Beyond pregnancy: development of cardiovascular disease after preeclampsia

Gerbrand Zoet

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De zwangerschap voorbij: ontwikkeling van hart- en vaatziekten na preëclampsie

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(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 14 november 2017 des middags te 2.30 uur

door

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geboren op 28 september 1986 te Ede

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"Work it harder
Make it better
Do it faster
Makes us stronger"

Bangalter, Birdsong, De Homem-Christo
Daft Punk - Harder, Better, Faster, Stronger (2001)

Contents


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Chapter 1

Introduction

Cardiovascular disease in women

Cardiovascular disease (CVD) is the most common cause of mortality worldwide with an estimated 17.3 million deaths worldwide.[1] Despite a 22% decline in death rate attributable to CVD since the 1990s, the total number of deaths due to CVD increased by 41% due to population growth and aging.[2] In women, CVD is the most important cause of death. In total, 34% of all deaths among females are attributed to CVD.[3] There is a wide variety of clinical manifestations in which CVD can present. Ischemic heart disease (IHD), consisting of coronary artery disease (CAD), cardiac microvascular dysfunction and myocardial oxygen imbalance, is the most prevalent cause of death (3.8 million women yearly globally), followed by cerebrovascular disease including ischemic and hemorrhagic stroke.[3]

Although CVD risk in women is partially explained by genetic or constitutional factors, so called modifiable CVD risk factors are commonly accepted as the major determinants in CVD risk.[4] As shown in the INTERHEART study, an international cohort study among ~15,000 cases and an equal number of controls, nine factors are responsible for the vast majority (over 90%) of CHD cases: dyslipidemia, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, insufficient consumption of fruit and vegetables, no alcohol intake, and physical inactivity.[5] Although the total effect of these factors is consistent in men and women, gender differences on specific items can be identified. Hypertension and diabetes for instance, contribute more to the risk in women than in men.[5] Based on these findings and data from others, preventive strategies to improve modifiable risk factors have been developed, as shown in Table 1.

Female-specific risk factors for CVD

In addition to modifiable risk factors, female-specific risk factors for CVD may be identified. Of these female-specific risk factors, a group of conditions specific to women can be viewed as early manifestations of CVD during reproductive life and child birth, mostly summarized under the terms 'reproductive disorders' and 'pregnancy disorders'.[6] The effect of these different female-specific risk factors varies for each condition and for each specific cardiovascular outcome, as shown in Table 2. Female-specific risk factors are now even addressed in guidelines aimed at prevention of CVD in women specifically.[7-10]

Pregnancy poses specific challenges to the cardiovascular system, which may translate to clinical disorders related to cardiovascular function. In fact, in many pregnancy-specific disorders, including pre-eclampsia, placental abruption, fetal growth restriction and preterm birth, a vascular origin can be identified as a contributor or risk factor. During pregnancy major hemodynamic and endothelial alterations occur, allowing for increase in total circulating blood volume, increased cardiac output and an elevated heart rate. Conversely, peripheral resistance and arterial blood pressure decrease in the first half of pregnancy.[11-14] How this cardiovascular adaptation during pregnancy influences long-term maternal health, both after uncomplicated and com-

Table 1. Modifiable ASCVD Risk factors: Sex-Based Differences and Recommendations

Risk factor	Sex-based differences	Recommendation
Diabetes mellitus 	- Diabetic women have 3-times higher risk of fatal CAD than nondiabetic women. - Earlier occurrence of MI & higher mortality in diabetic women than diabetic men. - DM stronger risk factor for stroke in women than men.	- Diabetic women and men should have aggressive management of their CVD risk factors. - Women may require greater frequency/intensity of physical activity than men to reduce CVD events.
Hypertension 	- Higher prevalence of HTN in women > 60 years than in men. - Less well controlled in women than in men	- Encourage optimal BP through diet, exercise, and avoidance of excess alcohol and sodium. - BP >140/90mmHg→pharmacotherapy
Dyslipidemia 	- Among women, dyslipidemia has the highest PAR at 47.1%, compared with all other known risk factors for CVD. - Atheroma regression and LDL lowering may be even greater among women on statins than in men.	- Statins are equally effective for secondary CVD prevention in both men and women; greater likelihood of developing DM and myalgias in women - Statins are recommended for primary prevention in women.
Obesity 	The impact of obesity on the development of CAD greater in women than in men.	- Women should maintain or lose weight through balance of physical activity and diet. - Women who need to lose weight should: accumulate minimum of 60-90min of moderate-intensity physical activity every day
Physical inactivity 	The prevalence of inactivity and sedentary behaviors is higher among women than men.	- Regular physical activity is one of most powerful health-promoting practices - Women should accumulate at least 150min/wk of moderate exercise, 75min/wk of vigorous exercise, or an equivalent combination.
Smoking 	Women had a significant 25% increased risk for CAD conferred by cigarette smoking compared with men, with the exception of the youngest age group (30–44 y).	- Smoking associated with decade of lost life, cessation reduces that loss ~ 90%. - Women should be advised not to smoke and to avoid environmental tobacco smoke.

BP, blood pressure; CAD, coronary artery disease; CVD, cardiovascular disease; DM, diabetes mellitus; FHS, Framingham Heart Study; HF, heart failure; HTN, hypertension; IHD, ischemic heart disease; LDL, low-density lipoprotein; MI, myocardial infarction; PAD, peripheral arterial disease; and PAR, population attributable risk.
Adapted from Garcia et al. (2011) *Circulation Research*.

plicated pregnancy, is not yet fully understood.[15-17] Several pregnancy disorders are considered female-specific risk factors for CVD. The most extsively described pregnancy disorders in the perspective of CVD later in life include hypertensive pregnancy disorders, gestational diabetes en preterm birth.[18] The focus of this thesis will be on hypertensive pregnancy disorders, and therefore other pregnancy disorders will not be discussed in depth.

Hypertensive pregnancy disorders (HPD) have for long been known to be associated with an increased risk for IHD, stroke and overall CVD.[19–24] PIH is defined as de novo hypertension, affecting around 10% of all pregnancies. However, many women with PIH in fact have chronic hypertension, which has not been diagnosed before as hypertension may be masked during the

Table 2. Female-specific risk factors associated with CVD

Female-specific risk factor	CVD	CHD	HT	Stroke	T2DM
PCOS	●	–	●	–	●●●
POI	●●	●●	–	–	–
PIH	●●	●	●●●	●	●●
Preeclampsia	●●	●●	●●●	●●	●●
GDM	●●	●●	●●●	–	●●●
Parity ≥1	●●	–	–	–	–
Parity ≥5	●●●	–	–	–	–
Miscarriage ≥1	–	●	–	–	–
Miscarriage 2+/3+	–	●●	–	–	–
Preterm birth	●●	●	●	●●	●●
SGA <10th percentile	●●	●●	–	●●	–
Stillbirth	–	●●	–	–	–

● weak association, Relative Risk (RR) 1–1.5 in cohort studies ●● moderate association, RR 1.5–2.5 in cohort studies ●●● strong association, RR ≥2.5 in cohort studies.
PCOS, polycystic ovary syndrome; POI, primary ovarian insufficiency; PIH, pregnancy induced hypertension; GDM, gestational diabetes mellitus; SGA, small-for-gestational-age; CVD, cardiovascular disease; CHD, coronary heart disease; HT, hypertension; T2DM, type 2 diabetes mellitus.
Adapted from Appelman et al. (2015) *Atherosclerosis*.

first half of pregnancy by the then physiological fall of blood pressure levels below hypertension cut-off levels. Preeclampsia affects 1-5% of all pregnancies and is characterized by de novo hypertension and proteinuria, maternal organ dysfunction, and is frequently associated with uteroplacental dysfunction resulting in fetal growth restriction.[25-27] The hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome occurs in 10-20% of the cases of severe preeclampsia.[28] Generally, women with a history of HPD have a two-fold increased risk of developing IHD, stroke and overall CVD and an almost four-fold increased risk of developing hypertension.[23,24,29] In addition, increasing severity of preeclampsia is associated with increasing CVD risk, with a relative risk on CVD of 7 for women with early-onset preeclampsia.[21]

These associations might indicate that both HPD and CVD are manifestations of a common vascular pathological process, although causality could not be proven by these cross-sectional studies. Following this hypothesis, pregnancy serves as a 'stress test' for cardiovascular health later in life.[30] Women, who experience HPD during pregnancy, 'fail' this stress test. Although the temporary vascular compromise subsides after pregnancy in most women, it reappears with aging as CVD in many. Pregnancy complications such as preeclampsia may be considered a 'red flag' for future health.

The exact mechanism behind the increased CVD risk after HPD is not fully understood, just as

the cause of preeclampsia remains unclear.[31] The pathophysiology of preeclampsia is characterized by the interplay of pre-pregnancy maternal factors (such as vascular impairment), placental factors (such as impaired trophoblast invasion and spiral artery remodeling) and fetal factors, ultimately leading to endothelial activation and release of pro-inflammatory cytokines which results in the development of systemic maternal disease.[31] Onset of preeclampsia is thus a result of various vascular and inflammatory triggers, which might also play a role in the onset of CVD.

Following the abovementioned epidemiological studies on the relation between preeclampsia and CVD, our group showed that the increased CVD risk might be mediated by an increased prevalence of modifiable CVD risk factors, such as hypertension, dyslipidemia and obesity.[32,33] The presence of these changes during pregnancy, is seen in many women months to years after pregnancy complicated by preeclampsia.[32–35] In addition, the onset of CVD risk factors appears to be accelerated as well. This is well illustrated by our data on 22.265 ever pregnant women in the EPIC-NL cohort study, in which age of hypertension onset after HPD and age of T2DM onset after gestational diabetes was 7-8 years earlier compared to women without pregnancy complications.[36] Apart from these modifiable CVD risk factors, previous studies showed a pro-inflammatory state in women several years after preeclampsia, which may further contribute to the increased CVD risk.[37,38] Thus, inflammatory factors (such as CRP, interleukins, and fibrinogen) might serve as CVD risk markers specifically in these women. Apart from these inflammatory markers, possible female-specific biomarkers for CVD have been identified in the past years. These circulating factors include angiogenic factors (such as soluble fms-like tyrosine kinase-1 (sFlt-1) and pro-relaxin (pro-RLX2)) and the hormone pro-neurotensin (pro-NT).[39,40] However, the predictive performance of these markers as CVD risk markers in women after preeclampsia specifically needs to be further investigated.

We have previously shown that atherosclerosis possibly plays a role in the increased CVD risk in women with a history of preeclampsia as well. In a cross-sectional study in 491 healthy postmenopausal women he risk of coronary calcifications was ~60% increased in those who reported a history of hypertensive pregnancy disorders compared with women who reported a normotensive pregnancy.[41] These findings were recently confirmed in a small prospective cohort study among women 3 decades after a confirmed diagnosis of preeclampsia.[42] Apart from the atherosclerotic lesions predisposing to obstructive arterial disease, coronary microcirculatory dysfunction is now believed to be involved in the development of IHD as well.[43] Coronary microcirculatory dysfunction, either through functional or structural alterations of the very small coronary pre-arterioles, is highly prevalent in women suspected for CAD and associated with an adverse cardiovascular outcome.[44,45]

Endothelial dysfunction, resulting in stiffening of the arteries due to decrease in 'endothelial-derived relaxing factor' nitric oxide, is major determinant in atherogenesis.[46,47] Arterial stiffness is related to adverse cardiovascular outcome and could serve as CVD risk predictor.[48,49] Preeclamp-

sia is associated with postpartum vascular dysfunction, such as increased arterial stiffness, endothelial dysfunction and subclinical atherosclerosis, as is previously shown by our group.[50] However, studies regarding the role of endothelial dysfunction and arterial stiffness in CVD development in women after preeclampsia showed conflicting results: some report increased arterial stiffness after preeclampsia, while others could not confirm this.[51–53] The use of different operator-dependent techniques to measure arterial stiffness might explain these conflicting results. State-of-the-art, non-invasive imaging might provide opportunities to assess vascular dysfunction more reliable.[54,55]

Knowledge gaps

CVD risk factor prevalence within the first decade after HPD has been studied extensively.[33-36,56,57] In addition, CVD events after HPD at the age of 60 have been subject of considerable research.[21,23,58,59] A cross-sectional study from our group showed an increased risk of coronary calcifications in postmenopausal women with a history of hypertensive pregnancy disorders.[41] However, very limited information is available on development of CVD and specifically CAD between the age of 40 and 60 years.[60,61] This is important, because preclinical development of atherosclerosis is thought to start years before the actual event. Therefore, for women within this age group in particular, preventative strategies to reduce the risk of a first coronary event or stroke should be sought. In the study by Bokslag et al. women with precious preeclampsia showed common occurrence of CVD risk factors, i.e. hypertension and metabolic syndrome.[60] It remains however largely unknown to what extend premature signs of CVD, such as atherosclerotic lesions, have already developed by then. Especially the improved computed tomography (CT) and magnetic resonance imaging (MRI) techniques could provide valuable opportunities in timely identification of women at risk for CVD. Furthermore, the pathophysiological mechanisms behind the increased risk of CVD after HPD remains unsolved.

In this thesis we focus on arterial damage and atherosclerosis after pregnancy at the fourth, fifth and sixth decade, which we assessed both after uncomplicated pregnancy and after pregnancy complicated by hypertensive pregnancy disorders.

Objectives

- To review the present knowledge on commonly used cardiovascular screening modalities available to women with a history of preeclampsia, and it discusses recent developments in early detection of CVD using state-of-the-art cardiovascular imaging **(Chapter 2)**.

Part I – CVD risk after pregnancy

- To describe the time-dependent changes in body composition and parameters of metabolic health in parous women as compared with nulliparous controls, stratified for number of children, in the well-defined longitudinal prospective PREVEND-cohort study **(Chapter 3)**.
- To assess the impact of parity on aortic characteristics as early markers of atherosclerosis and arterial stiffness in asymptomatic women between 25 and 35 years of age by cardiac magnetic resonance imaging **(Chapter 4)**.

Part II – CVD risk after hypertensive pregnancy disorders

- To compare the development of traditional CVD risk factors over time between women with a history of HPD and women with normotensive pregnancies, in order to identify optimal timing for cardiovascular screening and prevention **(Chapter 5)**.
- To investigate the eligibility of pro-NT and pro-RLX2 as CVD biomarkers for women with a history of preeclampsia in the PREVFEM cohort **(Chapter 6)**.
- To assess the occurrence of pregnancy and fertility disorders, including preeclampsia, among women with a history of ischemic stroke **(Chapter 7).**

Part III – Imaging of coronary and carotid artery atherosclerosis

- To evaluate coronary artery atherosclerotic lesions, both coronary artery calcium and plaques, in asymptomatic women at age 45 to 55 years with a history of preeclampsia by CT imaging as part of the CREW-IMAGO study **(Chapter 8)**.
- To describe the distribution of arterial calcifications and to determine the relation among arterial calcifications, arterial stiffness and traditional CVD risk factors in women with a history of preeclampsia in the fifth and sixth decade of life **(Chapter 9)**.

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Chapter 2

Determinants of future cardiovascular health in women with a history of pre-eclampsia

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Maturitas 2015;82(2):153-61

Abstract

Women who develop preeclampsia have an increased risk of cardiovascular disease (CVD) later in life. However, current guidelines on cardiovascular risk assessment and prevention are unclear on how and when to screen these women postpartum, and about the role of a positive history of preeclampsia in later-life CVD risk management. The aim of this review is to discuss the present knowledge on commonly used cardiovascular screening modalities available to women with a history of preeclampsia, and to discuss recent developmen ts in early detection of CVD using cardiovascular imaging.

Furthermore, we explore how female-specific risk factors may have additional value in cardiovascular screening, in particular in relatively young women, although their implementation in clinical practice is challenged by inconsistent results and lack of long-term outcome data. Non-invasive imaging techniques, e.g. coronary artery intima-media thickness (CIMT), can be helpful to detect subclinical atherosclerotic disease, and coronary artery calcium scoring (CACS) has shown to be effective in early detection of cardiovascular damage. However, whilst more short-term and long-term follow-up studies are becoming available, few studies have investigated women with a history of preeclampsia in the fourth and fifth decade of life, when early signs of premature CVD are most likely to become apparent. Further studies are needed to inform new and improved clinical practice guidelines, and provide long-term strategies to effectively prevent CVD, specifically targeted at women with a history of preeclampsia. Additionally, evaluation of feasibility, cost-effectiveness and implementation of CVD screening and prevention initiatives targeted at former preeclampsia patients are needed.

Introduction

Preeclampsia is a leading cause of maternal and neonatal morbidity and mortality that affects up to 2-5% of all pregnancies. After delivery, preeclampsia usually resolves within a few days. However, the focus of research on preeclampsia is slowly shifting towards its long-term complications. In particular, a well-established association exists between preeclampsia and an increased risk of CVD later in life.[1–7] However, routine cardiovascular screening in women who have had preeclampsia is hindered by conflicting results on the prevalence of CVD risk factors postpartum and uncertainty about optimal timing, and the relatively unexplored role of female-specific risk factors. The aim of this review is to discuss the present knowledge, opportunities for and concerns of cardiovascular screening in women with a history of preeclampsia, in particular in view of new developments in risk factor assessment and cardiovascular imaging.

Preeclampsia: prevalence and definitions

Preeclampsia is defined as a syndrome consisting of gestational hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg after 20 weeks of gestation) coinciding with one or more of the following new-onset conditions: de novo proteinuria, maternal organ dysfunction or placental dysfunction.[8] About 3-5% of pregnancies are affected, and besides peripartum hemorrhage, preeclampsia is the second most important direct cause of maternal mortality worldwide.[9,10]

Preeclampsia as an early indicator of cardiovascular risk

For most women affected by preeclampsia, including those cases with severe early-onset disease, clinical features resolve within days after delivery of the baby and placenta. In spite of the short-term clinical recovery, recent evidence consistently shows that long-term cardiovascular health in former preeclamptic patients is compromised.[1,2,4–7] Original cohort studies that have investigated the incidence of CVD events after preeclampsia are listed in Table 1. Outcomes of these studies have now been the subject of a number of excellent systematic reviews and meta-analyses.

In summary, women who have been diagnosed with preeclampsia in any of their pregnancies have an approximated twofold risk of developing major CVD events (i.e. myocardial infarction and stroke) and an almost fourfold increased risk of developing hypertension compared with women who do not develop preeclampsia. It appears that CVD events following preeclampsia generally occur at a much younger age than in other women within the same population.[1,4,5,11] In a recent study by our group, we estimated the onset of hypertension, type 2 diabetes mellitus, myocardial infarction and stroke after preeclampsia to be on average 8-10 years earlier than in women with normal pregnancy outcomes.[12] The risk of CVD events is more pronounced in the subgroup of women with so-called early-onset preeclampsia (generally defined as preeclampsia occurring before 34 weeks of gestation).[4,13] In these women, there is a 7- to 8-fold increased

Table 1: overview of original cohort studies assessing CVD after preeclampsia

First author, year	Total study-population	Cases	Controls	Primary outcome	Follow up
Bhattacharya, 2011	34854	PE (n=2026), PIH (n=8891)	Normal BP (n=23937)	CV event, IHD, stroke, HT (all fatal and non-fatal)	26–48 years
Callaway, 2011	2112	HDP (n=191)	No HDP (n=1921)	Hypertension	21 years
Callaway, 2007	3639	HDP (n=333)	No HDP (n=3306)	DM, anthropometrics	21 years
Carr, 2009	31463	PE (n=2032)	No PE (n=29431)	DM	Median 8.2 years (IQR 5–13 years)
Cassidy, 2009	498	HDP (n=52)	No HDP (n=446)	CACS, CVD risk factors (HT, dyslipidemia), T2DM, CHD, stroke	Mean 27 years
Engeland, 2011	226832	PE (n=8832)	No PE (n=215988)	DM	Mean 3.7 years (IQR 0–6 years)
Freibert 2011	3909	HDP (n=222) Preterm birth (n=324)	Uncomplicated pregnancy (n=2558)	Non-fatal MI, AP, HF, arrhythmia	Unknown (age ≥ 50 years)
Funai, 2005	37061	PE (n=1070)	No PE (n=35991)	Fatal CV event	Median 30 years (25–37 years)
Garovic, 2010	4782	HDP (n=643)	Normotensive pregnancy (n=3421)	Fatal/non-fatal IHD, Non-fatal stroke, DM, HT, dyslipidemia	Unknown (median age ≥38 years)
Hannaford, 1997	214356	Toxemia (n=3000)	No toxemia (n=18451)	Fatal/ non-fatal IHD & stroke, HT	Unknown
Haukkamaa, 2009	767	PE (n=35), PIH (n=61)	Healthy parous (n=489) and nulliparous (n=182) controls	Non-fatal IHD, HT IMT, lipids, T2DM	Unknown (≥30 years, mean age 55–57 years))
Henriques, 2014	60	PIH (n=30)	Uncomplicated pregnancy (n=30)	FMD,anthropometric variables, metabolic variables	Mean 15.2 years (SD 3.5 years)
Irgens, 2001	626272	PE term (21506), PE preterm (2649)	No PE, term (n=576099)	IHD & stroke (fatal)	0–25 years
Jonsdottir, 1995	7543	HDP (n=374)	General population (n=7169)	Fatal IHD	0–59 years
Kaaja, 2005	3559	PE (n=397)	No PE (n=3162)	T2DM, dyslipidemia, HT, heart failure, AP	28 years
Kestenbaum, 2003	124141	PIH (n=10687), mild PE (n=15508), severe PE (n=5044)	Normotensive pregnancy (n=92902)	Fatal/non-fatal CV event	Mean 7.8 years

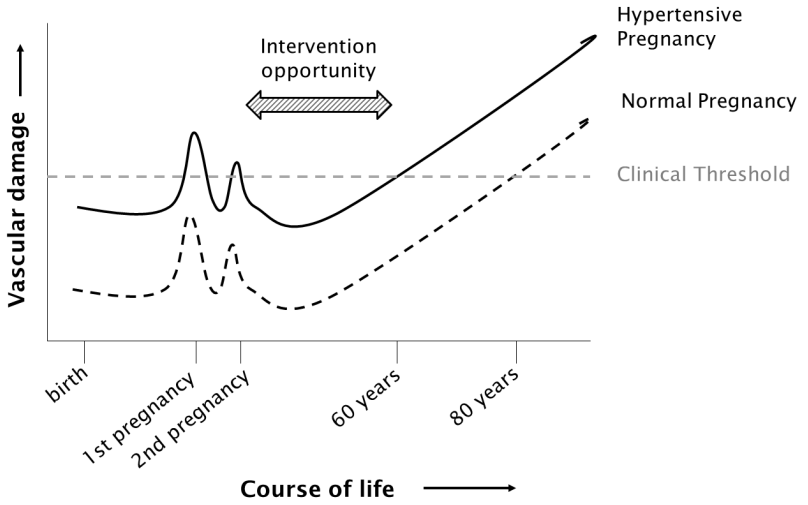
Table 1 (continued)

First author; year	Total study-population	Cases	Controls	Primary outcome	Follow up
Libby; 2006	7178	PE (n=810)	No PE (n=6377)	T2DM	Unknown (median age 71 years)
Lin; 2011	1132064	PE/eclampsia	No PE/eclampsia	Fatal/non-fatal CV event; MI; IHD	1–6 years
Lykke; 2010	782287	PIH (n=7449); Mild PE (26810); Severe PE (n=7016)	No HDP (n=741012)	CV event (fatal); IHD & stroke (fatal/ non-fatal); HT; DM	Median 15 years (0–30 years)
Magnussen; 2009	15065	HDP (n=1433)	No HDP (n=13632)	HT; T2DM; dyslipidemia	Mean 16–17 years (SD 8.2 years)
Mongraw; 2010	14403	PE (n=481)	No PE (n=13922)	Fatal CV event	Median 37 years
Ray; 2005	1026265	PE (n=36982); PIH (n=20942)	No MPS (n=950885)	Fatal/non-fatal CV event	Median 9 years
Shalom; 2013	22814	HDP (n=2072)	No HDP (n=20742)	HT; any relevant hospitalization	10–12 years
Skjaerven; 2012	836147	All PE (n=34824) - Term PE (n=26708) - Preterm PE (n=5886)	All no PE (n=801323) / Term no PE (n=712181)	Fatal CV event	7–42 years
Smith; 2001	129920	PE (n=22781)	No PE (n=107139)	Fatal/non-fatal IHD	15–19 years
Wang; 2011	5807	HDP (n=1092)	No HDP (4715)	Fatal/non-fatal stroke	Mean 7 years (SD 2 years)
Wikstrom; 2005	403550	Hypertensive disease (n=20469) - PIH (n=9718) - mild PE (n=9718) - severe PE (n=2815)	Uncomplicated pregnancy (n=347870)	Fatal/non-fatal IHD	14 years
Wilson; 2003	2790	PIH (n=951) PE (n=1043)	No HDP (n=796)	Fatal/non-fatal IHD; stroke; HT; VTE and kidney disease	10–48 years
Wu; 2014	944474	HDP (n=13633) - PIH (n=2361) - chron HT (n=731) - PE (n=8609) - superimposed PE (n=594)	No HDP (n=13633)	ESRD; T2DM	Median 9 years (IQR 7–10 years)

AP, angina pectoris; BP, blood pressure; CACS, coronary artery calcium score; CHD, coronary heart disease; CV, cardiovascular; CVD, cardiovascular disease; DM, diabetes mellitus; ESRD, end-stage renal disease; FMD, flow-mediated dilatation; HDP, hypertensive disorders of pregnancy; HT, hypertension; IHD, ischemic heart disease; IMT, intima media thickness; IQR, inter quartile range; MI, myocardial infarction; MPS, maternal placental syndrome; PE, preeclampsia; PIH, pregnancy induced hypertension; SD, standard deviation; T2DM, type 2 diabetes mellitus; VTE, venous thromboembolism.

incidence of ischemic heart disease, cerebrovascular disease, and peripheral arterial disease later in life.[1,6] The mechanisms underlying this increase in life-time cardiovascular risk are complex and much debated. Preeclampsia and atherosclerosis are likely to share common pathological features, including similar contributing risk factors (e.g. hypertension, obesity, inflammation), characteristic alterations of the vessel wall (intimal thickening, fat accumulation in middle to large arteries) and endothelial cell dysfunction.[14] One could argue that pregnancy serves as a "stress test" for cardiovascular health, and that preeclampsia is associated with temporary vascular compromise, which subsides after pregnancy but reappears with ageing as CVD later in life (see Figure 1). Following this hypothesis, preeclampsia may therefore be considered as a "red flag" and offer opportunities for early-life identification of high risk individuals, susceptible to premature atherosclerosis and CVD events, and serve as a potent risk marker to select a target population eligible for intervention trials at a young age to prevent further development of CVD. However, given the complexity and interaction of risk factors leading up to long-term increased CVD risk, as well as limited data on development of CVD risk over time (in particular in the fourth and fifth decade of life), the question arises how this information can best be used to design cardiovascular risk screening and prevention programs.

Figure 1. Preeclampsia and CVD



Adapted from Sattar & Greer, *BMJ*, 2002

Screening for subclinical cardiovascular disease after preeclampsia

Estimation of global cardiovascular risk

Both the American Heart Association (AHA) and the European Society of Cardiology (ESC) recommend cardiovascular risk assessment in men and women from the age of 40 years onwards. Although the two guidelines agree on this recommendation, their proposed risk estimation algorithms differ: AHA promotes the use of the race- and sex-specific Pooled Cohort Equations, whereas ESC recommends the use of the Systematic Coronary Risk Evaluation Project (SCORE).[15,16] These risk assessment tools overlap, apart from the parameters: race, high-density lipoprotein (HDL) cholesterol, stratification for the use of blood pressure lowering medication, which are only used in the Pooled Cohort Equations. Most CVD risk factor screening programs are based on these well-established algorithms, although there is growing evidence for sex-specific differences in risk factor prevalence and in their contribution to development of CVD.[17,18] In their latest recommendations, both U.S. and European guidelines now do include statements on cardiovascular risk assessment in women with a history of preeclampsia. The 2011 AHA guideline on prevention of CVD in women recommends to obtain a detailed obstetric history when a woman presents for the first time, and recommends to monitor and control CVD risk factors in women after a pregnancy complicated by preeclampsia.[19] However, no recommendations are made with respect to the questions of when to start screening, what targets to use, and what potential value a positive history of preeclampsia may have in improving risk classification.[15,16,19] Similar, the 2014 AHA guideline on stroke also points towards the role of preeclampsia as a potential identifier of stroke risk. However, the practical recommendations are no different from the AHA guideline for CVD prevention in women.[20] More recently, in a multidisciplinary guideline from the Netherlands focused on cardiovascular risk management after reproductive disorders current evidence for the association between reproductive disorders – amongst which PE – and the development of CVD has been updated and evaluated.[21] The authors advise on specific screening after preeclampsia based on current global CVD risk assessment protocols and blood pressure measurement at regular intervals postpartum, but note a lack of strong evidence and absence of longitudinal studies addressing the development of cardiovascular risk over time.

Major and contributing CVD risk factors

Current evidence suggests that women with a history of preeclampsia show a high prevalence of major traditional CVD risk factors, as well as other contributing factors and non-traditional risk factors.[22–25] An overview of studies on established and novel CVD risk factors in women with a history of preeclampsia, including anthropometric measures, circulating markers and imaging modalities, is presented in supplemental Table 2. In a recent meta-analysis by Hermes et al. several traditional risk factors for CVD (glucose, insulin, triglycerides, total cholesterol, HDL-cholesterol, low-density lipoprotein (LDL) cholesterol and homocysteine levels) were confirmed to be associated with previous preeclampsia in comparison to same-age women with a history of an uncomplicated pregnancy.[26] Moreover, in a recent study, we found that it is not uncommon

to find the presence of a combination of multiple independent major CVD risk among women with a history of early-onset preeclampsia within the first few years postpartum, with over half of women exhibiting 2 or more major risk factors and up to 20% of women with 3 or more major risk factors.[22] Despite these high prevalences, however, the estimated 10-year absolute risk of a cardiovascular event calculated by the Framingham Risk Score (FRS) was low for virtually all women.[22] This is explained by their relative young age, as these women are still premenopausal and CVD event rates are low. It can be speculated that assessment of CVD risk based on FRS 10-year predictions is likely to substantially underestimate the actual risk and estimations of lifetime risk may be more appropriate for these women.[27–30] In general, there is increasing support for the concept of using lifetime risk rather than the 10-year CVD risk, or the relative risk scores, in CVD screening programs, comparing individual CVD risk with the "ideal risk" of age-matched controls, to facilitate early identification of women at an increased risk of premature CVD. In addition, studies using surrogate endpoints of CVD, e.g. elevated carotid intima-media thickness (cIMT) and coronary artery calcium scoring (CACS), show more progression of subclinical atherosclerosis in women with a high lifetime cardiovascular disease risk compared with women with a low lifetime cardiovascular disease risk.[31,32] Indeed, the recent update of the SCORE algorithm includes a specific relative risk chart for women to estimate lifetime risk, which can be helpful for clinicians.[28]

In summary, in spite of increased attention for long-term follow-up after preeclampsia, effective and timely identification of women at risk of CVD remains a challenge. Tracking of CVD risk factor profiles after preeclampsia from the initial screening in the first years postpartum into the later stages of life is needed, and novel risk models that incorporate preeclampsia as a risk factor for CVD need to be developed.

