130.2 (13.4)	130.1 (13.5)	132.3 (13.0)	133.2 (13.1)	0.19
Diastolic blood pressure, mean (sd), mmHg	73.3 (10.3)	73.2 (10.4)	75.0 (10.7)	75.7 (9.3)	0.14

Table 1. Continued

	Total population n=2,502	Normotensive pregnancy n=2,322	Gestational hypertension n=87	Preeclampsia n=45	p-value*
<u>Birth and infant characteristics</u>					
Sex, n female (%)	1,303 (52.1)	1,204 (51.9)	39 (44.8)	31 (68.9)	0.03
Gestational age at birth, median (IQR), weeks	40.1 (39.1, 41.0)	40.3 (39.3, 41.0)	40.1 (39.0, 41.1)	38.4 (36.6, 39.8)	<0.01
Weight at birth, median (IQR), grams	3,460 (3120, 3800)	3,480 (3135, 3808)	3,340 (2955, 3750)	2,935 (2320, 3310)	<0.01
<u>Child characteristics</u>					
Age, median (IQR), years	9.9 (9.8, 10.4)	9.9 (9.8, 10.3)	10.0 (9.8, 11.0)	10.0 (9.8, 10.5)	<0.01
BMI, median (IQR), kg/m ²	16.9 (15.6, 18.7)	16.9 (15.6, 18.7)	17.4 (16.2, 20.2)	17.7 (15.2, 19.9)	<0.01
Body surface area, median (IQR), m ²	1.2 (1.1, 1.2)	1.1 (1.1, 1.2)	1.2 (1.1, 1.3)	1.2 (1.1, 1.3)	<0.01
Systolic blood pressure, mean (sd), mmHg	103.1 (7.9)	103.0 (7.8)	105.6 (8.0)	104.8 (9.5)	<0.01
Diastolic blood pressure, mean (sd), mmHg	58.5 (6.4)	58.4 (6.4)	60.5 (7.5)	59.7 (7.0)	<0.01
<u>Cardiac structure</u>					
Left ventricular mass, median (IQR), grams	47.6 (41.7, 55.2)	47.5 (41.7, 55.0)	50.7 (43.1, 63.0)	47.8 (43.1, 55.8)	<0.01
Left ventricular end-diastolic volume, median (95% range), ml	98.9 (88.5, 111.2)	98.8 (88.4, 111.1)	100.2 (90.9, 116.5)	100.6 (86.8, 114.2)	0.03
Right ventricular end-diastolic volume, median (95% range), ml	98.5 (86.3, 111.6)	98.4 (86.2, 111.3)	100.2 (91.7, 117.5)	100.9 (84.7, 113.9)	0.03
Left ventricular mass to volume ratio, median (IQR), grams/ml	0.48 (0.44, 0.53)	0.48 (0.44, 0.53)	0.49 (0.44, 0.56)	0.47 (0.44, 0.51)	0.10
<u>Cardiac function</u>					
Left ventricular ejection fraction, median (IQR), %	58.3 (55.1, 61.6)	58.3 (55.1, 61.5)	57.8 (54.7, 61.5)	57.9 (55.7, 62.6)	0.53
Right ventricular ejection fraction, median (IQR), %	58.1 (54.8, 61.4)	58.1 (54.8, 61.5)	57.6 (53.8, 60.4)	56.6 (53.9, 59.6)	0.13

IQR, inter quartile range. BMI, body mass index. MAP, mean arterial pressure. Values are mean (sd), median (IQR), or number (valid %). *P-values were obtained by ANOVA for continuous variables and by χ^2 for categorical variables.

RESULTS

Population characteristics

Table 1 shows the population characteristics. Gestational hypertension occurred in 87 pregnancies, while preeclampsia occurred in 45 pregnancies. Offspring exposed to preeclampsia had a higher left ventricular mass compared to children exposed to gestational hypertension or unexposed children (p-value <0.01), but no other differences in cardiac structure and function outcomes were present. **Supplementary Table S1** shows that compared to the population for analysis, mothers of offspring without follow-up at the age of 10 years, were younger, lower educated and more often from non-European descent.

Gestational hypertensive disorders with offspring cardiac outcomes

Table 2 shows that offspring from pregnancies affected by preeclampsia had lower right ventricular ejection fraction as compared to offspring from normotensive pregnancies after adjustment for sociodemographic and lifestyle factors (difference: -0.31 SDS (95% CI -0.60, -0.02)), but no differences in offspring left ventricular mass, left and right ventricular end-diastolic volume, left ventricular mass to volume ratio and left ventricular ejection fraction were present. This association was not explained by gestational age and weight at birth, breastfeeding status, child adiposity and systolic blood pressure (**Supplementary Table S2**). For offspring from mothers who developed gestational hypertension, no differences in any offspring cardiac outcomes were present as compared to offspring from normotensive pregnancies.

Table 2. Associations of gestational hypertension and preeclampsia with offspring cardiac outcomes at median 10 years (n=2,454)

Offspring outcomes	Normotensive pregnancy	Gestational hypertension		Preeclampsia	
		Basic	Confounder	Basic	Confounder
LVM, SDS	Reference	0.11 (-0.07, 0.30)	0.12 (-0.07, 0.31)	-0.07 (-0.32, 0.19)	-0.06 (-0.31, 0.20)
LVEDV, SDS	Reference	0.01 (-0.18, 0.20)	-0.00 (-0.19, 0.19)	-0.06 (-0.32, 0.20)	-0.05 (-0.31, 0.21)
RVEDV, SDS	Reference	0.03 (-0.16, 0.22)	0.03 (-0.16, 0.22)	0.03 (-0.23, 0.29)	0.05 (-0.21, 0.31)
LVMVR, SDS	Reference	0.16 (-0.06, 0.37)	0.16 (-0.06, 0.38)	-0.00 (-0.30, 0.29)	-0.01 (-0.30, 0.29)
LVEF, SDS	Reference	-0.09 (-0.31, 0.12)	-0.10 (-0.32, 0.11)	0.09 (-0.21, 0.38)	0.08 (-0.21, 0.38)
RVEF, SDS	Reference	-0.02 (-0.23, 0.19)	-0.05 (-0.27, 0.16)	-0.30 (-0.59, -0.01)*	-0.31 (-0.60, -0.02)*

LVM, left ventricular mass. LVEDV, left ventricular end-diastolic volume. RVEDV, right ventricular end-diastolic volume. LVMVR, left ventricular mass to volume ratio. LVEF, left ventricular ejection fraction. RVEF, right ventricular ejection fraction. *P-value <0.05. Values are regression coefficients (95% confidence interval) that were obtained from regular linear regression models, and reflect the differences in offspring LVM, LVEDV, RVEDV, LVMVR, LVEF and RVEF in SDS for gestational hypertension and preeclampsia. Groups are compared to women with a normotensive pregnancy as reference. Estimates are from multiple imputed data. Basic models are adjusted for child's age and sex, time difference with BSA measurement and CMR. Confounder model is basic model additionally adjusted for prepregnancy body mass index, maternal educational level, maternal ethnicity, folic acid supplementation, smoking and alcohol consumption during pregnancy.

Maternal blood pressure throughout pregnancy with offspring cardiac outcomes **Table 3** shows the associations of maternal systolic and diastolic blood pressure in each period with offspring cardiac outcomes per SDS change using regular linear regression models. A higher late-pregnancy maternal systolic blood pressure was associated with a higher left ventricular mass to volume ratio (all p-values <0.05), but this association attenuated into non-significance after additional adjustment for gestational age and weight at birth, breastfeeding status, child's body mass index and systolic blood pressure (**Supplementary Table S3**). No other associations for maternal systolic blood pressure with offspring cardiac outcomes were present. After adjustment for sociodemographic and lifestyle factors, a higher maternal diastolic blood pressure in early and late-pregnancy were associated with lower left ventricular end-diastolic volumes (differences: -0.06 SDS (CI 95% -0.10, -0.01) and -0.05 SDS (CI 95% -0.09, -0.02) per SDS increase in diastolic blood pressure in early and late-pregnancy, respectively) and right ventricular end-diastolic volumes (differences: -0.07 SDS (CI 95% -0.11, -0.02) and -0.04 SDS (CI 95% -0.08, -0.01) per SDS increase in diastolic blood pressure in early and late-pregnancy, respectively). Similar tendencies were present from diastolic blood pressure in mid-pregnancy. These associations were not explained by gestational age and weight at birth, breastfeeding status, child's body mass index and systolic blood pressure (**Supplementary Table S3**). No associations were present with offspring left ventricular mass, left and right ventricular ejection fraction.

Figure 2 shows the independent associations of maternal blood pressure in early, mid and late-pregnancy with offspring cardiac outcomes from conditional linear regression analyses (effect estimates with CI 95% from conditional change analyses are also shown in **Supplementary Table S4**). Higher maternal diastolic blood pressure in early-pregnancy, but not in mid and late-pregnancy, was independently associated with lower offspring left and right ventricular end-diastolic volume, and higher offspring left ventricular mass to volume ratio (all p-values <0.05). Higher maternal systolic blood pressure in late-pregnancy, but not in early and mid-pregnancy, was independently associated with higher offspring left ventricular ejection fraction (p-value <0.05). No independent associations were present for maternal diastolic and systolic blood pressure throughout pregnancy with other offspring cardiac outcomes.

Paternal blood pressure with offspring cardiac outcomes and parental comparison Paternal systolic blood pressure was not associated with offspring cardiac outcomes. Paternal diastolic blood pressure was only associated with a higher offspring left ventricular mass to volume ratio in the basic and confounder model (p-values <0.05),

Table 3. Associations of maternal systolic (upper panel) and diastolic blood pressure (lower panel) with offspring cardiac outcomes at median 10 years (n=2,497)

Offspring outcomes	Early-pregnancy maternal SBP n=2,009		Mid-pregnancy maternal SBP n=2,385		Late-pregnancy maternal SBP n=2,432	
	Basic model	Maternal model	Basic model	Maternal model	Basic	Maternal
LVM, SDS	0.02 (-0.02, 0.06)	0.03 (-0.01, 0.08)	0.01 (-0.02, 0.05)	0.02 (-0.02, 0.06)	0.03 (-0.01, 0.06)	0.03 (-0.00, 0.07)
LVEDV, SDS	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)	0.01 (-0.02, 0.05)	0.00 (-0.03, 0.04)	0.01 (-0.02, 0.05)	0.00 (-0.04, 0.04)
RVEDV, SDS	-0.00 (-0.04, 0.04)	0.00 (-0.04, 0.04)	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.04)	0.02 (-0.02, 0.05)	0.01 (-0.02, 0.05)
LVMVR, SDS	0.03 (-0.01, 0.07)	0.04 (-0.01, 0.09)	0.02 (-0.02, 0.06)	0.03 (-0.01, 0.07)	0.03 (-0.01, 0.07)	0.05 (0.00, 0.09)*
LVEF, SDS	-0.01 (-0.05, 0.03)	-0.02 (-0.07, 0.02)	-0.02 (-0.06, 0.02)	-0.02 (-0.07, 0.02)	0.03 (-0.01, 0.07)	0.03 (-0.01, 0.07)
RVEF, SDS	0.01 (-0.04, 0.05)	-0.02 (-0.06, 0.03)	-0.02 (-0.05, 0.03)	-0.03 (-0.07, 0.01)	0.01 (-0.03, 0.05)	-0.01 (-0.05, 0.04)

Offspring outcomes	Early-pregnancy maternal DBP n=2,009		Mid-pregnancy maternal DBP n=2,384		Late-pregnancy maternal DBP n=2,432	
	Basic model	Maternal model	Basic model	Maternal model	Basic	Maternal
LVM, SDS	-0.02 (-0.06, 0.02)	-0.01 (-0.05, 0.02)	-0.01 (-0.05, 0.02)	0.00 (-0.04, 0.04)	-0.02 (-0.05, 0.02)	-0.01 (-0.05, 0.03)
LVEDV, SDS	-0.05 (-0.09, -0.01)*	-0.06 (-0.10, -0.01)*	-0.04 (-0.07, -0.00)*	-0.03 (-0.07, 0.01)	-0.05 (-0.08, -0.01)*	-0.05 (-0.09, -0.02)*
RVEDV, SDS	-0.06 (-0.10, -0.03)*	-0.07 (-0.11, -0.02)*	-0.04 (-0.08, -0.01)*	-0.03 (-0.07, 0.01)	-0.05 (-0.08, -0.01)*	-0.04 (-0.08, -0.01)*
LVMVR, SDS	0.04 (-0.01, 0.08)	0.05 (-0.00, 0.09)	0.03 (-0.01, 0.07)	0.03 (-0.01, 0.08)	0.04 (-0.00, 0.08)	0.04 (0.00, 0.09)*
LVEF, SDS	-0.02 (-0.06, 0.03)	-0.03 (-0.08, 0.02)	-0.02 (-0.06, 0.02)	-0.03 (-0.08, 0.01)	0.00 (-0.04, 0.04)	0.00 (-0.04, 0.04)
RVEF, SDS	0.01 (-0.04, 0.05)	-0.01 (-0.06, 0.03)	-0.02 (-0.06, 0.02)	-0.04 (-0.08, 0.00)	0.00 (-0.04, 0.04)	-0.01 (-0.05, 0.03)

LVM, left ventricular mass. LVEDV, left ventricular end-diastolic volume. RVEDV, right ventricular end-diastolic volume. LVMVR, left ventricular mass to volume ratio. LVEF, left ventricular ejection fraction. RVEF, right ventricular ejection fraction. SBP, systolic blood pressure. DBP, diastolic blood pressure. *P-value < 0.05. Values are regression coefficients (95% confidence interval) that were obtained from regular linear regression models, and reflect the differences in offspring LVM, LVEDV, RVEDV, LVMVR, LVEF and RVEF in SDS per SDS change in maternal blood pressure. Estimates are from multiple imputed data. Basic model is adjusted for gestational age at the time of blood pressure measurements, child's age and sex, time difference with BSA measurement and CMR. Confounder model is basic model additionally adjusted for prepregnancy body mass index, maternal educational level, maternal ethnicity, folic acid supplementation, smoking and alcohol consumption during pregnancy.

but this association disappeared after additional adjustment for birth and child factors (**Supplementary Table S5**).

When we included both maternal and paternal blood pressure in the same model, the associations of maternal diastolic blood pressure with offspring left and right ventricular end-diastolic volume remained present (all p-values <0.05), while no associations were present for paternal blood pressure with any offspring cardiac outcomes (**Supplementary Table S6**).

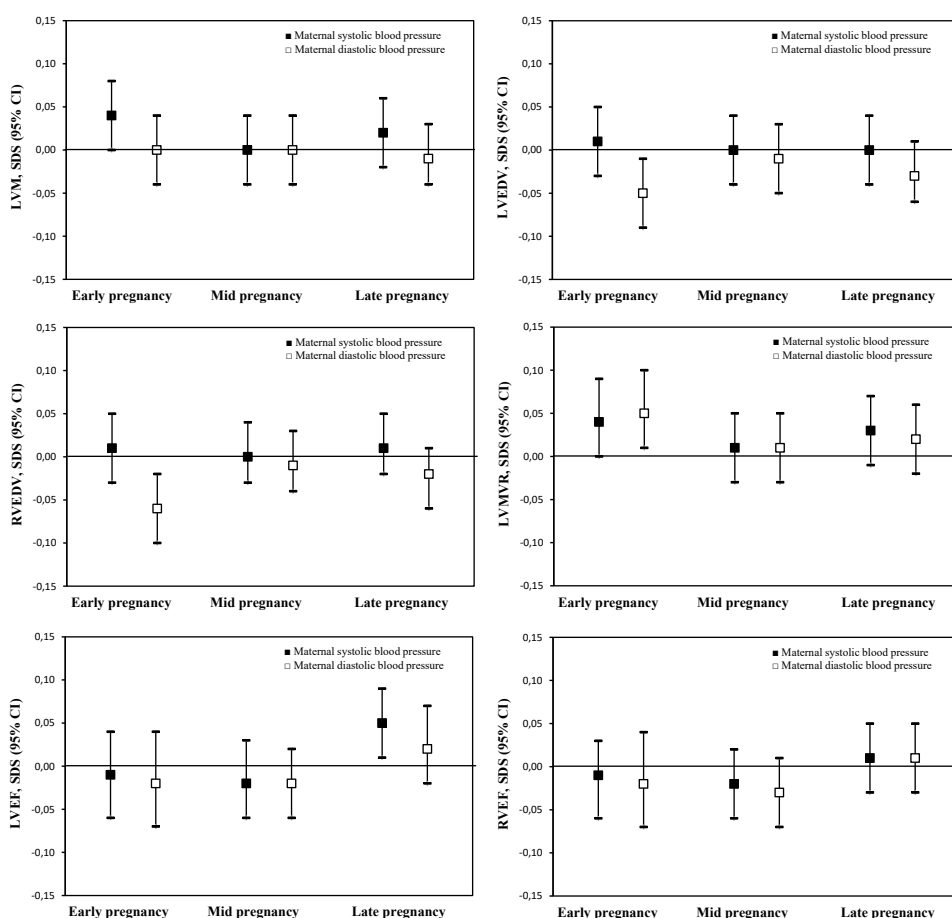


Figure 2. Associations of maternal blood pressure during pregnancy with child cardiac structure and function from conditional change analyses (n=2,497). Values are regression coefficients (95% confidence interval) that reflect the differences in offspring cardiac outcomes in SDS, per SDS change in maternal early-pregnancy blood pressure, and per SDS change in standardized residual change in maternal blood pressure in mid and late-pregnancy from conditional change models. Estimates are from multiple imputed data. Maternal blood pressure was additionally imputed for women with at least one blood pressure measurement in pregnancy. Models are adjusted for gestational age at intake, child's age and sex, time difference with BSA measurement and CMR, prepregnancy body mass index, maternal educational level, maternal ethnicity, folic acid supplementation, smoking and alcohol consumption during pregnancy. LVM, left ventricular mass. LVEDV, left ventricular end-diastolic volume. RVEDV, right ventricular end-diastolic volume. LVMVR, left ventricular mass to volume ratio. LVEF, left ventricular ejection fraction. RVEF, right ventricular ejection fraction.

DISCUSSION

No consistent associations of gestational hypertensive disorder status with offspring cardiac outcomes at the age of 10 years were present. Higher maternal diastolic blood pressure throughout pregnancy was associated with lower childhood left and right ventricular end-diastolic volumes, with the strongest effect for early-pregnancy. These findings were not explained by maternal, birth or child factors. The observed maternal-offspring associations were stronger compared to paternal-offspring associations, suggesting that these associations may be partly driven by a direct intrauterine effect on cardiac development rather than genetic predisposition or shared lifestyle within a family.

Interpretation of main findings

It is unclear whether exposure to gestational hypertensive disorders during pregnancy is associated with subclinical alterations in cardiac structure and function in the offspring. Few studies have investigated these associations using echocardiographic measures with a primary focus on the left ventricle. A British prospective study among 1,592 adolescents found that offspring exposed to gestational hypertension or preeclampsia had a greater relative left ventricular wall thickness⁵. Exposure to preeclampsia was additionally associated with a decreased left ventricular end-diastolic volume, reflecting a concentric type of cardiac remodeling that has also been observed in small-for-gestational-age infants and preterms⁵. As the assessment of the right ventricle is technically challenging, only one previous case-control study among 134 term-born infants was able to assess right ventricular measures using automated volumetric estimates from echocardiography⁴. At birth, infants exposed to preeclampsia or gestational hypertension had decreased right ventricular end-diastolic volume. During assessment at three months they also developed increased left and right ventricular mass, of which the right ventricular structural changes were most evident and correlated with the severity of the gestational hypertensive disorder⁴. The same investigators found that preterms of pregnancies affected by hypertension showed a pronounced increase in the right ventricular mass, as compared to preterms of normotensive pregnancies²⁹. These findings suggest a specific effect on the development of the right ventricle in offspring from pregnancies affected by gestational hypertensive disorders. Contrary to these previous studies, we did not find consistent associations of maternal gestational hypertensive disorders with offspring cardiac structure and function. We observed that offspring exposed to preeclampsia, but not gestational hypertension, had a lower right ventricular ejection fraction only. This isolated finding most likely reflects a chance finding, but may suggest a slightly lower right ventricular

global function during childhood in offspring of preeclamptic pregnancies. Differences in findings with previous studies may be explained by the timing of assessment as we assessed cardiac structure and function during childhood, while the other studies focused on infancy and adolescence^{4, 5}. Changes in cardiac structure and function observed in infancy may be transient, while long-term cardiac adaptations may not yet be present during childhood but only first detectable from adolescence onwards. Compared to the previous British prospective study among adolescents we had a smaller number of gestational hypertensive disorder cases, and therefore we had a relatively lower statistical power in these analyses. Concluding, we did not observe consistent associations of exposure to gestational hypertensive disorders and offspring cardiac outcomes.

Gestational hypertension and preeclampsia represent the extremes of the gestational hypertensive disorder spectrum. Also, already higher gestational blood pressure across the full range reflects an inability of the maternal cardiovascular system to adequately adapt to pregnancy and reflects poorer placental vascular function during pregnancy^{30, 31}. Previous large observational studies have shown that higher maternal blood pressure during pregnancy is associated with higher blood pressure and microvasculature narrowing in the offspring. Especially, early-pregnancy may be a critical period for exposure to suboptimal maternal blood pressure levels^{6-8, 32}. However, not much is known about the direct influence of maternal blood pressure in different pregnancy-periods on offspring cardiac development. We observed that a higher maternal diastolic blood pressure throughout pregnancy was associated with decreased offspring left- and right ventricular end-diastolic volumes, with early-pregnancy as a critical period. Ventricular end-diastolic volume is a structural measure that describes the cardiac filling capacity during diastole. A restrictive filling pattern can be the result of a decreased ability for ventricular relaxation and increased ventricular stiffness due to structural myocardial adaptations. Our findings are partly in line with the previously mentioned British observational study, they observed among 1,592 adolescents that a smaller decrease in maternal systolic blood pressure between 8 and 18 weeks gestation was associated with an increased left ventricular mass and end-diastolic volume during adolescence⁵. Together these findings suggest a potential adverse effect of higher maternal blood pressure especially during early-pregnancy on offspring structural cardiac measures.

The observed associations within the current study, may be explained by several underlying mechanisms. Gestational hypertensive disorders as well as a higher gestational blood pressure below the diagnostic cut-off for gestational hypertensive disorders are risk factors for delivering small-size-for-gestational-age and premature infants, both conditions that are associated with cardiac structural and functional changes in these

children¹². The associations in our study were not explained by gestational age and weight at birth. This is in line with findings from previous studies, that suggest that gestational hypertensive disorders independent of these adverse birth outcomes influence offspring cardiac development^{4, 33, 34}. Child's body mass index and child's blood pressure are also strong predictors for cardiac structure and function, but did not explain the observed associations. We observed associations of a higher maternal diastolic blood pressure with lower offspring left- and right ventricular end-diastolic volumes, while paternal-offspring associations were not present. This suggests that these associations may be partly explained by direct intrauterine mechanisms, rather than genetic predisposition or shared lifestyle within a family. Higher maternal diastolic blood pressure has been associated with placental vascular maladaptations which is likely to lead to an environment of hypoxia from early-pregnancy onwards^{11, 31}. In experimental studies uteroplacental hypoxia has shown to increase levels of cardiac collagen and alterations in the extracellular matrix with decreased ability for ventricular relaxation and increased ventricular stiffness^{35, 36}. Preeclampsia is particularly characterized by inadequate trophoblast invasion with increased placental resistance against which the right fetal ventricle ejects against¹⁶. This could explain why we found an effect on right ventricular ejection fraction in children from preeclamptic mothers, however this isolated finding most likely reflects chance. Further studies are needed to investigate the influence of gestational blood pressure and placental vascular maladaptations on fetal cardiac development.

The observed effect estimates within the current study were relatively small, but our findings are still important from an etiological perspective. The found effects might be stronger within higher risk populations with a higher prevalence of gestational hypertensive disorders and higher average blood pressure levels. Already, tracking of structural ventricular cardiac measures from infancy through adolescence has been observed^{37, 38}. However, whether the found cardiac alterations during childhood persist and influence the development of cardiovascular disease during adulthood, needs further investigation through long-term follow-up studies. Our findings should be considered hypothesis generating and the pathophysiological mechanisms behind these observations need further investigation. Future observational studies should collect detailed measurements on maternal hemodynamic parameters from preconception throughout the course of pregnancy, and provide long-term offspring cardiovascular follow-up.

Strengths and limitations

The main strengths of this study were the prospective design of the study with data collection already from early-pregnancy onwards and the use of highly detailed measurers

of childhood cardiac development without geometrical assumptions by CMR. However, we were not able to measure the right ventricular mass because at the age of 10 the right ventricular wall is too thin and due to its complex shape prone to measurement error^{39, 40}. The generalizability of our results may be affected by a selection toward a relatively healthy, high-educated study population. Our sample of women with gestational hypertension or preeclampsia was relatively small. This could have led to reduced statistical power for the gestational hypertensive disorder analyses. Only a subgroup of children was invited to participate in the CMR follow-up study. However, we do not expect that this affected our effect estimates, as maternal blood pressure and the prevalence of gestational hypertension and preeclampsia did not differ between children with and without CMR measurements. We examined the associations of maternal gestational hypertensive disorders and blood pressure throughout pregnancy with multiple measures of offspring cardiac structure and function. As these cardiac measures are strongly correlated, we did not correct our analyses for multiple testing. However, chance findings cannot be excluded and our findings need to be replicated in further studies among higher-risk populations. Finally, the analyses were adjusted for a large number of confounders. However, we did not have information on child's exercise habits for example. As in any observational study, residual confounding may still be present.

Conclusions

We observed no consistent associations of gestational hypertensive disorder status with offspring cardiac outcomes at the age of 10 years. Higher maternal diastolic blood pressure throughout pregnancy was associated with lower childhood left and right ventricular end-diastolic volumes, with the strongest effect for early-pregnancy. The observed maternal-offspring associations were stronger compared to the paternal-offspring associations, which may suggest that cardiac development is at least partly influenced by maternal hemodynamic adaptations through direct intrauterine mechanisms. Our findings should be considered hypothesis generating and the pathophysiological mechanisms behind the observed associations need further investigation.

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SUPPLEMENTARY MATERIAL

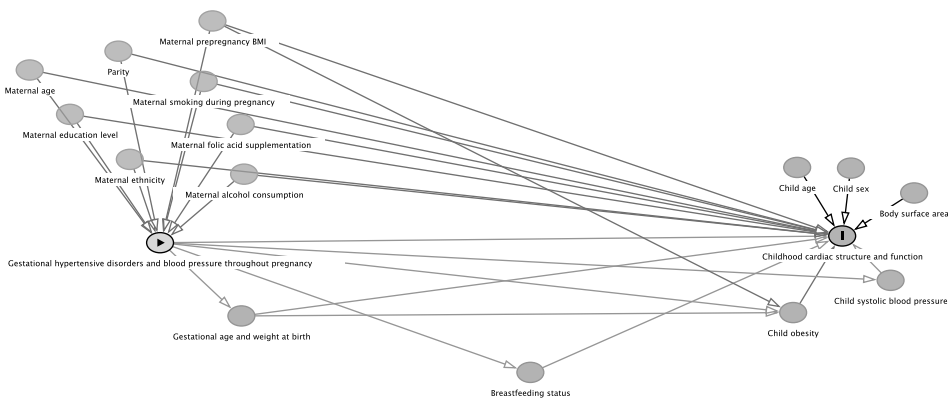


Figure S1. Directed acyclic graph to identify potential confounders and mediators.

Table S1. Non-response analyses for offspring with vs. without cardiovascular follow-up at 10 years

	CMR at 10 years n=2,502	No CMR at 10 years [*] n=5,995
<u>Maternal characteristics</u>		
Maternal age, mean (sd), years	31.0 (4.8)	29.0 (5.4)
Prepregnancy BMI, median (IQR), kg/m ²	22.5 (20.8, 25.0)	22.6 (20.7, 25.5)
Parity, n nulliparous (%)	1,466 (58.9)	3,195 (54.2)
Education level, n higher (%)	1,262 (53.1)	1,978 (37.1)
Ethnicity, n European (%)	1,600 (65.1)	2,949 (53.1)
Folic acid supplement use, n yes (%)	1,540 (80.0)	2,882 (66.2)
Smoking during pregnancy, n yes (%)	316 (14.2)	1,061 (20.5)
Alcohol consumption during pregnancy, n yes (%)	946 (43.0)	1,743 (34.2)
Preeclampsia, n yes (%)	45 (1.8)	124 (2.2)
Gestational hypertension, n yes (%)	87 (3.5)	218 (3.8)
Systolic blood pressure, mean (sd), mmHg		
Early-pregnancy	115.7 (11.8)	115.0 (12.1)
Mid-pregnancy	116.6 (11.8)	116.3 (12.0)
Late-pregnancy	118.1 (11.3)	118.0 (12.2)
Diastolic blood pressure, mean (sd), mmHg		
Early-pregnancy	68.0 (9.2)	67.9 (9.3)
Mid-pregnancy	67.0 (9.2)	66.9 (9.2)
Late-pregnancy	68.9 (9.0)	68.8 (9.2)
<u>Paternal characteristics</u>		
Age, mean (sd), years	33.5 (5.3)	32.3 (5.9)
BMI, median (IQR), kg/m ²	24.9 (23.0, 27.2)	25.0 (22.9, 27.3)
Education level, n higher (%)	994 (56.2)	1,569 (47.9)
Ethnicity, n European (%)	1,568 (65.0)	2,706 (54.1)
Systolic blood pressure, mean (sd), mmHg	130.2 (13.4)	130.0 (13.5)
Diastolic blood pressure, mean (sd), mmHg	73.3 (10.3)	73.2 (10.8)
<u>Birth and infant characteristics</u>		
Sex, n female (%)	1,303 (52.1)	2,887 (48.5)
Gestational age at birth, median (95% range), weeks	40.1 (39.1, 41.0)	40.1 (39.0, 41.0)
Weight at birth, median (95% range), grams	3,460 (3,120, 3,800)	3,410 (3,061, 3,755)
Breastfeeding status, n ever (%)	1,991 (93.1)	3,461 (91.7)

BMI, body mass index. Values are mean (sd), median (95% range), or number (valid %). ^{*}No attendance at the visit at 10 years, or no CMR done during the visit at 10 years, or poor quality CMR. Children with cardiac abnormalities are excluded from this analyses.

Table S2. Mediator models: associations of gestational hypertension and preeclampsia with offspring cardiac outcomes at median 10 years (n=2,454)

Offspring outcomes	Normotensive pregnancy	Gestational hypertension		Preeclampsia	
		Birth	Child	Birth	Child
Left ventricular mass, SDS	Reference	na	na	na	na
Left ventricular end-diastolic volume, SDS	Reference	na	na	na	na
Right ventricular end-diastolic volume, SDS	Reference	na	na	na	na
Left ventricular mass to volume ratio, SDS	Reference	na	na	na	na
Left ventricular ejection fraction, SDS	Reference	na	na	na	na
Right ventricular ejection fraction	Reference	na	na	-0.35 (-0.65, -0.06)*	-0.35 (-0.65, -0.06)*

Na, not applicable because no significant association in confounder model. *P-value <0.05. Values are regression coefficients (95% confidence interval) that were obtained from regular linear regression models, and reflect the differences in offspring cardiac outcomes for gestational hypertension and preeclampsia. Groups are compared to women with a normotensive pregnancy as reference. Estimates are from multiple imputed data. Birth model is confounder model additionally adjusted for gestational age and weight at birth. Child model is birth model additionally adjusted for offspring breast feeding status, body mass index and systolic blood pressure.

Table S3. Mediator models: Associations of maternal blood pressure with offspring cardiac outcomes at median 10 years (n=2,497)

Offspring outcomes	Early-pregnancy maternal SBP n= 2,009			Mid-pregnancy maternal SBP n=2,385			Late-pregnancy maternal SBP n=2,432		
	Birth	Child		Birth	Child		Birth	Child	
LVM, SDS	na	na		na	na		na	na	
LVEDV, SDS	na	na		na	na		na	na	
RVEDV, SDS	na	na		na	na		na	na	
LVMVR, SDS	na	na		na	na	0.05 (0.00, 0.09)*	0.03 (-0.02, 0.07)	na	
LVEF, SDS	na	na		na	na		na	na	
RVEF, SDS	na	na		na	na		na	na	
Offspring outcomes	Early-pregnancy maternal DBP n=2,009			Mid-pregnancy maternal DBP n=2,384			Late-pregnancy maternal DBP n=2,432		
	Birth	Child		Birth	Child		Birth	Child	
LVM, SDS	na	na		na	na		na	na	
LVEDV, SDS	-0.05 (-0.09, -0.01)*	-0.05 (-0.09, -0.01)*		na	na		-0.04 (-0.08, -0.01)*	-0.04 (-0.08, -0.01)*	
RVEDV, SDS	-0.06 (-0.10, -0.02)*	-0.06 (-0.10, -0.02)*		na	na		-0.04 (-0.07, 0.00)*	-0.03 (-0.07, 0.00)*	
LVMVR, SDS	na	na		na	na		0.04 (-0.00, 0.08)	na	
LVEF, SDS	na	na		na	na		na	na	
RVEF, SDS	na	na		na	na		na	na	

LVM, left ventricular mass. LVEDV, left ventricular end-diastolic volume. RVEDV, right ventricular end-diastolic volume. LVMVR, left ventricular mass to volume ratio. LVEF, left ventricular ejection fraction. RVEF, right ventricular ejection fraction. SBP, systolic blood pressure. DBP, diastolic blood pressure. Na, not applicable because no significant association in maternal model. *P-value <0.05. Values are regression coefficients (95% confidence interval) that were obtained from regular linear regression models, and reflect the differences in offspring cardiac outcomes per SDS change in maternal blood pressure. Estimates are from multiple imputed data. Birth model is confounder model additionally adjusted for gestational age and weight at birth. Child model is birth model additionally adjusted for offspring breast feeding status, body mass index and systolic blood pressure.

Table S4. Associations of maternal blood pressure with offspring cardiac outcomes at median 10 years from conditional change analyses (n=2,497)

Offspring outcomes	Maternal SBP		
	Early-pregnancy	Mid-pregnancy	Late-pregnancy
LVM, SDS	0.04 (-0.00, 0.08)	-0.00 (-0.04, 0.04)	0.02 (-0.02, 0.06)
LVEDV, SDS	0.01 (-0.03, 0.05)	0.00 (-0.04, 0.04)	0.00 (-0.04, 0.04)
RVEDV, SDS	0.01 (-0.03, 0.05)	0.00 (-0.03, 0.04)	0.01 (-0.02, 0.05)
LVMVR, SDS	0.04 (-0.00, 0.09)	0.01 (-0.03, 0.05)	0.03 (-0.01, 0.07)
LVEF, SDS	-0.01 (-0.06, 0.04)	-0.02 (-0.06, 0.03)	0.05 (0.01, 0.09)*
RVEF, SDS	-0.01 (-0.06, 0.03)	-0.02 (-0.06, 0.02)	0.01 (-0.03, 0.05)
Offspring outcomes	Maternal DBP		
	Early-pregnancy	Mid-pregnancy	Late-pregnancy
LVM, SDS	-0.00 (-0.04, 0.04)	-0.00 (-0.04, 0.04)	-0.01 (-0.04, 0.03)
LVEDV, SDS	-0.05 (-0.09, -0.01)*	-0.01 (-0.05, 0.03)	-0.03 (-0.06, 0.01)
RVEDV, SDS	-0.06 (-0.10, -0.02)*	-0.01 (-0.04, 0.03)	-0.02 (-0.06, 0.01)
LVMVR, SDS	0.05 (0.01, 0.10)*	0.01 (-0.03, 0.05)	0.02 (-0.02, 0.07)
LVEF, SDS	-0.02 (-0.08, 0.03)	-0.02 (-0.06, 0.02)	0.02 (-0.02, 0.07)
RVEF, SDS	-0.02 (-0.07, 0.04)	-0.03 (-0.07, 0.01)	0.01 (-0.03, 0.05)

LVM, left ventricular mass. LVEDV, left ventricular end-diastolic volume. RVEDV, right ventricular end-diastolic volume. LVMVR, left ventricular mass to volume ratio. LVEF, left ventricular ejection fraction. RVEF, right ventricular ejection fraction. SBP, systolic blood pressure. DBP, diastolic blood pressure. *P-value <0.05. Values are regression coefficients (95% confidence interval) that reflect the differences in offspring LVM, LVEDV, RVEDV, LVMVR, LVEF and RVEF in SDS, per SDS change in maternal early-pregnancy blood pressure, and per SDS change in standardized residual change in maternal blood pressure in mid and late-pregnancy from conditional change models. Estimates are from multiple imputed data. Maternal blood pressure was additionally imputed for women with at least one blood pressure measurement in pregnancy. Models are adjusted for gestational age at intake, child's age and sex, time difference with BSA measurement and CMR, prepregnancy body mass index, maternal educational level, maternal ethnicity, folic acid supplementation, smoking and alcohol consumption during pregnancy.

Table S5. Associations of paternal blood pressure with offspring cardiac outcomes at median 10 years (n=1,915)

Offspring outcomes	Paternal SBP		
	Basic model	Confounder model	Fully adjusted model
LVM, SDS	0.02 (-0.02, 0.06)	0.01 (-0.03, 0.05)	na
LVEDV, SDS	-0.02 (-0.06, 0.02)	-0.03 (-0.07, 0.01)	na
RVEDV, SDS	-0.01 (-0.05, 0.03)	-0.01 (-0.05, 0.03)	na
LVMVR, SDS	0.05 (0.00, 0.09)	0.04 (-0.01, 0.09)	na
LVEF, SDS	0.03 (-0.02, 0.07)	0.05 (-0.00, 0.09)	na
RVEF, SDS	0.03 (-0.01, 0.08)	0.04 (-0.01, 0.08)	na

Offspring outcomes	Paternal DBP		
	Basic model	Confounder model	Fully adjusted model
LVM, SDS	0.02 (-0.02, 0.06)	0.02 (-0.02, 0.06)	na
LVEDV, SDS	-0.03 (-0.07, 0.01)	-0.04 (-0.08, 0.00)	na
RVEDV, SDS	-0.03 (-0.06, 0.01)	-0.03 (-0.07, 0.01)	na
LVMVR, SDS	0.06 (0.01, 0.10)*	0.05 (0.00, 0.10)*	0.04 (-0.01, 0.09)
LVEF, SDS	-0.01 (-0.05, 0.04)	0.00 (-0.04, 0.05)	na
RVEF, SDS	-0.00 (-0.05, 0.04)	-0.01 (-0.05, 0.04)	na

LVM, left ventricular mass. LVEDV, left ventricular end-diastolic volume. RVEDV, right ventricular end-diastolic volume. LVMVR, left ventricular mass to volume ratio. LVEF, left ventricular ejection fraction. RVEF, right ventricular ejection fraction. SBP, systolic blood pressure. DBP, diastolic blood pressure. Na, not applicable because no significant association in maternal model. *P-value <0.05. Values are regression coefficients (95% confidence interval) that were obtained from regular linear regression models, and reflect the differences in offspring LVM, LVEDV, RVEDV, LVMVR, LVEF and RVEF in SDS per SDS change in paternal blood pressure. Estimates are from multiple imputed data. Basic model is adjusted for child's age and sex, time difference with BSA measurement and CMR. Confounder model is basic model additionally adjusted for paternal BMI during the blood pressure measurement, paternal educational level, paternal ethnicity, folic acid supplementation, smoking and alcohol consumption during pregnancy. Fully adjusted model is the confounder model additionally adjusted for child's gestational age and weight at birth, offspring breastfeeding status and body mass index and systolic blood pressure at time of the measurements.

Table S6. Combined associations of early-pregnancy maternal and paternal blood pressure with offspring cardiac outcomes at median 10 years (n=1,622)

Offspring outcomes	Maternal and paternal SBP		
	Basic model	Combined confounder model	Fully adjusted model
LVM, SDS	Maternal SBP 0.03 (-0.02, 0.07)	0.04 (-0.01, 0.08)	na
	Paternal SBP 0.01 (-0.04, 0.05)	-0.00 (-0.05, 0.05)	na
LVEDV, SDS	Maternal SBP 0.01 (-0.04, 0.05)	0.00 (-0.04, 0.05)	na
	Paternal SBP -0.01 (-0.06, 0.03)	-0.02 (-0.06, 0.02)	na
RVEDV, SDS	Maternal SBP -0.01 (-0.05, 0.03)	-0.01 (-0.05, 0.04)	na
	Paternal SBP 0.00 (-0.04, 0.04)	0.00 (-0.04, 0.05)	na
LVMVR, SDS	Maternal SBP 0.04 (-0.01, 0.08)	0.05 (-0.01, 0.10)	na
	Paternal SBP 0.03 (-0.02, 0.08)	0.02 (-0.03, 0.07)	na
LVEF, SDS	Maternal SBP -0.03 (-0.08, 0.02)	-0.04 (-0.09, 0.01)	na
	Paternal SBP 0.04 (-0.01, 0.09)	0.06 (0.01, 0.11)*	0.05 (-0.00, 0.10)
RVEF, SDS	Maternal SBP -0.01 (-0.05, 0.04)	-0.03 (-0.08, 0.02)	na
	Paternal SBP 0.04 (-0.00, 0.09)	0.05 (-0.00, 0.10)	na

Offspring outcomes	Maternal and paternal DBP		
	Basic model	Combined confounder model	Fully adjusted model
LVM, SDS	Maternal DBP -0.02 (-0.07, 0.02)	-0.01 (-0.06, 0.04)	na
	Paternal DBP 0.03 (-0.02, 0.07)	0.02 (-0.03, 0.06)	na
LVEDV, SDS	Maternal DBP -0.06 (-0.10, -0.01)*	0.06 (-0.10, -0.01)*	0.06 (-0.10, -0.01)*
	Paternal DBP -0.02 (-0.06, 0.02)	0.03 (-0.07, 0.02)	na
RVEDV, SDS	Maternal DBP -0.07 (-0.11, -0.03)*	-0.07 (-0.11, -0.02)*	-0.07 (-0.11, -0.02)*
	Paternal DBP -0.01 (-0.06, 0.03)	-0.01 (-0.06, 0.03)	na
LVMVR, SDS	Maternal DBP 0.04 (-0.01, 0.09)	0.05 (-0.01, 0.10)	na
	Paternal DBP 0.05 (-0.00, 0.10)	0.04 (-0.01, 0.09)	na
LVEF, SDS	Maternal DBP -0.02 (-0.07, 0.03)	-0.03 (-0.09, 0.02)	na
	Paternal DBP 0.00 (-0.05, 0.05)	0.01 (-0.04, 0.06)	na
RVEF, SDS	Maternal DBP -0.00 (-0.05, 0.05)	-0.02 (-0.08, 0.03)	na
	Paternal DBP -0.00 (-0.05, 0.05)	-0.00 (-0.05, 0.05)	na

LVM, left ventricular mass. LVEDV, left ventricular end-diastolic volume. RVEDV, right ventricular end-diastolic volume. LVMVR, left ventricular mass to volume ratio. LVEF, left ventricular ejection fraction. RVEF, right ventricular ejection fraction. SBP, systolic blood pressure. DBP, diastolic blood pressure. Na, not applicable because no significant association in maternal model. *P-value <0.05. Values are regression coefficients (95% confidence interval) that were obtained from regular linear regression models, and reflect the differences in offspring LVM, LVEDV, RVEDV, LVMVR, LVEF and RVEF in SDS per SDS change in maternal (early-pregnancy) and paternal blood pressure. Estimates are from multiple imputed data. Basic model is adjusted for child's age and sex, time difference with BSA measurement and CMR. Combined confounder model is adjusted for maternal and paternal confounders, maternal and paternal BMI, maternal and paternal educational level, maternal and paternal ethnicity, maternal folic acid supplementation, maternal smoking and maternal alcohol consumption during pregnancy. Fully adjusted model is the combined model additionally adjusted for child's gestational age and weight at birth, offspring breastfeeding status and body mass index and systolic blood pressure at time of the measurements.

CHAPTER

3.2

Childhood blood pressure, carotid intima media thickness and distensibility after in utero exposure to gestational hypertensive disorders

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BACKGROUND Offspring exposed to gestational hypertensive disorders have higher blood pressure and increased risk of stroke in later life. Gestational hypertensive disorders might influence vascular development in the offspring, predisposing them to a higher blood pressure and stroke in later life.

METHODS In a population-based cohort among 4,777 mother-offspring pairs, we examined whether gestational hypertension, preeclampsia, and higher gestational blood pressure across the full blood pressure spectrum were associated with offspring blood pressure, carotid intima media thickness (IMT) and distensibility at 10 years.

RESULTS Offspring exposed to gestational hypertension, but not preeclampsia, had higher systolic and diastolic blood pressure (0.17 (95% CI 0.02, 0.31) and 0.23 (95% CI 0.08, 0.38) increase in standard deviation score, respectively), while no associations with IMT and distensibility were present. Higher maternal systolic and diastolic blood pressure in early, mid and late-pregnancy were associated with higher offspring systolic and diastolic blood pressure, and lower carotid distensibility (p-values <0.05), but not with IMT. The associations were not explained by maternal, birth or child-factors. Paternal systolic and diastolic blood pressure were also associated with these offspring outcomes (p-values <0.05), with a comparable strength as maternal-offspring associations.

