Cardiovascular Health in Pregnancy and Beyond

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Chapter 1

General introduction

Nowadays, cardiovascular disease (CVD) is the leading cause of death amongst women worldwide.[1,2] For certain cardiovascular risk factors, such as hypertension and diabetes, there is a tremendous global awareness. However, pregnancy related risk factors are often overseen, in particular hypertensive disorders of pregnancy. These include gestational hypertension and preeclampsia; disorders that are characterized by the occurrence of new onset hypertension in pregnancy, with our without proteinuria.[3] Women with a hypertensive disorder of pregnancy have a higher risk to develop CVD compared to women with a normotensive pregnancy.[4] For comparison, these women are 16 times more likely to die from CVD than from breast cancer.[5]

Even though awareness for women's health increased over the last decennium, the management of cardiovascular risk factors after a hypertensive disorder of pregnancy is still not clearly defined in cardiovascular prevention guidelines.[6-8] The first large studies that showed a higher risk of CVD after preeclampsia were conducted 13 years ago.[4,9] Since then, numerous studies examined the relation between hypertensive disorders of pregnancy, cardiovascular risk factors and CVD.[10-14] Results showed that these women are more likely to develop cardiovascular risk factors, such as hypertension, obesity, dyslipidemia and insulin resistance after pregnancy. However, it remains unknown whether hypertensive disorders of pregnancy are a risk factor for CVD or if they just share common risk factors. Also, at what age and to what extend cardiovascular risk factors become apparent remains unclear.

Several scores, such as the Framingham score and the Atherosclerotic Cardiovascular Disease score, combine cardiovascular risk factors in to one model to predict 10-year CVD risk.[15,16] Though women with a previous hypertensive disorder of pregnancy have a substantially increased lifetime risk of CVD, their 10-year risk, depicted by these cardiovascular risk prediction models, is low.[11] Consequently, it remains a challenge to provide optimal cardiovascular counseling to these women at a younger age. Moreover, guidelines addressing the cardiovascular follow-up of women with a previous hypertensive disorder of pregnancy are not uniform in their recommendations.[17-26] This raises concerns for the health of these women as the prevalence of hypertensive disorders of pregnancy increased with 25% in the last decennia and will most likely continue to grow with the rise of risk factors such as diabetes and obesity and the trend to conceive at older age.[27-30] Identifying those women most at risk for future CVD can help to determine and treat cardiovascular risk factors at an early stage.

In this thesis we discuss cardiovascular biomarkers and cardiovascular risk factors after pregnancy, both short term and long term, in women with a hypertensive disorder of pregnancy and women with a previous uncomplicated pregnancy. The aims of this thesis can be summarized as follows:

1. To determine biomarkers in pregnancy to predict the risk of developing a hypertensive disorder of pregnancy and a suboptimal cardiovascular risk profile after pregnancy.
2. To determine cardiovascular risk factors after pregnancy in women with a previous hypertensive disorder of pregnancy, women with a previous normotensive pregnancy as well as their offspring.
3. To determine cardiovascular health in and after pregnancy in women with a hypertensive disorder of pregnancy.

Outline of this thesis

The outline of this thesis is discussed in three main parts. Part I is focussed on biomarkers in pregnancy and cardiovascular risk factors after pregnancy (Chapter 2 and 3). In **Chapter 2**, we examine the association between PlGF in mid-pregnancy and cardiovascular risk factors after pregnancy. In **Chapter 3** associations are being presented between the lipid profile in early pregnancy and the risk of a hypertensive disorder of pregnancy and blood pressure throughout pregnancy. Part II describes cardiovascular risk factors after a hypertensive disorder of pregnancy (Chapter 4-9). In **Chapter 4**, we examine blood pressure profile one year after pregnancy in women with severe preeclampsia. In **Chapter 5** associations are being presented between a hypertensive disorder of pregnancy and the maternal lipid profile six years after pregnancy. In **Chapter 6**, we examine whether cardiovascular risk factors track from mother to child. In **Chapter 7** associations are being presented between a hypertensive disorder of pregnancy and the maternal retinal microvasculature six years after pregnancy. In **Chapter 8** and **9** the risk of coronary artery calcification in women with a previous hypertensive disorder of pregnancy is being examined. Part III describes cardiovascular health in and after pregnancy in women with a hypertensive disorder of pregnancy (Chapter 9 and 10). In **Chapter 10** we examine the association between cardiovascular health in pregnancy and the carotid intima-media thickness and cardiovascular health after pregnancy. In **Chapter 11**, we examine cardiovascular health in women with previous severe preeclampsia and the association with carotid intima-media thickness as a measure of vascular age. Lastly, in **Chapter 12** we provide a general discussion.

General design

The research presented in this thesis was based on three study populations: the Generation R Study, the Follow-Up Preeclampsia (FUPEC) population and the Cardiovascular RiskprofilE: IMaging And Gender-specific disOrders (CREw-IMAGO) study.

The Generation R Study is a population-based prospective cohort from early pregnancy onwards. Pregnant women from the city Rotterdam in the Netherlands with an expected delivery date between April 2002 and January 2006 were eligible. In total, 8880 women were included during pregnancy. Women visited the research center in early (< 18 weeks), mid- (18 – 25 weeks) and late (> 25 weeks) pregnancy, and six and nine years after pregnancy. Measurements and information in pregnancy included: anthropometrics, blood pressure, blood samples, medical files and questionnaires. Follow-up measurements six and nine years after pregnancy in both mother and child included: anthropometrics, blood pressure, blood samples, cardiac ultrasound, pulse wave velocity, carotid intima-media thickness, retinal vascular imaging and questionnaires.

FUPEC is a prospective hospital based population of women with previous severe preeclampsia who receive multidisciplinary long-term cardiovascular follow-up after pregnancy. The outpatient clinic is located in Erasmus MC, Rotterdam, the Netherlands. All women throughout the Netherlands with previous severe preeclampsia are eligible and inclusion is still ongoing. Women (N = 636) attended the outpatient clinic six weeks, three months and one year after

pregnancy between April 2011 and September 2017. Cardiovascular follow-up included: anthropometrics, glucose and lipid profile, 24-hour ambulatory blood pressure monitoring, carotid intima-media thickness, medical files and questionnaires.

The CREw-IMAGO study is a retrospective hospital based multicenter study (Erasmus MC, Rotterdam and University Medical Center Utrecht) of women with gestational hypertension and preeclampsia. Women with a previous hypertensive disorder of pregnancy between the age of 40 and 55 and (a)symptomatic of cardiovascular disease were eligible. These women (N = 269) were recruited from three cohorts (Utrecht Cohort, Preeclampsia Risk Evaluation in FEMales cohort and Hypitat Risk Assessment Study cohort) and the FUPEC population. Between February 2016 and January 2018 women underwent coronary computed tomography angiography (CCTA) and cardiovascular screening.

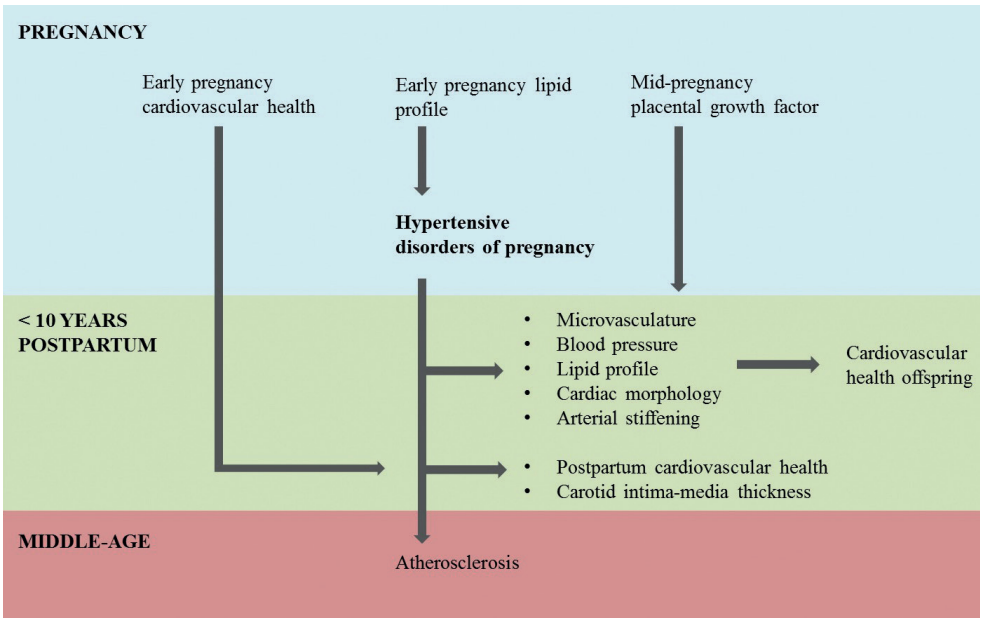

FIGURE 1 | Overview of the cardiovascular biomarkers and risk factors studied in this thesis.

Overview of the cardiovascular biomarkers and risk factors studied in this thesis

Placental growth factor

Placental growth factor (PlGF) is a pro-angiogenic factor, released by the syncytiotrophoblast of the placenta, and is enquired for proper remodeling of the maternal spiral arteries in the placenta. Suboptimal spiral artery remodeling, as seen in preeclampsia, can lead to abnormal placental perfusion and fetal growth restriction. Lower concentrations of circulating PlGF in mid-

pregnancy are associated with subnormal cardiovascular remodeling in pregnancy. It remains unclear whether PlGF concentrations in pregnancy are also associated with cardiovascular risk factors and CVD after pregnancy. If so, PlGF in pregnancy could be used as a biomarker to identify those women at risk for a suboptimal cardiovascular risk profile after pregnancy.

Blood pressure

Hypertension is a major risk factor for CVD and can explain up to 50 percent of an individual's cardiovascular risk.[31] After pregnancy, women with preeclampsia have on average a higher blood pressure and more often hypertension than women with a normotensive pregnancy.[32] They also develop hypertension at a younger age, which gives them a longer lifetime exposure to high blood pressure resulting in more endothelial damage. In current practice, women with preeclampsia undergo a single blood pressure measurement at the physician's office six weeks after pregnancy. However, some forms of hypertension, such as masked hypertension, white-coat hypertension, night-time hypertension, and a disadvantageous systolic night-to-day dipping pattern will remain undetected without 24-hour ambulatory blood pressure monitoring. All four are clinically relevant by serving as independent cardiovascular risk predictors. In the general population, one in four hypertension diagnoses are missed without a 24-hour ambulatory blood pressure monitoring.[33] Measuring 24-hour blood pressure pattern after pregnancy will help to objectify the actual hypertension percentages in these women.

Lipid profile

An atherogenic lipid profile, characterized by elevated total-cholesterol, low density lipoprotein (LDL)-cholesterol triglycerides, lipoprotein (a) and low high density lipoprotein (HDL)-cholesterol, can lead to atherosclerosis in later life. Previous studies showed that women with a hypertensive disorder of pregnancy are more often affected by an atherogenic lipid profile after pregnancy than women with uncomplicated pregnancies.[11, 34-36] Possibly, a hypertensive disorder of pregnancy leads to a more atherogenic lipid profile after pregnancy or an atherogenic lipid profile precedes the hypertensive disorder. Obtaining better insight in this mechanism will help to improve cardiovascular risk prevention strategies.

Microvasculature

The microvasculature in the eye can be visualized through non-invasive retinal vascular imaging. A suboptimal microvasculature is characterized by smaller retinal arteries and wider retinal venules and is associated with an increased risk of future CVD. A previous study showed that endothelial dysfunction is associated with a suboptimal microvasculature in the general population.[37] Women with a hypertensive disorder of pregnancy show endothelial dysfunction both in pregnancy and long after.[38, 39] Therefore, they might be more at risk for a suboptimal microvasculature and consequently future CVD. Visualizing the microvasculature after pregnancy might help to understand which women are most at risk for future CVD and understand the pathophysiological mechanisms involved.

Cardiac measurements and arterial stiffness

Acquired cardiac abnormalities, such as a larger left ventricular mass, larger left atrial diameter and larger aortic root diameter, are associated with an increased risk of CVD. A larger pulse wave velocity or carotid intima-media thickness, measures of arterial stiffness and subclinical atherosclerosis which often coincide with hypertension, are also risk factors of CVD. Identifying early markers of these aberrations can help to detect those women most at risk for CVD and to start treatment for underlying risk factors.

Coronary artery calcification

Coronary artery calcification is an important precursor of ischemic heart disease. Underlying risk factors, such as hypertension, diabetes, obesity and renal dysfunction, are prevalent in women with a previous hypertensive disorder of pregnancy. This might explain their increased risk of ischemic heart disease later in life.[12] Coronary artery calcification progression can be potentially halted or even attenuated by adapting a healthy lifestyle and implementing blood pressure and cholesterol control.[40] Early detection of coronary artery calcification could help to identify women at increased cardiovascular risk before they present with symptomatic CVD.

Cardiovascular health scores

The cardiovascular health score was created by the American Heart Association to improve cardiovascular health of all Americans.[41] The score consists of seven metrics: three health factors (blood pressure, and total-cholesterol and glucose concentration) and four health behaviors (body mass index, smoking habit, diet and physical activity). Healthier metrics result in a higher score, which is associated with a lower risk of CVD.[42, 43] It remains unclear whether the increased risk associated with a hypertensive disorder of pregnancy results from the disorder itself or predisposing risk factors. The cardiovascular health score in early pregnancy can visualize these risk factors in a single score, which can be reassessed after pregnancy. The latter might be a better approach in cardiovascular risk assessment of premenopausal women with a previous hypertensive disorder of pregnancy than applying conventional cardiovascular risk prediction models, such as Framingham and SCORE, which classify these women into a low risk category.[11]

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PART I

Biomarkers in pregnancy

PART II

Cardiovascular risk factors

Chapter

4

Blood pressure profile one year after severe preeclampsia

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Abstract

Background: Preeclampsia increases the long-term risk of cardiovascular disease, possibly through occurrence of hypertension after delivery such as masked hypertension, night-time hypertension and an adverse systolic night-to-day blood pressure ratio. These types of hypertension are often unnoticed and can only be detected with ambulatory blood pressure monitoring. We aimed to determine hypertension prevalence and 24-hour blood pressure pattern with ambulatory blood pressure monitoring and office blood pressure measurements in women one year after severe preeclampsia.

Methods: This is a retrospective cohort study. As part of a follow-up program after severe preeclampsia 200 women underwent ambulatory blood pressure monitoring and an office blood pressure measurement one year after delivery. We calculated hypertension prevalence (sustained hypertension, masked hypertension and white-coat hypertension) and systolic night-to-day blood pressure ratio (dipping pattern). Medical files and questionnaires provided information on pre-existing hypertension and antihypertensive treatment.

Results: One year after delivery, 41.5% of women had hypertension (sustained hypertension, masked hypertension or white-coat hypertension) with ambulatory blood pressure monitoring. Masked hypertension was most common (17.5%), followed by sustained hypertension (14.5%) and white-coat hypertension (9.5%). With sheer office blood pressure measurement only 24.0% of women would have been diagnosed hypertensive. Forty-six percent of women a disadvantageous dipping pattern.

Conclusions: Hypertension is common one year after experiencing severe preeclampsia. Masked hypertension and white-coat hypertension are risk factors of future cardiovascular disease and can only be diagnosed with ambulatory blood pressure monitoring. Therefore, ambulatory blood pressure monitoring should be offered to all these women at high risk for developing hypertension and possibly future cardiovascular disease.

Introduction

Preeclampsia affects three to five percent of pregnancies in the developed world and is characterized by hypertension and new onset of proteinuria or organ dysfunction after 20 weeks of gestation.[1, 2] Severe preeclampsia is characterized by organ damage and/or fetal growth restriction.[3] Preeclampsia increases not only the short-term risk of morbidity and mortality for mother and child but also the lifetime risk of cardiovascular disease (CVD). Women with severe preeclampsia can be seven times more susceptible to develop future CVD compared to women with a normotensive pregnancy.[4] It is unclear whether this is a direct result of only preeclampsia or the constitutional risk factors of preeclampsia and CVD. Recent studies have shown that women with preeclampsia more often have hypertension after pregnancy, which contributes considerably to their lifetime CVD and stroke risk.[5-9] Accurate and early hypertension diagnosis and treatment is important, as hypertension can explain up to half of an individual's risk for developing CVD.[10] Previous studies examining blood pressure (BP) and hypertension prevalence after preeclampsia, usually only measured office BP. [5, 7, 9] However, 24-hour ambulatory blood pressure monitoring (ABPM) is the gold standard to rule out masked hypertension or white-coat hypertension (WHC) and to assess systolic night-to-day BP ratio, also known as dipping pattern. Dipping pattern carries important prognostic information for future CVD risk; a disadvantageous dipping pattern (insufficient fall in systolic BP during night-time compared to daytime), even in combination with a normotensive ABPM, carries a similar CVD risk as an elevated ABPM.[11] Sustained hypertension, masked hypertension, WCH and a disadvantageous dipping pattern are all independent risk factors for future CVD and therefore clinically important.[12-14, 11, 15-17] In the general population, 25% of hypertension diagnoses are missed when solely using office BP and when ABPM is used as the reference standard.[18] We hypothesize that this percentage is even higher in women with previous severe preeclampsia. Therefore, ABPM might help to improve diagnosis and management of hypertension after severe preeclampsia. The aim of this study was to determine the prevalence of hypertension and 24-hour BP pattern in women one year after severe preeclampsia.

Methods

Design and study population

The authors declare that all supporting data are available within the article and its online supplementary files. This descriptive study included women referred to the Follow-Up Preeclampsia (FUPEC) outpatient clinic in Erasmus Medical Center (EMC), the Netherlands between April 2011 and September 2017. This multidisciplinary clinic provides a specifically designed program for long-term cardiovascular follow-up of women with previous severe preeclampsia, including ABPM one year after delivery. For the present study we included women with previous severe preeclampsia and data available on ABPM and office BP measured within nine to 15 months after delivery. Women were excluded when they were diagnosed with acute fatty liver disease or mild preeclampsia during the index pregnancy, or when they were pregnant during follow-up or had been pregnant between index pregnancy and follow-up. The final population for analysis comprised 200 women (Figure 1). This non-interventional study was approved by the EMC Medical Ethics Committee.

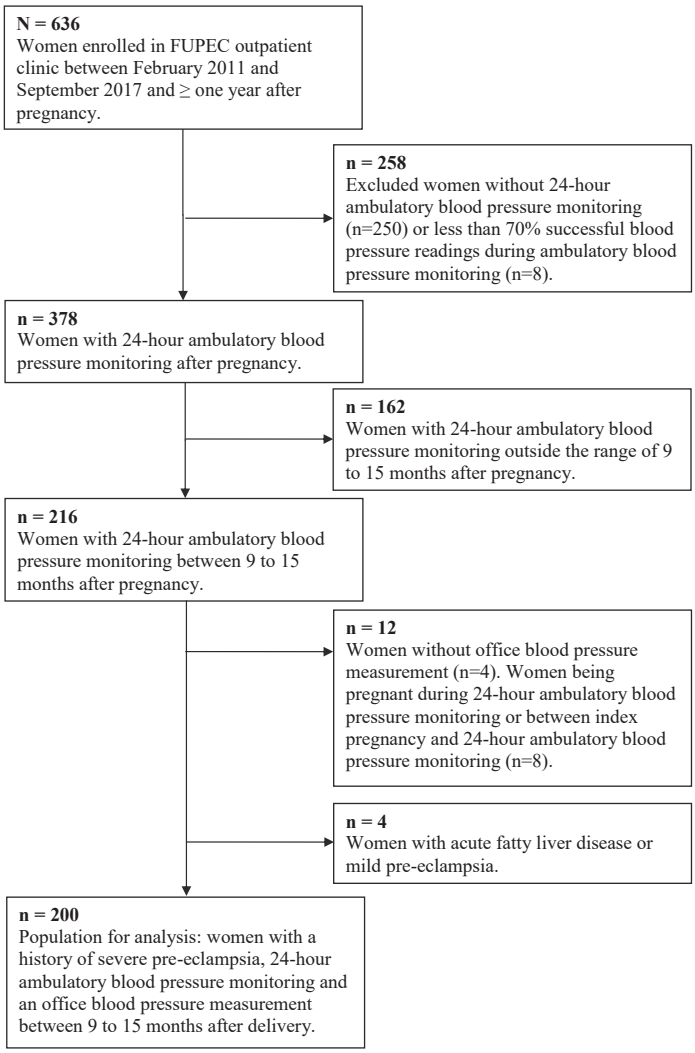

FIGURE 1 | Flowchart.

Severe preeclampsia

We used the ACOG 2002 criteria that were in effect at the time of inclusion to define preeclampsia as new onset hypertension (systolic blood pressure [SBP] ≥ 140 mmHg and/or diastolic blood pressure [DBP] ≥ 90 mmHg) after 20 weeks of gestation, and the presence of proteinuria with no evidence of urinary tract infection in a random urine sample.[3] Severe preeclampsia was accordingly classified as preeclampsia including one or more of the following criteria: SBP ≥ 160 mmHg and/or DBP ≥ 110 mmHg on two occasions at least six hours apart, proteinuria ≥ 5 g/24-hours or ≥ 3+ on two urine samples collected at least 4 hours apart, oliguria (<500 mL/24 hours), cerebral or visual disturbances, pulmonary edema or cyanosis, epigastric or right upper-quadrant pain, impaired

liver function (aspartate [AST] >70 U/L), thrombocytopenia (platelets <100 x 10^9/L), or fetal growth restriction (less than the 10th percentile). Severe preeclampsia for women with pre-existing hypertension was defined as hypertension before 20 weeks of gestation with an acute exacerbation in the second half of pregnancy combined with new-onset proteinuria (≥ 0.3 g/24h), or a sudden increase (doubling) in prior proteinuria, and supportive features of multisystem and/or fetal manifestations (e.g. neurological symptoms, HELLP syndrome or fetal growth restriction).[19]

Pregnancy and follow-up information

We obtained information on maternal characteristics during pregnancy and pregnancy outcomes from medical files and midwifery charts. Women received a questionnaire three months and one year after delivery which provided details on: Ethnicity, education, pre-existing hypertension (based on doctor diagnosis or antihypertensive medication prescription before pregnancy), previous diagnosis of hypertension (prior to ABPM and office BP assessment during follow-up), smoking (at any moment during pregnancy and during follow-up), breastfeeding (directly after delivery and during follow-up), intervening pregnancies (pregnancies between the index pregnancy and follow-up) and medication prescription (during follow-up).[20, 21] We cross-checked information from the questionnaire with the information from medical files and midwifery charts.

HELLP syndrome (hemolysis, elevated liver enzymes and low platelet count) was defined according to the class I and II 2006 Mississippi criteria (platelet count ≤ 100·10^9/L, AST or alanine [ALT] ≥ 40 IU/L and lactic acid dehydrogenase [LDH] ≥ 600 IU/L).[22]

We used the Niklasson growth curve to define a birth weight less than the 10th percentile, adjusted for gestational age and child's sex, as small for gestational age.[23] To calculate body mass index (BMI) (kg/m^2) we used the formula weight/(height * height). Weight, height and BP in early pregnancy (<15 weeks of gestation) were obtained from medical files or midwife charts. One year after delivery, maternal weight and height were measured by a trained nurse or research assistant.

Kidney function was evaluated at the time of ABPM and office BP measurement through the CKD-EPI glomerular filtration rate ([GFR] with GFR above 90 ml/min/1.73m^2 defined as a normal kidney function and GFR 60-90 ml/min/1.73m^2 as a mildly decreased kidney function) and albuminuria (microalbuminuria <30 mg/g as normal albuminuria, microalbuminuria 30-300 mg/g as moderately increased albuminuria and microalbuminuria >300 mg/g as severely increased albuminuria).[24]

Blood pressure at follow-up

A trained nurse or research assistant measured office BP in the upright sitting position after at least five minutes of rest. The appropriate arm cuff was placed around the right upper arm in order to measure BP with a validated oscillometric device. Women were not allowed to speak during the BP measurement.

We carried out 24-hour ABPM through portable BP devices with an embedded oscillometric technique (SpaceLabs Healthcare 90207-1Q and 90217-90, SpaceLabs Inc, Redmons, USA; Oscar 2, SunTech Medical Inc, Morrisville, USA; ABPM 6100 Monitor, Welch Allyn Inc, Skaneateles Falls, USA; Mobil-O-Graph NG Classic and Mobil-O-Graph 24h PWA, I.E.M. GmbH, Stolberg, Germany; WatchBP® O3, Microlife, Hoofddorp, the Netherlands; Reynolds Medical Tracker NIBP, Del Mar

Reynolds Medical, Ltd, Hertford, UK; BOSO TM2430, Bosch and Sohn GMBH U.CO.KG, Jungingen, Germany). Over the course of 24 hours, ABPM was carried out at 30 minute intervals during daytime (between 7:00 AM and 23:00 PM) and at 60 minute intervals during night-time. Beforehand, women received instructions to continue with their normal daily activities during ABPM. The analyses were restricted to women with at least 70% successful ABPM readings (both day and night-time), no more than 1 hour intervals of lacking ABPM data and a night-time sleep period between 6-12 hours during ABPM. Women received a diary to report their activities and their bedtime and awakening time.

Hypertension was defined according to the international guidelines of the ESH and ESC: Office hypertension (average SBP ≥140 mmHg and/or average DBP ≥ 90 mmHg), daytime hypertension with ABPM (average SBP ≥135 mmHg and/or average DBP ≥ 85 mmHg), nighttime hypertension with ABPM (average SBP ≥120 mmHg and/or average DBP ≥ 70 mmHg), sustained hypertension (office hypertension in combination with daytime hypertension measured with ABPM), masked hypertension (normotensive office BP in combination with daytime hypertension measured with ABPM) and WCH (office hypertension in combination with normotensive daytime ABPM).[25] Overall SBP and DBP were defined as the average SBP and DBP over a 24 hour period measured with ABPM.