Non-traditional markers of CVD risk

Because preeclampsia and CVD share common pathophysiological pathways, biomarkers used in prediction of preeclampsia might be useful in predicting CVD later in life. Novel cardiovascular biomarkers include markers associated with endothelial dysfunction and inflammation (intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-10 (IL-10), E-selectin), thrombosis (homocysteine, von Willibrand factor (VWF), fibrinogen, fibronectin, D-dimer, plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (tPA)), vasoconstriction (endothelin) and angiogenesis (vascular endothelial growth factor (VEGF), soluble Fms-like tyrosine kinase-1 (sFLT-1) and tumor necrosis factor alpha (TNF-a)).[33] Several of these markers have been shown to be elevated up to 20 years after pregnancy and may be involved in the pathogenesis of both preeclampsia and atherosclerosis, although the data are somewhat conflicting and heterogeneous.[34,35] In a recent meta-analysis on biomarker levels in women with a history of preeclampsia, for most of these novel biomarkers a trend towards higher levels was found in women with a history of a hypertensive pregnancy compared with controls with normotensive pregnancies, although only homocysteine levels were shown to be significantly higher.[33] Despite initial promising observations in

prospective cohort studies, the implementation of novel biological markers in addition to the repertoire of traditional cardiovascular risk factors is still much debated and is not routinely recommended in clinical practice.[32,36] It appears that for women, the contribution of novel markers to CVD risk stratification may be more promising than for men, as demonstrated by e.g. the recently developed Reynolds Risk Score for women that incorporates baseline CRP levels into the estimated CVD risk algorithm.[37] In a short-term follow-up study that included mostly term and mild cases of hypertensive disease in pregnancy tested 2.5 years postpartum, the Reynolds Risk Score and the more traditional risk algorithms (SCORE and Framingham Risk Scores), we more or less equivalent in estimating predicted 10-year CVD risk.[38] It will be interesting the see whether or not these risk algorithms perform differently in cohorts with longer-term (>10 years) postpartum follow-up, and whether or not novel risk markers (in particular inflammatory markers) may prove to be beneficial in improving CVD risk prediction in models specifically designed to predict CVD in women with previous preeclampsia.

Cardiovascular imaging

Recent advances in noninvasive cardiovascular imaging have enabled early detection of signs of subclinical atherosclerosis and indirect measures of arterial compliance.[39-41] This may be helpful as surrogate endpoints for intervention studies, as well as potentially add to global CVD risk assessment and guide treatment decisions.[42–46] Subclinical atherosclerosis is commonly assessed by carotid intima-media thickness (cIMT), coronary artery calcium score (CACS), coronary computed tomography angiography (CCTA) and cardiac magnetic resonance imaging (CMR).[5,45–55] In recent years, some groups have started to evaluate these imaging techniques in former preeclampsia patients, as discussed below.

1. Carotid intima-media thickness

CIMT is associated with the development of atherosclerosis and serves as an early indicator of CVD risk.[56–60] In a recent study in adults aged under the age of 45 years variation in cIMT was shown to be an early independent marker of later-life first-time myocardial infarction or stroke, although with modest discriminative power (hazard ratio (HR) 1.40 per standard deviation (SD) increase in cIMT, 95% confidence interval (CI) 1.11 – 1.76).[61] Since preeclampsia appears to be associated with premature atherosclerosis, cIMT may be of particular interest for early detection of vascular abnormalities in these women. Current studies exploring cIMT in women with previous preeclampsia have shown mixed results. In most studies, cIMT is increased in women after an episode of a hypertensive pregnancy,[5,48–50,52,54] although numbers are small and one study could not confirm these findings.[53] Furthermore, the added value of including cIMT in the current risk profiles in the general population is uncertain and it is not likely to improve risk classification.[15,62] Given the limited data available, it is unknown to what extend cIMT contributes to risk classification in formerly preeclamptic women.

2. Coronary artery calcium score

Coronary artery calcium score (CACS) is a non-invasive measurement of subclinical coronary atherosclerosis using low-dose (1 milliSievert) computed tomography (CT) scanning of the coronary arteries without administration of an intravenous contrast medium. CACS is a strong and independent predictor of cardiovascular events.[63,64] The additional value of CACS for CVD risk classification has mostly been demonstrated in asymptomatic persons with an intermediate risk of CVD, i.e. an estimated 10-year event risk of 5%-20% based on traditional cardiovascular screening.[45,47,65–70] Two retrospective cohort studies have evaluated CACS in women with a history of hypertensive pregnancy disorders, and both found a positive association between CACS and self-reported hypertension in pregnancy.[71,72] There are no published prospective studies yet to evaluate CACS in previous preeclamptic patients. Although CACS is a non-invasive measurement, holds great promise as a CVD risk marker, and provides the most direct evidence for cardiovascular damage, radiation dose and costs should be taken into account when considering CACS for risk assessment. The value of CACS will probably only be evident in individuals above the age of 45 years, as calcification of atheromatous plaques occurs relatively late in the development of atherosclerosis. More recently, evaluation of early-stage coronary artery atherosclerotic lesions by coronary computed tomography angiography (CCTA) has been suggested. This technique may have the advantage over CACS of being able to identify non-calcified plaques, and estimate the total atherosclerotic burden of the coronary artery tree.[73–75] In a number of retrospective cohort studies, it was shown that with CCTA, even in persons with very low CACS, a substantial presence of non-calcified plaques (or "plaque burden") can be found.[73,75,76] However, CCTA requires a higher radiation dose (3-4 milliSievert) and the use of intravenous contrast. To our knowledge, studies evaluating CCTA in women with a history of PE have not been conducted so far. Radiation dose, use of intravenous contrast material, and extra costs may limit the use of CCTA in younger age groups. In summary, there is growing interest in CACS and possibly low dose CCTA for CVD risk assessment in the general population.[43] CACS seems to be the most promising imaging marker and the AHA guideline now recommends considering CACS if the treatment decision is inconclusive based on global CVD risk assessment.[15]

3. Cardiac magnetic resonance imaging

Cardiac magnetic resonance (CMR) is a non-invasive imaging technique that enables detailed soft tissue characterization and can assess different parameters of cardiovascular function, as well as macrovascular and microvascular features of CVD without using ionizing radiation.[44] In addition, enhancement of CMR with adenosine perfusion MRI (also called 'adenosine stress MRI') can be used to identify both ischemic coronary artery disease as well as non-obstructive coronary disease in symptomatic women without major plaques.[77] It is unclear whether or not there is a role for adenosine stress MRI in the detection of cardiac dysfunction in asymptomatic women. However, CMR can be used to identify macroscopic fibrosis with late gadolinium enhancement, and evaluate early (preclinical) myocardial fibrosis with so-called T1 mapping.[78,79] In addition, CMR may be used as an alternative technique to evaluate aortic stiffness, which is

strongly related to systolic hypertension and is associated with future cardiovascular events.[80,81] Although sensitivity of CMR is high, the specificity is moderate for detecting major coronary artery lesions, and the availability and costs of CMR equipment currently limit large-scale use.[44] Another problem is the uncertainty of translating abnormal CMR findings observed at a young age to actual later-life CVD event risks, as longitudinal studies with sufficient follow-up time are not available. The use of CMR for screening purposes in asymptomatic, high-risk populations, such as women with a history of preeclampsia, needs to be further explored to establish a more conclusive role for CMR in the screening of CVD risk after preeclampsia.

Opportunities for prevention

Guidelines for CVD risk management increasingly include recommendations for cardiovascular prevention in women with a history of preeclampsia. However, and as demonstrated in this review, optimal screening and prevention in this high-risk group of young – apparently healthy – women still needs to be evaluated further.[16,19,82] In current practice, women who experienced preeclampsia are considered as "cured" after delivery and referred back to primary care without a plan for cardiovascular follow-up or prevention.[83] Question arises as to whether these women should be offered specific prevention strategies. Important to the debate on screening in this population is the observation that estimated 10-year CVD risks is low in this young age group despite multiple modifiable risk factors being present shortly postpartum. It seems rational to implement CVD screening and prevention on the basis of the expected high 'lifetime' risk of CVD in these women. However, uncertainties exist about the development and contribution of risk factors over time, and further efforts are needed to evaluate progression of early-life risk exposures with ageing, in particular *after* the first 10 years postpartum (or roughly from the age of 40 years onwards), when actual signs of CVD are expected to occur. Another question arises as how to organize effective screening and intervention programs in these women. Currently, a few clinics have initiated postpartum CVD risk assessment and counseling for women with who experience (mostly severe) preeclampsia at six to twelve months postpartum, offering global CVD risk assessment and an advice on lifestyle modifications.[84] A recent report from Cusimano et al. (2014) describing the experiences of a recently set-up maternal health clinic for CVD risk assessment after pregnancy complications (including gestational hypertension and preeclampsia), suggests that women are highly motivated to optimize lifestyle in the postpartum period, although only 40% of the booked patients showed up at the initial appointment.[85–87] It may be useful to consider targeted clinics that incorporate self-management (and eHealth) applications to improve adherence to postpartum prevention programs in women with reproductive disorders. A multidisciplinary approach, frequent interactions, and a more integrated women's health approach to simultaneously target young women with reproductive disorders associated with increased CVD risk, e.g. women with polycystic ovary syndrome (PCOS), preeclampsia and premature ovarian failure, can be considered.[88,89]

Summary and conclusions

In spite of a call for increased attention for long-term CVD risks after preeclampsia, translating this knowledge to clinical practice and population health initiatives remains a challenge. In this review, we set out to provide an overview of current data on CVD risk screening after preeclampsia and have aimed to discuss new and promising screening modalities and important caveats in CVD risk stratification and implementation. Importantly, in our view, identification of women with high risk, i.e. those women who will benefit most from early screening and prevention measures, remains the key to successful postpartum intervention studies. Routine use of biomarkers and modern CVD imaging techniques holds promise in research settings, but needs to be further evaluated before being implemented in clinical practice. Improved lifestyle interventions programs, developed for the general population, in particular those making use of smart technologies, merit further investigation. Continuing awareness of the high risk of premature CVD after preeclampsia should be raised among patients, specialists, and general practitioners to promote healthy cardiovascular lifestyles and ensure timely detection of CVD.

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Table 2: items used in cardiovascular screening

Screening method	Short term (< 5 years)	Long term (> 5 years)
Items commonly used in CVD risk assessment		
Gender	n.a.	n.a.
Age	n.a.	n.a.
Race	Unchanged[1]	Unknown[2]
Total cholesterol	Increased in most studies[1; 3-5]	Unchanged or marginally increased[3; 6-13]
HDL cholesterol	Decreased in most studies[1; 3-5]	Unchanged or marginally decreased[3; 6-14]
Systolic BP	Increased in most studies[1; 3-5; 15-17]	Increased in most studies[2; 3; 6-13; 18]
BP medication	Increased[15]	Increased[3; 6-13]
Diabetes	Unchanged[1]	Unchanged[2]
Smoking	Unchanged[4; 15; 17]	Inconclusive (unchanged / decreased)[2; 3; 6-13]
Metabolic syndrome	Increased[1]	Increased[6]
Family history of CVD	Unchanged[4; 15]	Increased in most studies[3; 6-13]
BMI	Increased in most studies[1; 4; 5; 15; 17]	Inconclusive (unchanged / increased)[2; 3; 6-13]
Triglycerides	Unchanged in most studies[1; 4; 5]	Unchanged or marginally increased[2; 3; 6-14]
Glucose	Increased in most studies[1; 3-5]	Inconclusive (unchanged / increased)[2; 3; 6-13]
HbA1c	Unknown	Inconclusive (unchanged / increased)[3; 6; 12]
Non-classic biomarkers		
CRP	Inconclusive (unchanged / increased)[3; 5]	Inconclusive (unchanged / increased)[2; 3; 6-11; 11-14]
Fibrinogen		Unchanged[6]
ICAM *	Unchanged[19]	Unchanged[19]
VCAM *	Unchanged[19]	Inconclusive (unchanged / increased)[12; 19]
Homocysteine *	Increased[4; 19]	Increased[19]
VWF *	Inconclusive (unchanged / increased)[19]	Inconclusive (unchanged / increased)[19]
Fibrinogen *	Inconclusive (unchanged / increased)[19]	Inconclusive (unchanged / increased)[12; 19]
Imaging modalities		
IMT	Increased[4; 5]	Inconclusive (unchanged / increased)[8-10; 12; 20; 21]
FMD	Decreased[5]	Unchanged[12]
CACS	Not performed	Increased[13; 22]
cCTA	Not performed	Not performed
CMR	Not performed	Not performed
Other modalities		
ECG		Inconclusive (unchanged / increased)[6; 10]

Level or occurrence of items compared to healthy women.

* used in meta-analysis.

BP, blood pressure; CACS, coronary artery calcium score; CCTA, coronary computed tomography angiography; CMR, cardiac resonance imaging; CVD, cardiovascular disease; BMI, body-mass index; CRP, c-reactive protein; ECG, electrocardiography; FMD, flow-mediated dilatation; HbA1c, hemoglobin A1c; HDL-cholesterol, high-density lipoprotein cholesterol; ICAM, intercellular adhesion molecule; IMT, intima-media thickness; VCAM, vascular cell adhesion molecule; VWF, von Willibrand factor.

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Part I

Cardiovascular disease risk after pregnancy


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Chapter 3

Association between parity and cardio-metabolic risk profile: a longitudinal follow-up study

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Submitted

Abstract

Objective: Physiological adaptations during pregnancy may persist postpartum and thereby contribute to an increased prevalence of metabolic syndrome (MetS) and an unfavorable cardiovascular disease (CVD) risk profile in parous women. Very limited data are available on the development of CVD risk factors over time and the quantification of this effect per child. The aim of the current study is to assess time-dependent changes in body composition and parameters of cardiometabolic health in parous women as compared with nulliparous controls, and stratified for number of children, in a well-defined longitudinal prospective cohort study.

Methods: We studied data of 2459 women who participated in the PREVEND study, a longitudinal population-based cohort for the assessment of CVD and renal disease in the general population. We selected women aged 40 years or older at the first visit, who reported no new pregnancies during the follow-up visits. All women were categorized according to parity (nulliparous, n=464; para 1, n=277; para 2, n=1021; para > 2, n=697), and stratified for age (40–49; 50–59, ≥ 60 years old). We compared the course of cardiometabolic profile in the course of six years using generalized estimating equation (GEE) models, all adjusted for age. Effect of parity on presence of CVD risk factors was assessed as well.

Results: We found BMI to be significantly higher with increasing parity in all age categories and this association was constant during follow-up. BMI increased up to 1.0 kg/m^2 per pregnancy, corresponding with 3.0 kg weight gain per pregnancy. Increasing parity was negatively associated with HDL cholesterol levels for women aged 40–49 and 50–59 years old, which was constant over time. Each extra pregnancy is associated with up to 0.09 mmol/L decrease in HDL cholesterol. MAP did not differ among parity groups in any of the age categories.

Conclusion: Our findings indicate an effect of parity itself on CVD risk profile that is constant during follow-up. Increasing parity, from para 2 onward, is associated with higher BMI, lower HDL cholesterol levels and higher prevalence of CVD risk factors.

Introduction

Pregnancy is associated with major alterations to the cardiovascular system and metabolic profile.[1–4] Increases in weight gain, lipid levels, blood plasma volume and cardiac output are needed to maintain a healthy, physiological pregnancy. Postpartum, this maternal adaptation reverses to its pre-pregnancy state, although several changes, (e.g. increased body weight and hypercholesterolemia) may persist for several months or indefinitely. It has been hypothesized that these persisting changes contribute to an increased prevalence of metabolic syndrome (MetS) and an unfavorable cardiovascular disease (CVD) risk profile in parous women.[5–7] Gestational weight gain (GWG) is of interest in this aspect, as GWG influences postpartum weight retention and BMI is one of the most important determinants in the development of CVD.[8–11] Cross-sectional cohort follow-up studies assessing long-term effects of excessive GWG in Caucasian women, showed a 3–4 kg/m^2 increase in BMI up to 21 years after pregnancy in these women.[12–14] However, it is unclear to what extent changes in metabolic health and blood pressure in parous women are driven by weight gain, or increased propensity to obesity.

Previous studies assessing parity and the relation with CVD showed conflicting results and even the association between parity and obesity is questioned in some studies.[15–18] However, a recent large cross-sectional study among Hispanic women in the US demonstrated that multiparity was associated with obesity, low HDL cholesterol and elevated fasting glucose, also after adjustment for sociodemographic and lifestyle factors.[7] Results from the cross-sectional Rotterdam study showed an inverse association between parity and HDL cholesterol and a positive association with total cholesterol and glucose/insulin ratios in Caucasian women at 70 years of age.[5] Cohort studies so far have not assessed the effect of parity on metabolic health longitudinally. Long-term effects have only scarcely been investigated, since most studies had a follow-up of only 1 to 3 years postpartum.[19,20]

Very limited data are available on the development of CVD risk factors over time and the quantification of this effect per child. Some studies suggested a linear association between number of children and adverse CVD risk profile, while others stated that only multiparity (i.e. more than 4 or more than 6 children) is associated with an increased CVD risk.[5–7,21,22] The aim of the current study was to assess time-dependent changes in body composition and parameters of (cardio)metabolic health in parous women as compared with nulliparous controls, and stratified for number of children, in a well-defined albuminuria-enriched longitudinal prospective cohort study.

Methods

Participants

The Prevention of Renal and Vascular End-stage Disease (PREVEND) study is a longitudinal cohort follow-up study for assessment of cardiovascular and renal disease in the general population. Details of this study have previously been published elsewhere.[23,24] In summary, all inhabit-

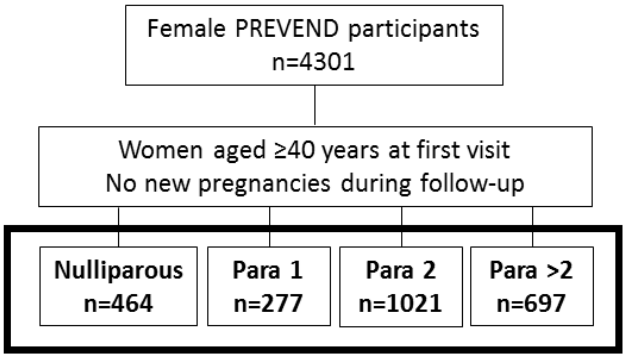
ants of Groningen, the Netherlands, at age 28–75 years (n = 85,421) were invited to participate by filling out a questionnaire and collecting a first-morning void urine sample. Pregnant women and subjects with type 1 Diabetes Mellitus were excluded. The urinary albumin concentration was assessed in a total of 40,856 (47.8%) responders. A total of 6,000 participants were enrolled out of 7,768 subjects with a urinary albumin concentration ≥ 10mg/L. In addition, 2,592 participants were enrolled out of 3,394 subjects with a urinary albumin concentration < 10mg/L. Thereby, the PREVEND study was enriched by subjects with an elevated albumin excretion.

In total, 4,301 women were enrolled in the PREVEND study (see Flowchart). For the current analysis, only women aged 40 years or older at the first visit and who reported no new pregnancies during the follow-up visits were included. Women who reported no children, were categorized as nulliparous (n = 464; 18.9%). Women who reported one child, two children or more than two children, were categorized as para 1 (n = 277; 11.3%), para 2 (n = 1021; 41.5%) and para > 2 (n = 697; 28.3%). The PREVEND study has been approved by the medical ethics committee of the University Medical Centre Groningen. Written informed consent was obtained from all participants.

Measurements and visits

Between 1997 and 2012, the screening visits took place, consisting of questionnaires, physical examinations, blood samples and 24-hour urine samples. The questionnaires included questions regarding parity. Participants reported the number of children they had, which was used as a proxy for the number of pregnancies. Except self-reported data from the questionnaires, BMI, HDL cholesterol, mean arterial pressure (MAP) and fasting glucose measurements were used for the current analysis. Details of clinical and laboratory measurements have previously been described elsewhere.[23] Prescription data from pharmacies was used to assess the use of antihypertensive and lipid lowering medication. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg

Flowchart. PREVEND parity cohort



or a diastolic blood pressure ≥ 90 mmHg or the use of antihypertensive medication. Type 2 diabetes mellitus (T2DM) was defined using the criteria previously described by Abbasi et al. (2013): fasting plasma glucose ≥ 7.0 mmol/L; random sample plasma glucose ≥ 11.1 mmol/L; self-reported physician diagnosis of type 2 diabetes mellitus; and/or use of glucose-lowering medication.[25] Data selection for analyses was based on a fixed median time interval of six years between the visits.

Statistical analysis

Data was arranged per subject per visit. Parametric variables are presented as mean ± standard deviation (SD) and analysed using Student *t*-test or One-Way ANOVA followed by Tukey post-hoc analysis. Non-parametric variables were expressed as median with 25th–75th percentile and analysed using Mann-Whitney U test or Kruskall Wallis. Categorical variables were analysed using Pearson Chi square or Fisher's exact, where appropriate. For longitudinal assessment (time factor) of the outcome measures among the different parity groups (group factor), a generalized estimating equations (GEE) analysis was performed, including the interaction term group*visit (interaction factor). All analyses were performed using an autoregressive correlation matrix structure. This assumes a variable correlation between measurements depending on the time between measurements, as was expected in the current analysis. For GEE analyses of continuous dependent variables (BMI, HDL cholesterol and MAP), participants were further stratified in three age categories (40 – 49 years old, 50 – 59 years old, and ≥ 60 years old) and in addition corrected for age. GEE analysis was corrected for age for dichotomous dependent variables (obesity, low HDL cholesterol, hypertension and T2DM). Data were analyzed using SPSS 22.0 (SPSS Inc. Chicago, IL, USA) and GraphPad prism 5.01 (GraphPad Software Inc. San Diego, CA, USA). In all analyses, a p-value <0.05 was considered statistically significant.

Results

Study population

Table 1 provides an overview of the baseline characteristics of women who were nulliparous, para 1, para 2 and para > 2 at the first PREVEND visit. The mean age was significantly higher in women who were para > 2. The vast majority of all women were Caucasian and the median follow-up time was 6 years in all groups. The cardiovascular risk profile among the groups differed significantly on BMI, blood pressure, smoking and use of alcohol. The cardiovascular comorbidity however, was equal among all groups. Unfavorable laboratory glucose measurements and cholesterol profiles were related to higher parity. The use of antihypertensive medication was higher in women who were para > 2 compared to the other groups, but the use anti-diabetic medication and lipid lowering medication did not differ among the groups. Women who were para > 2 less often used oral contraceptives compared to women who were nulliparous, para 1 or para 2.

Table 1. At entry characteristics

	No children (N=464) ‡	One child (N=277) ‡	Two children (N=1021) ‡	More than two children (N=697) ‡	*P-value*
General characteristics					
Age (years)	52.2 (10.1)	52.2 (9.0)	52.3 (8.4)	56.9 (9.6)	**<0.001**
Follow-up time (years)	11 (11-12)	11 (11-12)	11 (11-12)	11 (11-12)	0.71
Caucasian (n (%))	445 (96.9%)	264 (96.4%)	995 (98.0%)	663 (95.7%)	**0.04**
Job (n (%))	315 (68.3%)	127 (47.4%)	467 (46.5%)	264 (38.6%)	**<0.001**
Cardiovascular risk profile					
BMI (kg/m^2)	24.6 (22.5-27.7)	25.3 (22.5-28.6)	25.8 (23.4-28.8)	26.9 (24.0-30.2)	**<0.001**
SBP (mmHg)	120 (109-138)	121 (110-140)	122 (111-138)	130 (115-146)	**<0.001**
DBP (mmHg)	71 (65-78)	71 (67-77)	71 (66-78)	73 (67-79)	**0.004**
Current smoker (n (%))	139 (30.0%)	105 (37.9%)	326 (31.9%)	195 (28.0%)	**0.02**
Alcohol use (n (%))	203 (44.0%)	137 (49.6%)	503 (49.4%)	378 (54.5%)	**0.007**
Cardiovascular comorbidity (n (%))	11 (2.4%)	11 (4.1%)	24 (2.4%)	24 (3.6%)	0.30
Renal disease requiring dialysis (n (%))	1 (0.2%)	0 (0%)	3 (0.3%)	1 (0.1%)	0.78
Laboratory results					
Glucose (mmol/L)	4.6 (4.3-5.0)	4.7 (4.3-5.1)	4.7 (4.3-5.0)	4.8 (4.4-5.2)	**0.002**
Insulin (mmol/L)	7.0 (4.9-10.7)	7.6 (5.1-11.5)	7.8 (5.6-11.2)	8.5 (6.0-13.4)	**<0.001**
HOMA	1.4 (1.0-2.4)	1.6 (1.0-2.5)	1.6 (1.1-2.4)	1.8 (1.2-3.0)	**<0.001**
Total Cholesterol (mmol/L)	5.7 (5.0-6.4)	5.7 (5.1-6.5)	5.8 (5.0-6.7)	6.0 (5.3-6.7)	**<0.001**
HDL Cholesterol (mmol/L)	1.5 (1.2-1.8)	1.5 (1.2-1.8)	1.4 (1.2-1.7)	1.4 (1.2-1.7)	**<0.001**
Triglycerides (mmol/L)	1.0 (0.8-1.5)	1.1 (0.9-1.7)	1.1 (0.9-1.6)	1.2 (0.9-1.6)	**<0.001**
Medication use					
Total anti-HTN (n (%))	65 (16.4%)	39 (16.5%)	140 (15.6%)	151 (24.4%)	**<0.001**
ACEi/ARB (n (%))	19 (4.7%)	8 (3.3%)	41 (4.5%)	44 (7.0%)	0.07
Anti-diabetic (n (%))	7 (1.7%)	2 (0.8%)	12 (1.3%)	12 (1.9%)	0.63
Lipid lowering (n (%))	19 (4.7%)	15 (6.3%)	32 (3.5%)	37 (5.9%)	0.11
Oral contraceptive use (n (%))	79 (18.2%)	50 (19.5%)	198 (21.1%)	90 (14.6%)	**0.01**

Data are presented as median (25th – 75th percentile) unless otherwise stated. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; anti-HTN: antihypertensive medication; ACEi/ARB: angiotensin converting enzyme inhibitor and/or angiotensin receptor blocker; HOMA: homeostatic model assessment index.

‡ Total number that participated at visit 1 of the study, not all variables were available for each participant at baseline

Metabolic profiles in relation to parity and age

During the 6 year study period, there was a constant, significant difference in BMI among the parity groups at all age categories (Figure 1A). BMI was 0.7–2.0 kg/m^2 higher in the para 2 and para > 2 groups compared to nulliparous group. In addition, women who were para > 2 had a significantly higher BMI (1.0–1.8 kg/m^2) compared to women who were para 1 or para 2 aged 50–59 years old at both visits. Taken together, the mean increase in BMI was 0.6 kg/m^2 for each extra pregnancy, corresponding with 1.5–2.0 kg weight gain for each pregnancy. There was no significant time-effect on BMI and the change in BMI over time was similar among all age groups (40-50 years $p_{interaction}$=0.794; 50-60 years $p_{interaction}$=0.945; ≥60 years $p_{interaction}$=0.662).

HDL cholesterol levels differed among the groups, except for participants aged ≥ 60 years (Figure 1B). Both the para 2 group and the para > 2 group had significant lower HDL cholesterol levels at both measurements compared to nulliparous women aged 40–49 years (0.08–0.12 mmol/L) and 50–59 years (0.10–0.17 mmol/L). Each extra pregnancy is associated with 0.05 mmol/L decrease in HDL cholesterol at age 40–49 and 50–59 years. The differences among the age groups were similar over time ($p_{interaction}$=0.171–0.520) and HDL cholesterol levels increased in women 50–59 years by 0.04–0.07 mmol/L during the study period (p_{time}<0.040), but not in other age categories.

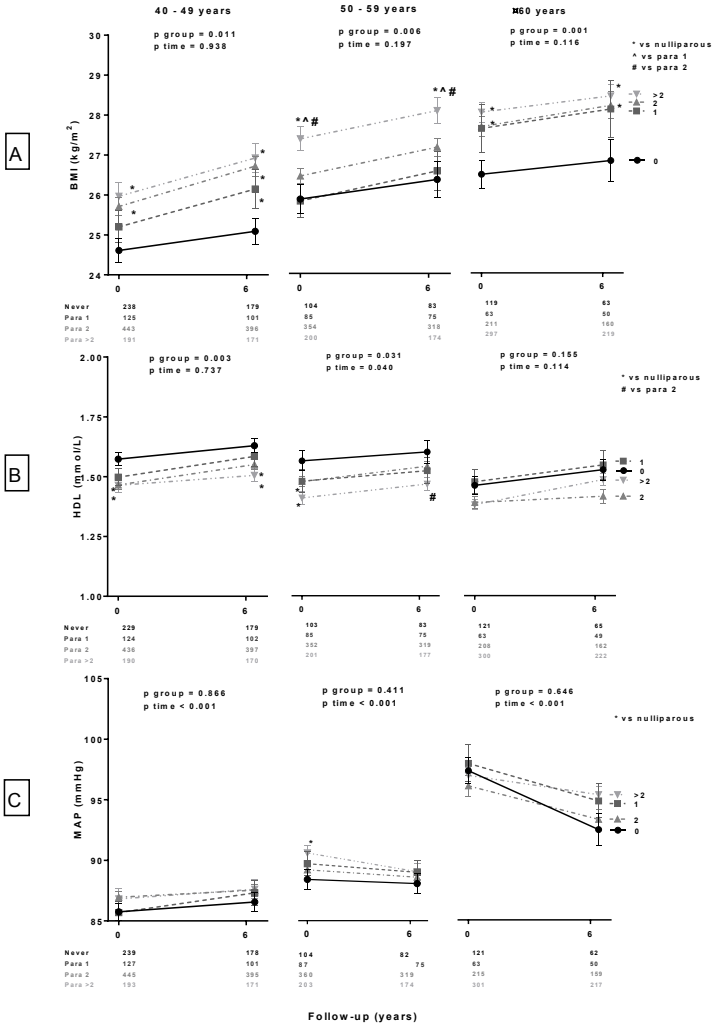
There were no differences among the parity groups over time in MAP at all ages, although MAP increased in all groups at age 40–49 years and 50–59 years and decreased in all groups at age ≥ 60 years. The differences among the parity groups were similar over time ($p_{interaction}$=0.407–0.779).

CVD risk factors in relation to parity

The prevalence of obesity increased with increasing parity at entry ($p_{for\ trend}$<0.001) and at 6 year follow up ($p_{for\ trend}$<0.001; Figure 2). At visit one, 15% of the nulliparous women was obese, compared to 26% of the women para >2. After the course of six years, this was increased to 16% of the nulliparous women compared to 30% of the para >2. The increase in prevalence over time was similar among the groups (p=0.450).

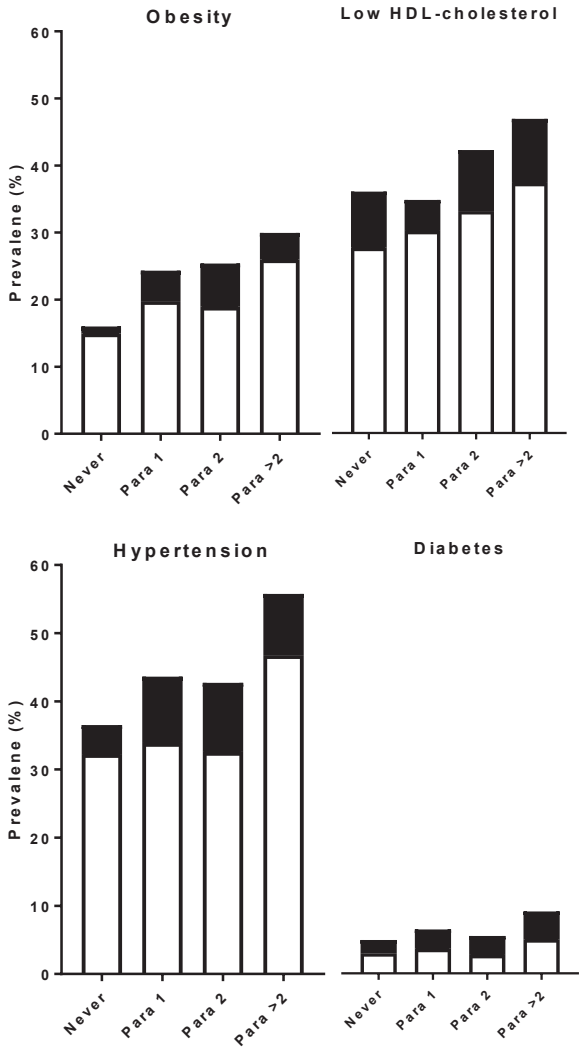
Low HDL cholesterol (<1.29 mmol/L) prevalence differed among the groups at visit one ($p_{for\ trend}$<0.001) and at follow up visit ($p_{for\ trend}$=0.006); low HDL cholesterol was more common when parity increased. Low HDL cholesterol prevalence inclined similar in all groups over time (p=0.160). Occurrence of T2DM did not differ among the groups at entry ($p_{for\ trend}$=0.094), although a positive association was found between T2DM prevalence and parity after six years ($p_{for\ trend}$=0.018). The increase in T2DM over time was comparable at all groups (p=0.336). T2DM prevalence was < 10% at all groups at both visits. Prevalence of hypertension increased with parity both at entry visit ($p_{for\ trend}$<0.001) and at follow up ($p_{for\ trend}$<0.001). Hypertension prevalence increased similar in all groups over time by 4–10% (p=0.761).

