HEMODYNAMICS IN WOMEN WITH A HISTORY OF PREECLAMPSIA

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Contents

CHAPTER 1

General introduction

Preeclampsia is a hypertensive disease in pregnancy and an important cause of maternal and fetal morbidity and mortality worldwide. After a pregnancy complicated by preeclampsia women have an increased risk to develop cardiovascular disease. This thesis aims to improve our understanding of the underlying circulatory risk profile for cardiovascular disease that is characterized by low plasma volume in women with a history of preeclampsia and to examine the effects of aerobic exercise training on this profile.

Preeclampsia is a hypertensive disorder of pregnancy unique to primates. It is characterized by the combination of hypertension and proteinuria and complicates approximately 2-5% of pregnancies[1]. The ultimate treatment of preeclampsia is delivery of the fetus and placenta, which can be postponed while giving antihypertensive treatment in an effort to improve fetal outcomes if gestational age at time of diagnosis is still limited. After delivery, the clinical syndrome of preeclampsia usually resolves within days[1].

Many women who experienced pregnancy complicated by preeclampsia are puzzled why they developed this condition during pregnancy despite their supposed good health and are concerned about their physical recovery after the complicated pregnancy. Thereafter, questions follow regarding recurrence risk in future pregnancies and implications for their future health. In an effort to answer these questions, studies have been performed to characterize women with a history of preeclampsia[2-6] and estimated recurrence risk based on the presence or absence of specific risk factors[7-10]. The least we can learn from these studies is, that there exists considerable heterogeneity among formerly preeclamptic women. Various risk factors are nowadays linked to preeclampsia, one more consistent than the other. So far no single risk factor could uniformly explain why these women developed preeclampsia in previous pregnancy. This made most clinicians and scientist believe that preeclampsia has a multifactorial aetiology. Gestational age at onset of preeclampsia is the strongest predictor for recurrent disease; the earlier women developed preeclampsia the higher the recurrence risk in next pregnancy[9].

After pregnancy complicated by preeclampsia, traditional risk factors for cardiovascular disease or, in other words, components of the metabolic syndrome (hypertension, obesity, hyperinsulinemia, dyslipidemia and/or micro-albuminuria) are among the most consistently demonstrated abnormalities in these women. This suggests that preeclampsia and cardiovascular disease may have disease mechanisms in common[4]. The scientific interest in the cardiovascular risk profile in women with a history of preeclampsia was fuelled by a meta-analysis by Bellamy in 2007[11]. This study clearly demonstrated an increased lifetime risk to develop cardiovascular

disease after a pregnancy complicated by preeclampsia. Women with a history of preeclampsia have a 4-fold higher risk of chronic hypertension and a 2-fold higher risk for subsequent cardiovascular disease over the 5 to15 years after preeclampsia[11, 12]. The life expectancy after preeclampsia is reduced by approximately 6 years, mainly due to cardiovascular death.

In this perspective, pregnancy can be considered as a unique cardiovascular "stress test"; and therefore preeclampsia in apparently healthy women may unmask a previously unrecognized cardiovascular risk profile. (Figure 1A)[13]. Whether preeclampsia itself has a direct effect contributing to the development of cardiovascular disease is still matter of debate[4].

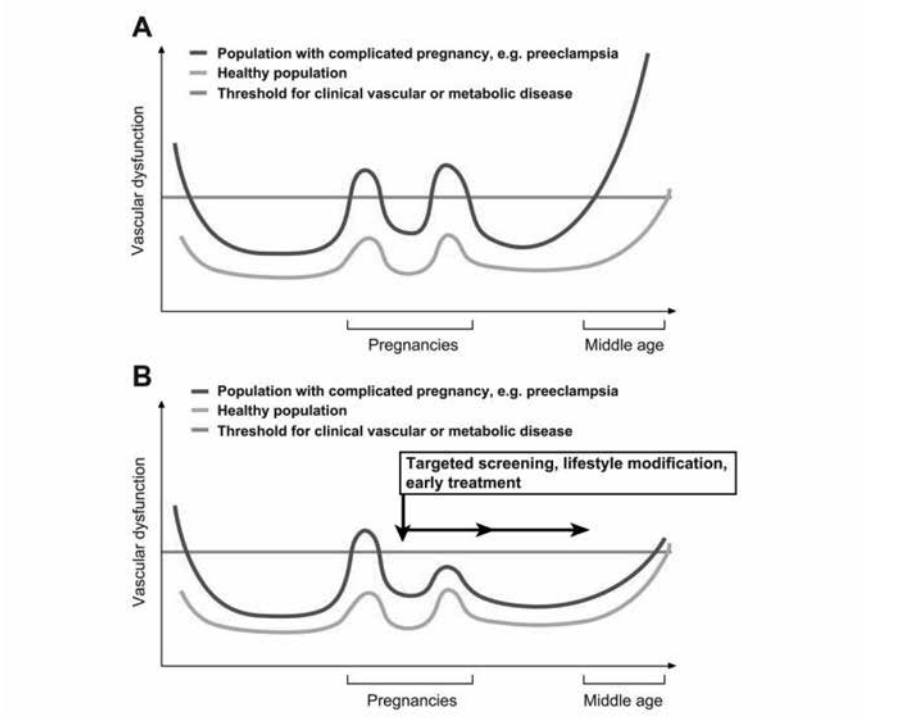

Figure 1 Schematic diagrams showing how pregnancy can be a window to future health by uncovering predispositions for cardiovascular diseases in women who develop pregnancy complications **(A)**, and how early intervention after these women are identified following their pregnancy can improve outcome of future pregnancy and prevent long term diseases **(B)**. *From J.W.Rich-Edwards et al. Hypertension 2010;56:331-334. Used with permission from BMJ publishing group and Wolters Kluwer Health Inc.*