We constructed systolic night-to-day BP ratios from the ABPM readings and categorized these in four dipping patterns: Reverse dippers (ratio > 1.0), non-dippers (ratio > 0.9 and ≤ 1.0), dippers (ratio > 0.8 and ≤ 0.9) and extreme dippers (ratio ≤ 0.8).[12] Thereafter, we combined the reverse dipping and non-dipping categories in the disadvantageous dipping pattern group, and the dipping and extreme dipping categories in the normal dipping pattern group.

Statistical analyses

First, a non-response analysis was carried out to compare baseline characteristics between women included and excluded from this study. Differences in baseline characteristics were tested using Students t-test and chi-square tests. Second, baseline and follow-up characteristics were examined (Table 1 and Table 2). Third, percentages were determined of women with sustained hypertension, masked hypertension and WCH (Figure 2). Fourth, the percentage of women within each dipping category (dippers and extreme dippers vs. non-dippers and reverse dippers) was determined amongst women with a normotensive or hypertensive ABPM (Figure 3). Fifth, a sensitivity analysis was performed in women with a normal to mildly decreased kidney function (Supplemental Table 1 and Supplemental Figures 1 and 2) and a second sensitivity analysis in women measured with the ABPM SpaceLabs device (Supplemental Table 2 and Supplemental Figures 3 and 4). In a third sensitivity analysis we examined the overlap between pre-existing hypertension (hypertension before the onset of pregnancy) and postpartum hypertension (Supplemental Table 3). Sixth, prior to logistic regression analyses we performed multiple imputation procedures in order to reduce potential bias in covariates due to missing data. Logistic regression analyses were carried out to determine potential risk factors for postpartum hypertension. Covariates in the regression models were selected based on: their association with the outcome of interest, previous studies or a change in effect estimate of more than 10%. The basic regression model included maternal age during ABPM. The full regression model included: maternal age during ABPM, small for gestational age below the 10th percentile, education, ethnicity, first trimester diastolic blood pressure and first trimester BMI. All analyses were performed with Statistical Package of Social Sciences version 21.0 for Windows (IBM Corp., Armonk, NY, USA).

TABLE 1 | Baseline characteristics.

Outcomes	Women N = 200
Maternal characteristics during index pregnancy	
Maternal age in years, mean (SD)	31.6 (4.8)
Ethnicity, n (%)	
Caucasian	166 (83.0)
African descent	21 (10.5)
Asian/South-Asian	13 (6.5)
Pre-existing hypertension, n (%)	29 (14.6)
Antihypertensive medication intake before pregnancy, n (%)	14 (7.2)
Nulliparity, n (%)	140 (70.0)
Multiple pregnancy (twin or triplet pregnancy), n (%)	16 (8.0)
§Gestational age at diagnosis of preeclampsia in weeks, median (IQR)	30.5 (5.0)
Time in days between diagnosis and delivery, median (90% range)	3.0 (0.0, 22.1)
HELLP syndrome class I and II of Mississippi criteria, n (%)	27 (14.2)
Early onset preeclampsia (<34 weeks of gestation), n (%)	146 (73.0)
Recurrent preeclampsia among multiparous women, n (%)	14 (23.3)
First trimester BMI (kg/m2), median (90% range)	24.4 (19.0, 35.6)
§First trimester systolic BP (mmHg), median (IQR)	120.0 (16.0)
§First trimester diastolic BP (mmHg), median (IQR)	73.5 (10.0)
Smoking at any moment during pregnancy, n (%)	24 (12.6)
Breastfeeding directly after delivery, n (%)	120 (83.9)
Pregnancy outcomes	
Gestational age at delivery, mean (SD)	31.7 (3.7)
Boys, n (%)	96 (48.0)
Neonatal death, n (%)	6 (3.0)
Birth weight gram, median (90% range)	1350 (515, 3210)
*Small for gestational age (less than the 10th percentile), n (%)	127 (69.0)
Mode of delivery, n (%)	
Spontaneous	28 (14.0)
Assisted delivery	6 (3.0)
Elective cesarean	5 (2.5)
Emergency cesarean	161 (80.9)
Maternal characteristics during follow-up	
Time interval in years between delivery and ABPM, median (90% range)	1.0 (0.90, 1.2)
Time interval in days between office BP measurement and ABPM, median (90% range)	16.0 (0.05, 49.0)
Education, n (%)	
None/primary	5 (3.5)
Lower secondary	62 (43.7)
Upper secondary	6 (4.2)
Academic	69 (48.6)
BMI (kg/m2), median (90% range)	25.5 (19.3, 36.6)
Smoking, n (%)	23 (13.5)
Breastfeeding, n (%)	14 (7.6)
Antihypertensive medication prescription, n (%)	41 (20.5)
Previously diagnosed hypertension, n (%)†	48 (24.0)

Outcomes	Women N = 200
Normal GFR, n (%)	
Normal albuminuria	118 (66.3)
Mildly increased albuminuria	25 (14.0)
*Severely increased albuminuria	4 (2.2)
Mildly decreased GFR, n (%)	
Normal albuminuria	26 (14.6)
Mildly increased albuminuria	3 (1.7)
Severely increased albuminuria	2 (1.1)

Abbreviations: Ambulatory blood pressure monitoring, ABPM; Body mass index, BMI; Blood pressure, BP; Glomerular filtration rate, GFR; Hemolysis, elevated liver enzymes and low platelet count, HELLP; Interquartile range, IQR. Values are valid percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians with 90% range or IQR§ for continuous variables with a skewed distribution.
*Based on weight - gestational age (24-40weeks) / Sweden 2008 (Sweden) / Niklasson.
†Occurred prior to ABPM and office BP assessment during follow-up and was based on doctor diagnosis or antihypertensive medication prescription. ǂ Women with severely increased albuminuria had pre-existing hypertension (n = 3), preeclampsia or HELLP syndrome in a previous pregnancy (n = 2), gestational diabetes in the index pregnancy (n = 1) or no previous disease (n = 1).

TABLE 2 | Blood pressure one year after pregnancy.

Outcomes	Women N = 200
Systolic blood pressure (mmHg), median (IQR)	
Office	120.5 (21.0)
Daytime ABPM	124.0 (19.0)
Night-time ABPM	111.0 (17.0)
Overall ABPM	121.0 (19.0)
Diastolic blood pressure (mmHg), median (IQR)	
Office	78.0 (13.0)
Daytime ABPM	79.0 (12.0)
Night-time ABPM	66.0 (13.0)
Overall ABPM	75.5 (13.0)
Hypertension, n (%)	
Daytime hypertension with ABPM (135/85 mmHg)	64 (32.0)
Night-time hypertension with ABPM (120/70 mmHg)	85 (42.5)
Hypertension with office BPM (140/90 mmHg)	48 (24.0)
Systolic night-to-day BP ratio, n (%)	
Reverse dippers	12 (6.0)
Non-dippers	79 (39.5)
Dippers	99 (49.5)
Extreme dippers	10 (5.0)

Abbreviations: Ambulatory blood pressure monitoring, ABPM; Interquartile range, IQR. Values are numbers (n) and valid percentages for categorical variables and medians with interquartile range for continuous variables.

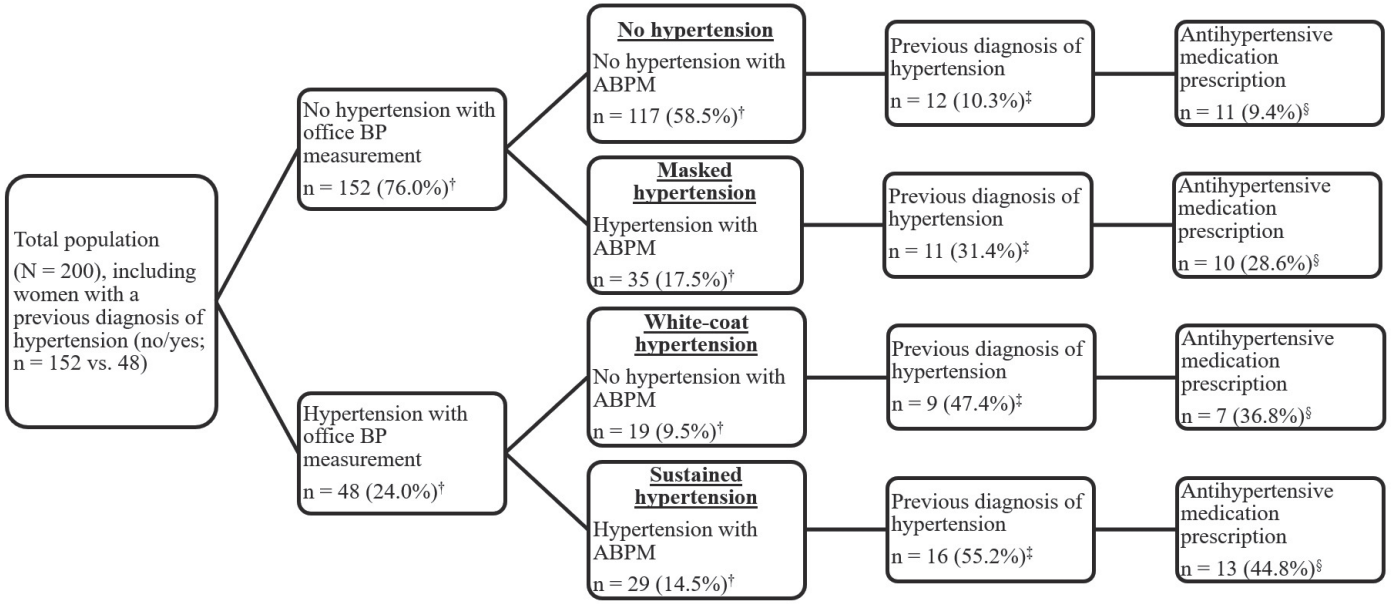

FIGURE 2 | The association between office hypertension prevalence, hypertension prevalence with ambulatory blood pressure monitoring, previous diagnosis of hypertension and antihypertensive medication prescription (N = 200).

Abbreviations: Blood pressure, BP; ambulatory blood pressure monitoring, ABPM. Hypertension with office BP: Systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg. Hypertension with daytime ABPM: Systolic blood pressure ≥135 mmHg and/or diastolic blood pressure ≥85 mmHg. †Values are calculated as percentages of the total population (N = 200). ‡Values are calculated as percentages of the number of women in the previous box. The diagnosis of hypertension occurred prior to ABPM and office BP assessment during follow-up and was based on doctor diagnosis and/or antihypertensive medication prescription. §Values are calculated as percentages of the number of women in the third row of boxes.

Results

Table 1 shows pregnancy outcomes, and women's characteristics during the index pregnancy and follow-up. During the index pregnancy, women were on average 31.6 years old (standard deviation [SD] 4.8) and mostly nulliparous (70.0%). The average gestational age at diagnosis was 30.5 weeks (SD 5.0) with the majority of women having an early onset preeclampsia (73.0%). Slightly more girls (52.0%) than boys were born and children were mostly small for gestational age (69.0%). A large percentage (48.6%) of women was academically educated. At the time of ABPM and office BP measurement, 20.5% of women were already receiving treatment for previous diagnosed hypertension. Kidney function was determined through GFR and albuminuria levels at the time of ABPM and office BP measurement (Table 1). A small number of women (n = 6) had severely increased albuminuria one year after pregnancy of which the majority (83%) had pre-existing comorbidity associated with impaired renal function (data not shown). Of women with moderately increased albuminuria (n = 28), 40% had pre-existing comorbidity associated with impaired renal function (data not shown). Excluding women with moderately and severely increased albuminuria from our analyses did not change the results substantially (Supplemental Table 1 and Supplemental Figures 1 and 2).

Associations between office hypertension prevalence, hypertension prevalence with ABPM, previous diagnosis of hypertension and antihypertensive medication prescription is shown in Figure 2. In total, 41.5% of women had some form of hypertension (sustained hypertension [14.5%], masked hypertension [17.5%] or WCH [9.5%]). A percentage of women with sustained hypertension, masked hypertension or WCH had been diagnosed with hypertension prior to ABPM and office BP measurement (55.2%, 31.4% and 47.4%, respectively) or prior to the onset of pregnancy (Supplemental Table 3). Figure 2 also shows that 75.0% of women (36 out of 48) with a diagnosis of hypertension prior to ABPM and office BP measurement either did not receive optimal antihypertensive drug treatment or were non-adherent to their antihypertensive treatment.

Table 2 shows mean blood pressures, hypertension prevalence and dipping pattern one year after delivery. The hypertension prevalence was higher during night-time ABPM (42.5%) than during daytime ABPM (32.0%) or office BP measurement (24.0%). Systolic night-to-day BP ratio showed a reverse dipping pattern or non-dipping pattern in 45.5% of women.

We examined the association between all characteristics mentioned in Table 1 and the risk of any type of hypertension after pregnancy through multivariate logistic regression analysis (data not shown). Only pre-existing hypertension before pregnancy and BMI were associated with hypertension after pregnancy. Pre-existing hypertension was associated with an increased risk for: daytime hypertension with ABPM (Odds ratio [OR] 2.8; 95% Confidence Interval [CI] 1.0, 7.7, *P*-value 0.048), office hypertension (OR 3.1; 95% CI 1.0, 9.3, *P*-value 0.042) and sustained hypertension (OR 7.5; 95% CI 1.7, 32.0, *P*-value 0.007). First trimester BMI was solely associated with an increased risk of office hypertension (OR 1.1; 95% CI 1.0, 1.2, *P*-value 0.04).

Figure 3 shows the association between systolic night-to-day BP ratio dipping pattern and hypertension status defined with daytime ABPM. A disadvantageous dipping pattern was seen in 45.6% of women with a normotensive daytime ABPM and in 45.3% of women with a hypertensive daytime ABPM.

Non-response analysis showed that women excluded from this study (due to missing ABPM) were on average slightly older at the time of follow-up (34.1 years [SD 7.7]) compared to women included in the study (32.7 years [SD 4.8], p-value 0.02). No differences were observed in ethnicity and educational level between women included and excluded from the study. Lastly, sensitivity-analysis showed that the use of various ABPM devices (other than the SpaceLabs device) did not change our results substantially (Supplemental Table 2 and Supplemental Figures 3 and 4).

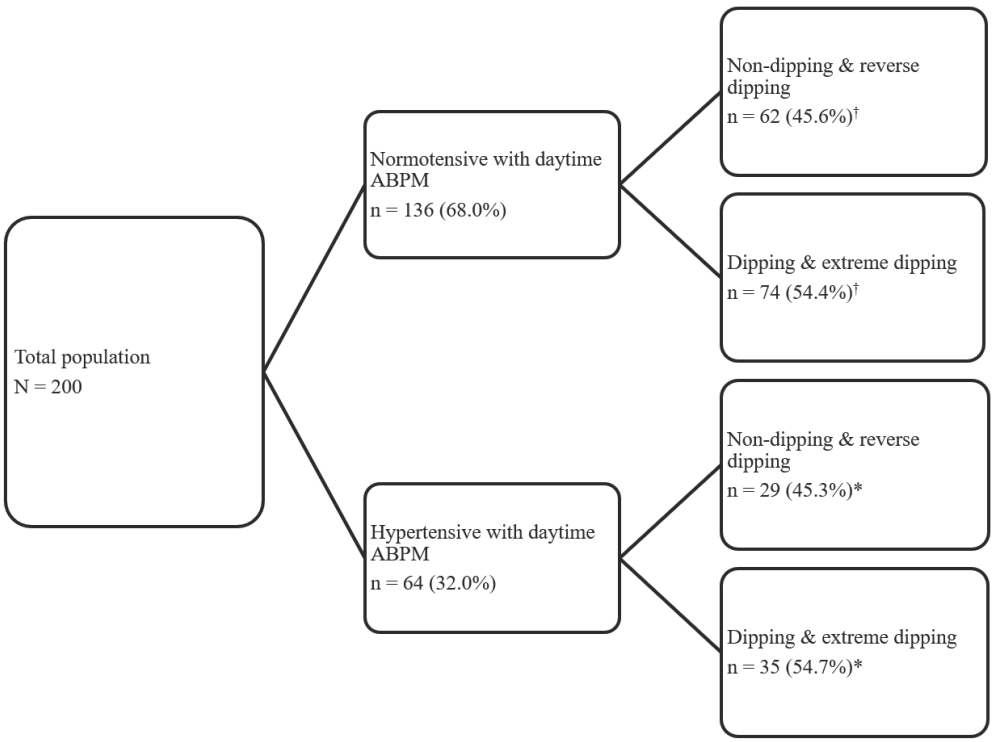

FIGURE 3 | The association between systolic night-to-day blood pressure ratio (dipping pattern) and hypertension prevalence with ambulatory blood pressure monitoring (N = 200).
Abbreviations: Ambulatory blood pressure monitoring, ABPM. Hypertension is defined as daytime hypertension with ambulatory blood pressure monitoring (≥135/85 mmHg). †Values are calculated as percentages of women with a normotensive daytime ambulatory blood pressure monitoring. *Values are calculated as percentages of women with hypertension during daytime ambulatory blood pressure monitoring.

Discussion

Our study of 200 women with previous severe preeclampsia shows that 41.5% of women have some form of hypertension one year after delivery based on ABPM. ABPM was essential to diagnose masked hypertension (17.5% of the total population) and WCH (9.5% of the total population) in 27% of women. Additionally, 45.5% of women had a disadvantageous systolic night-to-day BP ratio dipping pattern. This percentage was equal between women with a normotensive and hypertensive daytime ABPM.

It is well known that women with previous preeclampsia have an increased risk of CVD later in life,[1, 2, 4] especially in women with early-onset and/or severe preeclampsia [4]. The exact pathophysiological mechanism leading to this increased CVD risk remains unknown and might in fact be multifactorial. A previous study showed increased sensitivity to angiotensin II during and after a hypertensive pregnancy.[26] This increases the risk of persistence or development of hypertension after delivery.[5-9, 26] Clinical guidelines on hypertensive disorders in pregnancy and guidelines on the prevention of stroke therefore recommend BP screening in women with previous preeclampsia.[27, 28] However, specific recommendations regarding the appropriate method and timing to measure BP after preeclampsia is not provided, possibly because there are very few studies available examining 24-hour BP pattern after preeclampsia.[29] With regard to the method of BP measurement, we show that ABPM after a severe preeclampsia provides important additional information aside of office BP. Various types of hypertension (e.g. masked hypertension, WCH and night time hypertension) can be diagnosed with ABPM which would otherwise remain unnoticed. These types of hypertension and systolic night-to-day BP ratio dipping pattern are predictors of future CVD, independent of office hypertension.[17]

In our study 17.5% of women had masked hypertension, which has been associated in other studies with an increased risk of developing sustained hypertension, cardiovascular events and cardiovascular mortality, independent of office BP.[30-33] The prevalence of masked hypertension amongst women in the general population is 9.5%.[34] A known risk factor for masked hypertension is pre-hypertension (BP 130–139/85–89 mmHg), which affected 15.0% of women in our study. Seventeen to 30.3% of individuals with pre-hypertension will eventually develop masked hypertension. A large percentage (38%) of women with previous severe preeclampsia will ultimately be diagnosed with hypertension (based on office BP measurement) after nine to 16 years after delivery.[5] Possibly, these women are still in a pre-hypertensive state during the first decade after delivery, which makes them more vulnerable to develop masked hypertension.

The percentage of women with WCH in our study is comparable to percentages described in a large study of 115,708 Spanish patients with treated and untreated hypertension.[35] WCH is an important diagnosis because these individuals have an increased risk of developing sustained hypertension and target organ damage (e.g. microalbuminuria and left ventricular hypertrophy) compared to normotensive controls.[31, 35-37] Therefore, BP should be monitored after diagnosis of WCH for timely diagnosis of the development of sustained hypertension. However, WCH should not be treated with antihypertensive medication as this can lead to hypotension.[12, 38]

Night-time hypertension affected 42.5% of women in our study. Even a minor rise in night-time BP increases the risk of cardiovascular events, stroke, and non-cardiovascular and cardiovascular mortality.[39] Accordingly, progression towards a disadvantageous dipping pattern (from dipping, to non-dipping to reverse dipping pattern) also progressively increases the risk of future CVD by inducing subclinical target organ damage.[39, 40] Several pathophysiological mechanisms have been suggested to explain the insufficient reduction of night-time BP, including deficient decrease of night-time sympathetic activity.[40, 41] Sympathetic activity is inversely associated with the difference in day-to-night BP, suggesting that it may influence 24-hour BP pattern in hypertensive individuals.[41] In women with preeclampsia, sympathetic over-activity is described both during and after pregnancy.[29, 42-44] This might explain the high prevalence of night-time hypertension and disadvantageous dipping patterns in our study population compared to other study cohorts.[45, 46] The prevalence of a disadvantageous dipping pattern, especially the percentage of women with a non-dipping pattern was higher in our study population compared to that of a large meta-analysis conducted in European, Japanese, Chinese and South-American women (39.5% vs. 24.4%).[39] Interestingly, women in the aforementioned study were more than 20 years older than women in our study (mean age 56.8 years [SD 13.9] vs 32.7 years [SD 4.8]).

Results of our study should be interpreted within the context of some limitations. First, due to the descriptive design of this study differences in hypertension prevalence and dipping pattern between women with previous preeclampsia and women with a previous normotensive pregnancy or other gestational hypertensive disorder (e.g. gestational hypertension or mild preeclampsia) cannot be examined. Second, findings may not be generalizable to all women with previous severe preeclampsia because the majority of women were Caucasian and highly educated. Further analyses showed that women excluded from this study, due to missing ABPM, were on average slightly older at the time of follow-up compared to women included in the study. Third, 15.7% and 3.4% of women had moderately increased and severely increased albuminuria one year after pregnancy, which could affect BP. Nevertheless, our results did not change substantially when these women were excluded from our analyses (Table S1 and Figures S1 and S2). Fourth, we measured office BP and ABPM once, which might have reduced the diagnostic accuracy of true BP values. Previous studies showed that BP obtained from ABPM can be more accurately reproduced than BP obtained from office measurement[47, 48] and that the diagnoses of masked hypertension and WCH are well reproducible in short-term but tend to shift towards sustained hypertension in the long-term.[49] Limited evidence suggests that repeated measurements of office BP improve the diagnosis of hypertension [50]. It seems therefore unlikely that we overestimated the percentage of women with sustained hypertension. Lastly, ABPM was not standardized for device type. Though the majority of women (73.6%) were examined with one device brand (SpaceLabs Healthcare 90207-1Q and 90217-90, SpaceLabs Inc, Redmons, USA) and all devices used the oscillometric technique with identical time intervals, we should take intra-device BP reproducibility in to account. Sensitivity analysis in women measured with the SpaceLabs devices showed similar results to those of the total population (Table S2 and Figures S3 and S4). Therefore, the use of various ABPM devices did not seem to affect our results.

Conclusion

We show that 41.5% of women with previous severe preeclampsia have some form of hypertension one year after delivery (sustained hypertension, masked hypertension or WCH) and that 45.5% of women have a disadvantageous systolic night-to-day BP ratio dipping pattern, which cannot be diagnosed without ABPM. Current clinical guidelines on the prevention of CVD and stroke after a hypertensive pregnancy disorder lack advice on ABPM after delivery. We believe that ABPM should be offered to all women who experienced severe preeclampsia for more accurate BP assessment. By doing so, hypertension management can be improved which eventually might reduce the risk of future CVD.

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Novelty and significance

What is new?

We examined 24-hour blood pressure profile and hypertension prevalence (sustained hypertension, masked hypertension or white-coat hypertension) through ambulatory blood pressure monitoring (ABPM) and an office blood pressure (BP) measurement in women with previous severe preeclampsia.

What is relevant?

Unrecognized masked hypertension and a disadvantageous systolic night-to-day BP dipping pattern are associated with an increased risk of cardiovascular disease (CVD). This might at least partly explain the increased risk of CVD after preeclampsia.

Summary

Not only is hypertension common (41.5%) one year after a severe preeclampsia, 17.5% of women suffer from masked hypertension and 45.5% of women have a disadvantageous systolic night-to-day BP dipping pattern. These are diagnoses that would remain undetected when only office BP measurement is performed.

Supplemental material

SUPPLEMENTAL TABLE 1 | Blood pressure one year after pregnancy in women with normal to mildly increased albuminuria .

Outcomes	Women n = 149
Systolic blood pressure (mmHg), median (IQR)	
Office	120.0 (22.0)
Daytime ABPM	122.5 (18.0)
Night-time ABPM	109.5 (18.0)
Overall ABPM	120.0 (18.00
Diastolic blood pressure (mmHg), median (IQR)	
Office	78.0 (13.0)
Daytime ABPM	78.6 (11.0)
Night-time ABPM	65.5 (13.0)
Overall ABPM	75.0 (12.0)
Hypertension, n (%)	
Daytime hypertension with ABPM (135/85 mmHg)	40 (26.8)
Night-time hypertension with ABPM (120/70 mmHg)	58 (38.9)
Hypertension with office BPM (140/90 mmHg)	33 (22.1)
Systolic night-to-day BP ratio, n (%)	
Reverse dippers	6 (4.0)
Non-dippers	58 (38.9)
Dippers	76 (51.0)
Extreme dippers	9 (6.0)

Abbreviations: Ambulatory blood pressure monitoring, ABPM; Interquartile range, IQR. Values are numbers (n) and valid percentages for categorical variables and medians with interquartile range for continuous variables.

SUPPLEMENTAL TABLE 2 | Blood pressure one year after pregnancy of the total population and women measured with the ABPM SpaceLabs device.