Figure 1. Metabolic profiles in relation to parity and age



Development over time of BMI (A), HDL-cholesterol (B) and MAP (C), stratified for age.

Figure 2. CVD risk factors at entry



Hypertension = RR ≥140/90 mm/Hg and/or use of antihypertensive medication;
Obesity = BMI ≥30kg/m2; Low HDL cholesterol = HDL cholesterol <1.29 mmol/L;
Diabetes = fasting plasma glucose ≥7.0 mmol/L, self-report of a physician diagnosis and/or use of glucose-lowering medication.
□ = first visit; ■ = follow-up visit

Discussion

In this longitudinal cohort study, higher parity is associated with a higher BMI and lower HDL cholesterol levels in the course of 6 years follow-up in different age categories. This could already be shown from para 2 onwards. Our findings suggest a consistent relationship between number of children and BMI and HDL cholesterol, without an accelerated increase of BMI or decrease of HDL cholesterol levels over time. MAP did not differ among groups in any of the age categories. Increasing parity was associated with higher prevalence of CVD risk factors, including obesity, low HDL cholesterol and hypertension, which was constant over time. As analyses were stratified and/or corrected for age, these results suggest an effect of parity itself on BMI, HDL cholesterol levels and CVD risk factor prevalence.

Especially the effect of parity on BMI is of great interest, since BMI appears to be one of the most important CVD risk factors. This is not only due to the direct effect on CVD onset, but also due to its adverse effect on lipid profile and blood pressure.[26–29] Results from a population-based cohort study among 4699 women suggested that weight or weight chances might be an important mediator in the effect of parity on metabolic syndrome, thus confirming the importance of BMI in regard to cardiometabolic health.[21] Roughly, each extra pregnancy is associated with 1.5–2.0 kg weight gain. Comparable, each extra pregnancy is associated with 0.05 mmol/L decrease in HDL cholesterol at age < 60 year.

Parallel to these metabolic differences among the groups, occurrence of several CVD risk factors, such as obesity and hypertension, differed among the groups as well. This is in line with cross-sectional studies assessing metabolic profile in relation to the number of children.[5–7,21] However, some studies could not confirm the relation between parity and metabolic health, especially after adjustment for covariates such as lifestyle.[16,17,21,30] As we expected age to have an influence on BMI, HDL cholesterol and MAP, we stratified our analyses for age. However, we chose not to correct for other covariates either because these were considered part of the causal chain (total cholesterol levels and glucose) or because these were not measured in the PREVEND study (lifestyle factors). Since full adjustment was therefore not possible, our findings should be interpreted with caution as it has been stated that lifestyle changes following childbirth might have more impact on cardiovascular health than parity itself.[30] Moreover, lifestyle effects of family life and the protective effect of lactation could explain the influence of parity on cardiometabolic health.[31–33] Another possible explanation behind the mechanism of this relationship between parity and CVD risk factors could be found in the effect of disturbances by pregnancy itself that continue to last postpartum. For example, the ovarian peptide hormone relaxin has emerged as cardiovascular modulator involved in vasodilatation and inducing angiogenesis.[34,35] Moreover, relaxin was associated with insulin and lipid profile in women with type 2 diabetes mellitus as well.[36] The role of breastfeeding remains controversial and a recent meta-analysis showed no significant effect of breastfeeding on postpartum weight retention.[37–40]

Previous cohort studies showed a 'J-shaped' association between parity and coronary heart disease, with lowest prevalence in women with two children, or stated that only multiparity (i.e. more than four or five children) was associated with increased CVD risk.[5–7] However, our results indicate that the effect of parity on cardiometabolic health is earlier present; having two children or more than two children already affect BMI, HDL cholesterol levels and CVD risk factor prevalence.

Our paper is the first study providing detailed assessment of CVD risk factor development over time in relation to parity. This longitudinal study comprised a large, well-phenotyped cohort with uniform assessment of all measurements during a median follow-up of 6 years. The GEE analysis which was performed, allowed us to assess differences among groups over time, focusing on group effects. However, several limitations need to be discussed. The mean age of women who were para >2 was significantly higher than women who were nulliparous, para 1 or para 2. In addition, women who were para >2 less often used oral contraceptives and more often used antihypertensive medication. This might result in a slightly different metabolic profile and thereby influence the results. Despite extensive phenotyping, age at first delivery, interpregnancy interval and lactation have not been assessed in the PREVEND study and therefore, adjustment of the analyses for these factors was not possible. However, since the mean age of our study population is > 50 years of age and because of the abovementioned conflicting evidence regarding breastfeeding, we do not believe that this significantly affects our results. Additionally, pre-pregnancy BMI and gestational weight gain have not been assessed in the PREVEND study either, although their role on postpartum weight retention seemed limited in a recent publication.[20,41] The PREVEND study was enriched with subjects with an elevated albumin excretion, which mostly results in an unfavorable CVD risk profile compared to the general population. However, albuminuria did not significantly differ among the groups within our analyses. In addition, correction for albuminuria did not change the results (data not shown). As no CVD endpoints such as myocardial infarction and stroke, have been assessed in the PREVEND study, it is unclear what effect the results of our analyses will have on CVD onset. Lastly, since causality could not be assessed, it is not known if the differences might have been present before pregnancy.

Conclusion

In this longitudinal cohort study, we found a positive linear association between parity and increasing BMI, but no accelerated response over time. For each extra child, BMI increases with 0.6 kg/m^2, translating into a weight gain of 1.5–2.0 kg per child. Furthermore, a negative linear association was found between parity and HDL cholesterol levels and a significant difference in occurrence of CVD risk factors among the groups over time. These findings indicate an effect of parity itself on CVD risk profile.

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Chapter 4

Effect of pregnancy on MRI-derived aortic characteristics: the AMBITYON study

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Submitted

Abstract

Background: Pregnancy and pregnancy complications have been associated with increased arterial stiffness even at young age. In this study we aim to assess the impact of parity on CMR - derived aortic characteristics as early markers of atherosclerosis and arterial stiffness in healthy women between 25 and 35 years.

Methods: we studied 68 women who participated in the AMBITYON study, a prospective population-based cohort study for assessment of atherosclerotic burden by MRI and traditional CVD risk factors in healthy, young adults. Of these women, forty (58.8%) were nulliparous, whereas 13 women (19.1%) were primiparous and 15 women (22.1%) were multiparous. Descending aortic wall thickness and thoracic aortic PWV were measured with 3.0T CMR.

Results: Aortic wall thickness measurements were comparable between nulliparous women and primi- or multiparous women (1.58 mm ± 0.17 mm vs. 1.59 mm ± 0.21 mm; p=0.79). Correction for age and systolic blood pressure did not change these results. Applying percentile based cut-off values showed a non-significant, limited increase in aortic wall thickness in parous women. PWV measurements did not differ between nulliparous women and primi- or multiparous women (4.48 m/s ± 0.71 m/s vs. 4.53 mm ± 0.84 mm; p=0.78). Correction for age and systolic blood pressure did not influence these results. Using percentile based cut-off values, showed an increasing likelihood of higher PWV-values in parous women, although this was not statistically significant.

Conclusions: direct measurement of aortic PWV and aortic wall thickness by CMR showed no difference between nulliparous and parous women, indicating a limited effect of pregnancy on arterial stiffness and early markers of atherosclerosis.

Introduction

Pregnancy is associated with cardiovascular alterations and poses a considerable challenge to the maternal cardiovascular system.[1–3] During normal pregnancy the total circulating blood volume, cardiac output and heart rate increase markedly whereas peripheral vascular resistance and mean arterial blood pressure decrease.[1,3] Some of the maternal adaptations during pregnancy, such as increased body weight and hypercholesterolemia, persist during the postpartum period and may endure for extended periods of time.[4] Women who experience such changes may exhibit an unfavorable cardiovascular disease (CVD) risk profile following parity.[5,6]

Vascular dysfunction, including both arterial stiffness and subclinical atherosclerosis, has been suggested to play a key role in the development of CVD in women.[7] This has been extensively investigated in relation to hypertensive pregnancy disorders (HPD), such as preeclampsia and gestational hypertension.[8,9] HPD are associated with increased arterial stiffness and early development of atherosclerosis.[8,10] However, the influence of uncomplicated pregnancy on these early markers of CVD is less clearly understood.

Previous studies have used operator-dependent techniques to assess vascular dysfunction, such as carotid-femoral pulse wave velocity (cfPWV) by tonometry measurement and carotid intima-media thickness (cIMT) by ultrasound, both requiring trained operators and specialized equipment.[8,11] Recent advances in cardiovascular magnetic resonance (CMR) imaging, however, provide a unique opportunity to assess aortic arterial stiffness and subclinical atherosclerosis both non-invasively and with low operator dependency.[12–14] CMR–derived PWV has been established as measure for arterial stiffness and aortic wall thickness (AWT) as measure for subclinical atherosclerosis.[14–17] CMR measures of vascular dysfunction have been validated and shown to be in good agreement with invasive measurements.[18,19]

In this study we assessed the impact of parity on CMR – derived aortic characteristics as early markers of atherosclerosis and arterial stiffness in asymptomatic women between 25 and 35 years.

Methods

Population

We used data of the AMBITYON (Atherosclerosis-Monitoring-and-Biomarker-measurement-In-The-Young) study (Netherlands National Trial Register number: 4742). The AMBITYON study is a prospective mono-center cohort study, which included 131 healthy young adults.[14] The participants were recruited from Leidsche Rijn, a region in the city of Utrecht, The Netherlands. Potential participants were randomly selected from the municipality population registry and approached with an invitation letter. Healthy young adults between 25 and 35 years were considered suitable for inclusion in the AMBITYON study. Individuals with symptomatic CVD, history of CVD, cardiac arrhythmias, contra-indications to MRI (i.e. pregnancy) and use of cardiovascular

protective medication were excluded from the study. CMR imaging was performed in all participants of the AMBITYON cohort.

For the current study we included all female participants (n = 68) from the AMBITYON study. The institutional review board of the University Medical Center Utrecht approved the AMBITYON study (reference number: 13/397). The AMBITYON study was carried out according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment in the study.

Measurements

1. Demographic and clinical measurements

Details regarding demographic and anthropometric data have been published previously.[14]. In short, all participants filled out a standardized questionnaire upon inclusion in the AMBITYON study. This questionnaire comprised items regarding general health, presence of CVD risk factors and obstetric history. Measurements of body mass index (BMI), waist circumference, hip circumference and blood pressure were standardized and obtained upon inclusion. In addition, a venous blood sample was drawn for assessment of blood lipid profile, glucose, C-reactive protein and white blood cell count.

2. CMR Imaging protocol and analysis

All participants underwent CMR imaging in supine position on a 3.0T multi-transmit clinical MRI system (Achieva, Software Release 5.1.7.2, Philips Healthcare, Best, the Netherlands). Images were acquired using a 32-channel phased-array cardiac receive coil. Total CMR imaging examination time was approximately 60 minutes per participant.

AWT: Images of the descending thoracic aorta were acquired in the sagittal orientation using a non-contrast-enhanced isotropic 3 dimensional (3D) black-blood (BB), T1-weighted, turbo-spin-echo (TSE) sequence with variable flip angles (3D-T1-BB-VISTA) sequence. Spectral Attenuated Inversion Recovery (SPAIR) fat suppression was used as well as sensitivity encoding (SENSE) parallel imaging algorithm, during free breathing without electrocardiogram gating to reduce acquisition duration.[20] Blood signal suppression was achieved by intrasequence flow related dephasing. The field of view (FOV) ranged from the top of the aortic arch and the most distal boundary of the cardiac coil, covering approximately 35cm of the descending thoracic aorta. Aortic wall geometry, including AWT, was assessed using a validated software program specifically designed for measuring vessel wall characteristics (Vessel Mass, release 5.1, Laboratory for Clinical and Experimental Image processing (LKEB), the Netherlands).[21] Image analysis was carried out according to standardized protocol, which has been published previously.[14,22]

PWV: To assess stiffness of the thoracic aorta, global PWV was assessed over the entire thoracic

aorta. To depict the full extent of the thoracic aorta, a double oblique single-slice SENSE balanced turbo field gradient-echo survey image was acquired using retrospective ECG gating and a single end-expiratory breath-hold. This resulting image was then used to plan two velocity-encoded phase contrast acquisitions perpendicular to the center lumen line of the aorta. One acquisition was positioned in the ascending aorta at the level of the pulmonary trunk to obtain the through-plane flow velocity in the ascending and proximal descending aorta. Subsequently, a second acquisition plane was positioned in the descending aorta near the dome of the liver to obtain the through-plane flow velocity in the distal descending thoracic aorta. All flow measurements were obtained in the transverse orientation perpendicular to the center lumen line using a one-directional through-plane, segmented, gradient echo pulse sequence with velocity encoding (VE) set to 1.50 m/s, retrospective ECG gating and free breathing. Quantification of PWV was performed in two steps. First, aortic velocity maps were generated by using validated customized software (MASS version 5.1, LKEB, Leiden, The Netherlands). Subsequently, absolute PWV values for the total thoracic aorta were generated by using a validated PWV measurement software program (PwvAppStatic, LKEB, Leiden, the Netherlands).[18,23]

Data Analysis

Continuous characteristics of the participants were presented as the mean with standard deviation (SD). Categorical variables were expressed as numbers and percentages. Participants were categorized according to parity (nulliparous versus primi-or multiparous) and nulliparous women were considered the reference group. CMR–derived PWV and AWT were compared between parity groups by linear regression analysis with and without correction for age and systolic blood pressure to control for possible confounding. Based on distribution of PWV and AWT in the reference group; 50th, 75th, 90th and 95th percentile cut-off values for PWV and AWT were computed, which were used for further analyses, using Pearson Chi square or Fisher's exact, where appropriate. A two-sided probability (p) value <0.05 was considered statistically significant. Data were analyzed using SPSS 22.0 (SPSS Inc. Chicago, IL) and GraphPad prism 5.01 (GraphPad Software Inc. San Diego, CA).

Results

Out of the total study population of 68 women, 40 women (58.8%) were nulliparous and 28 women (41.2%) were primi-or multiparous (see Table 1). The mean age of the study population was 31 years. Mean BMI was 23.0 kg/m^2 whereas mean blood pressure was 123/78 mmHg. The most prevalent CVD risk factor was smoking, which was reported by 17 women (25.0%).

Aortic Wall Thickness

AWT measurements were comparable between nulliparous and parous women (1.58 mm ± 0.17 mm vs. 1.59 mm ± 0.21 mm; Figure 1B). Linear regression analysis showed no significant difference in AWT between nulliparous women and primi- or multiparous women (p=0.79, β=0.03,

Table 1. Baseline characteristics

	AMBITYON women (N = 68)	
Patient characteristics		
Age (y)	31.0	3.2
Caucasian ethnicity (no, %)	61	89.7%
History of pregnancy (no, %)	28	41.2%
Para 1	*13*	*19.1%*
Para ≥2	*15*	*22.1%*
Clinical measurements		
Systolic blood pressure (mmHg)	123	13
Diastolic blood pressure (mmHg)	78	8
BMI (kg/m^2)	23.0	3.2
Waist circumference (cm)	75.6	8.3
Total cholesterol (mmol/l)	4.6	0.8
Triglycerides (mmol/l)	1.1	0.6
HDL-cholesterol (mmol/l)	1.59	0.35
LDL-cholesterol (mmol/l)	2.5	0.7
Glucose (mmol/l)	5.1	0.8
CVD risk factors		
Hypertension, self-reported (no, %)	3	4.4%
Diabetes, self-reported (no, %)	1	1.5%
Hypercholesterolemia, self-reported (no, %)	3	4.4%
Current smoking (no, %)	17	25.0%
Obesity (no, %)	3	4.4%

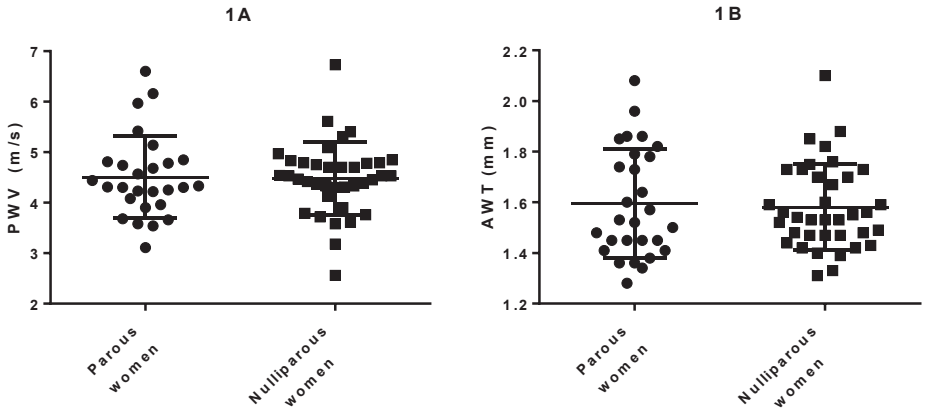
Data are presented as mean ± standard deviation, unless otherwise stated.
BMI, Body Mass Index; CVD, cardiovascular disease; HDL-cholesterol, high density lipoprotein cholesterol; LDL-cholesterol, low density lipoprotein cholesterol.

95% CI: -0.09–0.11). Correction for age and systolic blood pressure did not significantly change the results (p=0.77, β=-0.05, 95% CI: -0.15–0.11). Determining the 50th, 75th, 90th and 95th AWT percentile cut off, showed a non-significant increase in higher AWT-measurements with increasing percentile cut-off values in parous women compared with nulliparous women (Table 2).

Aortic Pulse Wave Velocity

Similar to AWT measurements, PWV values did not differ between the parity groups (4.48 m/s ± 0.71 m/s vs. 4.53 mm ± 0.84 mm; Figure 1A). Linear regression analysis showed no significant difference in PWV between nulliparous women and primi- or multiparous women (p=0.78, β=0.04, 95% CI: -0.35–0.46). Correction for age and systolic blood pressure did not significantly change the results (p=0.72, β=0.06, 95% CI: -0.39–0.56). Using percentile based cut-off values (50th, 75th, 90th and 95th percentile), showed an increasing likelihood of higher PWV-values in

Figure 1: scatterplot of PWV measures (1A) and AWT measures (1B) between parity groups



PWV, pulse wave velocity; AWT, aortic wall area

primi- or multiparous women with increasing percentile based cut-off value, although this was not statistically significant (Table 3).

Discussion

In this prospective cohort study assessing early signs of atherosclerosis and arterial stiffness in asymptomatic young adults we found no difference between nulliparous and parous women in CMR – derived direct PWV and AWT measures. Using percentile based cut-off values for AWT and PWV showed a non-significant increase in higher AWY- and PWV-measurements with increasing percentile cut-off values in parous women compared with nulliparous women.

The concept of pregnancy serving as a cardiovascular 'stress test' has now long been established.[24] According to this paradigm, pregnancy complications, such as hypertensive pregnancy disorders, serve as a red flag regarding future cardiovascular health and increase the risk of CVD later in life.[27,28] This is reflected in an increased prevalence of CVD risk factors already shortly after complicated pregnancy.[29,31] Despite extensive research regarding pregnancy complications and future maternal health, there is scarce data on the effect of parity itself on CVD risk and CVD risk factor development.[5,6,32] Previous studies assessing the effect of parity per se focused on long-term effects, i.e. follow-up of at least 10 years postpartum. In addition, only hard cardiovascular events, such as onset of CVD, onset of CHD or CVD mortality, have been assessed as outcome measures.[33–35] These cohort studies showed conflicting results regarding the effect of parity on future CVD and CVD

Table 2. Percentile based cut-off values AWT

Cut-off percentile	Cut-off AWT-value	Nulliparous (n=37)		Parous (n=24)		OR	95%CI	p-value
50th percentile	1.54	19	51.4%	13	46.4%	0.82	0.31–2.20	0.80
75th percentile	1.71	9	24.3%	10	35.7%	1.73	0.59–5.08	0.41
90th percentile	1.82	3	8.1%	5	17.9%	2.46	0.54–11.33	0.28
95th percentile	1.82	1	2.7%	2	7.1%	2.77	0.24–31.18	0.57

95%CI, 95% confidence interval; AWT, aortic wall thickness; OR, odds ratio; PWV, pulse wave velocity

Table 3. Percentile based cut-off values PWV

Cut-off percentile	Cut-off PWV-value	Nulliparous (n=37)		Parous (n=24)		OR	95%CI	p-value
50th percentile	4.53	18	48.6%	10	41.7%	0.75	0.27–2.13	0.59
75th percentile	4.79	9	24.3%	6	25.0%	1.04	0.32–3.41	0.95
90th percentile	5.32	3	8.1%	4	16.7%	2.27	0.46–11.18	0.42
95th percentile	5.72	1	2.7%	3	12.5%	5.14	0.50–52.67	0.29

95%CI, 95% confidence interval; AWT, aortic wall thickness; OR, odds ratio; PWV, pulse wave velocity

mortality. In the study by Jaffe et al., a J-shaped between parity and CVD mortality was found, which was confirmed in a recent meta-analysis with the lowest relative risk at para 3 to 5.[34,36] Short-term effects of parity on CVD risk factor development in young women however, have been overlooked in medical research. AWT and PWV have proven useful markers in predicting future cardiovascular events.[16,37] As vascular dysfunction is an important mechanism in the development of CVD in women, AWT and PWV might be extremely eligible in CVD risk assessment in young women.[7] Especially since CMR imaging emerged as non-invasive and operator-independent technique to assess AWT and PWV, more reliable assessment of vascular dysfunction is possible. In 1644 multiethnic women from the Dallas Heart Study, the number of live births was associated with subclinical aortic atherosclerosis measure by AWT by MRI.[38] In our study, we could not confirm these findings, as AWT and PWV measured by CMR did not differ between nulliparous and parous women. This might be explained by the >10 year age difference between women in our study compared with the Dallas Heart Study. Since age is an important determinant in development of atherosclerosis, it might be speculated that parity-effects manifest clinically at a later age.

Previous studies assessing arterial stiffness in relation to parity showed a decrease in PWV following pregnancy compared with nulliparous women.[39,40] This could not be confirmed in our current study. An explanation for this remarkable difference with the existing literature might be found in the application of state-of-the-art CMR imaging, allowing for direct and accurate assessment of aortic AWT and PWV, which has shown the best agreement with invasively measured gold standard.[18,19] We therefore question previous findings based on indirect measures, since our results suggest only a very limited effect of pregnancy itself on arterial stiffness. The use of CMR to assess subclinical signs of arterial stiffness and atherosclerosis is the major strength of our study. In addition, our study population comprises the largest sample of young adults to assess the effect of parity on CVD risk factor development by CMR. However, several limitations need to be addressed. Despite having the largest study sample, our cohort is still relatively small. Especially in the percentile-based cut off value analyses, the non-significant results might reflect insufficient power. Lastly, it would have been of great interest to measure PWV indirect as well, e.g. by applanation tonometer, to compare direct and indirect PWV measurements within the same subjects.

Conclusions

Direct measurement of aortic AWT and PWV by CMR showed no difference between nulliparous and parous women, indicating a limited impact of pregnancy upon arterial stiffness and subclinical atherosclerosis in the aorta. Validation of these results in a larger study sample is warranted.

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Part II

Cardiovascular disease risk after hypertensive pregnancy disorders


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Chapter 5

Trajectory of cardiovascular risk factors after hypertensive pregnancy disorders

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In preparation

Abstract

Background: Accumulating evidence consistently demonstrates an increased risk and accelerated development of cardiovascular disease (CVD) in women with a history of hypertensive disorders of pregnancy (HDP). Guidelines emphasize the emerging need for prevention of CVD in these women, but fail to provide uniform recommendations on commencement and time interval of cardiovascular risk assessment in these patients.

Objective: The aim of this study is to investigate development of blood pressure, lipids and fasting glucose levels over time in women with a history of HDP in order to identify a window of opportunity for starting cardiovascular risk factor management.

Methods: We identified women with self-reported normotensive pregnancy (NP, n=1811) and HDP (HDP, n=1005) within the PREVEND study, a Dutch population-based cohort study with a follow-up of 5 visits with 3-year intervals. Development of blood pressure, lipids, fasting glucose and 10-year CVD risk scores was longitudinally assed using generalized estimating equations.

Results: Women with a history of HDP presented with overall higher prevalence of hypertension ($p < .0001$), dyslipidaemia ($p = 0.003$) and glucose disturbances ($p < 0.0001$). Sub-analysis among women below 40 years of age at visit 1 showed that blood pressure at young age represents future status. Significantly more HDP women surpassed the 7.5% 10-years CVD risk threshold after the age of 50.

Conclusion: Women with a history of HDP showed a different development of classical cardiovascular risk factors. Shortly postpartum, blood pressure can identify women that are possibly at increased risk. The exact moment for screening and intervention remains a point for discussion, but our data imply this should not be held off until the fifth decade of life.

Introduction

Pregnancy induces an extensive adaptation of the circulatory system, including a major increase in cardiac output and glomerular changes.[1–4] Pregnancy complications, such as hypertensive disorders of pregnancy (HDP), might be an indication of limited cardiovascular capacities and thereby offer an opportunity to identify women at increased risk for cardiovascular diseases (CVD).[5–7] Accumulating evidence consistently demonstrates an increased risk and accelerated development of CVD risk factors and events later in life in women with a history of HDP.[8–12] Modification of vascular risk factors is effective in reducing vascular morbidity and mortality in patients with CVD.[13–15] Current guidelines suggest identification and evaluation of individual risk factors following tailored management, but have so far failed to provide uniform recommendations on how and when to screen these patients.[16–18] Previous observational studies showed a common occurrence of hypertension after HDP.[19–21] Persistent hypertension at 6 weeks post-partum was associated with hypertension at 2.5 years follow-up.[22,23] This persistent elevated blood pressure after delivery, suggests a hypertension-driven pathway in the development of CVD after HDP.[24] In addition, the duration of hypertension postpartum has been related to the severity of the HDP.[25] In contrast to prevalent hypertension after HPD, dyslipidemia and diabetes are much less often reported.[20,21] Despite the common occurrence of hypertension after HDP, current practice does not include standardized cardiovascular assessment after HDP to identify women that might benefit stringent follow-up or intervention strategies to prevent CVD onset. One of the factors involved is insufficient insight in the development of cardiovascular risk factors over time.[16,26]

The aim of this study is to investigate development of blood pressure, lipids and fasting glucose levels over time in women with and without HDP in order to identify a window of opportunity for screening and intervention.

Methods

The PREVEND study

The Prevention of Renal and Vascular End-stage Disease (PREVEND)-study is a prospective cohort study on long term natural course of renal, cardiac and peripheral vascular events with a follow-up to 75 years of age as described previously.[27,28] In short, starting in 1997-1998, all inhabitants of the city of Groningen (The Netherlands) aged 28-75 years, at that time 85.421, were asked to participate in the study. Participants were asked to fill in a questionnaire; collect a first-morning void urine sample and have venous blood samples taken. A total of 40.856 (47.8%) subjects responded to the call. Subjects with type 1 diabetes mellitus and women pregnant at the initiation of the study were excluded. The urinary albumin concentration was assessed in 40,856 responders. Subjects with a urinary albumin concentration ≥10mg/L (n=7,768) were invited to participate, of whom 6,000 were enrolled. In addition, a randomly selected group with a urinary albumin concentration <10 mg/l (n=3,394) was invited to participate in the cohort, of

whom 2,592 were enrolled. Five screening moments took place between 1997 and 2012, consisting of a questionnaire, physical examination and a blood and urine sample. The questionnaire included questions regarding reproductive disorders, such as HDP. The PREVEND study has been approved by the medical ethics committee of the University Medical Centre Groningen. Written informed consent was obtained from all participants.

Description of the cohort

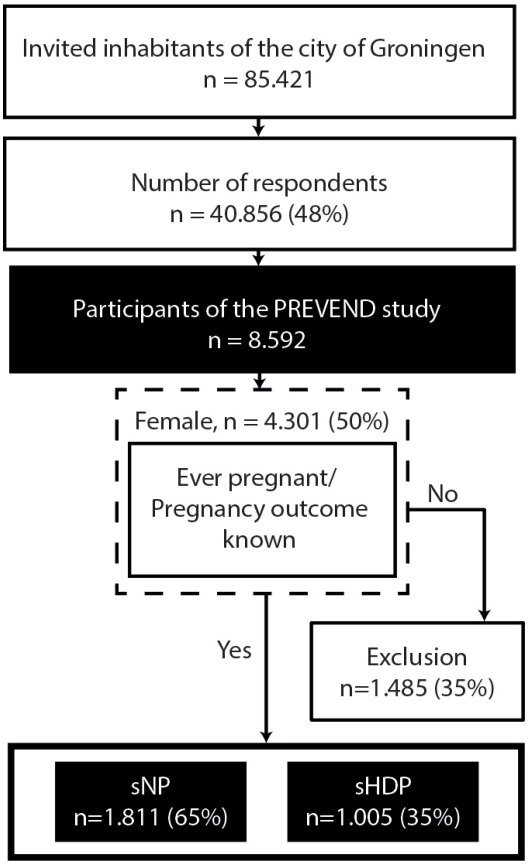
In total, 4,301 women were included in the PREVEND study. For the current study, only women who reported a history of pregnancy and who answered the questions on hypertension during pregnancy at the first and/or second visit were included (n=2,816). Based on these questions, women were categorized as either self-reported hypertensive disorder of pregnancy (HDP) or self-reported normotensive pregnancy (NP). Data were collected in five consecutive screening moments over a period of 15 years. Blood pressure, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and fasting glucose levels, were measured and used for current analysis. TC/HDL ratios were calculated dividing TC values by HDL-c values. Exact methods of clinical and laboratory measurements have previously been described elsewhere.[27] Prescription data from pharmacies was reviewed to assess the use of blood pressure and lipid lowering medication. The 10-year cardiovascular risk scores were calculated according to the Pooled Cohort Equations (PCE).[29]released in fall 2013, provide a long-anticipated update to the recommendations of the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III Patients were considered to be at "elevated" risk if the predicted risk was ≥ 7.5%.[30] For HDL-c was not measured at the second screening, the mean HDL-c of measurement 1 and 3 was taken. Micro-albuminuria was defined as urine protein of 30-300mg/24u, macro-albuminuria as >300mg/24u.[31] A subanalysis on a selection of women younger than 40 at visit 1 was conducted to provide additional insight on development of CVD risk factors in younger ages. We stratified results based on hypertension at visit 1: a blood pressure above 140/90mmHg at visit 1 or use of blood pressure lowering medication according to the questionnaire was defined as hypertension. Based on reported pregnancy data and presence of hypertension at visit 1, women were categorized as non-hypertensive NP (n=1,078), hypertensive NP (n=15), non-hypertensive HDP (n=185) and hypertensive HDP (n=23).