CONCLUSIONS Gestational hypertension and higher gestational blood pressure across the full blood pressure spectrum, are associated with higher offspring blood pressure and lower carotid distensibility. No associations were found for preeclampsia with offspring vascular outcomes. As maternal-offspring and paternal-offspring associations were comparable, these associations are more likely driven by genetic predisposition and shared lifestyle, rather than by a direct intrauterine effect.

INTRODUCTION

Gestational hypertensive disorders occur in 5-10% of pregnancies, and are associated with adverse long-term cardiovascular outcomes in both mothers and offspring. Offspring of pregnancies affected by gestational hypertensive disorders seem to have a ~2 mmHg increased systolic blood pressure and ~1 mmHg increased diastolic blood pressure during childhood and adolescence¹⁻³. One follow-up study among 6,410 subjects exposed to maternal preeclampsia or gestational hypertension showed a nearly twofold increased risk of stroke in adulthood⁴. The clinical manifestations of gestational hypertensive disorders are at the extreme end of the blood pressure spectrum during pregnancy. Already small increases across the full blood pressure spectrum, even below the clinical cut-off value of 140/90 mmHg for the diagnosis of gestational hypertensive disorders, may influence offspring cardiovascular outcomes⁵⁻⁷. Previous studies have shown that a higher gestational blood pressure across the full blood pressure spectrum is associated with higher blood pressure levels and an increased risk of hypertension in the offspring⁵.

The mechanisms underlying these associations remain to be elucidated. Gestational hypertensive disorders and already a higher maternal blood pressure during pregnancy may lead to an adverse intrauterine environment that initiates fetal developmental adaptations, leading to a suboptimal cardiovascular risk profile in later life. Animal studies suggest that uterine perfusion abnormalities, an intrauterine systemic hypoxic state and increased antiangiogenic factors, features present in the development of gestational hypertensive disorders, lead to fetal vascular remodeling⁸. This could lead to early development of atherosclerosis, and predispose offspring to hypertension and increased risk of stroke in later life⁸. Within these animal models, offspring alterations in vascular structure and blood pressure predominantly occurred if these adverse circumstances were already present from early to mid-pregnancy⁸. However, the development of an early atherosclerotic phenotype in the offspring of affected pregnancies could also reflect shared genetic predisposition or lifestyle factors in mother-offspring pairs, especially since the mother herself also has an increased risk of cardiovascular diseases in later life after gestational hypertension or preeclampsia. Thus far, only few studies investigated the associations of gestational hypertensive disorders with atherosclerotic changes in the offspring in human populations, which can be evaluated non-invasively by measurement of the carotid intima media thickness (IMT) and distensibility, and reported inconsistent findings^{2, 9-12}.

We hypothesized that gestational hypertensive disorders, and higher gestational blood pressure across the full blood pressure spectrum, adversely influence vascular development in the offspring which predisposes them to a higher blood pressure in later

life. In a population-based prospective cohort study among 4,777 mother-offspring pairs, we examined the associations of gestational hypertensive disorder status with offspring blood pressure, carotid IMT and distensibility at the age of 10 years. Next, we further examined the associations of maternal gestational blood pressure on the full continuous scale with offspring blood pressure, carotid IMT and distensibility, independent of gestational hypertensive disorder status. We further explored whether critical periods for the associations of maternal gestational blood pressure with these offspring outcomes were present. Lastly, to obtain further insights into potential underlying mechanisms, we examined whether these associations were explained by maternal, birth or child factors. We also compared the strength of the associations of maternal blood pressure and paternal blood pressure with these offspring outcomes, as stronger maternal-offspring association would support a direct intra-uterine mechanism.

METHODS

Design and study population

This study was embedded in the Generation R study, a population-based prospective cohort from early-pregnancy onwards in Rotterdam, The Netherlands¹³. All participants gave written informed consent. The study complies with the declaration of Helsinki and was approved by the local Medical Ethical Committee (MEC 198.782/2001/31). In total, 8,879 women were enrolled during pregnancy. We excluded women with missing data on the exposures (n=5), pre-existent hypertension (n=141) and non-singleton non-live births (n=201). Of the 5,081 children who participated in the general follow-up visit at median 10 year, 286 did not have any outcome data available and another 18 children were excluded due to a cardiac abnormality. The total population for analysis consisted of 4,777 mother-offspring pairs. Blood pressure measurements were available for 4,745 children. Ultrasonographic measurements of carotid IMT and distensibility were available for 4,403 and 4,225 children, respectively (**Figure 1**).

Parental blood pressure and gestational hypertensive disorders

Maternal blood pressure was measured in early, mid and late-pregnancy (medians, 95% range: 13.2 (9.6, 17.5), 20.4 (18.5, 23.6), 30.2 (28.4, 32.9) weeks gestation, respectively), as described previously¹⁴. Of the 4,771 women, 3,532 women had three blood pressure measurements, 1,044 women had two blood pressure measurements and 195 women had one blood pressure measurement over the course of pregnancy. Paternal blood pressure

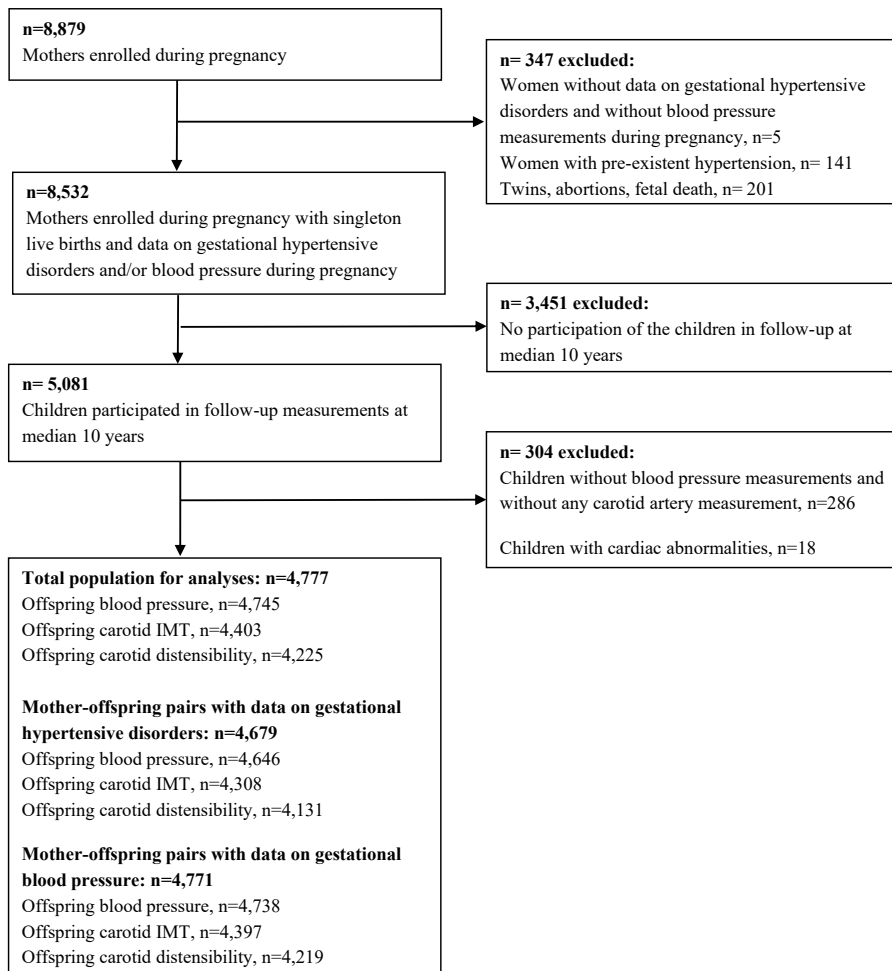


Figure 1. Flowchart of the study population.

was measured at study enrollment. An Omron 907 automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe BV, Hoofddorp, The Netherlands) was used for the maternal and paternal blood pressure measurements, while the participant was seated in upright position after a minimum of 5 minutes rest¹⁵. The mean of two measurements with a 60 second-interval was used for further analysis. We constructed standard deviation scores (SDS) of maternal and paternal blood pressure, to assess the associations of maternal and paternal blood pressure on the full continuous scale with offspring vascular outcomes.

Information on clinically diagnosed gestational hypertensive disorders was obtained from medical records that were cross-checked with the original hospital charts¹⁶. The clinical definition of gestational hypertension was defined as a systolic blood pressure of at least 140 mmHg and/or diastolic blood pressure of at least 90 mmHg after 20 weeks of gestation in previously normotensive women. Preeclampsia was defined as gestational hypertension with the addition of proteinuria¹⁷. A normotensive pregnancy was defined as systolic blood pressure below 140 mmHg and diastolic blood pressure below 90 mmHg throughout the entire course of pregnancy.

Childhood blood pressure, carotid IMT and distensibility

Children were invited to our research center at the median age of 9.7 years (95% range 9.4, 10.7). Systolic and diastolic blood pressure were measured with the child supine position. We measured blood pressure four times at the right brachial artery with a 1-minute interval, using an automated sphygmomanometer Datascope Accutor Plus TM (Paramus, NJ)¹⁸. The mean systolic and diastolic blood pressure were calculated using the last three measurements.

To make ultrasonographic recordings of the common carotid artery for the carotid IMT and distensibility measurements, we used the Logiq E9 (GE Medical Systems, Wauwatosa, WI, USA). Children were in the supine position with the head tilted in the contralateral direction. The common carotid artery was identified in a longitudinal plane, ~10 mm proximal from the carotid bifurcation. We obtained three recordings on both sides that included the coinciding cardiac cycles. Measurements were performed offline in a semi-automatic manner using Carotid Studio (Cardiovascular Suite (Quipu srl, Pisa Italy))¹⁹. The recording was frozen on each R-wave of the ECG, the carotid IMT was then measured at the far wall as the average distance between the lumen-intima and the media-adventitia interfaces and the average of all frames was computed. Carotid distensibility was defined as the relative change in lumen area during systole for a given pressure change. The lumen diameter was automatically computed as the average distance between the far and near media-adventitia interfaces for each frame of the acquired image sequence. The distension was calculated as the difference between the diastolic and systolic lumen diameter for each cardiac cycle in the recording. The average distension and diameter were used to calculate the distensibility. In a reproducibility study performed among 47 subjects, the interobserver and intraobserver intraclass correlation coefficient were greater than 0.85 for distensibility and 0.94 for IMT. We included all children with at least one successful carotid IMT or distensibility measurement and the mean values were used for further analyses. We calculated the overall mean carotid IMT (mm) and

distensibility ($\text{kPa}^{-1} \cdot 10^{-3}$). For the final analyses, distensibility was log-transformed to deal with a skewed distribution.

Covariates

At enrollment, we collected information on parental age, education level, ethnicity and maternal folic acid supplementation²⁰. Maternal prepregnancy weight, parity, smoking and alcohol consumption during pregnancy were obtained by prenatal questionnaires. Prepregnancy body mass index (BMI) was calculated using height measured at the intake appointment. Information on gestational age at birth, birth weight and child sex were obtained from medical records^{21, 22}. Small for gestational age (SGA) was defined as gestational age and sex-adjusted standard deviation scores for birth weight below the 10th percentile, and extremely SGA below the 3rd percentile within our population²². Prematurity was defined as onset of labor before 37 weeks (either spontaneous or induced). Breastfeeding status was collected by postnatal questionnaires. Child height and weight were measured during the research visit and used to calculate BMI. Age and sex-adjusted Body Mass Index Standard Deviation were calculated²³, and childhood overweight and obesity were classified using the IOTF criteria²⁴.

Statistical analysis

We examined population characteristics by maternal gestational hypertensive disorder status. We performed a non-response analysis to compare characteristics of children with any cardiovascular follow-up data available to those without. We constructed standard deviation scores (SDS) for all continuous exposures and outcomes. These SDS were calculated based on the variability in the current study population, and represent the equivalent of z scores. We did this to assess the continuous associations of maternal blood pressure per 1 SDS increase with offspring vascular outcomes in SDS, and to enable comparison of effect estimates for all the analyses. First, we examined the associations of gestational hypertension and preeclampsia with offspring blood pressure, carotid IMT and distensibility using linear regression models. Potential confounders and mediators were identified based on previous literature, and relationships were visualized using a directed acyclic graph (**Supplementary Figure S1**). To explore the effect of confounders and mediators we constructed four different adjustment models: 1) *Basic model*: adjusted for child's age and sex; 2) *Confounder model*: basic model additionally adjusted for maternal age, parity, prepregnancy BMI, educational level, ethnicity, folic acid supplementation, smoking and alcohol consumption during pregnancy; 3) *Birth model*: confounder model

additionally adjusted for child's gestational age and weight at birth to explore whether observed associations are explained by these adverse birth outcomes; 4) *Child model*: birth model additionally adjusted for breastfeeding status and child body mass index at the time of measurements to explore whether observed associations are explained by these child factors. We consider the *confounder model* as the main model, which included covariates selected on their association with exposure and outcome or a change in effect of >10%. Second, we used conditional linear regression analyses to explore the independent associations of maternal blood pressure in early, mid and late-pregnancy with offspring outcomes^{25, 26}. These models take the correlation between maternal blood pressure measurements at different time-points throughout pregnancy into account. Using standardized residuals from linear regression models of maternal blood pressure regressed on all the previous blood pressure measurements, maternal systolic and diastolic blood pressure variables were constructed that are statistically independent of each other. This approach allows inclusion of all maternal blood pressure measures simultaneously in one regression model. Thus, associations of maternal systolic and diastolic blood pressure in each period with childhood outcomes can be assessed adjusted for, and compared with, maternal systolic and diastolic blood pressure in other periods of pregnancy. We also examined the associations of maternal systolic and diastolic blood pressure in early, mid and late-pregnancy with offspring outcomes separately using regular linear regression models, and explored the role of confounders and potential mediators. Third, as a secondary analysis, we examined the associations of paternal early-pregnancy systolic and diastolic blood pressure with offspring outcomes and compared the strength of these paternal-offspring associations with the strength of the maternal-offspring associations. Stronger associations for maternal blood pressure with offspring outcomes would suggest direct intrauterine mechanisms, while similar or stronger associations for paternal blood pressure with offspring outcomes would suggest that these associations are more likely to be driven by genetic predisposition or shared lifestyle factors²⁷. Furthermore, we tested for interactions of gestational hypertensive disorder status and maternal blood pressure with offspring sex, gestational-age-adjusted-birth weight and gestational age at birth for all childhood outcomes, but none were significant (p -values>0.05) and no stratified analyses were performed. We performed two sensitivity analyses: 1) We repeated the maternal blood pressure analyses restricting to a population of normotensive pregnancies, to assess whether the found associations are also present for a higher maternal blood pressure across the normal range; 2) We repeated all analyses excluding children born small for gestational age below the 3rd percentile, to explore whether associations were driven by severe placental insufficiency as part of the underlying mechanism. We performed

multiple imputations using the Fully Conditional Specifications (FCS) method²⁸. We created five independent datasets, that were analyzed together and presented the pooled effect estimates. Missing data on covariates was <10% of missing values for covariates, except for folic acid supplementation (23%), breastfeeding status (19%) and prepregnancy BMI (17%). Analyses were performed using IBM SPSS version 25 (SPSS Inc., Chicago, Illinois).

RESULTS

Population characteristics

Table 1 shows the population characteristics for the total population and by gestational hypertensive disorder status. For the total population, the systolic blood pressure mean (sd) was 115.5 (11.8), 116.7 (11.7) and 118.4 (11.5) mmHg in early, mid and late-pregnancy, respectively. The diastolic blood pressure mean (sd) was 68.0 (9.1), 67.0 (9.1) and 69.1 (9.1) mmHg in early, mid and late-pregnancy, respectively. In total, there were 184 women diagnosed with gestational hypertension and 85 women with preeclampsia. Women with gestational hypertension or preeclampsia had higher blood pressure levels in each pregnancy-period when compared to women with a normotensive pregnancy. **Supplementary Table S1** shows that compared to the population for analysis, mother of offspring without follow-up at the age of 10 years, were younger, lower educated and more often from non-European descent. They had a slightly lower systolic and diastolic blood pressure, a slightly higher prevalence of preeclampsia and lower prevalence of gestational hypertension.

Gestational hypertensive disorders with offspring blood pressure, carotid IMT and carotid distensibility

Table 2 shows that offspring of mothers who developed gestational hypertension had a higher systolic and diastolic blood pressure as compared to offspring from mothers with a normotensive pregnancy in the confounder model (difference: 0.17 SDS (95% CI 0.02, 0.31) and 0.23 SDS (95% CI 0.08, 0.38) in offspring systolic and diastolic blood pressure, respectively), but no differences in offspring carotid IMT and distensibility were present. Additional adjustment for gestational age and weight at birth, breastfeeding or child adiposity did not explain these findings. Offspring of mothers who developed preeclampsia only had a higher systolic blood pressure as compared to offspring from mothers with a normotensive pregnancy (difference: 0.23 (95% CI 0.02, 0.44) SDS in offspring systolic

Table 1. Characteristics of the total study population (n=4,777)

	Total population n=4,777	Normotensive pregnancy n=4,410	Gestational hypertension n=184	Preeclampsia n=85	p-value*
Maternal characteristics					
Maternal age, mean (sd), years	30.7 (4.9)	30.7 (4.9)	30.8 (4.8)	29.8 (5.0)	0.20
Prepregnancy BMI, median (95% range), kg/m ²	22.5 (18.1, 34.1)	22.4 (18.0, 33.1)	25.2 (19.4, 42.5)	23.6 (18.7, 39.8)	<0.001
Parity, n nulliparous (%)	2,769 (58.3)	2,516 (57.3)	136 (73.9)	70 (82.4)	<0.001
Education level, n higher (%)	2,274 (50.2)	2,117 (50.6)	83 (46.1)	32 (39.5)	0.07
Ethnicity, n European (%)	3,028 (64.6)	2,767 (64.0)	145 (78.8)	53 (63.9)	<0.001
Folic acid supplement use, n yes (%)	2,861 (77.9)	2,630 (77.6)	122 (85.3)	54 (76.1)	0.09
Smoking during pregnancy, n yes (%)	651 (15.3)	601 (15.3)	30 (17.9)	6 (7.7)	0.11
Alcohol consumption during pregnancy, n yes (%)	1,828 (43.3)	1,693 (43.5)	75 (45.2)	33 (41.8)	0.87
Systolic blood pressure, mean (sd), mmHg					
Early-pregnancy	115.5 (11.8)	115.5 (11.5)	124.7 (12.9)	119.7 (12.3)	<0.001
Mid-pregnancy	116.7 (11.7)	116.2 (11.4)	127.57 (12.6)	120.4 (12.7)	<0.001
Late-pregnancy	118.4 (11.5)	117.8 (11.2)	130.0 (12.4)	125.9 (12.1)	<0.001
Diastolic blood pressure, mean (sd), mmHg					
Early-pregnancy	68.0 (9.1)	67.6 (8.9)	75.4 (10.5)	72.4 (8.8)	<0.001
Mid-pregnancy	67.0 (9.1)	66.5 (8.8)	76.4 (9.7)	73.3 (9.1)	<0.001
Late-pregnancy	69.1 (9.1)	68.4 (8.7)	79.4 (9.8)	77.0 (10.0)	<0.001
Paternal characteristics					
Age, mean (sd), years	33.4 (5.5)	33.0 (5.5)	33.4 (5.4)	33.6 (5.8)	0.34
BMI, median (95% range), kg/m ²	24.9 (19.6, 32.8)	24.9 (19.6, 32.6)	26.0 (19.2, 34.0)	24.7 (19.1, 35.7)	0.00
Education level, n higher (%)	1,820 (54.7)	1,692 (55.3)	71 (46.7)	26 (44.8)	0.04
Ethnicity, n European (%)	2,921 (65.0)	2,676 (64.4)	136 (77.3)	51 (67.1)	0.00
Systolic blood pressure, mean (sd), mmHg	130.4 (13.5)	129.0 (12.4)	130.3 (13.5)	132.4 (13.3)	0.09
Diastolic blood pressure, mean (sd), mmHg	73.4 (10.5)	73.0 (9.1)	73.3 (10.5)	75.3 (11.0)	0.03

Table 1. Continued

Birth and infant characteristics		Total population n=4,777	Normotensive pregnancy n=4,410	Gestational hypertension n=184	Preeclampsia n=85	p-value*
Sex, n female (%)		2,420 (50.7)	2,215 (50.2)	98 (53.3)	51 (60.0)	0.15
Gestational age at birth, median (95% range), weeks		40.1 (35.9, 42.3)	40.1 (36.0, 42.4)	40.1 (35.3, 42.3)	38.4 (29.4, 41.7)	<0.001
Prematurity, n (%)		214 (4.5)	172 (3.9)	8 (4.3)	23 (27.1)	<0.001
Weight at birth, median (95% range), grams		3,455 (2,556, 4,470)	3,475 (2,321, 4,475)	3,315 (2,229, 4,553)	3,025 (1,015, 4,308)	<0.001
Birthweight z-score, mean (sd)†		-0.07 (0.98)	-0.05 (0.99)	-0.26 (1.12)	-0.51 (1.08)	<0.001
Small for gestational age <p10, n (%)		10.0 (4.7)	425 (9.6)	26 (14.1)	18 (21.2)	<0.001
Extremely small for gestational age <p3, n (%)		3.0 (1.4)	119 (2.7)	14 (7.6)	9 (10.6)	<0.001
Breastfeeding, n yes (%)		3,588 (93.0)	3,351 (93.2)	132 (88.0)	63 (92.6)	<0.001
Child characteristics						
Age, median (95% range), years		9.7 (9.4, 10.7)	9.7 (9.4, 10.7)	9.7 (9.3, 11.2)	9.7 (9.4, 10.8)	0.51
BMI, median (95% range), kg/m ²		17.0 (14.0, 24.9)	17.0 (14.0, 24.7)	17.5 (14.3, 25.6)	17.5 (13.7, 29.8)	<0.001
BMI z-score, median (95% range)‡		0.48 (-1.49, 3.05)	0.47 (-1.49, 2.99)	0.67 (-1.28, 3.21)	0.76 (-1.74, 4.07)	0.01
Overweight (IOTF classification), n (%)		700 (14.7)	636 (14.5)	32 (17.4)	18 (21.2)	0.02
Obese (IOTF classification), n (%)		180 (3.8)	153 (3.5)	11 (6.0)	8 (9.4)	0.02
Systolic blood pressure, mean (sd), mmHg		103.1 (7.9)	103.0 (7.9)	105.3 (7.8)	105.5 (9.3)	<0.001
Diastolic blood pressure, mean (sd), mmHg		58.6 (6.4)	58.5 (6.4)	60.3 (7.1)	59.65 (6.9)	<0.001
Carotid IMT, mean (sd), mm		0.46 (0.04)	0.46 (0.04)	0.46 (0.04)	0.46 (0.06)	0.96
Carotid distensibility, median (95% range), kPa*10 ⁻³		55.9 (39.5, 79.8)	56.0 (37.1, 85.6)	54.7 (36.3, 90.7)	54.4 (34.7, 91.9)	0.63

BMI, body mass index. IOTF, International Obesity Taskforce. IMT, intima media thickness. Values are mean (sd), median (95% range), or number (%). P-values were obtained by ANOVA for continuous variables and by χ^2 for categorical variables. †Birthweight gestational age and sex-adjusted standard deviation score. ‡BMI age and sex-adjusted standard deviation scores.

Table 2. Associations of gestational hypertension and preeclampsia with offspring blood pressure, carotid IMT and carotid distensibility at median 10 years (n=4,679)*

Offspring outcomes	Normotensive pregnancy	Gestational hypertension				Preeclampsia			
		Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child
SBP, SDS† (n=4,646)	Reference	0.28 (0.14, 0.43)**	0.17 (0.02, 0.31)*	0.17 (0.02, 0.31)*	0.16 (0.02, 0.30)*	0.32 (0.11, 0.53)*	0.23 (0.02, 0.44)*	0.19 (-0.03, 0.40)	0.12 (-0.08, 0.32)
DBP, SDS† (n=4,646)	Reference	0.27 (0.13, 0.42)**	0.23 (0.08, 0.38)*	0.22 (0.07, 0.37)*	0.22 (0.07, 0.36)*	0.17 (-0.04, 0.38)	0.12 (-0.09, 0.34)	0.08 (-0.14, 0.29)	0.06 (-0.16, 0.27)
IMT, SDS‡ (n=4,131)	Reference	-0.02 (-0.17, 0.13)	0.01 (-0.15, 0.16)	0.03 (-0.12, 0.19)	0.04 (-0.12, 0.19)	0.03 (-0.19, 0.24)	0.03 (-0.19, 0.24)	0.10 (-0.12, 0.32)	0.09 (-0.13, 0.31)
Distensibility, SDS§ (n=4,308)	Reference	-0.07 (-0.22, 0.09)	-0.02 (-0.18, 0.14)	-0.04 (-0.20, 0.12)	-0.05 (-0.20, 0.11)	-0.09 (-0.31, 0.14)	-0.06 (-0.29, 0.16)	-0.11 (-0.33, 0.12)	-0.08 (-0.30, 0.15)

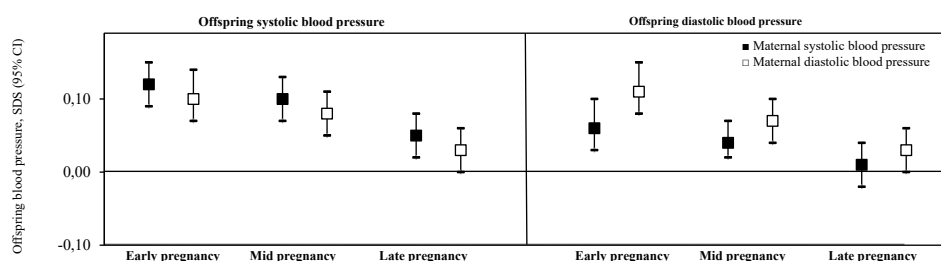
SBP, systolic blood pressure. DBP, diastolic blood pressure. IMT, intima media thickness. *P-value <0.05. **P-value <0.001. Values are regression coefficients (95% confidence interval) that were obtained from regular multivariable linear regression models, and reflect the differences in offspring blood pressure (SDS), carotid IMT (SDS) and carotid distensibility (SDS) for gestational hypertension and preeclampsia. Groups are compared to women with a normotensive pregnancy as reference. Estimates are from multiple imputed data. Basic models are adjusted for child's age and sex. Confounder model is basic model additionally adjusted for maternal age, parity, prepregnancy BMI, educational level, maternal ethnicity, folic acid supplementation and smoking and alcohol consumption during pregnancy. Birth model is confounder model additionally adjusted for child's gestational age and weight at birth. Child model is birth model additionally adjusted for offspring breastfeeding status and BMI at time of the measurements. †Study population for offspring blood pressure with 184 cases of gestational hypertension and 85 cases of preeclampsia. ‡Study population for offspring IMT with 165 cases of gestational hypertension and 77 cases of preeclampsia. §Study population for offspring distensibility with 174 cases of gestational hypertension and 83 cases of preeclampsia.

blood pressure), but no differences in offspring diastolic blood pressure, carotid IMT and distensibility were present. The association of preeclampsia with offspring systolic blood pressure attenuated towards the null after additional adjustment for birth and child factors.

Maternal blood pressure in different periods of pregnancy with offspring blood pressure, carotid IMT and carotid distensibility

Figure 2 shows the independent associations of maternal blood pressure in early, mid and late-pregnancy with offspring outcomes from conditional analyses (effect estimates with 95% confidence interval shown in **Supplementary Table S2**). Higher maternal systolic and diastolic blood pressure in early and mid-pregnancy but not late-pregnancy, were independently associated with higher offspring systolic and diastolic blood pressure and lower carotid distensibility (all p-values <0.05). No associations were present for maternal blood pressure in different periods of pregnancy with offspring carotid IMT.

A. Associations of maternal blood pressure with offspring systolic and diastolic blood pressure



B. Associations of maternal blood pressure with offspring carotid intima media thickness and distensibility

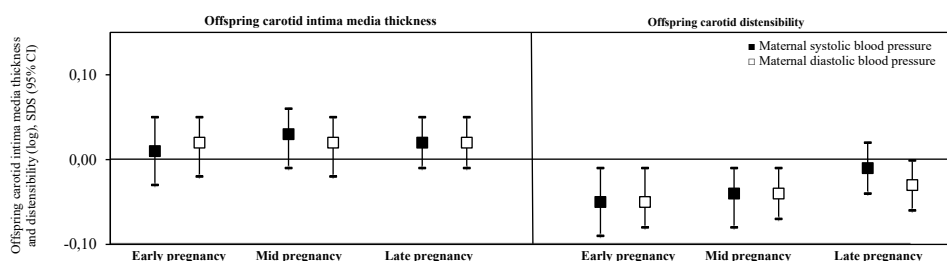


Figure 2. Associations of maternal blood pressure during pregnancy with child blood pressure (A) and, carotid IMT and distensibility (B) from conditional change analyses (n=4,771). Values are regression coefficients (95% confidence interval) and reflect the difference in offspring blood pressure (SDS), carotid IMT (SDS) and carotid distensibility (SDS) per SDS change in maternal early-pregnancy blood pressure, and per SDS change in standardized residual change in maternal blood pressure in mid and late-pregnancy from conditional models. Estimates are from multiple imputed data. Maternal blood pressure was additionally imputed for women with at least one blood pressure measurement in pregnancy. Models are adjusted for child's age and sex, gestational age at intake, maternal age, parity, prepregnancy BMI, educational level, maternal ethnicity, folic acid supplementation, smoking and alcohol consumption during pregnancy.

Role of maternal factors, birth outcomes, breastfeeding and childhood adiposity **Table 3** shows the associations of maternal early, mid and late-pregnancy systolic and diastolic blood pressure with offspring outcomes per SDS change using regular linear regression models, and the role of confounders and potential mediators. In the confounder model, higher maternal systolic and diastolic blood pressure in early, mid and late-pregnancy were associated with an increased offspring systolic and diastolic blood pressure and a decreased offspring carotid distensibility (all p-values <0.05), but not with offspring carotid IMT. For example, 1 SDS increase in maternal early-pregnancy systolic blood pressure, which corresponds to 11.8 mmHg, was related to 0.11 SDS increase in offspring systolic blood pressure, 0.06 increase in diastolic blood pressure and 0.04 decrease in carotid distensibility. Additional adjustment for gestational age and weight at birth, breastfeeding and child adiposity did not explain these associations. To investigate if the associations of maternal blood pressure with offspring blood pressure were explained by decreased distensibility, we additionally adjusted these analyses for offspring distensibility which led to a small attenuation of the effect estimates for systolic blood pressure only (**Supplementary Table S3**). To investigate if the associations of maternal blood pressure with offspring distensibility were explained by offspring blood pressure, we additionally adjusted these analyses for offspring mean arterial pressure which led to a small attenuation of the effect estimates (**Supplementary Table S4**).

Higher paternal early-pregnancy systolic and diastolic blood pressure were associated with increased offspring systolic and diastolic blood pressure, increased carotid IMT, and decreased distensibility (all p-values <0.05; **Supplementary Table S5**). The paternal associations with offspring blood pressure and carotid distensibility were comparable in strength to the maternal-offspring associations. When we included both maternal and paternal early-pregnancy blood pressure in the same model, both the maternal-offspring and paternal-offspring associations remained significant and the effect estimate was comparable in magnitude (all p-values <0.05; **Supplementary Table S6**).

Sensitivity analyses

When we restricted to a population of normotensive pregnancies we found similar associations for gestational blood pressure in early, mid and late-pregnancy with offspring outcomes (**Supplementary Table S7**). When we excluded children born small for gestational age below the 3rd percentile, we found similar associations for gestational hypertensive disorder status and gestational blood pressure in early, mid and late-pregnancy with offspring outcomes (**Supplementary Table S8 and S9**).

Table 3. Associations of maternal blood pressure with offspring blood pressure, carotid IMT and carotid distensibility at median 10 years (n=4,771)[†]

Offspring outcomes	Early-pregnancy maternal SBP				Mid-pregnancy maternal SBP				Late-pregnancy maternal SBP			
	Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child
SBP, SDS [‡]	0.13 (0.10, 0.17)**	0.11 (0.06, 0.14)**	0.11 (0.07, 0.14)**	0.10 (0.06, 0.13)**	0.16 (0.13, 0.19)**	0.14 (0.11, 0.17)**	0.14 (0.11, 0.17)**	0.12 (0.09, 0.15)**	0.14 (0.11, 0.17)**	0.11 (0.08, 0.14)**	0.11 (0.08, 0.14)**	0.09 (0.06, 0.12)**
DBP, SDS [‡]	0.07 (0.04, 0.10)**	0.06 (0.02, 0.09)*	0.06 (0.02, 0.09)*	0.05 (0.02, 0.09)*	0.08 (0.05, 0.11)**	0.07 (0.04, 0.10)**	0.07 (0.04, 0.10)**	0.06 (0.03, 0.09)*	0.05 (0.02, 0.08)**	0.04 (0.01, 0.07)**	0.04 (0.01, 0.07)**	0.03 (0.00, 0.06)*
IMT, SDS [‡]	-0.00 (-0.04, 0.03)	0.01 (-0.02, 0.05)	0.01 (-0.02, 0.05)	0.01 (-0.02, 0.05)	0.01 (-0.03, 0.04)	0.02 (-0.01, 0.06)	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)	0.01 (-0.02, 0.04)	0.03 (-0.00, 0.06)	0.03 (-0.00, 0.06)	0.03 (-0.01, 0.06)
Distensibility, SDS [§]	-0.05 (-0.10, -0.02)*	-0.04 (-0.08, -0.01)*	-0.04 (-0.09, 0.00)	-0.04 (-0.08, -0.00)*	-0.07 (-0.10, -0.04)**	-0.06 (-0.09, -0.03)**	-0.07 (-0.11, -0.03)**	-0.05 (-0.09, -0.02)*	-0.05 (-0.08, -0.02)*	-0.04 (-0.07, -0.00)*	-0.04 (-0.08, 0.00)	-0.03 (-0.06, 0.01)
Offspring outcomes	Early-pregnancy maternal DBP				Mid-pregnancy maternal DBP				Late-pregnancy maternal DBP			
	Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child
SBP, SDS [‡]	0.13 (0.10, 0.16)**	0.09 (0.06, 0.13)**	0.09 (0.06, 0.13)**	0.09 (0.06, 0.12)**	0.16 (0.13, 0.19)**	0.12 (0.09, 0.15)**	0.12 (0.09, 0.15)**	0.11 (0.08, 0.13)**	0.13 (0.10, 0.15)**	0.09 (0.06, 0.12)**	0.08 (0.05, 0.11)**	0.07 (0.04, 0.10)*
DBP, SDS [‡]	0.12 (0.09, 0.15)**	0.10 (0.07, 0.14)**	0.10 (0.07, 0.14)**	0.10 (0.07, 0.14)**	0.13 (0.10, 0.16)**	0.11 (0.08, 0.14)**	0.11 (0.07, 0.14)**	0.10 (0.07, 0.13)**	0.11 (0.08, 0.14)**	0.09 (0.06, 0.12)**	0.09 (0.06, 0.12)**	0.08 (0.05, 0.11)**
IMT, SDS [‡]	-0.00 (-0.03, 0.04)	0.02 (-0.02, 0.05)	0.02 (-0.01, 0.06)	0.02 (-0.01, 0.06)	0.01 (-0.02, 0.04)	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)	0.03 (-0.00, 0.06)	0.04 (0.00, 0.07)*	0.03 (0.00, 0.07)*
Distensibility, SDS [§]	-0.04 (-0.08, -0.01)*	-0.04 (-0.08, -0.00)*	-0.04 (-0.08, -0.00)*	-0.04 (-0.08, -0.00)*	-0.07 (-0.10, -0.04)**	-0.06 (-0.09, -0.03)**	-0.06 (-0.09, -0.02)**	-0.05 (-0.08, -0.01)*	-0.06 (-0.09, -0.03)**	-0.06 (-0.09, -0.03)**	-0.06 (-0.09, -0.03)**	-0.05 (-0.08, -0.02)*

SBP, systolic blood pressure. DBP, diastolic blood pressure. IMT, intima media thickness. *P-value <0.05. **P-value <0.001. Values are regression coefficients (95% confidence interval) that were obtained from regular multivariable linear regression models, and reflect the differences in offspring blood pressure (SDS), carotid IMT (SDS) and carotid distensibility (SDS) per SDS change in maternal blood pressure. Estimates are from multiple imputed data. Basic model is adjusted for child's age and sex, and gestational age at the time of blood pressure measurements. Confounder model is basic model additionally adjusted for maternal age, parity, prepregnancy BMI, educational level, maternal ethnicity, folic acid supplementation and smoking and alcohol consumption during pregnancy. Birth model is confounder model additionally adjusted for child's gestational age and weight at birth. Child model is birth model additionally adjusted for offspring breastfeeding status and BMI at time of the measurements. [†]Study population for offspring blood pressure: n=3,727 for early-pregnancy, n=4,487 for mid-pregnancy, n=4,577 for late-pregnancy. [‡]Study population for offspring IMT: n=3,448 for early-pregnancy, n=4,166 for mid-pregnancy, n=4,249 for late-pregnancy. [§]Study population for offspring distensibility: n=3,298 for early-pregnancy, n=3,990 for mid-pregnancy, n=4,076 for late-pregnancy.

DISCUSSION

We observed that offspring exposed to maternal gestational hypertension and already a higher maternal gestational blood pressure across the full blood pressure spectrum had a higher systolic and diastolic blood pressure and lower carotid distensibility at 10 years, but no differences in carotid IMT were present. Maternal systolic and diastolic blood pressure in early and mid-pregnancy, but not late-pregnancy, were independently associated with these offspring outcomes. No associations were present for preeclampsia. These findings were not explained by maternal, birth or child factors. However, as the maternal blood pressure and paternal blood pressure associations with these offspring outcomes were comparable in strength, these associations are more likely driven by genetic predisposition and shared lifestyle rather than by a direct intrauterine effect.

Methodological considerations

Strengths of our study are prospective data collection from early-pregnancy to school-age, a large sample size, repeated maternal blood pressure measurements during early, mid and late-pregnancy, and the availability of paternal blood pressure at study enrollment. From the mothers with singleton-life births and available information on the exposures during pregnancy, 56% of the children participated in the current study. Compared to the population for analysis, mothers of offspring without childhood follow-up had a slightly lower systolic and diastolic blood pressure blood pressure, a higher prevalence of preeclampsia, and a lower prevalence of gestational hypertension. These differences were only small and not of clinical relevance. Still, possible self-selection of children that are more healthy could have occurred, but we are not able to assess this with the information that we have available within our study. A selective non-response could have led to biased effect estimates if associations would be different between the included children and non-included children, but this does not seem likely. We had a relatively small number of gestational hypertension and preeclampsia, which might have led to reduced statistical power for the gestational hypertensive disorder analyses. Our prevalence of gestational hypertensive disorders was slightly lower when compared to the general Dutch population, this may be due to the exclusion of preexisting hypertension from the current study. Not all women had three blood pressure measurements during pregnancy available due to later enrollment or because they missed a physical examination. To avoid a reduction of statistical power for the conditional analyses, we imputed the maternal blood pressure measurements for these analyses only. When we compared the results of the imputed versus the complete-case analyses, the effect estimates were similar. Due to the design

of our cohort and limited time available during research visits, the child's blood pressure was measured during the ultrasound of the common carotid artery in supine position. Absolute blood pressure values might have been lower if they had been measured in seated position, which is the standard position for children's blood pressure measurement at the age of 10 years in clinical practice²⁹. Within our study we were interested in relative blood pressure differences by maternal gestational blood pressure levels among a group of children, which makes it unlikely that the method of measurement biased our results. Of the children 7.6% and 18.6% did not have three measurements on both sides of the common carotid artery for calculation of the carotid IMT and distensibility, respectively. This was due to low quality recordings or missing coinciding cardiac cycles. We included all children with at least one reliable carotid IMT or distensibility measurement in our main analyses. When we repeated the analyses among children with all three measurements on both sides available, we observed similar results (results not shown). We did not adjust for multiple testing since the childhood outcomes are strongly correlated. Finally, we had detailed information on a large number of covariates. Although we accurately tried to control for confounding, the observational nature of the study still leaves possibility for residual confounding because of unmeasured lifestyle factors or family history.

Interpretation of main findings

Gestational hypertensive disorders are an important risk factor for adverse birth outcomes and associated with a higher blood pressure in mothers and the offspring in later life. Results from animal studies suggest that exposure to an adverse intrauterine environment induced by impaired gestational hemodynamic adaptations might lead to atherosclerotic vascular alterations and higher blood pressure in the offspring, but only few studies investigated this among human populations. Carotid IMT and distensibility are sensitive markers to investigate atherosclerotic changes in pediatric and adult populations^{30, 31}. Carotid IMT primarily reflects the formation of fatty streaks by the accumulation of lipids in the intima media of the common carotid artery, while carotid distensibility is inversely related to arterial stiffness³⁰. Carotid IMT and distensibility are both strongly associated with systemic atherosclerosis³². These subclinical atherosclerotic markers have been associated with higher blood pressure in adulthood and an increased risk of all-cause cardiovascular mortality^{33, 34}. We hypothesized that offspring exposed to gestational hypertensive disorders, or already a higher maternal gestational blood pressure across the full spectrum are at risk of these adverse atherosclerotic changes, predisposing them to a higher blood pressure.

A recent systematic review of ten studies concluded that gestational hypertension is associated with higher offspring blood pressure during childhood and adolescence,

but these associations were inconsistent for offspring of pregnancies affected by preeclampsia³. Only few studies investigated the direct effects on offspring vascular development in response to maternal gestational hypertensive disorders. A study among 138 children at the age of 14 years, permanently living at high altitude in Bolivia, found that pulmonary artery pressure was higher and brachial artery flow-mediated dilation was smaller in offspring from pregnancies affected by preeclampsia compared to normotensive pregnancies¹². Likewise, a study from the United Kingdom among 71 subjects born preterm, found that those that were exposed to preeclampsia or gestational hypertension, had an increased carotid IMT and flow-mediated dilatation at the age of 20 years¹⁰. Two small studies found that neonates exposed to preeclampsia had an increased aortic IMT when compared to normotensive pregnancies^{9,11}. In these studies, no extensive adjustment for confounders was performed. Contrary, in a study among ~4,000 mother-offspring pairs from the United Kingdom no associations of gestational hypertensive disorders with brachial artery flow-mediated dilatation, brachial pulse wave velocity and brachial distensibility in children at the age of 9 to 12 years were observed². Partly in line with these previous studies, we observed that gestational hypertension, but not preeclampsia, was associated with a higher offspring blood systolic and diastolic pressure at the age of 10 years, independent of maternal, birth or childhood factors. We did not find any associations for gestational hypertension or preeclampsia with offspring carotid IMT and distensibility. Differences between our study and the previous studies may relate to the timing of vascular assessment. Neonatal aortic intima media thickening might only reflect a temporary alteration, in a response to insufficient placental flow in preeclamptic pregnancies, that does not persist into childhood^{11,35}. Furthermore, fatty deposits in the carotid intima media only first emerge during early adolescence and may not yet be detectable at the age of 10 years³⁶. Thus, we found that offspring exposed to gestational hypertension, but not preeclampsia, had an increased systolic and diastolic blood pressure at the age of 10 years when compared to offspring from normotensive pregnancies, but they did not display early signs of atherosclerotic vascular changes.

Gestational hypertension and preeclampsia represent the extremes of the gestational hypertensive disorder spectrum, but already a higher maternal blood pressure below the clinical threshold for gestational hypertensive disorders may be associated with a higher offspring blood pressure^{5,6}. In line with our findings for gestational hypertension, we observed that higher maternal gestational systolic and diastolic blood pressure across the full spectrum were associated with increased offspring systolic and diastolic blood pressure, and decreased carotid distensibility. These associations were also present when we restricted to a population of normotensive pregnancies. We observed the strongest and

independent effects for maternal early and mid-pregnancy systolic and diastolic blood pressure. This is in line with a previous study within our observational cohort that focused on the associations of maternal gestational blood pressure with childhood blood pressure among 6 year old children⁵. Similarly, a study among 6,619 mother-offspring pairs from the United Kingdom and a Danish study among 2,217 mother-offspring pairs, also found a positive association of early-pregnancy maternal systolic and diastolic blood pressure with offspring systolic and diastolic blood pressure in infancy, childhood and adolescence^{6, 37}. No previous study explored the direct effects of maternal gestational blood pressure on offspring vascular properties of large arteries. We observed that higher maternal gestational blood pressure across the full spectrum was associated with decreased carotid distensibility in the offspring, with the strongest effect in early and mid-pregnancy. When we adjusted the offspring blood pressure analyses for carotid distensibility, effect estimates for offspring systolic blood pressure partly attenuated. This suggests that early functional offspring vascular changes might represent steps in the pathophysiological pathway, predisposing offspring to a higher systolic blood pressure also later in life. However, the effect estimates for carotid distensibility also partly attenuated when these analyses were additionally adjusted for mean arterial pressure. As offspring blood pressure and carotid distensibility were measured at the same time, it is difficult to disentangle how arterial stiffness may influence offspring blood pressure and vice versa. Further studies should focus on the relation between blood pressure levels and arterial stiffness in children, and whether arterial stiffness is a cause or consequence of higher blood pressure levels. It is known that in an early stage of cardiovascular disease the formation of fatty streaks in the carotid intima media are preceded by functional vascular changes related to arterial stiffness, which may explain why we did not find an association with carotid IMT³⁰.