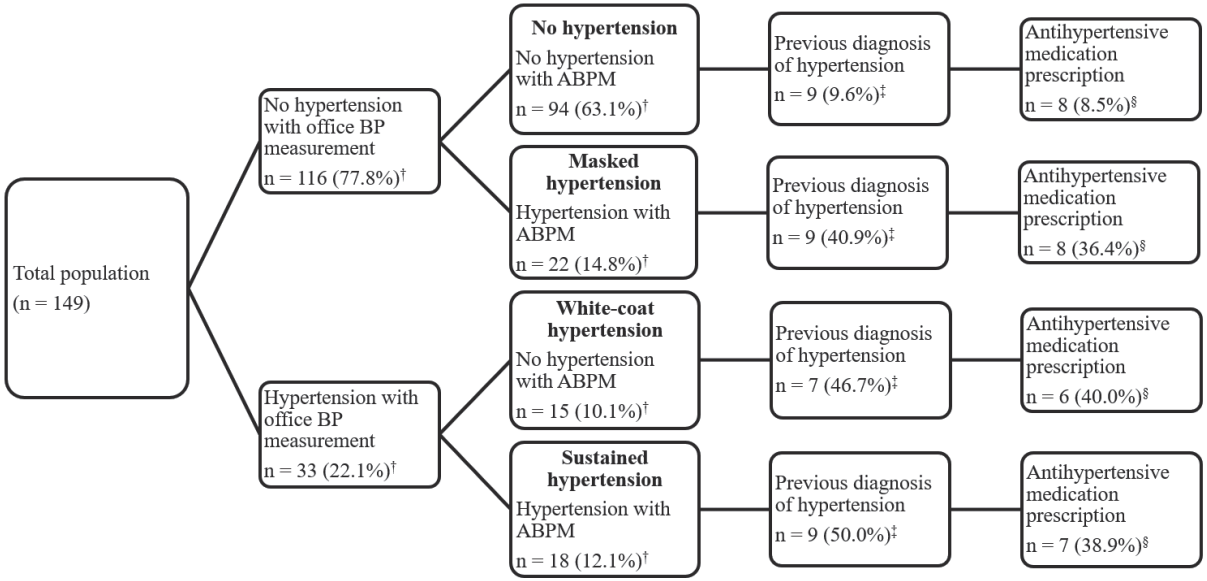
Outcomes	Total population N = 200	SpaceLabs device n = 134	*P*-value
Systolic blood pressure (mmHg), median (IQR)			
Office	120.5 (21.0)	121.5 (25.0)	0.79
Daytime ABPM	124.0 (19.0)	124.0 (17.0)	0.15
Night-time ABPM	111.0 (17.0)	111.0 (15.0)	0.15
Overall ABPM	121.0 (19.0)	120.0 (17.0)	0.15
Diastolic blood pressure (mmHg), median (IQR)			
Office	78.0 (13.0)	77.5 (14.0)	0.28
Daytime ABPM	79.0 (12.0)	79.0 (12.0)	0.71
Night-time ABPM	66.0 (13.0)	67.0 (13.0)	0.69
Overall ABPM	75.5 (13.0)	75.5 (11.0)	0.66
Daytime hypertension with ABPM (135/85 mmHg), n (%)	64 (32.0)	42 (31.3)	0.78
Night-time hypertension with ABPM (120/70 mmHg), n (%)	85 (42.5)	57 (42.5)	0.97
Hypertension with office BPM (140/90 mmHg), n (%)	48 (24.0)	34 (25.4)	0.53
Nightly blood pressure dippers, n (%)			0.43
Reverse dippers	12 (6.0)	6 (4.5)	
Non-dippers	79 (39.5)	58 (43.3)	
Dippers	99 (49.5)	66 (49.3)	
Extreme dippers	10 (5.0)	4 (3.0)	

Abbreviations: Ambulatory blood pressure monitoring, ABPM; Interquartile range, IQR. Values are numbers (n) with valid percentages for categorical variables and medians with interquartile range for continuous variables. Statistical testing was carried out through Student's t-test for continuous variables, and through chi-square test for categorical variables.

SUPPLEMENTAL TABLE 3 | Blood pressure one year after pregnancy in women with pre-existing hypertension.

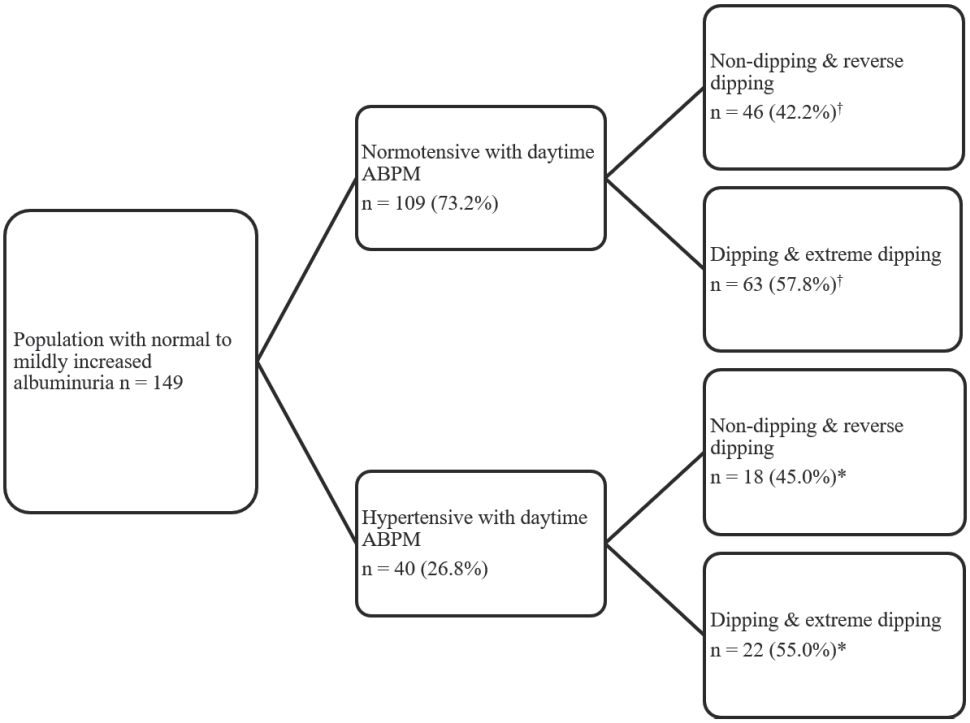
Outcomes	Total population N = 200	Women with pre-existing hypertension n = 29
Masked hypertension, n (%)	35 (17.5)	7 (24.1)
White-coat hypertension, n (%)	19 (9.5)	6 (20.7)
Sustained hypertension, n (%)	29 (14.5)	11 (37.9)
Daytime hypertension with ABPM (135/85 mmHg), n (%)	64 (32.0)	18 (62.1)
Night-time hypertension with ABPM (120/70 mmHg), n (%)	85 (42.5)	19 (65.5)
Hypertension with office BPM (140/90 mmHg), n (%)	48 (24.0)	17 (58.6)

Abbreviation: Ambulatory blood pressure monitoring, ABPM. Values are numbers (n) with valid percentages.

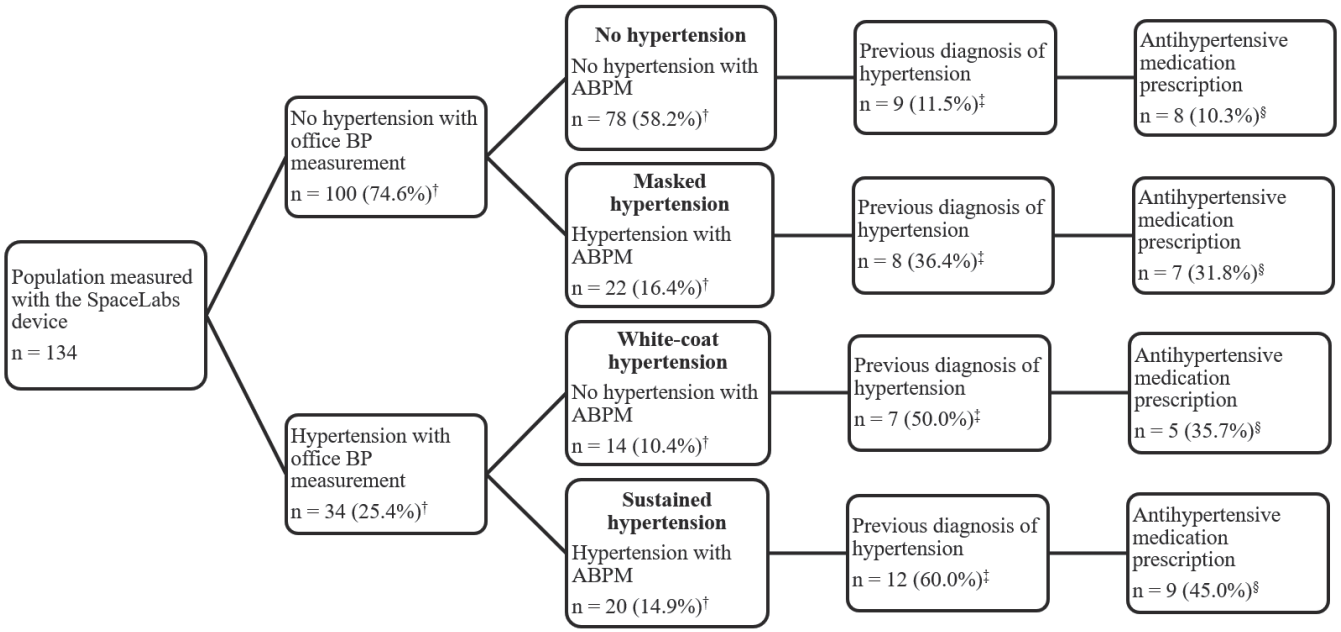


SUPPLEMENTAL FIGURE 1 | The association between office hypertension prevalence, hypertension prevalence with ambulatory blood pressure monitoring, diagnosis of hypertension and antihypertensive medication prescription in women with normal to mildly increased albuminuria (n = 149).

Abbreviations: Blood pressure, BP; ambulatory blood pressure monitoring, ABPM. Hypertension with office BP: Systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg. Hypertension with daytime ABPM: Systolic blood pressure ≥135 mmHg and/or diastolic blood pressure ≥85 mmHg. †Values are calculated as percentages of the total population (n = 149). ‡Values are calculated as percentages of the number of women in the previous box. The diagnosis of hypertension was based on doctor diagnosis and/or antihypertensive medication prescription previous to ABPM and office BP assessment during follow-up. §Values are calculated as percentages of the number of women in the third row of boxes.



SUPPLEMENTAL FIGURE 2 | The association between systolic night-to-day blood pressure ratio (dipping pattern) and hypertension rate with ambulatory blood pressure monitoring for women with normal to mildly increased albuminuria (n = 149).
Hypertension is defined as daytime hypertension with ambulatory blood pressure monitoring (≥135/85 mmHg). †Values are calculated as percentages of women with a normotensive daytime ambulatory blood pressure monitoring. *Values are calculated as percentages of women with hypertension during daytime ambulatory blood pressure monitoring.

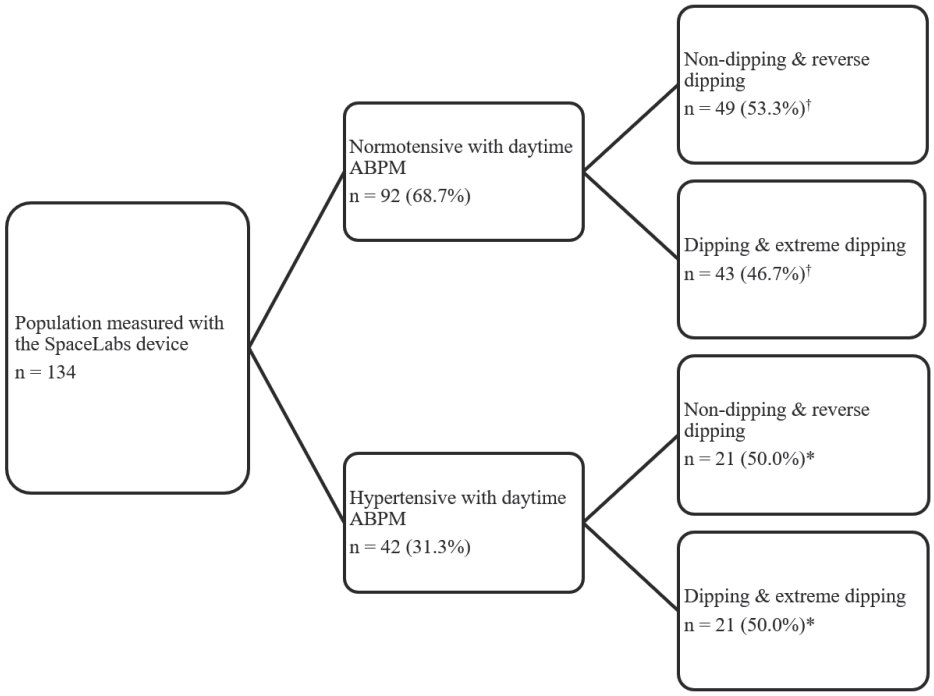


SUPPLEMENTAL FIGURE 3 | The association between office hypertension rate, hypertension rate with ambulatory blood pressure monitoring, previous diagnosis of hypertension and antihypertensive medication prescription for women measured with the SpaceLabs device (n = 134)

Abbreviations: Blood pressure, BP; ambulatory blood pressure monitoring, ABPM. Hypertension with office BP: Systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg. Hypertension with daytime ABPM: Systolic blood pressure ≥135 mmHg and/or diastolic blood pressure ≥85 mmHg.

†Values are calculated as percentages of the population measured with the SpaceLabs device (n = 134). ‡Values are calculated as percentages of the number of women in the previous box. The diagnosis of hypertension was based on doctor diagnosis and/or antihypertensive medication prescription previous to ABPM and office BP assessment during follow-up.

§Values are calculated as percentages of the number of women in the third row of boxes.



SUPPLEMENTAL FIGURE 4 | The association between systolic night-to-day blood pressure ratio (dipping pattern) and hypertension rate with ambulatory blood pressure monitoring for women measured with the SpaceLabs device (n = 134).

Hypertension is defined as daytime hypertension with ambulatory blood pressure monitoring (≥135/85 mmHg).

†Values are calculated as percentages of women with a normotensive daytime ambulatory blood pressure monitoring.

*Values are calculated as percentages of women with hypertension during daytime ambulatory blood pressure monitoring.

Chapter

5

Maternal lipid profile six years after a gestational hypertensive disorder

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Abstract

Background: Hypertensive disorders of pregnancy, including gestational hypertension and preeclampsia, are associated with an increased risk of cardiovascular disease (CVD) in later life, possibly through an atherogenic lipid profile. The objective of this study was to assess if women with a previous hypertensive disorder of pregnancy have a more atherogenic lipid profile six years after pregnancy compared to women with a previous normotensive pregnancy.

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

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

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

Introduction

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

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

Materials and methods

Design and study population

This study was embedded in the Generation R Study, a multi-ethnic and population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[26, 27] Approval has been obtained by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands.[28] For the present study we included women with: a live born singleton (exclusion of twin pregnancies), available information regarding postnatal development and information on the occurrence of a hypertensive disorder of pregnancy. Women were excluded when they had a history of chronic hypertension prior to enrollment in the Generation R Study, if they were pregnant during the follow-up visit or in case information on cholesterol

or glucose mediating medication at follow-up was missing. The final population for analysis comprised 4933 women (Figure 1). All women included in this study gave informed consent (MEC 198.782/2001/31).

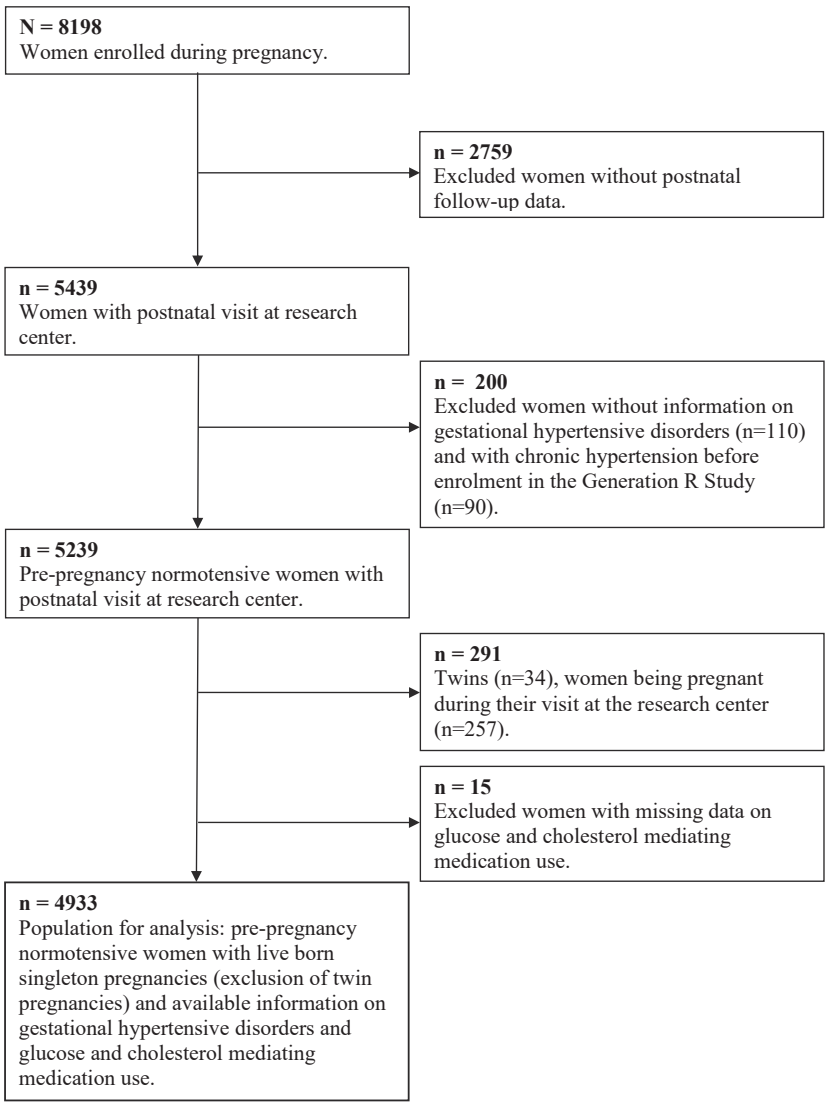

FIGURE 1 | Flowchart.

Blood pressure, hypertensive disorders of pregnancy and chronic hypertension

Trained research assistants wearing usual clothing (i.e. no white coats) measured blood pressure in early pregnancy (median 13.9 weeks of gestation [90% range 10.8, 22.5]) and six years after index pregnancy (median 6.0 years [90% range, 5.7 to 7.3]), with the validated Omron 907 automated

digital oscillometric sphygmomanometer (OMRON Healthcare Europe B.V., Hoofddorp, the Netherlands).[29] To prevent differences due to position, women sat in a standardized supine position with the cuff placed around the right upper arm. In case of an upper arm exceeding 33 centimeters a larger cuff (32 – 42 cm) was used. The mean value of two blood pressure readings over a five-minute interval was documented for each participant.

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

Maternal anthropometrics

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

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

Non-fasting blood samples were obtained six years after index pregnancy by trained research nurses and were temporarily stored at our research center at room temperature. Twice a day, these blood samples were transported to a dedicated laboratory facility of the regional laboratory in Rotterdam, the Netherlands (STAR-MDC) for further processing and storage at -80 °C. All collected EDTA plasma samples were processed within four hours after venous puncture.[27] Between 2013 and 2014 the samples were transferred from the STAR MC laboratory to the laboratory of Vascular Medicine of the Erasmus Medical Center Rotterdam. After thawing, the following lipids could be analyzed in the EDTA plasma samples: total-cholesterol (mmol/L), triglycerides (mmol/L), HDL-c (mmol/L), LDL-c (mmol/L), apoB (g/L) and Lp(a) (g/L). Also, plasma glucose (mmol/L) was determined. Samples were analyzed using the Vital Scientific (Merck) Selectra E Chemistry Analyzer (Vital Scientific N.V., Dieren, the Netherlands). Details of the mean range of the intra-assay and inter-assay precision with the coefficient of variation (CV) per lipid are provided in Supplemental Table 1. Remnant cholesterol was calculated as (total-cholesterol – LDL-c) – HDL-c and non-HDL-c level as total-cholesterol – HDL-c.

TABLE 1 | Subject characteristics by hypertensive disorder of pregnancy n = 4933.

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

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

Covariates

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

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA) and R version 3.0.0 (R foundation for Statistical Computing, Vienna, Austria, packages rmeta and metafor).[38] Maternal characteristics during pregnancy and follow-up were compared between women with a previous hypertensive disorder of pregnancy and women with a previous normotensive index pregnancy. We used Student's t-test to analyze continuous variables with a normal distribution (presented as means with

a standard deviation) and the Kruskal-Wallis test for variables with a skewed distribution (presented as medians with a 90% confidence interval [CI]). Categorical variables were analyzed with Chi-square tests. A *P*-value < 0.05 was considered statistically significant.

The association between a previous hypertensive disorder of pregnancy and lipid and glucose levels after pregnancy was assessed through linear and logistic regression analyses. Triglyceride levels were log transformed to obtain a normal distribution. Lp(a) could not obtain a normal distribution and was therefore analyzed with logistic regression analyses using a cut-off value above the 80th percentile. We also created cut-off values for lipid and glucose levels to define dyslipidemia and examine whether differences between women with a previous normotensive pregnancy and women with a previous gestational hypertensive disorder were driven by extreme values. Dyslipidemia was defined as exceeding the cut-off value of at least one of the lipids. The 90th percentile was used as the cut-off value for total-cholesterol, triglycerides, LDL-c, apoB and glucose. For Lp(a) we applied a cut-off value above the 80th percentile and for HDL-c a cut-off value below the 10th percentile. Differences were tested using chi-square tests. We repeated all linear and logistic regression analyses to test whether results differed between women with previous gestational hypertension and preeclampsia (Supplemental Tables 5 and 6). To perform regression analyses we selected potential confounders depending on their association with the outcome of interest and/or based on previous studies and/or based on a change in effect estimate of more than 10%. The final regression models included the following confounders; (1) basic model, adjusted for maternal age at intake, visit interval and time of blood sampling; (2) confounder model, which in addition to model (1) included ethnicity, educational level, smoking and gravidity at follow-up); (3) BMI model, which in addition to model (2) included pre-pregnancy BMI. Results of linear and logistic regression analyses are presented as regression coefficients (β) and odds ratios (OR) with a 95% CI. A *P*-value < 0.05 (*) or < 0.001 (**) was considered statistically significant.

For women included in our analyses, missing values of confounders that were used in the three regression models were imputed through multiple imputation procedures.[39] This procedure was carried out by taking five draws for each missing value which provided five complete data sets. Each dataset was analyzed separately and results were integrated into one final result by computing the mean over the five repeated analysis, including the variance, confidence interval and *P*-value. The following confounders had missing values (for women with a previous normotensive pregnancy and women with a previous hypertensive disorder of pregnancy respectively): pre-pregnancy BMI (17.5% and 13.6%), ethnicity (2.2% and 0.7%), educational level (15.2% and 12.3%), smoking in pregnancy (10.8% and 6.6%), gravidity at follow-up (0.6% and 0%) and blood sampling (13.3% and 14.9%). A sensitivity analysis was performed to observe differences in observed and expected values of confounders before and after imputation (Supplemental Table 4). Lastly, to test for non-response bias we compared subject characteristics between women with and without available follow-up data six years after index pregnancy (Supplemental Table 2).

Results

Tables 1 and 2 show maternal characteristics before, during and six years after index pregnancy. Compared to women with a previous normotensive pregnancy, women with a hypertensive disorder of pregnancy showed a higher BMI both before and after pregnancy and a higher systolic and diastolic blood pressure both at study enrollment and after pregnancy. In total, 0.4% and 0.5% of all women used cholesterol and glucose mediating medication after pregnancy respectively. The use of cholesterol and glucose regulating medication was similar between women with a previous normotensive pregnancy and women with a previous hypertensive disorder of pregnancy.

TABLE 2 | Subject characteristics by hypertensive disorder of pregnancy six years after pregnancy n = 4933.

	Normotensive Pregnancy n = 4631	**Hypertensive disorder of pregnancy** n = 302	***P*-value**
Maternal characteristics (follow-up)			
Visit interval, median (90% range), (years)	6.0 (5.7, 7.3)	6.1 (5.7, 7.5)	0.14
Medication use, n (%)			0.19
Cholesterol mediating medication	18 (0.4)	1 (0.4)	
Glucose mediating medication	22 (0.5)	4 (1.3)	
Smoking, n (%)	958 (20.7)	57 (18.9)	0.70
BMI, median (90% range), (kg/m2)	24.6 (19.7, 35.2)	27.6 (20.6, 43.5)	<0.001
SBP, mean (SD), (mmHg)	118.5 (12.1)	128.2 (17.5)	<0.001
DBP, mean (SD), (mmHg)	70.4 (9.5)	78.5 (12.2)	<0.001
Lipids at follow-up			
Total-cholesterol, mean (SD), (mmol/L)	4.85 (0.89)	4.95 (0.92)	0.08
Triglycerides, median (90% range), (mmol/L)	1.12 (0.61, 2.48)	1.21 (0.67, 2.48)	<0.001
HDL-c, mean (SD), (mmol/L)	1.37 (0.34)	1.32 (0.33)	0.02
LDL-c, mean (SD), (mmol/L)	2.64 (0.58)	2.73 (0.61)	0.02
Non-HDL-c, mean (SD), (mmol/L)	3.48 (0.90)	3.63 (0.95)	0.009
Remnant cholesterol, mean (SD), (mmol/L)	0.84 (0.49)	0.90 (0.50)	0.06
Lp(a), median (90% range), (mmol/L)	0.16 (0.0, 2.85)	0.17 (0.0, 1.26)	0.26
ApoB, mean (SD), (g/L)	0.80 (0.19)	0.83 (0.19)	0.005
Glucose, mean (SD), (mmol/L)	5.48 (0.98)	5.47 (0.83)	0.82

Abbreviations: ApoB, apolipoprotein B; BMI, body mass index; DBP, diastolic blood pressure; Lp(a), lipoprotein(a); N, number; SBP, systolic blood pressure. Values are numbers with valid percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution. Presented values are not imputed. Statistical testing was carried out through Student's t-test for continuous variables with a normal distribution and Kruskal-Wallis test for continuous variables with a skewed distribution. Chi-square tests were used for categorical variables.