Statistical analysis strategy

Normally distributed data are presented as mean ± standard deviation (SD). Skewedly distributed data were expressed as median with 25^{th}-75^{th} percentile. The data was collected and arranged per subject per visit. To assess risk factor patterns in association with age, measurements were restructured into age categories. Using Generalized Estimating Equations (GEE) the time factor during follow up, the difference between HDP and NP women and the interaction between time and group was assessed in an independent correlation structure. Some individuals would possibly remain in the same age category during two or even three con-

secutive visits, resulting in unequal assignment of weight to the measurements of certain participants. By distinguishing measurements based on the combination participant*visit, this was corrected for using analysis, without compromising the longitudinal data structure. Also the dependency of repeated or multiple observations from the same women was taken into account in the GEE. Analyses were corrected for smoking, BMI and medication use (blood pressure lowering, lipid lowering and antidiabetics) where appropriate. In the sub cohort of women aged 40 years or younger at visit 1, we performed the analysis per visit and reported additionally on mean age per visit, for all women (n=1,301) attended all visits. Statistical significance from GEE analyses was described in differences between the two groups in one age category ($p_{category}$), between the groups overall (p_{group}) and between the slopes of the two groups ($p_{interaction}$). All statistical analyses were conducted using SPSS 22.0 (SPSS Inc. Chicago, IL, USA). In all analyses, a p-value <0.05 was considered statistically significant. For visualisations GraphPad prism 5.01 (GraphPad Software Inc. San Diego, CA, USA) was used.

Figure 1. Flowchart



Results

Study population

In total, 1,005 women with HDP and 1,811 women with NP were included in the analysis (figure 1). The mean age at start of the PREVEND was 48 years (25^{th}–75^{th} percentile 41–58 years) in women with HDP and 47 (25^{th}–75^{th} percentile 39–59 years; table 1) with a median follow up of 12 years (25^{th}–75^{th} percentile 3–14 years). Most PREVEND participants were of Caucasian descent. Similar percentages of participants in both the NP and HDP group smoked and/or used alcohol. The prevalence of albuminuria was similar in both groups (10.7% vs 12.9%).

Classical risk factors and risk score

Estimated mean systolic blood pressure and mean diastolic blood pressure were calculated with adjustment for BMI, smoking and blood pressure lowering medication (figure 2A). Women with HDP showed an overall higher systolic and diastolic blood pressure compared to women after NP (p_{group}<0.0001). Until the age of 55-60 years, systolic pressure in HDP women was approximately 10mmHg higher than in women who experienced a NP (p_{group}<0.0001). Diastolic pressure differed +/- 5mmHg between the groups (p_{group}<0.0001). After the age of 30–35 years, significantly more blood pressure lowering medication was used by women after HDP compared with women after NP. Over time, a diverging increase of blood pressure lowering medication use in the HDP group occurred compared to the NP group: at 35–39 years old the difference

Table 1. Characteristics at entry of the PREVEND study

	NP n = 1811 (65%)	HDP n = 1005 (35%)
General characteristics		
Age (years)	49 (41-58)	48 (39-59)
Caucasian (n (%))	1706 (95)	968 (97)
Job (n (%))	725 (41)	375 (38)
Cardiovascular risk profile		
BMI (kg/m^2)	26 (5)	27 (5)
SBP (mmHg)	122 (20)	130 (22)
DBP (mmHg)	70 (9)	74 (9)
Current smoker (n (%))	643 (35.5)	314 (31)
Ever smoked (n (%))	1557 (86)	860 (86)
Current alcohol use (n (%))	802 (44.6)	449 (46.1)
Ever alcohol use (n (%))	1058 (59)	573 (57)
Renal disease requiring dialysis (n (%))	7 (0.4)	1 (0.1)
Laboratory results		
Glucose (mmol/l)	4.6 (4.2-5.0)	4.7 (4.3-5.1)
Total Cholesterol (mmol/l)	5.6 (4.8-6.8)	5.7 (4.9-6.5)
HDL Cholesterol (mmol/l)	1.4 (1.2-1.7)	1.4 (1.2-1.7)
Urine		
No micro-albuminuria	1590 (87.8)	854 (85)
Micro-albuminuria	179 (9.9)	119 (11.8)
Macro-albuminuria	15 (0.8)	11 (1.1)

Data are presented as mean ± SD or median (25^{th} – 75^{th} percentile) unless otherwise stated. BMI; body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein.

Figure 2. Metabolic profile per age category stratified for normotensive pregnancy and hypertensive disorders of pregnancy

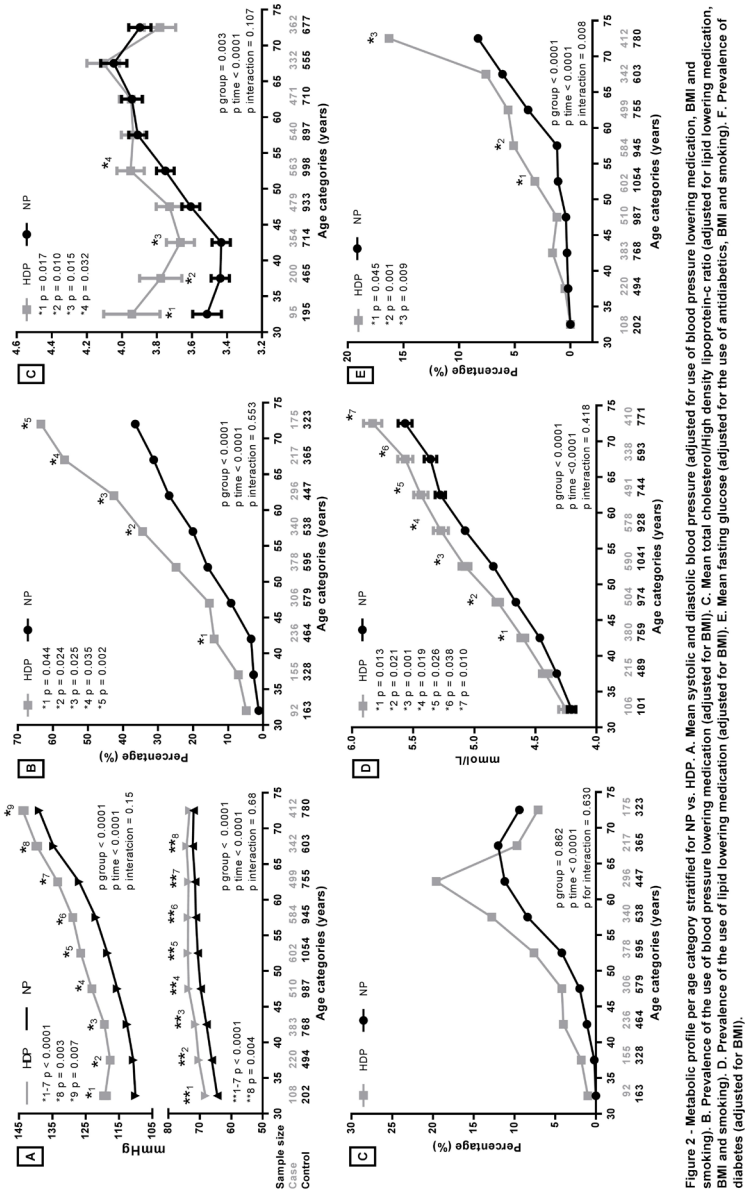


Figure 2 - Metabolic profile per age category stratified for NP vs. HDP. A. Mean systolic and diastolic blood pressure (adjusted for use of blood pressure lowering medication, BMI and smoking). B. Prevalence of the use of blood pressure lowering medication (adjusted for BMI). C. Mean total cholesterol/High density lipoprotein-c ratio (adjusted for lipid lowering medication, BMI and smoking). D. Prevalence of the use of lipid lowering medication (adjusted for BMI). E. Mean fasting glucose (adjusted for the use of antidiabetics, BMI and smoking). F. Prevalence of diabetes (adjusted for BMI).

Figure 3. Pooled Cohort Equations

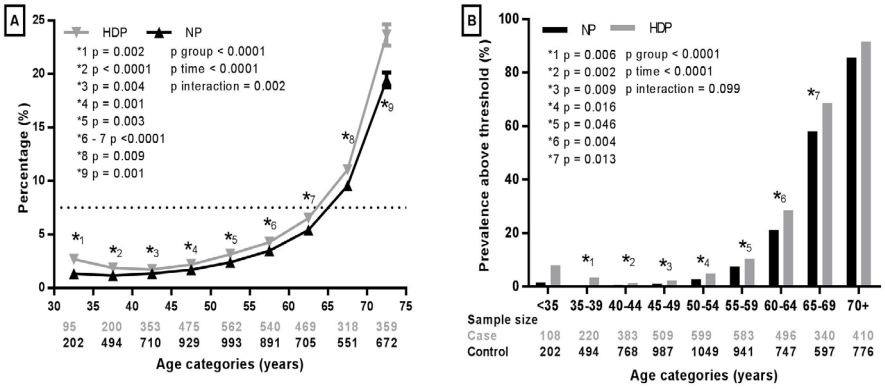


Figure 3 - A. Pooled cohort equations 10-years cardiovascular disease risk per age category stratified for NP vs. HDP. The dotted line at 7.5% emphasizes treatment threshold. B. The prevalence of women above the 7.5 % CVD risk.

Figure 4. Sub-analysis blood pressure

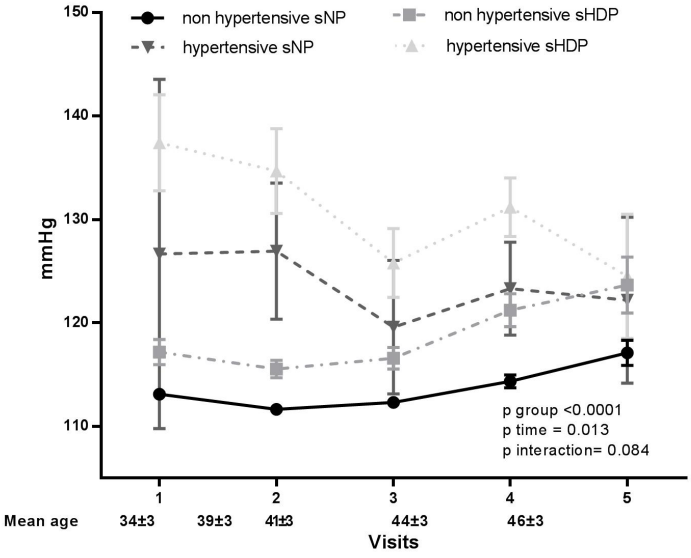


Figure 4 - Mean systolic blood pressure stratified by NP vs. HDP and hypertensive at visit 1 yes/no (adjusted for use of blood pressure lowering medication, BMI and smoking).
Sample size - hypertensive sHDP 23; non-Hypertensive sHDP 185; hypertensive sNP 15; non hypertensive sNP 1078.

was 7.5% in HDP's versus 2.7% in NP's. Overall there was a group difference ($p < 0.0001$), but no significant difference in slope ($p_{interaction}=0.553$). HDL-c and TC were at unfavorable levels in women after HDP before the age of 55–60 years, illustrated by significantly increase TC/HDL ratio in the younger age categories ($p_{category}=0.010$-0.032; figure 2C). The use of lipid lowering medication seems higher in post-HDP until the age of 65–70 years, but this difference does not hold over the entire follow up period ($p_{group}=0.862$; figure 2D). Fasting glucose levels were increased in women after HDP ($p_{group}<0.0001$; figure 2D). This difference developed after the age of 45 ($p_{category}=0.003$). At this time 9.5% of post-HDP women have glucose levels above 5.5mmol/L compared to 5.9% in women after NP ($p_{category}=0.003$). Overall, diabetes was more prevalent in women after HPD ($p_{group}=0.001$; figure 2F). Also the increase over time was steeper in women after HDP compared to NP ($p_{interaction}=0.008$). There was a significant difference in overall 10-years CVD risk between women after HDP and NP ($p_{group}<0.0001$; figure 3). The increase in absolute risk is also faster in women after HDP ($p_{interaction}=0.002$). There are significantly more HDP women that reach the clinical relevant threshold of 7.5% cardiovascular risk from the age of 50 years onwards ($p_{group}=0.023$).

Sub-analysis

Of the 1,301 women with an age of 40 years or younger at the first visit, 1,093 experienced a normal pregnancy and 208 a hypertensive pregnancy disorder. Hypertension prevalence at visit 1 was 11% versus 1.4% in women after HDP versus NP ($p<0.0001$). At all visits, blood pressure differed significantly between the four groups ($p_{group}<0.0001$, Figure 4). Women that were hypertensive at young age, thus shortly post index pregnancy, retained the highest blood pressure during follow up ($p_{group}<0.0001$). Lipid levels, glucose homeostasis parameters and corresponding medications did not show significant differences in this sub-analysis (data not presented in this manuscript).

Discussion

In this longitudinal cohort study we investigated the development of CVD risk factors in women after HDP compared to women after NP. We showed earlier development of hypertension, dyslipidemia and diabetes as possible determinants in this increased CVD risk. Shortly postpartum, blood pressure can identify women that are possibly at increased risk. Significantly more HDP women surpassed the 7.5% 10-years CVD risk threshold after the age of 50.

Our findings of an overall elevated blood pressure and lipid alterations in the HDP group 10–15 years ahead of NP group are in accordance with results from other large cohorts studies.[20,32–34] However, the expected natural increase of blood pressure in higher ages did surprisingly not occur.[35] Similar unexpected absence of deterioration over time is seen in the lipid analysis. This lack of deterioration might be explained by the increased use of blood pressure lowering treatment over time as shown in figure 2, despite our efforts to correct for this statistically. It is possible that

the effect of blood pressure lowering medication does not only cloud the moment of manifestation of a divergent alteration in the course of blood pressure, but also masks the severity of an increase in blood pressure. Another challenge in the interpretation our analysis is in the nature of the association. It is debated either HDP itself are in the causal chain to cardiovascular risk factors and disease, or HDP merely reflect a higher baseline CVD risk - and pregnancy serves subsequently as a cardiovascular stress test.[5] Increased lipid levels have been associated with the development of preeclampsia.[36] A longitudinal study among 3,225 women with singleton pregnancies assessing the risk factor patterns before and after pregnancy with HDP, showed a mitigated difference by 40–72% of CVD risk factors (BMI, blood pressure, TC, HDL-c) after adjustment for pre-pregnancy values.[37] Unfortunately, our analyses do not provide the opportunity to assess causality in the association between HDP and CVD risk factors. Based on common 10-year CVD risk estimation, one would not treat hypertension in post-HDP women before the age of 50.[17,30] But prediction models for disease risk are limited to one static measurements, while actual patients cardiovascular disease is a chronically evolving, progressive process, influenced by interventions, both pharmacological and non-pharmacological. Therefore, some clinicians plead to assess the indication based on gain in healthy life expectancy.[38]

The strength of our study is the precise insight in CVD risk factor development over time from 30 years up to 75 years of age in relation to HDP. This longitudinal study comprised a large cohort with uniform assessment of all measurements during a median follow-up of 11 years. The GEE analysis which was performed, allowed us to assess differences among groups over time, focusing on group effects. Some limitations need to be addressed. For assessment of obstetric complications, we relied on self-reported obstetric history. Thereby, our data could have been subject to recall bias, although validation analyses of self-reported history of HDP showed a specificity 98% and sensitivity of 87%.[39] In our cohort, 35% of the women reported a history of HDP, which is similar to another Dutch population based cohort study reporting approximately 30% of HDP based on self-reported obstetric history.[40] Our population was enriched with subjects with an elevated urinary albumin excretion, implying that our study population is at risk for CVD.[41,42] However, baseline analysis showed no significant difference between the groups and prevalence of micro- and macro-albuminuria in both groups was low. Therefore, we do not expect this to have a major effect on the generalizability of our results.

Conclusion

Women with a history of HDP showed a different development of classical cardiovascular risk factors. Already in the period shortly postpartum, blood pressure can identify women who might be at increased risk due to prolonged unfavorable cardiovascular risk profile. The exact moment for screening and intervention remains for discussion. Logically, our data imply this should not be held off until the fifth decade of life.

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Chapter 6

Similar pro-NT and pro-RLX2 levels after preeclampsia and after uncomplicated pregnancy

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Revisions in *Maturitas*

Abstract

Objective: Women are at increased risk of developing cardiovascular disease (CVD) after preeclampsia. Proneurotensin 1-117 (pro-NT) and prorelaxin 2 connecting peptide (pro-RLX2) have recently emerged as potent biomarkers for CVD risk prediction in women. We assessed pro-NT and pro-RLX2 levels in women with and without a history of preeclampsia.

Study design: We included 339 women with a history of early-onset preeclampsia and 327 women with an uncomplicated pregnancy for cardiovascular screening 10 years after delivery (the Preeclampsia Risk EValuation in FEMales (PREVFEM) cohort).

Main outcome measures: Pro-NT, a stable fragment of the neurotensin precursor, was assessed in the whole cohort. Pro-RLX2, the stable connecting peptide of the relaxin 2 prohormone, was assessed in a subset of this cohort, consisting of 27 women with a history of preeclampsia and 23 healthy controls. Associations between biomarker levels and traditional CVD risk factors in the preeclampsia and control group were assessed by Pearson's correlation.

Results: We found no differences in pro-NT and pro-RLX2 levels between the preeclampsia and control group. Pro-NT levels were associated with higher HbA1c (r=0.113, p-value 0.045) and with BMI (r=0.124, p-value 0.027), but only in the control group. Pro-RLX2 was related to current smoking and triglycerides in women with a history of preeclampsia and related to LDL-cholesterol in women with an uncomplicated pregnancy.

Conclusions: Pro-NT and pro-RLX2 levels were comparable between women 10 years after preeclampsia and women with an uncomplicated pregnancy. The role of pro-NT and pro-RLX2 in CVD development after preeclampsia should be further investigated.

Introduction

Female-specific risk factors such as pregnancy-related disorders are increasingly acknowledged as determinants in cardiovascular risk assessment in women.[1–6] A previous history of hypertensive pregnancy disorders (HPD) including preeclampsia is associated with a 2 to 7 fold increased risk of CVD compared to women with normotensive pregnancies.[7–9] The severity of the HPD appears to influence the CVD risk profile postpartum, with early onset preeclampsia having a less favorable risk profile.[10] Current CVD risk scores underestimate the actual risk of women affected by preeclampsia, since age is an important determinant and most women underwent CVD risk assessment at a relatively young age.[11–13] It is therefore desirable to identify biomarkers that can differentiate between women who are more susceptible of developing (premature) CVD and those who have a low risk. Previous studies reporting on novel CVD biomarkers in women after preeclampsia, such as soluble E-selectin (SE-selectin) and vascular cellular adhesion molecule (VCAM), showed inconsistent results.[14,15] However, proneurotensin 1-117 (pro-NT) and relaxin 2 prohormone (pro-RLX2) have recently emerged as a potential biomarker for CVD risk prediction in women specifically.[16,17]

Neurotensin is a 13-amino acid peptide involved in various biological processes throughout the body, and most extensively studied as a neurotransmitter and hormone in the central nervous system and the gastrointestinal tract.[18,19] Directly, circulating neurotensin is unstable and cannot be reliably measured in plasma or serum. A stable fragment of its precursor peptide, pro-NT, has recently been described as a promising novel CVD biomarker in women.[20] Relaxin, an ovarian peptide hormone with vasoactive properties, is a potential female-specific biomarker for components of CVD. Currently, relaxin is under evaluation for its potential role as therapeutic agent in both pregnancy-related disorders such as preeclampsia, and CVD such as heart failure.[21,22] In a cohort study among 63 women with newly diagnosed type 2 diabetes mellitus, relaxin was moderately correlated with fasting insulin and LDL-cholesterol, and weakly correlated with total cholesterol and C-peptide.[23]

Given the promising data on pro-NT and pro-RLX2 in general population, we assessed the eligibility of pro-NT and pro-RLX2 as biomarkers of cardiovascular health for women with a history of preeclampsia.

Methods

Patients

Our population consisted of women who participated in the Preeclampsia Risk Evaluation in FEMales (PREVFEM) study (trial registration number: 2668, The Netherlands National Trial Register). The aim of the PREVFEM study was to evaluate CVD risk factors 10 years postpartum in pregnancies complicated by early-onset preeclampsia. All women with early-onset preeclampsia who delivered between 1991 and 2007 at the obstetrics department of the Isala Klinieken

in Zwolle, the Netherlands, were invited to participate in the PREVFEM study. For the reference group, an equal number of age-matched controls with uncomplicated pregnancies in the same period were invited. Cases and controls were selected based on age and date of delivery, aiming at an average inclusion of 10 years postpartum and an equal distribution of these among the groups, although no specific restrictions to age at enrolment were applied. Since a lower response in the reference group was expected, 3 women with a history of a normotensive pregnancy were invited to participate for every 2 women with a history of preeclampsia. If a larger number of reference women was available, the women with the best match for age and delivery date were chosen. Exclusion criteria for the reference group included preterm birth, hypertensive pregnancy complications, and/or placental pathology. In total, 339 out of 515 invited women with a history of preeclampsia (response rate 64%) and 332 out of 810 invited women with uncomplicated pregnancies (response rate 41%) were eligible and consented to participate. A total number of 338 blood samples of cases and 327 blood samples of controls were suitable for this analysis. Early-onset preeclampsia was defined as a diastolic blood pressure ≥90 mmHg with proteinuria of ≥0.3g/24h between 20 and 32 weeks of gestation, in accordance with the International Society for the Study of Hypertension in Pregnancy (ISSHP). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Approval for the study was obtained from the institutional review board of the Isala Klinieken in Zwolle. All participants gave written informed consent upon inclusion in the study to undergo cardiovascular screening and to deliver a blood sample for later biomarker analysis. The study protocol, definitions of cardiovascular disease risk factors and baseline data have previously been described.[15,24]

Biomarker analysis

An overnight fasting venous blood sample was drawn at the scheduled cardiovascular screening visit. Blood lipid profile, glucose, CRP, fibrinogen and various cardiovascular biomarkers were analyzed. These results have been described elsewhere.[15,24] Participants with CRP values > 20 mg/L (i.e. indicating infection) were excluded from the analysis to prevent interference of high outliers in our analysis. Assessment of the biomarkers pro-NT and pro-RLX2 was performed batchwise on recoded samples in a central lab (sphingotec GmbH, Hennigsdorf, Germany). Pro-NT was analyzed as previously described using a one-step sandwich immunoassay based on a chemiluminescence-label and coated-tube technique (sphingotec GmbH, Hennigsdorf, Germany).[16] The assay had a sensitivity of 10 pmol/L and an inter-assay coefficient of variance of 5-9% in the concentration range observed in the present study. Pro-RLX2 was analyzed with a newly developed test [Rehfeldt et al., 2017, accepted] in a sandwich immunoluminometric assay format using two monoclonal antibodies. The assay had a sensitivity of 1.6 pmol/L, and an inter-assay coefficient of variance of below 15% for concentrations above 5 pmol/L. Pro-NT levels were assessed in the whole cohort, 338 cases and 327 controls, whereas it was decided to draw a random sample of 50 women for pro-RLX2 analysis.

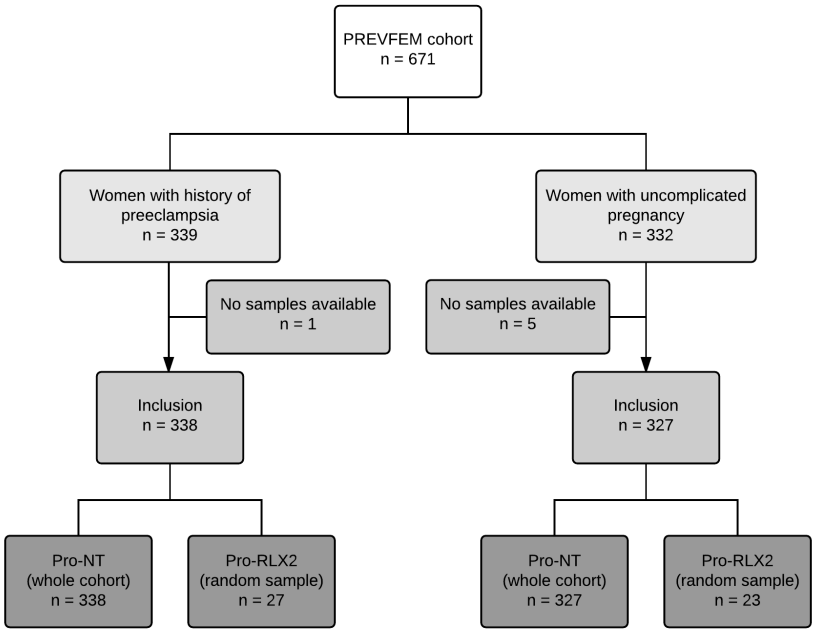
Data analysis

Data were expressed as means and standard deviation (SD) for parametric variables or as medians and 25th to 75th percentiles for non-parametric variables. Categorical variables were expressed as number and percentage. Differences in variables between women with a history of preeclampsia and controls with uncomplicated pregnancies were analyzed by student T-test for independent groups, Mann-Whitney U or by Chi-square, as appropriate. Menopausal status was assessed by self-report in a questionnaire, using dichotomous variables ('still having natural menstruations' (yes/no) and 'still having regular menstruations' (yes/no)). Normality of pro-NT and pro-RLX2 levels was assessed by the Shapiro-Wilk test. As both levels were not normally distributed, we performed natural log transformation for pro-NT and pro-RLX2. Associations between biomarkers and traditional CVD risk factors were assessed by Pearson's correlation. Correlation coefficients were interpreted as follows: 0–0.15 as poor, 0.15–0.30 as weak, 0.30–0.50 as moderate, 0.50–0.70 as strong and > 0.70 as very strong. We expected at least 10% difference in mean pro-NT levels between cases and controls. To detect this difference, we needed to include at least 311 women per group to reach power = 0.90 at α = 0.05. All data analyses were performed using the Statistical Package for Social Sciences (SPSS) version 22.0.

Results

In total, we included 338 women with a history of preeclampsia and 327 women with uncomplicated pregnancies in the current analysis (Figure 1).

Figure 1. Flowchart



Pregnancy data and baseline follow-up data are shown in Table 1. Mean age of participants at follow-up was 39.0 years (SD 4.7 years) and mean years post index-pregnancy was 9.9 years (SD 3.5 years). Women with a history of preeclampsia more often had hypertension (odds Ratio [OR] 3.65; 95% confidence interval [CI] 2.53-5.24) and metabolic Syndrome (MetS; OR 1.96 95% CI 1.28-3.01). We found no differences in lipid levels and C-reactive protein (CRP) between groups. In both groups, the vast majority of the women were still premenopausal (90.8% in the pre-eclampsia group and 87.3% in the control group).

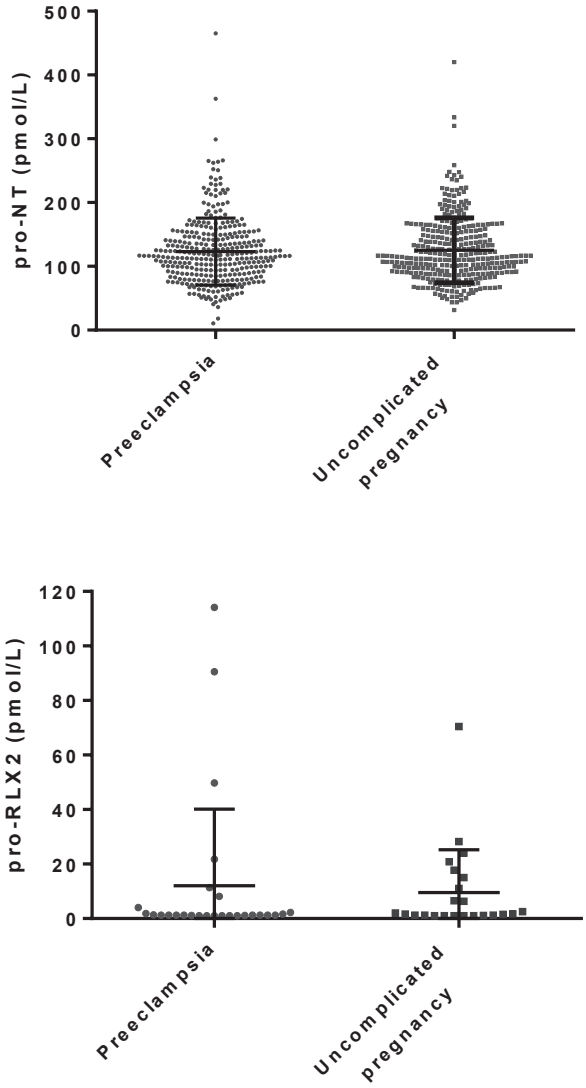
There were no differences in pro-NT levels between women with previous preeclampsia and the control group of women with only uncomplicated pregnancies (Figure 2). In our study, we did not observe any significant correlations between pro-NT levels and CVD risk factors, including age, blood pressure, BMI, lipids, glucose, HbA1c and insulin among women with a history of preeclampsia (Supplemental table). However, in women with uncomplicated pregnancies, we observed weak, but statistically significant associations between pro-NT levels and HbA1c (r=0.113, p-value 0.045) and BMI (r=0.124, p-value 0.027), although no significant correlations

Table 1. Pregnancy data and baseline follow-up data

	Women with history of preeclampsia (n = 338)*	Women with uncomplicated pregnancy (n = 327)*	p-value
Age (years)	38.8 ± 4.9	39.2 ± 4.4	0.28
Years postpartum after index pregnancy	9.1 ± 3.7	10.7 ± 3.1	< 0.01
Menopausal status			
Premenopausal (%)	307 (90.8%)	276 (87.3%)	0.15
Perimenopausal (%)	7 (2.1%)	5 (1.7%)	0.06
Postmenopausal (%)	24 (7.1%)	36 (11.0%)	0.05
Current smoking	53 (15.6%)	58 (18.4%)	0.36
Systolic blood pressure (mmHg)	127 ± 17	119 ± 15	< 0.01
Diastolic blood pressure (mmHg)	86 ± 12	79 ± 10	< 0.01
Antihypertensive use (%)	70 (20.7%)	6 (1.9%)	< 0.01
BMI (kg/m^2)	26.9 ± 5.7	26.3 ± 5.0	0.21
Waist circumference (cm)	86 ± 13	84 ± 11	< 0.01
LDL-cholesterol (mmol/L)	2.9 ± 0.8	2.9 ± 0.8	0.50
HDL-cholesterol (mmol/L)	1.5 ± 0.4	1.5 ± 0.3	0.75
Triglycerides (mmol/L)	1.0 ± 0.5	1.0 ± 0.5	0.18
Total cholesterol (mmol/L)	4.8 ± 0.8	4.9 ± 0.8	0.68
CRP (mh/L)	3.6 ± 5.4	3.2 ± 3.8	0.28
Glucose (mmol/L)	4.9 ± 1.1	4.9 ± 0.6	0.93
HbA1c (%)	5.3 ± 0.5	5.2 ± 0.3	0.31
Diabetes Mellitus (%)	3 (0.9%)	1 (0.3%)	0.35
Insuline (mIU/L)	12.4 ± 9.4	11.8 ± 11.2	0.45
Metabolic syndrome (%)	138 (41.1%)	67 (21.3%)	< 0.01
Pro-NT [pmol/L]; median (quartiles)	116 (88 – 147)	115 (91 – 155)	0.62
Pro-RLX2** (pmol/L); median (quartiles)	1.2 (1.1 – 4.1)	1.8 (1.1 – 15.0)	0.33

Data are presented as mean ± standard deviation unless otherwise stated. BMI: body mass index, LDL-cholesterol: low-density lipoprotein cholesterol, HDL-cholesterol: high-density lipoprotein cholesterol, CRP: C-reactive protein, pro-NT: pro-neurotensin 1-117, pro-RLX2: prorelaxin 2 connecting peptide.
* Total number of participants, not all variables were available for each participant at baseline
** In subset

Figure 2. Pro-NT and pro-RLX2 levels



with other markers of CVD risk were found. Similarly, pro-RLX2 levels, tested in a subset of this cohort (n=50), did not differ between the groups (Figure 2). In the whole subset, there was a significant moderate correlation between pro-RLX2 and triglycerides (r=0.398, p-value <0.01), which was also found in women with a history of preeclampsia (r=0.469, p-value 0.014). In addition, there was a strong correlation between pro-RLX2 and total-cholesterol (r=0.653, p-value <0.01) and LDL-cholesterol (r=0.636, p-value < 0.01) in women with an uncomplicated pregnancy.