Our findings suggest that maternal gestational hypertension and higher gestational blood pressure, even below the diagnostic threshold for gestational hypertensive disorders, might influence offspring blood pressure and arterial stiffness at the age of 10 years. These observed associations may be explained by several mechanisms. The associations for gestational hypertension and maternal gestational blood pressure with offspring outcomes were not explained by maternal socio-demographic and lifestyle factors or mediated by gestational age and weight at birth, breastfeeding or child adiposity. Contrary, the only observed effect of preeclampsia with offspring systolic blood pressure attenuated towards the null after additional adjustment for gestational age at birth and birth weight. Preeclampsia is a well-known risk factor for preterm birth and small for gestational age at birth, both birth outcomes that are associated with increased blood pressure in later life. Our findings suggest that the associations of preeclampsia with higher offspring

systolic blood pressure, are explained by these adverse birth outcomes. This is in line with the findings from other large observational studies^{3, 38}. Animal studies suggest that fetal exposure to an adverse intrauterine environment from early gestation, may lead to atherosclerotic vascular remodeling in the offspring⁸. However, atherosclerotic changes in the offspring can also be explained by shared genetic predisposition or lifestyle factors in mother-offspring pairs. Especially as mothers who suffered gestational hypertension or preeclampsia also have an increased risk of cardiovascular disease in later life. When we compared the strength of the maternal-offspring and paternal-offspring associations with offspring blood pressure and distensibility, the associations for maternal and paternal blood pressure were similar. This suggests that the associations of maternal gestational blood pressure with offspring blood pressure and arterial stiffness are more likely to be driven by shared genetic predisposition or lifestyle factors between mother and child, rather than by a direct intrauterine effect. We found similar associations when we repeated the analyses excluding children born extremely small for gestational age, these findings further contradict a direct intrauterine effect as the underlying mechanism for the found associations. Despite animal studies identifying early-pregnancy as a critical period for fetal vascular developmental adaptations, maternal blood pressure levels during early and mid-pregnancy might also reflect maternal genetic predisposition to a higher blood pressure, while this is reflected less by late-pregnancy blood pressure when more gestational hemodynamic adaptations have taken place³⁹. In a previous study among 3,748 children within our cohort, we found that gestational hypertensive disorders and gestational blood pressure influence offspring retinal vessel calibers at the age of 6 years, with stronger maternal-offspring than paternal-offspring associations⁷. Based on findings from this previous study and our current study, higher maternal blood pressure levels in pregnancy might have a direct effect on offspring microvasculature development, but to a lesser extent on offspring vascular properties of large arteries. Further observational and experimental studies need to focus on disentangling the underlying mechanisms for micro- and macrovascular changes in the offspring in response to maternal gestational blood pressure, and critical periods for exposure to a higher maternal blood pressure during pregnancy.

Perspectives

Maternal gestational hypertension and higher maternal gestational blood pressure across the full blood pressure spectrum are associated with a higher childhood blood pressure and lower carotid distensibility. This suggests that differences in arterial stiffness may already be present in their offspring from childhood onwards. No associations were

found for preeclampsia with offspring vascular outcomes. The strongest effects were present for maternal blood pressure in early and mid-pregnancy. These findings were not explained by maternal, birth or child factors. As the strength of the associations of maternal and paternal blood pressure with offspring vascular outcomes were comparable, these associations are most likely driven by shared genetic predisposition and lifestyle factors between mothers and offspring, rather than a direct intrauterine effect.

Even though the observed associations are relatively small, our findings are important on a population level and from a public health perspective. Higher blood pressure is known to track from childhood into adulthood⁴⁰. Higher blood pressure and increased arterial stiffness during adulthood are strong independent predictors for hypertension, myocardial infarction, stroke and all-cause cardiovascular mortality^{33, 34, 41, 42}. Our study suggests that the maternal gestational blood pressure profile might be useful for early identification of offspring at increased risk of an adverse cardiovascular risk profile in later life. These children may benefit from prevention strategies focused on reducing risk factors for cardiovascular diseases from early life onwards. Further studies are needed to investigate the long-term offspring cardiovascular consequences, and the potential of using maternal gestational blood pressure in screening tools for early-identification of children at increased risk of cardiovascular diseases.

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SUPPLEMENTAL MATERIAL

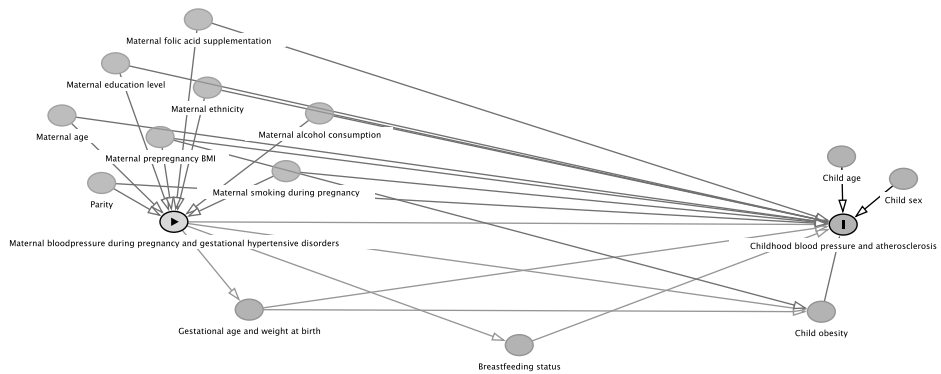


Figure S1. Directed acyclic graph with potential confounders and mediators.

Table S1. Non-response analysis: Baseline characteristics for the total study population with offspring with blood pressure and carotid ultrasound follow-up vs. baseline characteristics of population without offspring cardiovascular follow-up at 10 years

	Follow-up at 10 years n=4,777	No follow-up at 10 years n=3,737
<u>Maternal characteristics</u>		
Maternal age, mean (sd), years	30.7 (4.9)	28.2 (5.5)
Prepregnancy BMI, median (95% range), kg/m ²	22.5 (18.1, 34.1)	22.7 (17.7, 35.5)
Parity, n nulliparous (%)	2,769 (58.3)	1,903 (52.2)
Education level, n higher (%)	2,274 (50.2)	970 (30.4)
Ethnicity, n European (%)	3,028 (64.6)	1,532 (46.0)
Folic acid supplement use, n yes (%)	2,861 (77.9)	1,571 (60.0)
Smoking during pregnancy, n yes (%)	651 (15.3)	729 (19.5)
Alcohol consumption during pregnancy, n yes (%)	1,828 (43.3)	868 (23.2)
Preeclampsia, n yes (%)	85 (1.9)	84 (2.5)
Gestational hypertension, n yes (%)	184 (4.0)	121 (3.5)
Systolic blood pressure, mean (sd), mmHg		
Early-pregnancy	115.5 (11.8)	114.8 (12.3)
Mid-pregnancy	116.7 (11.7)	115.9 (12.1)
Late-pregnancy	118.4 (11.5)	117.4 (12.4)
Diastolic blood pressure, mean (sd), mmHg		
Early-pregnancy	68.0 (9.1)	67.8 (9.6)
Mid-pregnancy	67.0 (9.1)	66.9 (9.4)
Late-pregnancy	69.1 (9.1)	68.5 (9.3)
<u>Paternal characteristics</u>		
Age, mean (sd), years	33.4 (5.5)	31.6 (6.0)
BMI, median (95% range), kg/m ²	24.9 (19.6, 32.8)	25.1 (19.2, 33.8)
Education level, n higher (%)	1,820 (54.7)	746 (43.2)
Ethnicity, n European (%)	2,921 (65.0)	1,363 (46.4)
Systolic blood pressure, mean (sd), mmHg	130.4 (13.5)	129.5 (13.5)
Diastolic blood pressure, mean (sd), mmHg	73.4 (10.5)	73.0 (10.9)
<u>Birth and infant characteristics</u>		
Sex, n female (%)	2,420 (50.7)	1,774 (48.1)
Gestational age at birth, median (95% range), weeks	40.1 (35.9, 42.3)	40.0 (35.3, 42.3)
Prematurity, n (%)	214 (4.5)	223 (6.0)
Weight at birth, median (95% range), grams	3,455 (2,556, 4,470)	3,390 (2,217, 4,500)
Breastfeeding, n yes (%)	3,588 (93.0)	1,877 (90.8)

BMI, body mass index. Values are mean (sd), median (95% range), or number (%). *Baseline characteristics of the population that enrolled during pregnancy but did not attend at the follow-up visit at 10 year (n=3,451), or no measurements done during the visit at 10 years (n=286). Children with cardiac abnormalities are excluded from this analyses.

Table S2. Associations of maternal blood pressure with offspring blood pressure, carotid intima media thickness and carotid distensibility at median 10 years from conditional change analyses (n=4,771)^a

Offspring outcomes	Maternal SBP		
	Early-pregnancy	Mid-pregnancy	Late-pregnancy
SBP, SDS n=4,738	0.12 (0.09, 0.15)**	0.10 (0.07, 0.13)**	0.05 (0.02, 0.08)*
DBP, SDS n=4,738	0.06 (0.03, 0.10)**	0.04 (0.02, 0.07)*	0.01 (-0.02, 0.04)
IMT, SDS n=4,397	0.01 (-0.03, 0.05)	0.03 (-0.01, 0.06)	0.02 (-0.01, 0.05)
Distensibility, SDS n=4,219	-0.05 (-0.09, -0.01)*	-0.04 (-0.08, -0.01)*	-0.01 (-0.04, 0.02)
Offspring outcomes	Maternal DBP		
	Early-pregnancy	Mid-pregnancy	Late-pregnancy
SBP, SDS n=4,738	0.10 (0.07, 0.14)**	0.08 (0.05, 0.11)**	0.03 (-0.00, 0.06)
DBP, SDS n=4,738	0.11 (0.08, 0.15)**	0.07 (0.04, 0.10)**	0.03 (0.00, 0.06)
IMT, SDS n=4,397	0.02 (-0.02, 0.05)	0.02 (-0.02, 0.05)	0.02 (-0.01, 0.05)
Distensibility, SDS n=4,219	-0.05 (-0.08, -0.01)*	-0.04 (-0.07, -0.01)*	-0.03 (-0.06, 0.00)

SBP, systolic blood pressure. DBP, diastolic blood pressure. IMT, intima media thickness. *P-value <0.05. ** P-value <0.001. ^aValues are regression coefficients (95% confidence interval) that reflect the differences in offspring blood pressure (SDS), carotid IMT (SDS) and carotid distensibility (SDS) per SDS change in maternal early-pregnancy blood pressure, and per SDS change in standardized residual change in maternal blood pressure in mid and late-pregnancy from conditional change models. Estimates are from multiple imputed data. Maternal blood pressure was additionally imputed for women with at least one blood pressure measurement in pregnancy. Models are adjusted for child's age and sex, gestational age at intake, maternal age, parity, prepregnancy BMI, educational level, maternal ethnicity, folic acid supplementation, smoking and alcohol consumption during pregnancy.

Table S3. Associations of maternal blood pressure with offspring blood pressure adjusted for offspring distensibility (n=4,219)^a

Offspring outcomes	Maternal SBP		
	Early-pregnancy	Mid-pregnancy	Late-pregnancy
SBP, SDS	0.09 (0.06, 0.12)**	0.10 (0.08, 0.13)**	0.08 (0.05, 0.11)**
DBP, SDS	0.07 (0.03, 0.10)**	0.06 (0.03, 0.09)**	0.04 (0.00, 0.06)*
Offspring outcomes	Maternal DBP		
	Early-pregnancy	Mid-pregnancy	Late-pregnancy
SBP, SDS	0.07 (0.04, 0.11)**	0.09 (0.06, 0.12)**	0.06 (0.03, 0.09)*
DBP, SDS	0.10 (0.06, 0.14)**	0.11 (0.08, 0.14)**	0.09 (0.05, 0.11)**

SBP, systolic blood pressure. DBP, diastolic blood pressure. *P-value <0.05. ** P-value <0.001. ^aValues are regression coefficients (95% confidence interval) that were obtained from regular multivariable linear regression models with maternal blood pressure as SDS, and reflect the differences in offspring blood pressure (SDS) per SDS change in maternal blood pressure. Estimates are from multiple imputed data (distensibility not imputed). Models are adjusted for child's age and sex, gestational age at the time of maternal blood pressure measurements, maternal age, parity, prepregnancy BMI, educational level, maternal ethnicity, folic acid supplementation, smoking and alcohol consumption during pregnancy, child's gestational age and weight at birth, breastfeeding status, offspring BMI and distensibility. Study population with data on offspring blood pressure and carotid distensibility: n=3,288 for early-pregnancy, n=3,972 for mid-pregnancy, n=4,059 for late-pregnancy.

Table S4. Associations of maternal blood pressure with offspring distensibility adjusted for offspring mean arterial pressure (n=4,219)

Offspring outcomes	Maternal SBP		
	Early-pregnancy	Mid-pregnancy	Late-pregnancy
Distensibility, SDS	-0.04 (-0.07, 0.00)	-0.05 (-0.08, -0.01)*	-0.02 (-0.05, 0.01)
Offspring outcomes	Maternal DBP		
	Early-pregnancy	Mid-pregnancy	Late-pregnancy
Distensibility, SDS	-0.03 (-0.07, 0.00)	-0.05 (-0.08, -0.01)*	-0.04 (-0.08, -0.01)*

*P-value <0.05. Values are regression coefficients (95% confidence interval) that were obtained from regular multivariable linear regression models with maternal blood pressure as SDS, and reflect the differences in offspring distensibility (SDS) per SDS change in maternal blood pressure. Estimates are from multiple imputed data (mean arterial pressure not imputed). Models are adjusted for child's age and sex, gestational age at the time of maternal blood pressure measurements, maternal age, parity, prepregnancy BMI, educational level, maternal ethnicity, folic acid supplementation, smoking and alcohol consumption during pregnancy, child's gestational age and weight at birth, breastfeeding status, offspring BMI and mean arterial pressure. Study population with data on offspring carotid distensibility and mean arterial pressure: n=3,288 for early-pregnancy, n=3,972 for mid-pregnancy, n=4,059 for late-pregnancy.

Table S5. Associations of paternal blood pressure with offspring blood pressure, carotid intima media thickness and carotid distensibility at median 10 years (n=3,518)

Offspring outcomes	Paternal SBP			
	Basic model	Confounder model	Birth model	Child model
SBP, SDS n=3,518	0.13 (0.10, 0.16)**	0.11 (0.07, 0.14)**	0.11 (0.07, 0.14)**	0.12 (0.09, 0.15)**
DBP, SDS n=3,518	0.04 (0.01, 0.07)*	0.04 (0.01, 0.08)*	0.04 (0.01, 0.08)*	0.05 (0.01, 0.08)*
IMT, SDS n=3,276	0.04 (0.01, 0.07)*	0.04 (0.01, 0.08)*	0.04 (0.00, 0.08)*	0.04 (0.01, 0.08)*
Distensibility, SDS n=3,124	-0.08 (-0.11, -0.05)**	-0.05 (-0.10, -0.02)*	-0.06 (-0.09, -0.02)*	-0.06 (-0.10, -0.03)*
Offspring outcomes	Paternal DBP			
	Basic model	Confounder model	Birth model	Child model
SBP, SDS n=3,518	0.09 (0.06, 0.13)**	0.08 (0.05, 0.11)**	0.08 (0.05, 0.11)**	0.10 (0.06, 0.13)**
DBP, SDS n=3,518	0.09 (0.06, 0.12)**	0.10 (0.07, 0.14)**	0.10 (0.07, 0.14)**	0.11 (0.07, 0.14)**
IMT, SDS n=3,276	0.03 (-0.00, 0.07)	0.03 (-0.01, 0.07)	0.03 (-0.00, 0.07)	0.04 (0.00, 0.07)*
Distensibility, SDS n=3,124	-0.07 (-0.10, -0.03)**	-0.04 (-0.08, -0.01)*	-0.05 (-0.08, -0.01)*	-0.05 (-0.09, -0.02)*

SBP, systolic blood pressure. DBP, diastolic blood pressure. IMT, intima media thickness. *P-value <0.05. **P-value <0.001. Values are regression coefficients (95% confidence interval) from regular multivariable linear regression models and reflect the differences in offspring blood pressure (SDS), carotid IMT (SDS) and carotid distensibility (SDS) per SDS change in paternal blood pressure. Estimates are from multiple imputed data. Basic models are adjusted for child's age and sex. Confounder model is adjusted for child's age and sex, paternal age, parity, BMI of the father during blood pressure measurement, paternal educational level, paternal ethnicity, maternal folic acid supplementation, maternal smoking and maternal alcohol consumption during pregnancy. Birth model is confounder model additionally adjusted for child's gestational age and weight at birth. Child model is birth model additionally adjusted for offspring breastfeeding status and BMI at time of the measurements.

Table S6. Combined associations of maternal and paternal blood pressure with offspring blood pressure, carotid intima media thickness and carotid distensibility at median 10 years (n=2,929)^a

Offspring outcomes		Maternal and paternal SBP		
		Basic model	Combined confounder model	Fully adjusted model
SBP, SDS n=2,930	Maternal SBP	0.12 (0.09, 0.16)**	0.10 (0.06, 0.14)**	0.09 (0.05, 0.12)**
	Paternal SBP	0.10 (0.07, 0.13)**	0.10 (0.06, 0.13)**	0.11 (0.07, 0.14)**
DBP, SDS n=2,930	Maternal SBP	0.07 (0.04, 0.11)**	0.06 (0.02, 0.10)*	0.05 (0.02, 0.09)*
	Paternal SBP	0.03 (-0.01, 0.07)	0.04 (0.01, 0.08)*	0.04 (0.01, 0.08)*
IMT, SDS n=2,720	Maternal SBP	-0.02 (-0.06, 0.02)	-0.00 (-0.04, 0.04)	-0.00 (-0.04, 0.04)
	Paternal SBP	0.06 (0.02, 0.10)*	0.06 (0.02, 0.10)*	0.06 (0.02, 0.10)*
Distensibility, SDS n=2,583	Maternal SBP	-0.04 (-0.08, -0.01)*	-0.04 (-0.08, 0.00)	-0.03 (-0.07, 0.01)
	Paternal SBP	-0.06 (-0.11, -0.04)**	-0.06 (-0.10, -0.02)*	-0.06 (-0.10, -0.02)*

Offspring outcomes		Maternal and paternal DBP		
		Basic model	Combined confounder model	Fully adjusted model
SBP, SDS n=2,930	Maternal DBP	0.11 (0.07, 0.14)**	0.08 (0.04, 0.11)*	0.07 (0.04, 0.11)**
	Paternal DBP	0.07 (0.03, 0.10)**	0.06 (0.02, 0.10)*	0.08 (0.04, 0.11)**
DBP, SDS n=2,930	Maternal DBP	0.11 (0.07, 0.14)**	0.09 (0.05, 0.13)**	0.09 (0.05, 0.12)**
	Paternal DBP	0.07 (0.03, 0.11)**	0.09 (0.05, 0.13)**	0.09 (0.06, 0.13)**
IMT, SDS n=2,720	Maternal DBP	-0.01 (-0.05, 0.03)	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)
	Paternal DBP	0.04 (0.00, 0.08)*	0.04 (-0.00, 0.08)	0.04 (0.00, 0.08)*
Distensibility, SDS n=2,583	Maternal DBP	-0.03 (-0.07, 0.01)	-0.02 (-0.06, 0.02)	-0.03 (-0.07, 0.01)
	Paternal DBP	-0.06 (-0.10, -0.02)*	-0.04 (-0.08, 0.00)	-0.04 (-0.08, -0.00)*

SBP, systolic blood pressure. DBP, diastolic blood pressure. IMT, intima media thickness. *P-value <0.05. **P-value <0.001. Values are regression coefficients (95% confidence interval) from regular multivariable linear regression models and reflect the differences in offspring blood pressure (SDS), carotid intima media thickness (SDS) and carotid distensibility (SDS) per SDS change in maternal (early-pregnancy) and paternal blood pressure. Estimates are from multiple imputed data. Basic models are adjusted for child's age and sex. Combined confounder model is adjusted for maternal and paternal confounders, maternal and paternal age, parity, maternal and paternal BMI, maternal and paternal educational level, maternal and paternal ethnicity, maternal folic acid supplementation, maternal smoking and maternal alcohol consumption during pregnancy. Fully adjusted model is the combined model also adjusted for child's gestational age and weight at birth, offspring breastfeeding status and BMI at time of the measurements.

Table S7. Associations of maternal blood pressure with offspring blood pressure, carotid IMT and carotid distensibility at median 10 years in normotensive pregnancies (n=4,410)^a

Offspring outcomes	Early-pregnancy maternal SBP				Mid-pregnancy maternal SBP				Late-pregnancy maternal SBP			
	Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child
SBP, SDS ^b	0.13 (0.10, 0.17)**	0.11 (0.06, 0.14)**	0.11 (0.08, 0.15)**	0.10 (0.07, 0.13)**	0.16 (0.13, 0.19)**	0.13 (0.10, 0.17)**	0.13 (0.10, 0.16)**	0.12 (0.08, 0.15)**	0.13 (0.10, 0.16)**	0.11 (0.08, 0.14)**	0.11 (0.08, 0.14)**	0.09 (0.06, 0.12)**
DBP, SDS ^b	0.07 (0.04, 0.10)**	0.06 (0.02, 0.10)*	0.06 (0.02, 0.09)*	0.06 (0.02, 0.09)*	0.04 (0.01, 0.07)**	0.06 (0.02, 0.09)**	0.06 (0.03, 0.09)**	0.05 (0.02, 0.09)*	0.04 (0.01, 0.07)**	0.03 (0.00, 0.07)*	0.03 (0.00, 0.06)	0.03 (-0.00, 0.06)
IMT, SDS ^b	-0.00 (-0.04, 0.03)	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)	0.00 (-0.03, 0.03)	0.02 (-0.02, 0.05)	0.01 (-0.02, 0.05)	0.01 (-0.02, 0.05)	0.01 (-0.02, 0.04)	0.02 (-0.01, 0.06)	0.02 (-0.01, 0.06)	0.02 (-0.02, 0.05)
Distensibility, SDS ^b	-0.06 (-0.09, -0.02)*	-0.05 (-0.09, 0.02)*	-0.05 (-0.09, 0.02)*	-0.05 (-0.09, -0.01)*	-0.07 (-0.10, -0.04)**	-0.07 (-0.10, -0.03)**	-0.07 (-0.11, -0.03)**	-0.06 (-0.09, -0.02)*	-0.05 (-0.08, -0.02)*	-0.04 (-0.08, -0.01)*	-0.04 (-0.08, -0.01)*	-0.03 (-0.06, 0.00)
Offspring outcomes	Early-pregnancy maternal DBP				Mid-pregnancy maternal DBP				Late-pregnancy maternal DBP			
	Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child
SBP, SDS ^b	0.12 (0.09, 0.16)**	0.09 (0.06, 0.13)**	0.09 (0.05, 0.13)**	0.09 (0.06, 0.13)**	0.15 (0.12, 0.18)**	0.12 (0.08, 0.15)**	0.11 (0.08, 0.15)**	0.11 (0.08, 0.14)**	0.12 (0.09, 0.15)**	0.09 (0.06, 0.12)**	0.08 (0.05, 0.11)**	0.07 (0.04, 0.10)*
DBP, SDS ^b	0.13 (0.09, 0.16)**	0.11 (0.08, 0.15)**	0.11 (0.07, 0.14)**	0.11 (0.07, 0.14)**	0.13 (0.10, 0.16)**	0.11 (0.08, 0.14)**	0.11 (0.07, 0.14)**	0.10 (0.07, 0.14)**	0.11 (0.08, 0.14)**	0.09 (0.06, 0.12)**	0.09 (0.05, 0.12)**	0.08 (0.05, 0.12)**
IMT, SDS ^b	-0.00 (-0.04, 0.03)	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)	0.01 (-0.02, 0.05)	0.01 (-0.03, 0.04)	0.01 (-0.02, 0.05)	0.02 (-0.02, 0.05)	0.02 (-0.02, 0.05)	0.02 (-0.02, 0.05)	0.03 (-0.01, 0.06)	0.03 (-0.00, 0.07)	0.03 (-0.00, 0.06)
Distensibility, SDS ^b	-0.04 (-0.07, -0.00)*	-0.03 (-0.07, 0.01)	-0.04 (-0.07, -0.00)*	-0.04 (-0.07, 0.00)	-0.07 (-0.10, -0.03)**	-0.06 (-0.09, -0.02)**	-0.06 (-0.09, -0.02)**	-0.06 (-0.09, -0.02)*	-0.06 (-0.09, -0.03)**	-0.06 (-0.09, -0.02)**	-0.06 (-0.09, -0.02)*	-0.05 (-0.09, -0.02)*

SBP, systolic blood pressure. DBP, diastolic blood pressure. IMT, intima media thickness. *P-value <0.05. **P-value <0.001. Values are regression coefficients (95% confidence interval) that were obtained from regular multivariable linear regression models, and reflect the differences in offspring blood pressure (SDS), carotid IMT (SDS) and carotid distensibility (SDS) per SDS change in maternal blood pressure. Estimates are from multiple imputed data. Basic model is adjusted for child's age and sex, and gestational age at the time of blood pressure measurements. Confounder model is basic model additionally adjusted for maternal age, parity, prepregnancy BMI, educational level, maternal ethnicity, folic acid supplementation and smoking and alcohol consumption during pregnancy. Birth model is confounder model additionally adjusted for child's gestational age and weight at birth. Child model is birth model additionally adjusted for offspring breastfeeding status and BMI at time of the measurements. ^aStudy population for offspring blood pressure: n=3,440 for early-pregnancy, n=4,143 for mid-pregnancy, n=4,232 for late-pregnancy. ^bStudy population for offspring IMT: n=3,173 for early-pregnancy, n=3,836 for mid-pregnancy, n=3,919 for late-pregnancy. ^cStudy population for offspring distensibility: n=3,037 for early-pregnancy, n=3,676 for mid-pregnancy, n=3,762 for late-pregnancy.

Table S8. Associations of gestational hypertension and preeclampsia with offspring blood pressure, carotid IMT and carotid distensibility at median 10 years excluding small for gestational age below the 3rd percentile (n=4,502)^{*}

Offspring outcomes	Normotensive pregnancy	Gestational hypertension				Preeclampsia			
		Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child
SBP, SDS [†] n=4,502	Reference	0.29 (0.14, 0.44)**	0.17 (0.02, 0.32)*	0.17 (0.02, 0.32)*	0.16 (0.02, 0.30)*	0.39 (0.17, 0.62)*	0.29 (0.07, 0.51)*	0.24 (0.02, 0.47)*	0.17 (-0.04, 0.39)
DBP, SDS [†] n=4,502	Reference	0.26 (0.11, 0.42)*	0.21 (0.06, 0.37)*	0.21 (0.05, 0.36)*	0.20 (0.05, 0.36)*	0.14 (-0.08, 0.37)	0.08 (-0.14, 0.31)	0.04 (-0.19, 0.27)	0.02 (-0.20, 0.25)
IMT, SDS [‡] n=4,171	Reference	-0.03 (-0.19, 0.13)	-0.00 (-0.16, 0.16)	0.01 (-0.15, 0.17)	0.02 (-0.12, 0.19)	0.02 (-0.21, 0.25)	0.02 (-0.21, 0.25)	0.09 (-0.15, 0.32)	0.08 (-0.15, 0.31)
Distensibility, SDS [§] n=4,002	Reference	-0.08 (-0.24, 0.08)	-0.03 (-0.20, 0.13)	-0.05 (-0.21, 0.12)	-0.05 (-0.21, 0.11)	-0.16 (-0.39, 0.08)	-0.13 (-0.36, 0.11)	-0.17 (-0.41, 0.07)	-0.14 (-0.37, 0.10)

SBP, systolic blood pressure. DBP, diastolic blood pressure. IMT, intima media thickness. *P-value <0.05. **P-value <0.001. Values are regression coefficients (95% confidence interval) that were obtained from regular multivariable linear regression models, and reflect the differences in offspring blood pressure (SDS), carotid IMT (SDS) and carotid distensibility (SDS) for gestational hypertension and preeclampsia. Groups are compared to women with a normotensive pregnancy as reference. Estimates are from multiple imputed data. Basic models are adjusted for child's age and sex. Confounder model is basic model additionally adjusted for maternal age, parity, prepregnancy BMI, educational level, maternal ethnicity, folic acid supplementation and smoking and alcohol consumption during pregnancy. Birth model is confounder model additionally adjusted for child's gestational age and weight at birth. Child model is birth model additionally adjusted for offspring breastfeeding status and BMI at time of the measurements. [†]Study population for offspring blood pressure with 170 cases of gestational hypertension and 76 cases of preeclampsia. [‡]Study population for offspring IMT with 161 cases of gestational hypertension and 74 cases of preeclampsia. [§]Study population for offspring distensibility with 152 cases of gestational hypertension and 69 cases of preeclampsia.

Table S9. Associations of maternal blood pressure with offspring blood pressure, carotid IMT and carotid distensibility at median 10 years excluding small for gestational age below the 3rd percentile (n=4,626)[†]

Offspring outcomes	Early-pregnancy maternal SBP				Mid-pregnancy maternal SBP				Late-pregnancy maternal SBP			
	Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child
SBP SDS [†]	0.14 (0.11, 0.17)**	0.21 (0.08, 0.15)**	0.11 (0.08, 0.15)**	0.10 (0.07, 0.13)**	0.17 (0.14, 0.19)**	0.14 (0.11, 0.17)**	0.14 (0.11, 0.17)**	0.12 (0.09, 0.15)**	0.15 (0.12, 0.17)**	0.12 (0.09, 0.15)**	0.12 (0.09, 0.15)**	0.10 (0.07, 0.13)**
DBP SDS [†]	0.07 (0.04, 0.11)**	0.06 (0.03, 0.10)**	0.06 (0.03, 0.10)**	0.06 (0.02, 0.09)**	0.08 (0.05, 0.11)**	0.06 (0.03, 0.09)**	0.06 (0.03, 0.09)**	0.06 (0.03, 0.09)**	0.05 (0.02, 0.08)**	0.04 (0.01, 0.07)**	0.04 (0.01, 0.07)**	0.03 (0.00, 0.07)*
IMT, SDS [†]	-0.00 (-0.04, 0.03)	0.01 (-0.02, 0.05)	0.01 (-0.02, 0.05)	0.01 (-0.03, 0.05)	0.01 (-0.02, 0.04)	0.03 (-0.01, 0.06)	0.02 (-0.01, 0.06)	0.02 (-0.01, 0.05)	0.02 (-0.02, 0.05)	0.03 (-0.00, 0.07)	0.03 (-0.00, 0.06)	0.03 (-0.00, 0.06)
Distensibility, SDS [‡]	-0.05 (-0.09, -0.01)*	-0.04 (-0.08, -0.01)*	-0.04 (-0.08, -0.01)*	-0.04 (-0.07, -0.00)*	-0.07 (-0.10, -0.04)**	-0.06 (-0.09, -0.03)**	-0.06 (-0.09, -0.03)**	-0.05 (-0.09, -0.02)*	-0.05 (-0.08, -0.02)*	-0.04 (-0.08, -0.01)*	-0.04 (-0.07, -0.01)*	-0.03 (-0.06, 0.00)
Offspring outcomes	Early-pregnancy maternal DBP				Mid-pregnancy maternal DBP				Late-pregnancy maternal DBP			
	Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child	Basic	Confounder	Birth	Child
SBP, SDS [†]	0.14 (0.10, 0.17)**	0.10 (0.07, 0.13)**	0.10 (0.06, 0.13)**	0.09 (0.06, 0.13)**	0.16 (0.13, 0.19)**	0.12 (0.09, 0.15)**	0.12 (0.09, 0.15)**	0.11 (0.08, 0.14)**	0.14 (0.11, 0.17)**	0.10 (0.07, 0.13)**	0.10 (0.06, 0.13)**	0.08 (0.05, 0.11)**
DBP, SDS [†]	0.12 (0.09, 0.16)**	0.11 (0.07, 0.14)**	0.11 (0.07, 0.14)**	0.11 (0.07, 0.14)**	0.13 (0.10, 0.16)**	0.11 (0.08, 0.14)**	0.11 (0.08, 0.14)**	0.10 (0.07, 0.14)**	0.11 (0.08, 0.14)**	0.09 (0.06, 0.12)**	0.09 (0.06, 0.12)**	0.08 (0.05, 0.11)**
IMT, SDS [†]	0.01 (-0.03, 0.04)	0.02 (-0.02, 0.06)	0.03 (-0.01, 0.06)	0.03 (-0.01, 0.06)	0.01 (-0.02, 0.04)	0.02 (-0.01, 0.05)	0.03 (-0.01, 0.06)	0.02 (-0.01, 0.06)	0.02 (-0.01, 0.05)	0.03 (0.00, 0.07)	0.04 (0.01, 0.07)*	0.04 (0.01, 0.07)*
Distensibility, SDS [‡]	-0.04 (-0.08, -0.01)*	-0.04 (-0.07, -0.00)*	-0.04 (-0.08, -0.00)*	-0.04 (-0.07, -0.00)*	-0.06 (-0.09, -0.03)**	-0.05 (-0.08, -0.02)*	-0.05 (-0.09, -0.02)*	-0.05 (-0.08, -0.02)*	-0.07 (-0.10, -0.04)**	-0.06 (-0.09, -0.03)**	-0.06 (-0.10, -0.03)**	-0.06 (-0.09, -0.02)*

SBP, systolic blood pressure. DBP, diastolic blood pressure. IMT, intima media thickness. *P-value <0.05. **P-value <0.001. Values are regression coefficients (95% confidence interval) that were obtained from regular multivariable linear regression models, and reflect the differences in offspring blood pressure (SDS), carotid IMT (SDS) and carotid distensibility (SDS) per SDS change in maternal blood pressure. Estimates are from multiple imputed data. Basic model is adjusted for child's age and sex, and gestational age at the time of blood pressure measurements. Confounder model is basic model additionally adjusted for maternal age, parity, prepregnancy BMI, educational level, maternal ethnicity, folic acid supplementation and smoking and alcohol consumption during pregnancy. Birth model is confounder model additionally adjusted for child's gestational age and weight at birth. Child model is birth model additionally adjusted for offspring breastfeeding status and BMI at time of the measurements. [†]Study population for offspring blood pressure: n=3,616 for early-pregnancy, n=4,343 for mid-pregnancy, n=4,438 for late-pregnancy. [‡]Study population for offspring IMT: n=3,343 for early-pregnancy, n=4,030 for mid-pregnancy, n=4,118 for late-pregnancy. [§]Study population for offspring distensibility: n=3,200 for early-pregnancy, n=3,861 for mid-pregnancy, n=3,953 for late-pregnancy.

CHAPTER

3.3

Associations of fetal and infant growth patterns with early markers of arterial health in school-age children

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BACKGROUND Fetal life and infancy might be critical periods for predisposing individuals to cardiovascular disease in adulthood. We examined the associations of fetal and infant weight growth patterns with early markers of arterial health.

METHODS A population-based prospective cohort study from fetal life onwards. Estimated fetal weight was measured in the second and third trimester of pregnancy. Weight and gestational age at birth were collected from midwives. Infant weight was measured at 6, 12, and 24 months. The common carotid artery intima media thickness (IMT) (mm) and distensibility ($\text{kPa}^{-1} \times 10^{-3}$) were measured as early markers of arterial health in children aged 10 years ($n=4,484$).

RESULTS Gestational age at birth was not associated with markers of arterial health. A 500 grams higher birth weight was associated with a 0.08 SDS (95% Confidence Interval (CI): 0.05, 0.10) higher carotid IMT and a -0.05 SDS (95% CI: -0.08, -0.03) lower distensibility. Compared to children with a birth weight of 2,500-4,500 grams, those with a birth weight of $>4,500$ grams had the lowest carotid distensibility (difference -0.22 SDS (95% CI: -0.42, -0.02)). Conditional regression analyses showed that higher third trimester fetal weight, birth weight and weight at 6, 12 and 24 months were all independently associated with higher carotid IMT, whereas higher weight at 6, 12 and 24 months were all independently associated with lower carotid distensibility (all p -values <0.05). Compared to children with normal fetal and infant growth, children with normal fetal growth followed by infant growth acceleration had the highest carotid IMT (0.19 SDS; 95% CI: 0.07, 0.31) and lowest distensibility (-0.16 SDS; 95% CI: -0.28, -0.03). The observed associations were partly explained by childhood body mass index.

CONCLUSIONS Both higher fetal and infant weight growth are associated with early markers of impaired arterial health in children aged 10 years. Childhood body mass index seems to be involved in the underlying pathways of the observed associations.

INTRODUCTION

Children with low birth weight, high birth weight and subsequent high infant growth rates seem to be at risk for cardiovascular disease in adulthood^{1,2}. Findings from recent studies suggest that growth variation in both fetal life and infancy is associated with an adverse body fat distribution and cardiovascular risk profile from school-age onwards³⁻⁵. These findings suggest that altered growth in early-life might predispose individuals to atherosclerosis and subsequent cardiovascular disease in adulthood⁶. Results from post-mortem pathological studies show atherosclerosis of large arteries of fetuses, children and adolescents⁷⁻⁹. Also, studies show that an adverse fetal environment is associated with non-invasive markers of arterial health and atherosclerosis, such as carotid intima media thickness (IMT) and distensibility, already in adolescence^{10, 11}. In a recent systematic review assessing risk factors in the first 1,000 days of life, a consistent association was reported between small size for gestational age at birth with higher carotid IMT in individuals aged 0-18 years¹⁰. However, another study reported a positive association of higher birth weight with carotid IMT, independent of childhood obesity¹². These findings suggest a non-linear association of birth weight with childhood carotid IMT. Identification of critical periods in fetal life and infancy related to development of arterial health and atherosclerosis might contribute to novel prevention strategies¹³⁻¹⁸.

In a population-based prospective cohort study among 4,484 children, we examined the associations of fetal and infant weight growth patterns with carotid IMT and distensibility at the age of 10 years. We were specifically interested in the identification of critical periods and combinations of fetal and infant weight growth patterns.

METHODS

Study population

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onward¹⁹. Pregnant women with a delivery date between April 2002 and January 2006, living in Rotterdam, the Netherlands, were eligible for participation. Details on response and follow-up have been described previously¹⁹. Information on fetal or infant growth was available in 8,625 singleton births. Analyses were restricted to a subgroup of 4,484 children for whom we had information on early markers of arterial health. The flowchart of participants is given in the **Supplemental Figure 1**. Written informed consent was provided by the parents for all children. The Medical Ethics Committee of Erasmus Medical Center approved the study (MEC-2012-

165). This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline²⁰.

Fetal and infant growth measures

As previously described, fetal ultrasound examinations were performed in the first trimester (median 12.4, range 10.1-13.9 weeks), second trimester (median 20.5, range 18.0-25.0 weeks), and third trimester (median 30.4, range 25.3-39.2 weeks). We measured second and third-trimester fetal head circumference, abdominal circumference, and femur length to the nearest millimeter by using standardized ultrasound procedures^{19, 21}. We used the Hadlock formula to calculate gestational age adjusted estimated fetal weight and calculated standard deviation scores (SDS)^{22, 23}. We constructed gestational age adjusted standard deviation scores (SDS) for first-trimester fetal crown to rump length²³. First-trimester fetal growth was assessed in a subgroup of mothers who had a fetal first-trimester crown to rump measurement within the range of 10 weeks 0 days to 13 weeks 6 days and had a reliable gestational age estimate based on the last menstrual period and a regular menstrual cycle (n=941).

Sex, gestational age at birth, and birth weight were collected from midwives. Gestational age and sex-adjusted SDS for birth weight were constructed using World Health Organization fetal growth charts²⁴. Gestational age was categorized into preterm (<37 weeks), term (37-42 weeks) and post term (>42 weeks). Birth weight was categorized in low birth weight (<2,500 grams), normal birth weight (2,500-4,500 grams) and high birth weight (>4,500 grams). Children born small size for gestational age (SGA) were defined as gestational age and sex-adjusted SDS for birth weight below the tenth percentile and those born large size for gestational age (LGA) were defined as gestational age and sex-adjusted SDS for birth weight above the 90th percentile of our study population. Infant weight was measured in community health centers with a mechanical personal scale around age 6 months (median 6.2, 95% range 5.2-8.3 months), 12 months (median 11.1, 95% range 10.1-12.5 months), and 24 months (median 24.8, 95% range 23.4-28.2 months)¹⁹. We created age and sex-adjusted SDS using Dutch reference growth charts in Growth Analyzer 4.0¹⁹.

We prospectively constructed nine categories of fetal and infant weight change variables. Fetal weight change was defined as growth in SDS between the second trimester and birth. Infant weight change was defined as growth in SDS from birth to 24 months (2,929 of 3,651 children). If weight at 24 months was not available, we used weight at 11 months (587 of 3,651 children) and if weight at 11 months was not available, we used weight at 6 months (135 of 3,651 children). We considered an increase of more

than 0.67 SD between time points as growth acceleration and a decrease of more than 0.67 SD between time points as growth deceleration, reflecting the difference between 2 percentile lines on the growth charts.

Childhood common carotid artery intima media thickness and distensibility

At the median age of 9.7 years (95% range, 9.3-10.5 years), we measured carotid IMT and distensibility using the Logiq E9 device (GE Medical Systems, Wauwatosa, WI, USA). We obtained six recordings. Carotid IMT was computed at the ‘far wall’ as the average distance between lumen-intima and media-adventitia borders. Distensibility was defined as the relative change in lumen area during systole for a given pressure change. Children with at least one successful carotid IMT or distensibility measurement were included. The overall mean IMT (mm) and distensibility ($\text{kPa}^{-1} \cdot 10^{-3}$) were used as main outcomes of interest. For the final analyses, distensibility was log-transformed to deal with a skewed distribution. We constructed standard deviation score values $((\text{observed value} - \text{mean}) / \text{SD})$ for the childhood outcome measures to enable comparison of effect estimates.

Covariates

Information on maternal age, pre-pregnancy weight, parity, ethnicity, educational level, smoking, folic acid supplementation and gestational hypertensive disorders was obtained by questionnaires and registries¹⁹. Maternal height was measured and pre-pregnancy body mass index (BMI) (kg/m^2) was calculated. At median 10 years of age, child height and weight were measured. We calculated sex- and gestational age adjusted body mass index (BMI) (kg/m^2) SDS. The roles of the covariates of interest are presented in a directed acyclic graphs (DAG) (**Supplemental Figure 2**).

Statistical analysis

First, we described maternal, fetal and childhood characteristics. We performed a non-response analysis by comparing characteristics of children with and without outcome assessments by using Independent Student T-test, Mann-Whitney U and χ^2 tests. Second, we assessed the associations of gestational age at birth, birth weight and size for gestational age at birth with carotid IMT and distensibility at age 10 years using linear regression models. Third, we assessed the associations of weight measurements at different fetal and infant ages with carotid IMT and distensibility outcomes at the age of 10 years using linear regression models. Additionally we explored the associations of first trimester fetal growth with markers of arterial health. To identify independent critical weight periods, we

performed conditional regression analysis that take into account the correlations between the weight measurements^{4,25}. We constructed weight variables, statistically independent from weight at earlier time points, using standardized residuals resulting from linear regression models of weight regressed on all prior weights²⁶. This approach allows simultaneous inclusion of all weight measurements in one linear regression model to identify critical periods of growth on childhood carotid measurements²⁶. For these analyses participants were included if they had data available for weight at all time-points. The first trimester measurements were not taken into account due to the limited sample size. Fourth, we categorized fetal (second trimester to birth) and infant (birth to 24 months) weight change into 3 groups (growth deceleration, normal growth, and growth acceleration), and created a combined variable that reflects 9 different growth patterns. We used multivariable linear regression models to explore associations of fetal and infant weight changes combined with carotid measurements. For all analyses, the basic models were adjusted for child's sex and age at outcome measurement. The confounder model, which we considered the main model was additionally adjusted for maternal age, pre-pregnancy body mass index, educational level, ethnicity, folic acid use, smoking and gestational hypertensive disorders. The mediator model additionally included childhood body mass index. Potential confounders were identified based on previous literature and we selected those that fulfilled the graphical criteria for confounding in a DAG and changed the effect estimates >10% after addition to the crude model. We tested for statistical interaction of sex and ethnicity in these associations but no statistically significant interactions were observed ($p > 0.05$). As exposures were correlated, we did not correct for multiple testing and present significance levels at both $p < 0.05$ and $p < 0.001$. Missing data in covariates (ranging from 0 to 23%) were multiple imputed using the Markov Chain Monte Carlo method. Ten imputed datasets were created and analyzed together²⁷. Statistical analyses were performed using the Statistical Package of Social Sciences version 25.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Participant characteristics

Table 1 shows the subject characteristics from imputed data. **Supplementary Table S1** shows subjects characteristics from observed data. **Supplementary Table S2** shows that compared to the study population, mothers of children without outcome measurements were more often lower educated and of non-European descent, more often smoked during

pregnancy and less often used folic acid supplementation during pregnancy (all p-values <0.01). Children not participating in our study were slightly more often born preterm and had lower third trimester estimated fetal weight (all p-values <0.01).