Nowadays many women undergo cardiovascular and metabolic testing after their preeclamptic pregnancy to identify risk factors for future cardiovascular disease, based on the assumption that these risk factors are modifiable[14]. It is hypothesized that targeted screening, lifestyle modification and early treatment in women with a history of preeclampsia can improve outcome of future pregnancy and prevent long term diseases (Figure 1B).

The observed relationship between preeclampsia and future cardiovascular disease can only be partly explained by the increased prevalence of traditional cardiovascular risk factors alone. In an effort to explain this "risk factor gap" additional risk factors have been linked to both preeclampsia and cardiovascular disease, often referred to as non-traditional cardiovascular risk factors. Examples of non-traditional risk factors are markers of systemic inflammatory responses, thrombophilia[15], hyperhomocysteinemia[15], signs of renal disease[16] and specific circulatory abnormalities like cardiac dysfunction[17], endothelial dysfunction[18-20] and low plasma volume[21]. In women with a history of preeclampsia the hemodynamic profile characterized by low plasma volume is particularly interesting because low plasma volume status was demonstrated in almost 50% of formerly preeclamptic women; more frequent than any other studied cardiovascular risk factor so far[21].

Plasma volume refers to the total intravascular blood volume minus the cellular components of blood. In resting conditions, two-thirds of the plasma volume is localized in the venous system[22]. The venous system acts as a volume reservoir and if needed extra volume can be mobilized into the circulation by means of sympathetically mediated venoconstriction resulting in an increase of cardiac preload. Consequently, low plasma volume status reflects a reduced venous reserve capacity of the circulation and therefore a reduced ability to increase cardiac preload in times of increasing demands, like in pregnancy for example.

Previous studies have demonstrated that formerly preeclamptic women with prepregnant low plasma volume demonstrate a smaller increase of plasma volume during pregnancy compared with healthy parous control subjects[23]. Reduced plasma volume expansion in pregnancy provokes abnormal compensatory hemodynamic changes in an effort to cope with the increasing demands of advanced pregnancy. These changes can be characterized by a sympathetically driven hyperdynamic circulation with consequential increased shear stress on the vascular endothelium. These abnormal hemodynamic changes are thought to contribute to the hypertensive deterioration of pregnancy and eventually preeclampsia.

Low plasma volume is also linked with latent chronic hypertension[24]. Longitudinal studies in individuals with low plasma volume, other than formerly preeclamptic women, demonstrated a gradual change in hemodynamics from a hyperdynamic/low resistance circulation into a more hypodynamic/high resistance circulation with a consequent gradual increase in blood pressure over the years[25]. Resultantly low plasma volume is regarded as a form of latent hypertension in normotensive or borderline hypertensive individuals.

Based on the observed relationships between low plasma volume and both preeclampsia and chronic hypertension, studying plasma volume in women with a history of preeclampsia may advance our pathophysiological knowledge of cardiovascular disease in women. Ultimately, in order to translate and implement this knowledge into possible strategies of cardiovascular risk management in formerly preeclamptic women, it is necessary to test if low plasma volume and the associated hemodynamic consequences are amenable to modification.

The cornerstone of primary cardiovascular risk management is lifestyle intervention. Exercise training induces plasma volume expansion and represents a potent strategy to reduce the risk of future cardiovascular events in asymptomatic subjects and those with pre-existing disease[26]. Despite the overall health benefits of exercise training, recent studies have described heterogeneous cardiovascular adaptation to training[27]. In subjects who undertook similar exercise training interventions, some demonstrated large improvements in parameters such as cardiopulmonary fitness, blood pressure, and cholesterol, while others exhibited smaller increases or even "adverse" responses[28, 29]. In formerly preeclamptic women it is unknown to what extent plasma volume and associated hemodynamic characteristics change with exercise training. Based on the maternal maladaptation to the cardiovascular stimulus of pregnancy, formerly preeclamptic women may as well be maladaptive to the cardiovascular stimulus of exercise training.