Discussion

In this cross-sectional cohort study we assessed the associations of pro-NT and pro-RLX2 levels in women with a history of preeclampsia compared with controls who had uncomplicated pregnancies. In conclusion, average pro-NT and pro-RLX2 levels did not differ between these groups. We did observe weak associations between pro-NT levels and HbA1c and BMI among women within the control group. Pro-RLX2 was moderately related to triglycerides in women with a history of preeclampsia and strongly related to total-cholesterol and LDL-cholesterol in women with an uncomplicated pregnancy.

Pro-NT, which has previously been described as a promising risk marker for the development of diabetes mellitus and CVD in women, was only related to HbA1c and BMI in women with uncomplicated pregnancies in our cohort.[16] This is in line with the associations in the population-based cohort study by Melander et al., in which pro-NT was related to diabetes and CVD development in women specifically and also to several metabolic parameters (i.e. insulin and glucose) and smoking.[16] Surprisingly, this association was not found in the group of women with a history of preeclampsia. This might be due to the limited metabolic disturbance in these women, as is reflected by the low rate of diabetes mellitus and obesity in our study sample. We hypothesize that pro-NT might be a more relevant prediction marker in women who show more marked cardiometabolic changes, such as women within higher age groups and women with metabolic disorders e.g. polycystic ovary syndrome (PCOS), obesity, or other women at risk of type 2 diabetes. We suspect that in this particular cohort of women with a history of preeclampsia, the shared predisposition of these women for both preeclampsia and subsequent CVD may be predominantly driven by the 'hypertension pathway', rather than metabolic changes. This is further supported by the relatively high proportion of women found to be on antihypertensive drugs at follow-up (21.2% in the women with a history of preeclampsia). In other cohorts of women with previous preeclampsia higher rates of cardiometabolic risk factors may be found.[10,25] This study therefore cannot rule out a potential use for pro-NT and pro-RLX2 testing in these women as an additive marker predictive of early metabolic change after preeclampsia, although this needs to be confirmed in further studies.

Pro-RLX2 was moderately correlated with triglycerides in women with a history of preeclampsia and strongly correlated with LDL-cholesterol and total cholesterol in women with an uncomplicated pregnancy. This is in line with the study by Szepietowska et al (2008) in which relaxin was weakly correlated with LDL-cholesterol and moderately total-cholesterol in women newly diagnosed with type 2 diabetes mellitus, although relaxin was measured instead of pro-RLX2.[23] On the other hand, the correlations between relaxin and insulin, insulin sensitivity and age – which were described by Szepietwoska as well – could not be confirmed in our cohort. Only very limited data regarding CVD risk prediction by pro-RLX2 is currently available. Long-term follow-up data including cohorts of an older age are warranted to draw conclusion about the additional value of pro-NT and pro-RLX2 as cardiovascular biomarkers.

In summary, our paper provides new data on pro-NT and pro-RLX2 in women with a history of preeclampsia. In this cross-sectional analysis, pro-NT and pro-RLX2 did not differ between women with a history of preeclampsia compared with controls who had uncomplicated pregnancies.

Strengths of this study include the detailed, well-defined large cohort of women with early-onset preeclampsia. The follow-up period was approximately 10 years, thereby allowing for long-term CVD risk assessment without confounding of temporary changes in cardiometabolic measurements early after pregnancy. A few limitations need to be addressed. Despite the large number of participants, the PREVFEM study was originally designed to assess traditional CVD risk factors after preeclampsia and the current study is a secondary analyses of pro-NT levels in the whole cohort. However, Pro-RLX2 was only available for a random subset of participants, consisting of 27 preeclampsia patients and 23 controls. Therefore, a lack of power could play a role in these measurements and these results should be interpreted with caution. Despite the 10 years follow-up period, cardiovascular and metabolic changes due to menopause were not yet present, as the mean age of the participants was 39 years and only a few had reported to be postmenopausal. As CVD risk factors are increasingly apparent after menopause, associations between pro-NT and pro-RLX2 might be found in postmenopausal women. Due to the lack of occurrence of CVD endpoints within this cohort, we could not assess the value of pro-NT and pro-RLX2 in predicting these CVD endpoints. Studies comprising a larger study sample, including postmenopausal women and assessing CVD endpoints are warranted, before conclusions about the eligibility of pro-NT and pro-RLX2 as cardiovascular biomarkers can be made.

Conclusion

In this cross-sectional cohort study, pro-NT and pro-RLX2 levels were comparable between women 10 years after preeclampsia and women with an uncomplicated pregnancy. No significant correlations between pro-NT levels and CVD risk factors in women after preeclampsia were found. A moderate correlation was found between pro-RLX2 and triglycerides in women after preeclampsia. Further research assessing biomarkers to address CVD development after preeclampsia is warranted and should focus on the relation with CVD endpoints.

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Supplemental table 1. Associations between Pro-NT and traditional cardiovascular risk factors

	All participants (n = 665)		Preeclampsia (n=338)		Uncomplicated pregnancy (n= 327)	
	R-value	p-value	R-value	p-value	R-value	p-value
Age	0.008	0.83	0.006	0.91	0.010	0.86
BMI	0.036	0.35	-0.033	0.54	0.124	**0.03**
Systolic blood pressure (mmHg)	-0.006	0.88	0.001	0.99	-0.009	0.88
Diastolic blood pressure (mmHg)	0.004	0.92	-0.002	0.97	0.019	0.74
Total cholesterol (mmol/L)	-0.013	0.74	0.039	0.48	0.041	0.46
LDL-cholesterol (mmol/L)	0.002	0.96	-0.017	0.75	0.023	0.69
HDL-cholesterol (mmol/L)	-0.036	0.35	-0.08	0.74	-0.060	0.29
Triglycerides (mmol/L)	0.004	0.92	-0.006	0.91	0.017	0.76
Insuline (mIU/L)	0.031	0.45	-0.042	0.45	0.106	0.08
Glucose (mmol/L)	-0.015	0.70	-0.051	0.35	0.060	0.29
HbA1c (%)	0.022	0.57	-0.025	0.65	0.113	**0.05**

Supplemental table 2. Associations between Pro-RLX2 and traditional cardiovascular risk factors

	All participants (n = 50)		Preeclampsia (n=27)		Uncomplicated pregnancy (n=23)	
	R-value	p-value	R-value	p-value	R-value	p-value
Age	0.032	0.82	0.084	0.68	-0.105	0.63
BMI	0.056	0.70	0.138	0.49	-0.199	0.36
Systolic blood pressure (mmHg)	0.019	0.90	0.118	0.56	-0.184	0.40
Diastolic blood pressure (mmHg)	0.036	0.22	0.069	0.73	-0.077	0.73
Total cholesterol (mmol/L)	0.216	0.13	-0.032	0.87	0.653	**<0.01**
LDL-cholesterol (mmol/L)	0.133	0.36	-0.174	0.39	0.636	**<0.01**
HDL-cholesterol (mmol/L)	-0.054	0.71	-0.094	0.63	0.120	0.59
Triglycerides (mmol/L)	0.398	**<0.01**	0.469	**0.01**	0.081	0.71
Insuline (mIU/L)	0.013	0.931	0.012	0.95	0.016	0.95
Glucose (mmol/L)	0.017	0.91	-0.018	0.93	0.116	0.60
HbA1c (%)	0.002	0.99	0.026	0.90	-0.178	0.42


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Chapter 7

Stroke after Pregnancy Disorders

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Brief communication

Women with hypertensive pregnancy disorders are at risk of developing stroke, possibly mediated by female-specific risk factors. Pregnancy is considered to be a stress test for cardiovascular health later in life.[1] In the current study we assessed the occurrence of pregnancy disorders, among women with a history of ischemic stroke who participated in the Dutch acute stroke study (DUST) and related these risk factors to age of stroke onset, stroke subtype, radiological characteristics and clinical outcome. Details and results of the prospective, multicenter DUST study have previously been published elsewhere.[2] Out of the 429 living female participants in DUST with ischemic stroke and clinical outcome at 3 months follow-up, 166 women consented to participate in the current questionnaire follow-up study, assessing female-specific risk factors for cardiovascular diseases.

In total, 144 participants reported one or more pregnancies (86.7%). The most common reported pregnancy disorders were miscarriage (31 participants, 22.5%) and hypertensive pregnancy disorders (49 participants, 35.3%,). No differences were observed for age of stroke onset, stroke subtype, or clinical outcome when comparing the separate groups of patients with a history of miscarriage, preterm delivery, hypertensive pregnancy disorders or placental abruption with patients without these disorders. However, a difference was observed for age of stroke onset (mean difference 10.2 years, 95% CI 2.6–17.8 years, see Table 1) when comparing participants with and without a history of pregnancy complications associated with vascular disease (pre-eclampsia, HELLP syndrome and placental abruption). Six (50%) of these participants with a history of these pregnancy complications had a stroke before the age of 50 years. Logistic regression resulted in an odds-ratio for having stroke onset < 50 years of 4.71 (95% CI 1.38 – 16.06) for women with a history of pregnancy complications associated with vascular disease compared with women without a history of such pregnancy complications. No differences were observed for age of stroke onset, stroke subtype, radiological characteristics or clinical outcome when comparing the separate groups of patients with a history of miscarriage, preterm delivery, hypertensive pregnancy disorders or placental abruption with patients without these disorders. An increased risk of ischemic stroke in women with a history of pregnancy complications has been described in large retrospective and prospective cohort studies.[3,4] The relation of age at stroke onset and history of pregnancy disorders has not been previously reported. The 10 years earlier age of stroke onset in participants with a history of pregnancy complications is consistent with our previous observations of an earlier onset of hypertension and type 2 diabetes mellitus in women with adverse pregnancy outcome.[5] Pregnancy complications and ischemic stroke share common risk factors, such as obesity and hypertension. This might explain the accelerated stroke development after pregnancy complications.

Some limitations of our study need to be addressed. Firstly, our conclusions are based on relatively few women who experienced pregnancy complications within the cohort and information on pregnancy complications was based on self-reporting and could not be checked in medical records. Therefore, our findings need to be confirmed in a larger study sample. Secondly, selection bias may have occurred due to the retrospective nature of our sub-study and the use

of self-reporting.

In conclusion, pregnancy disorders are common among women who experience ischemic stroke. We found that the mean age of stroke onset was about 10 years earlier in participants with a history of pregnancy complications associated with vascular disease (preeclampsia, HELLP syndrome and placental abruption), compared with women without such a history.

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Table 1: stroke characteristics in patients with and without pregnancy complications

	Pregnancy complications (n=12)*		No pregnancy complications (n=119)*		p-value
Age at stroke, mean (SD)	53.9	(13.4)	64.1	(12.8)	0.009
BMI at stroke, mean (SD)	29.8	(9.1)	26.0	(4.3)	0.057
Stroke classification (TOAST)					0.795
Large-artery atherosclerosis	3	25.0%	29	24.4%	
Cardioembolism	1	8.3%	18	15.1%	
Small-vessel occlusion	3	25.0%	22	18.5%	
Stroke of other determined etiology	2	16.7%	9	7.6%	
Stroke of undetermined etiology	3	25.0%	41	34.5%	
Stroke classification (Oxfordshire)					0.497
PACI	5	41.7%	48	40.3%	
TACI	0	0.0%	11	9.2%	
LACI	3	25.0%	25	21.0%	
POCI	2	16.7%	23	19.3%	
Unclear	1	8.3%	12	10.0%	
Pre-stroke disability					
mRS ≥ 2	0	0.0%	9	7.6%	0.602
NIHSS score admission ≥ 5	6	50.0%	60	50.4%	1.000
Follow up at 3 months					
mRS ≥ 2	4	33.3%	53	44.5%	0.549
EQ5D ≥ 6	6	50.0%	75	63.0%	0.681
Barthel index ≤ 17	0	0.0%	11	9.2%	0.594

* Total number of women, not all variables were available for each participant.
BMI, body-mass index; PACI, partial anterior circulation infarcts; TACI, total anterior circulation infarcts; LACI, lacunar infarcts; POCI, posterior circulation infarcts; mRs, modified Rankin scale; NIHSS, National Institutes of Health Stroke Severity Scale, EQ5D, EuroQol five dimensions questionnaire.


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Part III

Imaging of coronary and carotid artery atherosclerosis


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Chapter 8

Subclinical coronary artery disease assessed by coronary computed tomography among asymptomatic women with a history of preeclampsia aged 45 to 55 years

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Submitted

Abstract

Background: Preeclampsia is associated with increased risk of coronary artery disease (CAD). However, the development of subclinical CAD as measured by coronary artery calcifications and plaques over time is unknown. In this study we aim to prospectively evaluate coronary artery atherosclerosis in asymptomatic women aged 45–55 years with a history of preeclampsia by computed tomography (CT).

Methods: In this cross-sectional part of a multicenter, prospective cohort study women with a history of preeclampsia were recruited for cardiovascular risk assessment. Data were compared with women of similar age and ethnicity in a population-based cohort (Multi-Ethnic Study of Atherosclerosis (MESA)). Coronary artery calcium score (CACS) was calculated and contrast-enhanced coronary CT angiography (CCTA) was performed in addition to routine cardiovascular risk assessment. Primary outcome was the number of women with CACS >0 Agatston Units (AU) and/or any coronary atherosclerotic plaques on CCTA. Significant stenosis was defined as luminal stenosis ≥50% on CCTA and subclinical CAD was defined as CACS ≥100 AU and/or significant stenosis. CACS were converted to MESA percentiles with adjustments for age, gender and race.

Results: 164 asymptomatic women (mean age 48.4 ± 2.9 (SD) years at inclusion) with a history of preeclampsia participated. Overall, 31% presented with a CACS >0 and 17% had CACS above the 95th MESA percentile. Compared to MESA-participants, women with a history of preeclampsia showed an increased prevalence of CACS >0 AU (OR 2.0, 95% CI 1.3-3.0), CACS >100 AU (OR 4.0, 95% CI (1.5-10.7) and CACS >95th MESA percentile (OR 4.0; 95% CI 2.2-7.4). In the preeclampsia group, 47% presented coronary atherosclerotic plaques on CCTA and 4.3% showed significant stenosis. Subclinical CAD was present in 8.7% of the participants.

Conclusions: Women with a history of preeclampsia have an increased prevalence of coronary artery atherosclerosis at age 45–55 years. These findings suggest accelerated and more severe development of subclinical CAD following preeclampsia.

Introduction

Preeclampsia affects 1–5% of all pregnancies and is characterized by de novo hypertension and proteinuria, maternal organ dysfunction or uteroplacental dysfunction resulting in fetal growth restriction.[1–3] Preeclampsia is a major cause of severe maternal and fetal morbidity and mortality.[4,5] Women with a history of preeclampsia are at increased risk of developing premature cardiovascular disease (CVD) including coronary artery disease (CAD).[6–9] CAD is commonly recognized as a major health problem in women and the association between preeclampsia and clinically manifest CAD endpoints is well established.[10–13] Timely identification of women at increased risk for CAD is important, as this might offer an opportunity for prevention, including lifestyle or pharmacological intervention.[14,15] However, it remains difficult to identify which women are truly at highest risk of developing CAD, as development of CAD over time varies among individuals and is currently unknown. Furthermore, there is yet insufficient data to substantiate optimal timing and strategy for preventive interventions in these women. Nevertheless, most recent clinical guidelines recommend risk assessment and preventive interventions in women with preeclampsia.[16–19]

Pathophysiological mechanisms leading to CAD in women with preeclampsia are mediated by traditional CVD risk factors such as hypertension, family history, smoking status and obesity, and other female-specific risk factors, such as angiogenic factors inflammation, leading to prolonged endothelial dysfunction.[20–23] A large cross-sectional study from our group among 491 postmenopausal women indicated increased prevalence of plaque in women with a history of high blood pressure during pregnancy as well.[24] Nevertheless, it has not been investigated to what extent CAC or non-calcified plaque formation as a precursor of calcified plaque, play a role in CAD development at a younger age, and if so, by which time line. It is also unclear if CAD in these relatively young women relates to traditional CVD factors.

The aim of the present study was to assess coronary artery atherosclerotic lesions, both CAC and coronary plaques, prospectively in asymptomatic women with a history of preeclampsia and women from a reference group by coronary computed tomography (CCT) in addition to routine cardiovascular risk assessment.

Methods

Design

The rationale and design of the Cardiovascular RiskprofilE: IMaging And Gender-specific disOrders (CREW-IMAGO) have been published previously.[25] In brief, CREW-IMAGO is a prospective, multicenter, cross-sectional cohort study in women asymptomatic of CVD with a history of reproductive disorders. Here, we report on women with a history of preeclampsia included in different ongoing cohorts in the Netherlands: the Utrecht Cohort, the Rotterdam Follow-up Preeclampsia (FUPEC) cohort, the Preeclampsia Risk Evaluation in FEMales (PREVFEM) cohort and

the Hypitat Risk Assessment Study (HyRAS) cohort.[22,26,27] Participants underwent CCT following their cardiovascular screening in two study centers in the Netherlands: the University Medical Center Utrecht and the Erasmus Medical Center Rotterdam. The study has been approved by the medical ethics committee of the University Medical Center Utrecht. All participants gave written informed consent upon inclusion in the study.

Participants

Asymptomatic women, aged 45 to 55 years, with a history of preeclampsia were included in this study between February 2016 and February 2017. Medical records, with information regarding pregnancy characteristics and hospital admission, were available for all participants. Preeclampsia was defined according to international criteria as hypertension developing after 20 weeks of gestation in combination with proteinuria, maternal organ dysfunction or uteroplacental dysfunction.[3,28] Women with any serious illness that can compromise study participation were excluded from the study, as well as patients with high risk for contrast nephropathy (renal dysfunction with an estimated glomerular filtration rate <60 ml/min/1.73 m2) or patients with a history of myocardial infarction. We compared our participants with an age-equivalent sample of female participants of Caucasian descent from the Multi-Ethnic Study of Atherosclerosis (MESA).[29] MESA is a prospective cohort study in which CAC and traditional cardiovascular risk factors were measured in 6110 multiethnic participants without a history of physician-diagnosed heart attack, angina, stroke, transient ischemic attack, or heart failure; current atrial fibrillation; taking nitroglycerin; or having undergone angioplasty, coronary artery bypass graft, valve replacement, pacemaker or defibrillator implantation, or any surgery on heart or arteries. MESA data used for comparison were restricted to women from Caucasian descent aged 45 to 55 years, resulting in 387 MESA participants included in our analyses.

Measurement CAC and plaque

CCT is performed using a multislice CT scanner with prospective ECG-triggering. An experienced cardiovascular radiologist (BKV and RPJB) in both centers assessed all cardiac CT scans in a standardized way. Heart rate was controlled <65/min by administration of oral and/or intravenous beta-blocker (metoprolol) before the scan. First, a non-contrast CCT was performed to calculate the coronary artery calcium score (CACS) with the Agatston scoring method.[30] The total Agatston score was categorized as no calcification (CACS=0), minimal calcifications (CACS >0 and <10); mild calcifications (CACS ≥10 and <100); moderate calcifications (CACS ≥100 and <400); and severe calcifications (CACS ≥400). In addition, absolute CACS were converted to percentiles adjusted for age, gender and race, based on MESA.[29] Radiographic settings were adapted from the MESA protocol.

Second, coronary CT angiography (CCTA) was performed after sublingual nitroglycerine and injection of non-ionic contrast (Iopromide, Ultravist, Bayer Healthcare, Berlin, Germany), and

reconstructed with an iterative reconstruction algorithm**.** Semi-automated vessel analysis was used to create multiple curved multiplanar reconstructions (MPR) of all coronary arteries. Plaque burden and luminal stenosis were assessed in all 17 coronary segments, according to the modified American Heart Association classification, and a segmental involvement score (SIS) was computed.[31–33] SIS ≥5 indicates increased cardiovascular event risk.[33] Luminal stenosis was categorized as absent, minimal (1–24 %), mild (25–49 %), moderate (50–69 %) or severe (≥70 %) narrowing, based on diameter measurements comparing diameters of the maximal stenosis to a reference diameter proximal and distal to the stenotic area.[34] Luminal stenosis ≥50% was considered a significant stenosis. CAD was defined as CACS ≥100 AU on non-contrast CCT and/or ≥50% luminal stenosis on CCTA.

Primary outcome was defined as CACS >0 AU and/or presence of any coronary atherosclerotic plaques measured by CCTA. Secondary outcomes included CACS ≥100 AU, CACS ≥95th MESA percentile, CACS ≥300 AU or ≥75th MESA percentile, and presence of a significant stenosis on CCTA. According to 2016 European guidelines on cardiovascular disease prevention a CACS ≥300 AU or ≥75th percentile corrected for age, sex and ethnicity is considered to indicate increased cardiovascular risk.[19]

Cardiovascular risk assessment

Traditional cardiovascular risk factors were assessed. Measurements included age, body-mass index (BMI), waist circumference, and blood pressure. In addition, medical history (cardiovascular history, smoking status, medication use, family history) was obtained and a venous blood sample was drawn to assess lipid profile, fasting plasma glucose, glycated hemoglobin and high-sensitivity C-reactive protein. CVD risk factors were defined as follows: hypertension (systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or current use of antihypertensive treatment), obesity (BMI ≥30 kg/m^2), and metabolic syndrome (MetS; defined according to ATP III criteria by at least three of the following criteria: waist circumference >88cm, triglycerides ≥1.7 mmol/l, blood pressure ≥130/85 mmHg, HDL cholesterol <1.29 mmol/L and fasting glucose ≥5.7mmol/l). According to the NCEP-ATP III guideline, participants were categorized as low-risk (0-1 risk factor), intermediate risk (≥2 risk factors and 10-year CVD risk ≤20%), or high risk (10-year CVD risk >20%).[35] In addition, the 10-years CVD risk was estimated according to the Framingham Risk Score (FRS) and the frequency of an intermediate or high (≥10%) CVD risk score was calculated.[36]

Statistical analysis

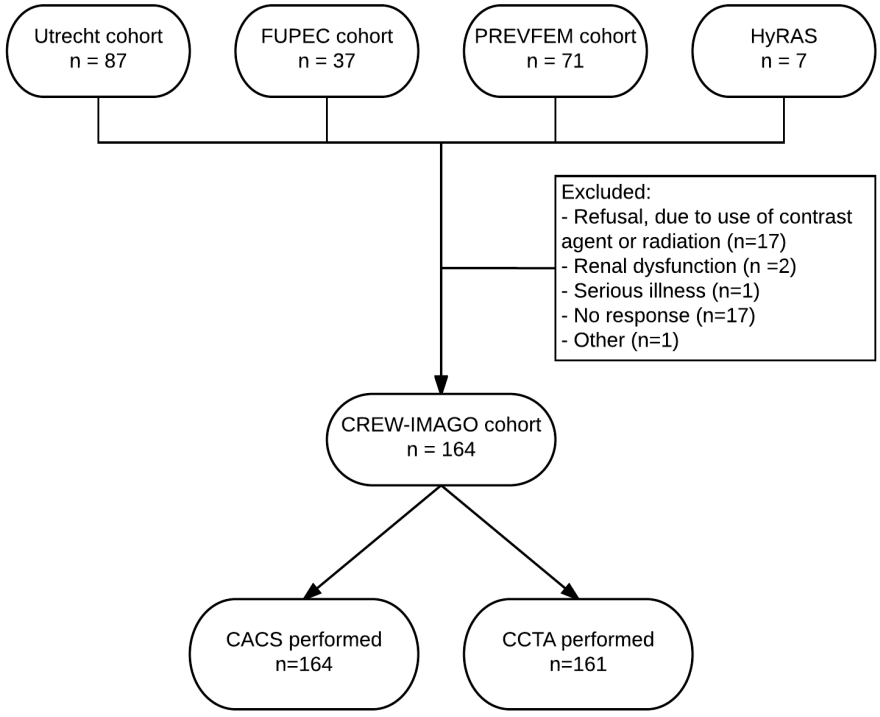
Patient characteristics, clinical measurements and CVD risk factors were described using means and standard deviations (SDs) or medians and interquartile range for continuous variables and frequencies and proportions for categorical variables. Comparisons were made between CREW-IMAGO and MESA participants and within CREW-IMAGO between participants at different gestational age at delivery by Student t-tests, ANOVA or Mann-Whitney-U tests (continuous data),

and chi-square or Fisher's exact tests (categorical data). In order to estimate the association of gestational age at onset of preeclampsia with coronary atherosclerotic lesions participants were categorized in equal tertiles based according to gestational age (GA) at delivery: GA delivery <208 days, GA delivery 208–234 days, GA delivery 234 days. All p-values were two-sided with significance level set at p <0.05. All analyses were conducted using Statistical Package for Social Sciences (SPSS) version 22.0 (SPSS Inc. Chicago, IL, USA).

Results

Out of 202 women with a history of preeclampsia who underwent regular cardiovascular screening, a total of 164 women (81.2%) consented to participate in the current study (Flow-chart). In total, 28 women were either unwilling to participate after counseling due to the use of contrast agent or radiation, or did not respond to our invitation. Except for a higher BMI in the participants compared to non-participants (28.1 kg/m^2 vs 24.7 kg/m^2, p=0.015), there were no significant differences between women who participated and those who did not. Compared

Figure 1. Flowchart



FUPEC, Follow-up Preeclampsia; PREVFEM, Preeclampsia Risk Evaluation in FEMales; HyRAS, Hypitat Risk Assessment Study; CREW-IMAGO, Cardiovascular RiskprofilE: IMaging And Gender-specific disOrders; CACS, coronary artery calcium score; CCTA, coronary computed tomography angiography.

to women aged 45-55 years participating in MESA, women with a history of preeclampsia were younger, more often had hypertension and MetS, and had significantly higher levels of total cholesterol, LDL-cholesterol and glucose (Table 1). In contrast, current smoking and a family history of premature CVD were higher in the MESA group. Obesity and diabetes prevalence were not different between groups. An intermediate or high CVD risk, defined as FRS ≥10%, was equally present in both groups.

Table 1. Baseline characteristics

	Preeclampsia (N = 164)		MESA (N = 387)	
Patient characteristics				
Age (y)	48.4	2.9	50.0	3.0
Caucasian ethnicity (no, %)	162	98.8%	187	100%
History of pregnancy	164	100%	288	74.6%
Clinical measurements				
Systolic blood pressure (mmHg)	130	15	110	16
Diastolic blood pressure (mmHg)	81	10	66	9
BMI (kg/m^2)	28.1	6.2	27.6	6.7
Waist circumference (cm)	89.4	13.3	92.8	18.1
Total cholesterol (mmol/L)	5.4	1.0	5.1	1.0
Triglycerides (mmol/L)	1.2	0.5	1.4	1.1
HDL-cholesterol (mmol/L)	1.52	0.34	1.49	0.41
LDL-cholesterol (mmol/L)	3.4	0.9	3.0	0.8
Glucose (mmol/L)	5.5	1.1	4.7	1.0
CVD risk factors				
Family history of premature CVD (no, %)	31	18.9%	114	31.8%
Hypertension [a] (no, %)	89	54.3%	88	22.8%
Diabetes (no, %)	5	3.1%	10	2.6%
Current smoking (no, %)	12	7.5%	69	17.7%
Obesity (no, %)	49	29.9%	114	29.3%
Metabolic syndrome [b] (no, %)	54	32.9%	75	20.3%
CVD risk scores				
≥2 risk factors [b] (no, %)	29	18.1%	68	19.1%
Intermediate–high risk, FRS ≥10% (no, %)	15	9.4%	20	5.2%

Data are presented as mean ± standard deviation, unless otherwise stated.
Legend: MESA, multi-ethnic study of atherosclerosis; GA, gestational age; BMI, body mass index; ABI, ankle-brachial index; IMT, intima-media thickness; HbA1c, hemoglobin A1c; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; CVD, cardiovascular disease; NCEP, national cholesterol education program; FRS, Framingham Risk Score.
[a] Blood pressure ≥140/90 mmHg or current use of antihypertensive treatment
[b] According to NCEP ATP-III criteria

CACS was available for all participants. Compared to MESA-participants, women in the pre-eclampsia group had an increased risk for CACS >0 AU (OR 2.0, 95% CI 1.3-3.0), and CACS >100 AU (OR 4.0, 95% CI (1.5-10.7) (Table 2). In the preeclampsia group 17% had a CACS ≥95th MESA percentile compared to 5% in the MESA-group (OR 4.0, 95% CI 2.2-7.4). The risk of CACS ≥300

AU or CACS >75th MESA percentile was higher for women with a history of preeclampsia (OR 2.0, 95% CI 1.3-3.0). Comparing the women in the preeclampsia group only to MESA-participants who had one or more pregnancies did not influence these results (data not shown).

CCTA was performed in 161 of the 164 participants in the preeclampsia group (98.2%). CCTA was omitted in three women because of a possible allergy for the contrast agent (one woman) and BMI >45 kg/m^2 (two women) that precluded a low-dose scan. Seventy-six of the 161 women showed plaques in their coronary arteries (47.2%), of which 7 (4.3%) had a significant stenosis (Table 3). SIS ≥5 was present in 28 women (17.4%). All women with significant stenosis and/or

Table 2. Radiological characteristics – CACS

	Preeclampsia (N = 164)		MESA (N = 387)		OR (95% CI)
CACS >0 AU	50	30.5%	70	18.1%	1.99 (1.30-3.03)
CACS 0.1 – 9 AU	*14*	*8.5%*	*33*	*8.5%*	*1.18 (0.61-2.28)*
CACS 10 – 99 AU	*26*	*15.9%*	*30*	*7.8%*	*2.41 (1.37-4.25)*
CACS ≥100 AU	*10*	*6.1%*	*7*	*1.8%*	*3.97 (1.48-10.68)*
MESA ≥95th percentile	28	17.1%	19	4.9%	3.99 (2.16-7.38)
CACS ≥300 AU and/or MESA ≥75th percentile	50	30.5%	70	18.1%	1.99 (1.30-3.03)

Data are presented as frequencies and percentages.
CACS, coronary artery calcium score; MESA, multi-ethnic study of atherosclerosis; OR, Odds ratio, CI; confidence interval.

Table 3. Radiological characteristics – CCTA

	Preeclampsia (N = 161)	
CCTA		
Any plaque	76	47.2%
Significant stenosis[a]	7	4.3%
SIS (median, quartiles)	0	(0-3)
SIS ≥5	28	17.4%
CAD[b]	14	8.7%
Maximum stenosis of any plaque		
No stenosis	89	55.3%
Minimal, <25%	40	24.8%
Mild, 25 – 49%	25	15.5%
Moderate, 50 – 69%	4	2.5%
Severe, 70 – 99%	3	1.9%

Data are presented as mean ± standard deviation, unless otherwise stated.
CCTA, coronary computed tomography angiography SIS, segmental involvement score; CAD, coronary artery disease
[a] Luminal stenosis ≥50%
[b] CACS >100 AU and/or luminal stenosis ≥50%

CACS >100 AU were referred to a cardiologist. Two of the 7 women with a significant stenosis underwent additional invasive coronary angiography. Only one woman required coronary angioplasty while the other woman was treated with statins. The five other women with a significant stenosis (>50%) and 7 other women with CACS ≥100 AU received lifestyle recommendations and were prescribed statins.

Trend-analyses of the three gestational age groups with regard to CACS and CCTA results showed a significant inverse relation between gestational age at delivery and the prevalence of positive CACS and the prevalence of CACS ≥300 AU or CACS >75th MESA percentile. There was no correlation between gestational age at delivery and the other radiologic characteristics.

Discussion

In this multicenter prospective cohort study, we found coronary atherosclerotic lesions in a substantial proportion of asymptomatic women with a history of preeclampsia at age 45–55 years. Compared to the MESA population as a reference, women with a history of preeclampsia have double the risk of positive CACS and CACS ≥300 AU or CACS ≥75th MESA percentile; which is considered to indicate increased cardiovascular risk according to the European guidelines on cardiovascular disease prevention. Furthermore, there was a four times elevated risk of moderate–severe CACS (≥100 AU) and CACS ≥95th MESA percentile. Almost half of the participants showed coronary plaques on CCTA. These data strongly suggest that preeclampsia is associated with accelerated atherosclerosis, leading to detectable coronary artery calcifications and plaques at an early age.