Table 1. Participant characteristics of the study population n= 4,484

Characteristics		Value
Maternal	Age at enrolment, median (95% range), years	31.2 (20.3, 39.6)
	Pre-pregnancy BMI, median (95% range), kg/m ²	22.7 (17.8, 34.1)
	Parity, n nulliparous (%)	2,628 (58.6)
	Education level, n higher education (%)	2,187 (48.8)
	Ethnicity, n European (%)	2,908 (64.9)
	Smoking during pregnancy, n continued (%)	1,081 (24.1)
	Folic acid supplement use, n did not use (%)	1,072 (23.9)
Fetal	First-trimester	
	Gestational age, median (95% range), weeks	12.4 (10.6, 13.8)
	Crown rump length, mean, mm	61.0 (11.6)
	Second trimester	
	Gestational age, median (95% range), weeks	20.5 (18.6, 23.3)
	Estimated fetal weight, median (95% range), grams	364 (246, 624)
	Third trimester	
Birth	Gestational age, median (95% range), weeks	30.4 (28.5, 33.0)
	Estimated fetal weight, median (95% range), grams	1,605 (1,179, 2,218)
	Child sex, n female (%)	2,260 (50.4)
	Gestational age at birth, median (95% range), weeks	40.1 (35.9, 42.3)
	< 37 weeks, n (%)	207 (4.6)
	37-42 weeks, n (%)	4,060 (90.5)
	>42 weeks, n (%)	217 (4.8)
	Birth weight, median (95% range), grams	3,450 (2,255, 4,485)
	<2,500 grams, n (%)	196 (4.4)
	2,500-4,500 grams, n (%)	4,182 (93.4)
	>4,500 grams, n (%)	101 (2.3)
	Sex- and gestational age adjusted birth weight	
	Small (<10 th percentile), n (%)	447 (10.0)
Infant	Appropriate (10 th -90 th percentile), n (%)	3,582 (80.0)
	Large (>90 th percentile), n (%)	447 (10.0)
	At 6 months visit	
	Age at visit, median (95% range), months	6.2 (5.2, 8.3)
	Weight, median (95% range), kg	7.8 (6.2, 9.8)
	At 12 months visit	
	Age at visit, median (95% range), years	11.1 (10.1, 12.5)
	Weight, median (95% range), kg	9.6 (7.7, 11.8)
	At 2 year visit	
	Age at visit, median (95% range), years	24.8 (23.4, 28.2)
	Weight, median (95% range), kg	12.8 (10.3, 16.2)
Childhood	Age at follow-up, median (95% range), years	9.7 (9.3, 10.5)
	BMI median (95% range), kg/m ²	17.0 (14.0, 24.9)
	Carotid intima media thickness, mean (SD), mm	0.46 (0.04)
	Carotid distensibility, median (95% range), kPa ⁻¹ *10 ⁻³	55.9 (37.1, 85.5)

BMI, body mass index. Values are mean (SD), median (95% range), or number (valid %).

Table 2. Associations of birth outcomes with childhood carotid measurements

Birth outcomes	Difference in standard deviation scores (95% CI)			
	Carotid intima media thickness (n=4,484)		Carotid distensibility (n=4,304)	
	Confounder model	BMI model	Confounder model	BMI model
Birth weight				
<2,500 grams (n=196)	-0.06 (-0.20, 0.09)	-0.05 (-0.2, 0.09)	0.11 (-0.04, 0.25)	0.10 (-0.05, 0.24)
2,500-4,500 grams (n=4,182)	Reference	Reference	Reference	Reference
>4,500 grams (n=101)	0.15 (-0.05, 0.35)	0.14 (-0.06, 0.33)	-0.22 (-0.42, -0.02)*	-0.18 (-0.37, 0.02)
Continuously (per 500 grams)	0.08 (0.05, 0.10)**	0.07 (0.04, 0.10)**	-0.05 (-0.08, 0.03)**	-0.04 (-0.07, -0.01)*
Size for gestational age at birth				
Small <10 th percentile (n=447)	-0.14 (-0.24, -0.14)*	-0.13 (-0.23, -0.03)*	0.12 (0.02, 0.22)*	0.10 (-0.00, 0.20)
Appropriate 10 th -90 th percentile (n=3,582)	Reference	Reference	Reference	Reference
Large >90 th percentile (n=447)	0.11 (0.12, 0.21)*	0.10 (0.00, 0.20)*	-0.10 (-0.20, -0.00)*	-0.07 (-0.17, 0.03)
Continuously (per 1 SD grams)	0.08 (0.05, 0.11)**	0.07 (0.04, 0.10)**	-0.07 (-0.10, -0.04)**	-0.05 (-0.08, -0.02)*
Gestational age at birth				
<37 weeks (n=207)	-0.08 (-0.22, 0.06)	-0.08 (-0.22, 0.06)	0.06 (-0.08, 0.20)	0.06 (-0.08, 0.20)
37-42 weeks (n=4,060)	Reference	Reference	Reference	Reference
>42 weeks (n=217)	0.07 (-0.07, 0.21)	0.07 (-0.07, 0.21)	0.03 (-0.12, 0.17)	0.03 (-0.11, 0.17)
Continuously (per week)	0.02 (-0.00, 0.03)	0.02 (-0.00, 0.03)	-0.01 (-0.02, 0.01)	-0.01 (-0.02, 0.01)

CI, Confidence interval. BMI, body mass index. *P-value <0.05. **P-value <0.001. Values are regression coefficients (95% confidence interval) that were obtained from multivariable linear regression models and reflect the differences in carotid intima media thickness (SDS) and carotid distensibility (SDS) for birth outcomes. Estimates are from multiple imputed data. The confounder model is adjusted for child age at outcome visit and sex, maternal age, pre-pregnancy body mass index, educational level, ethnicity, folic acid use, smoking and gestational hypertensive disorders. The BMI model is the confounder model additionally adjusted for sex-and gestational age adjusted child body mass index at outcome measurement.

Birth outcomes with offspring carotid IMT and distensibility

Table 2 shows the associations of birth outcomes with childhood carotid measurements adjusted for confounders and childhood BMI (basic models are shown in **Supplementary Table S3**). Gestational age was not associated with childhood carotid measurements. An increase in birth weight of 500 grams was associated with a 0.08 SDS (95% CI 0.05, 0.10) higher carotid IMT and a -0.05 SDS (95% CI -0.08, -0.03) lower distensibility. As compared to children with a birth weight of 2,500-4,500 grams, those with a birth weight of >4,500 grams had the lowest carotid distensibility (difference -0.22 SDS (95% CI -0.42, -0.02)). Similarly, a 1-SDS increase in gestational age adjusted birth weight was associated with a 0.08 SDS (95% CI 0.05, 0.11) higher carotid IMT and a -0.07 SDS (95% CI -0.10, -0.04) lower distensibility. Being small size for gestational age was associated with a -0.14 SDS (95% CI -0.24, -0.14) lower carotid IMT and 0.12 SDS (95% CI 0.02, 0.22) higher distensibility. The associations of birth weight >4,500 grams, small- and large size for gestational age with carotid distensibility attenuated into non-significance after additional adjustment for childhood BMI.

Critical fetal and infant periods with carotid IMT and distensibility

Results from conditional regression analyses showed that higher third trimester fetal weight, birth weight and weight at 6, 12 and 24 months were all independently associated with higher carotid IMT, and these associations remained after additional adjustment for childhood BMI. Higher weight at 6, 12 and 24 months were all independently associated with lower distensibility (all p-values <0.05) (**Table 3**). After additional adjustment for

Table 3. Associations of fetal and infant growth with childhood carotid measurements from conditional analyses

Infant and fetal weight SDS	Difference in standard deviation scores (95% CI)			
	Carotid intima media thickness (n=2,249)		Carotid distensibility (n=2,137)	
	Confounder model	BMI model	Confounder model	BMI model
At fetal weight 20 weeks	0.04 (-0.01, 0.08)	0.03 (-0.01, 0.08)	-0.01 (-0.05, 0.04)	-0.00 (-0.05, 0.04)
At fetal weight 30 weeks	0.08 (0.04, 0.12)**	0.08 (0.04, 0.12)**	-0.03 (-0.08, 0.01)	-0.03 (-0.07, 0.02)
At birth	0.05 (0.01, 0.09)*	0.05 (0.00, 0.09)*	-0.01 (-0.05, 0.04)	0.01 (-0.04, 0.05)
At 6 months	0.05 (0.01, 0.10)*	0.05 (0.00, 0.09)*	-0.04 (-0.09, -0.00)*	-0.01 (-0.06, 0.03)
At 12 months	0.06 (0.02, 0.10)*	0.05 (0.01, 0.09)*	-0.05 (-0.09, -0.01)*	-0.03 (-0.07, 0.02)
At 24 months	0.07 (0.03, 0.11)*	0.06 (0.02, 0.10)*	-0.10 (-0.15, -0.06)**	-0.08 (-0.12, -0.03)**

CI, Confidence interval. BMI, body mass index. *P-value <0.05. **P-value <0.001. Values are regression coefficients (95% confidence interval) and reflect the differences in carotid intima media thickness (SDS) and carotid distensibility (SDS) per SDS change in infant and fetal weight from conditional models. Estimates are from multiple imputed data. The confounder model is adjusted for child age at outcome visit and sex, maternal age, pre-pregnancy body mass index, educational level, ethnicity, folic acid use, smoking and gestational hypertensive disorders. The BMI model is the confounder model additionally adjusted for sex-and gestational age adjusted child body mass index at outcome measurement.

Table 4. Associations of fetal and infant growth patterns with childhood carotid measurements

	Difference in standard deviation scores (95% CI)			
	Carotid intima media thickness (n=3,485)		Carotid distensibility (n=3,316)	
	Confounder model	BMI model	Confounder model	BMI model
Fetal and infant growth patterns				
Fetal growth deceleration				
Infant growth deceleration (n=122)	-0.02 (-0.20, 0.17)	0.00 (-0.18, 0.19)	0.12 (-0.08, 0.32)	0.06 (-0.13, 0.26)
Infant normal growth (n=382)	-0.06 (-0.18, 0.06)	-0.05 (-0.17, 0.07)	0.02 (-0.11, 0.14)	-0.01 (-0.13, 0.12)
Infant growth acceleration (n=397)	0.05 (-0.07, 0.17)	0.03 (-0.09, 0.15)	-0.08 (-0.20, 0.05)	-0.04 (-0.16, 0.09)
Fetal normal growth				
Infant growth deceleration (n=336)	-0.00 (-0.13, 0.12)	0.01 (-0.11, 0.14)	0.10 (-0.03, 0.23)	0.06 (-0.07, 0.18)
Infant normal growth (n=812)	Reference	Reference	Reference	Reference
Infant growth acceleration (n=400)	0.19 (0.07, 0.31)*	0.17 (0.05, 0.28)*	-0.16 (-0.28, -0.03)*	-0.09 (-0.21, 0.04)
Fetal growth acceleration				
Infant growth deceleration (n=422)	-0.00 (-0.12, 0.11)	-0.00 (-0.12, 0.12)	-0.03 (-0.15, 0.09)	-0.03 (-0.15, 0.09)
Infant normal growth (n=469)	0.12 (0.01, 0.23)*	0.11 (-0.00, 0.22)	-0.04 (-0.16, 0.07)	-0.01 (-0.13, 0.10)
Infant growth acceleration (n=145)	0.10 (-0.08, 0.27)	0.07 (-0.11, 0.24)	-0.09 (-0.27, 0.09)	-0.01 (-0.18, 0.17)

Confidence interval. BMI, body mass index. *P-value <0.05. Values are regression coefficients (95% confidence interval) and reflect the differences in carotid intima media thickness (SDS) and carotid distensibility (SDS) for fetal and infant growth patterns from multivariable linear regression models. Estimates are from multiple imputed data. The confounder model is adjusted for child age at outcome visit and sex, maternal age, pre-pregnancy body mass index, educational level, ethnicity, folic acid use, smoking and gestational hypertensive disorders. The BMI model is the confounder model additionally adjusted for sex-and gestational age adjusted child body mass index at outcome measurement.

childhood BMI, only the associations of higher infant weight at 24 months and lower distensibility remained (p -value <0.05). The basic models are given in **Supplementary Table S4**. **Supplementary Table S5** shows that no associations were present for first trimester length and fetal weight at 20 weeks of gestation with markers of arterial health.

Fetal and infant growth patterns with carotid IMT and distensibility

Table 4 shows that as compared to children with normal fetal and infant growth those with normal fetal growth followed by infant growth acceleration had the highest carotid IMT (difference: 0.19 SDS (95% CI 0.07, 0.31)) and lowest distensibility (difference: -0.16 SDS (95% CI -0.28, -0.03)). The association of normal fetal growth followed by infant growth acceleration and carotid distensibility attenuated into non-significance after additional adjustment for childhood BMI. No other consistent associations were observed. The corresponding basic models are shown in **Supplementary Table S6**. **Supplementary Table S7** shows maternal pre-pregnancy BMI (median, 95% range) for the different fetal and infant weight growth patterns.

DISCUSSION

In this population-based prospective cohort study, we observed that higher fetal and infant weight growth are associated with higher carotid IMT and lower distensibility. Children with a normal fetal growth, followed by infant growth acceleration have the highest carotid IMT and lowest distensibility. Childhood body mass index seems to be involved in the pathways underlying the observed associations and partly explained the observed associations.

Early-life development might predispose individuals to develop atherosclerosis and subsequent cardiovascular disease in adulthood^{3-5, 7-9, 25, 28}. We hypothesized that fetal life and infancy are critical for development of an adverse arterial health. We aimed to identify critical periods by prospectively assessing fetal and infant growth in combination with carotid IMT and distensibility in children aged 10 years.

Birth weight was positively associated with carotid IMT and negatively associated with distensibility. Also, children born with a large size for gestational age had a higher carotid IMT. A higher gestational age adjusted birth weight was associated with a higher carotid IMT and lower distensibility. Gestational age at birth was not associated with carotid IMT or distensibility. Our findings seem to be in concordance with other studies. A study in California among 670 children aged 11 years reported that higher birth weight was associated

with an increased carotid IMT, whereas no association was found for lower birth weight²⁹. Another study among 696 participants in Finland reported that adults who were born at term and large size for gestational age had a higher carotid IMT and higher risk of obesity at the age of 24–45 years¹². A study among 2,281 adults aged 24–45 years reported higher carotid IMT in children born preterm or fetal growth restricted³⁰. In a British retrospective cohort study among 181 people around 70 years old, lower birth weight was associated with a higher risk of carotid atherosclerosis¹⁸. In contrast to these studies, no associations of low birth weight or small size for gestational age with the development of adverse arterial health were observed. Because of the changes in prevalence of underweight, overweight and obesity in the last decades the associations might differ between birth cohorts. Overall, our results suggest that in contemporary children a higher birth weight, followed by a higher childhood BMI, is associated with an increased risk of adverse arterial health.

Since fetal and infant weight growth are strongly correlated, it is important to study the associations fetal and infant weight combined. Higher fetal weight, birth weight and infant weight at 6, 12 and 24 months were independently from prior weights associated with increased carotid IMT. Infant weight at 6, 12 and 24 months were also associated with decreased carotid distensibility. To our best knowledge, our study is the first that assessed the independent critical periods for weight development from fetal life to infancy and the association with markers of arterial health. Both fetal life and infancy seem to be critical.

Normal fetal growth followed by infant growth acceleration was associated with the highest childhood carotid IMT even after adjustment for childhood body mass index. However an Australian cohort study of 140 children aged 14 years old showed no association of BMI trajectory in early-life and carotid IMT³¹. In line with our findings, a Brazilian prospective cohort study among 5,914 participants aged 30 years old, reported that weight gain in the first 2 years of life was positively associated with carotid IMT¹⁵. Similarly, results from an Australian prospective cohort study among 395 8-years children, showed that excessive weight gain between 0 to 18 months was positively associated with carotid IMT¹⁶. Thus, body weight changes in the first two years of life seem to be critical in the development of atherosclerotic changes of the carotid arteries.

Higher birth weight and weight gain in childhood is associated to obesity later in life^{4, 32–37}. Obesity is a major risk factor in the development of atherosclerosis and arterial stiffness, and subsequently cardiovascular disease^{36, 37}. Weight gain in infancy and early childhood are strongly associated with the risk of developing obesity, high blood pressure, cardiovascular disease and its precursors in adulthood^{14, 32–35, 38}. Our effect estimates were partly explained by childhood body mass index suggesting that childhood body mass index is involved in the pathways underlying the observed associations.

The results of our study suggest that weight growth during fetal life and infancy is critical for arterial health in adulthood. Optimizing growth in early-life, and especially preventing childhood obesity might be beneficial for arterial health. The observed effect estimates were small and more important on a population-level rather than individual level.

Strengths of this study include the population-based prospective cohort study design, large number of participants, detailed data on weight measurements from birth up to 2 years of age and information on carotid atherosclerotic markers at an early age. This study also has some limitations. Of the 8,631 singleton live births with information on fetal or infant growth, 4,484 children had data on childhood carotid measurements. Mothers of children not included in the analyses were more often nulliparous, of non-European ethnicity, smoked and did not use folic acid supplements. Children not included were slightly more often born preterm and had lower estimated fetal weight in the third trimester of pregnancy. These differences may affect the generalizability of our results. Although, we demonstrated high reproducibility in measuring the carotid IMT and distensibility, we cannot completely rule out observer bias. Furthermore, for infant weight change patterns we used weight at 24 months, if unavailable we used weight at 11 months or 6 months. By doing so we might have introduced bias, though this approach allowed for adequate statistical power and sample size in our analysis. Last, we adjusted for a large number of potential confounders, but residual confounding might still be a possibility due to the observational nature of the study.

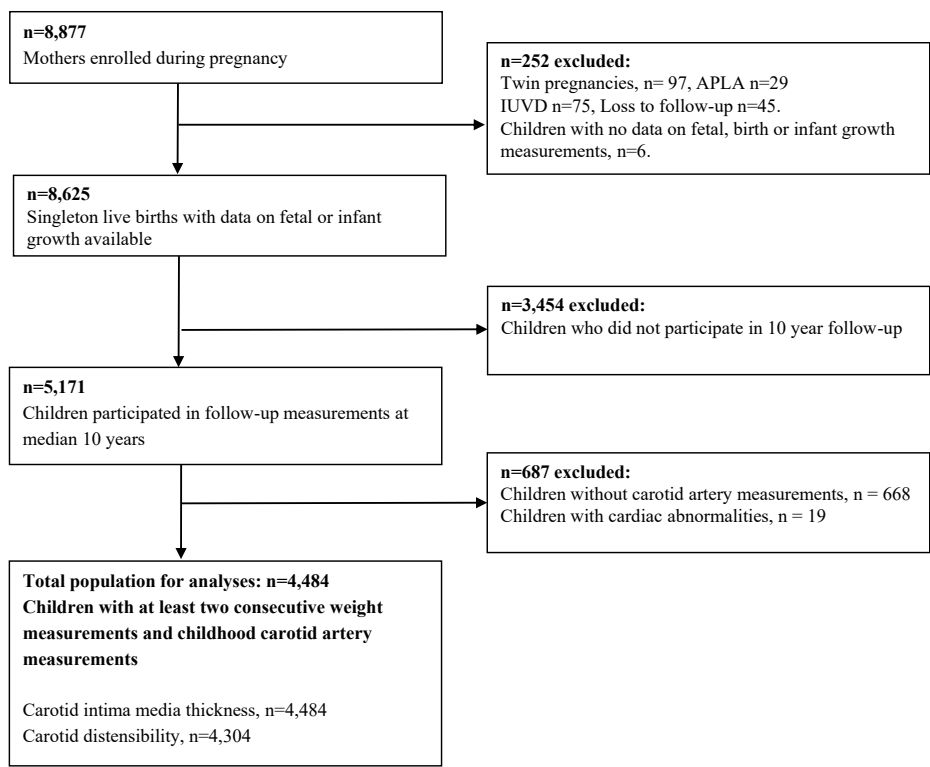
In conclusion, both higher fetal and infant weight growth are associated with early markers of impaired arterial health in children aged 10 years. Childhood body mass index seems to be involved in the underlying pathways of the observed associations. Future studies are needed to assess potential causal pathways and to study how these associations are linked to the development of early atherosclerotic changes in later life.

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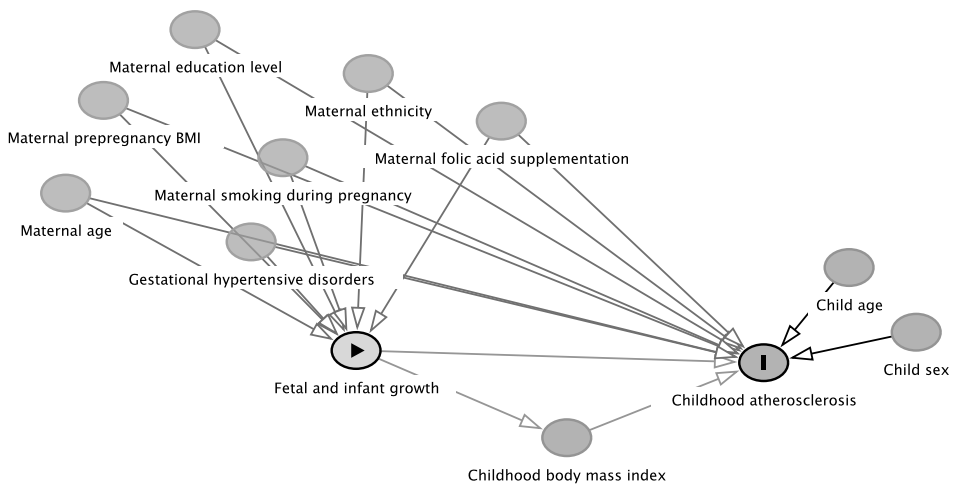
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SUPPLEMENTAL MATERIAL



Supplementary Figure S1. Flowchart of the study population.



Supplementary Figure S2. Directed acyclic graph depicting the relationships between fetal and infant weight measurements, carotid intima-media thickness and distensibility at 10 years and potential covariates, confounders and mediators.

Supplementary Table S1. Observed participant characteristics of the study population (n=4,484)

Characteristics		Value
Maternal	Age at enrolment, median (95% range), years	31.2 (20.3, 39.6)
	Pre-pregnancy BMI, median (95% range), kg/m ²	22.6 (18.1, 34.6)
	Parity, n nulliparous (%)	2,613 (58.6)
	Education level, n higher education (%)	2,134 (50.1)
	Ethnicity, n European (%)	2,869 (65.2)
	Smoking during pregnancy, n continued (%)	961 (23.9)
	Folic acid supplement use, n did not use (%)	752 (21.8)
Fetal	First-trimester	
	Gestational age, median (95% range), weeks	12.4 (10.6, 13.8)
	Crown rump length, mean, mm	61.0 (11.6)
	Second trimester	
	Gestational age, median (95% range), weeks	20.5 (18.6, 23.3)
	Estimated fetal weight, median (95% range), grams	364 (246, 624)
	Third trimester	
Birth	Gestational age, median (95% range), weeks	30.4 (28.5, 33.0)
	Estimated fetal weight, median (95% range), grams	1,605 (1,179, 2,218)
	Child sex, n female (%)	2,260 (50.4)
	Gestational age at birth, median (95% range), weeks	40.1 (35.9, 42.3)
	< 37 weeks, n (%)	207 (4.6)
	37-42 weeks, n (%)	4,060 (90.5)
	>42 weeks, n (%)	217 (4.8)
	Birth weight, median (95% range), grams	3,450 (2,255, 4,485)
	<2,500 grams, n (%)	196 (4.4)
	2,500-4,500 grams, n (%)	4,182 (93.4)
	>4,500 grams, n (%)	101 (2.3)
Infant	Sex- and gestational age adjusted birth weight	
	Small (<10 th percentile), n (%)	447 (10.0)
	Appropriate (10 th -90 th percentile), n (%)	3,582 (80.0)
	Large (>90 th percentile), n (%)	447 (10.0)
	At 6 months visit	
	Age at visit, median (95% range), months	6.2 (5.2, 8.3)
	Weight, median (95% range), kg	7.8 (6.2, 9.8)
	At 12 months visit	
	Age at visit, median (95% range), years	11.1 (10.1, 12.5)
	Weight, median (95% range), kg	9.6 (7.7, 11.8)
	At 2 year visit	
Childhood	Age at visit, median (95% range), years	24.8 (23.4, 28.2)
	Weight, median (95% range), kg	12.8 (10.3, 16.2)
	Age at follow-up, median (95% range), years	9.7 (9.3, 10.5)
	BMI median (95% range), kg/m ²	17.0 (14.0, 24.9)
	Carotid intima media thickness, mean (SD), mm	0.46 (0.04)
	Carotid distensibility, median (95% range), kPa ⁻¹ ·10 ⁻³	55.9 (37.1, 85.5)

BMI, body mass index. Values are mean (SD), median (95% range), or number (valid %).

Supplementary Table S2. Non-response analysis in singleton live births with and without outcome measurements

Characteristics	Children included in the analysis (n=4,484)	Children not included in the analysis* (n=4,141)	p-value
Maternal			
Age at enrolment, median (95% range), years	31.2 (20.3, 39.6)	28.8 (18.5, 38.7)	<0.001
Pre-pregnancy BMI, median (95% range), kg/m ²	22.6 (18.1, 34.6)	22.7 (17.7, 35.5)	0.06
Parity, n nulliparous (%)	2,613 (58.6)	2,132 (52.5)	<0.001
Education level, n higher education (%)	2,134 (50.1)	1,161 (32.3)	<0.001
Ethnicity, n European (%)	2,869 (65.2)	1,767 (47.1)	<0.001
Smoking during pregnancy, n continued (%)	961 (23.9)	1,090 (31.0)	<0.001
Folic acid supplement use, n did not use (%)	752 (21.8)	1,125 (38.3)	<0.001
Fetal			
First-trimester			
Gestational age, median (95% range), weeks	12.4 (10.6, 13.8)	12.4 (10.8, 13.9)	0.97
Crown rump length, mean (SD), mm	61.0 (11.6)	60.8 (11.1)	0.76
Second trimester			
Gestational age, median (95% range), weeks	20.5 (18.6, 23.3)	20.5 (18.5, 23.8)	0.40
Estimated fetal weight, median (95% range), grams	364 (246, 624)	364 (240, 656)	0.81
Third trimester			
Gestational age, median (95% range), weeks	30.4 (28.5, 33.0)	30.3 (28.0, 33.0)	0.01
Estimated fetal weight, median (95% range), grams	1,605 (1,179, 2,218)	1,580 (1,141, 2,200)	<0.001
Birth			
Child sex, n female (%)	2,260 (50.4)	2,015 (48.7)	0.10
Gestational age at birth, median (95% range), weeks	40.1 (35.9, 42.3)	40.0 (35.3, 42.3)	0.02
<37 weeks, n (%)	207 (4.6)	250 (6.0)	0.01
37-42 weeks, n (%)	4,060 (90.5)	3,678 (88.9)	0.01
>42 weeks, n (%)	217 (4.8)	210 (5.1)	0.01
Birth weight, median (95% range), grams	3,450 (2,255, 4,485)	3,400 (2,210, 4,490)	<0.001
<2,500 grams, n (%)	196 (4.4)	219 (5.4)	0.10
2,500-4,500 grams, n (%)	4,182 (93.4)	3,766 (92.3)	0.10
>4,500 grams, n (%)	101 (2.3)	95 (2.3)	0.10
Sex- and gestational age adjusted birth weight			
Small (<10 th percentile), n (%)	447 (10.0)	455 (11.2)	0.18
Appropriate (10 th -90 th percentile), n (%)	3,582 (80.0)	3,228 (79.3)	0.18
Large (>90 th percentile), n (%)	447 (10.0)	388 (9.5)	0.18
Infant			
At 6 months visit			
Age at visit, median (95% range), months	6.2 (5.2, 8.3)	6.2 (5.2, 8.3)	0.45
Weight, median (95% range), kg	7.8 (6.2, 9.8)	7.9 (6.2, 9.9)	0.00
At 12 months visit			
Age at visit, median (95% range), years	11.1 (10.1, 12.5)	11.1 (10.1, 12.6)	0.38
Weight, median (95% range), kg	9.6 (7.7, 11.8)	9.7 (7.7, 12.1)	0.00
At 2 year visit			
Age at visit, median (95% range), years	24.8 (23.4, 28.2)	24.8 (23.4, 28.2)	0.21
Weight, median (95% range), kg	12.8 (10.3, 16.2)	12.9 (10.2, 16.3)	0.17

BMI, body mass index. *Children who did not participate in the 10-year follow-up or did not have childhood carotid measurements or had cardiac abnormalities for which exclusion was indicated. Values are mean (SD), median (95% range), or number (valid %). Differences in subject characteristics between the groups were evaluated using Independent Student T-test and Mann-Whitney U for continuous variables and χ^2 tests for categorical variables.

Supplementary Table S3. Basic models: Associations of birth outcomes with childhood carotid measurements

Birth outcomes	Difference in standard deviation scores (95% CI)	
	Carotid intima media thickness (n=4,484)	Carotid distensibility (n=4,304)
	Basic Model	Basic Model
Birth weight		
<2,500 grams (n=196)	-0.04 (-0.18, 0.10)	0.10 (-0.05, 0.25)
2,500-4,500 grams (n=4,182)	<i>Reference</i>	<i>Reference</i>
>4,500 grams (n=101)	0.13 (-0.06, 0.33)	-0.24 (-0.44, -0.04)*
<i>Continuously (per 500 grams)</i>	0.06 (0.04, 0.09)*	-0.05 (-0.08, -0.03)*
Size for gestational age at birth		
Small <10 th percentile (n=447)	-0.12 (-0.22, -0.02)*	0.12 (0.02, 0.22)*
Appropriate 10 th -90 th percentile (n=3,582)	<i>Reference</i>	<i>Reference</i>
Large >90 th percentile (n=447)	0.09 (-0.01, 0.18)	-0.11 (-0.21, -0.01)*
<i>Continuously (per 1 SD grams)</i>	0.07 (0.04, 0.09)*	-0.07 (-0.10, -0.04)*
Gestational age at birth		
<37 weeks (n=207)	-0.08 (-0.22, 0.06)	0.04 (-0.10, 0.18)
37-42 weeks (n=4,060)	<i>Reference</i>	<i>Reference</i>
>42 weeks (n=217)	0.06 (-0.07, 0.20)	0.02 (-0.12, 0.16)
<i>Continuously (per week)</i>	0.02 (-0.00, 0.03)	-0.00 (-0.02, 0.01)

CI, Confidence interval. *P-value <0.05. Values are regression coefficients (95% confidence interval) that were obtained from multivariable linear regression models and reflect the differences in carotid intima media thickness (SDS) and carotid distensibility (SDS) for birth outcomes. Estimates are from multiple imputed data. The basic model is adjusted for child age at outcome visit and sex.

Supplementary Table S4. Basic models: associations of fetal and infant growth with childhood carotid measurements from conditional analyses

Infant and fetal weight standard deviation scores	Difference in standard deviation scores (95% CI)	
	Carotid intima media thickness (n=2,249)	Carotid distensibility (n=2,137)
	Basic model	Basic model
At 20 weeks	0.03 (-0.02, 0.07)	-0.01 (-0.06, 0.03)
At 30 weeks	0.07 (0.03, 0.11)**	-0.04 (-0.08, 0.01)
At birth	0.04 (0.00, 0.08)*	-0.01 (-0.05, 0.04)
At 6 months	0.05 (0.01, 0.09)*	-0.05 (-0.09, -0.01)*
At 12 months	0.05 (0.01, 0.09)*	-0.06 (-0.10, -0.01)*
At 24 months	0.06 (0.02, 0.10)**	-0.11 (-0.15, -0.06)**

CI, Confidence interval. *P-value <0.05. **P-value <0.001. Values are regression coefficients (95% confidence interval) and reflect the differences in carotid intima media thickness (SDS) and carotid distensibility (SDS) per SDS change in infant and fetal weight from conditional models. Estimates are from multiple imputed data. The basic model is adjusted for child age at outcome visit and sex.

Supplementary Table S5. Basic models: Associations of fetal and infant weight measurements with childhood carotid measurements

Infant and fetal weight standard deviation scores	Difference in standard deviation scores (95% CI)					
	Carotid intima media thickness (n=4,484)			Carotid distensibility (n=4,304)		
	Basic model	Confounder model	BMI model	Basic model	Confounder model	BMI model
At first-trimester (n=942)	-0.01 (-0.07, 0.06)	-0.00 (-0.07, 0.06)	-0.00 (-0.07, 0.07)	-0.03 (-0.10, 0.04)	-0.03 (-0.10, 0.04)	-0.03 (-0.10, 0.03)
At 20 weeks (n=4,238)	0.02 (-0.01, 0.05)	0.03 (-0.01, 0.06)	0.02 (-0.01, 0.05)	-0.03 (-0.06, -0.00)*	-0.03 (-0.06, 0.00)	-0.03 (-0.06, 0.01)
At 30 weeks (n=4,352)	0.05 (0.03, 0.08)*	0.06 (0.03, 0.09)*	0.06 (0.03, 0.09)*	-0.05 (-0.08, -0.02)*	-0.05 (-0.08, -0.02)*	-0.04 (-0.07, -0.01)*
At birth (n=4,476)	0.07 (0.04, 0.09)*	0.08 (0.05, 0.11)*	0.07 (0.04, 0.10)*	-0.07 (-0.10, -0.04)*	-0.07 (-0.10, -0.04)*	-0.05 (-0.08, -0.02)*
At 6 months (n=3,385)	0.10 (0.07, 0.14)*	0.11 (0.07, 0.14)*	0.09 (0.06, 0.13)*	-0.07 (-0.11, -0.04)*	-0.07 (-0.10, -0.03)*	-0.02 (-0.06, 0.02)
At 12 months (n=3,103)	0.12 (0.08, 0.15)*	0.13 (0.09, 0.17)*	0.12 (0.08, 0.16)*	-0.11 (-0.15, -0.07)*	-0.10 (-0.14, -0.06)*	-0.05 (-0.09, -0.01)*
At 24 months (n=2,930)	0.13 (0.09, 0.16)*	0.14 (0.10, 0.18)*	0.13 (0.09, 0.17)*	-0.16 (-0.19, -0.12)*	-0.14 (-0.19, -0.11)*	-0.10 (-0.14, -0.08)*

CI, Confidence interval. *P-value <0.05. Values are regression coefficients (95% confidence interval) that were obtained from multivariable linear regression models and reflect the differences in carotid intima media thickness (SDS) and carotid distensibility (SDS) for birth outcomes. Estimates are from multiple imputed data. The basic model is adjusted for child age at outcome visit and sex. The confounder model is the basic model additionally adjusted for maternal age, pre-pregnancy body mass index, educational level, ethnicity, folic acid use, smoking and gestational hypertensive disorders. The BMI model is the confounder model additionally adjusted for sex and gestational age adjusted child body mass index at outcome measurement.

Supplementary Table S6. Basic models: Associations of fetal and infant growth patterns with childhood carotid measurements

Fetal and infant growth patterns	Difference in standard deviation scores (95% CI)	
	Carotid intima media thickness (n=3,485)	Carotid distensibility (n=3,316)
	Basic model	Basic model
Fetal growth deceleration		
Infant growth deceleration (n=122)	-0.00 (-0.19, 0.18)	0.12 (-0.08, 0.32)
Infant normal growth (n=382)	-0.06 (-0.18, 0.06)	0.01 (-0.11, 0.14)
Infant growth acceleration (n=397)	0.05 (-0.07, 0.17)	-0.09 (-0.22, 0.03)
Fetal normal growth		
Infant growth deceleration (n=336)	0.00 (-0.12, 0.13)	0.10 (-0.03, 0.23)
Infant normal growth (n=812)	<i>Reference</i>	<i>Reference</i>
Infant growth acceleration (n=400)	0.19 (0.07, 0.31)*	-0.17 (-0.29, -0.05)*
Fetal growth acceleration		
Infant growth deceleration (n=122)	-0.01 (-0.12, 0.11)	-0.04 (-0.16, 0.08)
Infant normal growth (n=383)	0.11 (0.00, 0.22)*	-0.06 (-0.17, 0.06)
Infant growth acceleration (n=397)	0.10 (-0.07, 0.27)	-0.11 (-0.28, 0.07)

CI, confidence interval. *P-value <0.05. Values are regression coefficients (95% confidence interval) and reflect the differences in carotid intima media thickness (SDS) and carotid distensibility (SDS) for fetal and infant growth patterns from multivariable linear regression models. Estimates are from multiple imputed data. The basic model is adjusted for child age at outcome visit and sex.

Supplementary Table S7. Maternal pre-pregnancy BMI for fetal and infant growth patterns (n=3,485)^a

Fetal and infant growth patterns	Pre-pregnancy BMI, median (95% range), kg/m ²
Fetal growth deceleration	
Infant growth deceleration (n=122)	22.6 (17.7, 34.0)
Infant normal growth (n=382)	22.5 (17.9, 34.3)
Infant growth acceleration (n=397)	22.3 (17.6, 36.7)
Fetal normal growth	
Infant growth deceleration (n=336)	22.7 (17.8, 32.2)
Infant normal growth (n=812)	22.2 (18.0, 33.9)
Infant growth acceleration (n=400)	22.5 (18.0, 34.5)
Fetal growth acceleration	
Infant growth deceleration (n=422)	22.7 (18.5, 35.8)
Infant normal growth (n=469)	22.8 (18.4, 34.6)
Infant growth acceleration (n=145)	23.4 (18.6, 36.3)

BMI, body mass index. Values are median (95% range). ^aCharacteristics are based on the pooled results after multiple imputations.

CHAPTER



NOVEL PARAMETERS OF FIRST-TRIMESTER FETAL DEVELOPMENT 4

CHAPTER

4.1

First-trimester fetal proportion volumetric measurements using a Virtual Reality approach

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Adapted from Prenatal Diagnosis, 2021.

OBJECTIVE To establish feasibility and reproducibility of fetal proportion volumetric measurements, using three-dimensional (3D) ultrasound and a Virtual Reality (VR) system.

METHODS Within a population-based prospective birth cohort, 3D ultrasound datasets of 50 fetuses in the late first-trimester were collected by three sonographers in a single research center. V-scope software was used for volumetric measurements of total fetus, extremities, head-trunk, head, trunk, thorax, and abdomen. All measurements were performed independently by two researchers. Intraobserver and interobserver reproducibility were analyzed using Bland and Altman methods.

RESULTS Intraobserver and interobserver analyses of volumetric measurements of total fetus, head-trunk, head, trunk, thorax and abdomen showed ICCs above 0.979, CV below 7.51% and mean difference below 3.44%. The interobserver limits of agreement were within the $\pm 10\%$ range for volumetric measurements of total fetus, head-trunk, head and trunk. The interobserver limits of agreement for extremities, thorax and abdomen were -26.09 to 4.77%, -14.14% to 10.00% and -14.47% to 8.83%, respectively.

CONCLUSION First-trimester fetal proportion volumetric measurements using 3D ultrasound and VR are feasible and reproducible, except volumetric measurements of the fetal extremities. These novel volumetric measurements may be used in future research to enable detailed studies on first-trimester fetal development and growth.

INTRODUCTION

The first-trimester of pregnancy is a crucial period for growth and the initial arrangement of organs¹. Observational studies suggest that impaired first-trimester growth, measured by traditional and relatively simple two-dimensional ultrasound parameters, might be associated with increased risks of adverse birth outcomes, and adverse cardiovascular and respiratory risk profile in childhood²⁻¹⁰. Ongoing developments in obstetric two- and three-dimensional (3D) transvaginal ultrasound techniques provide opportunities for improved evaluation of early fetal growth and development¹¹. Detailed studies on first-trimester fetal development are needed to enable better understanding of developmental adaptation mechanisms in early-pregnancy, leading to adverse outcomes in later life.

The combination of 3D transvaginal ultrasound with offline analyses using a Virtual Reality (VR) system enables more advanced measurement of first-trimester volumetric markers compared to the traditional crown rump length and biometric measures¹². Previously, embryonic volume measurements using this technique have shown to be feasible, and seem related to fetal growth and birth outcomes^{2, 3, 11, 13}. Additionally, segmentation of the various parts of the fetal body (extremities, trunk, head, thorax and abdomen) could increase the knowledge on early fetal growth and organ development in early-pregnancy¹⁴. These novel volumetric measurements could have great potential in observational research settings in the field of Developmental Origins of Health and Disease focused on fetal developmental adaptations.

Therefore, we developed novel volumetric measurements of first-trimester fetal body parts, from this stage forward *fetal proportions*, using 3D ultrasound datasets combined with a VR system. We assessed the intraobserver and interobserver reproducibility and agreement for volumetric measurements of the total fetus, extremities, head-trunk, head, trunk, thorax and abdomen of 50 fetuses in the late first-trimester.

METHODS

Study sample

This study was embedded in the Generation R *Next* study, a population-based prospective cohort study from preconception onwards in Rotterdam, the Netherlands. Recruitment started in August 2017 and is still ongoing. Pregnant women were invited to the research center for three appointments in the first-trimester of pregnancy, from 7 to 13 weeks of gestational age, with an interval of approximately two weeks. During these 30-minute visits 3D ultrasound datasets were obtained to assess embryonic, early fetal and placental

development. Around 30 weeks of gestational age, participants were invited back to the research centre for a follow-up visit. All participating women gave written informed consent. The medical ethics committee of the Erasmus University Medical Center approved of this study (MEC-2016-589, December 2016). For the current analysis, we focused on 3D ultrasound datasets collected in the late first-trimester (during the last appointment in the first-trimester of pregnancy). We selected 50 participants who visited the research center at the Erasmus MC from March 2019 to May 2019, in whom all the 3D ultrasound data according to the ultrasound study protocol was acquired.

Gestational age assessment

Gestational age was calculated from the first day of the last menstrual period (LMP) in spontaneous pregnancies, or from oocyte pick-up plus 14 days in IVF pregnancies. Gestational age was based on crown rump length in five subjects, because the LMP was unknown or gestational age determined by crown rump length differed more than 7 days from the LMP¹².

First-trimester fetal ultrasound examination

All ultrasound scans were performed by three experienced ultra-sonographers using a Voluson E10 System (GE Healthcare, Zipf, Austria) with a 5-13 MHz transvaginal transducer (RIC6-12D). Ultrasound settings were predefined to create uniformity (gain=0, line filter = low, persistence filter = 2, enhance = 2, dynamic contrast = 6, enhance = 2). The 3D ultrasound dataset acquisition of the total fetus was performed under a 90-110° volume angle. To assure at least one good quality 3D ultrasound dataset would be available for offline analysis, multiple 3D ultrasound dataset acquisitions were performed. The fetus was preferably facing towards the transducer in the mid-sagittal plane to provide detailed imaging of the fetal anatomy. The ultra-sonographer ensured that the fetus was not moving during the 3D ultrasound dataset acquisition. The 3D ultrasound datasets were stored in Cartesian volume files for offline analysis.

Fetal proportion volumetric measurements

We used the BARCO I-Space, a CAVETM-like VR system for offline analysis of the 3D ultrasound datasets¹⁵. V-Scope software enables accurate semi-automatic volumetric measurements due to improved depth perception using VR displays^{16, 17}. Multiple 3D ultrasound dataset acquisitions of a single fetus were stored. The dataset that was used for further offline analyses was selected by the first observer (C.W.) based on completeness

and quality of the 3D dataset. In preparation of the measurements, the surrounding uterine wall and the umbilical cord were manually erased using a brusher that can be adjusted in size to enable accurate deletion of voxels. Initially, we measured the volume of the total fetus as described previously¹⁸. To perform the volumetric measurement of the total fetus, fully automatic segmentation of hyperechoic structures was performed using strict preset thresholds. This was followed by manual segmentation of hypoechoic parts (e.g. brain ventricles, stomach, bladder and to a minimal extent artefacts due to acoustic shadowing) to obtain a whole-body segmentation of the fetus^{17, 18}.

Subsequently, we performed the novel volumetric measurements of extremities, head-trunk, head, trunk, thorax and abdomen. First, we manually deselected the segmented voxels of the extremities, from hands to axillae and feet to groins using a spherical brusher with the same diameter as the extremity, to perform the volumetric measurements of the extremities and head-trunk. Second, the volumetric measurement of the head and trunk were obtained by manually deselection of the segmented voxels of the head using a brusher with the size of the occipital- frontal diameter. The base of the chin and the fourth ventricle in the midsagittal plane are used as reference points, as described previously¹⁹. Third, we performed the volumetric measurements of the thorax and abdomen by manually deselection of the segmented part below the diaphragm using a spherical brusher with the same diameter as the trunk. During the fetal proportion volumetric measurements, we used a transparent segmentation color to enable identification of the fourth ventricle and the diaphragm. To obtain reproducible measurements, the measurements were performed using a detailed technical measurement protocol with instructions about the size of the brusher, the alignment of the fetus and the plane in which the measurement should be performed (for a detailed description see **Supplementary material: Protocol for first-trimester fetal proportion volumetric measurements using the V-Scope software**. **Figure 1** shows a step-by-step approach for the fetal proportion volumetric measurements. All fetal proportion volumetric measurements were performed independently by two researchers (C.W. and J.E.) to obtain intraobserver and interobserver reproducibility. Both researchers were experienced ultrasonographers and had previous experience with performing volume measurements in the BARCO I-Space using V-scope software. Both researchers performed the offline measurements twice, with an interval of at least one week to prevent recall bias. The measurements were performed in a blinded setting.