Outline of this thesis

This thesis is based on five main research questions:

1. To what extent does nonpregnant plasma volume relate to the onset of preeclampsia and to the co-occurrence of other cardiovascular risk factors in women with a history of preeclampsia? (Chapter 2)
2. To what extent is the adult plasma volume determined at birth? (Chapter 3)
3. To what extent does nonpregnant plasma volume relate to the recurrence rate of preeclampsia and to the development of chronic hypertension after a pregnancy complicated by preeclampsia? (Chapter 4 and 5)
4. To what extent can plasma volume in formerly preeclamptic women be improved by means of aerobic exercise training in comparison with healthy parous control subjects? (Chapter 6)
5. What are the hemodynamic consequences of aerobic exercise training aimed at increasing plasma volume in women with a history of preeclampsia compared with healthy parous controls? (Chapter 6, 7 and 8)

In summary, this thesis aims to explore the implications of low plasma volume in normotensive women with a history of preeclampsia. We studied in these women the effects of low plasma volume status on outcomes of the subsequent pregnancy and the occurrence of chronic hypertension in the years following the complicated pregnancy. To test if plasma volume can be increased in women with a history of preeclampsia we examined the effects of 12-weeks aerobic exercise training on plasma volume and associated hemodynamic parameters like venous compliance, autonomic balance, endothelial function and shear characteristics of the arterial blood flow.

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CHAPTER 2

Co-occurrence of cardiovascular and prothrombotic risk factors in women with a history of preeclampsia

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Abstract

Formerly preeclamptic women are at increased risk for remote cardiovascular and thrombotic diseases. We studied co-occurrence of cardiovascular and prothrombotic risk factors within a cohort of formerly preeclamptic women, and tested if prevalence of these risk profiles related to onset of preeclampsia in previous pregnancy.

We evaluated 1297 nonpregnant formerly preeclamptic women (6-12 months postpartum) for the presence of four risk profiles: 1. circulatory risk profile (hypertension or latent hypertension [low plasma volume, increased vascular resistance or both]); 2. metabolic syndrome (World Health Organization criteria); 3. thrombophilia (factor V Leiden, prothrombin mutation or protein C/S deficiency); and 4. hyperhomocysteinemia. Trends between prevalence of these four profiles and onset of preeclampsia were studied using linear regression analysis.

After exclusion of 63 women (4.9%), because of incomplete data, 1234 women were included. One or more risk profiles were detected in 958/1234 (77.6%) formerly preeclamptic women. Circulatory risk profile was more prevalent (66.1%) than hyperhomocysteinemia (18.7%), metabolic syndrome (15.1%) or thrombophilia (10.8%). Prevalence of circulatory risk profile, metabolic syndrome and hyperhomocysteinemia decreased significantly with gestational age at delivery, whereas thrombophilia did not ($p=0.22$). There was minimal overlap ($<2\%$) between metabolic syndrome, thrombophilic profile and hyperhomocysteinemia.

Circulatory risk profile is present in two thirds of formerly preeclamptic women. Metabolic syndrome, thrombophilia and hyperhomocysteinemia are prevalent in 10-20%. There is considerable overlap between circulatory risk profile and other profiles, but not among the three other profiles. Prevalence of these risk factors, except thrombophilia, decreases with gestational age at delivery in preceding pregnancy.

Introduction

Preeclampsia complicates 2 to 7% of all pregnancies and is associated with considerable maternal and fetal morbidity[1]. Studies in the past decade show that women with a history of preeclampsia have an increased risk of development of thrombotic and cardiovascular disease later in life[2-6]. Preeclampsia and cardiovascular diseases are thought to have disease mechanisms in common[7-9]. Traditional cardiovascular risk factors are present in nulliparous women who subsequently develop preeclampsia[8,9]. Therefore, the cardiovascular risk profiles in formerly preeclamptic women are likely to reflect the pre-existing constitution rather than a consequence of hypertensive pregnancy itself[8,9].

Early onset of preeclampsia is clinically considered as the most important indicator of severity of preeclampsia[1]. The remote risk for cardiovascular disease is higher the earlier the onset of preeclampsia, and is sevenfold increased if preeclampsia occurs at less than 32 weeks of gestation compared with term onset preeclampsia[5].

Preeclampsia has been linked with a circulatory risk profile (hypertension or latent hypertension)[7,10,11], metabolic syndrome[12-14], thrombophilia[15-18], and hyperhomocysteinemia[15-18]. Most studies involved a single risk profile in relation to preeclampsia in the preceding pregnancy. Therefore, any possible interrelation between these profiles within women with a history of preeclampsia is currently unclear.

The extent to which the prevalence of underlying cardiovascular and prothrombotic risk factors increases after earlier onset of preeclampsia is unknown. In the present study we have attempted to elucidate this question. A secondary aim of this study was to estimate the possible overlap between separate risk profiles within women with a history of preeclampsia. We therefore evaluated the prevalence of four risk profiles (circulatory risk profile, metabolic syndrome, thrombophilia and hyperhomo-cysteinemia) in a cohort of women 6 to 12 months after a pregnancy complicated by preeclampsia.