This is confirmed when comparing our findings with general population cohorts. Positive CACS was rarely seen in women aged <55 years of a healthy reference sample based on 3238 participants free from clinical CVD in the Framingham Heart Study.[37] Moderate-severe CACS (>100 AU) was found in only 0.9% of the participants, compared to 6.5% in our study. In 3043 participants from the population-based CARDIA study, positive CAC was found in 13.3% of those 40-45 years old and less common in women than in men.[38] A cross-sectional study from our group showed 57% increased risk of CAC in 491 postmenopausal women aged 66.8 ± 5.4 years with self-reported high blood pressure during pregnancy.[24] A recent small case-control study compared CACS between 40 women with a history of preeclampsia and 40 women without such history showed a comparable difference in occurrence of coronary calcifications as in our study.[39] In both studies however, women were 10–20 years older than women in the current study. Since age is an important determinant in development of coronary artery calcifications, one would expect that positive CACS was less common in our study sample because of this 10-year age difference.[40] In addition, further stratification in age categories did not reveal any differences with regard to CACS and CCTA measures (data not shown).

In 6772 MESA-participants free of clinical CVD with a median follow-up of 3.9 years, CAC 1–100 AU was associated with a 4 times higher risk and CAC >100 AU with a 7–10 times increased risk of coronary events.[41] Comparable associations between positive CACS and higher risks of coronary heart disease were found in the population-based Dallas Heart Study among 2084 participants aged 44.4 ± 9.0 years with a median follow-up of 9 years.[42] In our study, women with a history of preeclampsia had double the risk of CACS ≥300 AU or CACS ≥75th MESA percentile, which is considered to indicate increased cardiovascular risk according to international guidelines.[19] CACS is not only an important independent predictor of future cardiovascular events, but also improves risk classification of asymptomatic individuals when added to traditional CVD risk models.[41,43] This is of great interest for participants in our study, since traditional risk models classify most of these women as low risk due to their relatively young age.[22] Adding CACS to specific patient groups with elevated lifetime risk of CVD, such as women with a history of preeclampsia, might improve risk classification and timely identification of high-risk women.[44]

The role of plaque detection by CCTA in assessment of cardiovascular risk, has not been extensively investigated in asymptomatic populations.[45] This may be due to increased radiation exposure compared to CACS; necessity of a contrast agent and higher costs compared with CACS, whereas the additional value compared with CACS might be insignificant. [45,46] Although a population based reference cohort regarding CCTA results is lacking, we exploratory assessed coronary plaques by CCTA in this study. A large, retrospective cohort study from South Korea including 1282 self-referred, low-risk, asymptomatic women aged 50.0 ± 8.4 years demonstrated a lower prevalence of any plaque and significant stenosis: 6.7% and 0.5% respectively.[47] Another South-Korean cohort study including 374 self-referred asymptomatic women (79% low-risk) aged 51.0 ± 9.3 years showed plaques in 38 women (10.2%) of which only 4 women (1.1%) had significant stenosis.[46] Despite comparable risk profiles, both plaque occurrence and significant stenosis were far more common in our study (47.2% and 4.3% respectively). Although there are clear ethnic and probably also lifestyle differences between the South Korean and our groups, these findings suggest that risk profiles based on traditional risk factors underestimate cardiovascular risk in women with a history of preeclampsia.

CCTA can, like CACS, improve risk classification of asymptomatic individuals when added to traditional CVD risk models.[48] SIS is a measure for extent of coronary plaque burden on CCTA. SIS ≥5 was associated with an increased risk of major adverse cardiac events in a prospective cohort study among 711 asymptomatic patients with a high 'a priori' risk of CAD.[49] In this study, 13.9% of these high-risk participants had SIS ≥5, comparable to the 17.4% in the present study of women with a history of preeclampsia.

In women affected by preeclampsia requiring delivery at an early gestational age (<37 weeks) the risk of ischemic heart disease was higher (HR 4.5, 2.7-7.4) compared with those women with a delivery beyond 37 weeks (HR 2.0, 1.5-2.5).[10] In our study, we found a similar inverse relationship between gestational age at delivery and CACS and CCTA measures. Positive CACS and CACS

≥300 AU or CACS >75th MESA percentile were associated with an earlier age at delivery, indicating more extended vascular impairment and consequently higher CVD risk after earlier onset of preeclampsia. However, atherosclerotic lesions are not confined to a subgroup of women with early onset preeclampsia, as positive CACS and coronary plaques are commonly seen in women with late-onset preeclampsia as well.

Our study is the largest prospective published cohort thus far assessing risk factors and signs of subclinical atherosclerosis assessed by CCTA in well phenotyped women with a history of preeclampsia. The study population consisted of relatively young women between 45 and 55 years of age, providing a unique opportunity to identify high-risk patients at an early age to enable timely cardiovascular prevention. Based on CCTA, risk stratification could be performed to identify high-risk women, e.g. those with CACS ≥300AU or CACS ≥75th MESA percentile, those with significant stenosis or those with SIS ≥5, since these are associated with an increased cardiovascular risk. High-risk women might profit from cardiovascular prevention, including lifestyle recommendations, blood pressure control, treatment of dyslipidemia with statins, or – as one of our patients showed – even angioplasty.[49]

Several potential limitations of our study need to be addressed. For the CCTA results we had no reference data from MESA, as CCTA was not performed in MESA. The MESA cohort consists of participants free from clinical CVD, which could have led to an overestimation of our results. At present, no reliable population-based data for asymptomatic individuals are available, as exposing healthy individuals to radiation burden required for CCTA (approximately 3.0-3.5 mSv) for research purposes is not permitted due to regulatory and ethical restrictions. Women with any serious illness were excluded from study participation. As only two women were excluded for this reason, we assume that this has not led to serious selection bias. Lastly, due to the cross-sectional design of the study, we could not study 'hard' endpoints such as cardiovascular disease events. Such data are however crucial to appreciate the significance of CACS, and possibly CCTA, in women with a history of preeclampsia. Future research should also focus on assessment of women younger than 45 years to further unravel the time course of the development of coronary atherosclerosis and, consequently, the optimal time for screening.

Conclusion

In conclusion, a higher proportion (30 tot 50%) of women with a history of preeclampsia show substantial subclinical coronary atherosclerosis on vascular imaging between 45 and 55 years of age compared to women from a reference group. Subclinical coronary atherosclerosis is usually not detected by using traditional CVD risk assessment. In the future, CCT may play a role in the identification of formerly preeclamptic women who are at high risk for developing CVD. Earlier identification may provide opportunities for more timely preventive measures to lower CVD risk.

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Supplemental table. Comparison CACS and CCTA between preeclampsia subgroups based on gestational age at delivery

CACS	**Tertile 1, GA <208 days (n = 54)**		**Tertile 2, GA 208-234 days (n = 54)**		**Tertile 3, GA >234 days (n = 56)**		**P for trend**
Gestational age at delivery in days (median, quartiles)	196	(185–201)	220	(214–225)	260	(246–276)	<0.001
CACS >0 AU	20	37.0%	19	35.2%	11	19.6%	0.047*
CACS 0.1 – 9 AU	5	9.3%	6	11.1%	3	5.4%	0.166
CACS 10 – 99 AU	10	18.5%	9	16.7%	7	12.5%	0.225
CACS ≥100 AU	5	9.3%	4	7.4%	1	1.8%	0.070
CCTA	**Tertile 1, GA <208 days (n = 53)**		**Tertile 2, GA 208-234 days (n = 54)**		**Tertile 3, GA >234 days (n = 54)**		**P for trend**
Gestational age at delivery in days (median, quartiles)	195	(185–201)	220	(214–225)	260	(247–277)	<0.001
Any plaque	26	49.1%	31	57.4%	19	35.2%	0.147
Stenosis	25	46.3%	29	53.7%	18	32.1%	0.057
Minimal, <25%	10	18.9%	19	35.2%	11	20.4%	0.672
Mild, 25 – 49%	12	22.6%	7	13.0%	6	11.1%	0.089
Moderate-severe, 50 –99%	3	5.7%	3	5.6%	1	1.9%	0.260
SIS ≥5	11	20.8%	12	22.2%	5	9.3%	0.116
CAD [a]	20	37.0%	19	35.2%	11	19.6%	0.047*

Data are presented as Odds ratios and 95% Confidence Intervals, unless otherwise stated.
CCACS, coronary artery calcium score; MESA, multi-ethnic study of atherosclerosis; CCTA, coronary computed tomography angiography; SIS, segmental involvement score; CAD, coronary artery disease; GA, gestational age.
[a] CACS >100 AU and/or luminal stenosis ≥50%
* Significant at p <0.05


GUSTAV
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Chapter 9

Distribution of arterial calcifications in women after preeclampsia

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In preparation

Abstract

Background: Preeclampsia is associated with long-term hypertension and accelerated development of atherosclerosis, which may lead to premature arterial calcifications and arterial stiffness. We evaluated the distribution of arterial calcifications by computed tomography (CT) and assessed the association between arterial calcifications and cardiovascular risk factors including arterial stiffness after preeclampsia.

Methods: Subclinical arterial disease was assessed in 143 asymptomatic women after preeclampsia (mean age 48.5 ±2.9 years, range 45-55) in a prospective multicenter evaluation of cardiovascular health. Of these women, 53.8% had hypertension and 34.3% fulfilled criteria for metabolic syndrome. Calcium scores of coronary arteries (CACS), thoracic aorta (TACS), heart valves (VCS) and carotid siphon (CSCS) were assessed by non-contrast CT. Arterial stiffness was measured by pulse wave velocity using a TensioMed Arteriograph. Correlations among vascular calcifications and the relationship with cardiovascular risk factors were assessed.

Results: Arterial calcifications were seen in 102 participants (71.3%): 46 (32.2%) had CACS >0; 18 (12.6%) had TACS >0; 4 (2.8%) had VCS >0 and 87 (68.5%) had CSCS >0. TACS correlated strongly with CACS (R=0.91, p<0.001) and moderately with CSCS (R=0.45, p<0.001). No other correlations were observed among calcification scores. A positive association was only found between CACS >0 and hypertension (OR 2.3, 95%CI 1.1-4.8). Calcifications were not related to other cardiovascular risk factors including arterial stiffness.

Conclusion: Arterial calcifications are common in women with a history of preeclampsia aged 45-55 years. Only CACS >0 appears to be associated with chronic hypertension, but not with increased arterial stiffness or other cardiovascular risk factors.

Introduction

Cardiovascular disease (CVD), including coronary heart disease (CHD) and ischemic stroke, is mainly caused by the development of calcified atherosclerosis in arterial vessels with age. Measurement of arterial calcifications in different locations by radiographic imaging has increasingly gained interest, since these calcifications might be helpful in early detection of atherosclerosis and potentially aid CVD risk assessment. Best example of this is the coronary artery calcium score (CACS), which has been shown to be a strong and independent predictor for future CHD and CVD.[1,2] In addition, CACS improves CVD risk classification when added to common used CVD risk models in asymptomatic subjects.[3,4] Apart from CACS, the presence and predictive value of arterial calcifications at other arterial sites have been assessed in the general population.[2,5–8] Calcifications at other (non-coronary) arterial sites, including the thoracic aorta, abdominal aorta, carotid artery and carotid siphon, appear to be frequently inter-related and may also be predictive of CVD events.

Previous studies showed a positieve relation between arterial stiffness and the presence and extent of arterial calcifications, also after adjustment for common CVD risk facors, although the strength of the correlation differed among the studies.[9–13] Arterial stiffness has not only been associated with atherosclerotic burden, but also with cardiovascular events.[14] Nevertheless, some studies showed conflicting results regarding the correlation between arterial stiffness and arterial calcifications or could not relate arterial stiffness to incident CHD.[15,16]

Women with a history of preeclampsia are at increased risk of long-term chronic hypertension and accelerated development of atherosclerosis, which may lead to arterial stiffness and premature arterial calcifications.[17–20] Clinical data from our own group and from others also showed that women who had preeclampsia are at increased risk for coronary calcifications.[21,22] However, the distribution of arterial calcifications in these women is unknown and the relation between traditional CVD risk factors, arterial stiffness and arterial calcifications has not been investigated before.

The aim of this study was to assess the distribution of arterial calcifications and to determine the relation among arterial calcifications, arterial stiffness and traditional CVD risk factors in women with a history of preeclampsia in the fifth and sixth decade of life.

Methods

Participants

This monocenter, cross-sectional study is part of the multicenter prospective Cardiovascular RiskprofilE: IMaging And Gender-specific disOrders (CREw-IMAGO) cohort study. Details regarding the rationale and design of this ongoing study have been published elsewhere.[23] Women with a history of preeclampsia aged 45–55 years old (n=143) were recruited after regular cardiovascular screening to participate in the CREw-IMAGO study. Preeclampsia was defined according to international guidelines as hypertension during pregnancy developing after 20 weeks of

gestation in combination with proteinuria, maternal organ dysfunction or uteroplacental dysfunction (ISSHP 2014). Women were excluded from participation in the study in case of serious illness that can compromise study participation, in case of high risk for contrast nephropathy (renal dysfunction with an estimated glomerular filtration rate <60 ml/min/1.73 m^2) or in case of previous myocardial infarction. The study has been conducted according to the principles of the declaration of Helsinki and the medical ethics committee of the University Medical Center Utrecht approved this study. All participants gave written informed consent upon inclusion in the study.

CT Imaging

Participants were screened for subclinical arterial disease using low-dose CT and pulse wave velocity (PWV) analysis. The coronary CT (CCT) was performed using a 256-slice CT scanner (Philips Healthcare, Best, The Netherlands) with prospective ECG-triggering. An experienced cardiovascular radiologist assessed all cardiac CT scans. Heart rate was controlled <65/min by administration of oral and/or intravenous beta-blocker (metoprolol) before the scan. A cardiac non-contrast CCT (scan parameters 120 kV, 50 mAs, 3 mm reconstructed slice thickness,)was performed to calculate the coronary artery calcium score (CACS), thoracic aortic calcium score (TACS) excluding the aortic arch, and valvular calcium score (VCS). Carotid siphon calcium score (CSCS) was assessed on a separate non-contrast carotid siphon scan (scan parameters 20 mm coverage, 120 kV, 100 mAs, 1 mm reconstructed slice thickness). CACS, TACS, CVS and CSCS were measured with the Agatston scoring method on a workstation (IntelliSpace Portal, Philips Healthcare, The Netherlands).[24] For CACS, the total Agatston score was categorized as no calcification (CACS = 0), minimal calcifications (CACS >0 and <10); mild calcifications (CACS ≥10 and <100); moderate calcifications (CACS ≥100 and <400); and severe calcifications (CACS ≥400). In addition, absolute CACS were converted to percentiles with adjustments for age, gender and race, based on the MESA.[25] All other calcification measures were used as continuous variables.

CVD risk factor assessment

The standardized cardiovascular screening in our center included a questionnaire regarding medical history, physical examination, laboratory measurements and assessment of arterial stiffness. The questionnaire contained items assessing cardiovascular history, smoking status, medication use, reproductive characteristics and family history. Physical examination included measurement of body-mass index (BMI), waist circumference and blood pressure. Laboratory measures comprised lipid profile, fasting plasma glucose, glycated hemoglobin and high-sensitivity C-reactive protein. Pulse wave velocity (PWV) was assessed as a measure of arterial stiffness by the Arteriogaph system (Tensiomed, Budapest, Hungary). The Arteriograph is an operator-independent device using oscillometric pressure curves to determine the PWV.[26,27] PWV was measured twice in each patient in supine position using a blood pressure cuff on the left arm after several minutes of rest. Two measurements were performed and the mean PWV of these two measurements was used for further analysis.

Table 1. Baseline table

	All participants (N = 143)	
Patient characteristics		
Age (y)	48.5	2.9
Premenopausal (no, %)	79	60.8%
Clinical measurements		
Systolic blood pressure (mmHg)	130	15
Diastolic blood pressure (mmHg)	80	10
BMI (kg/m^2)	28.3	6.3
Waist circumference (cm)	89.8	13.5
ABI	1.19	0.08
IMT (mm)	0.63	0.09
PWV (m/s)	9.0	1.7
Glucose (mmol/l)	5.5	1.1
Total cholesterol (mmol/l)	5.4	1.0
Triglycerides (mmol/l)	1.2	0.5
HDL-cholesterol (mmol/l)	1.50	0.32
LDL-cholesterol (mmol/l)	3.4	0.8
CVD risk factors		
Family history of premature CVD (no, %)	31	21.7%
Hypertension[a] (no, %)	77	53.8%
Diabetes (no, %)	5	3.5%
Current smoking (no, %)	11	7.8%
Obesity (no, %)	44	30.8%
Metabolic syndrome[b] (no, %)	49	34.3%
PWV		
PWV (m/s)	9.0	1.7
PWV >8.3 m/s (no, %)	78	58.7%
PWV >7.5 m/s (no, %)	102	78.5%

Data are presented as mean ± standard deviation, unless otherwise stated. Abbreviations: GA, gestational age; BMI, body mass index; ABI, ankle-brachial index; IMT, intima-media thickness; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; CVD, cardiovascular disease; NCEP, national cholesterol education program; PWV, pulse wave velocity.
[a] Blood pressure >140/90 mmHg or current use of antihypertensive treatment.
[b] According to ATP-III criteria

Statistical analysis

Descriptive data of patient characteristics, CT results and CVD risk factors are presented as means and standard deviations or medians and interquartile range for continuous variables as appropriate. Categorical variables are presented as frequencies and proportions.

Possible association of arterial calcifications at different arterial sites with each other was assessed by Pearson's correlation coefficient. Presence of arterial calcifications was compared with the presence of CVD risk factors including arterial stiffness by using the Chi-square test. CVD risk factors were defined as follows: hypertension (systolic blood pressure >140 mmHg, diastolic

Table 2. Radiological characteristics

	All participants (n = 143)	
Coronary calcifications		
CACS > 0 AU	46	32.2%
CACS 0.1 – 10 AU	*13*	*9.1%*
CACS 10 – 100 AU	*25*	*17.5%*
CACS >100 AU	*8*	*5.6%*
CACS (median, quartiles)	0.0	0.0–5.5
Thoracic aortic calcifications		
Aortic diameter in cm (mean, SD)	29.9	3.6
TACS > 0 AU	18	12.6%
TACS (median, quartiles)	0.0	0.0–0.0
Valvular calcifications		
VCS > 0 AU	4	2.8%
VCS (median, quartiles)	0.0	0.0–0.0
	All participants (n = 128)	
Carotid siphon calcifications		
CSCS > 0 AU	87	68.5%
CSCS (median, quartiles)	0.1	0.0–1.7

Data are presented as frequency and percentage, unless otherwise stated.
Legend: CACS, coronary artery calcium score; TACS, thoracic aortic calcium score; VC, valvular calcium score CSC, carotid siphon calcium score.

Table 3. Correlations of calcifications at different arterial sites

	CACS		TACS		CSCS		VCS	
	R-value	**p-value**	**R-value**	**p-value**	**R-value**	**p-value**	**R-value**	**p-value**
CACS	-	-	**0.907**	**<0.001**	0.068	0.513	-0.001	0.992
TACS	**0.907**	**<0.001**	-	-	**0.446**	**<0.001**	-0.015	0.856
CSCS	0.068	0.513	**0.446**	**<0.001**	-	-	-0.045	0.662
VCS	-0.001	0.992	-0.015	0.856	-0.045	0.662	-	-

Abbreviations: CACS, coronary artery calcium score; TACS, thoracic aortic calcium score; CSCS, carotid siphon calcium score; VCS, valvular calcium score.

blood pressure >90 mmHg, or current use of antihypertensive treatment), obesity (BMI >30 kg/m^2), metabolic syndrome (Three or more of the following criteria, according to ATP II criteria: waist circumference >88cm, triglycerides ≥1.7mmol/l, RR ≥130/85, HDL cholesterol <1.29 mmol/L and fasting glucose ≥5.7mmol/l). Two cut-off values for the PWV measurement have been used; the normal value of 8.3 m/s (established by carotid-femoral PWV measurements) and the normal value of 7.5 m/s, which corrects for the 0.8 m/s lower measurements as conducted by the Arteriograph system.[28] Associations between PWV measurements as continuous variable

Table 4. Presence of vascular calcifications and relationship to CVD risk factors

	CAC presence (N = 143)		TAC presence (N = 143)		VC presence (N = 143)		CSC presence (N = 128)	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Hypertension	**2.29**	**1.10-4.77**	1.87	0.66-5.31	2.64	0.27-25.96	1.22	0.58-2.59
Obesity	1.52	0.72-3.20	2.02	0.74-5.56	2.31	0.32-16.95	0.79	0.35-1.78
Metabolic syndrome	1.37	0.66-2.84	0.96	0.34-2.75	0.63	0.06-6.24	1.04	0.47-2.31
Smoking	2.91	0.84-10.10	0.68	0.08-5.68	-[a]	-[a]	1.28	0.32-5.11
Diabetes	1.42	0.23-8.83	1.71	0.18-16.18	-[a]	-[a]	1.39	0.14-13.82
Family history of premature CVD	1.45	0.63-3.31	1.39	0.45-4.26	-[a]	-[a]	1.80	0.66-4.89
Increased PWV >8.3m/s	1.37	0.65-2.87	1.13	0.41-3.13	-[a]	-[a]	1.46	0.66-3.23
Increased PWV >7.5m/s	2.73	0.96-7.78	1.98	0.42-9.31	0.54	0.05-6.18	1.43	0.54-3.78

CAC, coronary artery calcifications; TAC, thoracic aortic calcifications; CSC, carotid siphon calcifications; VC, valvular calcifications; CVD, cardiovascular disease; PWV, pulse wave velocity; OR, odds ratio; 95% CI, 95% confidence interval.

[a] Odds ratio could not be computed due to low frequency of VC

with various CVD risk factor variables and different arterial calcification scores were assessed by Pearson's correlation coefficient. A p-value < 0.05 was considered statistically significant. All analyses were performed using Statistical Package for Social Sciences (SPSS) version 22.0.

Results

In total, 143 women with a history of preeclampsia were included in the current study with a mean age of 48.5 years (standard deviation (SD) 2.9 years), as shown in table 1. CAC, TAC and VC have been assessed in all participants, whereas CSC could be assessed in 128 participants (89.5%). PWV was available for 134 participants (93.7%). CVD risk factors were common of which hypertension (53.8%), MetS (34.3%) and obesity (30.8%) were most prevalent. Mean PWV was 9.0 m/s (SD 1.7 m/s) and 78 participants (58.7%) had a PWV above the 8.3 m/s cut-off value and 102 (78.5%) had a PWV >7.5 m/s.

Arterial calcifications were visible on low-dose CT in 102 participants (71.3%) and most often seen in the carotid siphon (87 women, 68.5%) and the coronary arteries (46 women, 32.2%), as shown in table 2. Thoracic aortic and valvular calcifications were far less common. The calcifications at different arterial sites were partially related to each other, as shown in table 3. TACS was strongly related to CACS (R-value 0.901, p-value < 0.001) and moderately related to CSCS (R-value 0.446, p-value < 0.001). CACS and CSCS however were not associated with each other nor was VCS related to any of the other arterial calcifications.

Comparing the presence of arterial calcifications with the presence of CVD risk factors showed that CACS >0AU was associated with hypertension (Odds Ratio (OR) 2.29, 95% Confidence Interval (CI) 1.10-4.77), as shown in table 4. Other CVD risk factors however, were not related to the presence of coronary calcifications. Although not statistically significant, there was a trend towards an increased risk of coronary calcifications when PWV was increased >7.5m/s (OR 2.73, 95% CI 0.96-7.78). Thoracic aortic calcifications, valvular calcifications and carotid siphon calcifications were not related to any of the CVD risk factors including increased PWV.

In addition, assessment of a possible correlation between the PWV measurement as continuous variable and CVD risk factor variables showed a significant association between PWV and age, systolic blood pressure, diastolic blood pressure, waist-hip ratio, intima-media thickness and triglycerides, as shown in the supplemental table. However, no association was found between the PWV measurement and CACS, TACS, CSCS and VCS.

Discussion

In this cross-sectional cohort study assessing the distribution of arterial calcifications among women with a history of preeclampsia aged 45-55 years, arterial calcifications were common.

The presence of calcifications at different arterial sites seemed to be partially related to each other; thoracic aortic calcifications were associated with coronary and carotid siphon calcifications. Participants with hypertension had an increased prevalence of coronary calcifications, but not of other calcifications. Other CVD risk factors including arterial stiffness were not related to the presence of arterial calcifications.

Large population-based cohort studies assessing the presence and distribution of arterial calcifications, showed a large variety in occurrence of arterial calcifications at different sites.[2,7,10,29] Thoracic aortic calcifications are found in 5–60% of asymptomatic adults.[7,10,29] Coronary calcifications have been reported in 28–68% and carotid siphon calcifications in 63–81% of the general population.[2,7,10,30,31] Possible explanations for this variance in calcification prevalence might be the ratio of men and women in these studies and the mean age of participants, which ranges from 45 years op to 70 years of age. Since age is an important determinant for calcifications, this might influence the prevalence of arterial calcifications.[7,32] The arterial calcifications are not confined to the coronary arteries, but are very frequent in the carotid siphon and common in the thoracic aorta as well. This might be clinically relevant, since carotid siphon calcifications are associated with ischemic stroke.[4,35] Valvular calcifications were much less common and seen in only a minority of the participants, which is in accordance with current literature.[2,36]

Arterial stiffness, assessed by PWV using the Arteriograph, was increased in the majority of our study population. In addition, hypertension was commonly seen in our participants. We hypothesized that the onset of arterial calcifications is mediated by hypertension and increased arterial stiffness. PWV was indeed related to blood pressure, which has been described by others as well.[37,38] In addition, hypertension was related to the presence of coronary artery calcifications. However, arterial stiffness was not related to any of the arterial calcifications. This is in contrast to a large population-based cohort study, which showed a relation between PWV and both thoracic aortic calcifications and coronary artery calcifications.[10] A possible explanation for this difference might be found in the variability of the PWV measurements and in the large sample size of the study by Tsao et al. The relation between hypertension and coronary calcifications suggests a hypertension-driven pathway in development of CVD after preeclampsia, as has been suggested by others as well.[17,18]

This cross-sectional cohort study is the first study describing the distribution of arterial calcifications in women with a history of preeclampsia. All participating women underwent extensive cardiovascular screening as well. Thereby, our cohort consists of well-characterized women with a history of preeclampsia. The relative young age of 45-55 years of the participants in this study offers a unique insight in development of atherosclerotic disease in women after preeclampsia. However, several limitations need to be addressed. This is a cross-sectional study and therefore, causality of the associations we found could not be determined. In addition, follow-up for assessment of CVD events is lacking as well. Longitudinal follow-up studies performing repeated

measurements might elucidate the onset and development of arterial calcifications after preeclampsia and could allow for evaluation of an association between arterial calcifications and cardiovascular events. Furthermore, CSC could not be assessed in the whole cohort, but only in 128 of the participants (89.5%). Visualization of the aortic arch was not part of our scan protocol, although this might have been of interested as the volume aortic arch calcifications have been related to ischemic stroke.[34] Lastly, due to ethical and regulatory restrictions it was not possible to include a control group, as exposing healthy individuals to radiation burden required for CT angiography for research purposes is not permitted under Dutch law.

Conclusion

In conclusion, women with a history of preeclampsia show a high prevalence of arterial calcifications at age 45-55 years. Calcifications at different arterial sites appear to be partially related to each other. Hypertension was associated with presence of coronary artery calcifications, but arterial stiffness and other CVD risk factors were not related to any arterial calcifications in the current study. These results indicate accelerated development of systemic atherosclerotic disease after preeclampsia.

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Supplemental table 5. Correlations of PWV with baseline characteristics

	Participants (N = 143)	
	R-value	p-value
Baseline characteristics		
Age	0.180	**0.039**
Systolic blood pressure	0.338	**<0.001**
Diastolic blood pressure	0.263	**0.002**
BMI	0.045	0.603
Waist circumference	0.221	**0.008**
ABI	0.079	0.362
IMT (mm)	0.175	**0.045**
Glucose	0.052	0.536
Total cholesterol	0.132	0.129
Triglycerides	0.167	0.054
HDL-cholesterol	-0.108	0.213
LDL-cholesterol	0.144	0.097
Vascular calcifications		
CACS	0.010	0.913
TACS	0.068	0.441
CSCS	0.179	0.092
VCS	-0.073	0.403

PWV, pulse wave velocity; BMI, body-mass index; ABI, ankle-brachial index; IMT, intima-media thickness; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; CACS, coronary artery calcium score; TACS, thoracic aortic calcium score; CSCS, carotid siphon calcium score; VCS, valvular calcium score.


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Chapter 10

Summary and general discussion

Summary

In this thesis, we aimed to explore the cardiovascular health of women in the fifth and sixth decade of life in relation to pregnancy outcome, with a specific focus on women with preeclampsia. Additionally, we investigated the effect of uncomplicated pregnancy on arterial and metabolic health later in life. In particular, we assessed the impact of pregnancy on arterial stiffness and markers associated with arterial function, i.e. pro-NT and pro-RLX2.

To explore these relationships, we investigated combined data of a number of well-established cohort studies, and conducted a newly established, multicenter prospective cohort study as part of a Dutch national consortium to promote CardiovasculaR hEalthy aging in Women (CREW consortium).

Our results indicate accelerated atherosclerosis in coronary and carotid arteries after preeclampsia. Women with a history of preeclampsia showed cardiovascular abnormalities and ischemic stroke at a younger age. Further, we found that pregnancy itself is associated with increased BMI and an unfavorable CVD risk profile still present in the fifth and sixth decade of life. The main findings of this thesis are further discussed below.

Main findings

First, we set out to assimilate existing knowledge on the association between preeclampsia and subsequent cardiovascular risk and risk factor prevalence. Current clinical practice and opportunities for cardiovascular risk assessment in women with a history of preeclampsia are reviewed in **chapter 2**. Early identification of women at risk for CVD is based on assessment of common modifiable risk factors including lipids, blood pressure, BMI and markers of insulin resistance. Recently, new test modalities have been evaluated to attempt improved CVD risk assessment and detect subclinical disease based on advanced imaging. Non-invasive imaging techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI), might be helpful in this aspect, as they show the ability to recognize subclinical atherosclerotic lesions and might also be effective in risk classification.[1–3]

Cardiovascular risk after uncomplicated pregnancy

We concentrated on cardiovascular risk factors after uncomplicated pregnancy in the first part of this thesis, both by measuring modifiable ('traditional') CVD risk factors and by using state-of-the-art imaging techniques. In **chapter 3** we described the effect of pregnancy on BMI using parity data from the Prevention of Renal and Vascular End-stage Disease (PREVEND) study. The PREVEND cohort is a well-defined longitudinal prospective cohort study for assessment of cardiovascular and renal disease in the general population. Women aged 40 years or older were included and categorized based on their number of children, as proxy for parity. We found a positive linear association between parity and increasing BMI. BMI increased 0.6 kg/m^2 for each extra pregnancy, corresponding with a 1.5–2.0 kg weight gain per pregnancy. Furthermore, a negative linear association was found between parity and HDL cholesterol levels and a signifi-

cant difference in occurrence of CVD risk factors among parity groups. This indicates an association between parity itself and BMI, which further mediates a small but significant effect on lipid levels and blood pressure. These results might have clinical impact, as not only complicated pregnancy increases CVD risk, but also parity itself modulates metabolic and cardiovascular health even in the fifth and sixth decade of life.

We assessed the impact of parity on aortic characteristics in asymptomatic women between 25 and 35 years of age by state-of-the-art cardiac magnetic resonance imaging in **chapter 4**. These aortic characteristics include pulse-wave velocity (PWV), as measure for arterial stiffness, and aortic wall thickness, as measure for atherosclerosis. All women from the prospective Atherosclerosis-Monitoring-and-Biomarker-measurement-In-The-Young (AMBITYON) cohort study were included in this analysis. Both PWV and aortic wall thickness were equally distributed in nulliparous and multiparous women, also after adjustment for possible confounders. In contrast to long-term follow-up studies of cardiovascular health after pregnancy, this study showed no relation between parity and both arterial stiffness and subclinical atherosclerosis in the aorta early after pregnancy.