Statistical analysis

We performed statistical analysis described by Bland and Altman^{20, 21}. For the intraobserver analysis, the first measurement was compared with the second measurement for each

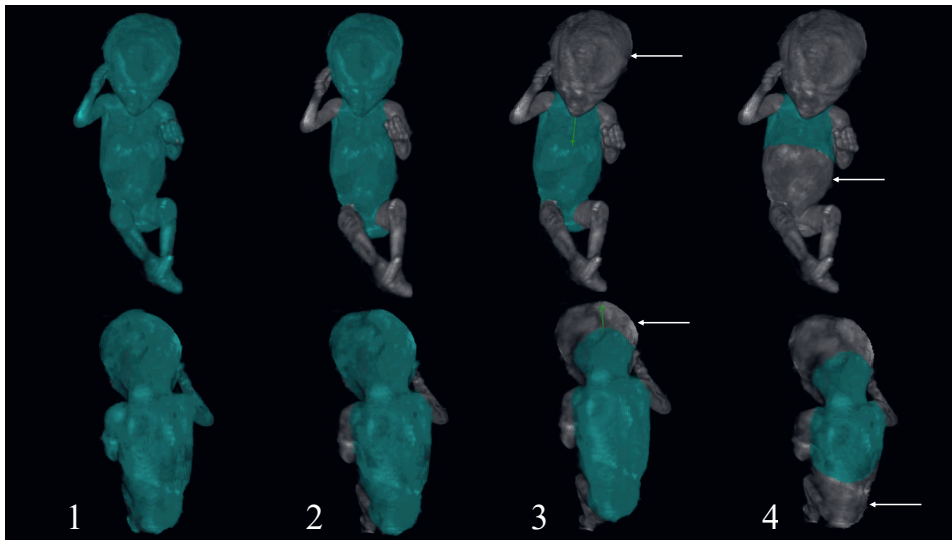


Figure 1. Anterior and posterior view of a fetus at 12 weeks and 5 days of gestation in the BARCO I-Space. A step-by-step approach for volumetric measurement of the total fetus and fetal proportions is shown. Volumetric measurements from left to right: 1) Segmentation of the total fetus in cyan; 2) Segmentation of head-trunk in cyan with indirect measurement of the extremities in grey; 3) Segmentation of the trunk cyan with indirect measurement of the head in grey indicated by the arrow (reference line through chin and fourth ventricle in the midsagittal plane); 4) Segmentation of the thorax in cyan with indirect measurement of the abdomen in grey indicated by the arrow.

observer. For the interobserver analysis, the mean of the two measurements of the first observer was compared to the mean of the two measurements of second observer using similar calculations.

First, we plotted the measurements with the line of equality to give an initial sense of the degree of agreement²¹. Second, intraclass correlation coefficients (ICC) with a 95% confidence interval and the coefficients of variation (CV) were calculated for each measurement to evaluate consensus within each observer and between observers²¹. Third, intraobserver and interobserver variability was quantified calculating the mean difference in percentage measurement error with the 95% limits of agreement (mean difference (%) $\pm 1.96SD$) for all the fetal proportion volumetric measurements. Within the limits of agreement the measurements within and between observers can be assumed to be interchangeable¹². Lastly, we plotted the mean differences in percentage measurement error with the 95% limits of agreement. These so called Bland and Altman plots were specifically provided to visualize that the agreement for the volumetric measurements does not depend on fetal size.

We consider the ICC, CV, mean difference and the limits of agreement as our main outcomes of interest. We decided that an ICC >90%, a CV <10%, a mean difference <10%

and limits of agreement within $\pm 10\%$ were considered to be proof of good agreement²². Importantly, an acceptable mean difference and limits of agreement are not a statistical but a clinical and more subjective consideration¹². To establish that the measurements are useable for future association studies, we decided that the limits of agreement should deviate a maximum of 10% from the mean difference, which indicates that 95% of all differences should be within the $\pm 10\%$ measurement error range²⁰. Statistical analyses were performed using IBM SPSS, version 25.

RESULTS

Participant characteristics

Participants and pregnancy characteristics are shown in **Table 1**. The median gestational age was 12 weeks and 3 days. **Figure 2** shows the different fetal proportion volumetric measurements plotted against the fetal crown rump length.

Table 1. Participant characteristics (n=50)

	Median (95% range) / n (%)
Maternal age (years)	31.5 (25.7, 38.3)
Maternal BMI (kg/m ²)	22.5 (18.5, 34.6)
Gestational age (weeks, days)	12.3 (10.9, 13.2)
Crown Rump Length (mm)	60.90 (44.60, 74.57)
Reproduction method	
Spontaneously conceived	45 (90)
IVF	1 (2)
Ovulation induction	4 (8)

BMI, Body Mass Index; IVF, In Vitro Fertilization.

Intraobserver reproducibility analyses

Table 2 presents the mean volumes, ICCs, CVs, mean differences and corresponding limits of agreement for intraobserver agreement for volumetric measurements of the total fetus, extremities, head-trunk, head, trunk, thorax and abdomen. All measurements of both observers lie in close proximity to the line of equality suggesting small intraobserver differences, except for the volumetric measurements of the extremities (**Supplementary Figures S1 and S2**). Intraobserver ICCs were higher than 0.980, and CVs were below 9.43% for each measurement. The observed mean differences ranged from -0.76% to 0.04 for intraobserver differences of observer 1 and from 1.44% to 1.07% for observer 2. **Supplementary Figures S3 and S4** depicts the Bland and Altman plots for intraobserver

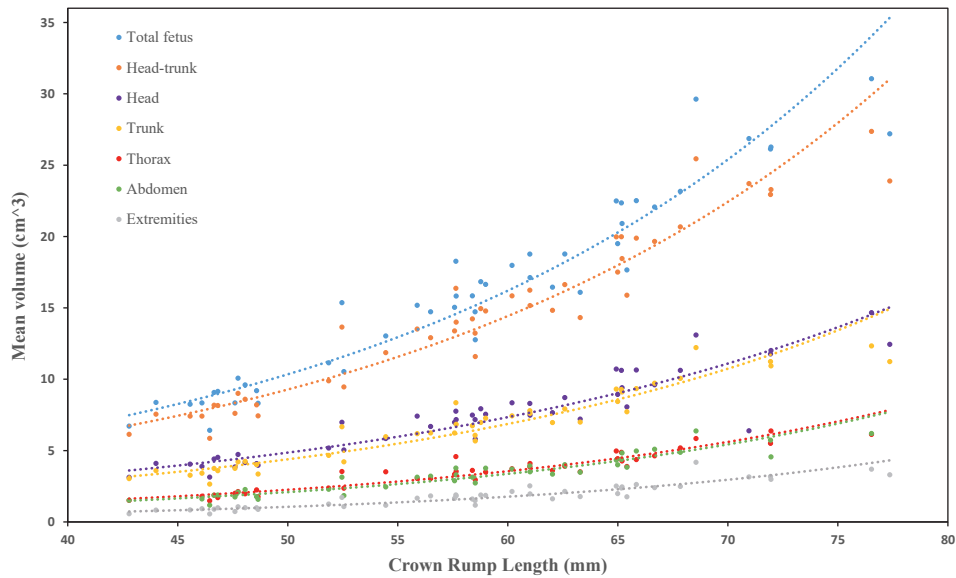


Figure 2. Fetal crown rump length and fetal total and body proportion volume measurements. In the graph the colored dots indicate the measurements, the mean value is indicated by the accompanying colored dotted line.

Table 2. Intraobserver agreement of fetal proportion volumetric measurements for both observers (n=50)

Volumetric measurement	Observer	ICC (95% CI)	CV	Mean difference	Mean difference (LLOA, ULOA)
			%	cm³	%
Total fetus	1	0.998 (0.996, 0.999)	2.70	0.01	0.04 (-4.60, 4.67)
	2	0.999 (0.999, 1.000)	1.34	-0.01	-0.16 (-2.99, 2.67)
Extremities	1	0.980 (0.982, 0.994)	9.43	0.03	0.76 (-17.42, 18.94)
	2	0.983 (0.97, 0.991)	8.88	-0.01	-0.89 (-16.77, 14.98)
Head-Trunk	1	0.998 (0.996, 0.999)	2.67	-0.02	-0.00 (-4.61, 4.52)
	2	0.999 (0.998, 0.999)	1.71	-0.00	-0.11 (-3.04, 2.82)
Head	1	0.996 (0.994, 0.998)	3.29	-0.03	-0.00 (-6.70, 5.89)
	2	0.996 (0.994, 0.998)	3.38	-0.01	-0.13 (-6.09, 5.83)
Trunk	1	0.996 (0.993, 0.998)	3.34	-0.02	-0.00 (-6.42, 6.25)
	2	0.997 (0.995, 0.998)	3.08	-0.01	-0.17 (-6.23, 5.89)
Thorax	1	0.989 (0.980, 0.994)	5.48	-0.03	-0.01 (-11.19, 8.85)
	2	0.991 (0.983, 0.995)	5.08	-0.06	1.07 (-8.90, 11.03)
Abdomen	1	0.992 (0.992, 0.985)	5.04	-0.01	0.01 (-10.44, 11.47)
	2	0.985 (0.973, 0.992)	7.06	-0.06	-1.44 (-12.44, 9.56)

CI, confidence interval; ICC, intraclass correlation coefficient; CV, coefficient of variation; LLOA, lower limit of agreement; ULOA, upper limit of agreement.

agreement for each measurement of both observers, in which the mean difference is plotted against the mean of the assessments accompanied with the limits of agreement. We observed that the limits of agreement for volumetric measurements of the total fetus, head-trunk and trunk were within $\pm 10\%$ for both observers, but slightly exceeded the

$\pm 10\%$ limits of agreement for volumetric measurements of thorax and abdomen. Limits of agreement for volumetric measurement of the extremities ranged between -17.42% and 18.94% .

Interobserver reproducibility analyses

Table 3 presents the mean volumes, success percentage, ICCs, CVs, mean differences and corresponding limits of agreement for volumetric measurements of the total fetus, extremities, head-trunk, head, trunk, thorax and abdomen. Not all measurements could be performed by both observers due to the lack of visibility of anatomic landmarks, and thus measurements were incomplete in 10 fetuses. The plots with the line of equality suggest small interobserver differences, but larger interobserver differences for the volumetric measurement of the extremities (**Supplementary Figure S5**). Interobserver ICCs for all measurements were higher than 0.951. CVs ranged from 3.35% to 6.44%, except for the volumetric measurements of the extremities (CV=10.86%). The observed mean differences were $<10\%$ and ranged from -3.44 to 2.84 , except for the volumetric measurements of the extremities (mean difference= -10.66%). **Supplementary Figure S6** depicts the Bland and Altman plots for interobserver agreement. We observed good agreement for volumetric measurement of the total fetus, head-trunk volume, head and trunk with limits of agreement within $\pm 10\%$. Interobserver limits of agreement for the volumetric measurements thorax and abdomen slightly exceeded the $\pm 10\%$ limits of agreement (lower limit of agreement, upper limit of agreement: -14.14% , 10.00% ; -14.47% , 8.83% , respectively). Limits of agreement for the volumetric measurement of the extremities were -26.09% and 4.77% .

Table 3. Interobserver agreement of fetal proportion volumetric measurements (n=50)

Volumetric measurement	Mean volume (SD)	n (%)	ICC (95% CI)	CV	Mean difference	Mean difference (LLOA, ULOA)
	cm ³			%	cm ³	%
Total fetus	16.32 (6.51)	46 (92)	0.991 (0.944, 0.997)	3.95	-0.58	-3.44 (-8.79, 1.91)
Extremities	1.82 (0.86)	46 (92)	0.951 (0.697, 0.983)	10.86	-0.19	-10.66 (-26.09, 4.77)
Head-Trunk	14.50 (5.67)	46 (92)	0.993 (0.973, 0.997)	3.75	-0.39	-2.57 (-7.90, 2.77)
Head	7.41 (2.89)	45 (90)	0.991 (0.977, 0.996)	4.51	-0.19	-2.51 (-10.00, 4.98)
Trunk	6.89 (2.69)	45 (90)	0.995 (0.984, 0.998)	3.35	-0.15	-2.14 (-8.76, 4.46)
Thorax	3.57 (1.34)	40 (80)	0.979 (0.960, 0.989)	7.51	-0.08	-2.07 (-14.14, 10.00)
Abdomen	3.40 (1.33)	40 (80)	0.985 (0.971, 0.992)	6.44	-0.07	2.82 (-14.47, 8.83)

CI, confidence interval; ICC, intraclass correlation coefficient; CV, coefficient of variation; LLOA, lower limit of agreement; ULOA, upper limit of agreement. *Number and percentage of datasets in which both observers could do the measurement.

Feasibility

A total of 112 3D ultrasound datasets of the whole-body fetus were available for offline analysis, on average 2.4 3D ultrasound datasets per participant. **Table 3** shows the number and percentage of late first-trimester fetuses, in which both observers could perform the fetal proportion volumetric measurements. Volumes of the total fetus, extremities and head-trunk could be obtained in 46 of 50 (92%) late first-trimester fetuses. Success percentages were 90% for head and trunk, and 80% for thorax and abdomen.

DISCUSSION

Main findings

Using 3D ultrasound datasets acquired in the late first-trimester of pregnancy, combined with a VR system, we observed good intraobserver and interobserver reproducibility for volumetric measurements of the total fetus, head-trunk, head, trunk, thorax and abdomen. We observed that volumetric measurements of extremities were feasible but with lower intraobserver and interobserver reproducibility.

Interpretation of main findings

Currently, first-trimester growth is assessed by crown rump length and biometric measurements, which are relatively simple two-dimensional ultrasound parameters. Advanced ultrasound techniques such as 3D ultrasound in combination with VR volumetric measurements can lead to more accurate first-trimester growth parameters when compared to the routine two-dimensional ultrasound measures¹¹. The use of VR, enables depth-perception in 3D ultrasound datasets and therefore offers the possibility to reliably conduct complex volumetric measurements. Due to detailed measurement protocols with predefined ultrasound and VR settings, this technique is highly reproducible²³. Assessment of 3D ultrasound datasets with a VR system has previously shown feasible and reproducible for several first-trimester measurements, including the measurement of embryonic volume^{18, 19, 24}. As the increase in volume during the first-trimester is much larger than the increase in length, it is suggested that these volumetric measurements may have higher sensitivity to assess deviations in first-trimester growth compared to customary biometric measurements¹⁸.

As the relative growth rate of the fetus is highest during the first-trimester of pregnancy, the fetus is most vulnerable during this period for stressors that can lead to early developmental adaptations. These developmental adaptations might translate into dissimilar growth rates of the different organ systems and fetal body parts¹. We developed

novel volumetric measurements of the various parts of the fetal body as an addition to already existing techniques for volumetric measurement of the total fetus and head using V-scope software^{18, 19}. We believe that these novel measurements could increase the knowledge on fetal growth and development in early-pregnancy, when applied in research settings focused on fetal developmental adaptations.

Before a novel measurement technique is introduced, it is important to assess the reliability of the measurements. To this purpose, we used a combination of statistical methods to allow a good impression of the reproducibility and agreement of fetal proportion volumetric measurements²⁰. We found good intraobserver and interobserver agreement as indicated by a high ICC accompanied by a low CV¹². As expected, we found slightly lower interobserver ICCs and higher interobserver CVs when compared to the intraobserver values. This indicates that different observers measure slightly different. Except for the volumetric measurements of the extremities, we found no bias between the observers as the mean differences were <10%, and the Bland and Altman plots do not show a larger measurement error with increasing volumes. The consideration to require limits of agreement within the $\pm 10\%$ measurement error was made to ensure that these novel measurements would be useful for future association studies within an observational setting, but these limits remain arbitrary. Therefore, we consider the reproducibility of the volumetric measurements of thorax and abdomen as good.

The volumetric measurements of the extremities had slightly lower ICC and CV values when compared to the other measurements. As the measurements of the extremities separate from the fetal body also had a mean difference >10% and limits of agreement exceeding $\pm 10\%$, we consider the current reproducibility of these volumetric measurement as suboptimal. The lower reproducibility can be explained by poorer visualization of the extremities when compared to other parts of the fetus. This is caused by the presence of acoustic shadowing caused by calcification of the bones in the upper and lower extremities that is visible during this stage of fetal development. Although these artefacts are minor, they can compromise the 3D interface between the fluid and fetal surface in such a way that the V-scope software cannot automatically recognize the interface at the level of the artefact. If the extent of the artefacts is only minor, the researcher can decide to manually extrapolate these parts of the segmentation of the extremities. We think this approach gives a slightly larger interobserver differences when compared to the automatic segmentations. Importantly, the small absolute volumes of the extremities only allow for very small absolute measurement errors. We hope to improve these measurements in the future.

Within our study, the success percentages ranged from 92% for volumetric measurement of the total fetus to 80% for volumetric measurements of the thorax and

abdomen. Some of the measurements could not be performed due to the inability to identify the anatomical landmarks that are necessary for the proposed measurements. The quality of the 3D ultrasound data is of extreme importance for feasibility of the fetal proportion volumetric measurements. To enable collection of high quality 3D ultrasound data, the data collection was done by three experienced ultrasonographers using a high-frequency transvaginal transducer, and 3D acquisition was done in the midsagittal fetal plane while the fetus was not moving. Unfortunately, factors such as maternal adiposity or fetal movements can still negatively influence ultrasound quality, leading to a lower success percentage of the fetal proportion volumetric measurements. Despite these limitations, we consider the success percentages in our study to be sufficiently high. Thus, we conclude that the fetal proportion volumetric measurements are feasible for application in research projects.

The observed reproducibility and agreement was similar to previous VR studies for volumetric measurement of the embryo and head^{18, 19}. Previously, one other study attempted to reconstruct volumes of extremities in early fetuses, using specialized software that allows to estimate volumes by drawing contours²⁵. Within this study it was found feasible to measure the extremities separately from the fetal body, but as in our study the measurement agreement seemed poor. To our knowledge, no previous studies have been conducted to assess volumetric measurements of the abdomen and thorax. In large scale population-based research settings like the Generation R *Next* Study, these measurements could be used for research within the field of Developmental Origin of Health and Disease research. The Generation R *Next* Study, is a population-based prospective cohort study from preconception onwards. The study has a specific focus on the consequences of maternal and paternal preconception lifestyle, diet and health, and embryonic development in relation to childhood growth, development and health. In the future, we will measure the fetal proportion volumetric measurements in a larger study sample and assess whether early fetal growth is influenced by preconception and early prenatal lifestyle, diet and health related factors. We will also investigate whether early fetal growth is related to adverse birth outcomes, and unfavorable outcomes in children. Volumetric measurements are expected to have higher sensitivity to assess deviations in early fetal growth compared to the traditional crown lump length that is used in earlier research investigating first-trimester growth restriction and adverse outcome. Thus, these novel measurements might give further insights in the influence of periconceptual exposures on early fetal growth, and the consequences for later health. The measurements of the thorax and abdomen can be used as surrogate markers for organ development of the cardiopulmonary system and the gastrointestinal system. Also, volumetric measurements

of organs in first-trimester fetuses could provide further and more specific knowledge on developmental adaptations and should be the focus of future studies.

Strengths and limitations

The technique we propose can be used on a large scale in research settings¹⁶. It is an easily comprehensible technique that is conducted following a detailed protocol. To ensure that the measurements are conducted according to protocol and in a reproducible manner, both researchers practiced the measurement method in a rehearsal setting. This training program is approximately 20-hours in duration and could be used to train other researchers within Generation R *Next* in the future. There are some limitations to this measurement approach. We compared the mean of the two measurements to achieve good interobserver reproducibility. This implies that in research settings with multiple observers, measurements have to be conducted twice by the same observer. Approximately 20-30 minutes are needed to conduct the fetal proportion volumetric measurements in a single 3D ultrasound dataset, which could be considered as time-consuming. In the current study, we only used 3D ultrasound datasets collected in the Generation R *Next* study during the visit in the late first-trimester. Because the proposed aim of these novel measurements is to give insights in early fetal growth and development within research settings, we do not think the narrow range of gestational age within our study influenced the generalizability of our findings.

Conclusion

In conclusion, we found that fetal proportion volumetric measurements in the late first-trimester using 3D ultrasound in combination with a VR system are feasible and reproducible, except for volumetric measurements of the fetal extremities. These novel volumetric measurements may be used in future research to enable detailed studies on first-trimester fetal development and growth. These studies may lead to better understanding of early developmental adaptation mechanisms leading to adverse birth outcomes, and unfavorable cardiovascular and respiratory risk profiles in later life.

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SUPPLEMENTARY MATERIAL

Protocol for first-trimester fetal proportion volumetric measurements using the V-Scope software

The three-dimensional dataset containing the whole-body fetus is visualized in the BARCO I-Space using the V-scope software^{15, 16}. In preparation of the measurements, the surrounding uterine wall and the umbilical cord were manually erased using a spherical brusher that can be adjusted in size to enable accurate deletion of voxels¹⁸. The fetus is then aligned in an upright position facing towards the operator (coronal plane). The volumetric measurements are executed in a semi-automated manner and in a fixed order: 1) total fetus, 2) head-trunk, 3) trunk, and 4) thorax are segmented. Volumetric measurements of extremities, head and abdomen are derived indirectly during the measurement process of the total fetus, head-trunk, trunk and thorax. If a measurement could not be performed due to the inability to identify anatomical land-marks or because of insufficient data quality, the subsequent other measurements could also not be performed. The segment that is being measured is checked in the coronal plane and sagittal plane after every volumetric measurement, using the V-scope slice option. This enables detection of segmentation errors that would indicate under- or overestimation of the volumetric measurement. If errors are detected by the operator, corrections can be made manually using the spherical brusher in combination with the 'deselect voxels' or 'select voxels' options in V-scope; or the segment can be reloaded and the protocol step can be repeated.

Step 1. Volume of the total fetus

Direct measurement: Volume of the total fetus. The measurement is executed according to Rousian et al.¹⁸

Anatomical margins: Prior to the semi-automatic volume measurement the fetal insertion of the umbilical cord and surrounding uterine wall are manually 'brushed' away with the spherical brusher to avoid segmentation of other parts than the total fetus. Important attention has to be paid that the surroundings of the embryo are thoroughly brushed away, and the total fetus stays intact, before the automatic segmentation option 'plant seed' is used.

Conditions: If the fetus is incomplete (due to extensive acoustic shadowing or incompleteness of the dataset), the volume measurement of the total fetus cannot be performed.

Process specification:

1. To measure the volume of the total fetus the hyperechoic structures are automatically segmented, followed by manual segmentation of the hypoechoic structures (for example the brain ventricles, stomach, and bladder).
2. **Automatic segmentation:** The option 'use blurred data' is selected. This uses smoothed data during the automatic segmentation, to prevent incomplete segmentation due to noise. The operator selects an upper threshold value of 255 (pure white) and a lower threshold of 60 (dark to medium grey) for the grey level, and a high (>60) value for the deviation threshold, to effectively disable this. The 'plant seed' option will automatically segment the hyperechoic structures. All voxels connected to the seedpoint or other segmented voxels, with a grey value between the lower and upper threshold will be automatically segmented. Voxels outside of this range that are an obvious part of the whole-body fetus should be manually segmented by the operator (e.g. brain ventricles, stomach, bladder, minor acoustic shadowing).
3. **Manual segmentation:** The operator manually segments the hypoechoic structures with the option 'select voxels'. Special attention has to be paid to:
 - All anatomic hypoechoic structures should be selected manually, e.g. brain ventricles, stomach and bladder.
 - Hypoechoic parts due to acoustic shadowing can be segmented manually. If the fetus is incomplete due to extensive acoustic shadowing the measurement will not be performed. This decision is up to the operator conducting the measurements.
 - Correct segmentation of the fluid-fetus interface, the area around the vertebral column and the extremities.
4. The obtained segmentation is saved.

*Step 2. Head-trunk volume (indirect derived measurement: extremities volume)***Direct measurement:** Volume of the head-trunk excluding the extremities**Indirect measurement:** Volume of the extremities (volume of the total fetus minus head-trunk volume)**Anatomical margins upper extremities:** The fetus is aligned in an upright position facing towards the operator. The upper extremities are deselected from hands to the axillae in the coronal plane (without using the V-scope slice option), afterwards the measurement is checked to detect segmentation errors in the coronal plane while using the V-scope slice option.**Anatomical margins lower extremities:** The fetus is aligned in a caudal position in the transverse plane. The lower extremities are deselected from feet to the groin (without using the V-scope slice option). The measurement is consecutively checked from a caudal position in the transverse plane using the slice option, and any necessary corrections are made.**Process specification:**

1. In the segmentation of the total fetus, the segmented voxels in the extremities are manually deselected using the option 'deselect voxels' without the V-scope slice option.
2. A brush radius of the same size as the diameter of the extremity is used.
3. The obtained segmentation is saved.

*Step 3. Trunk volume (indirect derived measurement: head volume)***Direct measurement:** Volume of the trunk.**Indirect measurement:** Volume of the head (head-trunk volume minus trunk volume). The measurement of the head was executed according to Koning et al.19**Anatomical margins:** The base of the chin and the base of the fourth ventricle are used as anatomical landmarks (in the midsagittal plane).**Conditions:** If the fourth ventricle is not recognizable, the measurement cannot be performed.**Process specification:**

1. The transparent appearance of the segmentation allows for recognition of the anatomical landmarks: the operator first identifies the base of the chin and the base of the fourth ventricle.
2. A line is drawn using the V-scope measure line option, through the base of the chin and the base of the fourth ventricle to ensure a sharp cutting plane for deselecting voxels.
3. The brush radius is the same size as the occipital frontal diameter is used.
4. The fetus is aligned in the midsagittal plane, the segmented voxels of the head are manually deselected using the option 'deselect voxels' without the V-scope slice option.
5. The cutting plane is used to check for segmentation errors within the sagittal plane, and any corrections should only be made from the midsagittal plain.
6. The obtained segmentation is saved.

*Step 4. Thorax volume (indirect derived measurement: Abdomen volume)***Direct measurement:** Volume of the thorax.**Indirect measurement:** Volume of the abdomen (trunk volume minus thorax volume).**Anatomical margins:** The diaphragm is used as the cutting plane.**Conditions:** If the entire diaphragm is not fully recognizable, the measurement cannot be performed.**Process specification:**

1. The transparent appearance of the segmentation allows for recognition of the anatomical landmarks: the operator first identifies the full course of the diaphragm.
2. The fetus is positioned in the coronal plane, facing towards the operator.

3. For deselection of segmented voxels a spherical brush with the same diameter as the trunk was used to follow the shape of the diaphragm.
4. The first step of deselecting the abdomen is performed from the coronal plane. Two thirds of the spherical brush is placed in the embryo just below the diaphragm in the coronal plain. The cutting plane is consecutively checked in the coronal plane, corrections can be made within the coronal plane.
5. If the cutting plane from a coronal point of view is correct, the next step is to check the cutting plane is in the sagittal plane and correct if necessary. The separation plane should follow the full course of the diaphragm. If this is not the case, the trunk segmentation volume is reloaded and the process is repeated. Special attention has to be paid to deselection of the complete lower part of the trunk.
6. The obtained segmentation is saved.

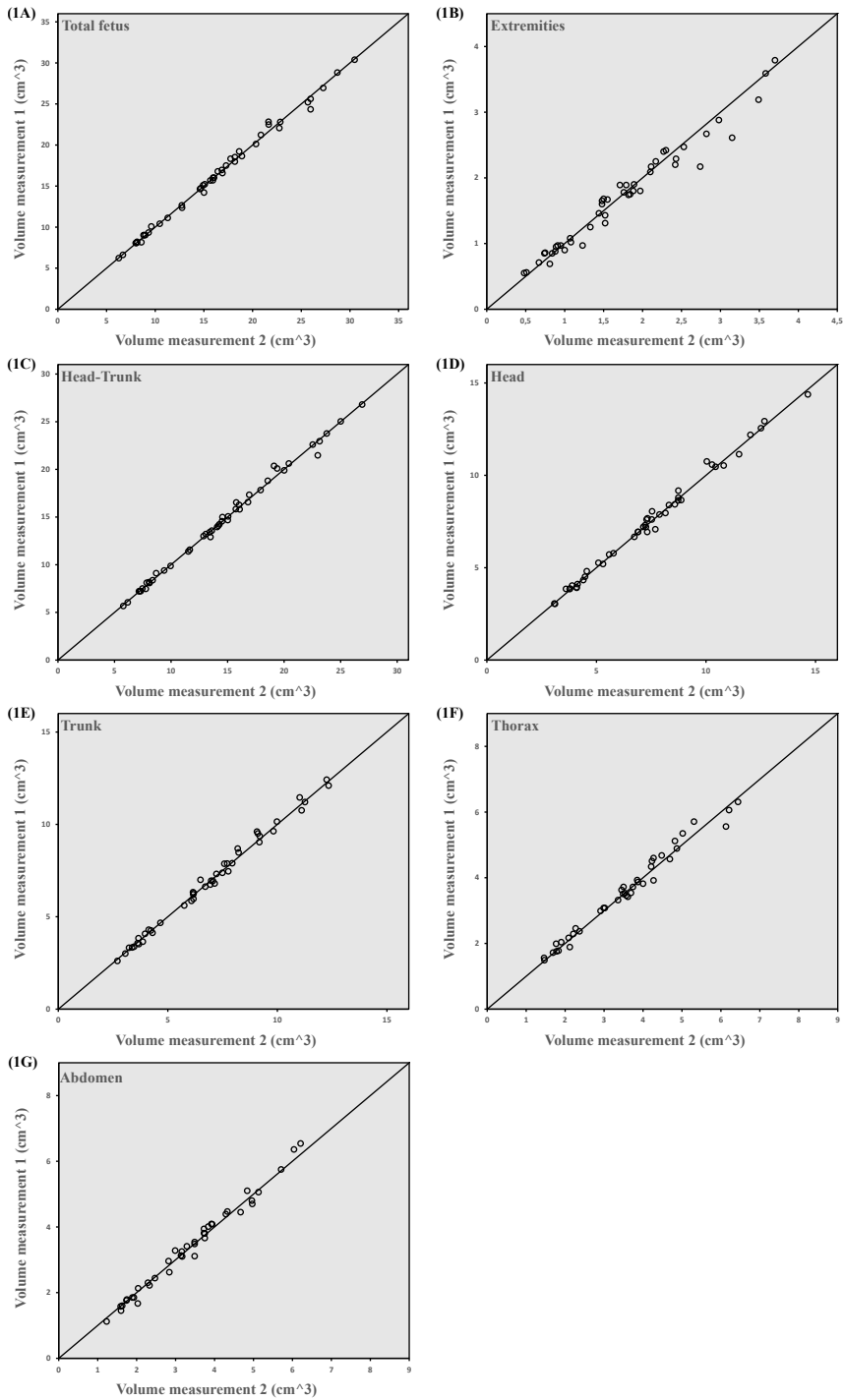


Figure S1. Measurements of the observer 1 plotted with line of equality for volumetric measurement of: (1A) Total fetus; (1B) Extremities; (1C) Head-trunk; (1D) Head; (1E) Trunk; (1F) Thorax; (1G) Abdomen.

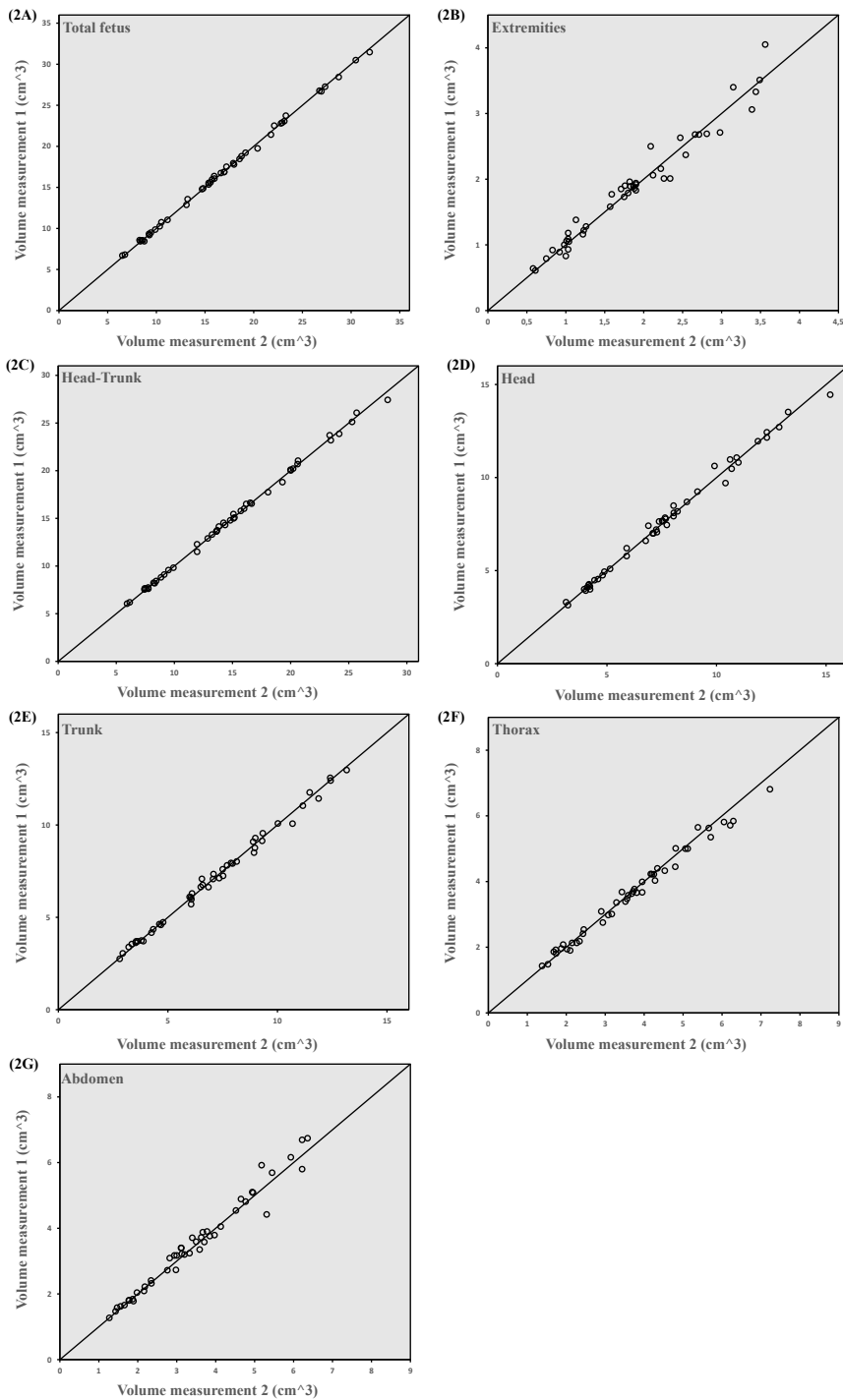


Figure S2. Measurements of observer 2 plotted with line of equality for volumetric measurement of: (2A) Total fetus; (2B) Extremities; (2C) Head-trunk; (2D) Head; (2E) Trunk; (2F) Thorax; (2G) Abdomen.

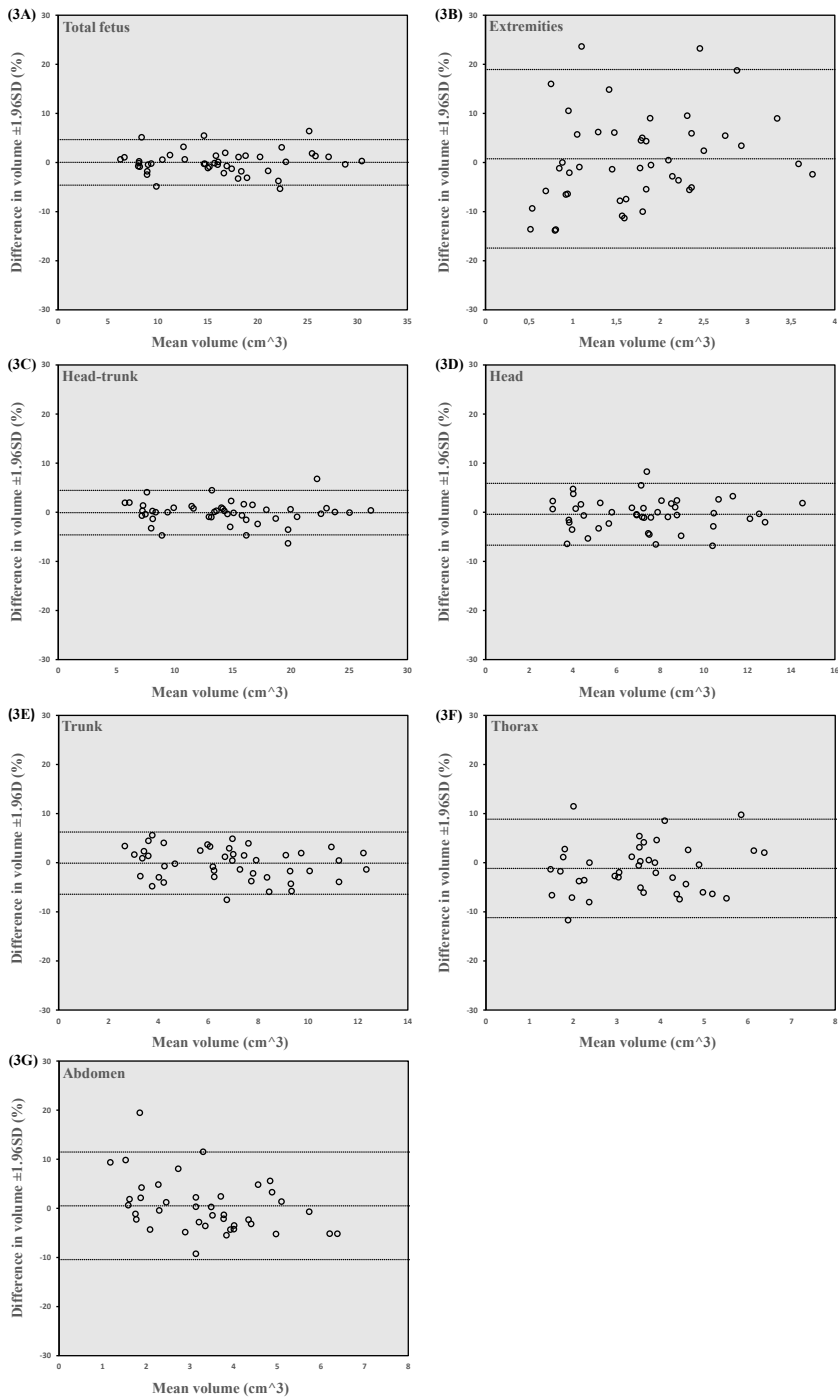


Figure S3. Bland and Altman plots of intraobserver agreement of observer 1 with corresponding limits of agreement in proportion of the mean ± 1.96 SD for volumetric measurement of: (3A) Total fetus; (3B) Extremities; (3C) Head-trunk; (3D) Head; (3E) Trunk; (3F) Thorax; (3G) Abdomen.

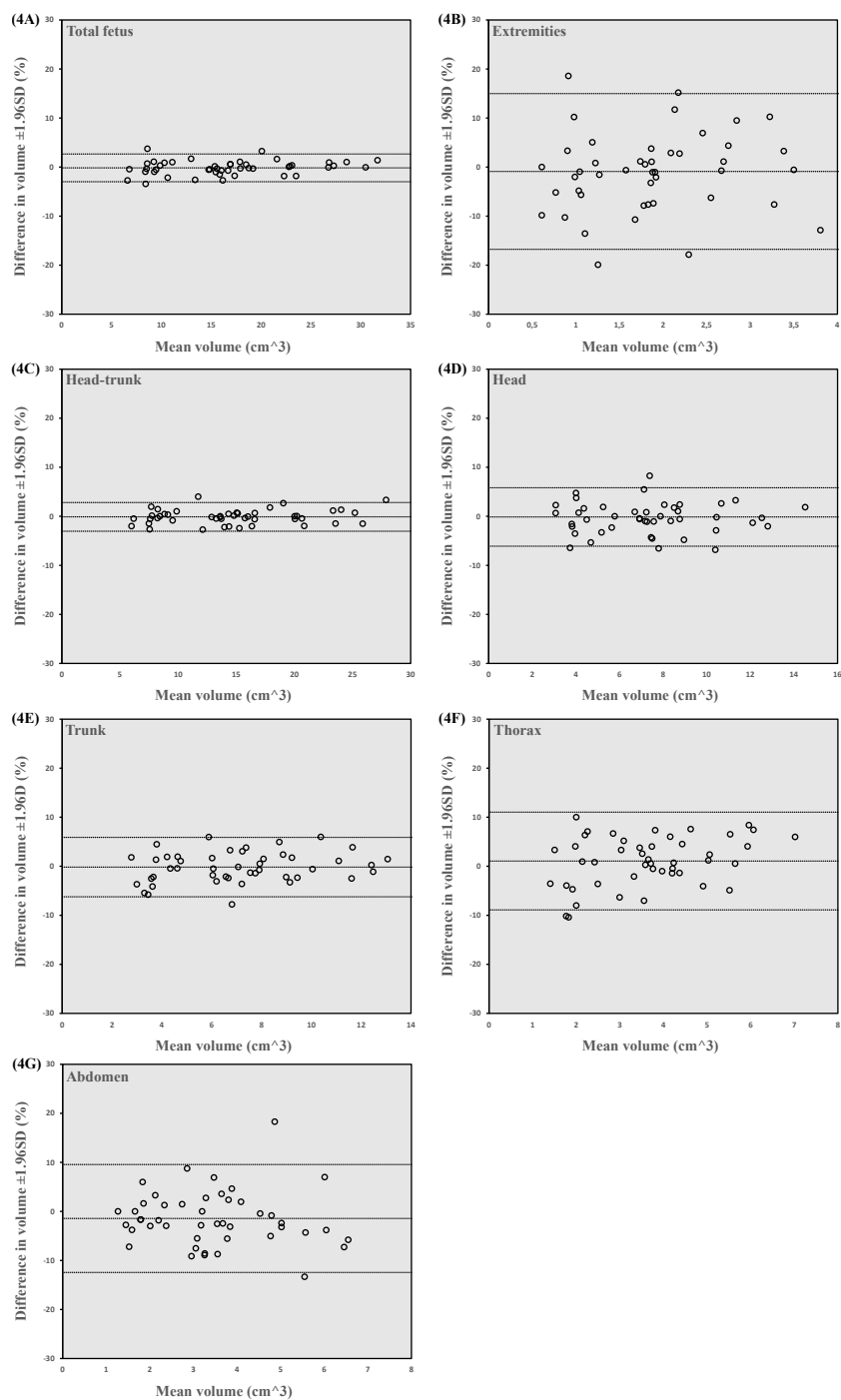


Figure S4. Bland and Altman plots of intraobserver agreement of observer 2 with corresponding limits of agreement in proportion of the mean ± 1.96 SD for volumetric measurement of: (4A) Total fetus; (4B) Extremities; (4C) Head-trunk; (4D) Head; (4E) Trunk; (4F) Thorax; (4G) Abdomen.

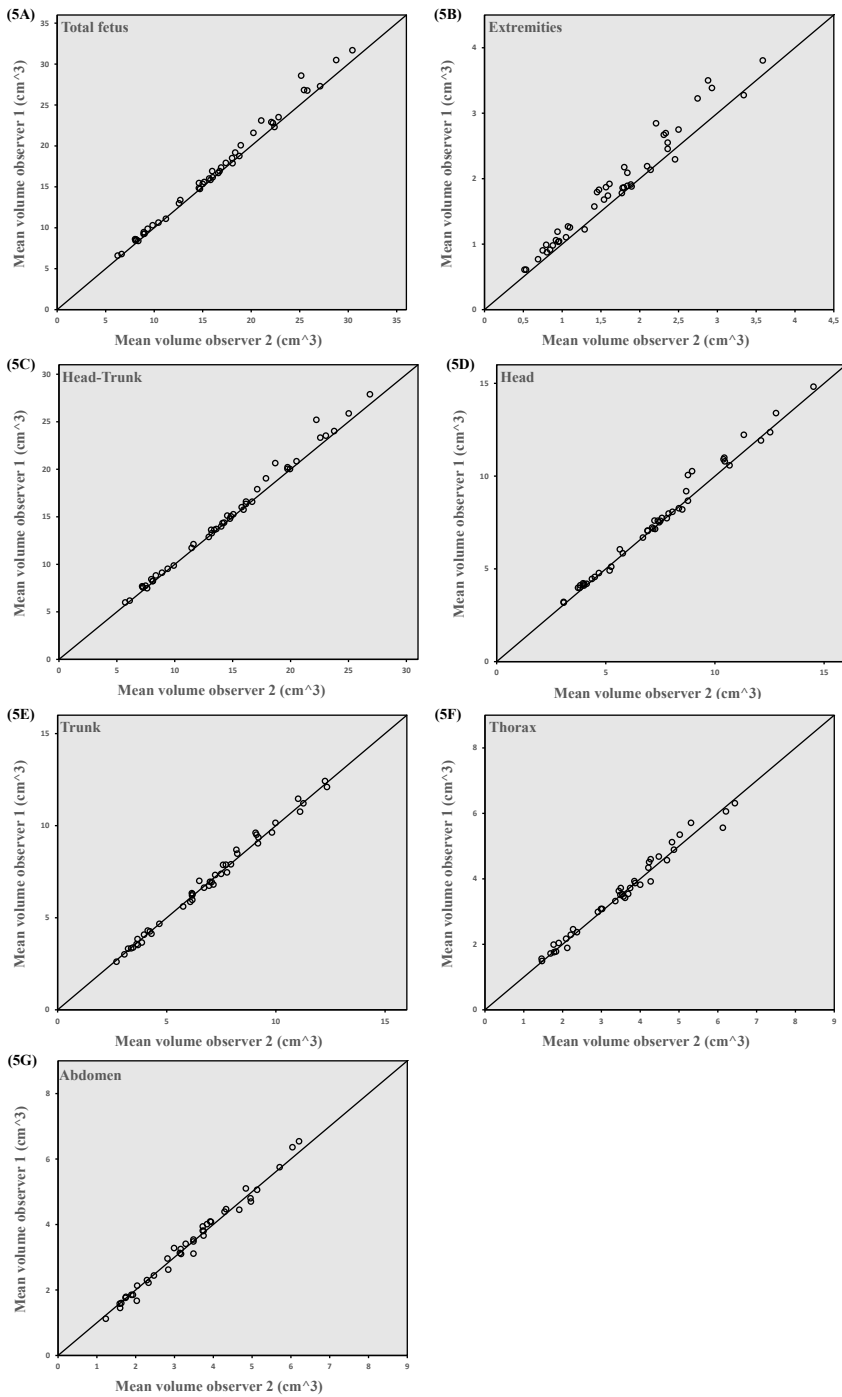


Figure S5. Measurements of observer 1 plotted against measurements of observer 2 with line of equality for volumetric measurement of: (5A) Total fetus; (5B) Extremities; (5C) Head-trunk; (5D) Head; (5E) Trunk; (5F) Thorax; (5G) Abdomen.