Materials and methods

We conducted a retrospective analysis of 1297 women with a history of preeclampsia who were consecutively screened for possible cardiovascular and prothrombotic risk factors (January 2004 and December 2010). All women were screened at our preconception clinic, a tertiary referral center of the Radboud University Nijmegen Medical Center. All measurements were performed in the nonpregnant state, 6 to 12

months after pregnancy complicated by preeclampsia. Preeclampsia was diagnosed if women had blood pressure of 140/90 mm Hg or higher, measured twice, six or more hours apart, and consistent proteinuria of 300 mg/24 hours or more after gestational week 20 in previously normotensive women[19]. HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome was defined according to the criteria of the International Society on the Study of Hypertension in Pregnancy[20]. Intrauterine growth restriction (IUGR) was defined as birth weight less than the10th centile of the national birth weight chart[21]. Gestational age at delivery was taken as a proxy for onset of preeclampsia. At the time of measurements, none of the women used hormonal contraceptives, nor were they breastfeeding. All women were native Dutch. The study was approved by the Medical Ethics Committee of the Radboud University Nijmegen Medical Center (2007/252).

At enrollment, all participants collected urine in the 24 hours preceding the measurements. The 24- hour urine sample was assayed for albumin, protein and creatinine to calculate the (micro) albuminuria corrected for creatinine output (g/mol creatinine) and total protein level (g/24 hours) (Aeroset).

All measurements were performed in the morning, in the fasting state (overnight). Height and body mass (Seca 888 scale) were measured. Body surface area (BSA) was calculated using the Du Bois and Du Bois formula[22] for the normalization of plasma volume.

Blood pressure and heart rate were measured oscillometrically (Dinamap, Vital Signs Monitor 1846) at 3-minute intervals for 30 minutes at the right upper arm in the upright position. We used the median of nine consecutive measurements. Measurements were performed with the cuff size recommended for the arm circumference. We recorded systolic and diastolic blood pressures (BPsys and BPdia, mm Hg), mean arterial blood pressure (MAP, mm Hg) and heart rate (HR, beats per minute).

Venous blood samples were taken from an antecubital vein and analyzed for metabolic parameters: glucose, insulin, total cholesterol, high and low density lipoproteins and triglycerides (Aeroset). To estimate insulin resistance (homeostasis model assessment index (HOMA)) was calculated (insulin [mU/L] x glucose [mmol/L]/22.5) as the product of fasting glucose and insulin[23]. Factor V Leiden (F5 R506Q) and prothrombin (G20210A) mutation analyses were performed by routine polymerase chain reaction techniques. Citrated blood was centrifuged and plasma was assayed for total protein S, free protein S and the protein C activity. Total homocysteine levels were measured using high-performance liquid chromatographic assay.

Plasma volume (PV, ml) was measured using the ^{125}I-Human Serum Albumin indicator dilution technique (^{125}I-HSA). Plasma volume was calculated by dividing the total injected radioactivity by the virtual volume-specific radioactivity at time zero, as described elsewhere[24]. Plasma volume was normalized by dividing total plasma volume by body surface area (mL/m^2).

Echocardiographic measurements were obtained by an experienced cardiology technician. Measurements were performed in left lateral position, using a cross-sectional phased array echocardiographic Doppler system (Vivid 7). Left ventricular outflow tract velocity was measured using pulse wave Doppler, and midsystolic left ventricular outflow diameter also was measured. Stroke volume was calculated by multiplying the left ventricular outflow tract velocity integral and the left ventricular outflow tract area (as calculated from the left ventricular outflow tract diameter (^{2}). Heart rate was determined as the reciprocal of the time interval between the R waves of the electrocardiogram measured during the Doppler measurements. This heart rate was used only for the calculation of cardiac output. Cardiac output (CO) was calculated as stroke volume multiplied by heart rate. Total vascular resistance (TPVR) was calculated as 80 times the mean arterial pressure, divided by the cardiac output (80 x MAP/CO).

Circulatory risk profile: Circulatory risk profile was defined as hypertension, or the use of antihypertensive medication, or signs of latent hypertension, *in the absence* of metabolic syndrome. Hypertension was defined as BPsys of 140mmHg or higher or BPdia 85 mmHg or higher; latent hypertension as reduced plasma volume (PV less than 1405 mL/m^2) or increased total peripheral vascular resistance (TPVR: more than 1600 dynes.sec/cm^5). Hypertensive women who additionally met the criteria for metabolic syndrome were allocated to the metabolic syndrome profile to prevent women to be counted twice on the basis of their blood pressure. Women who used antihypertensive medication were excluded from analysis of plasma volume and vascular resistance in order to prevent any confounding effect of medication.

Metabolic syndrome: Metabolic Syndrome was defined using World Health Organization criteria[25] by the concomitant presence of insulin resistance (fasting insulin 9.2 mU/L or higher or fasting glucose 6.1 mmol/L or higher or HOMA of 2.2 or more) and two or more of the following factors: hypertension (systolic blood pressure 140mm Hg or higher or diastolic blood pressure 85 mm Hg or higher or the use of antihypertensive medication); obesity (body mass index 30 kg/m^2 or higher); dyslipidemia (triglycerides 1.69 mmol/L or higher or high-density lipoprotein 0.9 mmol/L or less); or micro-albuminuria (urine albumin 0.30 g/mol creatinine or more or urine protein 0.30 g/ 24 hours or more).

Thrombophilia: Hereditary thrombophilia was defined as the presence of Factor V Leiden or prothrombin 20210A mutation or protein S levels or protein C activity below the normal range (free protein S less than 55%, protein C activity less than 70%).