The effect of pregnancy on cardiovascular health in the third and fourth decade of life seems therefore limited. Results from **chapter 3** on the other hand, showed an association between parity and metabolic health later in life. Both results have to be interpreted with caution, as causality could not be assessed in these cohort studies and correction for possible confounders, such as lactation and low social-economic status, was not possible. Future research should focus on CVD risk development over time after uncomplicated pregnancy, ideally also taking into account possible pre-pregnancy determinants of future health.

Cardiovascular risk after hypertensive pregnancy disorders

In the second part of this thesis, we shifted the focus from CVD risk after uncomplicated pregnancies towards CVD risk after hypertensive pregnancy disorders, including preeclampsia. Several CVD risk parameters were explored, among which modifiable ('traditional') risk factors, non-classical circulating biomarkers, arterial stiffness and imaging markers. These risk parameters will be separately discussed in more detail.

1. Modifiable risk factors

We compared the development of modifiable CVD risk factors, i.e. hypertension, dyslipidemia and diabetes, over time between women with a history of HPD and women with normotensive pregnancies in **chapter 5**. The aim was to distinguish the optimal timing for cardiovascular risk assessment. Female participants from the longitudinal PREVEND study were used for this purpose and women were categorized as having a history of normotensive pregnancies or having a history of hypertensive pregnancies, based on their self-report of obstetric history. We showed an unfavorable CVD risk profile in women with a history of hypertensive pregnancy disorders from the fourth decade of life already, as these women had overall higher blood pressure and

higher total-cholesterol at most time points. Sub analysis showed that women with hypertension early postpartum remained at higher mean blood pressure levels compared to women who were normotensive shortly postpartum, with the highest blood pressure levels found in women with a history of hypertensive pregnancy disorders who had hypertension early postpartum as well. Comparable results were found for lipid levels. These results endorse the current Dutch guideline on cardiovascular risk management after reproductive disorders, which advises to follow and treat these women until blood pressure normalizes.[4] The optimal timing for regular CVD risk assessment remains debated, as CVD risk is increased in women after hypertensive pregnancy disorders at all ages, but prevalence of increased absolute CVD risk as assessed by CVD risk models is still low.

2. Non-classical circulating biomarkers

Several circulating biomarkers have been investigated regarding their role in CVD onset after hypertensive pregnancy disorders. These markers include factors related to inflammation (such as CRP and interleukins), thrombosis (such as homocysteine and fibrinogen) and angiogenesis (such as vascular endothelial growth factor and soluble Fms-like tyrosine kinase).[5–7] In **chapter 6**, we described the eligibility of novel biomarkers, i.e. pro-neurotensin (pro-NT) and pro-relaxin (pro-RLX2), in CVD risk assessment for women with a history of preeclampsia. The hormone pro-NT is involved in obesity-related disease and coronary artery disease, which is mediated by increased LDL-cholesterol and seems to be partially female-specific.[8,9] Pro-RLX2 is an ovarian peptide hormone with vasoactive properties, which thereby appeared to be a promising biomarker for CVD in women as well.[10,11] Pro-NT and pro-RLX2 levels were comparable between women 10 years after preeclampsia and women with an uncomplicated pregnancy. Levels of pro-NT and pro-RLX2 did not differ between these groups. However, some associations between traditional CVD risk factors and pro-NT or pro-RLX2 in either women with a history of preeclampsia or women with a history of an uncomplicated pregnancy could be shown. The major limitations to this study are the small sample size and the relatively young age at which the measurements took place; women were on average 10 years postpartum at which cardiovascular events are still rare. Therefore, our results warrant further research assessing the role of pro-NT and pro-RLX2 in CVD development and the eligibility of CVD risk markers in a larger prospective cohort, and should include follow-up for assessment of CVD endpoints as well.

3. Arterial stiffness

Arterial stiffness could play a role in the development of atherosclerosis for it is associated with both atherosclerotic burden and cardiovascular events.[12–15] Arterial stiffness, mostly assessed non-invasive by pulse wave velocity (PWV) measurement, has been shown to improve CVD risk prediction when added to current CVD risk models.[14,16,17] A recent meta-analysis among women with hypertensive pregnancy disorders showed a persistence of arterial stiffness after pregnancy in these women compared to controls.[18] Arterial stiffness might prove a valuable cardiovascular marker for women with hypertensive pregnancy disorders. However, most PWV measurement

methods are operator-dependent and CVD risk models including PWV have yet to be validated.

In **chapter 4** we assessed PWV by cardiac magnetic resonance (CMR) imaging, an operator-independent and non-invasive method to assess arterial stiffness and related the PWV measurements to parity in young women. In contrary to other population-based cohort studies, we found no relationship between parity and arterial stiffness.[19,20] This might be explained by the use of CMR in our study, which has shown best agreement with invasively measured 'gold standard' arterial stiffness.[21] Our results therefore question previous findings based on operator-dependent measurements. Further prospective research including cardiovascular follow-up is warranted to confirm our findings in a larger study population and to assess possible associations between CMR derived PWV and the presence of CVD risk factors and cardiovascular events.

4. Imaging of coronary and carotid artery atherosclerosis

In part three of this thesis we concentrated on imaging of arterial disease in women with a history of preeclampsia specifically. In **chapter 8** we evaluated subclinical coronary artery atherosclerotic lesions, both coronary artery calcium and plaques, in asymptomatic women at age 45-55 years with a history of preeclampsia by CT. This prospective study was part of the ongoing multicenter, prospective Cardiovascular RiskprofilE: IMaging And Gender-specific disOrders (CREW-IMAGO) cohort study, assessing subclinical atherosclerosis in women after reproductive disorders. Coronary atherosclerotic lesions were commonly found in women with a history of preeclampsia at age 45–55 years. These women had a fourfold increased risk of elevated coronary artery calcium score compared to an age-and ethnicity matched reference group, consisting of 387 women from the multi-ethnic study of atherosclerosis (MESA). Importantly, almost half of participants showed coronary plaques on CCTA, and significant stenosis was found in 4.7% of the participants. These results confirm the long-standing presumption that women with preeclampsia develop premature atherosclerotic coronary artery disease, in the fifth and the sixth decade already. Of interest, CACS was not associated with age within the cohort. Based on these results, it seems validated to further assess the presence of coronary calcium and plaques in even younger women with a history of preeclampsia. This is currently conducted within the CREW consortium as part of the ongoing prospective cohort study in these women. In addition, follow-up of the participants is necessary to assess the association with CVD events.

Furthermore, in **chapter 9** we investigated the distribution of these arterial calcifications in the coronary arteries, aortic arch, cardiac valves and carotid siphon and we determined the relation among arterial calcifications, arterial stiffness and traditional CVD risk. In 143 asymptomatic women the vast majority (71.3%) showed arterial calcifications on CT, mostly coronary calcifications and carotid siphon calcifications. The development of arterial calcifications seemed to be hypertension-driven, although no other CVD risk factors, including arterial stiffness, were related to any arterial calcifications. These results reconfirm accelerated development of systemic atherosclerotic disease after preeclampsia.

This is further confirmed by the results we describe in **chapter 7**, as women with a history of pregnancy complications related to vascular function (preeclampsia, HELLP syndrome and pla-

cental abruption), were at increased risk of early ischemic stroke (i.e. stroke before the age of 50 years) compared with women without such a history. Apart from reconfirming the commonly reported association between specific hypertensive pregnancy complications and the onset of CVD events later in life, it showed in particular that cardiovascular event development is accelerated as well. This is in line with previous work from our group, showing accelerated onset of hypertension after hypertensive disorders of pregnancy and of T2DM after gestational diabetes.[22]

In clinical practice, coronary artery calcium score (CACS) is an important independent predictor of cardiovascular events and in addition CACS improves CVD risk classification when added to traditional CVD risk models.[1,23] Current CVD risk assessment guidelines advise to consider CACS after formal CVD risk assessment indicates an intermediate CVD risk.[24,25] CACS could provide an opportunity to reclassify patients and improve selection of those who might benefit risk reduction interventions. The role of CCTA on the other hand has not been extensively explored in the general population, due to the need of a contrast agent, the increased radiation exposure and higher costs compared with CACS.[26] In addition, the additional value over CACS is questioned and in the absence of a reference cohort, implementation of CCTA as screening tool seems not feasible.[27]

In conclusion, atherosclerotic lesions are commonly seen on CT after preeclampsia in several arterial sites in the fifth and sixth decade of life and significantly more prevalent among women after preeclampsia than in age and ethnicity equivalent controls. How to perform CVD risk assessment is still open for debate, although our data imply use of CT-imaging in these women after preeclampsia, as subclinical coronary atherosclerosis will not be detected by using traditional CVD risk assessment.

Implications and future perspectives

The results from this thesis confirm evidence for accelerated atherosclerosis in women after preeclampsia, as well as high prevalence of traditional and non-traditional cardiovascular risk factors. These results have several implications. First, the occurrence of early-age formation of coronary artery and carotid calcifications and plaques in women with previous preeclampsia confirms a shared etiological link between these two vascular disorders. Further research should be aimed at addressing the mechanisms behind this association, e.g. by phenotyping the type of atherosclerosis, and identification of associated biomarkers. Currently, several groups have started investigations into possible links, such as the Dutch Heart Foundation sponsored national research consortium 'Queen of Hearts (QOH)'.[28] Apart from atherosclerotic obstructive CAD, research should focus on the role of non-obstructive CAD including coronary micro-vascular dysfunction and endothelial dysfunction, since non-obstructive CAD appears to be more common in women than men.[29] Signs of vascular damage, such as endothelial dysfunction and the interplay with platelets and other soluble factors, may be further evaluated in these women.[30]

Secondly, preeclampsia appears to result in cardiovascular damage at a much younger age than women without such a history. This may provide opportunities for early prevention strategies to reduce risk. However, this is expected to be a challenging task, as we still lack information about adherence, effectiveness and costs of such strategies within this group of apparently healthy young women.

To appreciate the clinical relevance of our results, predictive CVD risk modeling is required. This will provide further answers to the questions of how and when CVD risk assessment should commence and if these women qualify for risk reduction strategies. In the past decade, guidelines specifically addressing CVD and CVD prevention in women have been adopted worldwide.[4,31–33] Although all these guidelines acknowledge the increased risk for CVD after hypertensive pregnancy disorders, they neither include clear and equivocal recommendations on when, how and how often to perform CVD risk assessment, nor which (specific) preventive strategies should be adopted. This is due to the low absolute 10-year risk of these women, which is explained by their relatively young age. Within the CREW-consortium, systematic prediction modeling will be carried out on the clinical data collected as part of this thesis. The modeling methods include development of risk prediction strategies, external validation, identification of specific target populations of women that might benefit interventions and impact- and scenario-analysis of these strategies.

Future directions include building of strong, population cohorts as well as evaluations of new intervention strategies to address this specific age group. Ideally, these studies should include pre-pregnancy measurements and interventions to prevent pregnancy complications, which will provide knowledge on the mechanisms behind the relation between hypertensive pregnancy disorders and CVD later in life. Examples of such intervention studies include the Hypertension and Preeclampsia Intervention Trial at Term (HYPITAT) and the Control of Hypertension in Pregnancy Study (CHIPS).[34,35] Both studies investigated the effect of interventions during pregnancy on pregnancy outcome and the HYPITAT study even performed a 10-year follow-up for assessment of cardiovascular health. The future challenge will be to conduct studies starting with pre-pregnancy measurements, with sufficient power to assess pregnancy outcome and long-term follow-up to investigate the relation with future cardiovascular health.

In conclusion, these results provide important new information on preeclampsia as a 'failed stress test' for cardiovascular disease, and appear to identify women with early damage to coronary arteries and other arterial atherosclerotic abnormalities. We hope these results will help to unravel paths towards early-age development of cardiovascular disease in women, and may improve care for women affected by preeclampsia.

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Chapter 11

Appendices


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Appendix I

Pitfalls in interpreting the relevance of studies on vascular function in women after hypertensive pregnancy disorders

Gerbrand A. Zoet, Nina D. Paauw, A. Titia Lely

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In the extensive meta-analysis by Grand'Maison et al. on vascular dysfunction after hypertensive pregnancy disorders (HPD), the authors reported that women after HPD have vascular dysfunction as reflected by an increased Augmentation index (AIx), higher carotid-femoral pulse wave velocity (cfPWV), and increased carotid intima-media thickness (cIMT).[1] A major limitation of the studies presented is the substantial heterogeneity of the results, which the authors try to explain by assessing differences in age, study design and elapsed time since index pregnancy between the studies. All of these factors are very relevant when interpreting data on vascular function after HDP, but the authors fail to note the important influence of external conditions, such as dietary sodium intake, on measures of vascular function.

High sodium intake is known to negatively influence arterial stiffness by establishing endothelial dysfunction and enhancement of vascular smooth muscle tone. In a recent publication by our group we report on the importance of assessment of sodium status in former preeclamptic women when interpreting measures of vascular function.[2] After measuring arterial stiffness under both low (50 mmol Na/day) and high (200 mmol Na/day) sodium conditions, we showed that former preeclamptic women had an impaired ability to adapt their arterial stiffness measured by AIx in response to sodium status compared to women with uncomplicated pregnancies. Other important conditions to consider when interpreting vascular function studies in women include hormonal related conditions such as menstrual cycle phas, presence of polycystic ovary syndrome (PCOS) and the onset of (early) menopause.[3–5]

One other important finding in this meta-analysis that we would like to underline is that vascular dysfunction as measured by AIx and IMT only differed between former preeclamptic women and controls before the age of 40 years. Another recent meta-analysis by Weissgerber et al. showed comparable age-dependent findings after preeclampsia after assessing the studies reporting on flow-mediated dilation with no differences between groups in studies performed >10 years after pregnancy.[6] Apparently, the initial signs of an unfavorable vascular profile in formerly preeclamptic women diminish at later age. Together, these findings indicate the impact of aging and presence of traditional cardiovascular risk factors from ~40 years onwards on vascular function is likely to prevail over factors like a history of HPD.[7,8] Therefore, we believe that recommendation on screening for cardiovascular disease (CVD) based on vascular function after HDP at young age should be made with caution.

Potential markers should preferably be independent predictors of CVD that are detectable at young age and persist over time. Recent studies on early markers of arterial stenosis such as coronary calcifications showed promising results.[9,10] We emphasize the need for studies with standardized conditions to enable identifying clinical markers for timely screening and prevention of CVD in former preeclamptic women.

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Appendix II

Cardiovascular RiskprofilE - IMaging And Gender-specific disOrders (CREW-IMAGO): rationale and design of a multicenter cohort study

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Abstract

Background: Reproductive disorders, such as polycystic ovary syndrome (PCOS), primary ovarian insufficiency (POI) and hypertensive pregnancy disorders (HPD) like pre-eclampsia (PE), are associated with an increased risk of cardiovascular disease (CVD). Detection of early signs of cardiovascular disease (CVD), as well as identification of risk factors among women of reproductive age which improve cardiovascular risk prediction, is a challenge and current models might underestimate long-term health risks. The aim of this study is to assess cardiovascular disease in patients with a history of a reproductive disorder by low-dose computed tomography (CT).

Methods: Women of 45-55 years, who experienced a reproductive disorder (PCOS, POI, HPD), are invited to participate in this multicenter, prospective, cohort study. Women will be recruited after regular cardiovascular screening, including assessment of classical cardiovascular risk factors. CT of the coronary arteries (both coronary artery calcium scoring (CACS), and contrast-enhanced coronary CT angiography (CCTA)) and carotid siphon calcium scoring (CSC) is planned in 300 women with HPD and 300 women with PCOS or POI. In addition, arterial stiffness (non-invasive pulse wave velocity (PWV)) measurement and cell-based biomarkers (inflammatory circulating cells) will be obtained.

Discussion: Initial inclusion is focused on women of 45-55 years. However, the age range (40-45 years and/or ≥55 years) and group composition may be adjusted based on the findings of the interim analysis. Participants can potentially benefit from information obtained in this study concerning their current cardiovascular health and expected future risk of cardiovascular events. The results of this study will provide insights in the development of CVD in women with a history of reproductive disorders. Ultimately, this study may lead to improved cardiovascular prediction models and will provide an opportunity for timely adjustment of preventive strategies. Limitations of this study include the possibility of overdiagnosis and the average radiation dose of 3.5 mSv during coronary and carotid siphon CT, although the increased lifetime malignancy risk is negligible.

Background

Reproductive disorders, including polycystic ovary syndrome (PCOS), primary ovarian insufficiency (POI) and hypertensive pregnancy disorders (HPD) such as pre-eclampsia (PE), are associated with an increased risk of cardiovascular diseases (CVD).

Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) has a prevalence of around 8 to 10% in Caucasian women and is the most common endocrine disorder in women of reproductive age.[1] According to the Rotterdam consensus criteria, PCOS is diagnosed when at least two of the following criteria are present: (i) oligo-/anovulation, (ii) clinical and/or biochemical hyperandrogenism, and (iii) polycystic ovaries on ultrasonography.[2] Insulin resistance, dyslipidemia and type 2 diabetes mellitus (T2DM) have been associated with PCOS.[3–7]

Increasingly PCOS has been associated with cardiovascular risk factors, such as impaired glucose tolerance, obesity, metabolic syndrome (MetS) and hypertension. Several studies have ascertained premature signs of subclinical arterial disease in women with PCOS, such as abnormal carotid intima media thickness on ultrasound or coronary artery calcification score (CACS) on computed tomography (CT).[8–10] Nevertheless, evidence on the potential association between PCOS and CVD endpoints is still limited.[11–13]

Primary Ovarian Insufficiency

Primary ovarian insufficiency (POI), formerly known as premature ovarian failure, is characterized by secondary amenorrhea for at least 4 months accompanied by elevated FSH levels above 40 IU/L, before 40 years of age.[14] The incidence of POI is reported to be 1-2%.[15,16] POI is associated with elevated gonadotropins, hypoestrogenemia and hypoandrogenemia.

Early age at menopause, including POI, is associated with an increased incidence of coronary heart disease and CVD mortality.[17–19] Epidemiological data showed that the relative risk (RR) on CVD was 1.03 (95% confidence interval (CI) 1.01 – 1.05) for each 1-year decrease in age at menopause.[19] Hypoandrogenemia in women has been associated with an increased risk of atherosclerosis, as measured by CIMT or catheter angiography[20–23] and CVD events.[24] A recent systematic review and meta-analyses identified POI as an independent, modest risk factor for developing or dying from IHD (ischemic heart disease) (hazard ratio (HR) 1.69, 95% CI 1.29-2.21, p = 0.0001) and total CVD (HR 1.61, 95% CI 1.22-2.12, p = 0.0007).[25] No relationship was found for POI and stroke (HR 1.03, 0.88-1.19, p = 0.74). These findings may implicate a decreased cardiovascular health in women with POI. However, like PCOS, it remains unclear to which extent POI is independently associated with CVD due to the paucity of data.

Hypertensive pregnancy disorders

HPD include pregnancy-induced hypertension (PIH), PE and the hemolysis, elevated liver en-

zymes, low platelets (HELLP) syndrome. Together, this group of disorders complicates 5-12% of all pregnancies worldwide,[26] while PE alone is seen in 3-5% of all pregnancies.[27,28] Several studies showed that both classical CVD risk factors and novel serum biomarkers for CVD were increased in former hypertensive pregnancies (PIH, late-onset PE and especially early-onset PE) compared to normotensive pregnancies in both premenopausal and postmenopausal women.[29,30] Major CVD risk factors (e.g. hypercholesterolemia, hypertension, diabetes and MetS) were 3-4 fold more prevalent in formerly pre-eclamptic patients when compared with healthy controls of the same age at one to three years after index pregnancy.[31–33] However, mainly due to the relative young age (mean 30.5 years), the 10-year absolute risk of a CVD event as estimated by the Framingham Risk Score (FRS) was still low (mean estimated 10-year cardiovascular disease risk 1.08%).[31]

Women who were diagnosed with pre-eclampsia have a twofold future CVD risk.[34–38] The relative risk of developing hypertension later in life in women with a history of pre-eclampsia is 3.74.[39] Moreover, the risk of developing diabetes later in life is also 2-3 times increased in women with a history of pre-eclampsia compared to women without such a history.[40–42] These findings have led to the hypothesis that pregnancy acts as a stress-test for CVD later in life.[43]

The sub-analyses of a longitudinal follow-up study of the HYPITAT trial – the HyRAS study – showed neither significant differences in hypertension and biochemical cardiovascular risk factors postpartum, nor a difference in the estimated 10- and 30 year Framingham cardiovascular event risk, between women with a history of late-onset pre-eclampsia compared to women with PIH.[32] On the other hand, the increased CVD risk does appear to be more pronounced in the subgroup of early-onset of pre-eclampsia (generally defined as pre-eclampsia occurring before 34 weeks of gestation), with a RR of 7 to 8 on IHD and death due to IHD. [34,35]

Despite recent advances in long-term follow-up after reproductive disorders, identifying women at increased risk for premature CVD remains a challenge. The use of the FRS and other risk models for IHD, like the Systematic Coronary Risk Evaluation (SCORE), Reynolds risk score and the Pooled Cohort Equations, are limited by their underestimation of lifetime CVD risk in young women. The recently published Dutch guideline "Cardiovascular Risk Management after Reproductive Disorders" recommends all women with a history of a reproductive disorder to optimize lifestyle factors.[44] Patients with a history of pre-eclampsia are advised to generate a risk profile at 50 years of age, as their risk of hypertension and diabetes mellitus is increased and the onset of these cardiovascular risk factors is up to 7 years earlier compared to women without pregnancy complications.[39,45] However, longitudinal follow-up data on biomarkers, signs and symptoms of premature subclinical atherosclerosis are needed to better identify the potential adverse effects of female-specific risk factors and life-events on CVD risk.

Serum biomarkers

Circulating endothelial cells, extracellular vesicles and circulatory inflammatory cells might lead

to discovery of new biomarkers for women with reproductive disorders which are at risk for CVD development.

Both HPD and CVD later in life share a pathophysiologic pathway of vascular (endothelium) damage. Pre-eclampsia is associated with an increased number of circulating endothelial cells due to a high degree of endothelial cell activation or injury. Extracellular vesicles (e.g. microvesicles, exosomes) reflect the disease state of pre-eclampsia patients compared to healthy pregnant women.[46,47] In addition, extracellular vesicles -associated polygenic immunoglobin receptor, cystatin C, and complement factor C5a are markedly increased in patients suspected of acute coronary syndrome.[48] As EVs might be involved in both reproductive disorders and CVD, they could possibly serve as a biomarker. The inflammatory profile of circulating cells is proven to be very different in women suffering from CVD.[47] For example, carotid plaques show sex-dependent inflammatory cell content, including neutrophils.[49]

Imaging

CACS acquired with CCT has been shown to have superior predictive value for CVD events to traditional risk factors, risk factor scores and serum biomarkers in asymptomatic persons.[50] Contrast-enhanced CCTA may have additional value over CACS as it can also identify non-calcified plaques, and thereby the total atherosclerotic burden, and assess the presence of coronary luminal narrowing.[51–54] As calcification of plaque occurs at a relatively late stage in atherosclerosis, significant coronary atherosclerosis may be visualized earlier by visualizing the non-calcified coronary plaque with CCTA. Data of CCT as a diagnostic tool, i.e. CACS or CCTA, is scarce and inconclusive for women with reproductive disorders.

Several studies have assessed the presence of coronary calcium in PCOS. Although some of these studies showed an increased CACS in women with PCOS, a recent large, cross-sectional study could not find an association with PCOS and CACS.[55–58]

In a retrospective cohort study published in 2007, the relation between HPD and coronary calcification later in life was assessed in 491 women (mean age 66.8 years, standard deviation 5.4 years).[59] Coronary calcifications (Agatston score ≥1) were found in 305 women (62.9%). This study showed that a self-reported history of hypertension during pregnancy is related to higher CACS in the 7th decade of life (Odds ratio (OR) 1.57, 95% CI 1.04 - 2.37). In an adjusted model correcting for age, BMI, waist:hip ratio, systolic blood pressure and diastolic blood pressure the relation did not reach statistical significance anymore (OR 1.52, 95% CI 0.96 - 2.39).[59] Other retrospective studies assessing coronary artery disease (CAD) by catheter coronary angiography in women suspected for CAD and with a history of HPD showed contrasting results.[60] A recent prospective cohort study conducted among 40 former pre-eclamptic women and 40 age- and parity-matched healthy controls showed increased CACS in former pre-eclamptic women at a mean age of 59.5 ± 4.6 years. The unadjusted OR for having higher CACS due to preeclampsia was 3.54 (95% CI 1.39 - 9.02), although the adjusted model correcting for BMI and hypertension did not reach statistical significance anymore.[62]

There is mounting evidence that intracranial carotid artery calcifications are associated with increased large artery and intracranial artery stiffness, as well as ischemic stroke, white matter abnormalities and cognitive impairment.[63,64] Recent studies found a relationship between carotid siphon calcium (CSC) and white matter hyperintensities and lacunar infarcts.[65–67] Other studies, however, could not confirm these findings.[68–70]

Collectively, previous studies indicate an association between women with reproductive disorders and CVD later in life. Current risk profiles are inadequate to establish future CVD risk in still relative young premenopausal women. The aim of this study is to assess the diagnostic value of cell-based biomarkers, CCT imaging (both non-contrast CACS and contrast enhanced CCTA) and non-contrast CSC in patients with a reproductive disorder to detect CVD.

Methods

Study design and study setting

In this multicenter, prospective, cross-sectional study of patients with a reproductive disorder (PCOS, POI or HPD) we aim to assess the diagnostic value of cell-based biomarkers and CT imaging of the coronary arteries and carotid siphon in the detection of CVD. Patients will be invited to participate at their regular cardiovascular screening, which is performed at two large University Medical Centers in Utrecht (UMC Utrecht) and Rotterdam (Erasmus MC) in the Netherlands.

Participant characteristics

All patients with a reproductive disorder undergo regular cardiovascular screening at a specialized vascular outpatient clinic in one of the participating hospitals as part of standard care for cardiovascular diseases. The study population consists of women aged 45-55 years within three different groups:

1. Women with PCOS defined by Rotterdam consensus criteria, requiring the presence of at least two of the following criteria: (i) oligo-/anovulation, (ii) clinical and/or biochemical hyperandrogenism, and (iii) polycystic ovaries on ultrasonography.
2. Women with POI defined as women with secondary amenorrhea for at least 4 months accompanied by elevated FSH levels above 40 IU/L, occurring prior to 40 years of age.
3. Women with a history of HPD (PIH, early-onset PE (i.e. delivery before 34 weeks of gestation) and late-onset PE (i.e. delivery after 34 weeks of gestation)) according to the ISSHP criteria, verified in clinical records.

Patients with any serious illness that can compromise study participation, patients with high risk for contrast nephropathy (renal dysfunction with an estimated glomerular filtration rate <60 ml/min/1.73 m2) or patients with a history of myocardial infarction are excluded from the study.

After written informed consent is obtained, patients will undergo cardiovascular imaging assessment by CCT imaging, biomarkers and a non-invasive vascular measurement.

Coronary CT imaging

CCT is performed using a multislice CT scanner (256 slice Philips CT, Philips Healthcare, Best, the Netherlands or dual source Somaton Force or Drive Siemens CT, Siemens, Forchheim, Germany) with prospective ECG-triggering. A non-contrast coronary CT is acquired first to calculate the CACS (scan parameters 120 kV, 50 mAs or reference mAs of 80 mAs). Participants with a heart rate >65 beats/min may receive an oral (25-50 mg) and/or intravenous (5-20 mg) beta-blocker (metoprolol, Selokeen AstraZeneca, Zoetermeer, the Netherlands) before the scan. All participants will receive sublingual nitroglycerine just before the CCTA. CCTA scan parameters will be as follows depending on the participant's weight:

1. For the Philips scanner a prospective ECG-triggered acquisition is performed at a mid-diastolic phase (78 %) with 80-120 kV; 195-210 mAs; and 90–115 ml non-ionic contrast material (Iopromide, 300 mg I/ml; Ultravist, Bayer Healthcare, Berlin, Germany) followed by 30–40 ml saline, both injected at a speed of 6–6.7 ml/s. A bolus-tracking technique is used to time the arrival of contrast in the coronary arteries. The CCTA scan is initiated once a threshold of 130 HU is reached in the descending aorta followed by a 7-second post-threshold delay. CCTA's are reconstructed with 0.9 mm slice thickness and iDose iterative reconstruction level 4 and 6.
2. For the Siemens scanners a sequential prospective ECG–triggered acquisition is performed with a pulsing window width depending on heart rate or a high-pitch acquisition timed to image the heart in diastole in case of low regular heart rate. KV and mAs are selected using automatic KV selection based on the topogram (range 70-120 kV) and a reference mAs setting of 230 mAs at 120 kV. At lower kVs reference mAs is automatically adapted accordingly. Either a bolus-tracking technique or test bolus injection with 10ml contrast is used to time the arrival of contrast in the coronary arteries at the discretion of the technician. Non-ionic contrast material (Iopromide, 370 mg I/ml; Ultravist, Bayer Healthcare, Berlin, Germany) is used followed by 30-40 ml saline, both injected at a speed of 5.4 ml/s. Total contrast volume is calculated as scan time +8 seconds multiplied by contrast flow rate. Mostly around 70 ml of contrast is injected for the CCTA. CCTA's are reconstructed with 0.6 mm slice thickness and ADMIRE iterative reconstruction level 3.

The total CCT radiation dose to which participants will be exposed is expected to be within 3.0 mSv.

CT scans are post processed on a workstation (IntelliSpace Portal, Philips Healthcare, QAngio CT software, Medis Medical Imaging or SyngoVia,Siemens) by experienced personnel. CACS is measured on the non-contrast CT with the Agatston scoring method.[71] Coronary artery calcium is defined as a density of >130 Hounsfield units (HU) in a coronary artery. Total CACS is calculated by the sum of all lesions in all four coronary arteries and their side branches. The total Agatston score will be categorized as no calcification (CACS = 0), mild (CACS >0 and <100), moderate (CACS ≥100 and <400) and severe (CACS ≥ 400) calcification; and compared with the MESA database.[72] Semi-automated vessel analysis is used to make multiple curved multiplanar reconstruc-

tions (MPR) of all coronary arteries on the CCTA data.

All cardiac CT scans will be assessed by an experienced cardiovascular radiologist in both academic hospitals. Image quality, plaque characteristics and coronary lumen stenosis will be analyzed on a 18-segment basis according to the modified American Heart Association classification.[73,74] Plaque composition will be evaluated in a qualitative manner as calcified, mixed (both calcified and non-calcified components) and non-calcified (plaques without calcium). Total atherosclerotic plaque burden will be measured with both the segmental involvement score (SIS) and the segment stenosis score (SSS) based on the 18-segment coronary artery model.[74] Luminal stenosis will be graded as absent, minimal (1–24 %), mild (25–49 %), moderate (50–69 %), and severe (≥70 %) narrowing on the basis of diameter measurements comparing the diameters of the maximal stenosis to a reference diameter proximal and distal to the stenotic area.[75] If severe calcifications are present and quantification of stenosis is difficult, the radiologist will refrain from stenosis quantification and score the segments involved as 'calcified, stenosis unclear'.

Carotid Siphon Calcification Imaging

A non-contrast CT with 20-40 mm coverage is planned around the sella turcica to include the intracranial carotid siphon and anterior clinoid process. The head is tilted with the chin towards the breast to avoid scanning the eye lens. The CSC radiation dose to which participants will be exposed is expected to be less than 0.5 mSv on average. Scan parameters will be as follows:

1. For the Philips scanner: 20 mm coverage from petrous apex to ICA top with 120 kV, 100 mAs, brain filter B and C, iDose iterative reconstruction level 3, 1mm and 2mm reconstruction thickness without overlap.
2. For the Siemens scanner (Somatom Force or Drive): 40 mm coverage from horizontal part of the petrous ICA to ICA top with 120 kV, 100 mAs reference, H31s and H45s head filter, 075 mm reconstruction thickness and 0.4 mm increment.