The association between a gestational hypertensive disorder, and lipid and glucose levels at follow-up is presented in Table 3 and Supplemental Table 3. Six years after index pregnancy, women with a previous hypertensive disorder of pregnancy had higher levels of triglycerides, LDL-c, non-HDL-c, remnant cholesterol and apoB, and lower levels of HDL-c than women

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

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

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

with a previous normotensive pregnancy. After additional adjustment for pre-pregnancy BMI these results attenuated to non-significant levels. No differences were observed in total-cholesterol, Lp(a) and glucose levels. Sensitivity analyses showed that especially women with previous gestational hypertension had higher lipid levels after pregnancy than women with a previous normotensive pregnancy ([higher levels of triglycerides, LDL-c, non-HDL-c, remnant cholesterol and apoB, and lower levels of HDL-c] Supplemental Table 5). Women with previous preeclampsia had higher triglyceride levels than women with a previous normotensive pregnancy. Results attenuated to non-significant levels after adjustment for pre-pregnancy BMI. The prevalence of dyslipidemia, defined as LDL-c levels > 90th percentile, was higher among women with previous hypertensive disorder of pregnancy than women with a previous normotensive pregnancy (Supplemental Table 3). This result remained significant after adjusting for confounders, including pre-pregnancy BMI (OR 1.6; 95% CI 1.1, 2.4, *P* 0.015 [data not shown]) and was most profound in women with previous gestational hypertension (Supplemental Table 6). Women with previous preeclampsia were more often affected by dyslipidemia resulting from triglyceride levels > 90th percentile (Supplemental Table 6).

An additional sensitivity analysis was performed to examine whether values of confounders used for linear and logistic regression analyses differed before and after multiple imputation (Supplemental Table 4). No major differences were observed. Linear and logistic regression analyses showed similar results when confounders were not imputed (data not shown).

Discussion

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

The results of this large prospective cohort study show that women with previous hypertensive disorder of pregnancy have higher levels of triglycerides, LDL-c, non-HDL-c, remnant cholesterol and apoB and lower levels of HDL-c after pregnancy compared to women with a previous normotensive pregnancy. Pre-pregnancy BMI largely explained these results as most lipid levels attenuated after adjustment for this factor. However, when pre-pregnancy BMI was taken into account, women with previous hypertensive disorder of pregnancy still had a higher risk of having LDL-c levels above the 90th percentile compared to women with a previous normotensive pregnancy. A smaller study, comparing lipid levels 7.8 years after pregnancy between women with a previous hypertensive disorder of pregnancy (gestational hypertension [n = 105] or preeclampsia [n = 63]) and women with a previous normotensive pregnancy, showed results similar to ours without adjusting for pre-pregnancy BMI.[15] Women with previous gestational hypertension showed higher levels of triglycerides, apoB and glucose, and lower levels of HDL-c. Women with previous preeclampsia showed higher levels of LDL-c and apoB than women with a previous normotensive pregnancy. In the study concerned, pre-pregnancy BMI was not taken in to consideration and might therefore have overestimated their results.[15] Other studies comparing lipid profile and glucose levels between women with only previous preeclampsia and women with a previous normotensive pregnancy show no differences within

the first 10 years after pregnancy (1.5 to 9.1 years).[19, 20, 22] However, after a longer time period (up to 16 years after pregnancy), women with previous preeclampsia do seem to have a more atherogenic lipid profile, but this is largely driven by BMI.[14, 21] These results are in line with our findings, suggesting pre-pregnancy BMI is an important factor contributing to an atherogenic lipid profile after a pregnancy affected by a hypertensive disorder of pregnancy.

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

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

There is an established association between gestational hypertensive disorders and the risk for CVD in later life.[6, 43] Depending on the type and the severity of the hypertensive disorder of pregnancy, the occurrence of CVD can be seven times larger compared to women with a previous normotensive pregnancy.[43] Currently, there are several guidelines in practice for the prevention of CVD after a hypertensive disorder of pregnancy.[23, 24, 44] Nevertheless, it remains controversial whether to assess a woman's lipid profile after a hypertensive disorder of pregnancy and a uniform recommendation of these clinical guidelines is not available. The guideline of the American Heart Association (AHA) for the prevention of stroke in women, as well as the NICE and ACOG guidelines on the management of hypertensive disorders during pregnancy advise to perform annual measurement of a lipid profile and glucose level after a hypertensive disorder of pregnancy.[23, 24, 44] Contrarily, the guidelines of the ESC on CVD prevention and of the AHA on the prevention of CVD in women do not address lipid profile assessment after a hypertensive disorder of pregnancy, although the former does recommend periodic screening for hypertension and diabetes in these women.[45, 46]

The association between an atherogenic lipid profile and the risk of future CVD is evident within the general population.[47, 48] Nevertheless, results from similar studies on women with a previous hypertensive disorder of pregnancy remain inconsistent, possibly due to a variety in study methodology. The most important methodological differences are: the time interval between pregnancy and lipid profile assessment (varying between 0.5 to 16 years),[10, 14, 15, 17, 19-21] incomplete lipid profile assessment,[10, 14, 17, 19-21] differences in statistical models to adjust for confounding[14, 20] and differences in the definition of gestational hypertensive disorders.[10, 21]

Interestingly, our study shows that differences in lipid profile between women with a previous normotensive pregnancy and women with previous gestational hypertensive disorders are most likely the result of a higher pre-pregnancy BMI in the latter. BMI and blood pressure are important constitutional risk factors for developing a hypertensive disorder of pregnancy as well as developing future CVD.[49]

Lipid profile assessment after a hypertensive disorder of pregnancy, especially in overweight women, might help to identify those women at risk for CVD. Based on our results we foremost suggest to encourage women to achieve a healthy weight status before pregnancy as pre-pregnancy weight is strongly associated with an atherogenic lipid profile after pregnancy and with an increased risk of developing a hypertensive disorder of pregnancy.[49]

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

Conclusion

Women with a previous hypertensive disorder of pregnancy have a more atherogenic lipid profile six years after pregnancy than women with a previous normotensive pregnancy. This is more likely resulting from a higher pre-pregnancy BMI than from the hypertensive disorder of pregnancy itself. Weight counseling before the onset of pregnancy might therefore be beneficial in reducing the risk of developing an atherogenic lipid profile after pregnancy and possibly CVD in later life.[50]

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Supplemental material

SUPPLEMENTAL TABLE 1 | Mean range of the intra-assay precision with the coefficient of variation (CV) per lipid.

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

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

Maternal characteristics	Follow-up six years after index pregnancy n = 5439	Loss to follow-up n = 2759	*P*-value
Age at intake (years)	30.2 (5.1)	28.2 (5.5)	<0.001
Gestational age at intake (weeks)	13.9 (10.8, 22.6)	14.5 (10.8, 23.8)	<0.001
Pre-pregnancy BMI (kg/m2)	22.9 (18.9, 32.6)	22.6 (18.2, 33.1)	0.02
SBP at intake (mmHg)	115.7 (12.3)	114.7 (12.4)	0.001
DBP at intake (mmHg)	68.2 (9.5)	67.5 (9.8)	0.002
Primigravida, n (%)	2338 (43.0)	1332 (48.3)	<0.001
Non-European ethnicity, n (%)	2192 (40.3)	1454 (52.7)	<0.001
Lower educational level, n (%)	533 (9.8)	455 (16.5)	<0.001
Smoking, n (%)	1446 (26.6)	849 (30.8)	<0.001
Gestational hypertension, n (%)	239 (4.4)	85 (3.1)	0.009
Preeclampsia, n (%)	114 (2.1)	80 (2.9)	0.04

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

SUPPLEMENTAL TABLE 3 | Maternal glucose and lipid profile six years after pregnancy (n = 4933).

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

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

SUPPLEMENTAL TABLE 4 | Observed and expected values for confounders.

	Normotensive Pregnancy n = 4631		Hypertensive disorder of pregnancy n = 302	
	Observed	**Expected**	**Observed**	**Expected**
Maternal characteristics (pregnancy)				
Age at intake, mean (SD), (years)	30.1 (5.1)	30.1 (5.1)	30.4 (5.1)	30.4 (5.1)
Pre-pregnancy BMI, median (90% range), (kg/m2)	22.7 (18.8, 31.6)	22.8 (18.8, 31.7)	24.9 (19.8, 38.5)	24.8 (19.8, 38.1)
Normal BMI (≥18.5 and < 25.0), n (%)	2790 (60.2)	3331 (71.9)	133(44.0)	154 (51.0)
High BMI (≥25.0), n (%)	1030 (22.2)	1300 (28.1)	128 (42.4)	148 (49.0)
Non-European ethnicity, n (%)	1828 (39.5)	1919 (41.4)	92 (30.5)	94 (31.1)
Lower educational level, n (%)	417 (9.0)	555 (12.0)	19 (6.3)	24 (7.9)
Smoking, n (%)	1095 (23.6)	1213 (26.2)	77 (25.5)	85 (28.1)
Primigravid, n (%)	318 (6.9)	406 (8.8)	41 (17.6)	48 (15.9)
Maternal characteristics (follow-up)				
Visit interval, median (90% range), (years)	6.0 (5.7, 7.3)	6.0 (5.7, 7.3)	6.1 (5.7, 7.5)	6.1 (5.7, 7.5)
Blood sampling before 10:00 h, n (%)	1129 (24.4)	1272 (27.5)	71 (23.5)	81 (26.8)

Abbreviations: BMI, body mass index; N, number. Values are numbers with percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution.

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

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

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

SUPPLEMENTAL TABLE 6 | Maternal glucose and lipid profile six years after pregnancy (n = 4933).

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

Abbreviations: ApoB, apolipoprotein B; Lp(a), lipoprotein(a); N, number. Values are numbers with percentages. Statistical testing was carried out through one-way ANOVA and chi-square tests. A: Significant (*P*-value < 0.05) differences in distribution between women with gestational hypertension and women with a normotensive pregnancy. B: Significant (*P*-value < 0.05) differences in distribution between women with preeclampsia and women with a normotensive pregnancy.

Chapter

6

Cardiovascular risk factors track from mother to child

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Abstract

Background: Cardiovascular risk factors can track from mother to child via several pathways: pregnancy complications, genetic inheritance and shared environmental risk factors after pregnancy. The degree of tracking and to which extend this is influenced by these pathways is unknown. We hypothesized that cardiovascular risk factors track from mother to child regardless of pregnancy complications and environmental risk factors. We determined the degree of tracking between maternal and offspring micro- and macrovascular cardiovascular risk factors after pregnancy and the extent to which this is influenced by pregnancy complications and shared environmental risk factors.

Methods: We included 5624 mother-offspring pairs from The Generation R Study; an ongoing prospective population-based birth cohort. Information on pregnancy complications (preeclampsia, small for gestational age and preterm birth) was obtained through hospital charts. Mother-offspring associations were assessed six years after pregnancy (central retinal arteriolar and venular calibers, body mass index, blood pressure, left atrial diameter, aortic root diameter, left ventricular mass, fractional shortening and pulse wave velocity) and nine years after pregnancy (body mass index and blood pressure).

Results: We observed that worse cardiovascular parameters in mothers were associated with worse cardiovascular parameters in their offspring six and nine years after pregnancy (*P*-values <.001). Results were similar when mother-offspring pairs with a previous pregnancy complication were excluded.

Conclusions: Six and nine years after pregnancy, an adverse cardiovascular profile in mothers is strongly associated with an adverse cardiovascular profile in their offspring. Results were not attenuated by environmental exposures or a previous pregnancy complication. This supports the hypothesis that cardiovascular risk factors (micro- and macrovascular) track from mother to child, regardless off the course of pregnancy.

Introduction

Cardiovascular disease is the leading cause of morbidity and mortality worldwide.[1] The prevalence of cardiovascular risk factors (e.g. obesity, hypertension and an atherogenic lipid profile) is not only rising among adults but also among children. Cardiovascular risk factors are most likely transmitted from parents to their offspring, which can be explained through three potential mechanisms; pregnancy complications, genetic inheritance and shared environmental risk factors after pregnancy. The degree to which cardiovascular risk factors track from mother to child and the extent to which this is influenced by pregnancy complications and shared environmental risk factors after pregnancy is still unknown. Unraveling these mechanisms may assist us in identifying at an early stage children at risk for cardiovascular disease later in life.

The first mechanism involves pregnancy complications, such as preeclampsia, which require adaptation in order for the fetus to survive to a stressful environment. This process can affect perinatal health as it is associated with a low birth weight and preterm birth, which themselves are risk factors for the development of cardiovascular risk factors during adolescence such as obesity and hypertension.[2] Secondly, environmental exposures (e.g. diet, exposure to smoking and physical activity) may be involved. These are often similar between parents and their offspring until they enter adulthood. Previous studies show that parents' diet and energy intake, smoking behavior and physical activity levels are positively associated with those of their offspring.[3-7] Thirdly, heritability studies have identified genetic factors as a determinant for certain cardiovascular risk factors such as body mass index (BMI) level.[8, 9] Obtaining better insight in the degree of tracking and the most important mechanisms involved will help to predict and reduce future cardiovascular disease in offspring. In this study, we hypothesized that cardiovascular risk factors track from mother to child regardless of pregnancy complications and environmental risk factors. We determined the degree of tracking between maternal and offspring microvascular (central retinal and arteriolar calibers) and macrovascular (blood pressure, left atrial diameter, aortic root diameter, left ventricular mass, fractional shortening and pulse wave velocity) cardiovascular risk factors after pregnancy and the extent to which this is influenced by pregnancy complications and shared environmental risk factors.

Materials and methods

Design and Study Population

This study was embedded in The Generation R Study, a multi-ethnic population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[10, 11] Mothers (N=8976) enrolled during pregnancy between April 2002 and January 2006. The response rate at baseline was 61% and 85% six years after pregnancy. The study has been approved by the Medical Ethics Committee of Erasmus Medical Center, Rotterdam, the Netherlands (MEC 198.782/2001/31) and the procedures followed were in accordance with institutional guidelines.[12] Written informed consent was obtained from all participants. For the present study, we included mother-offspring pairs with at least one measurement of interest available six or nine years after pregnancy (N = 5624 [Figure 1]). Supplemental Figure 1 presents an overview of our exposures, outcomes and covariates.

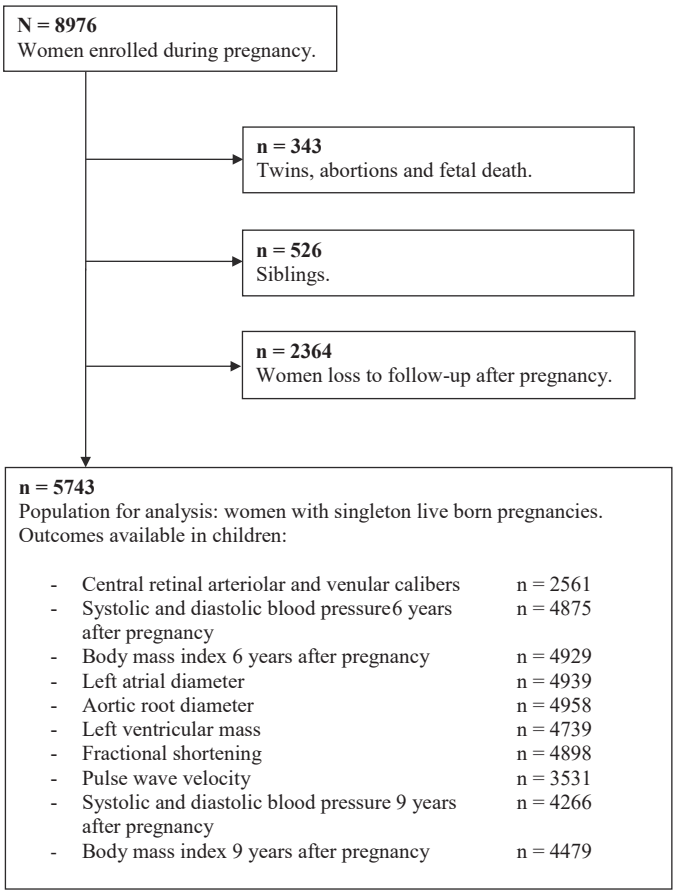

FIGURE 1 | Flowchart.

Pregnancy complications

The presence of a pregnancy complication (preeclampsia, a small for gestational age child or spontaneous preterm birth) was determined from the original hospital charts. Preeclampsia was defined as a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg after 20 weeks of gestation in previously normotensive women with concurrent new onset proteinuria in a random urine sample and no evidence of a urinary tract infection.[13,14] Small for gestational age was defined as a birth weight under the 10th percentile (based on our own cohort) and spontaneous preterm birth as the spontaneous onset of labor before 37 weeks of gestation.[15]

Environmental exposures and covariates

At study enrollment maternal height (cm) and weight (kg) without shoes were measured after which BMI (kg/m^2) was calculated. Identical measurements were obtained during follow-up six and nine years after pregnancy for both mother and child. Pre-pregnancy BMI was established

at enrollment through a questionnaire. Pre-pregnancy BMI was strongly correlated with BMI measured in early pregnancy (Pearson's correlation coefficient $r = 0.95$ ($P<0.001$)).[16]

Questionnaires were repeatedly applied during pregnancy to obtain information on maternal age, pre-pregnancy weight, gravidity, ethnicity, educational level, smoking and folic acid intake. Information on gestational age at birth, birth weight and child's sex was obtained from medical records.[15, 17] Six years after index pregnancy we obtained information on gravidity at follow-up and smoking through questionnaires.

Cardiovascular measurements six years after pregnancy

Central retinal arteriolar and venular calibers were assessed in mothers and offspring by taking digital retinal photographs six years after index pregnancy. Details of this procedure are described previously.[18-20]

Research assistants in non-medical clothing (i.e. no white coat) measured blood pressure during study enrollment (median 13.2 weeks of gestation [90% range 10.6, 17.0]) and six years after pregnancy (90% range, 5.7 to 7.4 years) with the validated Omron 907 automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe B.V., Hoofddorp, the Netherlands).[21] Blood pressure was measured in the right upper arm and in a standardized supine position to prevent differences due to position. The mean value of two systolic and diastolic blood pressure readings (SBP and DBP) was documented for each participant.

Carotid-femoral pulse wave velocity (PWV) was measured with an automatic non-invasive, validated device (Complior®; Artech Medical, Pantin, France) to assess arterial wall stiffness. The device measures the distance between the recording sites at the carotid (proximal) and femoral (distal) artery. Echocardiographic measurements were performed in two-dimensional M-mode using the ATL-Philips Model HDI 5000 (Seattle, WA, USA) or the Logiq E9 (GE Medical Systems, Wauwatosa, WI, USA) devices. Fractional shortening , aortic root diameter and left atrial diameter were measured. Left ventricular mass was calculated with the Devereux equation.[22] Intraobserver and interobserver intraclass correlation coefficients were described previously and demonstrated good repeatability and reproducibility.[23]

Blood pressure nine years after pregnancy

The validated automatic sphygmomanometer Datascope Accutorr Plus (Paramus, NJ, USA) was used to measure blood pressure in mothers and children 9.8 years after pregnancy (90% range 9.5, 10.3 years).[24] The average of the last three (out of four) blood pressure measurements was used for further analyses.

Statistical analyses

Baseline characteristics are presented in Table 1. The mean ± standard deviation is presented for data with a normal distribution and the median with 90% confidence interval for data with a skewed distribution. In the total population for analysis 17.7% had missing information on pre-pregnancy BMI, 6.9% on education, 11.0% on smoking during pregnancy, 24.1% on folic

acid intake during pregnancy, 1.0% on maternal systolic blood pressure at study intake, 2.5% on child's ethnicity, 13.3% and 9.0% on child's systolic and diastolic blood pressure six years after pregnancy, 5.4% on child's BMI after pregnancy. To reduce potential bias associated with missing data we imputed missing values in variables of interest through multiple imputation.[25] Complete case analysis showed similar results to those presented in Table 2 and Table 3 (data not shown). Multivariate linear regression analysis was used to examine the association between mother-offspring cardiovascular risk factors after pregnancy (Table 2 and 3) while adjusting for confounders. Regression models adjusted for confounding were: basic model (including maternal age at enrollment and child's age during follow-up) and four confounder models (including pre-pregnancy BMI, maternal age at enrollment, maternal SBP at study enrollment, educational level mother, smoking during pregnancy, folic acid intake during pregnancy, child's sex, child's ethnicity, child's age [six and nine years after pregnancy], child's weight and height [six and nine years after pregnancy], and mean SBP and DBP six years after pregnancy). These confounders were selected based on their associations with the exposures and outcomes of interest and based on previous studies. The effect estimates in Table 2 and 3 are unstandardized beta coefficients and represent an increase in the outcome measure for each unit increase in the exposure. We applied a Benjamin-Hochberg procedure controlling for false discovery rate at 0.05 level.[26] We tested whether there was effect modification in all mother-offspring associations by pregnancy complications through inclusion of the interaction term (exposure * pregnancy complication yes) in each regression model. To estimate the proportion of total variance in offspring attributable to variation in genotypes we calculated unadjusted heritability (h^2, expressed as percentage) through regression of offspring phenotype on the maternal phenotype.[27] Estimates are presented in the results section. Lastly, we examined baseline characteristics between women included and excluded from this study (Supplemental Table 1). Statistical analyses were performed with SPSS version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

This study included 5624 mother-offspring pairs (Figure 1). Mothers were on average 30.3 (SD 5.1) years of age at the start of pregnancy (Table 1). They were mostly European, highly educated and nulliparous. In total, 15.3% of pregnancies were affected by a pregnancy complication.

TABLE 1 | Baseline characteristics n = 5743.

Pregnancy	
Age at enrollment (years), mean (SD)	30.1 (5.1)
Non-European ethnicity (%)	42.1
No education/primary school (%)	11.7
Pre-pregnancy BMI (kg/m^2), median (5th, 95th percentile)	22.7 (18.7, 32.3)
Lean and normal (%)	71.2
Overweight (%)	20.3
Obese and morbidly obese (%)	8.5
SBP at enrollment (mmHg), mean (SD)	115.6 (12.1)
DBP at enrollment (mmHg), mean (SD)	68.1 (9.5)
Smoking (%)	27.1
Nulliparous (%)	60.1
No folic acid intake, (%)	28.3
Diabetes (%)	0.4
Gestational diabetes (%)	1.1
Pregnancy outcomes	
Spontaneous preterm birth, (%)	3.6
Small for gestational age (< p10), (%)	9.9
Preeclampsia (%)	2.1
Gestational hypertension (%)	4.2
Boys (%)	49.6
Non-European ethnicity child (%)	39.0
Maternal outcomes six years after pregnancy	
Follow-up interval (years), median (5th, 95th percentile)	6.0 (5.7, 7.4)
BMI (kg/m2), median (5th, 95th percentile)	24.8 (19.7, 36.0)
SBP (mmHg), mean (SD)	119.3 (13.0)
DBP (mmHg), mean (SD)	71.0 (10.1)
Cardiovascular medication (%)	2.1
Central retinal arteriolar caliber (µm), mean (SD)	145.3 (16.9)
Central retinal venular caliber (µm), mean (SD)	206.8 (22.5)
Smoking (%)	20.0
Primigravid (%)⁑	8.5
Child outcomes six years after pregnancy	
BMI (kg/m2), median (5th, 95th percentile)	15.9 (13.9, 19.8)
SBP (mmHg), mean (SD)	103.0 (8.3)
DBP (mmHg), mean (SD)	62.0 (8.6)
Central retinal arteriolar caliber (µm), mean (SD)	158.8 (14.9)
Central retinal venular caliber (µm), mean (SD)	218.8 (19.9)
Outcomes nine years after pregnancy	
BMI mother (kg/m2), median (5th, 95th percentile)	24.8 (20.0, 36.2)
SBP mother (mmHg), mean (SD)	114.6 (12.8)
DBP mother (mmHg), mean (SD)	68.6 (8.2)
Cardiovascular medication (%)	2.4
SBP child (mmHg), mean (SD)	103.2 (8.0)
DBP child (mmHg), mean (SD)	58.6 (6.4)
BMI child (kg/m2), median (5th, 95th percentile)	17.0 (14.4, 23.3)

Abbreviations: Body mass index, BMI; Central retinal arteriolar caliber, Diastolic blood pressure, DBP; Systolic blood pressure, SBP. Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (5^{th}, 95^{th} percentile) for continuous variables with a skewed distribution. ǂ Up until follow-up visit pregnancy had occurred no more than once.