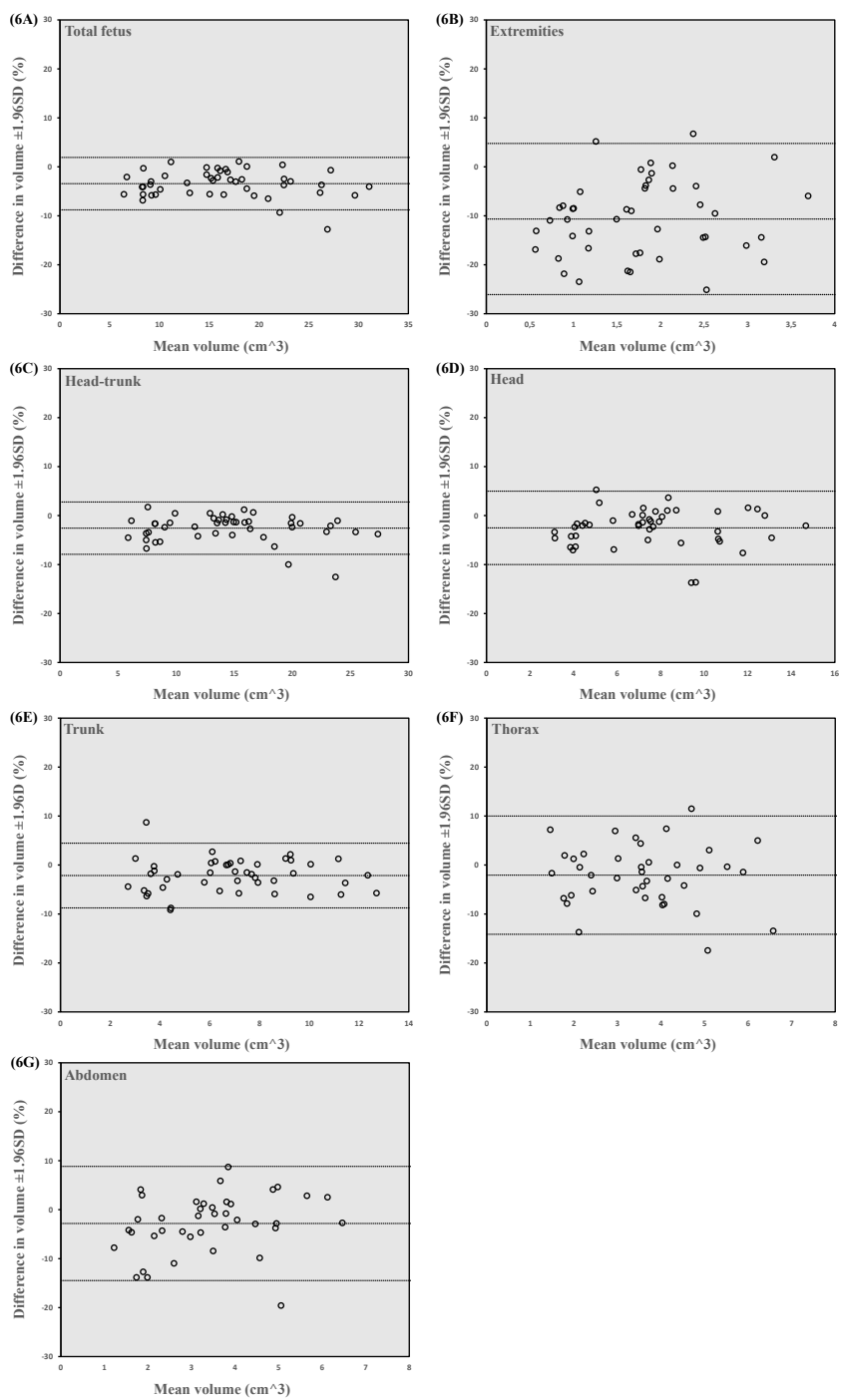


Figure S6. Bland and Altman plots of interobserver agreement with corresponding limits of agreement in proportion of the mean ± 1.96 SD for volumetric measurement of: (6A) Total fetus; (6B) Extremities; (6C) Head-trunk; (6D) Head; (6E) Trunk; (6F) Thorax; (6G) Abdomen.

CHAPTER

4.2

Innovative approach for first-trimester fetal organ volume measurements using a Virtual Reality system: The Generation R *Next* Study

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OBJECTIVE To investigate the reproducibility of first-trimester fetal organ volume measurements using three-dimensional (3D) ultrasound and a Virtual Reality system.

METHODS Within a population-based prospective cohort study, 3D ultrasound datasets of 25 first-trimester fetuses were collected by three sonographers. We used the V-scope application to perform Virtual Reality volume assessments of the fetal heart, lungs and kidneys. All measurements were performed by two independent researchers.

RESULTS Intraobserver analyses for volume measurements of the fetal heart, lungs and kidneys showed intraclass correlation coefficients ≥ 0.86 , mean differences $\leq 8.3\%$, and coefficients of variation $\leq 22.8\%$. Interobserver analyses showed sufficient agreement for right lung volume measurements, but consistent measurement differences between observers for left lung, heart and kidney volume measurements (p-values < 0.05).

CONCLUSION We observed sufficient intraobserver reproducibility, but overall suboptimal interobserver reproducibility for first-trimester fetal heart, lung and kidney volume measurements using an innovative Virtual Reality approach. In the current stage, these measurements might be promising for the use in research settings. The reproducibility of the measurements might be further improved by novel post-processing algorithms.

INTRODUCTION

Early fetal life is a crucial period for organ development¹. Previous studies have shown that suboptimal first-trimester development, measured by crown rump length in women with a known last menstrual period date, is associated with increased risks of adverse fetal and birth outcomes²⁻⁵. First-trimester fetal growth has also been associated with childhood cardiovascular and respiratory outcomes⁶⁻⁹. The mechanism underlying these associations might include structural adaptations in early organ development¹⁰. Detailed studies on first-trimester fetal organ development might improve understanding of mechanisms underlying fetal developmental adaptations that may lead to adverse cardiovascular and respiratory outcomes in later life. Thus far, studies on in-vivo first-trimester organ development are scarce. Three-dimensional (3D) transvaginal ultrasound allows detailed visualization of first-trimester anatomy. Virtual Reality approaches enable visualization of 3D ultrasounds as hologram, which offers opportunities for volumetric measurements of complex early-pregnancy fetal structures¹¹. Embryonic volume measurements using a region-growing segmentation algorithm in a Virtual Reality setting have previously shown to be feasible¹¹. These embryonic measurements seem related to fetal growth and birth outcomes^{12, 13}. Recently, we have reported that first-trimester fetal proportion volumetric measurements using a Virtual Reality approach are reproducible¹⁴. More detailed measurements of cardiovascular and respiratory tract related organs might be useful in research on fetal developmental adaptations and long term cardiovascular and respiratory consequences¹⁰.

We aimed to develop a novel method for fetal heart, lung and kidney volume measurements in the late first-trimester using a Virtual Reality approach. We assessed the intraobserver and interobserver reproducibility for volume measurements of fetal organs in 25 fetuses in the late-first-trimester.

METHODS

Study design

This study was embedded in the Generation R *Next* study, a population-based prospective cohort study from preconception onwards in Rotterdam, the Netherlands. Recruitment started in August 2017 and is still ongoing¹⁴. Pregnant women were invited to the research center for three appointments in the first-trimester of pregnancy, from 7 to 13 weeks of gestation, with an interval of approximately two weeks. During these 30-minute visits 3D ultrasound datasets were obtained to assess embryonic, early fetal and placental

development. Around 30 weeks of gestation, participants were invited back to the research centre for a follow-up ultrasound. All participating women gave written informed consent. The medical ethics committee of the Erasmus University Medical Center approved of this study (MEC-2016-589, December 2016). For the current analysis, we focused on 3D ultrasound datasets collected in the late first-trimester. From March to April 2019, we selected a random group of 25 participants who visited the research center at the Erasmus MC, in whom all the 3D ultrasound data according to the ultrasound study protocol were acquired.

Gestational age assessment

Gestational age was calculated from the first day of the last menstrual period in spontaneous pregnancies or from oocyte pick-up plus 14 days in IVF pregnancies. Gestational age was based on crown rump length if the last menstrual period was unknown or gestational age determined by crown rump length differed more than 7 days from the last menstrual period^{14, 15}.

Fetal ultrasound examination

All ultrasound scans were performed by three experienced ultra-sonographers using a Voluson E10 System (GE Healthcare, Zipf, Austria) with a 5-13 MHz transvaginal transducer (RIC6-12D). The ultrasound settings were predefined to collect high quality ultrasound data in a uniform manner (gain=0, line filter = low, persistence filter = 2, enhance = 2, dynamic contrast = 6, enhance = 2)¹⁴. We acquired 3D ultrasound dataset of the fetal trunk under a 40° volume angle while the fetus was not moving. Multiple 3D ultrasound datasets were collected during the examination. To allow proper imaging of the fetal heart and lungs, the 3D ultrasound datasets of the trunk were preferably acquired from the midsagittal plane while the fetus was facing towards the transducer. To allow proper imaging of the fetal kidneys, the 3D ultrasound datasets of the trunk were preferably acquired from the midsagittal plane while the fetus was facing away from the transducer. The 3D ultrasound datasets were stored in Cartesian volume files for offline analysis.

Fetal organ volume measurements

In the Barco I-Space, a CAVETM-like Virtual Reality system we used the V-scope volume rendering application for offline analysis of the 3D ultrasound datasets¹⁶. The 3D ultrasound datasets were first stored as a Cartesian volume files, and then converted to our own V-scope file format to allow offline analysis using the V-Scope application.

The V-Scope application enables accurate volumetric measurements due to detailed depth perception offered by Virtual Reality displays that create a hologram of the 3D ultrasound dataset^{17,18}. With a hand-held controller, the V-Scope slice option enables the operator to “slice through” the 3D ultrasound dataset in all preferred 3D planes. Within the current study, differences in grey scale values between the organ of interest and the surrounding tissues were used to visually identify the contours of the fetal heart, lungs and kidneys. We did not apply a smoothing module to produce a less pixelated image of the 3D ultrasound datasets, as this will reduce the already minimal differences in grey scale value of the various tissues. After identification of the organ contours, the operator manually selects voxels within the 3D ultrasound dataset using a brusher that is adjustable in size. This process results in a “volume segmentation” of the organ of interest. After manual segmentation, all volumes were post-processed using an algorithm that examines the greyscale of the voxels in a radius of 5 voxels of the segments border: if the voxels have a greyscale outside one standard deviation (SD) of the average greyscale value they were excluded, otherwise they were included. This automated post-processing algorithm was created to increase the accuracy of the delineation of the anatomical boundaries used for the segmentation. Finally, the organ volume segmentations were automatically calculated in mm³ and used for the final analyses. The organ volume measurements were performed using a detailed measurement protocol with instructions on the alignment of the fetus, the plane in which the volume segmentation should be performed, and the size of the brusher. For a detailed description of the measurement procedure, see **Figure 1** and **Supplementary document ‘Protocol for first trimester organ volume measurements using the V-Scope application’**. **Figure 2** shows a 360° 3D view of the segmented heart, lungs and kidneys of a fetus at 11 weeks and 6 days of gestation.

The volumetric measurements were performed in a blinded setting by two operators (C.W. and C.S.), who were experienced ultrasonographers with previous experience of performing VR volume measurements using the V-Scope application. The first operator (C.W.) assessed the overall quality of the datasets, whether movement artefacts or acoustic shadowing were present, and if the region of interest was complete. The dataset of the best quality was used for further offline analyses. Both operators performed the offline measurements twice, with an interval of at least one week to prevent recall bias.

Statistical analysis

We performed statistical analysis described by Bland and Altman^{19,20}. For the intraobserver analysis, the first measurement was compared with the second measurement for each observer. For the interobserver analysis, the mean of the two measurements of the first

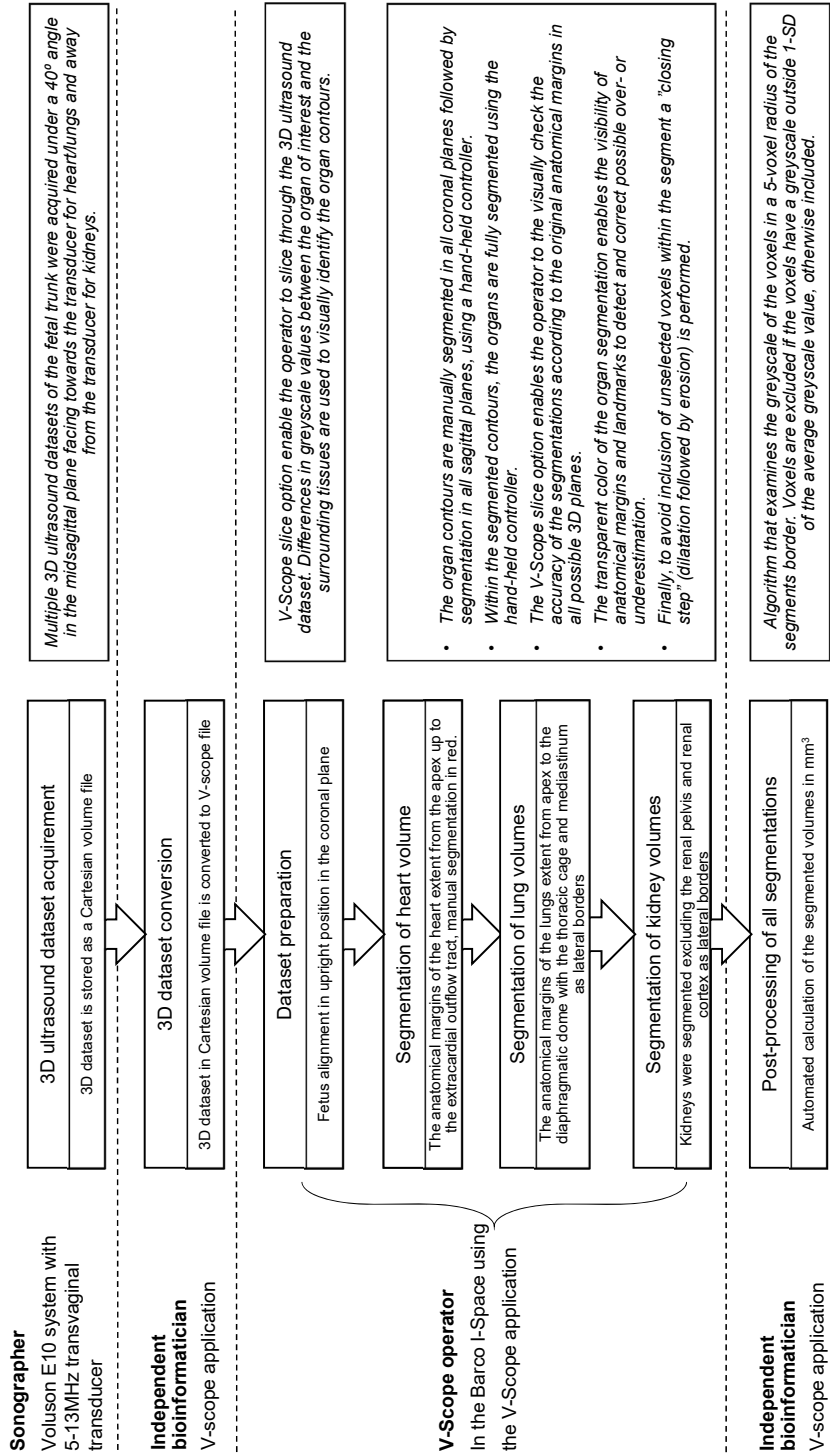


Figure 1. Protocol flowchart for segmentation of the organ volumes.

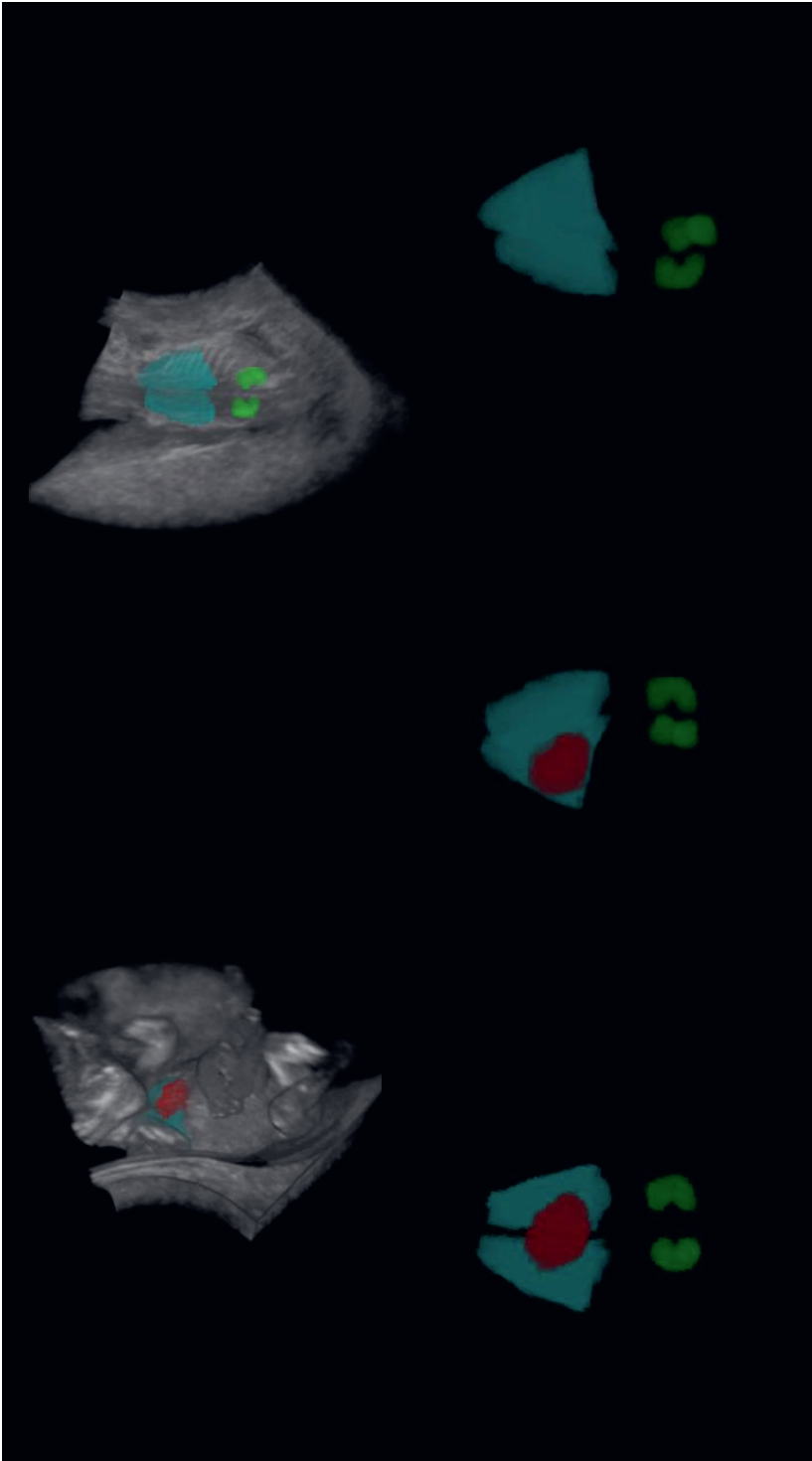


Figure 2. Three-dimensional view of the segmented heart (red), lungs (cyan) and kidneys (green) of a fetus at 11 weeks and 6 days of gestation in the BARCO I-Space obtained using the V-scope application. Upper row of images show the segmented organs within the 3D ultrasound dataset, left is anterior view and right is posterior view. Lower row of images show the segmented organs (supporting video file is provided as **supplementary video S1**).

observer was compared to the mean of the two measurements of second observer using similar calculations.

First, we plotted the measurements with the line of equality to give an initial sense of the degree of agreement²⁰. Second, intraclass correlation coefficients (ICC) with a 95% confidence interval and the coefficients of variation (CV) were calculated for each measurement to evaluate consensus within each observer and between observers²⁰. Third, intraobserver and interobserver variability was quantified calculating the mean difference in percentage measurement error with the 95% limits of agreement (mean difference (%) $\pm 1.96SD$) for all the fetal proportion volumetric measurements. Within the limits of agreement the measurements within and between observers can be assumed to be interchangeable¹⁵. Lastly, we plotted the mean differences in percentage measurement error with the 95% limits of agreement. These so called Bland and Altman plots were specifically provided to visualize that the agreement for the volumetric measurements does not depend on fetal size.

We consider the ICC, CV, mean difference and the limits of agreement as our main outcomes of interest. We decided that an ICC >90%, a CV <10%, a mean difference <10% and limits of agreement within $\pm 10\%$ were considered to be proof of good agreement²¹. Importantly, an acceptable mean difference and limits of agreement are not a statistical but a clinical and more subjective consideration¹⁵. To establish that the measurements are useable for future association studies, we decided that the limits of agreement should deviate a maximum of 10% from the mean difference, which indicates that 95% of all differences should be within the $\pm 10\%$ measurement error range¹⁹. Statistical analyses were performed using IBM SPSS, version 25.

RESULTS

Characteristics of the participants are shown in **Table 1**. The gestational age ranges between 10.7 weeks and 13.3 weeks. Descriptives of fetal organ volume measurements are shown in **Table 2**. In these 25 participants a total of 84 3D ultrasound datasets of the fetal trunk were available, on average 3.4 per participant. The number of datasets in which both observers were able to perform the fetal organ volume measurements were 22 out of 25 (88%) for heart and right lung, 21 out of 25 (84%) for left lung and 20 and 18 out of 25 (72-80%) for kidney volumes. **Figure 3** shows that the average fetal organ volumes increase with fetal crown rump length.

Table 1. Participant characteristics (n=25)

	Median (IQR) / n (%)
Maternal age (years)	32.0 (31, 36)
Maternal Body mass index (kg/m ²)	22.7 (20.8, 26.3)
Gestational age (weeks)	12.1 (11.4, 12.7)
Crown Rump Length (mm)	60.2 (50.0, 65.9)
Conception mode	
Spontaneously conceived (%)	23 (92)
In Vitro Fertilization (%)	1 (4)
Ovulation induction (%)	1 (4)

Table 2. Descriptives of fetal organ volume measurements for both observers (n=25)

Volumetric measurement	Observer	Measurement	Mean (minimum, maximum) mm ³
Heart	1	1	206 (39, 409)
		2	217 (58, 370)
	2	1	156 (29, 305)
		2	158 (39, 317)
Right lung	1	1	373 (92, 811)
		2	372 (119, 735)
	2	1	361 (103, 90)
		2	354 (90, 672)
Left lung	1	1	269 (74, 543)
		2	260 (67, 514)
	2	1	288 (99, 505)
		2	279 (78, 512)
Right kidney	1	1	102 (28, 245)
		2	102 (31, 227)
	2	1	65 (6, 138)
		2	65 (10, 120)
Left kidney	1	1	89 (20, 216)
		2	90 (21, 214)
	2	1	54 (10, 115)
		2	57 (13, 121)

CI, confidence interval; ICC, intraclass correlation coefficient; CV, coefficient of variation; LLOA, lower limit of agreement; ULOA, upper limit of agreement.

Reproducibility analyses

For the intraobserver analyses, the heart, lung, and kidney volumes measurements lie close to and evenly scattered around the line of equality for both observers, suggesting acceptable intraobserver differences (**Supplementary Figures S1 and S2**). **Table 3** shows the ICC, CV and mean difference with corresponding limits of agreement for the intraobserver analyses. For volumetric measurements of the heart, intraobserver ICCs were 0.86 and 0.90, CVs were 22.8% and 21.8% for observer 1 and 2, respectively. Intraobserver ICCs for all other measurement were above 0.95 and CVs below 19.6%. The

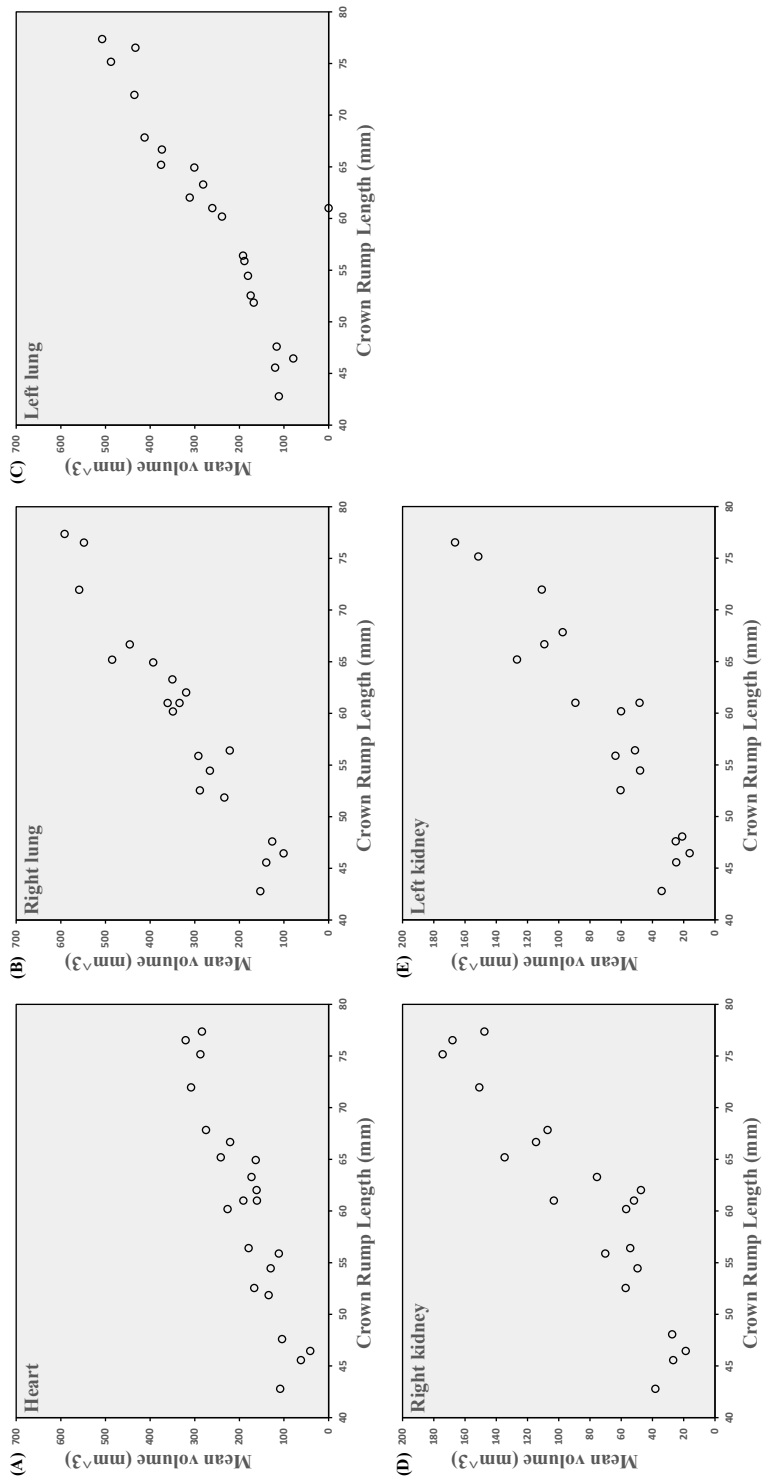


Figure 3. General characteristics of the study populations: volume measurements of (A) heart, (B) right lung, (C) left lung, (D) right kidney and (E) left kidney of the study subjects in relation to crown rump length. Plotted measurements are mean values of measurements of observer 1 and observer 2.

Table 3. Intraobserver agreement of fetal organ volume measurements for both observers (n=25)

Volumetric measurement	Observer	ICC (95% CI)	CV	Mean difference (LLOA, ULOA)	Paired sample t-test
					Intra-observer
			%	%	p-value
Heart	1	0.86 (0.71, 0.94)	22.8	-8.3 (-52.3, 35.8)	0.29
	2	0.90 (0.77, 0.96)	21.8	-0.1 (-41.6, 41.5)	0.77
Right lung	1	0.96 (0.91, 0.98)	14.4	-3.0 (-34.3, 28.4)	0.94
	2	0.98 (0.96, 0.99)	9.38	1.7 (-12.9, 16.3)	0.36
Left lung	1	0.96 (0.90, 0.98)	15.0	3.9 (-28.7, 36.4)	0.28
	2	0.98 (0.95, 0.99)	9.16	4.3 (-15.6, 24.3)	0.13
Right kidney	1	0.95 (0.88, 0.98)	19.6	-4.8 (-39.8, 30.3)	0.96
	2	0.97 (0.93, 0.99)	14.9	-2.0 (-46.0, 41.9)	0.90
Left kidney	1	0.96 (0.90, 0.99)	18.0	-1.6 (-38.3, 35.2)	0.83
	2	0.97 (0.92, 0.99)	14.8	-3.4 (-34.2, 27.3)	0.17

CI, confidence interval; ICC, intraclass correlation coefficient; CV, coefficient of variation; LLOA, lower limit of agreement; ULOA, upper limit of agreement.

p-values obtained from the paired sample t-tests for all measurements of both observers were >0.05 , suggesting no consistent intraobserver measurement differences. **Figure 4** shows that the agreement does not depend on organ size for all volumetric measurements for both observers. The mean differences for all measurements were below 8.3%. Limits of agreement ranged from -52.3% to 41.9%.

For the interobserver analyses, the measurements of the lung volumes lie in close proximity to the line of equality, suggesting acceptable interobserver differences for these volumetric measurements (**Supplementary Figure S3**). The measurements of the heart and kidney volumes all lie below the line of equality, suggesting structural interobserver differences for these volumetric measurements (**Supplementary Figure S3**). **Table 4** shows the ICC, CV and mean difference with corresponding limits of agreement for the interobserver analyses. Interobserver ICCs were 0.98 and 0.97, CVs were 10.6 and 10.3 for volumetric measurements of the right and left lung volumes, respectively. Interobserver ICCs for heart, left kidney and right kidney volumes ranged from 0.68 to 0.71. The p-values obtained from the paired sample t-tests for heart, left lung, right and left kidney were <0.05 , indicating consistent interobserver measurement differences for these volumetric measurements. **Figure 5** shows that the mean differences for the right and left lung volumes were below 10.0%, with limits of agreement ranging from -33.3% to 28.0%. The mean differences for heart, right and left kidney volume ranged from 30.3 to 47.4, with limits of agreement ranging from -33.3% to 71.6%.

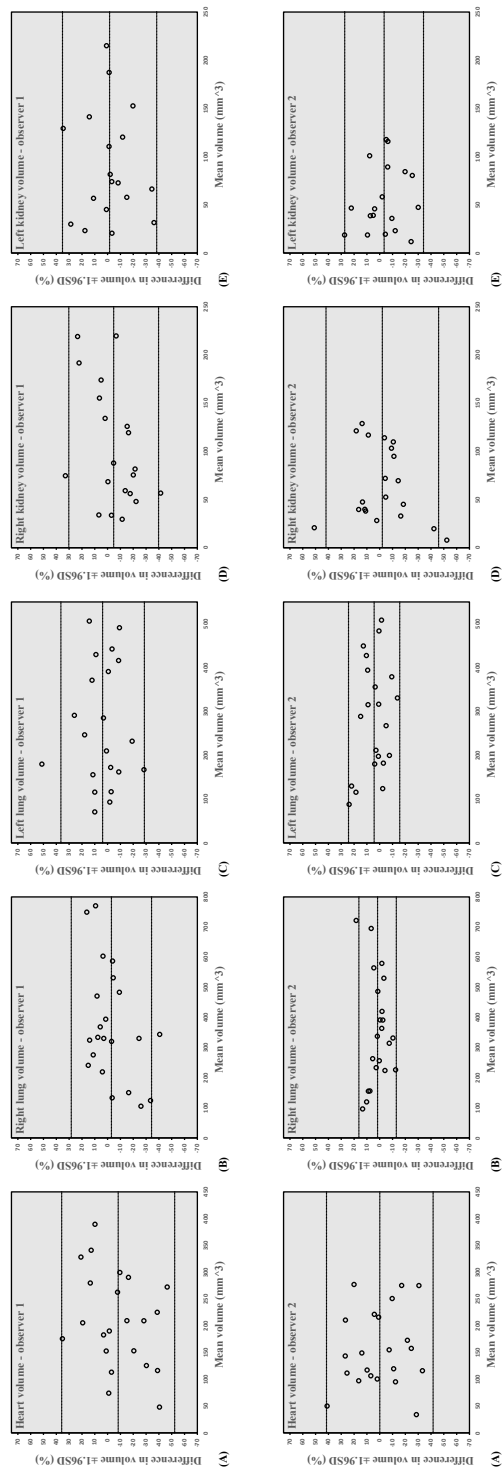


Figure 4. Bland and Altman plots of intraobserver agreement with corresponding limits of agreement in percentage of the mean ± 1.96 SD for (1) Observer 1 and (2) Observer 2 for volume measurements of: (A) Heart, (B) Right lung, (C) Left lung, (D) Right kidney, (E) Left kidney. First row intraobserver agreement for observer 1, second row intraobserver agreement for observer 2. Dotted grey lines indicate upper limit of agreement, mean difference in percentage and lower limit of agreement.

Table 4. Interobserver agreement of fetal organ volume measurements (n=25)

Volumetric measurement	n (%) [†]	ICC (95% CI)	CV	Mean difference (LLOA, ULOA)	Paired sample t-test
			%	%	p-value
Heart	22 (88)	0.71 (-0.03, 0.91)	22.7	30.3 (-5.6, 66.2)	<0.01
Right lung	22 (88)	0.98 (0.94, 0.99)	10.6	3.8 (-20.4, 28.0)	0.08
Left lung	21 (84)	0.97 (0.88, 0.99)	10.3	-10.0 (-33.3, 12.9)	0.01
Right kidney	20 (80)	0.69 (-0.06, 0.90)	32.8	47.4 (2.0, 92.7)	<0.01
Left kidney	18 (72)	0.68 (-0.06, 0.91)	34.5	47.0 (22.5, 71.6)	<0.01

CI, confidence interval; ICC, intraclass correlation coefficient; CV, coefficient of variation; LLOA, lower limit of agreement; ULOA, upper limit of agreement. [†]Number and percentage of datasets in which both observers could perform the measurements.

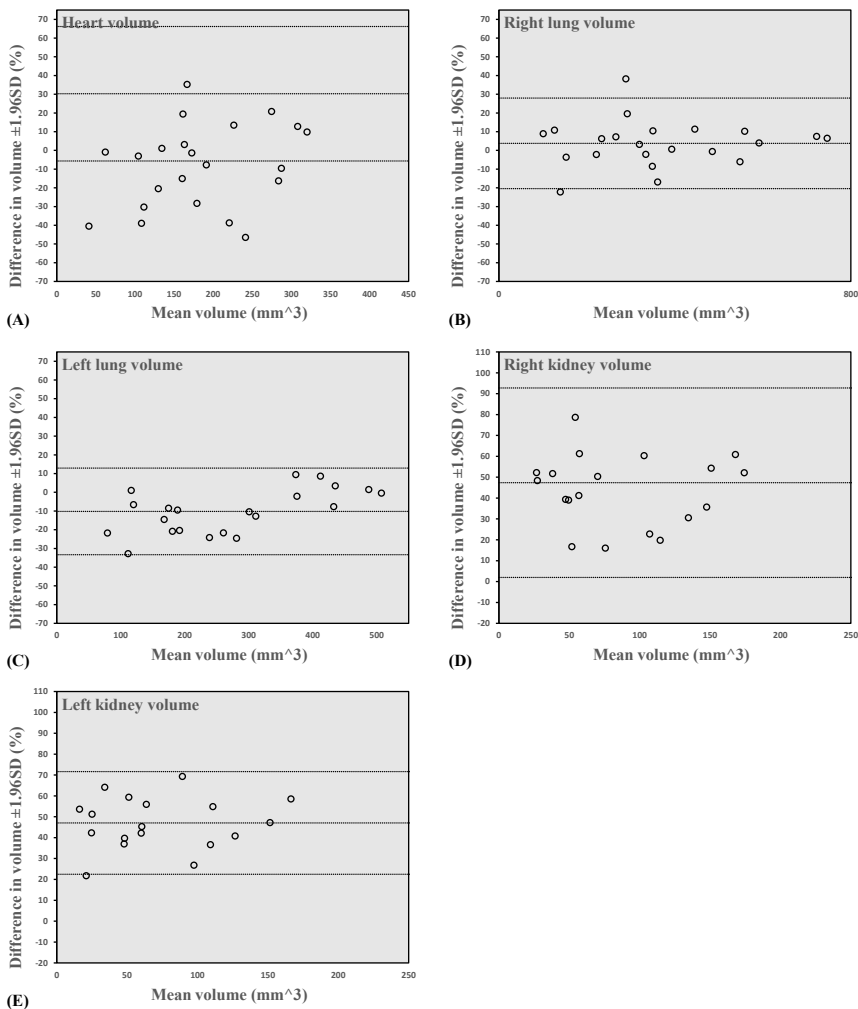


Figure 5. Bland and Altman plots of interobserver agreement with corresponding limits of agreement in percentage of the mean ± 1.96 SD for volume measurements of: (A) Heart, (B) Right lung, (C) Left lung, (D) Right kidney, (E) Left kidney. Dotted grey lines indicate upper limit of agreement, mean difference in percentage and lower limit of agreement.

DISCUSSION

Main findings

We evaluated a novel Virtual Reality approach to measure first-trimester fetal heart, lung and kidney volumes using 3D ultrasound datasets that were acquired in the late first-trimester. We observed sufficient intraobserver reproducibility for volumetric measurements of the fetal heart, lungs and kidneys. We also observed sufficient interobserver reproducibility for volume measurements of the fetal right lung, but suboptimal interobserver reproducibility for volume measurements of the fetal heart, left lung and both kidneys.

Interpretation of main findings

Quantitative estimation of first-trimester development is currently performed by traditional ultrasound length measures, most importantly using the crown rump length¹⁵. Volumetric measurements might be more sensitive parameters for first-trimester growth and development, since the increase in first-trimester fetal volume is cubic with a linear increase in fetal length¹¹. Over recent years, volume measurement techniques using 3D ultrasound datasets in combination with Virtual Reality have been introduced and seem to be feasible for several first-trimester volume measurements¹². This Virtual Reality technique offers depth perception in high-resolution and therefore improves the visualization of complex anatomical structures, when compared to regular two-dimensional displays¹⁷.

We have recently reported that first-trimester fetal proportion volumetric measurements are feasible and reproducible¹⁴. More detailed measurements on fetal organ systems might be useful for research in the field of Developmental Origins of Health and Disease, as early fetal developmental adaptations in response to an adverse intrauterine environment might directly affect long term health and disease⁸. We developed a novel approach for volume measurements of the first-trimester fetal heart, lungs and kidneys using 3D ultrasound datasets of the fetal trunk acquired in the late first-trimester of pregnancy. Our Virtual Reality approach shows promising results for one observer as indicated by high ICCs, CVs around 10% and mean differences <10% for all measurements, although the limits of agreement remain relatively broad. Despite good intraobserver agreement, our method provided inconsistent results in a setting with two observers. Interobserver analyses showed sufficient agreement for fetal right lung volume measurements but structural measurement differences for the heart, left lung and both kidney volume measurements. The interobserver agreement for the left lung volumetric measurement

appeared sufficient as indicated by an ICC of 0.97, and CV and mean difference around 10%, but as the interobserver differences seem consistent we do not consider the agreement for the volume measurements of the left lung as sufficient. The percentage of cases in which both observers could perform the measurements ranged from 72 to 88%, which can be considered a relatively low success percentage. We consider this success percentage sufficiently high for research purposes in large observational cohort studies, but currently insufficient for the use of these measurements in clinical practice.

The suboptimal interobserver reproducibility, most prominently for kidney volumes, can be explained by several factors. First, the measured absolute volumes are extremely small and therefore only allow for minor measurement differences. Second, a large part of this measurement technique involves manual segmentation as it is not possible to automate the recognition of these small but complex anatomical structures. Unfortunately, ultrasound data tends to be noisy which results in a lack of clear demarcation of the fetal organs, and therefore results in larger interobserver differences. For accurate segmentation of the lungs, the whole diaphragm, the thoracic cage and the heart needs to be clearly visible. This may explain the sufficient interobserver agreement for the fetal right lung, with a relatively large volume compared to the other organs and a clear demarcation at the borders with the diaphragm and ribcage. Although the kidneys are easily detectable in the late first-trimester, the demarcation from the surrounding intestines and liver remains difficult to distinguish in our ultrasound data. Similar difficulties are present regarding segmentation of the fetal heart during the late first-trimester²². These difficulties highlight the importance for acquisition of high-quality 3D ultrasound datasets. For optimal 3D ultrasound acquisition, we used a state-of-the-art ultrasound machine with a high-frequent transvaginal transducer. The acquisition was performed when the anatomical structures of interest were clearly visible without fetal movements. To our knowledge, only one previous study group developed a method to measure volumes of fetal heart and lungs in fetuses at 12 to 32 weeks gestation using the VOCAL method²³. Although the researchers indicate that the reproducibility for these measurement at 12 to 13 weeks gestation was sufficient, they only provide absolute measurement differences and limits of agreement in mL, and do not provide ICC and CV values. This makes it hard to establish if these measurements with VOCAL were truly reproducible in the first-trimester, and makes comparison to our study results difficult²³. We are not aware of any other studies focused on measurements of the kidney volume in the first-trimester.

In comparison to previous studies using a Virtual Reality approach for volumetric measurements in early-pregnancy, we found lower interobserver reproducibility^{11, 14, 18, 24}. This is most likely due to a larger role for automated segmentation in these previous

studies, compared to the current study. To perform the measurements described in this study, differences in grey scale values between the organ and the surrounding tissues were used to identify the organs, followed by manual segmentation of the organ volume. The operators in this study were experienced sonographers that had previous experience with the V-scope application, but they still considered this method difficult and time-consuming. The manual segmentation was followed by an automated post-processing method that takes into account greyscale differences of voxels at the borders of the segmentation. In the future, we hope to further improve automated post-processing steps to improve the utility of this method, and reduce intra- and interobserver measurement differences. Our primary step is the implementation of adaptive active contour tracking using a snake algorithm in the V-scope application²⁵. The snake algorithm starts with a rough manual delineation of the object to be segmented, and uses an automatic energy minimizing approach where this segmentation is pulled towards object contours, but at the same time resists deformation. This method is already used for image analysis purposes in other biomedical fields, and shows high segmentation accuracy²⁶⁻²⁹.

The method presented in this paper, does not seem suitable for clinical purposes in the current stage but might be adequate for the use in large-scale population based research settings such as the Generation R *Next* Study. Within this study, it is not specifically our aim to provide normative values for fetal organ growth but we aim to provide insights in the influence of periconceptional factors on early organ development. If the organ volume measurements are conducted by a small group of operators, operator adjusted *Z* scores of organ volumes can be calculated to reduce the problems with interobserver differences within the statistical analyses. As the potential measurement error would be non-differential, the use of these operator adjusted *Z* scores would not likely lead to biased estimates in association studies. Within the Generation R *Next* Study, we will assess whether early fetal organ size is associated with parental lifestyle and health during the preconception phase¹⁴. With the aim to do a long-term follow-up of the children that participate in the Generation R *Next* Study, these novel measurements might also be useful to investigate whether early fetal alterations in organ size influence cardiovascular and respiratory health during childhood.