The axial scans are visually assessed by a radiologist for presence or absence of carotid siphon calcification. The severity of CSC will be visually assessed and categorized according to Woodcock as 0 = absent, 1 = mild (thin, discontinuous), 2 = moderate (thin, continuous or thick, discontinuous) or 3 = severe (thick, continuous).[76,77] In addition, the CSC will be semi-automatically quantified as described in detail previously.[65]

Single center side study: serum biomarkers and non-invasive vascular measurements

Classical cardiovascular biochemical risk factor assessment (glucose, insulin, triglycerides, total cholesterol, low-density lipoprotein cholesterol and c-reactive protein) and a general hematological profile (total red and white blood cells and differential counts of nucleated cells) will both be determined.

As part of a single center side study in the UMC Utrecht, the neutrophil, monocyte, and lymphocyte cell numbers and subtype distribution will be examined based on established cell surface marker expression by flow cytometry analysis.[78,79] Peripheral blood mononuclear

cells and circulating endothelial cells will be isolated and sorted directly after drawing blood. To assess histone modification in inflammatory genes, monocytes are cultured in the presence of atherosclerosis associated antigens (oxLDL, HSP). Plasma will be used to isolate and detect (endothelial) microparticles. Proteomics on isolated microparticles will be performed on selected samples and analyzed. Lupus anticoagulants are phospholipid-dependent coagulation inhibitors that are detected with a functional assay based on a clotting test. Platelet reactivity can be assessed with the platelet activation test (PACT), a whole blood functional test of the total platelet response capacity.

Arterial stiffness measured by pulse wave velocity (PWV) is a predictor of cardiovascular events. The Arteriograph (TensioMed, Budapest, Hungar. EC Directive 93/42/EEC, ISO 13485:2003, ISO 13485:2004) is an oscillometric arterial stiffness measurement, which will be performed directly before the CCT in the UMC Utrecht.

Outcome

The primary outcome is the presence of relevant CAD that will be defined as one or more of the following on CT: a CACS ≥100 AU or luminal stenosis ≥50 %. All measurements will be discussed with participants individually under supervision of a vascular specialist. For management of cardiovascular risk factors, the current guideline on European Guidelines on cardiovascular disease prevention will be used.[80] All patients with relevant CAD are discussed with a cardiologist. Management and treatment decisions are left to the discretion of the cardiologist. As a general rule, participants with a CACS of 100-400 AU without obstructive CAD will receive lifestyle advice and may be recommended to initiate treatment with statins.[81,82] Participants with a CACS ≥400 AU or coronary artery stenosis ≥50% will be offered a consultation with a cardiologist to discuss management options. Other relevant cardiac findings will be discussed with the cardiologist. If incidental extra-cardiac findings, such as lung or liver lesions, are considered to be of clinical importance, recommendations of further testing, follow-up or referral to another specialist will be made.

Sample size calculation

No prospective studies have performed CCT (including both CACS and CCTA) in women with reproductive disorders as part of long-term cardiovascular follow up. The estimation of the expected necessary sample size is based on $\alpha = 0.05$ and desired power = 0.90. In addition, the background prevalence of coronary plaque (both non-calcified and calcified) in asymptomatic, healthy females ≥ 45 years old based on the CCTA study by Kim et al. (2013) is estimated 6.7%.[83] We presume a relative risk of around 2 for the development of CVD is women with reproductive disorders.[44] This leads to an (conservative) estimated prevalence of coronary artery disease (plaque) of 13.4 % (95% CI 10.0 - 17.6) and a minimal sample size of at least 261 CCT's in both the HPD and the PCOS/POI group.

Based on these results and given the radiation-induced health risks, initial inclusion will be

confined to women who are 45-55 years old and we will perform an interim analysis after 300 CCT's (100 in patients with HPD, 100 in patients with PCOS and 100 in patients with POI). If the prevalence of plaque as seen on CCTA is ≥ 10%, which is the lower bound of the 95% CI in our estimated prevalence of coronary artery disease, we expect to find significant differences compared to controls. In that case, we will perform the remaining 300 CCT's (200 in patients with HPD and 100 in patients with PCOS/POI), leading to a total of 300 CCT's in patients with HPD and 300 CCT's in patients with PCOS/POI. The age range (40-45 years and/or ≥55 years) and group composition for later inclusion may be adapted based on the findings of the interim analysis. If the prevalence of plaque as seen on CCT is ≤ 10%, we do not expect to find any significant differences compared to controls and therefore we will withdraw the remaining CCTA's and focus on CACS only.

Statistical analysis

Data analysis will be performed using SPSS. A probability (p) less than 0.05 will be considered significant. The characteristics of participants will be described using means +/- standard deviations for continuous variables using a two tailed independent t-test for comparison between groups (e.g. plaque burden). Categorical variables will be expressed as numbers (percentages) or medians (with inter quartile ranges) and compared with a chi-square test (e.g. CACS percentiles). Identical tests will be performed for circulating biomarkers. If several variables are identified to be statistically associated with cardiovascular risk factors, multiple linear regression or multiple logistic regression will be performed to identify the most important associations, appropriate to the endpoint chosen.

Ethical considerations

This study has been approved by the Medical Research Ethics Committee of the University Medical Center Utrecht (MEC number 15-508). This trial is registered in the Dutch Trial Register, NTR 5531, http://www.trialregister.nl, date of registration: October 21st, 2015.

Discussion

In this multicenter, prospective, cross-sectional study in patients with a reproductive disorder (PCOS, POI or HPD) we aim to assess CVD by CCT imaging (both CACS and CCTA) and CSC. Given the increased risk on CVD later in life, we believe the low radiation exposure is justified. Moreover, a substudy of the association of cell-based biomarkers and CCT will be performed.

The results of this study will provide insights in the added value CT imaging in the detection of cardiovascular disease. Ultimately, these insights can lead to improved cardiovascular prediction models in these women and may provide an opportunity for adjusted preventive strategies.

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GUSTAV
KLIMT

Chapter 12

Nederlandse samenvatting

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Dankwoord

Curriculum vitae

Nederlandse samenvatting

In dit proefschrift beschrijven wij de cardiovasculaire gezondheid van vrouwen in het vijfde en zesde decennium van het leven in relatie tot zwangerschapsuitkomst met specifieke aandacht voor vrouwen die zwangerschapsvergiftiging (preëclampsie) hebben gehad. Daarnaast hebben wij onderzocht wat het effect van één of meerdere ongecompliceerde zwangerschappen is op arteriële en metabole gezondheid later in het leven. Hierbij hebben wij ons met name gericht op de invloed van zwangerschap op arteriële stijfheid en indicatoren geassocieerd met arteriële functie zoals pro-neurotensin (pro-NT) en pro-relaxin (pro-RLX2).

Om deze associaties te onderzoeken hebben we gebruik gemaakt van data van een aantal bekende cohort studies en hebben we daarnaast een nieuwe multicenter prospectieve cohortstudie opgezet binnen het nationale onderzoeksconsortium "CardiovasculaR hEalthy aging in Women" (CREW consortium).

Onze resultaten laten zien dat slagaderverkalking (atherosclerose) in de kransslagaderen (coronair arteriën) en de halsslagaderen versneld optreedt na het doormaken van preëclampsie. Vrouwen met een preëclampsie in de voorgeschiedenis krijgen op een jongere leeftijd cardiovasculaire afwijkingen en een beroerte dan vrouwen met een ongecompliceerde zwangerschap. Daarnaast is zwangerschap zelf geassocieerd met een hoger BMI en ongunstig cardiovasculair risicoprofiel zelfs tot in het vijfde en zesde decennium van het leven. De belangrijkste bevindingen uit dit proefschrift worden hierna beschreven.

In **hoofdstuk 2** beschrijven wij de huidige kennis over de relatie tussen preëclampsie en cardiovasculaire gezondheid later in het leven, waarbij ook de huidige klinische praktijk en mogelijkheden voor cardiovasculaire screening bij vrouwen met preëclampsie in de voorgeschiedenis behandeld worden. Vroege identificatie van vrouwen met een verhoogd cardiovasculair risico is gebaseerd op het onderzoeken van veelvoorkomende beïnvloedbare ('traditionele') riscofactoren zoals bloeddruk lipidenprofiel BMI en indicatoren van insuline resistentie. Nieuwe indicatoren zijn echter recent onderzocht om de risicoschatting van cardiovasculaire gezondheid te verbeteren en om subklinische ziekte aan te tonen met behulp van geavanceerde beeldvorming. Niet-invasieve beeldvormende technieken, zoals computertomografie of computed tomography (CT) en magnetische resonantie of magnetic resonance imaging (MRI), kunnen hierbij behulpzaam zijn aangezien zij in staat zijn subklinische atherosclerose te herkennen en mogelijk kunnen bijdragen aan risicoclassificatie.

Het **eerste gedeelte** van dit proefschrift beschrijft cardiovasculaire risicofactoren na één of meerdere ongecompliceerde zwangerschappen, zowel door het meten van veelvoorkomende ('traditionele') risicofactoren als door het gebruiken van geavanceerde beeldvormende technieken.

Hoofdstuk 3 laat zien hoe traditionele cardiovasculaire risicofactoren zoals lichaamsbouw en

metabole gezondheid zich ontwikkelen over de tijd bij vrouwen die een zwangerschap hebben doorgemaakt in vergelijking met vrouwen die geen zwangerschap hebben doorgemaakt, waarbij is gecategoriseerd naar het aantal kinderen. Voor deze studie zijn vrouwen geselecteerd van 40 jaar of ouder uit de 'Prevention of Renal and Vascular End-stage Disease' (PREVEND) studie, een welomschreven prospectieve cohortstudie waarin de cardiovasculaire en renale gezondheid van de algemene populatie herhaaldelijk werd gemeten. Deze vrouwen werden gecategoriseerd op basis van het aantal kinderen dat zij hebben, als substituut voor het aantal doorgemaakte zwangerschappen (pariteit). Wij vonden een positieve lineaire associatie tussen pariteit en BMI. Zo nam BMI toe met 06 kg/m^2 per zwangerschap hetgeen overeen komt met een gewichtstoename van 15–20 kg per zwangerschap. Daarnaast vonden wij een negatieve lineaire associatie tussen pariteit en HDL cholesterol en een significant verschil in het voorkomen van cardiovasculaire risicofactoren tussen de pariteitsgroepen. Deze studie wijst op een mogelijk verband tussen zwangerschap op zich en BMI, wat verder een klein maar significant effect sorteert op lipidenprofiel en bloeddruk. Deze resultaten zijn klinisch van invloed aangezien niet alleen zwangerschapscomplicaties het risico op hart- en vaatziekten verhogen maar ook zwangerschap op zich de metabole en cardiovasculaire gezondheid in het vijfde en zesde decennium beïnvloedt.

In **hoofdstuk 4** beschrijven wij de invloed van zwangerschap op karakteristieken van de grote lichaamsslagader (aorta) bij asymptomatische vrouwen tussen de 25 en 35 jaar door middel van moderne cardiale MRI .Deze aorta karakteristieken omvatten polsgolfsnelheid (pulse wave velocity, PWV) als maat voor arteriële stijfheid en aoarta wanddikte als maat voor atherosclerose. Alle vrouwen die deelnamen aan de prospectieve 'Atherosclerosis-Monitoring-and-Biomarker-measurement-In-The-Young' (AMBITYON) cohort studie zijn hiervoor geselecteerd. Zowel PWV als aorta wanddikte verschilden niet tussen vrouwen die wel en vrouwen die niet zwanger waren geweest, ook niet na correctie voor mogelijke verstorende factoren. In tegenstelling tot lange termijn follow-up studie naar cardiovasculaire gezondheid na zwangerschap laat deze studie onder vrouwen kort na de zwangerschap geen relatie zien tussen zwangerschap en arteriële stijfheid en subklinische atherosclerose. Een mogelijke verklaring kan gevonden worden in het gebruik van niet-invasieve moderne cardiale MRI. Deze meetmethode komt het beste overeen met de 'gouden standaard': op invasieve wijze gemeten PWV. Eerdere studies zijn gedaan met meetmethoden die mogelijk minder betrouwbaar waren. Onze bevindingen trekken deze eerdere resultaten in twijfel. Grotere prospectieve studies met follow-up van cardiovasculaire gezondheid zijn nodig om onze bevindingen te bevestigen en om de relatie tussen aorta karakteristieken, gemeten met cardiale MRI, en het optreden van hart- en vaatziekten te onderzoeken.

In het **tweede gedeelte** van dit proefschrift wordt dieper ingegaan op het cardiovasculaire risico na hypertensieve zwangerschapscomplicaties waaronder preëclampsie. Hiertoe hebben wij verschillende cardiovasculaire risicofactoren onderzocht waaronder traditionele risicofactoren, nieuwe circulerende biomarkers, arteriële stijfheid en beeldvormingsindicatoren.

Hoofdstuk 5 presenteert de studie waarin we de ontwikkeling van beïnvloedbare traditionele cardiovasculaire risicofactoren zoals hypertensie dyslipidemie en diabetes vergelijken tussen vrouwen die een hypertensieve zwangerschapscomplicatie in de voorgeschiedenis hebben en vrouwen die een ongecompliceerde zwangerschap hadden. Het doel van deze studie was om hiermee de optimale timing van cardiovasculaire screening te bepalen. Hiervoor zijn vrouwen geselecteerd uit de eerder beschreven 'Prevention of Renal and Vascular End-stage Disease' (PREVEND) studie en gecategoriseerd op basis van zelf-gerapporteerde obstetrische voorgeschiedenis. Vrouwen met een voorgeschiedenis van hypertensieve zwangerschapscomplicaties bleken vanaf het vierde decennium een ongunstiger cardiovasculair risicoprofiel (hogere bloeddruk en hoger totaal cholesterol) te hebben dan vrouwen zonder zulke voorgeschiedenis. Sub-analyse liet zien dat vrouwen die kort na de bevalling een hoge bloeddruk hadden gedurende de follow-up een hogere bloeddruk hielden in vergelijking met vrouwen die kort na de bevalling een normale bloeddruk hadden. Vergelijkbare resultaten werden gevonden met betrekking tot het cholesterolgehalte. Deze resultaten bevestigen de aanbeveling in de huidige Nederlandse richtlijn 'cardiovasculair risicomanagement na een reproductieve aandoening', die adviseert om vrouwen met een hoge bloeddruk na de bevalling te vervolgen totdat de bloeddruk genormaliseerd is. De optimale timing van cardiovasculaire screening blijft echter onderwerp van discussie, aangezien het cardiovasculaire risico na een hypertensieve zwangerschapscomplicatie op elke leeftijd verhoogd is maar de prevalentie van een verhoogd absoluut risico op hart- en vaatziekte laag blijft.

In **hoofdstuk 6** beschrijven wij de mogelijkheid om nieuwe circulerende biomarkers te gebruiken in cardiovasculaire risicoschatting bij vrouwen met preëclampsie in de voorgeschiedenis. Het betreft de biomarkers pro-neurotensin (pro-NT), een deels vrouwspecifiek hormoon gerelateerd aan obesitas en ischemische hartziekte (coronairlijden), en pro-relaxin (pro-RLX2), een ovarieel hormoon met vasoactieve eigenschappen. Pro-NT en pro-RLX2 waarden waren vergelijkbaar bij vrouwen die gemiddeld 10 jaar geleden een preëclampsie hadden doorgemaakt en vrouwen met ongecompliceerde zwangerschap. Wij vonden echter wel associaties tussen bepaalde traditionele cardiovasculaire risicofactoren en pro-NT of pro-RLX2. De kleine studieomvang en de relatief jonge leeftijd waarop de metingen plaatsvonden zijn belangrijke beperkingen van deze studie, omdat het optreden van cardiovasculaire eindpunten zoals hartinfarct en beroerte daardoor in deze cohortstudie zeer weinig voorkomend zijn. De rol van pro-NT en prol-RLX2 in de ontwikkeling van hart- en vaatziekten en de geschiktheid van deze biomarkers als indicatoren van cardiovasculair risico dient daarom verder onderzocht te worden in grotere prospectieve studies, waarin follow-up plaatsvindt naar het optreden van hart- en vaatziekten als cardiovasculaire eindpunten.

De relatie tussen vasculaire zwangerschapscomplicaties zoals preëclampsie, HELLP-syndroom en loslating van de placenta en het optreden van ischemische beroerte wordt gepresenteerd in **hoofdstuk 7.** Vrouwen met een vasculaire zwangerschapscomplicatie in de voorgeschiede-

nis hebben een verhoogd risico op een vroege beroerte dat wil zeggen het optreden van een beroerte voor het 50e levensjaar in vergelijking met vrouwen zonder een dergelijke voorgeschiedenis. Deze studie bevestigt daarmee de al eerder gerapporteerde associatie tussen hypertensieve zwangerschapscomplicaties en het optreden van hart- en vaatziekten later in het leven, maar het laat ook zien dat de ontwikkeling van deze hart- en vaatziekten versneld is. Dit komt overeen met eerder studies van onze groep waarin een versneld optreden van hoge bloeddruk werd gezien bij vrouwen met een hypertensieve zwangerschapscomplicatie en versneld optreden van type 2 diabetes mellitus bij vrouwen met zwangerschapssuikerziekte.

Het **derde deel** van dit proefschrift concentreert zich op beeldvorming van (subklinische) arteriële ziekte bij vrouwen die specifiek preëclampsie in de voorgeschiedenis hebben.

In **hoofdstuk 8** beschrijven we de studie naar het voorkomen van subklinische coronaire atherosclerose, zowel coronaire verkalkingen (calcificaties) als coronaire plaques bij asymptomatische vrouwen tussen de 45 en 55 jaar die een preëclampsie hebben doorgemaakt. Deze studie was onderdeel van de lopende multicenter prospectieve 'Cardiovascular RiskprofilE: IMaging And Gender-specific disOrders (CREW-IMAGO)' cohortstudie, die subklinische atherosclerose bij vrouwen met een reproductieve aandoening onderzoekt middels een CT-scan. Coronaire atherosclerose werd vaak gezien bij de 164 vrouwen die deelnamen aan de studie. Vrouwen met preëclampsie hebben een vier keer zo hoog risico op een verhoogde coronaire calciumscore in vergelijking met een controlegroep van 387 vrouwen met vergelijkbare leeftijd en etniciteit uit de 'multi-ethnic study of atherosclerosis' (MESA). Bijna de helft van de vrouwen met preëclampsie had plaques in de coronair arteriën en bij 47% was sprake van significante stenose van de coronair arteriën. Deze resultaten bevestigen de lang bestaande aanname dat vrouwen met preëclampsie vroegtijdig atherosclerotische coronairlijden ontwikkelen. Interessant genoeg was de aanwezigheid van calcificaties niet gerelateerd aan leeftijd in onze studie. Gebaseerd op deze resultaten is verder onderzoek geïndiceerd naar de aanwezigheid van coronaire calcificaties en plaques bij jongere vrouwen die preëclampsie in de voorgeschiedenis hebben. Dit wordt momenteel uitgevoerd binnen het CREW consortium als onderdeel van de lopende prospectieve cohortstudie. Daarnaast is follow-up van deze vrouwen nodig om de mogelijke associatie tussen de gevonden subklinische afwijkingen en het optreden van klinische cardiovasculaire eindpunten te onderzoeken.

Hoofdstuk 9 laat zien hoe de verdeling van arteriële calcificaties is in de coronair arteriën, aortaboog, hartkleppen en carotis siphon bij vrouwen die preëclampsie in de voorgeschiedenis hebben. Daarnaast hebben we onderzocht of er een relatie is tussen deze arteriële calcificaties, arteriële stijfheid en traditionele cardiovasculaire risicofactoren. Arteriële calcificaties werden bij de meerderheid (713%) van deze vrouwen gezien op CT scan, waarbij calcificaties van de coronair arteriën en de carotis siphon het meest voorkomend waren. De ontwikkeling van deze calcificaties wordt mogelijk veroorzaakt door hoge bloeddruk, terwijl andere cardiovasculaire

risicofactoren waaronder arteriële stijfheid geen relatie hadden met deze calcificaties. Deze resultaten bevestigen de versnelde ontwikkeling van systemische atherosclerose na preëclampsie.

Conclusie

De resultaten van dit proefschrift bieden belangrijke nieuwe informatie over de relatie tussen preëclampsie en het risico op hart- en vaatziekten, waarbij zwangerschap kan worden gezien als cardiovasculaire stresstest. Vrouwen met preëclampsie zakken voor deze stresstest en hebben daarmee een verhoogd risico op vroege coronair atherosclerose en andere arteriële atherosclerotische afwijkingen. Deze resultaten dragen bij aan het begrip rondom de vroegtijdige ontwikkeling van cardiovasculaire aandoeningen bij vrouwen en kunnen mogelijk de zorg voor vrouwen die preëclampsie hebben gehad verbeteren.

List of publications

Arterial calcifications and arterial stiffness in women with a history of preeclampsia

GA Zoet, BB van Rijn, E Boersma, BCJM Fauser, CJM de Groot, A Franx, AHEM Maas, BK Velthuis on behalf of the CREW consortium

Manuscript in preparation

Trajectory of cardiovascular risk factors after hypertensive disorders of pregnancy

TKJ Groenhof, **GA Zoet**, A Franx, RT Gansevoort, ML Bots, H Groen, AT Lely

Manuscript in preparation

Effect of pregnancy on MRI-derived aortic characteristics: the AMBITYON study

GA Zoet, AK Sverrisdottïr, ALM Eikendal, A Franx, T Leiner, BB van Rijn

Submitted

Association between parity and cardiometabolic risk profile: a longitudinal follow-up study

GA Zoet, ND Paauw, TKJ Groenhof, A Franx, RT Gansevoort, H Groen, BB van Rijn, AT Lely

Submitted

Subclinical coronary artery disease on coronary computed tomography among asymptomatic women at age 45 to 55 years with a history of preeclampsia

GA Zoet, L Benschop, E Boersma, RPJ Budde, BCJM Fauser, Y van der Graaf, CJM de Groot, AHEM Maas, JE Roeters van Lennep, EAP Steegers, FL Visseren, BB van Rijn, BK Velthuis, A Franx on behalf of the CREW consortium

Submitted

Similar pro-NT and pro-RLX2 levels after preeclampsia and uncomplicated pregnancy

GA Zoet, BB van Rijn, M Rehfeldt, A Franx, AHEM Maas

Revisions in *Maturitas*

Preeclampsia and coronary plaque erosion: manifestiations of endothelial dysfunction resulting in cardiovascular events in women.

SCA de Jager, JAL Meeuwsen, FM van Pijpen, **GA Zoet**, AD Barendrecht, A Franx, G Pasterkamp, BB van Rijn, MJ Goumans, HM den Ruijter.

Eur J Pharmacol 2017; [epub ahead of print]

Cardiovascular RiskprofilE - IMaging And Gender-specific disOrders (CREw- IMAGO): rationale and design of a multicenter cohort study

GA Zoet, C Meun, L Benschop, E Boersma, RPJ Budde, BCJM Fauser, CJM de Groot, A van der Lugt, AHEM Maas, KGM Moons, JE Roeters van Lennep, JW Roos-Hesselink, EAP Steegers, BB van Rijn, JSE Laven, A Franx, BK Velthuis

BMC Womens' Health 2017;17:60-69

Stroke after pregnancy disorders

GA Zoet, KM Linstra, MLE Bernsen, MPH Koster, IC van der Schaaf, LJ Kappelle, BB van Rijn, A Franx, MJH Wermer, BK Velthuis on behalf of the DUST investigators

Eur J Obstet Gynecol Reprod Biol 2017,215:264-266

Pitfalls in interpreting the relevance of studies on vascular function in women after hypertensive pregnancy disorders (eLetter)

GA Zoet, ND Paauw, AT Lely

Hypertension, published online November 2016

Determinants of future cardiovascular health in women with a history of preeclampsia

GA Zoet, MPH Koster, BK Velthuis, CJM de Groot, AHEM Maas, BCJM Fauser, A Franx, BB van Rijn

Maturitas 2015,82:153-61

Varicocèle behandeling en de invloed op de androgene fertiliteit

F Amelung, **GA Zoet**, TE Vogelvang

NTOG 2013,126:461

Dankwoord

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Lieve onderzoekers van zowel de 'bright side' als de côte d'AZU; de afgelopen jaren hebben we

lief en leed gedeeld. Van begeleidingsfrustraties, METC-stress tot vlonder-lunches en festivalwerkbesprekingen: wat was het allemaal leuk met jullie! Dank voor de gezelligheid, de koffietjes, fietstochtjes, congressen en etentjes. Hopelijk zien we elkaar snel weer in de kliniek!

Dat werk en privé bij voorkeur door elkaar heen moet lopen bewijzen de allerliefste #bitchpleasers wel. Van AZU en WKZ naar Kopenhagen en Oxford en daarmee ook van collega's naar goede vrienden: het gebeurde in een oogwenk. Wat een verrijking zijn jullie en op naar het volgende weekendje, kusjes!

Lieve vrienden en vriendinnen, dank jullie wel voor de fijne vriendschap en de nodige ontspanning en gezelligheid tijdens deze promotietijd.

Op naar en Antibesitas, zoals mijn promotieteam me doordeweeks begeleidde, zo hebben jullie mijn weekenden begeleid. Zonder ontspanning geen inspanning en voor die ontspanning zorgden jullie zeker! In het weekend paste van de vroege tot de late uurtjes meestal net zoveel activiteiten als in een hele week; dubbel genieten dankzij blokkenschema's en waterijsjes met mooi weer, echt boffen met jullie. Lieve Noor; vanaf de gedeelde voorkeur voor Ali B, X-men en Twilight ben je een goede vriendin; ik bewonder je ongeorganiseerde laisser-fair mentaliteit waarmee je carrière, man, zwangerschap, kind en huishouden bestiert. Dank voor je onvoorwaardelijke steun in alles! Lieve Andrée, je bent mijn vrouwelijke wederhelft in liefde voor planning, organisatie, orde en regelmaat (en G&T's). Ik geniet van onze vriendschap; in al die jaren is die alleen maar sterker geworden. Ik kijk nu al uit naar onze traditionele weekendtrip: London baby!

Lieve nichtjes, ooit allemaal woonachtig en borrelend in Utrecht, maar inmiddels verspreid over de Amsterdam-Utrecht-as: wat een gayzellig en bont gezelschap zijn we. Wintersport, diner in Karel V en bruiloften in de stad, het platteland en op de piste: we zijn van alle markten thuis en ik hoop dat we dat nog lang blijven!

Meltteam en inmiddels Lowlandslieverds: wat een luchtbed, glitters, mollen, villatenten en plaktattoes wel niet kunnen doen. Vanaf minuut één leiden wij een heerlijk symbiotisch feestelijk festival bestaan! Rob & Mat, volgens mij is Berlin Calling; wanneer gaan we weer? Paultje, mag ik dan bij jou achterop de motor? Stiekemerd en Sjaantòl, nemen jullie de festivaldouche dan mee? Geven we Nico een podium om op te treden!

Annick, als achterbuurvrouw en wandelingetjesvriendin was je er altijd als het nodig was met een luisterend oor en afleiding in de vorm van koffietjes bij al onze koffiebuurtbarretjes. Wat mij betreft houden we dat er nu het proefschrift af is zeker in.

Meinie en Renée, al vanaf studiejaar één zijn we elkaars steun en toeverlaat. Ik ben blij met onze vriendschap, waarin de echtgenoten gelukkig ook enthousiast meedoen en zeer gewaardeerd worden. Naast drie bruiloften nu dus ook drie promoties; op naar de volgende drieslag!

Myrthe, waar waren we zonder elkaar? Van de brugklas tot een koophuis: jij bent de constante factor in mijn leven. Dank voor je onvoorwaardelijke vriendschap, zijn we inmiddels officieus geen familie?

Lieve paranimfen, de drie musgaytiers, wat fijn dat jullie me bijstaan tijdens de promotie; ik kan me geen beter team wensen. Tobs, vanaf dag één was je mijn maatje in de onderzoeksgroep en ik keek altijd uit naar onze weekendbespreking met koffie op maandag. Ik ben blij dat we elkaar ondanks de verschillende woonplaatsen en opleidingsclusters nog zo veel zien!

Jan, wat is het toch bijzonder dat we precies hetzelfde pad doorlopen en wat is het een eer om jouw onderzoek te mogen voortzetten! Behalve ontzettend prettig samenwerken, ben je ook een dierbare vriend met de traditionele citytripjes als hoogtepunt van ons vriendschapsjaar. Op naar de lustrumreis in 2019!

Lieve schoonfamilie, ik ben vanaf het begin altijd welkom geweest bij jullie. Ik voel me er dan ook zo thuis, dat ik in zonder gêne de studeerkamer inpikte om aan mijn proefschrift te werken. Dank voor jullie interesse, begrip en nuchtere Noord-Hollandse kijk op het leven.

Lieve Zoetjes, we kunnen niet zonder elkaar, ook al zijn we vier eigenwijze kinderen met net zulke eigenwijze ouders. Dank voor jullie interesse, steun en vertrouwen in mij. Pap, je bent een voorbeeld voor me en het is altijd verhelderend met jou te sparren over uitdagingen in werk en het leven. Mam, je hebt het hart op de tong, maar vooral ook een heel warm hart. Dank voor je liefde en betrokkenheid. Hannah, ik heb diep respect voor jouw doorzettingsvermogen, eindeloze energie en brede interesse; aanstekelijke girl power waar ik veel van kan leren! Florian, school en studie gingen niet altijd makkelijk, maar hoe je hiermee omgaat is een voorbeeld voor me. Ik ben trots op wat je allemaal hebt bereikt! Harmen, in jou zie ik veel van mezelf; onverstoorbaar en altijd vrolijk ga jij je eigen weg. Ik hoop op meer goede gesprekken en vaker boulderen!

Allerliefste Lars, mijn echtgenootje, wat moet ik zonder jou? Seneca zei: "zonder gezelschap is geluk onmogelijk" en jij bent het beste gezelschap dat ik me kan wensen. Al tien jaar houdt je mij dankzij je liefdevolle rust en nuchterheid met beide benen op de grond. Dankzij jouw onvoorwaardelijke steun en liefde én dankzij de ruimte en vrijheid die je me biedt, heb ik dit proefschrift kunnen schrijven. Na de marathon en de bruiloft kijk ik uit naar ons volgende hoogtepunt. Ik hou van jou!

Curriculum vitae

Gerbrand Zoet was born on the 28th of September 1986 after exactly 40 weeks of gestation at home in Lunteren, the Netherlands. He grew up in Amersfoort with his parents, sister and two brothers. After obtaining his gymnasium diploma at the Corderius College in 2004 he started medical school at the Utrecht University. His interest in Obstetrics and Gynaecology grew during clinical electives at the Hubert Kairuki Memorial Hospital in Dar-es-Salaam, Tanzania, and the Diakonessenhuis Utrecht. This was followed by a research internship at the Gynaecologic Oncology department of the University Medical Center Utrecht under supervision of prof. dr. R. Verheijen and drs. E. van Dorst. After graduating in 2011, he returned to the Diakonessenhuis Utrecht as a resident (not in training) and fertility doctor at the Obstetrics and Gynaecology department under supervision of dr. N. Schuitemaker and dr. M. van Haaften. In 2014 he commenced his PhD in Obstetrics at the University Medical Center Utrecht under supervision of prof. dr. A. Franx, prof. dr. B.K. Velthuis and dr. B.B. van Rijn, of which the results have been described in this thesis. Recently, he started his specialization in Obstetrics and Gynaecology at the St. Antonius Hospital in Nieuwegein under supervision of dr. J. Schagen van Leeuwen and dr. P. Graziosi. In June 2017 Gerbrand married Lars, the love of his life. Together with their cat and chickens they happily live in Utrecht.