Table 2 and Table 3 show the association between mother-offspring cardiovascular risk factors six and nine years after pregnancy for the total population and for mother-offspring pairs without a pregnancy complication during the index pregnancy. Maternal microvasculature (central retinal arteriolar and venular calibers), BMI, blood pressure (SBP and DBP), cardiac measurements (left atrial diameter, aortic root diameter, left ventricular mass and fractional shortening) and PWV are all positively associated with the corresponding measurements in offspring. Results did not change after environmental factors (confounders) were taken into consideration nor after excluding mother-offspring pairs affected by a pregnancy complication (Table 3 and Supplemental Figure 2) or when we corrected for multiple testing. Also, effect modification analysis showed no significant interaction in all mother-offspring associations by pregnancy complications (data not shown).

TABLE 2 | The association between mother-offspring cardiovascular risk factors six and nine years after pregnancy n = 5743.

Outcomes child	**Exposures mother**	**Basic model** Beta (95%CI)	***P*-value**	**Confounder model** Beta (95%CI)	***P*-value**
Six years after pregnancy					
Microvasculature					
Central retinal arteriolar caliber (SDS)	Central retinal arteriolar caliber (n = 2561)	0.12 (0.08, 0.16)	<0.001	0.11 (0.07, 0.14)*	<0.001
Central retinal venular caliber (SDS)	Central retinal venular caliber (n = 2561)	0.18 (0.14, 0.22)	<0.001	0.19 (0.15, 0.22)*	<0.001
BMI (kg/m2)	BMI (n = 4929)	0.11 (0.10, 0.12)	<0.001	0.10 (0.09, 0.11) †	<0.001
Blood pressure					
SBP (mmHg)	SBP (n = 4875)	0.11 (0.09, 0.13)	<0.001	0.08 (0.06, 0.10)†	<0.001
DBP (mmHg)	DBP (n = 4875)	0.10 (0.08, 0.12)	<0.001	0.08 (0.06, 0.10)†	<0.001
Cardiac measurements					
Left atrial diameter (mm)	Left atrial diameter (n = 4939)	0.14 (0.12, 0.16)	<0.001	0.13 (0.11, 0.15)ǂ	<0.001
Aortic root diameter (mm)	Aortic root diameter (n = 4958)	0.16 (0.14, 0.17)	<0.001	0.13 (0.12, 0.15)ǂ	<0.001
Left ventricular mass (g)	Left ventricular mass (n = 4739)	0.12 (0.11, 0.13)	<0.001	0.10 (0.09, 0.11)ǂ	<0.001
Fractional shortening (%)	Fractional shortening (n = 4898)	0.14 (0.12, 0.17)	<0.001	0.14 (0.12, 0.17)ǂ	<0.001
PWV (m/s)	PWV (n = 3531)	0.18 (0.15, 0.21)	<0.001	0.17 (0.14, 0.20)ǂ	<0.001

TABLE 2 | Continued.

Outcomes child	Exposures mother	Basic model Beta (95%CI)	*P*-value	Confounder model Beta (95%CI)	*P*-value
Nine years after pregnancy					
BMI (kg/m2)	BMI (n = 4479)	0.19 (0.18, 0.21)	<0.001	0.16 (0.14, 0.17) §	<0.001
SBP (mmHg)	SBP (n = 4266)	0.11 (0.09, 0.13)	<0.001	0.06 (0.04, 0.08)§	<0.001
DBP (mmHg)	DBP (n = 4266)	0.12 (0.10, 0.14)	<0.001	0.10 (0.07, 0.12)§	<0.001

Abbreviations: Body mass index, BMI; Confidence Interval, CI; Diastolic blood pressure, DBP; Pulse wave velocity, PWV; Standard Deviation Score, SDS; Systolic blood pressure, SBP. Values are betas with corresponding 95% CI and represent the mean difference in children per unit increase in maternal parameter.

Basic model: Age mother at intake and age child during follow-up.

*__Confounder model__: In addition to the basic model: Educational level mother at intake, ethnicity child, smoking during pregnancy, mean systolic blood pressure child six years after pregnancy and maternal systolic blood pressure during study intake.

†__Confounder model__: In addition to the basic model: Educational level mother at intake, ethnicity child, maternal systolic blood pressure during study intake, child's sex, child's weight and height six years after pregnancy.

‡__Confounder model__: In addition to the basic model: Educational level mother at intake, ethnicity child, maternal smoking during pregnancy, folic acid intake during pregnancy, pre-pregnancy BMI, child's weight and height and diastolic blood pressure six years after pregnancy.

§__Confounder model__: In addition to the basic model: Educational level mother at intake, ethnicity child, maternal systolic blood pressure during study intake, child's sex, child's weight and height nine years after pregnancy.

TABLE 3 | The association between mother-offspring cardiovascular risk factors six and nine years after pregnancy in women without a pregnancy complication during the index pregnancy n = 4536.

Outcomes child	Exposures mother	Basic model Beta (95%CI)	*P*-value	Confounder model Beta (95%CI)	*P*-value
Six years after pregnancy					
Microvasculature					
Central retinal arteriolar caliber (SDS)	Central retinal arteriolar caliber (n = 2044)	0.12 (0.08, 0.16)	<0.001	0.11 (0.07, 0.16)*	<0.001
Central retinal venular caliber (SDS)	Central retinal venular caliber (n = 2044)	0.18 (0.14, 0.23)	<0.001	0.19 (0.14, 0.23)*	<0.001
BMI (kg/m2)	BMI (n = 3899)	0.12 (0.10, 0.13)	<0.001	0.10 (0.09, 0.11) †	<0.001
Blood pressure					
SBP (mmHg)	SBP (n = 3865)	0.12 (0.09, 0.14)	<0.001	0.09 (0.07, 0.11)†	<0.001
DBP (mmHg)	DBP (n = 3865)	0.10 (0.08, 0.12)	<0.001	0.09 (0.06, 0.11)†	<0.001
Cardiac measurements					
Left atrial diameter (mm)	Left atrial diameter (n = 3898)	0.13 (0.11, 0.16)	<0.001	0.12 (0.10, 0.15)‡	<0.001
Aortic root diameter (mm)	Aortic root diameter (n = 3917)	0.15 (0.13, 0.17)	<0.001	0.13 (0.11, 0.15)‡	<0.001
Left ventricular mass (g)	Left ventricular mass (n = 3753)	0.12 (0.10, 0.13)	<0.001	0.10 (0.09, 0.11)‡	<0.001
Fractional shortening (%)	Fractional shortening (n = 3869)	0.14 (0.12, 0.17)	<0.001	0.14 (0.12, 0.17)‡	<0.001
PWV (m/s)	PWV (n = 2821)	0.19 (0.16, 0.22)	<0.001	0.18 (0.15, 0.22)‡	<0.001

TABLE 3 | Continued.

Outcomes child	Exposures mother	Basic model Beta (95%CI)	*P*-value	Confounder model Beta (95%CI)	*P*-value
Nine years after pregnancy					
BMI (kg/m2)	BMI (n = 3549)	0.19 (0.18, 0.21)	<0.001	0.16 (0.14, 0.18)	<0.001
SBP (mmHg)	SBP (n = 3382)	0.11 (0.09, 0.14)	<0.001	0.06 (0.04, 0.08)	<0.001
DBP (mmHg)	DBP (n = 3382)	0.12 (0.10, 0.15)	<0.001	0.10 (0.08, 0.13)	<0.001

Abbreviations: Body mass index, BMI; Confidence Interval, CI; Diastolic blood pressure, DBP; Pulse wave velocity, PWV; Standard Deviation Score, SDS; Systolic blood pressure, SBP. Values are betas with corresponding 95% CI and represent the mean difference in children per unit increase in maternal parameter. *†‡§Regression models were identical to those presented under Table 2.

Heritability varied between 9 and 20% (central retinal arteriolar caliber [12%], central retinal venular caliber [19%], BMI, SBP and DBP at the age of six [12%, 11% and 9%], left atrial diameter [15%], aortic root diameter [15%], left ventricular mass [12%], fractional shortening [14%], PWV [16%] and BMI, SBP and DBP at the age of nine [20%, 11% and 11%].

Supplemental Table 1 shows baseline characteristics of women included and excluded from this study. Excluded women were on average younger during study enrollment, more often non-European, lower educated, smoked more often in pregnancy and had more often children born small for gestational age.

Discussion

This large prospective study shows that adverse maternal cardiovascular risk factors (central retinal arteriolar and venular calibers, BMI, blood pressure, left atrial diameter, aortic root diameter, left ventricular mass, fractional shortening and PWV) are strongly associated with corresponding adverse cardiovascular risk factors in offspring at the age of six years, and for BMI and blood pressure also at the age of nine years, independent of pregnancy complications and environmental factors. This indicates that cardiovascular risk factors most likely track from mother to child via genetic and epigenetic pathways. Possibly, central retinal arteriolar and venular calibers, cardiac ultrasound measurements and PWV also track up to nine years after pregnancy. However, these measurements were not available nine years after pregnancy.

The association between mother-offspring retinal arteriolar and venular calibers has not been studied previously. Our study shows that the offspring retinal microcirculation is a representation of the maternal microcirculation and possibly also of the paternal microcirculation but we did not examine the latter. In adults, smaller retinal arteriolar calibers and larger retinal venular calibers are independent risk factors for cardiac heart disease and stroke death.[28]

Several studies have examined traits between mother and offspring, such as BMI and blood pressure.[29-31] Results indicate that tracking of these cardiovascular risk factors from mother to offspring likely continues into adult life. A large cross-sectional study in Norwegian parent-offspring pairs showed that a higher BMI in parents was associated with a higher BMI in their

adult offspring (mean age 38-41 years).[30] A similar tracking pattern was also observed for blood pressure. Compared to our study, effect estimates were larger for BMI but similar for blood pressure. A smaller study in mother-offspring pairs from Ohio also showed a positive association between mother-offspring BMI throughout childhood and adolescence. The tracking pattern was stronger from mother to daughter than from mother to son, which we did not observe in our study.[29] A similar study to ours, conducted in French school children (age 5-13 years), also showed a stronger association between maternal-offspring BMI than paternal-offspring BMI.[32] This might be due to pregnancy complications which the authors did not correct for. Genetic and epigenetic mechanisms might also be involved in the mother-offspring associations we examined in this study; previous studies have shown a transgenerational epigenetic inheritance of cardiovascular disease.[33]

Increased left atrial diameter is a risk factor for atrial fibrillation, thrombus formation and consequently stroke.[34, 35] BMI and blood pressure are two major determinants of left atrial diameter. Adjusting for these confounders did not attenuate our results significantly, indicating that other factors might be more important, such as pregnancy complications, heritability and epigenetics. Removing those pregnancies affected by a pregnancy complication from our analyses did not affect our results. We observed relatively small heritability (15%), which was also small in previous studies examining parents and their teenagers or adult offspring (2-7%).[36, 37]

Aortic root diameter increases with age and BMI and is a risk factor for aortic root dissection.[38, 39] The association between mother-offspring aortic root diameter has not been studied previously. Therefore, we cannot compare our results to other studies.

Previous population and twin studies that examined the association between parent-offspring cardiovascular risk factors have shown a strong heritability component for cardiac features, such as left ventricular mass.[40, 41] Left ventricular mass is a predictor of cardiac and cerebrovascular related morbidity and mortality.[42] Family-based studies showed a wide variation in left ventricular mass heritability estimates between different ethnicities: 12% to 41% for Caucasians, 22% to 44% for African-Americans, 15% for Chinese and 43% to 61% for Japanese.[43] Our heritability estimate of 12% is comparable to that found in previous studies examining Caucasians. Besides genetic variance, the variation in heritability estimates between studies might be due to several other factors including differences in study design, statistical modeling and characteristics of study participants. Left ventricular mass is strongly related to the efficacy of glucose and fatty acid oxidation by the mitochondria.[44] Mitochondrial DNA is inherited through the mother, which might explain why previous studies presented stronger mother-offspring left ventricular mass associations than father-offspring associations.[45]

This is the first study to examine the association between parent-offspring fractional shortening. Reduced fractional shortening is associated with left ventricular systolic dysfunction.

To our knowledge, PWV in children has been examined in one previous study of 291 American mother-offspring pairs (child's mean age 12 years).[46] Results showed a higher heritability estimate for PWV compared to our study (26% vs. 16%).[46] Similar to our results, findings were not affected by environmental factors such as age and BMI.

All our results were not driven by children born from pregnancies affected by a pregnancy complication. Therefore, the impact of intrauterine growth and development on cardiovascular risk factors in offspring seemed small.

Strengths and limitations

Some limitations need to be addressed. Retinal vascular imaging six years after pregnancy was introduced into the Generation R Study after recruitment of study subjects had already started. Therefore, retinal vascular images were not available of 52.6% of mothers and children who attended follow-up visit six years after pregnancy. This was independent of any subject characteristics, which makes it unlikely that selection bias occurred. Compared to women included in this study, those excluded were on average younger during study enrollment, more often non-European, lower educated, smoked more often in pregnancy and had more often children born small for gestational age. This may have led to some degree of selection bias as the included women were relatively healthy and may have led to an underestimation of our associations. Nevertheless, we still observed significant associations that support tracking from mother to child of cardiovascular traits. Data on fathers was not available. A large population-based study, examining cardiovascular risk factors (BMI, blood pressure, lipid profile and waist circumference), showed that offspring risk factors were positively associated with those in both parents.[31] This finding argues against a strong impact of pregnancy complications and is more in favor of a genetic or epigenetic predisposition of cardiovascular risk factors in offspring. Reported heritability estimates might be confounded by pregnancy complications and environmental risk factors as we did not adjust for these factors. Strengths of this study are the large sample size, the prospective data collection, the standardized procedures that were used for data collection and this is the first study to examine the mother-offspring microvasculature. Future studies should also examine tracking of the microvasculature and cardiac ultrasound measurements from father to offspring.

Conclusion

Six and nine years after pregnancy, an adverse cardiovascular profile in mothers is strongly associated with an adverse cardiovascular profile in their offspring. Results were not attenuated by environmental exposures or a previous pregnancy complication. This supports the hypothesis that cardiovascular risk factors (micro- and macrovascular) track from mother to child, regardless off the course of pregnancy. Besides focusing on children born from complicated pregnancies we should also focus our efforts on children whose mothers have a worse cardiovascular risk profile.

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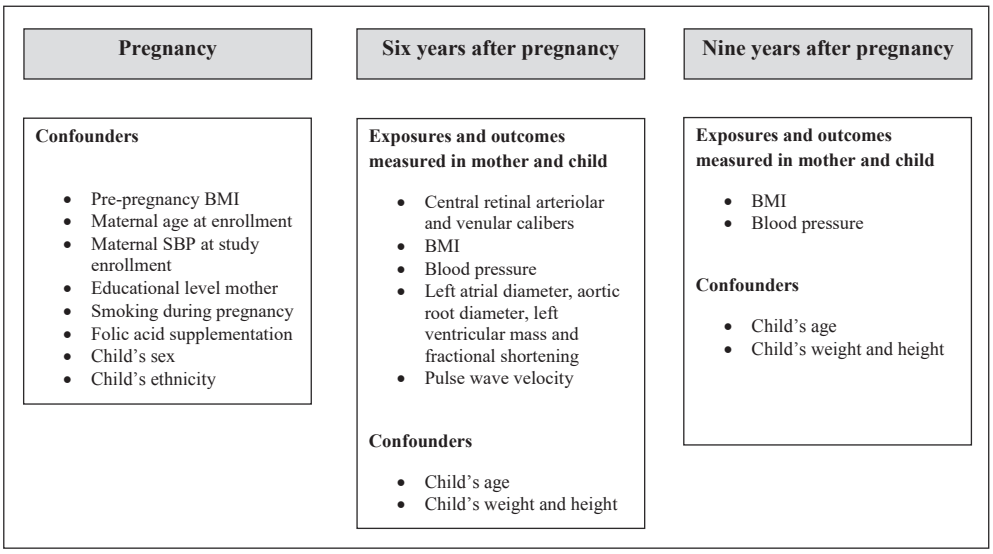
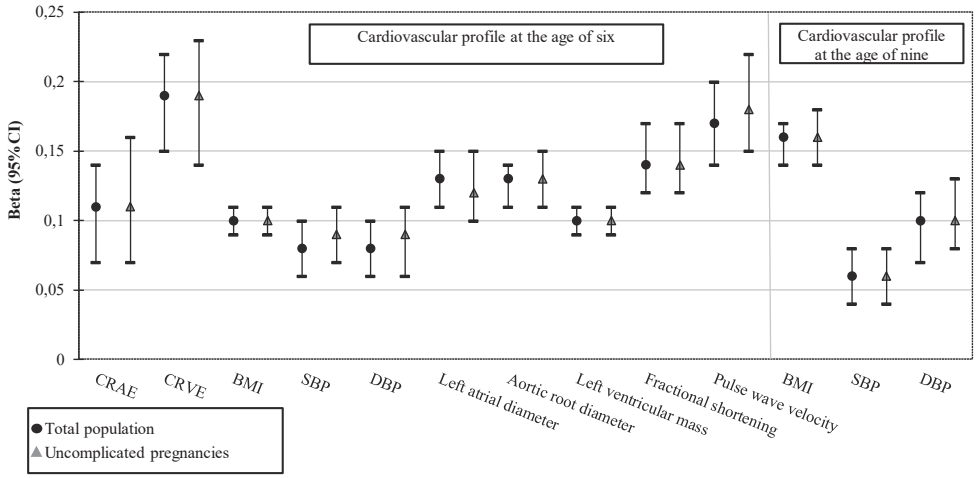
Supplemental material



SUPPLEMENTAL FIGURE 1 | Timeline of exposures, outcomes and confounders measured in mother and child. Abbreviations: BMI, body mass index; SBP, systolic blood pressure.



SUPPLEMENTAL FIGURE 2 | Overview of results in complicated and uncomplicated pregnancies.
Abbreviations: Body mass index, BMI; Central retinal arteriolar caliber, CRAE; Central retinal venular caliber, CRVE; Confidence Interval, CI; Diastolic blood pressure, DBP; Systolic blood pressure, SBP. Values are betas with corresponding 95% CI and represent the mean difference in children per unit increase in maternal parameter.

SUPPLEMENTAL TABLE 1 | Baseline characteristics of women included and excluded in this study n = 8107.

	Included n = 5743	**Excluded** n = 2364	***P*-value**
Pregnancy			
Age at enrollment (years), mean (SD)	30.1 (5.1)	27.9 (5.5)	<0.001
Non-European ethnicity (%)	42.1	60.6	<0.001
No education/primary school (%)	11.7	21.2	<0.001
Pre-pregnancy weight, median (5^{th}, 95^{th} percentile)	64.0 (50.0, 90.0)	63.0 (49.0, 92.0)	0.06
SBP at enrollment (mmHg), mean (SD)	115.6 (12.1)	114.8 (12.6)	0.01
DBP at enrollment (mmHg), mean (SD)	68.1 (9.5)	67.6 (9.9)	0.02
Smoking (%)	27.1	35.7	<0.001
Nulliparous (%)	60.1	54.9	<0.001
No folic acid intake, (%)	28.3	45.3	<0.001
Pregnancy outcomes			
Spontaneous preterm birth, (%)	3.6	4.3	0.12
Small for gestational age (< p10), (%)	9.9	11.6	0.03
Preeclampsia (%)	2.1	2.6	0.24
Gestational hypertension (%)	4.2	3.3	0.06
Boys (%)	49.6	52.6	0.01
Non-European ethnicity child (%)	39.0	55.6	<0.001

Abbreviations: Body mass index, BMI; Diastolic blood pressure, DBP; Systolic blood pressure, SBP. Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (5th, 95th percentile) for continuous variables with a skewed distribution.

Chapter

7

Gestational hypertensive disorders and retinal microvasculature

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Abstract

Background: Changes in the microvasculature associated with preeclampsia and gestational hypertension have been proposed as a potential pathway in the development of cardiovascular disease. We examined whether hypertensive disorders of pregnancy, such as preeclampsia and gestational hypertension, are related to the maternal retinal microvasculature status after pregnancy.

Methods: This study is part of an ongoing population-based prospective cohort study. During pregnancy and 6.2 years after index pregnancy (90% range 5.7, 7.4 years) we examined 3391 women with available information on preeclampsia, gestational hypertension and retinal vascular calibers. Retinal arteriolar and venular calibers were measured in the left eye from digitized retinal photographs.

Results: Women with preeclampsia had smaller retinal arteriolar calibers six years after pregnancy than women with a normotensive pregnancy (adjusted difference: -0.40 SDS; 95% confidence interval (CI): -0.62, -0.19). For women with previous gestational hypertension similar trends were observed (-0.20 SDS; 95% CI: -0.34, -0.05). With respect to retinal venular calibers, we did not observe consistent trends for women with previous preeclampsia. However, in women with previous gestational hypertension we observed larger venular calibers (0.22 SDS; 95% CI: 0.07, 0.36) than in women with a previous normotensive pregnancy. The association of hypertensive disorders of pregnancy with retinal vessel calibers was mediated through mean arterial pressure at the time of retinal imaging.

Conclusions: Compared to women with a previous normotensive pregnancy women with preeclampsia and gestational hypertension show an altered status of the microvasculature six years after index pregnancy. This is reflected by smaller retinal arteriolar calibers and wider retinal venular calibers. These microvascular changes may possibly contribute to the development of cardiovascular disease in later life.

Introduction

Hypertensive disorders of pregnancy (e.g. preeclampsia and gestational hypertension) affect seven percent of pregnancies today.[1, 2] Both disorders are characterized by new onset of hypertension after 20 weeks of gestation and, in the case of preeclampsia, proteinuria. All features of hypertensive disorders of pregnancy were previously believed to resolve after delivery. However, results from a large meta-analysis show that women with a history of a hypertensive disorders of pregnancy have a two to seven-fold increased risk of developing cardiovascular disease (CVD) in later life.[3] The exact pathophysiologic mechanism underlying this increased CVD risk remains unclear. Nevertheless, it has been shown that microvascular endothelial dysfunction as reflected by a reduced brachial artery flow-mediated dilatation persists in former preeclamptic women for many years after their index pregnancy.[4] These data suggest that the microvasculature may have been affected by a hypertensive disorder of pregnancy and therefore form an important link between hypertensive disorders of pregnancy and the development of CVD in later life.

In recent years, retinal vascular imaging has emerged as a non-invasive technique to visualize the microvasculature.[5-7] Using this technique in individuals over 50 years of age, several studies have shown that microvascular pathology is an independent contributor to the development of CVD in later life, especially in women.[8] Furthermore, a recent study examining the vasodilatory response of retinal vessels to flicker light showed that women with preeclampsia had reduced arteriolar vasodilatation during and after pregnancy compared to women with a normotensive pregnancy, indicating that microvascular dysfunction may already start early in life.[9] Examining the retinal microvasculature may provide further inside into why women with a previous hypertensive disorder of pregnancy have an increased risk for developing CVD later in life.

In the current study we investigated the association between a history of a hypertensive disorder of pregnancy and the status of the microvasculature six years after index pregnancy, as reflected by retinal vascular calibers, in women between the age of 24 to 36 years.

Materials and methods

Design and Study Population

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[10, 11] The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, University Medical Centre, Rotterdam, the Netherlands and the procedures followed were in accordance with institutional guidelines.[12] Written informed consent was obtained from all participants. For the present study we included women with a live born singleton with available information on hypertensive disorders of pregnancy and on postnatal follow-up data. Women were excluded from the main analyses when they had a history of chronic hypertension prior to enrollment in the Generation R Study. We also excluded women who were pregnant during follow-up and women without information on retinal vascular calibers during follow-up visit six years after index pregnancy. The final population for analysis comprised 3391 women (Figure 1).

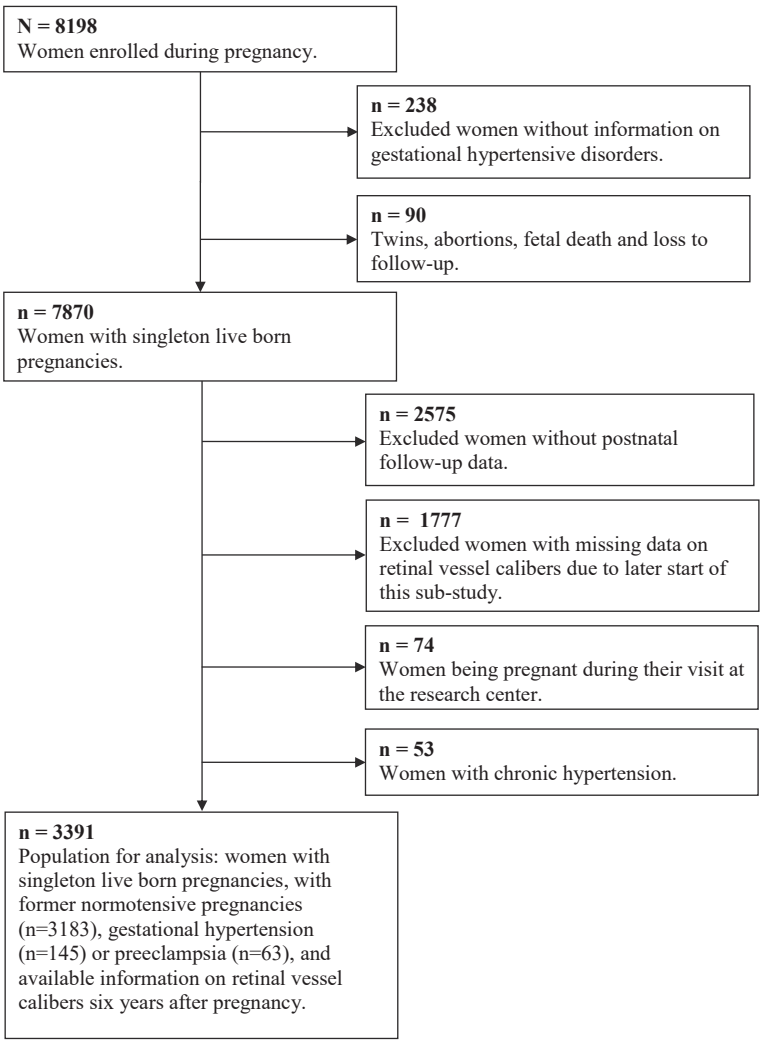

FIGURE 1 | Flowchart.