Hyperhomocysteinemia: Hyperhomocysteinemia was defined as a fasting homocysteine more than 12.1μmol/L[26].

Statistical analysis

To illustrate the relationship between gestational age at delivery in previous pregnancy and the prevalence of cardiovascular and prothrombotic risk factors, the study group was divided into four subgroups based on the gestational age at previous delivery (more than 22 to 28 weeks or less, more than 28 to 32 weeks or less, more than 32 weeks to 37 weeks or less, and more than 37 weeks of gestation). Outcome variables are presented as frequencies and percentage including 95% confidence interval (CI) for estimated proportions. For all risk factors, the relative risks with corresponding 95% CIs for each gestational age group were calculated, with the more than 37 weeks of gestation group used as reference. Trends in risk factors by onset of preeclampsia in the previous pregnancy were studied by linear regression analysis; gestational age at delivery was used as a continuous variable.

The change in cardiovascular and prothrombotic risks with each additional week of gestation in the preceding pregnancy was expressed by hazard ratios. Hazard ratios were estimated using multivariable regression analysis, with adjustments made for the following predefined factors: age, parity, smoking, or HELLP or IUGR in the preceding pregnancy. Statistical significance (two-sided *P*-value) was set at $P \leq .05$. The statistical analyses were performed using the standard statistical software package SPSS 16.0.

The inter-relation between the four risk profiles within formerly preeclamptic women was studied with the use of a scaled rectangle diagram similar to Venn diagrams in which each rectangle area is scaled according to the prevalence of the attribute[27]. Overlapping areas are proportional to the prevalence of joint occurrence of attributes.

Results

From the 1297 formerly preeclamptic women included in our study, 63 women had to be excluded because of one or more missing values in any of the studied risk profiles. The remaining 1234 women with a complete set of study variables were available for analysis. The characteristics of these women are shown in Table 1. Women delivered at a median of 33 weeks of gestation [interquartile range 29-36; range 19] in their previous pregnancy. The screening for possible risk factors was performed at a median of 7 months postpartum [interquartile range 6-10; range 6].

Table 1 General characteristics of the included formerly preeclamptic women

General characteristics	
N	1234
Age (years)	32 ± 4
BMI (kg/m²)	25.6 ± 5.3
Systolic blood pressure (mmHg)	120 ± 15
Diastolic blood pressure (mmHg)	73 ± 11
Mean arterial pressure (mmHg)	90 ± 12
Antihypertensive medication (n (%))	180 (15)
Smoking (n (%))	207 (17)
Primiparous (n (%))	991 (80)
Additional diagnoses in index pregnancy:	
- HELLP (n (%))	654 (53)
- Growth restricted infant (n (%))	432 (35)
Gestational age of delivery in previous pregnancy (weeks)	33 [29-36]
Screening, time after delivery (months)	7 [6-10]

HELLP, hemolysis, elevated liver enzymes, and low platelet count. Data are mean ± standard deviation, n (%), or median [range].

Any of the four risk profiles (circulatory profile, metabolic syndrome, thrombophilia, or hyperhomocysteinemia) was present in 958 of 1234 (77.6%; 95%CI 75.3-80.0%) formerly preeclamptic women (Table 2). The prevalence of having any risk profile decreased with gestational age at delivery in the preceding pregnancy (*P* for linear trend <.01). Absence of any of the four risk profiles occurred in 276 of 1234 (22.4%; 95%CI 20.0-24.7%) of the formerly preeclamptic women.

Table 2 Prevalence of any risk profile (either circulatory profile or metabolic syndrome or thrombophilia or hyperhomocysteinemia) in formerly preeclamptic women stratified by gestational age at delivery in preceding pregnancy.

		22-28 wks n=143	28-32 wks n=357	32-37 wks n=501	>37 wks n=233	Total n=1234	*P* for trend
Any risk profile	*n (%)*	123 (85.9)	288 (80.9)	394 (78.8)	154 (66.8)	958 (77.6)	
	RR [95%CI]	1.3 [1.2-1.4]	1.2 [1.1-1.3]	1.2 [1.1-1.3]	*reference*		<.01

Data are n(%) or relative risk [95% confidence interval]

Hypertension was present in 318 of 1234 formerly preeclamptic women (25.8%; 95%CI 23.3-28.2%). From these, 152 of 1234 women (12.4%; 95%CI 10.5-14.2%) had hypertension in the absence of metabolic syndrome; 166 of 1234 (13.4%; 95%CI 11.5-15.4%) additionally fulfilled the WHO criteria for metabolic syndrome and were analyzed as such.

Circulatory risk profile was present in 816 of 1234 women (66.1%; 95%CI 63.5-68.8%) (Table 3). The prevalence decreased with gestational age at delivery in the preceding pregnancy (*P* for linear trend <.01). In addition to the 12.4% women with overt hypertension, 664 of 1234 formerly preeclamptic women (53.8%; 95%CI 51.0-56.6%) had latent hypertension. In formerly preeclamptic women, not using antihypertensive medication 512 of 1054 (48.6%; 95%CI 45.6-51.6%) had low plasma volume and 247 of 1054 (23.4%; 95%CI 20.9-26.0%) had increased vascular resistance.