Conclusion

We observed sufficient intraobserver reproducibility for volume measurements of the fetal heart, lungs and kidneys using a novel Virtual Reality approach. The interobserver reproducibility seems suboptimal. In the current stage, these measurements might be promising for the use in research settings, but not for clinical purposes. The reproducibility of the measurements might be further improved by novel post-processing algorithms.

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SUPPLEMENTARY MATERIAL

Protocol for first-trimester fetal organ volume measurements using the V-Scope application

The three-dimensional dataset containing the trunk of the fetus is visualized in the BARCO I-Space using the V-scope application^{16, 17}. The first observer (C.W.) assesses the overall quality of the 3D ultrasound datasets, whether movement artefacts or acoustic shadowing are present, and if the region of interest is complete. The dataset of the best quality is used for further offline analyses. The volume measurements of the heart and lungs should be performed within the same dataset. The volume measurements of the kidneys can be performed within a different 3D ultrasound dataset, if visualization of the kidneys is better within this dataset. The fetus is aligned in an upright position facing towards the operator (coronal plane) before every measurement. The volumetric measurements are executed in a consecutive order: 1) heart, 2) right lung, 3) left lung, 4) right kidney, and 5) left kidney. Measurement could only be performed if the required anatomical margins can be visualized. Voxels can be manually selected and deleted using a brusher that is adjustable in size. A small brush radius is used for all segmentations to allow detailed tracing of the organ contour. The V-Scope slice option enables the observer to check if the voxels are selected according to the original anatomical margins. The transparent color of the segmentation enables the visibility of anatomical landmarks during the segmentation process. This enables detection of segmentation errors that would indicate under- or overestimation of the volumetric measurement. If errors are detected by the operator, corrections can be made accordingly. All volume measurements are post-processed to increase the accuracy of the delineation of the anatomical boundaries used for the segmentations.

Step 1. Volume of the fetal heart

Measurement: Volume of the fetal heart.

Segmentation colour: Red (opaque/transparent).

Anatomical margins: Cardial apex up to the extracardial outflow tract.

Conditions: If the anatomical margins of the fetal heart are not clearly visible due to extensive acoustic shadowing or low-quality data, the volume measurement cannot be performed.

Process specification:

1. **Manual segmentation:**
 - The 'red opaque' segmentation colour is selected.
 - The contour of the heart was manually drawn in the coronal planes, using a brush radius size 3.
 - The contour of the heart was manually drawn in the sagittal planes, using a brush radius size 3.
 - The heart volume is filled within the earlier drawn margins.
 - During the above described process the V-scope 'zoom option' should be used for detailed visualisation of anatomical margins.
 - The 'red transparent' segmentation colour is selected to enables the visibility of anatomical landmarks during the following steps.
 - The V-Scope 'slice option' is used to check if the voxels are selected according to the original anatomical margins.
 - Segmentation corrections can be made from different planes using the spherical brusher in combination with the 'deselect voxels' or 'select voxels' options in V-scope; or the segment can be reloaded and the protocol step can be repeated.
2. **Automatic segmentation:** The V-scope 'dilate option' and 'erode option' are used consecutively, to avoid inclusion of unselected voxels within the segment. Only after this automated processing step, the right lung volume can be segmented.
3. The obtained segmentation is saved.

Step 2. Volume of the fetal lungs

Measurements: Volumes of the right and left fetal lungs.

Segmentation colours: Cyan (opaque/transparent) for right lung, yellow (opaque/transparent) for left lung.

Anatomical margins: Apex to the diaphragmatic dome, with the thoracic cage and mediastinum as lateral borders.

Conditions: If the anatomical margins of the fetal lungs are not clearly visible due to extensive acoustic shadowing or low-quality data, the volume measurement cannot be performed.

Process specification:

1. **Manual segmentation:**

- The 'cyan opaque' segmentation colour is selected.
- The contour of the right lung was manually drawn in the coronal planes, using a brush radius size 3.
- The contour of the right lung was manually drawn in the sagittal planes, using a brush radius size 3.
- The heart volume is filled within the earlier drawn margins.
- During the above described process the V-scope 'zoom option' should be used for detailed visualisation of anatomical margins.
- The 'cyan transparent' segmentation colour is selected to enable the visibility of anatomical margins during the following steps.
- The V-Scope 'slice option' is used to check if the voxels are selected according to the original anatomical margins.
- Segmentation corrections can be made from different planes using the spherical brusher in combination with the 'deselect voxels' or 'select voxels' options in V-scope; or the segment can be reloaded and the protocol step can be repeated.

2. **Automatic segmentation:** The V-scope 'dilate option' and 'erode option' are used consecutively, to avoid inclusion of unselected voxels within the segment. Only after this automated processing step, the right lung volume can be segmented.

3. The obtained segmentation is saved.

4. The above described process is followed for the segmentation of the left lung using the 'yellow opaque' and the 'yellow transparent' segmentation colours.

Step 3. Volume of the fetal kidneys

Measurements: Volumes of the right and left fetal kidneys.

Segmentation colours: Magenta transparent for right kidney, green transparent for left kidney.

Anatomical margins: The margins of the renal capsule excluding the renal pelvis.

Conditions: If the anatomical margins of the fetal kidneys are not clearly visible due to extensive acoustic shadowing or low-quality data, the volume measurement cannot be performed.

Process specification:

1. **Manual segmentation:**

- The 'magenta transparent' segmentation colour is selected to enable the visibility of anatomical landmarks during the following steps.
- The contour of the right kidney was manually drawn in the coronal planes, using a brush radius size 3.
- The contour of the right kidney was manually drawn in the sagittal planes, using a brush radius size 3.
- The right kidney volume is filled within the earlier drawn margins.
- During the above described process the V-scope 'zoom option' should be used for detailed visualisation of anatomical margins.

- The V-Scope 'slice option' is used to check if the voxels are selected according to the original anatomical margins.
 - Segmentation corrections can be made from different planes using the spherical brusher in combination with the 'deselect voxels' or 'select voxels' options in V-scope; or the segment can be reloaded and the protocol step can be repeated.
2. **Automatic segmentation:** The V-scope 'dilate option' and 'erode option' are used consecutively, to avoid inclusion of unselected voxels within the segment. Only after this automated processing step, the right lung volume can be segmented.
 3. The obtained segmentation is saved.
 4. The above described process is followed for the segmentation of the left kidney using the 'green transparent' segmentation colour.

Step 4. Post-processing procedure

All segmentations are then post-processed using an optimization algorithm that examines the greyscale of the voxels in a radius of 5 voxels of the segments border: if the voxels have a greyscale outside one standard deviation (SD) of the average greyscale value they were excluded, otherwise they are included. The SD of the 26-connected neighborhood of the included border-voxels has to be equal or smaller than the average SD of all the voxels within the segment. This automated post-processing step increases the accuracy of the delineation of the anatomical boundaries used for the segmentation. The segmented volumes were automatically calculated in mm³ and used as final organ volumes.

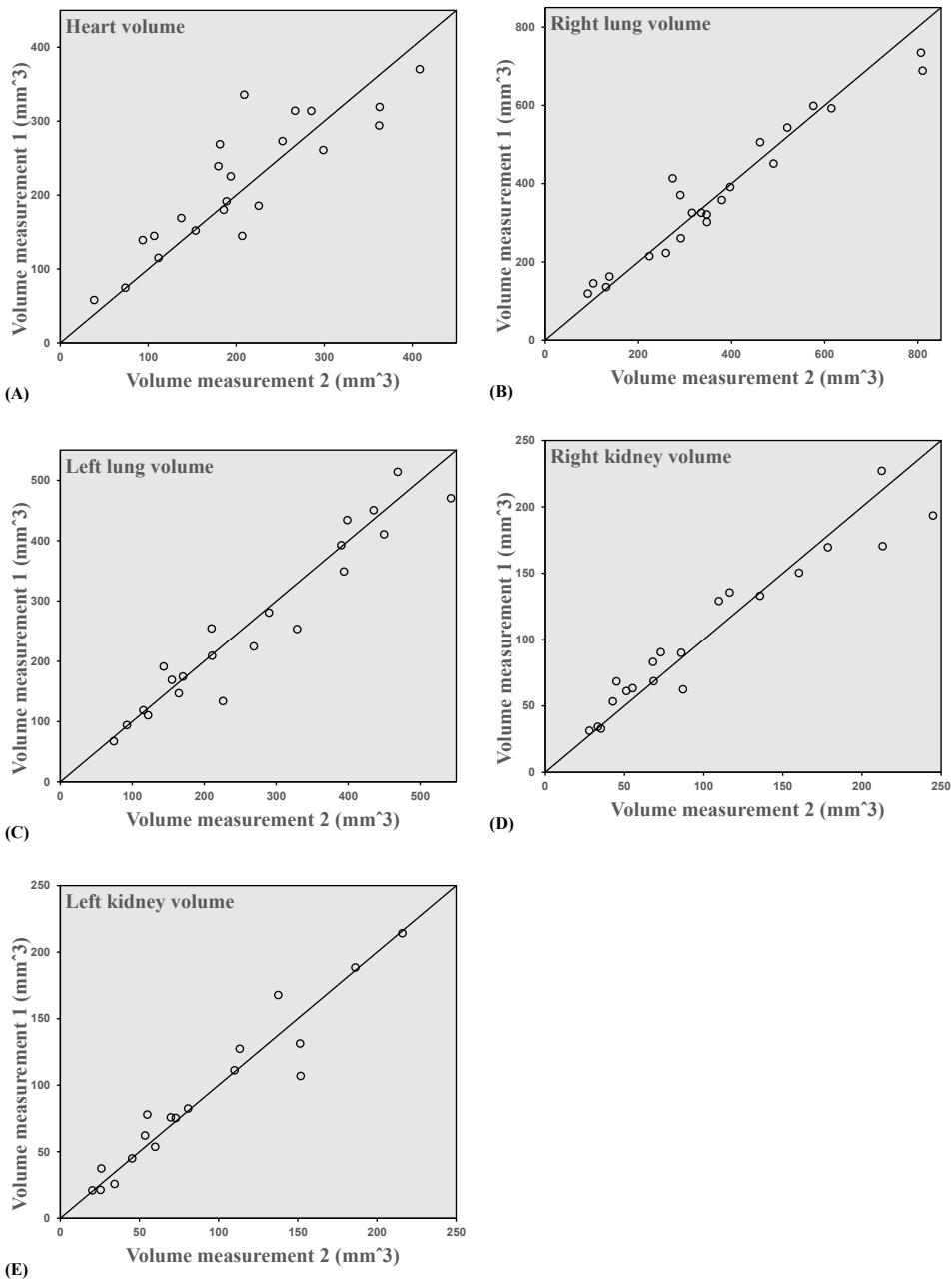


Figure S1. Measurements of the observer 1 plotted with line of equality for volumetric measurement of: (1A) Total fetus; (1B) Extremities; (1C) Head-trunk; (1D) Head; (1E) Trunk; (1F) Thorax; (1G) Abdomen.

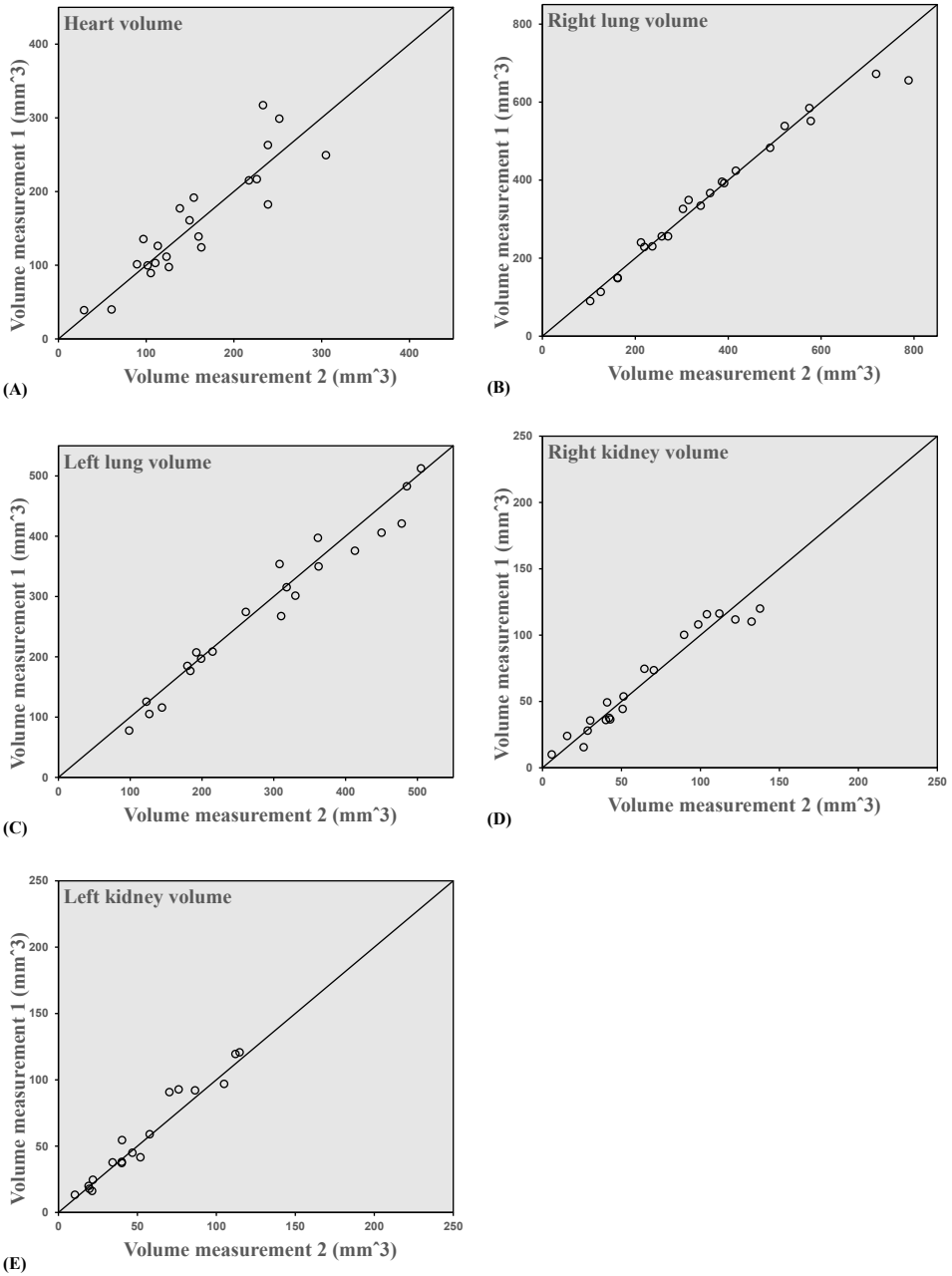


Figure S2. Measurements of observer 2 plotted with line of equality for volume measurement of: (A) Heart; (B) Right lung; (C) Left lung; (D) Right kidney; (E) Left kidney.

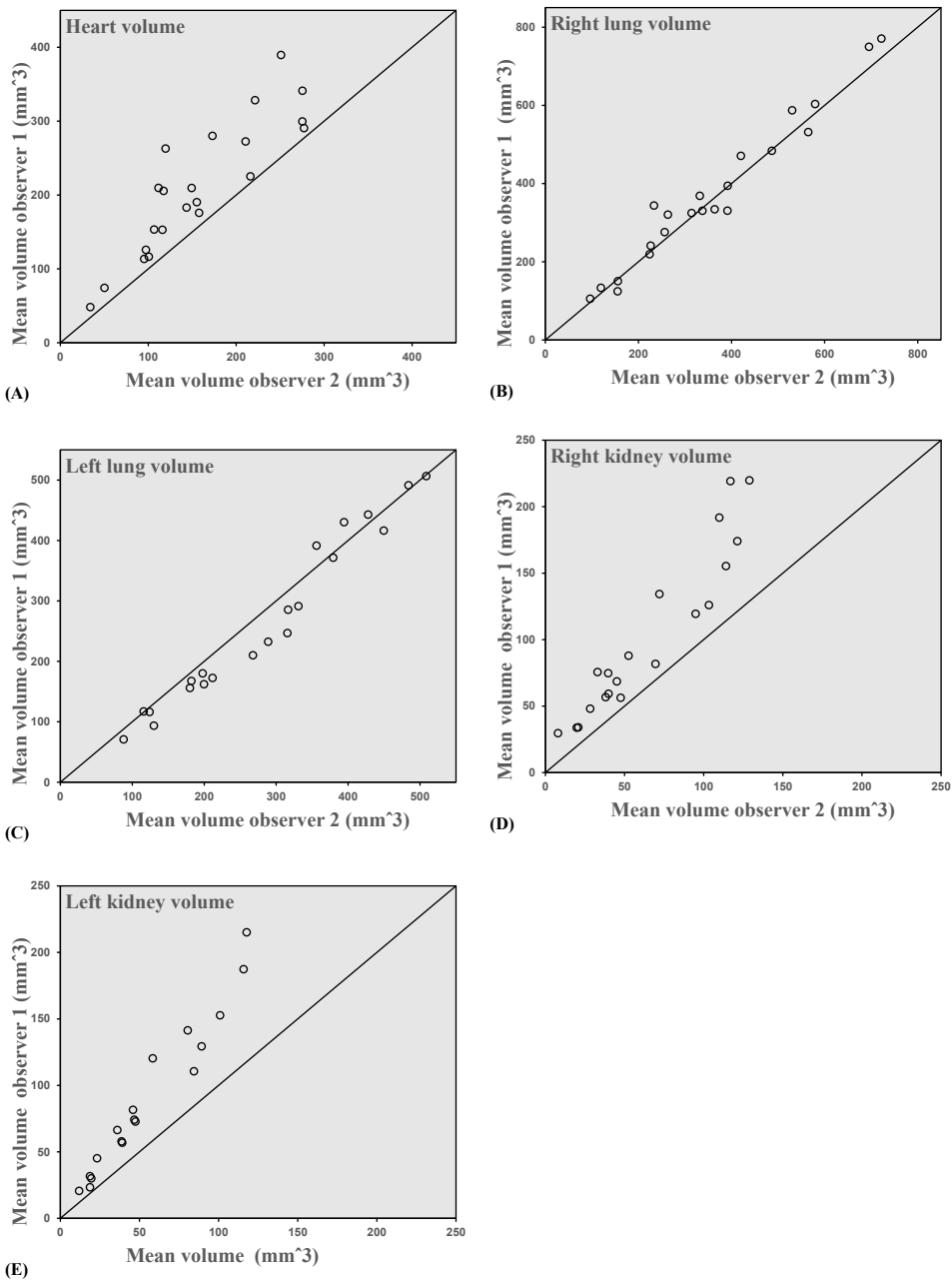
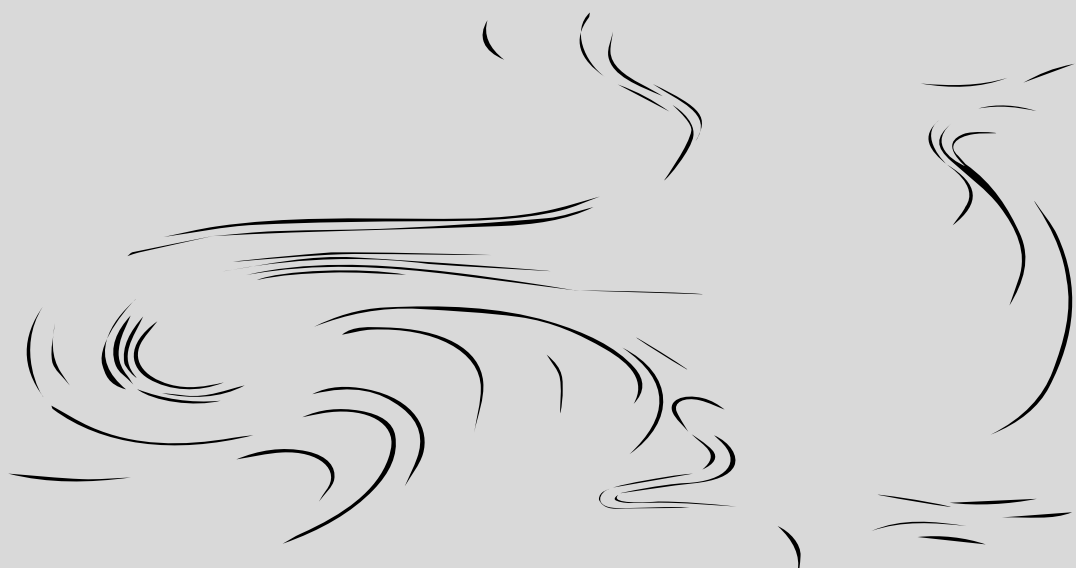


Figure S3. Measurements of observer 1 plotted against measurements of observer 2 with line of equality for volume measurement of: (A) Heart; (B) Right lung; (C) Left lung; (D) Right kidney; (E) Left kidney.

CHAPTER



GENERAL DISCUSSION



INTRODUCTION

Cardiovascular diseases are the leading cause of morbidity and mortality worldwide¹. Research on preventive strategies primarily focus on traditional cardiovascular risk factors that are present during late adulthood, but pregnancy-related and early-life risk factors that influence cardiovascular health in later life are often overlooked.

Maternal cardiovascular health status influences the ability to adequately adapt to the hemodynamic and metabolic changes that occur during pregnancy². Impaired maternal cardiovascular health may result in suboptimal placental vascular development, elevated blood pressure levels and ultimately the development of gestational hypertensive disorders as well as other adverse pregnancy outcomes². Maternal diet prior to and during early-pregnancy has been recognized to improve maternal cardiovascular health, and might also facilitate adequate hemodynamic responses to pregnancy that lead to a lower risk of gestational hypertensive disorders³.

Accumulating evidence has shown that women who suffered from any gestational hypertensive disorder are at increased risk of developing cardiovascular disease far beyond pregnancy^{4,5}. Likewise, the offspring of pregnancies affected by gestational hypertensive disorders may suffer long-term cardiovascular health consequences. The Developmental Origins of Health and Disease (DOHaD) hypothesis proposes that adverse exposures during different stages of fetal and early postnatal development initiate developmental adaptations^{6,7}. This may lead to permanent alterations in the structure and function of the cardiovascular system, which could predispose them to poorer cardiovascular health on the long-term^{6,7}. As the first trimester of pregnancy is critical for the proliferation and differentiation of cells into the fetal organ systems, the fetus might be most vulnerable for an adverse environment during early-pregnancy. To gain further insights in potential fetal developmental adaptation mechanisms, novel parameters of first-trimester fetal development are needed⁶.

The general aim of this thesis was to assess modifiable dietary factors that might influence maternal cardiovascular health during pregnancy, the influence of gestational hypertensive disorders and pre- and postnatal growth on offspring cardiovascular health, and to develop novel parameters of first-trimester fetal development. This chapter provides a general discussion in which we present the main findings, methodological considerations, and clinical implications of the studies described in this thesis. Furthermore, we will give suggestions for future research.

INTERPRETATION OF MAIN FINDINGS

Maternal diet in early-pregnancy and pregnancy outcomes

Accumulating evidence indicates that a healthy diet from preconception onwards is of great importance for maternal and offspring health⁸. The Dutch Health Council recently published guidelines with dietary recommendations for pregnant women for the very first time⁹. The Dutch Health Council aimed to translate scientific evidence into uniform dietary guidelines for health professionals, to provide women with clear dietary recommendations before and during their pregnancy⁹. However, evidence to provide specific dietary recommendations to lower the risk of gestational hypertensive disorders remains limited. Diet is defined as the sum of food components, macro- and micronutrients consumed. Within a diet these food components and nutrients have interactive and synergistic effects, therefore it is important to consider diet as a dietary pattern to comprehend complex diet-disease relationships^{10, 11}. However, pathophysiological mechanisms involved in the development of gestational hypertensive disorders can also be modified by specific properties of food components or nutrients¹². Identifying specific dietary patterns, food components or nutrients that reduce the risk of gestational hypertensive disorders, might improve future preventive strategies that can be translated into public health recommendations.

The Dietary Approaches to Stop Hypertension (DASH) is a dietary pattern high in fruits, vegetables, total grains, nuts, seeds, legumes and non-full-fat dairy products, and low in animal protein, sugar and sodium¹³. This dietary pattern is known for its blood pressure lowering properties in non-pregnant populations¹⁴. These results have been reproduced in numerous other intervention and observational studies that suggest beneficial effects on numerous cardiovascular risk factors and long-term cardiovascular outcomes¹⁵⁻¹⁹. The DASH diet is accordingly recommended by the American Heart Association to manage blood pressure, improve lipid profile and reduce the risks of heart attack and stroke²⁰. We hypothesized that maternal adherence to the DASH diet during pregnancy may also reduce the risk of gestational hypertensive disorders through its potential positive effects on blood pressure and vascular function. We observed that a higher DASH dietary score was associated with a lower diastolic blood pressure in mid-pregnancy and lower fetoplacental vascular resistance within a relatively low-risk population of Dutch women. We found no associations with systolic blood pressure and uteroplacental vascular resistance. Largely in line with these findings, an observational study in Ireland among 511 women with a large-for-gestational-age infant in their previous pregnancy, showed that higher maternal adherence to the DASH diet in their

second pregnancy was associated with a lower diastolic blood pressure and mean arterial pressure in early and late-pregnancy²¹. The observed associations may be explained by improved endothelial function, reduction of oxidative stress and potential positive effects on the renin-angiotensin-aldosterone system via sodium reduction^{17, 22, 23}. Through these mechanisms, the DASH diet may positively affect physiological hemodynamic adaptations in pregnancy, which could explain the strongest effect on mid-pregnancy diastolic blood pressure when the physiological diastolic blood pressure dip in pregnancy occurs^{3, 24}. No consistent associations with the risk of gestational hypertensive disorders were present, but we did observe a tendency for an association of a higher maternal DASH score with a lower risk of preeclampsia. A recent study among 11,535 women in the Nurses' Health Study II observed that a higher prepregnancy adherence to the DASH diet was associated with a lower risk of preeclampsia, while other studies that assessed diet later in pregnancy found weaker effects or no associations at all^{21, 24-26}. Partly in line with our findings, this might indicate that the periconceptional phase is a critical window for adherence to the DASH dietary pattern, possibly also through beneficial influence on placental development which seems related to preeclampsia. Our findings suggest that in a low-risk population adherence to the DASH dietary pattern during early-pregnancy might have small beneficial effects on maternal gestational hemodynamic adaptations. These findings are especially important from an etiological perspective and on a population level. Recommending the DASH dietary pattern from early-pregnancy onwards may be a future target for preventive strategies to improve gestational hemodynamic adaptations, and maternal cardiovascular health in general. Further studies are needed to assess whether maternal adherence to the DASH diet has more pronounced effects if women already adhere to the dietary pattern from the preconceptional phase onwards, and in higher-risk populations.

Besides dietary patterns, assessing diet on the level of food components using the dietary glycemic index and load has gained substantial attention over the recent years²⁷. The glycemic index and load are dietary measures to qualify carbohydrate intake, and provide information on the postprandial glycemic response to carbohydrate containing food products^{28, 29}. A low-glycemic index diet can be achieved by consuming carbohydrate containing food products that are less likely to increase blood sugar levels referred to as low-glycemic index products, while avoiding products with a high-glycemic index. For a low-glycemic load diet the daily quantity of carbohydrates is additionally taken into account. Among non-pregnant populations lowering dietary glycemic index and load has shown to have beneficial effects on traditional cardiovascular risk factors and to decrease all-cause cardiovascular mortality^{30, 31}. During pregnancy a low-glycemic index diet is suggested to have beneficial effects on glucose metabolism, lipid profile, gestational

weight gain and the risk of delivering a large-for-gestational-age-infant, especially among women at high-risk of glucose impairment³²⁻⁴⁰. While higher glucose levels are associated with a higher risk of gestational hypertensive disorders through oxidative stress and vascular inflammation, beneficial effects of lowering dietary glycemic index and load on the risk of gestational hypertensive disorders have been less studied⁴¹. To our knowledge, we are the first to examine the associations of dietary glycemic index and load with blood pressure and placental vascular resistance during pregnancy. A meta-analysis of randomized controlled trials among 1,097 healthy non-pregnant individuals indicated that a lower glycemic index or load was associated with a lower systolic and diastolic blood pressure³⁰. However, within our low-risk pregnant population we observed no consistent associations of early-pregnancy dietary glycemic index and load with gestational blood pressure and placental vascular resistance throughout pregnancy. We only observed that a higher dietary glycemic load was associated with a higher early-pregnancy diastolic blood pressure after adjustment for socio-demographic, lifestyle and other dietary factors, but the effect estimate was only small. The observed differences between this meta-analysis and our study may be explained by the overrepresentation of participants at high-risk of impaired glucose metabolism due to adiposity, and a greater magnitude of change in dietary glycemic index and load in the included intervention trials. As many of the studies also aimed to achieve weight reduction, it is hard to isolate the effect on blood pressure alone and to make the comparison with a pregnant population³⁰. In line with the absence of consistent associations with gestational blood pressure and placental vascular resistance we did not observe any associations of dietary glycemic index and load with the risk of gestational hypertensive disorders. One previous intervention study among 370 obese pregnant women found a lower incidence of gestational hypertension among women prescribed a customized low-glycemic index diet with physical activity counseling^{42, 43}. The difference of our findings with these two studies can probably be explained a lower-risk of glucose impairment within our study population, and that we were not able to take physical activity into account. Further studies need to assess whether the effects of dietary glycemic index and load on gestational hemodynamic adaptations and the risk of gestational hypertensive disorders are more pronounced within pregnant populations at higher risk of impaired glucose metabolism.

Lastly, we investigated the potential influence of iron status on gestational hemodynamic adaptations and the risk of gestational hypertensive disorders using early-pregnancy serum ferritin as a proxy for nutritional iron intake. Important dietary sources of readily available iron are meat and fish, while additional iron supplementation is advised to women with iron deficiency. Iron is an important micronutrient for physiological

processes during pregnancy and is especially abundant in the placenta⁴⁴. Importantly, both iron overload and iron deficiency can cause oxidative stress. While iron overload may lead to exceeding production of free radicals, iron deficiency can result in leakage of free radicals through mitochondrial damage^{44,45}. This can lead to endothelial dysfunction and impaired vasoreactivity, with impaired function in the placental and increased levels of blood pressure as a possible result⁴⁶. The extent of free radical damage that is present in pregnancies affected by gestational hypertensive disorders, further supports the role of oxidative stress in the development of these conditions⁴⁴. A dysregulated iron status from early-pregnancy onwards may therefore be a risk factor for the development of gestational hypertensive disorders⁴⁷⁻⁵¹. Two previous small observational studies reported that lower serum iron concentrations at 12 weeks gestation were associated with a higher risk of gestational hypertensive disorders, however the adjustment for confounders was limited^{52,53}. In a meta-analysis of four randomized controlled trials a tendency towards an increased risk of preeclampsia was found among women who used iron supplementation during pregnancy, however the number of cases was small, the heterogeneity was poor and iron parameters were often not assessed^{9, 54}. Only partly in line with previous studies, we observed no consistent associations of maternal early-pregnancy iron status with gestational blood pressure, placental vascular resistance and the risk of gestational hypertensive disorders after considering maternal inflammation, sociodemographic and lifestyle factors. Different findings in these previous studies and our study may be explained by our non-fasting blood samples that influence the measurement of serum iron, our extensive adjustment for sociodemographic and lifestyle factors and since most of these previous studies had a higher percentage of gestational hypertensive disorders cases. Thus, our findings suggest that within our relatively low-risk population, iron status in early-pregnancy was not associated with gestational hemodynamic adaptations and the risk of gestational hypertensive disorders. The interpretation of iron status is particularly difficult in pregnancy due to iron stores being increasingly mobilized with gestational age progression in a reaction to higher iron requirements to facilitate placental and fetal development⁵⁵. Repeated measurement of fasting blood samples within the same participant are needed to assess longitudinal changes of iron parameters in pregnancy and their effect on maternal gestational hemodynamic adaptations.

For the interpretation of our results it is important to note that participating women already adhered to components of the DASH diet, and glycemic index and load were within the normal range in our study population. Among pregnant populations with a larger variability in dietary intake, the influence of these dietary interventions on gestational hemodynamic adaptations and the risk of gestational hypertensive disorders might be

more apparent. Furthermore, our study population reflects a relatively healthy pregnant population that is at low-risk for gestational hypertensive disorders and impaired glucose metabolism, as we excluded women with preexistent hypertension and diabetes. The prevalence of iron deficiency in our study population was 7%, which is slightly lower compared to the general Dutch population⁵⁶. Together, these factors reflect a selection towards a relatively healthy and lower-risk pregnant population. Possibly the effects of the DASH diet, a lower dietary glycemic index and a dysregulated iron status are especially apparent in women at higher risk. We were not able to assess these associations, as we had a relatively small number of pregnant women with pre-existent hypertension, diabetes, and iron overload or deficiency within this population-based study.

Summary

- Early-pregnancy adherence to the DASH diet is associated with a lower mid-pregnancy diastolic blood pressure and improved fetoplacental vascular resistance in a low-risk pregnant population.
- Early-pregnancy adherence to the low-GI diet is not associated with gestational hemodynamic adaptations or the risk of gestational hypertensive disorders in a low-risk pregnant population.
- Early-pregnancy maternal iron status is not consistently associated with gestational blood pressure and placental vascular resistance or the risks of gestational hypertensive disorders.
- Recommending the DASH dietary pattern from early-pregnancy onwards may be a future target for preventive strategies focusing on improving gestational hemodynamic adaptations.

Cardiovascular outcomes in childhood

Cardiovascular risk factors such as blood pressure, lipid levels and adiposity are all strong independent predictors for all-cause cardiovascular mortality⁵⁷⁻⁶⁰. These risk factors are known to track from childhood throughout adulthood, thus risk factors in childhood might already predict cardiovascular disease in later life^{61, 62}. It is likely that the origin of cardiovascular disease may at least partly originate from earlier phases in life. Early-pregnancy is a critical period for cardiovascular development and adverse exposures during this period may have a direct effect on fetal cardiovascular development. Offspring of pregnancies affected by gestational hypertensive disorders seem to have increased blood pressure levels and nearly a twofold increased risk of stroke in adulthood⁶³⁻⁶⁶. Experimental studies indicate that features that are present in pregnancies affected by gestational hypertensive disorders, such as impaired uterine perfusion with altered pressure loads, intrauterine hypoxia and increased antiangiogenic factors, may negatively affect fetal cardiovascular development⁶⁷.

Cardiomyocytes proliferate predominantly during the first-trimester of pregnancy, while further differentiation takes place during later gestation. Cardiac growth during postnatal life is established through hypertrophy of cardiomyocytes and hyperplasia of non-cardiomyocytes. It has been suggested that cardiomyocytes formed during embryonic development are directly responsible for a substantial part of the myocardial performance during an individual's life^{68,69}. A previous study among 134 term-born infants, found that those exposed to preeclampsia or gestational hypertension had decreased right ventricular end-diastolic volume directly at birth. During assessment at three months they also developed increased left and right ventricular mass⁷⁰. A previous British prospective study among 1,592 adolescents found that offspring exposed to gestational hypertension or preeclampsia had a greater relative left ventricular wall thickness⁷¹. Exposure to preeclampsia was additionally associated with a decreased left ventricular end-diastolic volume, possibly reflecting a concentric type of cardiac remodeling that has also been observed in small-for-gestational-age infants and preterms⁷¹. Contrary to these previous studies, we did not find consistent associations of maternal gestational hypertensive disorders with offspring cardiac structure and function in childhood. We observed that offspring exposed to preeclampsia, but not gestational hypertension, had a lower right ventricular ejection fraction that might suggest a slightly lower right ventricular global function during childhood. This finding can be explained by increased uteroplacental vascular resistance in these pregnancies, that primarily influences right ventricular pressures during intrauterine life⁷². However, this isolated finding may as well reflect a chance finding. Differences in findings with previous studies may be explained by the timing of assessment as we assessed cardiac structure and function during childhood, while the other studies focused on infancy and adolescence^{70,71}. Changes in cardiac structure and function observed in infancy may be transient, while long-term cardiac adaptations may not yet be present during childhood but only first detectable from adolescence onwards. Gestational hypertension and preeclampsia represent the extremes of the gestational hypertensive disorder spectrum, and seem to reflect an overt inability of the maternal cardiovascular system to adequately adapt to pregnancy. However, also a higher maternal gestational blood pressure below the diagnostic threshold for gestational hypertensive disorders, has been associated with adverse cardiovascular outcomes in the offspring⁷³⁻⁷⁵. We observed that a higher maternal diastolic blood pressure throughout pregnancy was associated with decreased offspring left- and right ventricular end-diastolic volumes during childhood. In line with our expectation, this effect was strongest in early-pregnancy. Ventricular end-diastolic volume is a structural measure that describes the cardiac filling capacity during diastole. A restrictive filling pattern can be the result of a decreased

ability for ventricular relaxation and increased ventricular stiffness due to structural myocardial adaptations. Our findings are partly in line with the previously mentioned British observational study among 1,592 adolescents, in this study a smaller decrease in maternal systolic blood pressure between 8 and 18 weeks gestation was associated with increased left ventricular mass and end-diastolic volume during adolescence⁷¹. Together these findings suggest a potential adverse effect of higher maternal blood pressure especially during early-pregnancy on offspring structural cardiac measures. These findings should be considered hypothesis generating and need further replication.

During fetal life, the development of the conduit arteries and vascular beds is an accurately orchestrated process influenced by growth factor signaling. During the course of gestation, vascular development is further influenced by biophysical forces that initiate vascular remodeling⁷⁶. Already from early-postnatal life onwards, endothelial dysfunction and vascular damage can lead to further vascular remodeling. Carotid intima media thickness (IMT) and distensibility are sensitive markers to investigate these vascular changes in pediatric and adult populations^{77, 78}. Carotid IMT primarily reflects the formation of fatty streaks by the accumulation of lipids in the intima media or medial hypertrophy of the common carotid artery, while carotid distensibility is inversely related to arterial stiffness⁷⁷. Carotid IMT and distensibility are both strongly associated with systemic atherosclerosis, higher blood pressure and adverse cardiovascular outcomes in late life⁷⁹. In line with a previous systematic review of ten studies, we observed that gestational hypertension, but not preeclampsia, was associated with a higher offspring systolic and diastolic blood pressure at the age of 10 years⁶⁵. However, we did not find any associations for gestational hypertension or preeclampsia with offspring carotid IMT and distensibility. This is in line with a study among approximately 4,000 mother-offspring pairs from the United Kingdom, that observed no associations of gestational hypertensive disorders with brachial artery flow-mediated dilatation, brachial pulse wave velocity and brachial distensibility in children at the age of 9 to 12 years⁶⁴. On the contrary, smaller studies indicated that neonates exposed to preeclampsia had increased intima media thickness and arterial stiffness but no extensive adjustment for confounders was performed in these studies⁸⁰⁻⁸². Differences between our study and the previous studies may relate to the timing of vascular assessment, type of vascular measurement and differences in study population. Neonatal aortic intima media thickening might only reflect a temporary alteration, in a response to increased placental resistance in preeclamptic pregnancies, that does not persist into childhood^{82, 83}. In line with our findings for gestational hypertension, we observed that higher maternal gestational systolic and diastolic blood pressure across the full spectrum were associated with increased offspring systolic and diastolic blood

pressure, and decreased carotid distensibility. We observed the strongest effects for maternal early and mid-pregnancy systolic and diastolic blood pressure. Another study among 6,619 mother-offspring pairs from the United Kingdom and a Danish study among 2,217 mother-offspring pairs, found similar positive associations of early-pregnancy maternal systolic and diastolic blood pressure with offspring systolic and diastolic blood pressure in infancy, childhood and adolescence^{74, 84}. No previous study explored the direct effects of maternal gestational blood pressure on offspring vascular properties of large arteries. As offspring blood pressure and carotid distensibility were measured at the same time, it is difficult to disentangle how arterial stiffness may influence offspring blood pressure and vice versa. Further studies should focus on the relation between blood pressure levels and arterial stiffness in children. It is known that in an early stage of cardiovascular disease the formation of fatty streaks in the carotid intima media are preceded by functional vascular changes related to arterial stiffness, which may explain why we did not find an association with carotid IMT⁷⁷. Our findings suggest that maternal gestational hypertension and higher gestational blood pressure, even below the diagnostic threshold for gestational hypertensive disorders, might influence offspring blood pressure and arterial stiffness at the age of 10 years.

Understanding of the underlying mechanisms that influence childhood cardiovascular development is important to identify targets for future preventive strategies for cardiovascular disease. Gestational hypertensive disorders as well as a higher blood pressure during pregnancy are risk factors for delivering small-size-for-gestational-age and premature infants, both conditions that are associated with increased blood pressure, and cardiac structural and functional changes in these children⁸⁵. The observed associations of gestational hypertensive disorders and higher maternal blood pressure with offspring cardiovascular outcomes were not explained by maternal socio-demographic and lifestyle factors, or mediated by gestational age and weight at birth, and child factors such as body mass index. The differences in associations of preeclampsia and gestational hypertension with offspring outcomes, might be a reflection of different pathological pathways in the development of these pregnancy disorders. Animal studies suggest that fetal exposure to an adverse intrauterine environment from early gestation, may influence cardiac development and vascular remodeling in the offspring⁶⁷. However, these associations can also be explained by shared genetic predisposition or lifestyle factors in mother-offspring pairs. Especially as mothers who suffered gestational hypertension or preeclampsia also have an increased risk of cardiovascular disease in later life. To obtain further insight into potential underlying mechanisms, we compared the strength of the associations of maternal blood pressure and paternal blood pressure with these offspring outcomes^{73, 86, 87}. Stronger

maternal-offspring associations would support a direct intra-uterine mechanism assuming that both parents contribute equally to shared lifestyle characteristics^{73, 86, 87}. The strength of maternal-offspring and paternal-offspring associations were similar for offspring blood pressure and distensibility. However, the observed associations of a higher maternal diastolic blood pressure with lower offspring left- and right ventricular end-diastolic volumes while these paternal-offspring associations were not present. This suggests that the associations of maternal gestational blood pressure with offspring blood pressure and arterial stiffness are more likely to be driven by shared genetic predisposition or lifestyle factors between mother and child. The effect on cardiac development may at least partly be influenced by maternal hemodynamic adaptations through direct intrauterine mechanisms. It is plausible that maternal blood pressure and other hemodynamic adaptations directly influence fetal cardiac development, especially in early-pregnancy as cardiomyocytes are predominantly formed during the first-trimester of pregnancy^{68, 69}. Higher maternal diastolic blood pressure partly reflect placental vascular maladaptation⁸⁸. In pregnancies with impaired hemodynamic and placental vascular adaptations, fetal cardiac development may be negatively affected by increased placental resistance, relative intrauterine hypoxia and altered fetal cardiac pressure loads^{67, 85}. Utero-placental hypoxia may specifically lead to increased levels of cardiac collagen and alterations in the extracellular matrix which could induce cardiac fibrosis. As shown in experimental studies, this might lead to decreased ability for ventricular relaxation and increased ventricular stiffness with decreased ventricular end-diastolic volumes as a possible result^{89, 90}. Preeclampsia is particularly characterized by inadequate trophoblast invasion with increased placental resistance against which the right fetal ventricle ejects⁷². This could explain why we found an effect on right ventricular ejection fraction in children from preeclamptic mothers, however this isolated finding could still reflect chance. Based on findings from this previous study and our current study, higher maternal blood pressure levels in early-pregnancy might have a direct effect on offspring cardiac development, but to a lesser extent on offspring vascular properties of large arteries. Further observational and experimental studies need to focus on disentangling the underlying mechanisms for cardiac and vascular changes in the offspring in response to maternal gestational blood pressure, and critical periods for exposure to a higher maternal blood pressure during pregnancy.

Children with low birth weight, high birth weight and subsequent high infant growth rates also seem to be at risk for increased blood pressure levels and cardiovascular disease in later life^{91, 92}. We observed that higher fetal, birth and infant weight were associated with higher carotid IMT and lower distensibility. Children with normal fetal growth, followed by infant growth acceleration had the highest carotid IMT and lowest

distensibility. These associations were not explained by maternal or birth characteristics. These findings suggest that weight during fetal life and infancy is critical for arterial health in later life. Weight gain in infancy and early childhood are strongly associated with the risk of developing obesity, high blood pressure, cardiovascular disease and its precursors in adulthood⁹³⁻⁹⁸. Obesity is a major risk factor in the development of atherosclerosis and arterial stiffness, and subsequently the development of cardiovascular disease^{99, 100}. The observed associations were partly explained by childhood body mass index suggesting that childhood body mass index is involved in the pathways underlying the observed associations. Optimizing growth in early life, and especially preventing childhood obesity might be beneficial for arterial health.

Summary

- Gestational hypertension is associated with increased childhood blood pressure. Gestational hypertensive disorders are not associated with childhood cardiac development, carotid IMT and distensibility.
- Higher maternal blood pressure throughout pregnancy is associated with a higher childhood blood pressure, lower carotid distensibility, and lower childhood left and right ventricular end-diastolic volumes.
- Maternal-offspring versus paternal-offspring associations are stronger for childhood cardiac outcomes, but similar for childhood vascular outcomes. This suggests that suboptimal maternal gestational hemodynamic adaptations might affect offspring cardiac structure through direct intrauterine effects, while blood pressure and arterial stiffness are more likely driven by shared genetic predisposition or lifestyle factors between mother and child.
- Higher fetal, birth and infant weight are associated with higher carotid IMT and lower distensibility. Children with normal fetal growth, followed by infant growth acceleration had the highest carotid IMT and lowest distensibility. Childhood body mass index is involved in the pathways underlying these observed associations.