Hypertensive disorders of pregnancy

The presence of doctor diagnosed preeclampsia or gestational hypertension was retrieved from hospital charts and was determined on the basis of the former criteria described by the International Society for the Study of Hypertension in Pregnancy of 2001.[13, 14] Gestational hypertension was defined by a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg after 20 weeks of gestation in previously normotensive women. Preeclampsia was defined as de novo gestational hypertension with concurrent new onset proteinuria in a random urine sample with no evidence of urinary tract infection.[13]

Maternal blood pressure and anthropometrics

Blood pressure was measured at study enrollment (median 13.2 weeks of gestation [90% range 10.6, 17.0]) and six years after index pregnancy (90% range, 5.7 to 7.4 years) as the average of two systolic and diastolic blood pressure readings, with the validated Omron 907 automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe B.V., Hoofddorp, the Netherlands).[15] All participants were in a standardized supine position to prevent differences due to position. A cuff was placed around the right upper arm. In case of an upper arm exceeding 33 centimeters a larger cuff (32 – 42 cm) was used. The mean value of two blood pressure readings over a five minute interval was documented for each participant. Blood pressure was measured by trained research assistants wearing normal clothing (i.e. no white coat). Mean arterial pressure (MAP) six years after pregnancy was calculated as the average systolic blood pressure plus two times the average diastolic blood pressure, divided by 3. Hypertension six years after index pregnancy was defined by the average of two consecutive blood pressure readings, with systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or the use of antihypertensive medication [16]. Using an alternative cut-off, with systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg, did not change the direction of our results but attenuated our results to non-significant levels.

At study enrollment maternal height (cm) and weight (kg) without shoes were measured after which body mass index (BMI) (kg/m^2) was calculated. Identical measurements were obtained during follow-up six years after pregnancy. Pre-pregnancy BMI was established at enrollment through a questionnaire. Pre-pregnancy weight was highly correlated with the measured early pregnancy weight (Pearson's correlation coefficient r 0.95 (P<0.001)).[17]

Retinal vascular caliber assessment

Retinal vascular calibers were assessed by taking digital retinal photographs six years after index pregnancy. Unilateral and unfractionated photographs were taken of the left eye by a Topcon digital retinal camera (model TRC, NW300) while centered on the optic disc. The resolution of the images was set to 4096 and 3072 pixels. Additionally, a semi-automatic computer-imaging program was used to measure the six largest retinal arteriolar and venular calibers of the photos. These calibers were located one half to one disc diameter from the optic disc margin.[18] The average of the six largest retinal arteriolar and retinal venular calibers were then summarized as central retinal arteriolar and central retinal venular equivalents.[19] The semi-automatic computer-imaging program that was used for computation of the central retinal arteriolar and central retinal venular equivalents was operated by two graders who were blinded to participants' characteristics. Grader specific standard deviation scores were used for both central retinal arteriolar and central retinal venular equivalents. Intraclass correlation coefficients between both graders were excellent for both retinal arteriolar calibers (0.77; 95% CI: 0.69, 0.84) and retinal venular calibers (0.87; 95% CI: 0.81, 0.91). This suggests adequate reproducibility.

Covariates

Information on maternal characteristics during pregnancy including maternal age, self-reported pre-pregnancy weight, gravidity, parity, ethnicity, educational level, smoking and chronic hypertension was available from questionnaires repeatedly applied during pregnancy. Information on gestational age at birth, birth weight and child sex was obtained from medical records.[20, 21] Six years after index pregnancy we obtained information on gravidity and parity at follow-up, medication intake and smoking through questionnaires.

Statistical analyses

We performed four types of analyses. First, a non-response analysis was performed by comparing subject characteristics between mothers with and mothers without available follow-up data six years after index pregnancy (Additional file 1). Women with available follow-up data, but without information on retinal vascular calibers, were left out of the non-response analysis. Second, to reduce the possibility of potential bias associated with missing data, missing values in covariates were imputed using multiple imputation procedures. Five draws for each missing value were performed providing five substituted data points, which in turn created five completed data sets. Analyses were performed separately on each completed dataset and thereafter combined into one global result.[22] In the total population for analysis 19.4% had missing information on pre-pregnancy BMI, 2.3% on ethnicity, 6.7% on education, 11.3% on smoking during pregnancy, 29.5% on gravidity at follow-up, 29.9% on smoking during follow-up, 2.3% on blood pressure during follow-up and 0.2% on medication intake during follow-up. Third, differences in maternal characteristics during pregnancy and follow-up were compared between women with a hypertensive disorder of pregnancy and women with normotensive pregnancies using one-way ANOVA for continuous variables and chi-square tests for categorical variables. Fourth, associations between hypertensive disorders of pregnancy, normotensive pregnancies and retinal vascular calibers were assessed through linear regression. The linear regression model included covariates selected based on their associations with the outcome of interest based on previous studies or a change in effect estimate of >10% (maternal age at enrollment, ethnicity, educational level at enrollment, smoking during pregnancy, pre-pregnancy BMI and lastly when assessing retinal arteriolar caliber we additionally adjusted for retinal venular caliber and vice versa. Hypertensive disorders of pregnancy are known to increase blood pressure and blood pressure is known to be associated with smaller retinal arteriolar calibers.[23] To examine the mediating role of mean arterial blood pressure at the time of retinal imaging in the association of hypertensive disorders of pregnancy with retinal vascular calibers, we analyzed the direct and indirect causal mediation effects through mediation analyses.[24] We used the full model, as was used for linear regression analysis, to adjust for confounding. Statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA) and R version 3.3.2 (R foundation for Statistical Computing, Vienna, Austria [packages 'foreign', 'rms' and 'mediation']).[25]

Results

Table 1 and Table 2 show maternal characteristics during pregnancy and six years after index pregnancy. Women with hypertensive disorders of pregnancy had higher systolic and diastolic blood pressures at the start of pregnancy and six years later compared to women with a normotensive pregnancy. Additionally, women with a history of a hypertensive disorder of pregnancy had more often hypertension and were more likely to take cardiovascular or antihypertensive medication six years after pregnancy compared to women with a normotensive pregnancy.

TABLE 1 | Subject characteristics by hypertensive disorder of pregnancy n = 3391.

	Normotensive Pregnancy n = 3183	**Gestational hypertension** n = 145	**Preeclampsia** n = 63	***P*-value**
Maternal characteristics (pregnancy)				
Age at enrollment (years)	30.1 (5.1)	30.4 (5.1)	29.4 (5.5)	0.44
Gestational age at enrollment (weeks)	13.9 (10.9, 22.2)	13.4 (10.1, 22.9)	13.8 (10.3, 22.5)	0.15
Height (cm)	166.6 (7.4)	168.0 (7.3)	165.3 (6.8)	0.03
Pre-pregnancy weight (kg)	64.0 (50.0, 87.0)	70.0 (54.0, 114.4)	68.0 (53.2, 105.0)	<0.001
Pre-pregnancy BMI (kg/m2)	22.7 (18.7, 31.5)	25.2 (20.0, 40.2)	24.4 (19.2, 37.2)	<0.001
Weight at enrollment (kg)	67.0 (52.8, 91.1)	75.0 (56.3, 112.0)	70.0 (52.0, 110.4)	<0.001
SBP at enrollment (mmHg)	114.8 (11.7)	126.0 (13.1)	120.4 (12.9)	<0.001
DBP at enrollment (mmHg)	67.3 (9.0)	76.7 (11.3)	74.3 (9.9)	<0.001
Primiparous, n (%)	1894 (60.0)	110 (75.9)	51 (81.0)	<0.001
Non-European ethnicity, n (%)	1267 (40.8)	42 (29.0)	30 (47.6)	0.005
Lower educational level, n (%)	272 (9.2)	11 (7.9)	6 (10.0)	0.02
Smoking, n (%)	749 (26.6)	43 (32.1)	13 (21.3)	0.29
Pregnancy outcomes				
Gestational age at birth (weeks)	40.1 (37.1, 42.1)	40.1 (37.2, 42.1)	38.6 (30.9, 41.5)	<0.001
Birth weight (g)	3442.1 (539.8)	3432.8 (578.6)	2889.3 (877.4)	<0.001
Small for gestational age < p10, n (%)	296 (9.3)	20 (13.8)	17 (27.0)	<0.001
Male sex, n (%)	1624 (51.0)	76 (52.4)	25 (39.7)	0.19

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; N, number; SBP, systolic blood pressure. Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution. Differences in baseline characteristics were tested using Students t-test, ANOVA, Kruskal-Wallis and chi-square tests. Presented values are not imputed.

Table 3 and Supplemental Figures 1A and 1B show that women with preeclampsia had smaller retinal arteriolar calibers six years after pregnancy than women with a previous normotensive pregnancy (age-adjusted difference: -0.49 SDS; 95% confidence interval [CI] -0.74, -0.25). Additional adjustment for ethnicity, educational level, smoking, pre-pregnancy BMI and retinal venular caliber did not alter these results. Women with gestational hypertension also had smaller retinal arteriolar calibers than women with a previous normotensive pregnancy (fully adjusted difference: -0.20 SDS; 95% CI -0.34, -0.05). Women with previous preeclampsia did not show any difference in retinal venular calibers six years after pregnancy compared to those with a

normotensive pregnancy. However, retinal venular calibers were larger in women with gestational hypertension than in those with a previous normotensive pregnancy (0.22 SDS; 95% CI 0.07, 0.36).

TABLE 2 | Subject characteristics six years after index pregnancy by hypertensive disorder of pregnancy n = 3391.

	Normotensive Pregnancy n = 3183	**Gestational hypertension** n = 145	**Preeclampsia** n = 63	***P*-value**
Age (years)	36.7 (5.1)	37.1 (4.9)	26.0 (5.5)	0.38
Visit interval (years)	6.2 (5.7, 7.4)	6.1 (5.7, 7.6)	6.0 (5.7, 7.5)	0.30
SBP (mmHg)	118.1 (12.0)	128.4 (17.6)	126.5 (15.8)	<0.001
DBP (mmHg)	70.0 (9.3)	78.2 (12.0)	78.5 (12.4)	<0.001
Mean arterial pressure (mmHg)	86.0 (9.6)	95.0 (13.2)	94.5 (12.9)	<0.001
Hypertension, n (%)	203 (6.4)	39 26.9)	15 (23.8)	<0.001
Primigravid, n (%)	225 (10.1)	19 (17.0)	9 (22.0)	0.003
Medication, n (%)				
Cholesterol lowering medication	8 (0.3)	1 (0.7)	0 (0.0)	0.55
Glucose lowering medication	13 (0.4)	2 (1.4)	1 (1.6)	0.11
Anti-hypertensives	36 (1.1)	10 (6.9)	4 (6.3)	<0.001
Cardiovascular medication	43 (1.4)	10 (6.9)	4 (6.3)	<0.001
Combined	54 (1.7)	11 (7.6)	5 (7.9)	<0.001
Smoking, n (%)	420 (18.9)	21 (18.9)	3 (7.3)	0.17
Retinal arteriolar caliber (µm)	145.8 (16.9)	141.9 (17.8)	137.8 (14.4)	<0.001
Retinal venular caliber (µm)	207.1 (22.5)	208.6 (22.5)	203.8 (19.0)	0.36
Retinal arteriolar venular ratio	0.71 (0.07)	0.68 (0.07)	0.68 (0.06)	<0.001
Weight (kg)	68.6 (53.6, 97.8)	77.0 (58.8, 121.1)	73.5 (53.3, 120.7)	<0.001
BMI (kg/m2)	24.5 (19.7, 34.5)	27.7 (21.3, 45.5)	27.6 (19.6, 43.9)	<0.001

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; N, number; SBP, systolic blood pressure. Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution. Differences in baseline characteristics were tested using Students t-test, ANOVA, Kruskal-Wallis and chi-square tests. Values are not imputed.

TABLE 3 | Associations of hypertensive disorders of pregnancy with retinal vascular calibers six years after index pregnancy n = 3391.

	Normotensive pregnancy n = 3183	**Gestational hypertension** n = 145 Beta (95% CI)	***P*-value**	**Preeclampsia** n = 63 Beta (95% CI)	***P*-value**
Retinal arteriolar caliber (SDS)					
Full model	*Ref*	-0.20 (-0.34, -0.05)	0.007	-0.40 (-0.62, -0.19)	0.0002
Retinal venular caliber (SDS)					
Full model	*Ref*	0.22 (0.07, 0.36)	0.003	0.08 (-0.13, 0.30)	0.45

Abbreviations: Confidence Interval, CI; standard deviation scores, SDS. Values are regression coefficients (95% confidence interval) and are based on linear regression. Estimates are from multiple imputed data. Full model: adjusted for maternal age at enrollment, ethnicity, educational level at intake, smoking at intake and pre-pregnancy BMI. Additionally, we adjusted for retinal venular caliber when assessing the outcome retinal arteriolar caliber and vice versa.

TABLE 4 | The mediation role of mean arterial pressure at the time of retinal imaging in the association of hypertensive disorders of pregnancy with retinal vascular calibers six years after index pregnancy n = 3391.

	Normotensive pregnancy	**Gestational hypertension**	***P*-value**	**Preeclampsia**	***P*-value**
	n = 3183	n = 145		n = 63	
		Beta (95% CI)		Beta (95% CI)	
Retinal arteriolar caliber (SDS)					
Total effect	*Ref*	-0.20 (-0.35, -0.05)	0.01	-0.41 (-0.62, -0.21)	<0.001
Direct effect	*Ref*	-0.01 (-0.14, 0.13)	0.94	-0.21 (-0.41, -0.02)	0.03
Mediated effect	*Ref*	-0.20 (-0.26, -0.13)	<0.001	-0.20 (-0.29, -0.11)	<0.001
Retinal venular caliber (SDS)					
Total effect	*Ref*	0.22 (0.08, 0.36)	<0.001	0.08 (-0.09, 0.27)	0.39
Direct effect	*Ref*	0.16 (0.02, 0.30)	0.02	0.03 (-0.14, 0.22)	0.76
Mediated effect	*Ref*	0.06 (0.03, 0.09)	<0.001	0.05 (0.02, 0.09)	<0.001

Abbreviations: Confidence Interval, CI; body mass index, SDS. Values are regression coefficients (95% confidence interval) and are based on mediation analysis. Values are adjusted for maternal age at enrollment, ethnicity, educational level at intake, smoking at intake and pre-pregnancy BMI. Additionally, we adjusted for retinal venular caliber when assessing the outcome retinal arteriolar caliber and vice versa.

The results of mediation analyses for the mediating role of mean arterial pressure at the time of retinal imaging in the association of hypertensive disorders of pregnancy with retinal vessel calibers is presented in Table 4. There was mediation by mean arterial pressure in the association of both preeclampsia and gestational hypertension with retinal arteriolar and retinal venular calibers.

Finally, we tested whether the amount of previous pregnancies affected our results. No differences were observed in retinal vascular calibers in association to gravidity or parity.

Discussion

Our study shows that women with hypertensive disorders of pregnancy have an altered status of the retinal microvasculature six years after index pregnancy compared to women with a normotensive index pregnancy. In particular women with preeclampsia have smaller retinal arteriolar calibers than women with previous normotensive pregnancies. Additionally, women with gestational hypertension have wider retinal venular calibers than women with previous normotensive pregnancies. These associations may partly be related to concurrent blood pressure, since adjustment for mean arterial pressure attenuated most relationships.

During a normotensive pregnancy the maternal cardiovascular system undergoes imperative adaptations by increasing intravascular volume, heart rate and cardiac output.[26] Concomitantly with these cardiovascular adaptations there is a decrease in blood pressure accompanied by physiologic vasodilatation of the microvasculature.[27] Cross-sectional results from the GUSTO study support these adaptations showing that each 10 mmHg increase in MAP during pregnancy was associated with a significant reduction in retinal arteriolar caliber, especially when MAP ≥ 90mmHg.[28] Another study, using retinal images obtained throughout pregnancy and six months after pregnancy of 53 normotensive women, also demonstrated changes in the retinal

microvasculature over the course of a normotensive pregnancy in conjunction with blood pressure adaptations.[29] During mid-pregnancy, when blood pressure shows a physiologic decrease, retinal arteriolar and retinal venular calibers increased significantly. Nevertheless, retinal arteriolar and venular calibers returned to normal (early pregnancy) values in late pregnancy and six months after pregnancy. These results imply that changes in the retinal microvasculature during pregnancy are transient and mainly the consequence of concurrent physiologic blood pressure fluctuations. However, other studies in non-pregnant populations demonstrated that not only concurrent but also past elevated blood pressures are associated with retinal arteriolar narrowing.[30-32] Therefore, retinal arteriolar narrowing may not only be considered a transient response to an increased blood pressure, but also the result of cumulative exposure to hypertension over the life course. The underlying pathophysiology might be explained by accumulating endothelial damage, due to hypertension, eventually leading to endothelial dysfunction and microvascular impairment.[33, 34]

This might also explain the larger retinal venular calibers in women with previous gestational hypertension in our study and not in women with previous preeclampsia. Gestational hypertension women had a higher weight and blood pressure at the start of pregnancy than preeclampsia women. These factors have been associated with larger retinal venular calibers, possibly through local vascular inflammation and endothelial damage.[35, 36] Though we did not reach statistical significance, retinal venular calibers also tended to be larger in women with previous preeclampsia than in women with a previous normotensive pregnancy. Nevertheless, we need to be causative with interpreting this finding due to the small sample size of women with previous preeclampsia.

Even though previous studies did not examine retinal microvascular calibers both before and after a hypertensive disorder of pregnancy, it seems likely that damage to the endothelium as a result of the hypertensive disorder of pregnancy is not completely reversible and therefore enhances retinal microvascular changes after pregnancy. Previous studies showed through flickering response and laser Doppler perfusion imaging technique in women with preeclampsia that retinal microvascular function is impaired both during pregnancy up to 25 years after.[9, 37] Microvascular dysfunction therefore does not merely seem to be a disorder of pregnancy. For this reason, future research should aim to visualize the retinal microvasculature, before and at multiple time points after a hypertensive disorder of pregnancy, in order to detect if retinal microvascular abnormalities precede the onset of a hypertensive disorder of pregnancy and whether they progress after pregnancy.

Numerous studies have shown a relationship between retinal vascular changes (e.g. retinal arteriolar narrowing and retinal venular widening) and future CVD.[5, 32, 38-40] For instance, a large meta-analysis showed an increased risk of coronary heart disease (CHD) in women with wider retinal venular or narrower retinal arteriolar calibers.[41] The Atherosclerosis Risk in Communities Study (ARIC) assessed retinal microvascular calibers among men and women aged 49-73 showing that retinal arteriolar narrowing was associated with an increased risk of CHD, myocardial infarction, congestive heart failure and incident hypertension.[32, 38, 39] Additionally, the Beaver Dam Eye Study demonstrated that retinal arteriolar narrowing is associated with an increased

10-year risk of hypertension.[42] Our study provides evidence to support the concept that women with hypertensive disorders of pregnancy show more unfavorable retinal microvascular calibers six years after pregnancy than women with previous normotensive pregnancies. As a result, it is reasonable to speculate that women with hypertensive disorders of pregnancy might have an increased risk for future CVD.

Strengths and limitations

Several limitations of the present studies need to be discussed: First, we did not obtain retinal vascular imaging from 33.6% of all women who came for follow-up visit six years after pregnancy, because retinal vascular imaging was introduced into the Generation R Study after recruitment of study subjects had already started. As this was independent of any subject characteristics, we do not expect any additional selection bias. However, there may be some loss of power due to a smaller sample size available for our analysis and hence larger confidence intervals of the reported associations. Second, compared to non-responders (43%) study participants were on average older at study enrollment, more often primiparous and of European descent, higher educated, more often non-smokers, and had more often gestational hypertension. This may have led to some degree of selection bias as the included women were relatively healthy and may have led to an underestimation of the association between hypertensive disorders of pregnancy and retinal microvasculature. Third, due to unavailability of pre-pregnancy data on blood pressure (as is also the case in most other studies focusing on pregnant women and cardiovascular outcomes after pregnancy) we cannot exclude the possibility that microvascular changes and hypertension preceded the onset of hypertensive disorders of pregnancy. However, we coped with this by excluding women with chronic hypertension. Information on chronic hypertension before pregnancy was obtained through a questionnaire during pregnancy which was cross-checked with information from the original medical records and the Dutch obstetric database.[43] Our results did not change significantly after performing a sensitivity analyses excluding women with hypertension in early pregnancy (13.2 weeks of gestation, 95% CI; 11.1, 17.0) or women with hypertension at the time of retinal imaging. Hypertension in early pregnancy was defined as a systolic blood pressure of ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg. Hypertension at the time of retinal imaging was defined as the intake of antihypertensive medication and a systolic blood pressure of ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg. Lowering the cut-off for hypertension at the time of retinal imaging (systolic blood pressure of ≥ 130 mmHg and/or a diastolic blood pressure ≥ 80 mmHg) did not change the direction of our results, but did attenuate our results to non-significant levels. Fourth, retinal vascular calibers were not assessed before pregnancy. Therefore, microvascular changes might be due to hypertensive disorders of pregnancy or might have predated pregnancy. Fifth, the observational nature of this study does not allow for inference of causality and does not preclude the existence of residual confounding. Sixth, information on pregnancies and hypertensive disorders of pregnancy occurring after the index pregnancy was incomplete. The absence of these data might have affected our results. Finally, our study also has several strengths. First, this is a prospective cohort study from early pregnancy onwards with a large sample size of 3391 participants. Second, retinal images were taken and graded following standardized protocols.

Conclusion

Our study shows that in women with a hypertensive disorder of pregnancy the microvasculature is already affected early in life. Six years after index pregnancy women with a hypertensive disorder of pregnancy show smaller retinal arteriolar and wider retinal venular calibers than women with a normotensive pregnancy, suggesting that the changes in the microvasculature possibly represent the pathophysiological substrate linking hypertensive disorders of pregnancy to CVD in later life. Future research should therefore aim to investigate associations between the microvasculature and cardiovascular risk factors before and after the onset of hypertensive disorders of pregnancy and the long-term cardiovascular outcomes in these women.

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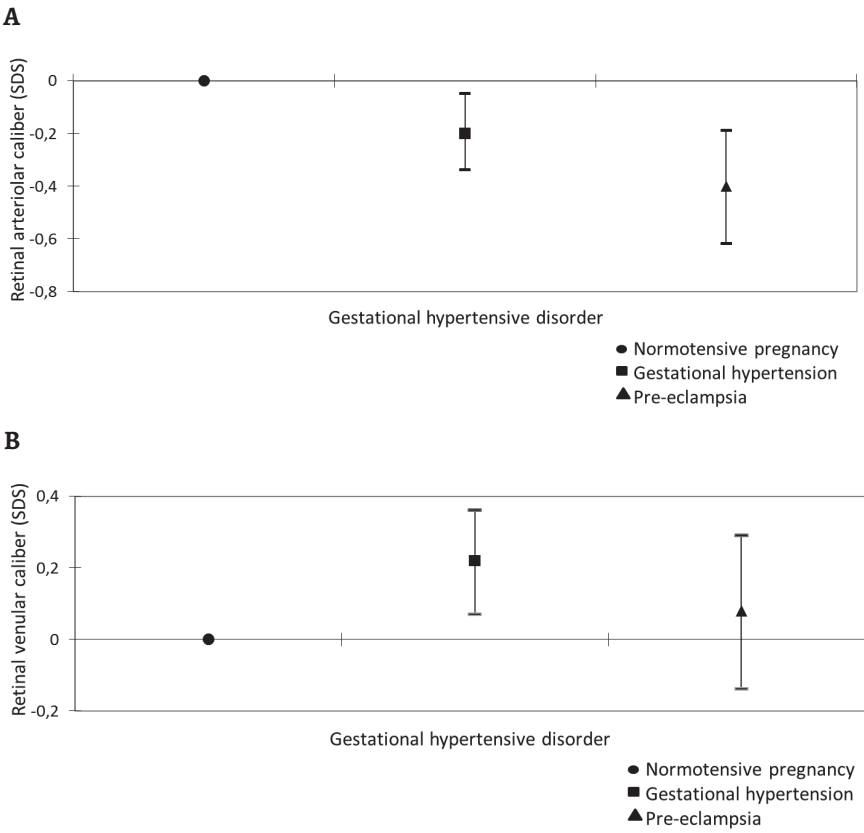
Supplemental material



SUPPLEMENTAL FIGURE 1 | The association of gestational hypertensive disorders with retinal arteriolar (A) and venular (B) calibers six years after index pregnancy.

Values are regression coefficients (95% confidence interval) and are based on linear regression models. Estimates are from multiple imputed data. We adjusted for maternal age at enrollment, ethnicity, educational level at enrollment, smoking during pregnancy, pre-pregnancy BMI and lastly when assessing retinal arteriolar caliber we additionally adjusted for retinal venular caliber and vice versa.

SUPPLEMENTAL TABLE 1 | Maternal and fetal characteristics stratified for loss to follow-up n=5966.