Metabolic syndrome was present in 191 of 1234 women (15.4%; 95%CI 13.5-17.5%) (Table 4). The prevalence decreased with gestational age at delivery in the preceding pregnancy (*P* for linear trend <.01). The prevalence of all components of the metabolic syndrome also decreased with gestational age at previous delivery (*P* for linear trend<.01), except for obesity. Hyperinsulinemia was present in 742 of 1234 (60.1%; 95%CI 57.4-62.9%) of formerly preeclamptic women. From these 206 of 742 (27.7%; 95%CI 24.5-31.0) met the criteria of metabolic syndrome; 536 of 742 (72.3%; 95%CI 69.0-75.5%) did not.

Thrombophilia was present in 133 of 1234 (10.8%; 95%CI 9.1-12.5) formerly preeclamptic women (Table 5). 130 of 133 (98%; 95%CI 95.2-99.2%) of the women with thrombophilia had only one thrombophilic factor. Women with either Factor V Leiden or prothrombine 20210A mutation all were heterozygous. The prevalence of

Table 3 Prevalence of circulatory risk profile in formerly preeclamptic women stratified by gestational age at delivery in preceding pregnancy.

		22-28 wks n=143	28-32 wks n=357	32-37 wks n=501	>37wks n=233	Total n=1234	*P* for trend
Circulatory risk profile	*n (%)* *RR [95%CI]*	104 (72.5) 1.3 [1.1-1.5]	245 (68.5) 1.2 [1.1-1.4]	334 (66.7) 1.2 [1.0-1.3]	132 (56.7) *reference*	816 (66.1)	<.01
- Hypertension (in absence of MetS)	*n (%)* *RR [95%CI]*	21 (14.7) 1.9 [1.1-3.4]	47 (13.2) 1.7 [1.0-2.9]	66 (13.2) 1.7 [1.0-2.8]	18 (7.8) *reference*	152 (12.4)	<.01
- Low plasma volume*	*n/N(%)* *RR [95%CI]*	69/128(53.9) 1.3 [1.1-1.7]	162/301(53.8) 1.3 [1.1-1.6]	199/424(46.9) 1.2 [0.9-1.4]	82/201(40.7) *reference*	512/1054(48.6)	.02
- Increased total peripheral vascular resistance*	*n/N(%)* *RR [95%CI]*	35/128(27.3) 1.7 [1.2-2.5]	78/301(25.9) 1.7 [1.2-2.3]	100/424 (23.6) 1.3 [1.0-1.9]	34/201(16.9) *reference*	247/1054 (23.4)	<.01

MetS, metabolic syndrome.

Data are n(%) or relative risk [95% confidence interval]

*Analyzed only in women who did not use antihypertensive medication.

Table 4 Prevalence of the metabolic syndrome and its' components in formerly preeclamptic women stratified by gestational age at delivery in preceding pregnancy.

		22-28 wks n=143	28-32 wks n=357	32-37 wks n=501	>37wks n=233	Total n=1234	*P* for trend
Metabolic Syndrome	*n (%)* *RR [95%CI]*	34 (24.0) 2.2 [1.3-3.5]	79 (22.2) 2.0 [1.3-3.1]	53 (10.5) 0.9 [0.6-1.5]	26 (11.1) *reference*	191 (15.4)	<.01
- **Hyperinsulinemia**	*n (%)* *RR [95%CI]*	98 (68.8) 1.3 [1.1-1.5]	239 (67.0) 1.2 [1.1-1.4]	277 (55.3) 1.0 [0.9-1.2]	126 (54) *reference*	742 (60.1)	<.01
- **Hypertension**	*n (%)* *RR [95%CI]*	46 (32.1) 1.8 [1.2-2.5]	107 (30.1) 1.6 [1.2-2.2]	122 (24.4) 1.3 [1.0-1.8]	43 (18.3) *reference*	318 (25.8)	<.01
- **Obesity**	*n (%)* *RR [95%CI]*	25 (17.7) 1.1 [0.7-1.8]	77 (21.7) 1.4 [1.0-2.0]	74 (14.7) 0.9 [0.7-1.4]	37 (15.7) *reference*	213 (17.3)	.97
- **Dyslipidemia**	*n (%)* *RR [95%CI]*	34 (24.1) 1.6 [1.1-2.4]	91 (25.5) 1.7 [1.2-2.5]	81 (16.2) 1.1 [0.7-1.6]	35 (14.9) *reference*	241 (19.5)	<.01
- **Microalbuminuria**	*n (%)* *RR [95%CI]*	44 (31) 1.9 [1.3-2.8]	99 (27.7) 1.7 [1.2-2.4]	93 (18.5) 1.1 [0.8-1.6]	38 (16.2) *reference*	274 (22.1)	<.01

Data are n(%) or relative risk [95% confidence interval]

Table 5 Prevalence of thrombophilia in formerly preeclamptic women stratified by gestational age at delivery in preceding pregnancy.