Novel parameters of first-trimester development

The first-trimester of pregnancy is a crucial period for organ development, with each organ having specific critical periods of development and growth¹⁰¹. Adverse exposures in the first-trimester of pregnancy may lead to permanent alterations in the structure and function of various organs, which might predispose the offspring to poorer health on the long-term^{6, 7}. More recently, studies have shown that suboptimal first-trimester development as measured by crown rump length, is associated with increased risks of adverse fetal, birth and child outcomes¹⁰²⁻¹⁰⁶. To gain further insights in potential fetal developmental adaptation mechanisms during early-pregnancy, novel parameters of first-trimester fetal development are essential⁶. With recent improvements in

conventional two-dimensional and novel three-dimensional ultrasound, more detailed ultrasonographic scans of first-trimester embryos and fetuses can be acquired. The combination of 3D transvaginal ultrasound with offline analyses using a Virtual Reality system enables advanced volumetric parameters of early fetal development^{107, 108}. Virtual Reality enables the measurement of complex structures that require depth perception and three-dimensional interaction. Additionally, the V-scope Virtual Reality application allows the operator to perform semi-automated volume measurements using a region growth algorithm and manual segmentation of voxel¹⁰⁹. Previously, embryonic volume measurements using this technique have shown to be feasible, and seem related to fetal growth and birth outcomes¹¹⁰. We aimed to develop novel three-dimensional ultrasound markers using V-scope to assess *in vivo* fetal growth and development in even more detail. Before novel measurement techniques are introduced, it is important to assess the reproducibility of the measurements. In this thesis we present the reproducibility studies of fetal body proportions and fetal organ volumetric measurements using statistical methods as described by Bland and Altman¹¹¹. We found good intra-observer and inter-observer reproducibility of fetal body proportion measurement. We found limits of agreement of approximately $\pm 10\%$ for the total embryonic volume, head, trunk, thorax and abdomen. This is important in the context of future association studies, as these narrow limits of agreement decrease the risk of biased effect estimates in association studies by the influence of measurement errors. The success percentages in which both observer could conduct the measurements was approximately 90%, which we consider to be sufficiently high for research purposes. Thus, we conclude that the fetal proportion volumetric measurements seem feasible for application in research projects. These measurements should be conducted in a larger study sample to establish its value for epidemiological research. Next, we focused on volumetric measurements of the fetal heart, lungs and kidneys as these direct measurements would allow to study organ-specific development. Despite good intra-observer agreement for these fetal organ volume measurements, our method provided insufficient reproducibility in a setting with two observers. The suboptimal interobserver reproducibility for these fetal organ volume measurement in the late first-trimester, can be explained by several factors. First, the measured absolute volumes are extremely small and therefore only allow for minor measurement differences. Second, a large part of this measurement technique involves manual segmentation as it is not possible to automate the recognition of these small but complex anatomical structures with current technology. We think that manual segmentation and an inability to properly demarcate the anatomical boundaries, increases the inter-observer differences for these measurements. This also highlights the importance for acquisition of high-quality 3D

ultrasound datasets. For optimal 3D ultrasound acquisition, we used a state-of-the-art ultrasound machine with a high-frequent transvaginal transducer. The acquisition was performed when the anatomical structures of interest were clearly visible without fetal movements. In the future, technological improvements in ultrasound equipment might further improve three-dimensional ultrasound dataset quality. It is important that future studies assess technical possibilities for automated pre- and post-processing steps to reduce measurement errors and improve utility for the operator, in order to successfully implement these measurements in research settings.

Summary

- First-trimester fetal proportion volumetric measurements using three-dimensional ultrasound and a Virtual Reality system are feasible and show good intraobserver and interobserver reproducibility. These measurements seem feasible for application in research projects.
- We observed sufficient intraobserver reproducibility, but overall suboptimal interobserver reproducibility for first-trimester fetal heart, lung and kidney volume measurements using a Virtual Reality approach. These measurements might be improved by novel pre- and post-processing algorithms.

METHODOLOGICAL CONSIDERATIONS

The studies within this thesis were conducted within the Generation R Study and the Generation R *Next* study. In the specific chapters, the strengths and limitations of these studies have been addressed. In the following paragraphs, general methodological considerations will be further discussed in detail.

Selection bias

Selection bias can arise from selective non-response at baseline or selective loss-to-follow-up. Selection bias at baseline is present if the association between the exposure and outcome of interest differs for participants as compared to eligible non-participants. Of all children that were eligible at birth in the catchment area, the participation rate was 61% in the Generation R Study¹¹². Women who participated in the Generation R Study had higher socioeconomic status and were less likely to be from ethnic minority groups when compared to the general population of Rotterdam¹¹³. Within the study population pregnancy complications occurred less than could be expected from numbers in the general population of Rotterdam. This indicates a selection towards a relatively healthier population, which might have led to lower prevalence rates of gestational hypertensive

disorders and subsequently to reduced statistical power. Therefore, this may have affected the generalizability of the findings to higher-risk populations. However, several studies have shown that non-response at baseline is not likely to lead to biased results in prospective cohort studies such as the Generation R Study^{114, 115}. Selection bias is more likely to occur due to selective loss to follow-up. This occurs when the association between the exposure and outcome of interest differ for participants included in the analyses as compared to participants that were loss to follow-up. In the studies described within this thesis, the percentage loss to follow-up at birth was low.

Children were invited for a follow-up visit at the median age of 10 years, to participate in detailed anthropometric and cardiovascular measurements including ultrasonography of the common carotid artery. For these follow-up visits 76% of the original cohort was invited, with a response rate of approximately 80%¹¹². A random subgroup was also invited for a separate follow-up visit to participate in detailed Cardiovascular Magnetic Resonance. Mothers from children who did not visit the research centre had a lower educational level and maintained an unhealthier lifestyle when compared to the total study population. It is possible that this selective loss to follow-up may have led to biased effect estimates in the studies described within this thesis, but it is difficult to quantify the magnitude and direction of this possible bias. To reduce the risk of selection bias due to missing values, multiple imputation was used in the analyses.

Two of the studies within this thesis were embedded in the Generation R *Next* Study. For these studies we used 3D-dimensional ultrasound datasets that were collected in a small and random sample of participants while inclusion was ongoing in an early-phase of the study. Participants who respond in an early-phase of a study might differ in characteristics from non-responders and participants that need more encouragement and only respond in a later-phase of the study¹¹⁶. It is unlikely that this caused a problem in the two studies that were embedded in the Generation R *Next* Study, as the general aim was to assess of the feasibility and reproducibility of novel first-trimester ultrasound parameters.

Information bias

Information bias can arise from misclassification or measurement error of the exposure or outcome, which can be classified as differential and non-differential misclassification. Differential misclassification occurs when the status of the exposure is related to the status of the outcome, or vice versa. This can result in a bias with either overestimation or under estimation of the effect estimates. It is unlikely that differential misclassification has occurred in the studies described within this thesis as data on the exposures was gathered before assessment of the outcomes, the data collector was unaware of the exposure status

while assessing the outcome, and both participants and data collectors were uninformed about the specific research questions. However, non-differential misclassification may have occurred. Non-differential misclassification occurs when misclassification of the exposure is unrelated to the status of the outcome, or vice versa. This usually leads to an underestimation of the effect estimates. In some of the studies described in this thesis we used self-administered food frequency questionnaires (FFQ) to assess information on dietary exposures. The FFQ that was used within our studies were validated against 3 day 24 hour recalls in 82 women of only Dutch ethnicity¹¹⁷. However this is a commonly used method, dietary measurement error can result in non-differential misclassification which may lead to reduced statistical power to detect associations¹¹⁸. Recall bias can arise because the questionnaires consider the dietary intake of the three months prior to administration. Bias may also occur when women that are overweight or obese are more likely to underestimate their dietary intake or exaggerate the intake of healthy food components. However this does not seem likely, we adjusted all our analyses for prepregnancy body mass index to deal with this. Additionally, we adjusted for total energy intake which may also resolve some of the measurement error induced due to under- or over-reporting of dietary intake¹¹⁹. The other exposures and outcomes that were used in the studies described within this thesis, were either measured following standardized measurement protocols or were obtained from medical records. All prenatal measurements were adjusted or standardized for gestational age at the time of assessment. Postnatal growth measures were standardized on both gender and age. Furthermore, high intra- and interobserver reproducibility was shown for fetal ultrasound measures, common carotid artery ultrasound measures and cardiac assessment using Magnetic Resonance Imaging.

Confounding bias

A confounding factor is associated with both the exposure and the outcome, while the factor is not in the causal pathway between the exposure and the outcome. Confounding bias may occur if the confounding factor is not properly considered in the statistical analyses. In the studies within this thesis, the risk of confounding bias was diminished by the adjustment for multiple potential confounders within the final statistical analyses. For example dietary exposures are prone to confounding by other maternal sociodemographic and lifestyle characteristics. Potential confounders were identified based on information in previous literature using a directed acyclic graph, on their association with the exposure and the outcome of interest, or a change in effect estimate of more than 10%. The observational nature of the study still leaves possibility for residual confounding caused by potential confounders that were not measured within the study. Although we accurately

tried to control for confounding, residual confounding might have still led to biased effect estimates. In some of the studies described in this thesis, we assessed exposures during pregnancy of both mother and father. Similar strength in mother-offspring and father-offspring associations would suggest that the association of maternal exposure with offspring outcome is most likely explained by unmeasured environmental factors, rather than a direct intrauterine effect.

FUTURE PERSPECTIVES

In this thesis we presented studies that describe the associations of early-pregnancy dietary factors with the risk of gestational hypertensive disorders, and the associations of gestational hypertensive disorders and blood pressure with offspring cardiovascular outcomes during childhood. In addition we developed novel markers for first-trimester fetal growth and development. However, we have recommendations for further research in which the following issues remain to be addressed (Table 1).

Despite the fact that we were able to adjust our analyses for numerous sociodemographic and lifestyle related confounding factors, further research is necessary to establish causality for the observed associations. The golden standard to assess causal relations are randomized controlled trials. In these studies differences in outcomes can be fully attributed to the intervention rather than to confounders, as participants are randomly assigned to control and intervention group. Large-scale randomized controlled trials among pregnant women aiming at improving diet, following the DASH and low-glycemic index diet, are warranted. The adherence to these dietary interventions should be strictly monitored to be able to assess causal relations with the development of gestational hypertensive disorders. Intervention studies that aim to improve overall maternal cardiovascular health already before the start of pregnancy are scarce. However, evidence on the importance of a healthy lifestyle already before conception on maternal and offspring health is now so compelling that future studies should focus on dietary interventions implemented before conception¹²⁰. In this way these studies will give additional insight on effectivity and optimal timing for these interventions. From the studies in this thesis, the DASH diet seems to have the most potential as a future target for preventive strategies to lower the risk of gestational hypertensive disorders, but the DASH diet and low-glycemic index diet might target different pathological pathways in the development of gestational hypertensive disorders. Depending on the presence or absence of higher baseline blood pressure or overt diabetes, beneficial effects of these specific dietary interventions might be more or less apparent. Therefore it is important

to not only focus on the general population, but also specific high-risk pregnancies to assess appropriate target populations for future interventions. Integrated randomized controlled trials could provide further insight on the cumulative effect of an overall healthy lifestyle. These studies should entail a combination of interventions focused on diet, supplementation of micronutrients, physical activity, stress reduction, and cessation of alcohol, drugs and smoking in preparation to pregnancy. In the new dietary guidelines from the Dutch Health Council, obstetric health care professionals are recommended to provide dietary advice at the start of pregnancy during the intake appointment⁹. It may be questionable whether such an approach will lead to sustainable dietary improvements. Therefore, another important focus of future studies should lie on the most effective implementation and dissemination of dietary advice to target populations, preferentially in preparation to pregnancy.

Not all exposures studied in this thesis are suitable to be investigated in a randomized controlled trial. Observational study designs that deal with confounding using more sophisticated statistical methods, could give further insights into causality. This is possible through parent-offspring comparison, sibling comparison and mendelian randomization studies. Within this thesis we compared the associations of maternal blood pressure and paternal blood pressure with offspring cardiovascular outcomes. Stronger maternal-offspring compared to paternal-offspring associations suggest a direct intrauterine effect, while stronger or similar paternal-offspring associations suggest a mechanism through genetic or lifestyle factors shared within a family. Our findings suggest that the effect of maternal blood pressure on offspring end-diastolic volumes may at least partly be explained by a direct intrauterine effect. Whereas the associations with offspring blood pressure and carotid distensibility seem more likely explained by genetic or lifestyle factors. Sibling comparison studies could be helpful to further to investigated causality of the observed associations. In sibling comparison studies confounding is controlled as it is assumed that confounders shared within a family remain similar among siblings. However, in these studies the effect of parity should be controlled for as parity is known to be related to the risk of gestational hypertensive disorders and placental function. Mendelian randomization studies could further be helpful to assess causality. In these studies genetic variants that are strongly associated with the exposure of interest are used as instrumental variable, which is less likely to be biased by confounding.

To design effective preventive strategies, disentangling underlying mechanisms for the found associations should be studied in more detail. More detailed assessments of exposures and outcomes might provide additional insights in these underlying mechanisms. Regarding studies on maternal dietary intake on gestational hemodynamic

adaptions, studies are needed that have repeated measurements on dietary intake before and throughout pregnancy to identify critical periods. Food frequency questionnaires need to be validated in multi-ethnic populations, to increase generalizability of study results to the general population. To obtain insights in diet-specific effects through which gestational hemodynamic adaptions might be influenced, it is important that parameters of glucose and lipid metabolism, endothelial function, oxidative stress, inflammation, nutrient status and repeated weight measurements before and during pregnancy are also taken into account in future studies. One of the mechanisms through which maternal diet might influence the risk of gestational hypertensive disorders, is through effects on early placental development. Therefore, future studies should not only focus on uteroplacental flow parameters but also on early placental development using more advanced ultrasound parameters such as trophoblast volume, placental volume, basal plate surface area and utero-placental vascular skeletons with the use of power-doppler, 3D ultrasound, Virtual Organ Computer-aided Analysis and Virtual Reality¹²¹⁻¹²³.

In studies with gestational hypertensive disorders as exposure, we were unfortunately not able to focus on different subtypes of preeclampsia as the prevalence of early-onset preeclampsia is relatively low. As early-onset preeclampsia is associated with the highest neonatal morbidity and a higher risk of cardiac abnormalities, it would be interesting to investigate whether intrauterine exposure to early-onset preeclampsia is more evidently associated with adverse offspring cardiovascular outcomes¹²⁴⁻¹²⁶. Therefore, large-scale collaborations of cohort studies are needed to conduct studies with sufficient power. Regarding all studies on offspring cardiovascular outcomes, we took gestational age and weight at birth and childhood body mass index into account. The associations of gestational hypertensive disorders and maternal blood pressure with offspring cardiovascular outcomes were not explained by these birth and child characteristics. Childhood body mass index did seem to be involved in the pathways underlying the associations of fetal and infant growth with carotid intima media thickness and distensibility. Future studies should focus on the influence of optimizing growth in early life, and especially preventing childhood obesity on arterial health. To investigate whether the associations of maternal blood pressure with offspring blood pressure, carotid distensibility and ventricular end-diastolic volumes were explained by direct intrauterine effect, we compared maternal-offspring and paternal-offspring associations. However, it is important to further investigate whether the observed associations are explained by a direct intrauterine effects or common predisposing cardiovascular risk factors, to provide accurate targets for future preventive strategies. The previously mentioned advanced placental parameters might serve as good proxies to examine whether impaired early placental development underlies

adverse offspring cardiovascular development. The studies in this thesis provide clues on the influence of maternal hemodynamic adaptations, and fetal and infant growth with offspring cardiovascular health from a developmental perspective. The observed effects were small and primarily of interest on a population level. Previous studies have shown that cardiovascular risk factors tend to track and that subclinical differences during childhood can lead to substantial cardiovascular risk in later life. However, the importance of our findings for later life health remain unclear. It is particularly important to investigate how the observed differences in blood pressure, carotid intima media thickness, arterial stiffness and ventricular structure during childhood relate to evident cardiovascular disease in later life. To understand the long-term consequences of maternal hemodynamic adaptations and early life growth, longitudinal offspring follow-up studies with repeated assessment of detailed cardiovascular measurements are needed.

Lastly, we proposed novel parameters for the assessment of early-pregnancy fetal growth and development using 3D ultrasound data in a Virtual Reality system. In large scale population-based research settings like the Generation R *Next* Study, these parameters might further aid in understanding underlying mechanisms of offspring developmental adaptations in response to an adverse intrauterine environment. As the relative growth rate of the fetus is highest during the first-trimester of pregnancy, the fetus is most vulnerable during this period to stressors that might lead to developmental adaptations. We found good reproducibility for first-trimester fetal proportion volumetric measurements. As the increase in volume during the first-trimester is much larger than the increase in length, it is to be expected that these fetal proportion volumetric measurements have higher sensitivity to assess deviations in first-trimester growth compared to customary length and biometric measurements¹¹⁰. In future studies these measurements should be conducted in a larger study sample to establish its value for epidemiological research. Furthermore, we aimed to develop a novel method for first-trimester fetal heart, lung and kidney volume measurements, as these volumetric measurements could provide further knowledge on organ-specific effects during this crucial stage of development. For these organ volume measurements we observed sufficient intraobserver reproducibility, but overall suboptimal interobserver reproducibility with considerable measurements errors. This is most likely due to a large role for manual segmentation in this measurement technique. It is important that future studies assess technical possibilities to reduce measurement errors and improve utility for the operator, in order to successfully implement these measurements in research settings. Efforts should be made towards more automated analyses of the 3D ultrasound datasets. This might be facilitated through atlas-based segmentation frameworks that achieve both automated alignment and segmentation as a preprocessing step in the

3D dataset analysis¹²⁷. Automated imaging post-processing steps might further aid in improving measurements of complex anatomical fetal structures, for example through implementation of adaptive active contour tracking using a snake algorithm in the V-scope application¹²⁸. This method is already used for image analysis purposes in other biomedical fields, and shows high segmentation accuracy¹²⁹⁻¹³². Furthermore, it is important to explore other imaging techniques to conduct volumetric measurements that assess first-trimester organ development. A novel imaging technique that can be proposed to assess the fetal heart and fetal cardiac function in the first-trimester is 3D/4D spatiotemporal image correlation (STIC) fetal echocardiography¹³³. Close collaborations with neighboring departments such as bioinformatics, medical imaging, prenatal medicine and neighboring technical universities are essential to facilitate these processes.

Table 1 Overview of recommendations for future research

Focus	Recommendations
Causality	<ul style="list-style-type: none">• Randomized controlled trials aiming at adherence to early-pregnancy DASH and low-GI diet to lower the risk of gestational hypertensive disorders in specific high-risk population
Underlying mechanisms	<ul style="list-style-type: none">• Repeated measurement of dietary intake before and throughout pregnancy to identify critical periods• Advanced and repeated measurements of the placenta using 3D ultrasound to assess effect of early-pregnancy dietary intake on placental development• Advanced and repeated measurements of first-trimester fetal development using 3D ultrasound to investigate early fetal adaptations
Long-term implications	<ul style="list-style-type: none">• Longitudinal offspring follow-up studies with repeated cardiovascular assessment to understand the long-term consequences of maternal hemodynamic adaptations and early life growth
Technical developments	<ul style="list-style-type: none">• Development of pre-processing and post-processing algorithms to reduce measurement error of three-dimensional volume measurements• Exploring innovative imaging techniques to assess first-trimester organ development, such as 3D/4D spatiotemporal image correlation• Close collaborations with neighboring departments and technical universities

RELEVANCE AND IMPLICATIONS FOR POLICY AND CLINICAL PRACTICE

Although the results from the association studies presented in this thesis do not prove causal relations, the findings provide suggestions for future prevention strategies. Stimulating adherence to the DASH diet may lead to small improvements in cardiovascular health during pregnancy. Although the effects of the DASH diet found in our study population were small, we consider these findings important from an etiological perspective and on a population level. Possibly, the effects on gestational hemodynamic adaptations on an individual level will be larger when more substantial dietary improvements are achieved and women already adhere to the diet in preparation to pregnancy. The periconceptional

period is an important period in which women and their partners are susceptible to make lifestyle changes, with the aim to take good care of themselves and their unborn child. Therefore it is important to make use of this unique window of opportunity through providing clear evidence-based recommendations that are accessible for all mothers to be. Improving awareness among health-care professionals and the general public about a healthy diet is the first step to achieve this. Identification of women who will benefit most from dietary improvements would further help to tailor personalized dietary interventions. Emphasis should especially be given to the importance of a healthy lifestyle already before conception. Providing preconceptional counselling on a more structural basis, through implementation of preconception care in the Dutch health care system, would be a key strategy to achieve this. This approach is in line with already existing strategies of the Dutch ministry of Health, Welfare and Sports focused on the first 1,000 days after conception to improve offspring outcomes¹³⁴.

This thesis also provides new clues regarding the influence of maternal hemodynamic adaptations during pregnancy and the child's growth during early-life on the development of the cardiovascular system. We identified that early-pregnancy blood pressure levels are independently associated with offspring cardiovascular outcomes during childhood. We highlight that cardiovascular development is partly influenced by a direct intra-uterine effect but also lifestyle factors shared between a mother and child seem to play a role. These findings provide potential targets to prevent cardiovascular disease in later life. The results of our study also suggests that weight during fetal life and infancy is critical for arterial health in childhood. Optimizing growth in early life, and especially preventing childhood obesity might be beneficial for arterial health later in life. Thus, both fetal life and infancy seem to be critical for cardiovascular development and potentially provide a critical window for preventive strategies. It is well established that alterations in cardiac structural and function measures, and increased intima media thickness and arterial stiffness are strong predictors for cardiovascular morbidity and mortality during adulthood. Childhood cardiovascular markers tend to track from infancy to adolescence, and therefore alterations in childhood may directly relate to cardiovascular health in adulthood^{62, 135, 136}. Our findings may be useful for early identification of offspring at increased risk of an adverse cardiovascular risk profile in later life. These children may benefit from prevention strategies focused on reducing risk factors for cardiovascular diseases from early life onwards.

Finally, in this thesis we presented novel markers for first-trimester fetal development using three-dimensional ultrasound datasets in a Virtual Reality setting. In large scale population-based research settings like the Generation R *Next* Study the value of these

novel markers for epidemiological research needs to be determined. These measurements might give further insights in the influence of periconceptional exposures on early fetal growth and developmental adaptations. This may lead to better understanding of early developmental adaptation mechanisms leading to adverse birth outcomes and adverse cardiovascular risk profile in later life, and provide targets for further preventive strategies.

Conclusions

Findings of this thesis suggest that maternal dietary pattern in early-pregnancy might have small beneficial effects on maternal hemodynamic adaptations during pregnancy in a low-risk population. Offspring cardiovascular development is partly influenced by early-pregnancy hemodynamic adaptations through a direct intra-uterine effect, but also lifestyle factors shared between a mother and child seem to play a role. These findings provide potential targets to prevent cardiovascular disease in later life. Future studies should further determine the critical role of early-pregnancy in adverse pregnancy outcomes and offspring development using novel 3D ultrasound for early placental and fetal development.

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CHAPTER



SUMMARY

6



SUMMARY

In **Chapter 1** we provide an introduction and rationale leading to the studies presented in this thesis. From early-pregnancy onwards, impaired maternal cardiovascular health may result in impaired uteroplacental circulation, elevated blood pressure levels and the development of gestational hypertensive disorders. Although gestational hypertensive disorders clinically manifest in later stages of pregnancy, the origin of this spectrum of disorders is assumed to be found in early-pregnancy. A healthy maternal diet has been recognized to benefit maternal cardiovascular health. Improvements in maternal diet prior to and during early-pregnancy might also facilitate adequate hemodynamic responses to pregnancy that lead to a lower risk of gestational hypertensive disorders. Women who suffered from any gestational hypertensive disorder are at increased risk of developing cardiovascular disease far beyond pregnancy. Likewise, the offspring of pregnancies affected by gestational hypertensive disorders may have long-term cardiovascular health consequences. Identifying pregnant women and children that are at risk from early-pregnancy onwards of adverse cardiovascular consequences, may help to develop strategies at earlier stages in life to prevent adverse cardiovascular health in later life. Specific focus to identify critical periods for adverse exposures and potential underlying mechanisms for adverse outcomes, might further aid in appropriate timing and specific targets for these preventive strategies. Novel markers on first-trimester fetal development may further help to elucidate mechanisms of fetal developmental adaptations in early-pregnancy that may result in adverse cardiovascular health in later life. The general aim of this thesis was to assess dietary factors that might influence maternal cardiovascular health during pregnancy, the influence of gestational hypertensive disorders and pre- and postnatal growth on offspring cardiovascular health, and to develop novel measurements of first-trimester fetal development. The studies presented in this thesis were embedded in the Generation R and Generation R *Next* studies. The Generation R Study is a population-based prospective cohort study from early-pregnancy onwards in Rotterdam, The Netherlands. The Generation R *Next* Study is population-based prospective cohort study with study enrollment from the preconception phase onwards. These studies were designed to identify early environmental and genetic determinants of growth, development and health in preconception, pregnancy and beyond focusing on both mother and child.

In **Chapter 2** studies on the associations of maternal dietary factors, hemodynamic adaptations in pregnancy and the risk of gestational hypertensive disorders are described. In **Chapter 2.1** we investigated the associations of the DASH diet in early-pregnancy with hemodynamic adaptations and the risk of gestational hypertensive disorders. The

DASH diet is a diet high in fruits, vegetables, total grains, nuts, seeds, legumes and non-full-fat dairy products and low in animal protein, sugar and sodium, and specifically developed to lower blood pressure and improve cardiovascular health. We observed that a higher DASH dietary score was associated with a lower diastolic blood pressure in mid-pregnancy and lower fetoplacental vascular resistance. We found a tendency of early-pregnancy DASH diet with a lower risk for preeclampsia. These findings suggest that adherence to the DASH diet during early-pregnancy might have small beneficial effects on the maternal gestational hemodynamic adaptations. In **Chapter 2.2** investigated whether adherence to a low-glycemic index diet was associated with beneficial effect on hemodynamic adaptations and the risk of gestational hypertensive disorders, however we found no associations in our population at low-risk for glucose impairment. In **Chapter 2.3** we found no consistent associations for maternal iron status in early-pregnancy with gestational blood pressure and placental vascular resistance or the risks of gestational hypertensive disorders.

In **Chapter 3** studies on the associations of in utero exposure to gestational hypertensive disorders and higher blood pressure within the normal range, on childhood cardiovascular development in the offspring were described. In **Chapter 3.1** we investigated whether maternal gestational hypertensive disorders were associated with alterations in offspring cardiac structure and function in childhood. No consistent associations of gestational hypertensive disorder status with childhood cardiac outcomes were present. When focusing on maternal blood pressure throughout pregnancy, we observed that a higher maternal diastolic blood pressure throughout pregnancy was associated with decreased offspring left- and right ventricular end-diastolic volumes with independent effect in early-pregnancy. In **Chapter 3.2** we observed that gestational hypertension, but not preeclampsia, was associated with a higher offspring systolic and diastolic blood pressure at the age of 10 years. In line with these findings for gestational hypertension, we observed that higher maternal gestational systolic and diastolic blood pressure across the full range were associated with increased offspring systolic and diastolic blood pressure and decreased carotid distensibility, but not with carotid IMT. We observed independent effects for maternal early and mid-pregnancy systolic and diastolic blood pressure. The found associations in **Chapter 3.1 and 3.2** were not explained by maternal socio-demographic and lifestyle factors or mediated by birth and child factors. The strength of maternal-offspring and paternal-offspring associations were similar for offspring blood pressure and distensibility. However, the observed associations of a higher maternal diastolic blood pressure with lower offspring left- and right ventricular end-diastolic volumes were stronger than the paternal-offspring associations. This suggests that the associations of

maternal gestational blood pressure with offspring blood pressure and arterial stiffness are more likely to be driven by shared genetic predisposition or lifestyle factors between mother and child. While the effect on cardiac development may at least partly be influenced by maternal hemodynamic adaptations through direct intrauterine mechanisms, rather than genetic predisposition or shared lifestyle within a family. In **Chapter 3.3** we also investigated the influence of fetal and postnatal growth on vascular development. We observed that higher fetal, birth and infant weight were associated with higher carotid IMT and lower distensibility. Children with normal fetal growth, followed by infant growth acceleration had the highest carotid IMT and lowest distensibility. Although these associations were partly explained by childhood body mass index, these findings suggest that growth during both fetal life and infancy are critical for arterial health in later life.

To gain further insights in potential fetal developmental adaptation mechanisms during early-pregnancy, novel parameters of first-trimester fetal development are essential. In **Chapter 4** we presented novel approaches of first-trimester fetal measurements using three-dimensional ultrasound and Virtual Reality. In **Chapter 4.1** we found good intra-observer and inter-observer reproducibility of fetal body proportion measurement. These measurements might give further insights in the influence of periconceptional exposures on early fetal growth and developmental adaptations. This may lead to better understanding of early developmental adaptation mechanisms leading to adverse birth outcomes and adverse cardiovascular risk profile in later life. In large scale population-based research settings like the Generation R *Next* Study the value of these novel markers for epidemiological research needs to be determined. In **Chapter 4.2** we focused on volume measurements of the fetal heart, lungs and kidneys as these direct measurements would allow to study organ-specific effects in future studies. Despite good intra-observer agreement for these fetal organ volume measurements, our method provided insufficient reproducibility in a setting with two observers. These measurements need further improvements using automated post-processing steps to aid in the utility of this method, and reduce intra- and interobserver measurement differences.

Finally, in **Chapter 5** we discussed the general conclusions in a broader context of existing literature, suggestions for future research, and implications for clinical practice.

SAMENVATTING

In **Hoofdstuk 1** geven we een introductie voor de studies gepresenteerd in dit proefschrift. Vanaf de vroege zwangerschap, kan verminderde maternale cardiovasculaire gezondheid resulteren in verhoogde placentaire weerstand, hogere bloeddruk en de ontwikkeling van hypertensieve aandoeningen in de zwangerschap. Ondanks dat hypertensieve aandoeningen zich in een later stadium van de zwangerschap manifesteren, lijkt de origine van deze aandoeningen in de vroege zwangerschap te liggen. Een gezond dieet heeft mogelijk voordelen voor de maternale cardiovasculaire gezondheid. Het is waarschijnlijk dat verbeteringen van het dieet vóór en tijdens de zwangerschap zorgen voor adequate hemodynamische aanpassingen tijdens de zwangerschap en zodoende leiden tot een lager risico op hypertensieve aandoeningen. Vrouwen die een hypertensieve aandoening tijdens de zwangerschap hebben gehad, hebben een groter risico later in het leven cardiovasculaire ziekten te ontwikkelen. Eveneens lijken hun nakomelingen een groter risico te hebben op een slechtere cardiovasculaire gezondheid op de lange termijn. Door zwangere vrouwen en kinderen met een verhoogd risico op een slechte cardiovasculaire gezondheid al vanaf de zwangerschap te identificeren, kunnen gerichte preventiestrategieën worden ontwikkeld om cardiovasculaire events op de lange termijn te voorkomen. Het specifiek identificeren van kritische periodes voor blootstellingen en onderliggende mechanismen, helpt verder om adequate timing en specifieke targets te bepalen voor toekomstige preventiestrategieën. Nieuwe parameters van eerste trimester foetale ontwikkeling kunnen daarnaast helpen om mechanismes van foetale adaptatie in de vroege zwangerschap te begrijpen. Het doel van dit proefschrift is te beoordelen hoe dieet factoren maternale cardiovasculaire gezondheid tijdens de zwangerschap kunnen beïnvloeden, hoe maternale hypertensieve aandoeningen tijdens de zwangerschap en perinatale groei de cardiovasculaire ontwikkeling van de nakomelingen beïnvloed en welke rol de vroege zwangerschap hierin speelt. Daarnaast wilden wij nieuwe metingen ontwikkelen van foetale ontwikkeling in het eerste trimester van de zwangerschap. De studies gepresenteerd in dit proefschrift werden verricht binnen de Generation R en de Generation R *Next* studies. De Generation R Studie is een prospectief populatie cohortstudie vanaf de vroege zwangerschap. De Generation R *Next* Studie is een populatie cohortstudie vanaf de preconceptie fase. Deze studies zijn opgezet om omgevingsfactoren en genetische determinanten die invloed hebben op ontwikkeling, groei en gezondheid te identificeren in de preconceptie fase, zwangerschap en daarna met de focus op zowel moeder als kind.

In **Hoofdstuk 2** onderzochten we de associaties van maternale dieet factoren met hemodynamische adaptaties en het risico op hypertensieve aandoeningen tijdens de

zwangerschap. In **Hoofdstuk 2.1** bestudeerden we het Dietary Approaches to Stop Hypertension (DASH) dieet. Het DASH dieet bestaat uit een hoge consumptie van fruit, groente, granen, noten, zaden, peulvruchten en magere zuivel producten, en lage consumptie van dierlijke proteïnes, suiker en zout. Dit dieet is specifiek ontwikkeld om bloeddruk te verlagen en cardiovasculaire gezondheid te verbeteren in de algemene populatie. In dit proefschrift was een hogere DASH dieet score geassocieerd met een lagere diastolische bloeddruk in het tweede trimester van de zwangerschap en een lagere foetoplacentaire weerstand. Daarnaast was er sprake van een tendens dat een hogere naleving van het DASH dieet geassocieerd is met een lager risico op pre-eclampsie. Deze bevindingen suggereren dat naleving van het DASH dieet in de vroege zwangerschap kleine voordelige effecten heeft op maternale hemodynamische aanpassingen in de zwangerschap. In **Hoofdstuk 2.2** onderzochten we of naleving van een laag glycemische index dieet geassocieerd was met voordelige effecten op hemodynamische aanpassingen en het risico op hypertensieve aandoeningen tijdens de zwangerschap, maar we vonden geen consistente associaties. In **Hoofdstuk 2.3** vonden we geen consistente associaties van maternale ijzer status in de vroege zwangerschap met hemodynamische aanpassingen en het risico op hypertensieve aandoeningen tijdens de zwangerschap.

In **Hoofdstuk 3** bestudeerden we de associaties van intra-uteriene blootstelling aan hypertensieve aandoeningen in de zwangerschap en een hogere maternale bloeddruk onder die diagnostische grenswaarde voor hypertensieve aandoeningen, met cardiovasculaire uitkomsten in de nakomelingen op tienjarige leeftijd. In **Hoofdstuk 3.1** onderzochten we de effecten op cardiale structuur en functie op de kinderleeftijd. We observeerden geen consistente associaties met maternale hypertensieve aandoeningen tijdens de zwangerschap en cardiale uitkomsten van het kind op tienjarige leeftijd. We observeerden ook dat een hogere maternale diastolische bloeddruk gedurende de gehele zwangerschap geassocieerd was met lagere eind-diastolische volumes van het linker en rechter ventrikel van het kind op tienjarige leeftijd. Dit effect was het sterkst en onafhankelijk in de vroege zwangerschap. In **Hoofdstuk 3.2** observeerden we dat blootstelling aan zwangerschapshypertensie, maar niet pre-eclampsie, geassocieerd was met een hogere systolische en diastolische bloeddruk in de nakomelingen op tienjarige leeftijd. In lijn met deze bevindingen observeerden we dat hogere maternale systolische en diastolische bloeddruk geassocieerd waren met een hogere systolische en diastolische bloeddruk en lagere carotis distensibiliteit in de nakomelingen op tienjarige leeftijd. De sterkste en onafhankelijke effecten werden geobserveerd in het eerste en tweede trimester van de zwangerschap. Er werden geen associaties gevonden van hypertensieve aandoeningen in de zwangerschap en een hogere maternale bloeddruk met carotis intima media dikte van

de nakomelingen. De gevonden associaties in **Hoofdstuk 3.1 en 3.2** werden niet verklaard door maternale socio-demografische status en leefstijl factoren, en niet gemedieerd door geboorte en kind factoren. De moeder-nakomeling en vader-nakomeling associaties waren vergelijkbaar voor bloeddruk en carotis distensibiliteit van de nakomelingen. Een hogere maternale diastolische bloeddruk was wel geassocieerd met lagere eind-diastolische volumes van het linker en rechter ventrikel, terwijl deze vader-nakomeling associaties niet aanwezig waren. Dit suggereert dat de associaties van maternale bloeddruk met bloeddruk en carotis distensibiliteit van de nakomelingen meest waarschijnlijk worden gedreven door genetische predispositie en ongemeten leefstijl factoren. Daarentegen lijkt het effect op hart ontwikkeling tenminste gedeeltelijk verklaard door maternale hemodynamische adaptaties op basis van een direct intra-uterien mechanisme. In **Hoofdstuk 3.3** onderzochten we ook de associaties van foetale en postnatale groei met carotis distensibiliteit en intima media dikte. We observeerden dat een hoger foetaal, geboorte en zuigeling gewicht geassocieerd was met een lagere carotis distensibiliteit en een hogere intima media dikte. Kinderen met een normale foetale groei, gevolgd door groei versnelling als zuigeling, hadden een lagere carotis distensibiliteit en een hogere intima media dikte. Deze associaties werden gedeeltelijk bepaald door adipositas op de kinderleeftijd. Deze bevindingen suggereren dat zowel foetale groei als groei op de zuigelingen leeftijd van belang zijn voor arteriële gezondheid op de kinderleeftijd.

Om meer inzichten te verkrijgen in potentiële foetale adaptatie mechanismen in de vroege zwangerschap, zijn nieuwe en innovatieve parameters voor eerste trimester foetale ontwikkeling van essentieel belang. In **Hoofdstuk 4** presenteren we nieuwe metingen voor eerste trimester foetale groei en ontwikkeling waarbij we gebruik hebben gemaakt van drie-dimensionale echo data en een Virtual Reality systeem. In **Hoofdstuk 4.1** vonden we goede intra-observer en inter-observer reproduceerbaarheid voor volume metingen van foetale lichaamsproporties. Deze innovatieve metingen zouden nieuwe inzichten kunnen geven over de invloed van periconceptionele blootstellingen op vroege foetale groei en ontwikkeling. Dit kan leiden tot beter begrip ten aanzien van vroege foetale adaptaties die leiden tot nadelige geboorte uitkomsten en cardiovasculaire uitkomsten later in het leven. In grootschalige populatie cohortstudies, zoals de *Generation R Next Studie*, moet de waarde van deze nieuwe metingen voor epidemiologisch onderzoek verder worden beoordeeld. In **Hoofdstuk 4.2** hebben we ons gefocust op nieuwe volume metingen van het foetale hart, de longen en de nieren. Deze metingen zouden helpen om orgaan-specifieke effecten te bestuderen in de vroege zwangerschap. Ondanks goede intra-observer reproduceerbaarheid, was er sprake van onvoldoende inter-observer reproduceerbaarheid in een setting met twee onderzoekers. Deze metingen moeten verder

worden verbeterd door geautomatiseerde post-processing algoritmes om meetfouten te verminderen.

In **Hoofdstuk 5** bespreken we de algemene conclusies van dit proefschrift in een bredere context van bestaande literatuur. Daarnaast bespreken we suggesties voor toekomstig onderzoek en implicaties van onze bevindingen voor de klinische praktijk.

CHAPTER



ADDENDUM

7

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LIST OF PUBLICATIONS

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Wiertsema CJ, Mensink-Bout SM, Duijts L, Mulders AGMGJ, Jaddoe VWV, Gaillard R. Associations of DASH diet in early-pregnancy with blood pressure patterns, placental hemodynamics and gestational hypertensive disorders. *Journal of the American Heart Association*, 2021 Jan 5;10(1):e017503. Doi: 10.1161/JAHA.120.017503.

Wiertsema CJ, Wahab RJ, Mulders AGMGJ, Gaillard R. Associations of dietary glycemic index and load during pregnancy with blood pressure, placental hemodynamic parameters and the risk of gestational hypertensive disorders. *European Journal of Nutrition*, 2022 Mar;61(2):703-716. Doi: 10.1007/s00394-021-02670-5.

Wiertsema CJ, Jaddoe VWV, Mulders AGMGJ, Gaillard R. Childhood blood pressure, carotid intima media thickness, and distensibility after in utero exposure to gestational hypertensive disorders. *Journal of the American Heart Association*, 2022 Feb;11(3):e023163. Doi: 10.1161/JAHA.121.023163.

Wiertsema CJ, Erkamp JS, Mulders AGMGJ, Steegers EAP, Duijts L, Koning AHJ, Gaillard R, Jaddoe VWV. First-trimester fetal proportion volumetric measurements using a Virtual Reality approach. *Prenatal Diagnosis*, 2021 Jun;41(7):868-876. Doi: 10.1002/pd.5947.

Wiertsema CJ, Sol CM, Mulders AGMGJ, Steegers EAP, Duijts L, Gaillard R, Koning AHJ, Jaddoe VWV. Innovative approach for first-trimester fetal organ volume measurements using a Virtual Reality system: The Generation R Next Study. *The Journal of Obstetrics and Gynaecology Research*, 2022 Mar;48(3):599-609. Doi: 10.1111/jog.15151.

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Taeubert MJ, **Wiertsema CJ**, Vermeulen MJ, Quezada-Pinedo H, Reiss IK, Muckenthaler MU, Gaillard R. Maternal iron status in early-pregnancy and blood pressure throughout pregnancy, placental hemodynamics and the risk of gestational hypertensive disorders. *The Journal of Nutrition*, 2022 Feb 8;152(2):525-534. Doi: 10.1093/jn/nxab368.

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Clarissa Wiertsema was born in the Ikazia Hospital in Rotterdam, The Netherlands on the January 13th 1990. She graduated from secondary school at Johannes Calvijn in Rotterdam. After this she started her medical education at the Erasmus University Medical Centre in Rotterdam, The Netherlands. During her medical education she went abroad to Zambia for a minor in Tropical Health and International Medicine and to Australia for her Master Research at the Royal Children's Hospital in Melbourne. During her clinical internships she found out that she had a specific interest in the field of Obstetrics & Gynecology. In 2016 she obtained her Master in Medicine and graduated *cum laude*. After her graduation she started working as an ANIOS (medical doctor not in training) at the Obstetrics & Gynecology department in the Ikazia Hospital in Rotterdam, The Netherlands. After 1,5 years of working as a medical doctor, she became a PhD student at the Generation R Study Group at the Erasmus University Medical Centre. She was involved in the project management, ultrasound data collection and Virtual Reality measurements for Generation R *Next* Study, a new prospective population-based cohort study from preconception onwards. During her PhD she also obtained her master in Clinical Epidemiology at the Netherlands Institute of Health Sciences. She finalized here PhD under supervision of dr. Romy Gaillard, dr. Annemarie Mulders, prof. dr. Vincent Jaddoe and prof. dr. Eric Steegers.

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Research school	Netherlands Institute for Health Sciences
PhD period	February 2018 – May 2022
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Co-promotors	Dr. R. Gaillard Dr. A.G.M.G.J. Mulders

1. PhD training	Year	ECTS
Courses		
Good Clinical Practice Training (Erasmus MC)	2018 2020	0.3
Systematic Literature Retrieval in multiple databases (Erasmus MC)	2019	1.0
Introduction in Endnote (Erasmus MC)	2019	0.3
Scientific Integrity	2019	0.3
Ultrasonography training – biometric measurement (EchoXpert)	2019	1.0
Scientific Writing	2020	0.3
Master Health Sciences – Clinical Epidemiology (NIHES)	2018 – 2021	70
Local research meetings		
Weekly Obstetrics research meeting	2017 – 2021	1.0
Weekly Generation R meetings	2017 – 2021	2.0
Monthly ACE Pregnancy & Childhood meeting	2018 – 2019	
Annual Wladimiroff Research Day	2018 2019	0.3
Seminars, conferences and presentations		
Developmental Origins of Health and Disease, The Netherlands	2017	0.6
Groot Stedelijke Perinatale Gezondheid, The Netherlands	2017	0.3
KNAW colloquium, The Netherlands	2019	0.3
Sophia Research Day, The Netherlands (oral presentation)	2021	0.3
Society for Pediatric and Perinatal Epidemiologic Research (SPER), online (oral presentations)	2021	0.3
Society for Reproductive Investigation (SRI), online (oral and poster presentation)	2021	0.3
WEON, Dutch Epidemiological conference, online (oral presentation)	2021	0.3
Reviewing manuscripts		
Diabetologica	2020	0.3
Nutrients	2021	0.3
Organization of meetings		
Organization ACE Pregnancy & Childhood research day	2018	2.0
Organization ACE Pregnancy & Childhood monthly meetings	2018 – 2019	2.0
Interviews		
Algemeen Dagblad Rotterdam, Rotterdam Rijnmond, Amazing Erasmus MC	2021	0.3
2. Teaching and supervisor of students		
Teaching ultrasound protocols and skills to PhD and ultrasound personnel	2018 – 2020	2.0
Generation R <i>Next</i> ultrasound training days	2019 2020	0.6
Daily supervisor of J. Taubert, Heidelberg University	2020 – 2021	1.0
Daily supervisor of R. Goncalves	2020 – 2021	1.0
Supervisor of minor students, TU Delft	2020 – 2021	0.6

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Early-pregnancy and cardiovascular health in pregnancy and childhood