	Included in analyses n = 3391	**Not included** n = 2575	***P*-value**
Maternal characteristics (prenatal)			
Age at enrollment (years)	30.1 (5.1)	28.2 (5.5)	<0.001
Gestational age at enrollment (weeks)	13.8 (10.8, 22.3)	14.4 (10.8, 23.8)	<0.001
Height (cm)	166.7 (7.4)	168.6 (7.4)	0.09
Pre-pregnancy weight (kg)	64.0 (50.0, 90.0)	63.0 (49.0, 92.0)	0.07
Pre-pregnancy BMI(kg/m2)	22.7 (18.8, 31.9)	23.3 (19.2, 30.9)	0.52
Weight at enrollment (kg)	67.0 (53.0, 93.0)	67.0 (51.0, 95.0)	0.92
SBP at intake (mmHg)	115.3 (12.0)	114.7 (12.3)	0.06
DBP at intake (mmHg)	67.8 (9.3)	67.5 (9.8)	0.25
Primiparous (%)	61.0	55.8	<0.001
Non-European ethnicity (%)	40.4	53.1	<0.001
Lower educational level (%)	9.1	16.9	<0.001
Smoking (%)	26.8	30.5	0.004
Birth and childhood characteristics			
Gestational age at birth (weeks)	40.1 (37.1, 42.1)	40.0 (36.5, 42.1)	<0.001
Birth weight (g)	3450.0 (2540.0, 4300.0)	3380.0 (2450.0, 4300.0)	<0.001
Small for gestational age (<p10), (%)	9.8	11.3	0.07
Male sex (%)	50.9	51.7	0.51
Pregnancy complications			
Gestational hypertension (%)	4.4	3.1	0.02
Preeclampsia (%)	1.9	2.7	0.07

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure. Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution. Differences in baseline characteristics were tested using Students t-test, ANOVA, Kruskal-Wallis and chi-square tests. Presented values are not imputed.

Chapter

8

Prevalence of subclinical coronary artery disease assessed by coronary computed tomography angiography among women with a history of preeclampsia aged 45 to 55 years

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Research letter
Circulation. 2018;137:877-879

Introduction

Preeclampsia is associated with an increased risk of coronary artery disease (CAD), although evidence on premature CAD development after preeclampsia is limited.[1] A cross-sectional study among 491 postmenopausal women with a mean age of 67 years showed an increased prevalence of coronary artery calcification (CAC) in participants with self-reported high blood pressure during any previous pregnancy compared with women without such history.[2] However, the association between hypertensive pregnancy, CAC and coronary plaque formation has not been reconfirmed in prospective studies, and there are no data on the timeline by which atherosclerosis develops in women with previous preeclampsia. The aim of this study is to compare the prevalence of coronary artery atherosclerosis of asymptomatic women aged 45 to 55 years with a history of preeclampsia to a population-based reference cohort.

Methods

The rationale and design of the Cardiovascular RiskprofilE: IMaging And Gender-specific disOrders (CREw-IMAGO) have been published previously.[3] Asymptomatic women, aged 45 to 55 years, with a history of preeclampsia 10 to 20 years earlier were included in this multicenter, prospective cohort study. Medical records, including pregnancy characteristics and hospital admission, were available for all women. Outcomes were compared with women of similar age and ethnicity who participated in the Multi-Ethnic Study of Atherosclerosis (MESA).[4] MESA is a prospective cohort study measuring CAC and traditional cardiovascular risk factors in 6814 multiethnic participants without a history of physician-diagnosed cardiovascular or neurovascular disease. For our analyses, MESA data were restricted to all participating women from Caucasian descent aged 45 to 55 years, resulting in a reference group of 387 women.

Routine cardiovascular risk assessment included medical and pregnancy history, age, body-mass index, waist circumference, blood pressure, and a venous blood sample assessed lipid profile, fasting plasma glucose, glycated hemoglobin and high-sensitivity C-reactive protein. CAC score (CACS) and contrast-enhanced coronary computed tomography angiography (CCTA) were performed in women with previous preeclampsia. In the MESA cohort only CACS was performed. CACS were converted to MESA percentiles adjusted for age, gender and ethnicity. The primary outcome was the prevalence of CACS >0 Agatston Units (AU) and/or any coronary atherosclerotic plaques on CCTA. Secondary outcomes included CACS ≥100AU, CACS ≥95th MESA percentile, and presence of a significant (≥50%) luminal stenosis on CCTA. The study was approved by the medical ethics committee of the University Medical Center Utrecht and all participants provided written informed consent. Analyses were performed with Statistical Package for Social Sciences (SPSS) version 22.0 (SPSS Inc).

Results

We included 164 asymptomatic women with a history of preeclampsia (mean age 48.4 ± 2.9 years at inclusion). Overall, 31% presented with a CACS >0 and 17% had CACS ≥95th MESA percentile. Compared with the MESA reference group, women with a history of preeclampsia showed a higher risk of CACS >0AU (RR 1.7, 95% CI 1.2-2.3), CACS >100AU (RR 2.8, 95% CI (0.4-19.3) and CACS ≥95th MESA percentile (RR 3.5; 95% CI 2.0-6.1). In addition, 47% of women with previous preeclampsia had coronary atherosclerotic plaques on CCTA and 4.3% showed significant stenosis. Compared to the MESA cohort, women with a history of preeclampsia had higher prevalence of hypertension and metabolic syndrome (Table 1).

Discussion

Our data are the first to demonstrate that preeclampsia is associated with accelerated atherosclerosis. CAC and plaques are detectable as early as the fifth decade of life, which may explain the higher rate of ischemic coronary events in these women as observed by Smith and colleagues in 2001.[1] We present new evidence for signs of subclinical atherosclerosis on CCTA in middle-aged women with a history of preeclampsia in a well-phenotyped prospective cohort study. CCTA reference data on coronary plaques in the general population with similar ethnicity are not available yet. However, prevalence of subclinical plaques on CCTA has been reported in a retrospective cohort study from South Korea.[5] The presence of plaques on CCTA in 1282 asymptomatic middle-aged women (50.0 ± 8.4 years) was substantially lower than the plaque burden detected in our cohort of women with previous preeclampsia (6.7% versus 47.2%).

Limitations of our study include uncertainty whether a positive CACS and CCTA result will progress to symptomatic CAD and/or ischemic coronary events, and the lack of data on women younger than 45 years or of nonwhite ethnicity. Future research should address these knowledge gaps.

Conclusion

In conclusion, 30% of women with a history of preeclampsia show signs of coronary atherosclerosis on vascular CT imaging compared with 18% of women from the reference group. Therefore, women with previous preeclampsia have an increased risk of subclinical coronary artery atherosclerosis at age 45 to 55 years. These findings demonstrate that early coronary atherosclerosis precedes the development of subclinical ischemic heart disease in women with previous preeclampsia. Early identification of these women at high risk may facilitate timely prevention to reduce future CAD events.

TABLE 1 | Baseline characteristics of the preeclampsia group and the reference group.

	Preeclampsia		MESA	*P*-value	Relative Risk
	n = 164	n = 161	n = 387		(95% CI)
Patient characteristics					
Age, y	48.4 (2.9)		50.0 (3.0)	<0.01	
White ethnicity, n (%)	162 (99%)		187 (100%)	0.09	
History of pregnancy, n (%)	164 (100%)		288 (75%)	<0.01	
Premenopausal, n (%)	78 (57%)		179 (46%)	<0.01	
Clinical measurements					
Systolic blood pressure, mmHg	130 (15)		110 (16)	<0.01	
Diastolic blood pressure, mmHg	81 (10)		66 (9)	<0.01	
BMI, kg/m^2	28.1 (6.2)		27.6 (6.7)	0.40	
Waist circumference, cm	89.4 (13.3)		92.8 (18.1)	0.01	
Total cholesterol, mmol/L	5.4 (1.0)		5.1 (1.0)	<0.02	
Triglycerides, mmol/L	1.2 (0.5)		1.4 (1.1)	0.02	
HDL-cholesterol, mmol/L	1.52 (0.34)		1.49 (0.41)	0.28	
LDL-cholesterol, mmol/L	3.4 (0.9)		3.0 (0.8)	<0.01	
Glucose, mmol/L	5.5 (1.1)		4.7 (1.0)	<0.01	
Cardiovascular disease risk factors					
Family history of premature CVD, n (%)	31 (19%)		114 (32%)	0.02	
Hypertension,* n (%)	89 (54%)		88 (23%)	<0.01	
Diabetes, n (%)	5 (3%)		10 (3%)	0.78	
Current smoking, n (%)	12 (8%)		69 (18%)	<0.01	
Obesity, n (%)	49 (30%)		114 (29%)	0.92	
Metabolic syndrome,† n (%)	54 (33%)		75 (20%)	<0.01	
Cardiovascular disease risk score					
≥2 risk factors,† n (%)	29 (18%)		68 (19%)	0.79	
Intermediate–high risk, FRS ≥10%, n (%)	15 (9%)		20 (5%)	0.07	
Coronary artery calcium score, AU, n (%)					
>0	50 (31%)		70 (18%)		1.7 (1.2-2.3)
0.1 – 9	14 (9%)		33 (9%)		1.2 (0.6-2.1)
10 – 99	26 (16%)		30 (8%)		2.2 (1.3-3.5)
≥100	10 (6%)		7 (2%)		2.8 (0.4-19.3)
MESA ≥95th percentile, n (%)	28 (17%)		19 (5%)		3.5 (2.0-6.1)
Coronary CT angiography					
Any plaque, n (%)		76 (47%)			
Significant stenosis,⁜ n (%)		7 (4%)			

Abbreviations: AU, Agatston units; BMI, body mass index; CACS, coronary artery calcium score; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; FRS, Framingham Risk Score; MESA, multi-ethnic study of atherosclerosis; CI, confidence interval. Data are presented as mean ± standard deviation, unless otherwise stated. Unadjusted relative risk and 95% CI's presented.

*Blood pressure ≥140/90 mmHg or current antihypertensive treatment.

†According to National Cholesterol Education Program Adult Treatment Panel III criteria.

⁜Luminal stenosis ≥50%.

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Chapter 12

General discussion

One in two women die due to cardiovascular disease (CVD), making it the largest contributor to global mortality.[1, 2] Traditional cardiovascular risk factors, such as hypertension, obesity, dyslipidemia and smoking explain up to 90% of CVD risk.[3] For this reason, the main focus of cardiovascular prevention guidelines lies in reducing these risk factors through the implementation of cost-effective population wide strategies. Recently, there has been more awareness for CVD in women and women's health.[4] More attention is however needed for a specific category within the female population. This includes women with a pregnancy complicated by a hypertensive disorder such as gestational hypertension and preeclampsia. Compared to women with a previous normotensive pregnancy, they are two to eight times more likely to develop CVD in later life and if so, at a younger age, depending on the severity of the hypertensive disorder of pregnancy.[5] Traditional cardiovascular risk factors are more frequently present in these women after pregnancy. These cardiovascular risk factors might be preexisting to pregnancy or they might be a direct consequence of the hypertensive disorder of pregnancy, resulting in a greater cardiovascular stress level after pregnancy than there would have been without the disease.[6]

The general aim of this thesis was to determine cardiovascular health and to identify early cardiovascular risk factors and cardiovascular risk biomarkers in women with a previous hypertensive disorder of pregnancy and compare these results to women with a previous normotensive pregnancy. This chapter reflects on our main findings, clinical practice and future implications.

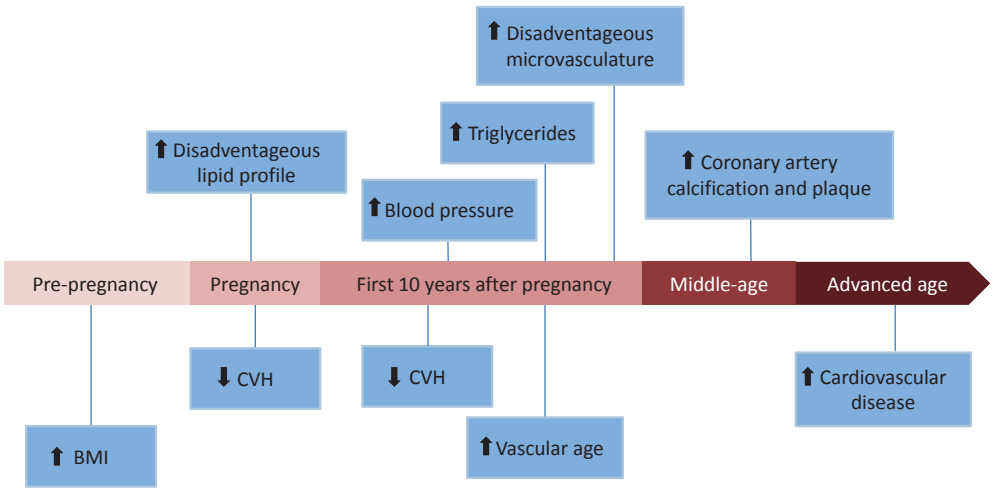

FIGURE 1 | Cardiovascular and metabolic outcomes over the life course in women with a hypertensive disorder of pregnancy.
Abbreviations: BMI, body mass index; CVH, cardiovascular health.

Biomarkers

In Part I of this thesis we aimed to determine biomarkers in pregnancy to predict the risk of developing a hypertensive disorder of pregnancy and a suboptimal cardiovascular risk profile after pregnancy. The World Health Organization (WHO) defines a biomarker as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease".[7] This definition provides a wide range of potential biomarkers in relation to hypertensive disorders of pregnancy, including biochemical tests (e.g. placental growth factor [PlGF], lipids and glucose), physical tests (e.g. blood pressure and body mass index [BMI]) or imaging tests (coronary artery calcification by coronary computed tomography angiography and carotid intima-media thickness by ultrasound).

Biomarkers are only relevant when they can be related to clinical endpoints.[8] All too often, clinical endpoints are considered to be the only relevant outcomes in research. However, they can be scarce in certain populations (e.g. death in a young population), resulting in a long and costly follow-up time. Biomarkers can then be used as surrogate endpoints to provide interim information on the risk of developing a clinical endpoint. This provides the opportunity to implement interventions and reduce the risk of a clinical endpoint such as cardiovascular events or death. In the context of hypertensive disorders of pregnancy, the ideal biomarker could be measured before pregnancy or in an early stage of pregnancy, predicts the risk of developing the pregnancy disorder while also predicting the risk of future CVD. Circulating PlGF is such a biomarker to predict the risk of preeclampsia.[9, 10] PlGF is a proangiogenic factor produced by the syncytiotrophoblast of the placenta.[11, 12] In complicated pregnancies affected by a placental disorder, such as preeclampsia or fetal growth restriction, the syncytiotrophoblast is stressed, produces lower concentrations of PlGF, leading to a suboptimal hemodynamic adaptation to pregnancy.[13-15] It is well known that these women more often have cardiovascular risk factors after pregnancy than women with an uncomplicated pregnancy. However, also women with an uncomplicated pregnancy might have had low PlGF concentrations in mid-pregnancy, as a sign of suboptimal hemodynamic adaptation to pregnancy, without clinically apparent disease. Consequently, they might also be more at risk for developing cardiovascular risk factors after pregnancy. We examined whether low PlGF concentrations in mid-pregnancy were associated with cardiovascular risk factors after pregnancy in women with and without a complicated pregnancy (**Chapter 2**). Results showed that low PlGF was independently associated with a larger left atrial diameter and left ventricular mass six years after pregnancy, regardless of the pregnancy course. In animal knockout studies, the absence of PlGF in pregnancy has also been linked to a larger left ventricular mass.[16] Low concentrations of mid-pregnancy PlGF therefore seem to be a biomarker of not only suboptimal hemodynamic adaptation to pregnancy but also of a suboptimal cardiovascular risk profile after pregnancy.

An atherogenic lipid profile is a common biomarker of atherosclerotic risk.[17, 18] We showed (**Chapter 3**) that an atherogenic lipid profile in early pregnancy is associated with an increased risk of developing a hypertensive disorder of pregnancy. This might be due to a higher degree of atherosis in the spiral arteries of the placental bed.[19, 20] Atherosis is histologically comparable to the early stage of atherosclerosis.[21] In theory, this could lead to a suboptimal functioning of the

placenta and consequently to a complicated pregnancy. In addition, women with a hypertensive disorder of pregnancy have an increased risk of coronary atherosclerosis later in life (**Chapter 8**). As such, an atherogenic lipid profile in early pregnancy might be a precursor of not only a hypertensive disorder of pregnancy but also coronary atherosclerosis later in life.

Cardiovascular follow-up

Health care providers should not merely focus on cardiovascular health in pregnancy but also be aware of the importance of cardiovascular follow-up afterwards. Table 12.1 provides an overview of the recommendations regarding cardiovascular follow-up of women after a hypertensive disorder of pregnancy, by the largest national and international guidelines on cardiovascular risk prevention.[22-30, 79] Evidently, there is no uniform recommendation for cardiovascular risk management in women with a previous hypertensive disorder of pregnancy. Current guidelines are largely developed by gynecologists and cardiologists. Gynecology guidelines mainly cover the diagnosis and treatment of hypertensive disorders of pregnancy and occasionally recommend postpartum cardiovascular follow-up. Because cardiovascular risk prevention is in general not the expertise of a gynecologist, most of the guidelines do not address who should be responsible for the organization of postpartum cardiovascular follow-up. In general, cardiovascular risk prevention is coordinated by cardiologists, general practitioners (GPs) and internists. Their guidelines mostly have a population based approach but do highlight certain specific populations. Some cardiovascular prevention guidelines acknowledge the cardiovascular follow-up of women with a previous hypertensive disorder of pregnancy.[22, 23, 79] However, they often focus only on women with preeclampsia and to a lesser extend or not at all on women with gestational hypertension. Though the risk of developing CVD is higher for women with preeclampsia than women with gestational hypertension, absolute risks are high amongst both groups.[31-33] Based on previous studies and the results discussed below, we like to emphasize that cardiovascular follow-up should be provided to all women with preeclampsia as well as those with gestational hypertension.

As providers of long-term care, general practitioners seem to be the most suitable group to provide cardiovascular follow-up to women with a previous hypertensive disorder of pregnancy. GPs generally have more insight in their patient's well-being, social network and living conditions than other medical specialists. Another possibility would be a 'shared care' model, as commonly used for the follow-up of oncology patients, where follow-up strategies are shared between specialists (e.g. gynecologist, internist and GP).[34, 35] The gynecologist could provide an initial cardiovascular follow-up plan, intended for the GP, on discharge of the patient. This plan should include an overview of the medical history and a timeline with the cardiovascular measurements that need to be performed.

We showed that cardiovascular risk factors after a hypertensive disorder of pregnancy were already apparent in the first year after pregnancy. Nearly half (41.5%) of the women with previous severe onset preeclampsia had some form of hypertension (sustained hypertension, masked hypertension, or white-coat hypertension) and a disadvantageous systolic night-to-day

dipping profile one year after pregnancy (**Chapter 4**). Their vascular age, measured by carotid intima-media thickness, was also more advanced than their chronological age (**Chapter 11**). Six years after pregnancy, women with any previous hypertensive disorder of pregnancy had a higher blood pressure and BMI and a more atherogenic lipid profile than women with a previous normotensive pregnancy (**Chapter 5**). Simultaneously, the retinal microvasculature of these women was more disadvantageous, reflected by smaller retinal arteriolar calibers and wider retinal venular calibers (**Chapter 7**). These microvascular changes may possibly contribute to the development of CVD in later life. From the age of 45 years onwards, asymptomatic CVD became apparent in women with a previous hypertensive disorder of pregnancy (**Chapter 8** and **9**). Forty-seven percent of these women had coronary plaque formation and their risk of having coronary artery calcification was twice as high compared to women with no previous hypertensive disorder of pregnancy. Therefore, initiating cardiovascular risk assessment around the age of 50, as suggested by the Dutch Obstetrical guideline (NVOG Richtlijn), is too late as a large percentage of women will already have established subclinical CVD.[30]

Based on our results and those of previous studies we suggest to start cardiovascular screening (blood pressure, BMI, lipid profile, glucose and lifestyle factors [smoking habit, dietary intake and physical activity]) one year after any hypertensive disorder of pregnancy.[36-38] Cardiovascular screening should include annual monitoring of blood pressure and BMI. Blood pressure should be measured at the office and by 24 hours ambulatory blood pressure monitoring (ABPM). The lipid profile could be monitored one year after pregnancy and, if normal, consecutively in larger time intervals such as every two to five years. Glucose should be monitored one year postpartum.[39, 40] Thereafter larger time intervals (every three years) should be sufficient. Glucose monitoring should be intensified if women have an increased risk for developing diabetes (e.g. women with gestational diabetes, obesity, a family history of early onset type 2 diabetes mellitus or a glucose intolerance).

To initiate treatment, it is important to understand which cut-offs of each cardiovascular risk factor are considered abnormal and whether treatment is appropriate. We will discuss the cut-offs and treatment of cardiovascular risk factors examined in this thesis (hypertension and dyslipidemia) and those of other cardiovascular risk factors that were not examined into detail in this thesis but were included in the cardiovascular health score (obesity, diabetes, smoking, unhealthy diet and sedentary lifestyle).

Hypertension and obesity

The presence of masked hypertension should be evaluated in women with an elevated office blood pressure (120-129/80 mm Hg). Lifestyle changes, including weight control, regular physical activity, smoking cessation and the Dietary Approaches to Stop Hypertension (DASH) diet, should be recommended to all these women.[41] The DASH diet is rich in vegetables, fruit and whole grains, while limiting highly saturated foods and sweets. Previous studies showed that the DASH diet, in combination with reduced sodium intake, reduces blood pressure in women with pre(hypertension).[41, 42] Regarding antihypertensive treatment, the European and American

guidelines on CVD prevention recommend to consider treating women with hypertension stage 1 (130-139/or 80-89 mm Hg).[22] For women with hypertension stage 2 (≥140/ ≥90 mm Hg [office] or ≥135/ ≥85 mm Hg [daytime ABPM]) antihypertensive treatment should be started and evaluated after one to three months.[23] These recommendations are not in line with the Dutch cardiovascular risk management guideline (NHG Standaard) for GPs, which recommends antihypertensive treatment if 10-year CVD risk is ≥20% and systolic blood pressure is ≥140 mm Hg.[43] This guideline aims on treating individuals with a substantial 10-year CVD risk and considers this risk low in young women with a previous hypertensive disorder of pregnancy. Despite their high risk of having cardiovascular risk factors shortly after pregnancy, their absolute 10-year CVD risk is indeed low. This is mostly due to their relatively young age after delivery. Consequently, these women do not receive any or no appropriate cardiovascular follow-up after pregnancy.

In this thesis, we suggest to focus on cardiovascular health rather than cardiovascular risk for the follow-up of these women (**Chapter 10** and **11**). Cardiovascular health (CVH), captured in the CVH score, does not include age and is therefore also applicable to younger women. The CVH score gives a clear overview of an individual's current CVH status and the cardiovascular risk factors that can be improved. Moreover, a better cardiovascular health score has previously been associated with a reduced risk of subclinical atherosclerosis (carotid intima-media thickening), CVD and all-cause mortality. We showed that the cardiovascular health score after pregnancy is lower in women with a previous hypertensive disorder of pregnancy compared to women with a previous normotensive pregnancy. Especially an unhealthy blood pressure and BMI attenuated their score. We suggest to use the CVH score for the cardiovascular follow-up of these women in a way to project their current CVH, to clearly communicate which cardiovascular risk factors can be improved and to follow their CVH status over time.

A blood pressure that scores on average 'poor' or 'intermediate' with the CVH score should be treated as advised by the aforementioned European and American guidelines on CVD prevention. Women with a 'poor' or 'intermediate' BMI have a higher risk of developing CVD than normal weight women.[44] For this reason it is important to promote a healthy weight (BMI 18.5-24.9). Overweight women should be encouraged to lose weight, e.g. through a high-intensity lifestyle intervention program.[45] These programs combine diet, increased physical activity and recommendations to adhere to the program.

Dyslipidemia

As previously described, women with a hypertensive disorder of pregnancy have a more atherogenic lipid profile after pregnancy than women with a normotensive pregnancy. This is largely explained by BMI. Therefore, achieving a healthy weight should be the first treatment goal for these women with a more atherogenic lipid profile. Some women might also have to start with medical (statin) treatment, although this might have to be stopped in a subsequent pregnancy. Overall, we cannot provide a clear advise on when to initiate statin treatment as it remains unclear which cut-off for LDL-c should be applied due to incoherent recommendations of current dyslipidemia guidelines.[46] Five out of six guidelines recommend statin treatment no sooner than after the age of 40 or 45, providing no therapy for these young women during the first 10 to 20 years after pregnancy.[47-50] The NHG Standaard advises lifestyle changes and statin treatment when 10 year CVD risk is >10% and LDL-c > 2.5 mmol/L.[43] The American College of Cardiology and American Heart Association advise statin treatment in women over the age of 21 with a LDL-c concentration of 4.9 mmol/L.[51] In addition to age, statin treatment relies on 10-year atherosclerotic CVD risk, which has to be at least 5% and in some guidelines even 20%. As previously mentioned, these guidelines are therefore not suitable for young women with a hypertensive disorder of pregnancy because their 10-year CVD risk is still low (**Chapter 11**).[52-54]

Glucose

The CVH score considers a fasting glucose between 5.55 and 7.0 mmol/L 'intermediate' and a glucose over 7.0 mmol/L as 'poor'. This is in line with the recommendations of the WHO.[55] In non-diabetics, an intermediate or poor glucose should be measured again on another day. When results remain the same, lifestyle changes should be promoted, including a healthy diet, achieving a healthy weight and increased physical activity.[55] Women with (newly diagnosed) diabetes mellitus should receive standard appropriate diabetic care.[56]

Smoking, unhealthy diet and sedentary lifestyle

Smoking cessation, a healthy diet and regular physical activity should be encouraged to all women. Healthcare providers should explain the benefits of these healthier lifestyle choices. Adapting a healthy lifestyle can be encouraged through shared decision making, motivational interviewing, eHealth (e.g. Smarter Pregnant), support in self-management or referral to a dietician or behavioral therapist.[43, 57-59]

TABLE 1 | Cardiovascular follow-up after a hypertensive disorder of pregnancy.