		22-28 wks n=143	28-32 wks n=357	32-37 wks n=501	>37wks n=233	Total n=1234	*P* for trend
Any thrombophilia	*n (%)* *RR [95%CI]*	13 (9.1) 1.1 [0.7-1.8]	49 (13.7) 1.2 [0.8-1.8]	50 (10.0) 1.3 [0.9-1.8]	21 (9.0) *reference*	133 (10.8)	 .22
- **Factor V Leiden**	*n (%)* *RR [95%CI]*	9 (6.5) 1.1 [0.5-2.7]	21 (5.8) 1.0 [0.5-2.1]	17 (3.4) 0.6 [0.3-1.3]	13 (5.7) *reference*	60 (4.9)	 .39
- **Prothrombin**	*n (%)* *RR [95%CI]*	2 (1.7) 3.1 [0.3-34.1]	12 (3.4) 6.3 [0.8-50.2]	18 (3.6) 6.6 [0.9-50.0]	1 (0.5) *reference*	33 (2.7)	 .59
- **Protein S deficiency**	*n (%)* *RR [95%CI]*	2 (1.4) 1.1 [0.2-6.8]	9 (2.5) 1.8[0.5-6.5]	8 (1.6) 1.1[0.3-4.2]	3 (1.3) *reference*	22 (1.8)	 .74
- **Protein C deficiency**	*n (%)* *RR [95%CI]*	0 (0) N/A	9 (2.6) 1.5 [0.5-4.6]	8 (1.6) 1.0 [0.3-3.0]	4 (1.8) *reference*	21 (1.7)	 .89

N/A: not applicable
Data are n(%) or relative risk [95% confidence interval]

thrombophilia did not change with gestational week at previous delivery (*P* for trend .22). Factor V Leiden was the most prevalent thrombophilic factor 60 of 1234 (4.9%; 95%CI 3.7-6.1%).

Hyperhomocysteinemia was present in 231 of 1234 (18.7%; 95%CI 16.5-20.9%) formerly preeclamptic women (Table 6). The prevalence decreased with gestational age at delivery in the preceding pregnancy (*P* for linear trend <.01).

Table 7 summarizes the hazard ratios for all four studied profiles associated with each additional week at delivery in the preceding pregnancy. Except for thrombophilia all profiles show a decreasing prevalence with each additional week at delivery in the preceding pregnancy (ie, the later onset of preeclampsia). These trends were preserved after adjustment for age, parity, smoking and the additional diagnoses of HELLP syndrome or IUGR as indicated by the adjusted hazard ratios.

Table 6 Prevalence of hyperhomocysteinemia in formerly preeclamptic women stratified by gestational age at delivery in preceding pregnancy.

		22-28 wks n=143	28-32 wks n=357	32-37 wks n=501	>37wks n=233	Total n=1234	*P* for trend
Hyperhomo-cysteinemia	*n (%)*	32 (22.6)	80 (22.3)	92 (18.4)	27 (11.5)	231(18.7)	
	RR [95%CI]	2.0 [1.2-3.2]	1.9 [1.3-2.9]	1.6 [1.1-2.4]	*reference*		<.01

Data are n(%) or relative risk [95% confidence interval]

Table 7 Hazard ratios for the prevalence of the risk profiles with each additional gestational week at delivery in preceding pregnancy.

	Hazard Ratio [95%CI]	Adjusted-Hazard Ratio [95%CI]*
Circulatory risk profile	0.96 [0.93-0.99] (*P* <.001)	0.96 [0.93-0.99] (*P* <.001)
Metabolic syndrome	0.92 [0.88-0.95] (*P* <.001)	0.91 [0.87-0.96] (*P* <.001)
Any thrombophilia	0.99 [0.95-1.02] (*P* =.81)	1.0 [0.96-1.03] (*P* =.68)
Hyperhomocysteinemia	0.95 [0.91-0.98] (*P* =.004)	0.95 [0.91-0.99] (*P* =.01)

* Hazard ratio after adjustments for age, parity, smoking and additional diagnosis of HELLP and growth restricted infant in the index pregnancy.

Figure 1 demonstrates the prevalence of the four studied risk profiles and their interrelation. In total 958 of 1234 (77.6%; 95%CI 75.3-80.0%) formerly preeclamptic women had one or more risk profiles. Three hundred seventeen of 1234 (25.7%; 95%CI 23.3-28.1%) women with a history of preeclampsia had more than one risk profile, more specifically 278 of 1234 (22.5%; 95%CI 20.2-24.9%) women had two concurrent risk profiles, 37 of 1234 (3%; 95%CI 2.1-4.0%) women had three concurrent risk profiles and only 2 of 1234 (0.2%; 95%CI 0.0-0.4%) women had all four risk profiles after a pregnancy complicated by preeclampsia. Metabolic syndrome, thrombophilia