Guideline	Year	Follow-up blood pressure	Follow-up CVD risk
WHO[24]	2011	In case of antihypertensive intake before pregnancy. In case of severe hypertension after pregnancy.	None
ACOG[25, 26]	2013 and 2018	Evaluation within 10 days after pregnancy. Treatment of persistent hypertension (BP ≥150/100 mm Hg) after pregnancy.	ASCVD risk assessment, no time period mentioned. Women with preterm delivery (<37 weeks) or recurrent preeclampsia: annual blood pressure, lipids, fasting glucose and BMI. No recommendation on starting time and which health care provider.
NICE[27]	2017	Antihypertensive management by GP.	Discuss future CVD risk 6-8 weeks after pregnancy with health care provider.
ISSHP	-	None	None
RCOG[28]	2006	Check proteinuria and blood pressure 6-8 weeks after pregnancy.	Inform about increased CVD risk in the future.
SOGC[29]	2014	Blood pressure monitoring 3-6 days after pregnancy. Maintain blood pressure < 160/110 mm Hg. Consider antihypertensive medication. In case of persistent hypertension after pregnancy or proteinuria/renal disease: referral to internal medicine. In case of pre-pregnancy hypertension or persistent hypertension after pregnancy: urinanalysis, lipid profile, fasting glucose and ECG 6 weeks after pregnancy.	Assessment of traditional cardiovascular risk markers may be beneficial. Encourage a healthy diet and lifestyle. Especially, for overweight women.
SOMANZ[60]	2014	Annual blood pressure monitoring	Cardiovascular risk assessment every 5 years.
AHA[23]	2017	None	None
ESC[22, 79]	2016 and 2018	Periodic screening for hypertension and diabetes mellitus	Annual check of blood pressure and metabolic factors by primary care physician.
NVOG[30]	2014	Follow-up after pregnancy till normotensive.	Cardiovascular risk assessment at the age of 50.

Abbreviations: ACOG, American College of Obstetricians and Gynecologists; AHA, American Heart Association; ASCVD, atherosclerotic cardiovascular disease; SOGC, Society of Obstetricians and Gynecologists of Canada; ESC, European Society of Cardiology; ISSHP, International Society for the study of Hypertension in Pregnancy; NICE, National Institute for Health and Care Excellence; NVOG, Nederlandse Vereniging voor Obstetrie en Gynaecologie; RCOG Royal College of Obstetricians and Gynaecologists; SOMANZ, Society of Obstetric Medicine Australia and New Zealand; WHO, World Health Organization.

Future research

The best prevention of CVD in women with a previous hypertensive disorder of pregnancy might be preventing the hypertensive disorder to occur in the first place. Though a large number of studies aimed to disentangle the mechanisms behind gestational hypertension and preeclampsia, the exact pathophysiology remains largely unknown. Also, it is still unclear whether hypertensive disorders of pregnancy are a risk factor for CVD, whether they share common risk factors or both. Gestational hypertensive disorders might merely be a sign of failed hemodynamic adaptation to pregnancy in women with an underlying predisposition to CVD, already present before pregnancy. As we show in this thesis, women with a gestational hypertensive disorder have a higher BMI before pregnancy and a higher blood pressure in early pregnancy compared to women with a normotensive pregnancy. In general, some women might have an underlying predisposition to CVD but never develop a hypertensive disorder of pregnancy, whereas they do show signs of suboptimal hemodynamic remodeling in pregnancy. This theory is supported by our findings in Chapter 2 which show that low PlGF concentrations in mid-pregnancy were associated with suboptimal hemodynamic remodeling in pregnancy and cardiovascular risk factors after pregnancy, regardless of pregnancy complications.

A well-known cause of CVD and mortality is biological ageing. As we showed in Chapter 11, biological age (presented through vascular age) can accelerate and consequently be more advanced than chronological age. Besides the important role of genetic inheritance, some risk factors for accelerated biological ageing include low educational level, obesity, smoking and physical inactivity.[61] Alongside, pregnancy also plays an important role in the ageing process of both mother and child.[62, 63] A recent study amongst 1954 American women showed that those with a history of life birth had accelerated biological ageing compared to women who never gave birth.[64] Moreover, the impact of pregnancy on the biological ageing process was greater than that of smoking or obesity, suggesting an independent role for pregnancy. As such, pregnancy might increase the risk of premature mortality and chronic disease.[65]

While pregnancy itself seems to influence the biological ageing process, chronological ageing also influences pregnancy. Advanced chronological age is associated with infertility and adverse pregnancy complications such as preeclampsia.[66, 67] The age-dependent decline in reproductive function seems to be the result of oocyte senescence as oocyte donation of younger to older women has shown to improve fertility.[68] The reproductive ageing phenotype is passed on via the nucleus after which multiple factors, including abnormal meiotic spindles and DNA damage, can mediate the reproductive ageing process and induce embryo arrest.[69]

What exactly influences the reproductive ageing process? Previous studies showed that age-dependent changes in the bovine reproductive tract (oviduct) are partly mediated by inflammatory pathways and markers of inflammation (cytokines). Pro-inflammatory cytokines, including interleukin (IL)-1β, are more active in older bovine oviducts. Interestingly, IL-1β is also involved in pregnancy complications, such as infertility, early pregnancy loss and preeclampsia.[70-72] In addition to the age-dependent increase in IL-1β, this cytokine induces cellular senescence by increasing the levels of senescence-associated proteins.[73] These findings suggest that, at least in animals, ageing stimulates an inflammatory response, which further accelerates cell senescence.

Signs of inflammation and increased oxidative stress are also typically found in women with preeclampsia.[74] The disease is characterized by local inflammation in the placenta and a generalized systemic inflammation response. The concentration of pro-inflammatory markers, including IL-1β, is also higher in preeclamptic pregnancies compared to uncomplicated pregnancies.[75, 76] To clarify whether preeclampsia is a risk factor of CVD or whether they both share common risk factors, we might have to explore the association between biological ageing and preeclampsia. It might well be that preeclampsia induces accelerated biological ageing, thereby increasing the risk of CVD, or that preeclampsia is induced through accelerated biological ageing. Future studies should evaluate these hypotheses to improve understanding of the pathophysiology and consequences of preeclampsia. Such a study could include various markers of biological ageing, including telomere length and DNA methylation patterns (epigenetic age).[77, 78]

If results of these studies will confirm the first hypothesis, where preeclampsia induces accelerated biological ageing, then this might form the missing link between preeclampsia and the increased risk of CVD in these women. Future research should aim to develop new cardiovascular risk prediction models, including preeclampsia as a determinant of advanced biological age. This might lead to better risk stratification of women with previous preeclampsia and ultimately improve cardiovascular healthcare in these young women.

Conclusion

Results of this thesis suggest that women with a hypertensive disorder of pregnancy have more cardiovascular risk factors and worse cardiovascular health after pregnancy than women with a normotensive pregnancy. The lipid profile in early pregnancy and PlGF concentration in mid-pregnancy might be useful biomarkers of cardiovascular health after pregnancy. We also recommend to initiate cardiovascular screening in women with a history of preeclampsia before the age of 45, preferably already one year after pregnancy. Additionally, we should not merely focus on cardiovascular screening after pregnancy in women with a history of preeclampsia but also in women with a history of gestational hypertension.

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Chapter

13

Summary

Samenvatting

Summary

In **Chapter 1** we explain the background and hypotheses of the studies presented in this thesis. Women with a hypertensive disorder of pregnancy have a higher risk to develop cardiovascular disease (CVD) later in life compared to women with a normotensive pregnancy. Cardiovascular risk factors after pregnancy, such as hypertension, obesity, dyslipidemia and insulin resistance, are more prevalent amongst women with a previous hypertensive disorder compared to women with a previous normotensive pregnancy. Nevertheless, it remains unclear at what age and to what extend cardiovascular risk factors become apparent. Consequently, cardiovascular risk management after pregnancy is still not clearly defined in current cardiovascular prevention guidelines.

In **Part I** we examine biomarkers in pregnancy for cardiovascular risk factors after pregnancy and the risk of a hypertensive disorder of pregnancy. In **Chapter 2** we examine placental growth factor (PlGF) in mid-pregnancy as a marker of cardiac morphology and blood pressure six and nine years after pregnancy. Lower PlGF levels are associated with a worse cardiovascular risk profile six and nine years after pregnancy. Lower PlGF concentrations in mid-pregnancy might help identify women who are more likely to manifest cardiovascular risk factors after pregnancy. In **Chapter 3** we show that an atherogenic lipid profile in early pregnancy is associated with a higher risk of preeclampsia and a higher blood pressure throughout pregnancy and years after pregnancy. As such, lipid levels in early pregnancy may help to identify women at risk for future hypertension.

In **Part II** we examine short-term and long-term cardiovascular risk factors after pregnancy. We show in **Chapter 4** that in women with previous severe preeclampsia, hypertension is common one year after pregnancy. Masked hypertension, white-coat hypertension, sustained hypertension, night-time hypertension and a disadvantageous dipping profile were prevalent amongst these women. These are all individual cardiovascular risk factors and can only be diagnosed with ambulatory blood pressure monitoring. Ambulatory blood pressure monitoring one year after pregnancy should therefore be offered to all these women at high risk for developing hypertension and possibly future cardiovascular disease. In **Chapter 5** we show that women with a previous hypertensive disorder of pregnancy have a more atherogenic lipid profile six years after pregnancy than women with a previous normotensive pregnancy. This might explain their higher CVD risk after pregnancy. In **Chapter 6** we examined the degree of tracking between maternal and offspring micro- and macrovascular cardiovascular risk factors after pregnancy and the extent to which this is influenced by pregnancy complications and shared environmental risk factors. An adverse cardiovascular profile in mothers is strongly associated with an adverse cardiovascular profile in their offspring. This was independent of environmental exposures or a previous pregnancy complication, which supports the hypothesis that cardiovascular risk factors (micro- and macrovascular) track from mother to child. Possibly, certain mothers (including women with a hypertensive disorder of pregnancy) also have a genetic predisposition to cardiovascular risk factors. In **Chapter 7** we show that women with a previous preeclampsia and gestational hypertension have an altered status of the microvasculature six years after index pregnancy compared to women with a previous normotensive pregnancy. These microvascular

changes may possibly contribute to the development of CVD in later life. In **Chapter 8** we examine the risk of coronary artery calcification (CAC) and coronary plaque formation in middle-aged women with a previous preeclampsia. Compared to women with a previous uncomplicated pregnancy, these women were two times more likely to have CAC and more than 40 percent had plaque formation. In **Chapter 9** we show that women with previous preeclampsia develop CAC approximately 5 years earlier than women without a history of preeclampsia. They also have more modifiable cardiovascular risk factors between the age of 40 to 63. These findings might explain why these women have a higher risk to develop CVD, but also at a younger age, compared to women with an uncomplicated pregnancy. Women with a previous preeclampsia might therefore benefit from regular cardiovascular screening and follow-up before the age of 45.

In **Part III** we examine cardiovascular health (CVH) in and after pregnancy in women with a hypertensive disorder of pregnancy and women with a normotensive pregnancy. In **Chapter 10** we show that better CVH in pregnancy is associated with less subclinical atherosclerosis, measured by carotid intima-media thickness, after pregnancy and better postpartum CVH in all women, but especially in those with a hypertensive disorder of pregnancy. As pregnancy is an incentive for women to improve lifestyle, future studies should evaluate whether the CVH score in pregnancy is useful for early cardiovascular counseling to optimize CVH. In **Chapter 11** we suggest that the CVH score is more suitable than the Framingham risk score for determining cardiovascular risk factors after severe preeclampsia. Additionally, the CVH score seems useful to assess postpartum vascular age in these women.

In **Chapter 12** we discuss the general conclusions, clinical practice and future implications. We conclude that women with a hypertensive disorder of pregnancy have more cardiovascular risk factors and worse cardiovascular health after pregnancy than women with a normotensive pregnancy. The lipid profile in early pregnancy and PlGF concentration in mid-pregnancy might be useful biomarkers of cardiovascular risk factors after pregnancy. The cardiovascular follow-up of women with a previous hypertensive disorder of pregnancy should be clearly and uniformly implemented in cardiovascular prevention guidelines. Additionally, it should be clearly stated who should provide this follow-up.

Samenvatting

Dit proefschrift beschrijft de relatie tussen aandoeningen in de zwangerschap waarbij de bloeddruk verhoogd is en risicofactoren voor hart- en vaatziekten later in het leven. Aandoeningen waarbij de bloeddruk in de zwangerschap verhoogd is zijn zwangerschapshypertensie en pre-eclampsie. Samen worden zij ook wel hypertensieve zwangerschapsaandoeningen genoemd. In vergelijking tot vrouwen met een normale bloeddruk in de zwangerschap hebben vrouwen met een hypertensieve zwangerschapsaandoening een verhoogd risico op het ontwikkelen van hart- en vaatziekten naarmate zij ouder worden. Na de zwangerschap komen bepaalde risicofactoren voor hart- en vaatziekten vaker voor bij vrouwen die een hypertensieve zwangerschapsaandoening hebben doorgemaakt. Voorbeelden van deze risicofactoren zijn: een hoge bloeddruk, zwaarlijvigheid (obesitas), een slecht gehalte aan vetten (lipiden) in het bloed en een verminderde gevoeligheid voor het hormoon insuline waardoor men vatbaarder wordt voor het ontwikkelen van diabetes. Ondanks deze kennis, blijft het onduidelijk rond welke leeftijd en in welke mate risicofactoren voor hart- en vaatziekten zichtbaar worden bij vrouwen die een hypertensieve zwangerschapsaandoening hebben doorgemaakt. Dit was de aanleiding voor het starten van dit onderzoek en wordt verder beschreven in **Hoofdstuk 1**.

In **Deel I** van dit proefschrift onderzoeken we biologische markers in de zwangerschap om daarmee het risico op het ontwikkelen van hypertensieve zwangerschapsaandoeningen te kunnen voorspellen. Daarnaast wilden we onderzoeken of we met deze biologische markers risicofactoren voor hart- en vaatziekten na de zwangerschap kunnen voorspellen. We hebben hiervoor de markers placental growth factor (PlGF) en enkele lipiden gemeten. PlGF wordt onder andere door de moederkoek (placenta) aangemaakt en is belangrijk voor het goed functioneren van de placenta. Dit is weer belangrijk bij de ontwikkeling van hypertensieve zwangerschapsaandoeningen. Vrouwen met een pre-eclampsie hebben een lagere PlGF concentratie in het bloed dan vrouwen met een normale bloeddruk in de zwangerschap. We hebben in **Hoofdstuk 2** onderzocht of een lage PlGF concentratie in de zwangerschap kan voorspellen welke vrouwen zes en negen jaar na de zwangerschap risicofactoren voor hart- en vaatziekten hebben. Het blijkt dat vrouwen met een lage PlGF concentratie vaker risicofactoren voor hart- en vaatziekten hadden, ongeacht of hun zwangerschap wel of niet in een hypertensieve zwangerschapsaandoening resulteerde. In **Hoofdstuk 3** hebben we de lipiden in de vroege zwangerschap als biomarker onderzocht. Vrouwen met een lipidenprofiel wat aderverkalking kan veroorzaken (een atherogeen lipiden profiel), hadden een verhoogd risico op het ontwikkelen van pre-eclampsie en een hoge bloeddruk, zowel tijdens als na de zwangerschap. Het lipidenprofiel in de vroege zwangerschap zou daarom kunnen helpen om vrouwen, die een verhoogd risico hebben op het ontwikkelen van pre-eclampsie en een hoge bloeddruk, al in een vroeg stadium op te sporen.

In **Deel II** van dit proefschrift onderzochten we korte en lange termijn risicofactoren voor hart- en vaatziekten. We tonen in **Hoofdstuk 4** aan dat vrouwen die één jaar geleden een pre-eclampsie hebben doorgemaakt vaak een hoge bloeddruk hebben. Er bestaan verschillende vormen van een hoge bloeddruk die allen een individueel risico met zich meedragen voor hart- en vaatziekten. Wanneer de bloeddruk op het spreekuur normaal is, maar thuis verhoogd dan spreken we van

een gemaskeerde hoge bloeddruk. Wanneer de bloeddruk op het spreekuur verhoogd is en thuis normaal dan is er sprake van een witte-jassen hoge bloeddruk. Wanneer beide metingen verhoogd zijn dan noemt men dit een aanhoudende hoge bloeddruk. Deze drie diagnoses, maar ook een verhoogde bloeddruk tijdens de nacht en een onvoldoende daling van de bloeddruk in de nacht ten opzichte van de dag, kwamen één jaar na de zwangerschap vaak voor bij vrouwen die een pre-eclampsie hadden doorgemaakt. Deze diagnoses kunnen enkel opgemerkt worden door de bloeddruk gedurende 24 uur te meten met een draagbare bloeddrukmeter. Op basis van de resultaten in dit proefschrift adviseren we om deze 24 uurs meting één jaar na de zwangerschap uit te voeren bij vrouwen die een pre-eclampsie hebben doorgemaakt. In **Hoofdstuk 5** laten we zien dat vrouwen die een hypertensieve zwangerschapsaandoening doormaken, zes jaar na de zwangerschap een ongunstiger profiel van de vetten in het bloed hebben dan vrouwen die een normale bloeddruk in de zwangerschap hebben. Dit zou een oorzaak kunnen zijn voor hun verhoogd risico op hart- en vaatziekten later in het leven. Risicofactoren voor hart- en vaatziekten kunnen zichtbaar zijn in de kleinste bloedvaten van het lichaam (de microvasculatuur). Een voorbeeld hiervan zijn de bloedvaten die door het netvlies lopen en welke gemakkelijk bekeken kunnen worden via het oog. In **Hoofdstuk 6** hebben we onderzocht of moeder haar risicofactoren voor hart- en vaatziekten, op zowel het niveau van de kleinste als dat van de grote bloedvaten, doorgeeft aan haar kind. Hier blijkt inderdaad sprake van te zijn ongeacht de omgevingsfactoren die moeder met haar kind deelde. Mogelijk is er sprake van een genetische overerving van deze risicofactoren tussen moeder en kind. In **Hoofdstuk 7** tonen we aan dat vrouwen die een pre-eclampsie of een zwangerschapshypertensie doormaken zes jaar na de zwangerschap een slechtere microvasculatuur hebben dan vrouwen die een normale bloeddruk hebben in de zwangerschap. Eerdere studies hebben aangetoond dat een slechtere microvasculatuur een risicofactor is voor het ontwikkelen van hart- en vaatziekten. De microvasculatuur zou daarom ook een rol kunnen spelen in het ontwikkelen van hart- en vaatziekten bij vrouwen die een hypertensieve zwangerschapsaandoening hebben doorgemaakt. In de kransslagaderen van het hart kan men een duidelijk beeld vormen van het risico op het ontwikkelen van een hartinfarct of een beroerte. Middels een speciale CT-scan van het hart vergeleken we kalk en vernauwingen in de kransslagaderen bij vrouwen die een pre-eclampsie hadden doorgemaakt met die van vrouwen die een normale zwangerschap doormaakten. Bijna de helft van de vrouwen met een doorgemaakte pre-eclampsie (namelijk 40%) bleek vaatvernauwing van de kransslagaderen te hebben. Na een pre-eclampsie hadden vrouwen ook een tweemaal verhoogd risico op kalk in de kransslagaderen ten opzichte van vrouwen met een normale zwangerschap. In **Hoofdstuk 9** laten we zien dat vrouwen met een doorgemaakte pre-eclampsie al vanaf het 45e levensjaar kalk vertonen in de kransslagaderen. Dit is vijf jaar eerder dan vrouwen die geen pre-eclampsie hebben doorgemaakt. Daarnaast hadden vrouwen na een pre-eclampsie ook meer risicofactoren voor hart- en vaatziekten tussen de leeftijd van 40 en 63 jaar. Deze resultaten zouden kunnen verklaren waarom deze vrouwen een hoger risico hebben op het ontwikkelen van hart- en vaatziekten en waarom ze deze risicofactoren eerder ontwikkelen dan vrouwen met een normale zwangerschap. Vrouwen die een pre-eclampsie hebben doorgemaakt zouden kunnen profiteren van reguliere screening op hart- en vaatziekten vooraf aan het 45ste levensjaar.

In **Deel III** onderzoeken we een score voor het meten van de gezondheid van het hart en de bloedvaten: de cardiovasculaire gezondheidsscore. We bestudeerden deze score tijdens en na de zwangerschap in vrouwen met een hypertensieve zwangerschapsaandoening en in vrouwen die een normale bloeddruk hadden tijdens de zwangerschap. In **Hoofdstuk 10** tonen we aan dat vrouwen met een slechtere cardiovasculaire gezondheidsscore in de zwangerschap tien jaar na de zwangerschap een dikkere vaatwand van de halsslagader hadden. Een dikkere vaatwand is een risicofactor voor aderverkalking en het ontwikkelen van een beroerte. Vrouwen met een betere gezondheidsscore in de zwangerschap hadden ook een betere cardiovasculaire gezondheidsscore na de zwangerschap. Deze verbanden waren het sterkst in vrouwen die een hypertensieve zwangerschapsaandoening hadden doorgemaakt. Toekomstige studies moeten evalueren of de cardiovasculaire gezondheidsscore in de zwangerschap gebruikt kan worden voor vroege counseling van risicofactoren voor hart- en vaatziekten en het optimaliseren van de score. Dit laatste zou onder andere kunnen door het optimaliseren van de bloeddruk, het gewicht, het cholesterolgehalte, de bloedsuiker, het dieet, de hoeveelheid dagelijkse lichamelijke beweging en door te stoppen met roken. De zwangerschap zou hier een goed moment voor kunnen zijn omdat vrouwen dan veelal gemotiveerd zijn om hun leefstijl te verbeteren ten behoeve van de zwangerschap. In de gezondheidszorg richt men zich veelal op het communiceren van risico's, zoals het risico op hart- en vaatziekten. Dit wordt bijvoorbeeld gedaan door middel van een 10-jaars risicoberekening op hart- en vaatziekten met de Framingham risico score. In **Hoofdstuk 11** tonen we aan dat de cardiovasculaire gezondheidsscore beter toepasbaar is dan de Framingham risico score in het bepalen van risicofactoren voor hart- en vaatziekten na een ernstige pre-eclampsie. Daarnaast lijkt de cardiovasculaire gezondheidsscore ook toepasbaar bij het bepalen van de vaatleeftijd na de zwangerschap in deze vrouwen.

In **Hoofdstuk 12** bespreken we de algemene conclusies van dit proefschrift en de klinische en toekomstige implicaties. We concluderen dat vrouwen met een hypertensieve zwangerschapsaandoening na de zwangerschap meer risicofactoren voor hart- en vaatziekten hebben en tevens een slechtere cardiovasculaire gezondheidsscore in vergelijking tot vrouwen met een normale bloeddruk tijdens de zwangerschap. Het lipiden profiel en de PlGF concentratie in de zwangerschap zouden gebruikt kunnen worden als biomarkers van risicofactoren voor hart- en vaatziekten na de zwangerschap. De follow-up van deze risicofactoren voor vrouwen die een hypertensieve zwangerschapsaandoening hebben doorgemaakt, moet duidelijk en uniform geïmplementeerd worden in richtlijnen voor de preventie van hart- en vaatziekten. Daarnaast moet duidelijk beschreven worden wie deze follow-up moet gaan verzorgen.

Chapter

14

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List of abbreviations

ABPM	Ambulatory blood pressure monitoring
AHA	American Heart Association
ALT	Alanine
ANOVA	Analysis of variance
AOD	Aortic root diameter
AST	Aspartate
AU	Agatston units
BMI	Body mass index
BP	Blood pressure
CAC	Coronary artery calcification
CACS	Coronary artery calcification score
CAD	Coronary artery disease
CCT	Coronary computer tomography
CCTA	Coronary computed tomography angiography
CI	Confidence interval
CIMT	Carotid artery intima-media thickness
CRAE	Central retinal arteriolar caliber
CREw-IMAGO	Cardiovascular RiskprofilE: IMaging And Gender-specific disOrders
CRVE	Central retinal venular caliber
CVD	Cardiovascular disease
CVH	Cardiovascular health
DBP	Diastolic blood pressure
EMC	Erasmus Medical Center
DAG	Directed Acyclic Graph
DNA	Desoxyrinonucleinezuur
FHS	Framingham Heart Study
FRS	Framingham risk score
FS	Fractional shortening
FUPEC	Follow-Up PreEClampsia
GFR	Glomerular filtration rate
HDL-c	High density lipoprotein cholesterol
HELLP	Hemolysis Elevated Liver enzymes and Low Platelets
IDF	International Diabetes Federation
LAD	Left atrial diameter
LDH	Lactic acid dehydrogenase
LDL-c	Low density lipoprotein cholesterol
LGA	Large for gestational age
Lp(a)	Lipoprotein(a)
LV mass	Left ventricular mass
MESA	Multi-Ethnic Study of Atherosclerosis
MoM	Multiple of the median

MPR	Multiplanar reconstructions
NA	Not applicable
NHANES	National Health and Nutrition Examination Survey
OR	Odds ratio
PE	Preeclampsia
PlGF	Placental growth factor
PWV	Pulse wave velocity
RR	Relative risk
SBP	Systolic blood pressure
SD	Standard deviation
SDS	Standard deviation score
sFlt-1	Soluble fms-like tyrosine kinase-1
SGA	Small for gestational age
SPSS	Statistical Package of Social Sciences
sPTB	Spontaneous preterm birth
STB	Syncytiotrophoblast
UtA-PI	Uterine artery pulsatility index
WCH	White-coat hypertension