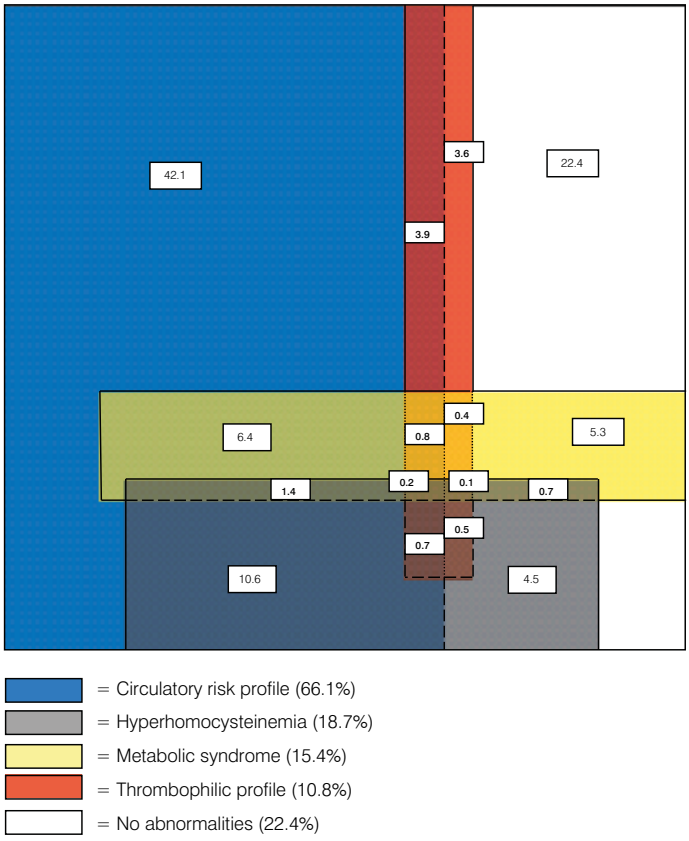

Figure 1 Scaled rectangle diagram indicating the prevalence and co-occurrence of circulatory risk profile, metabolic syndrome, thrombophilia and hyperhomocysteinemia in formerly preeclamptic women. Numbers represent percentages relative to the total study population. Each rectangle area is scaled according to the prevalence of the attribute. Overlapping areas are proportional to the prevalence of joint occurrence of attributes.

and hyperhomocysteinemia each co-occurred considerably with the highly prevalent circulatory risk profile. Co-occurrence of risk profiles other than the circulatory risk profile was uncommon (less than 2%).

Discussion

We have tested in each patient the four most common risk factors associated with preeclampsia. This allowed us to study the interrelationship between the risk profiles.

Two thirds of formerly preeclamptic women demonstrated a (latent) hypertensive hemodynamic profile 6 to 12 months post partum. One fourth of formerly preeclamptic women had hypertension and 50% of these hypertensive women additionally met the criteria for metabolic syndrome. The high prevalence of the circulatory risk profile (66%) was mainly attributable to reduced plasma volume status. Low plasma volume after preeclampsia predisposes to recurrent preeclampsia, IUGR and preterm birth in subsequent pregnancy[24]. Low plasma volume reflects reduced cardiovascular reserve capacity, a condition that, together with increased vascular resistance, relates to the development of chronic hypertension within the next decade. Timely blood pressure lowering leads to reductions in vascular disease risk and premature death[28,29]. Therefore, formerly preeclamptic women may well benefit from circulatory follow-up, most accessibly by blood pressure measurements, even in women who are normotensive in the first year after pregnancy. Circulatory follow-up does not necessitate the measurement of plasma volume status and total peripheral vascular resistance per se.

The prevalence of the metabolic syndrome in formerly preeclamptic women (15%) was approximately three times higher than that of the general Dutch female population of comparable age (5%)[30]. The prevalence of metabolic syndrome and all its components – except obesity – decreased with gestational age at delivery. This observation suggests that the other components relate to severity of preeclampsia, whereas obesity does not. Metabolic syndrome not only increases the risk of (recurrent) preeclampsia[13,14,31,32], but also increases the risk of later cardiovascular disease[33,34], and diabetes mellitus[33,35,36]. These risks can be reduced by weight management and physical activity, or by medical treatment when lifestyle adjustments are ineffective[37-39]. Recognizing the metabolic syndrome in formerly preeclamptic women is likely efficient and cost effective[40, 41].

The prevalences of Factor V Leiden (4.9%) and prothrombin 20210A (2.7%) mutation in our study were comparable to those in the Dutch general population[42,43], whereas

those of Protein C (1.7%) and S (1.8%) deficiency were slightly increased[44,45]. The added value of routine screening for these factors in formerly preeclamptic women seems limited. However a recent randomized trial in women with hereditable thrombophilia and previous early-onset hypertensive disease in pregnancy has demonstrated a reduction of recurrent preeclampsia by combined treatment with low molecular weight heparin and aspirin[46]. Our study was not designed to answer definitively the question of to what extent the association between thrombophilia and preeclampsia is causal or if women should be routinely screened and treated for possible thrombophilia after preeclampsia. If the decision to screen and treat is made, it would seem more reasonable to test all formerly preeclamptic women rather than only those with early-onset disease.

Almost one out of five formerly preeclamptic woman had hyperhomocysteinemia (19%), which is four times higher than in the general population. Hyperhomo-cysteinemia co-occurred with the circulatory risk profile in 69% of the cases. This evident overlap might be explained by increased vascular tone, given that hyperhomocysteinemia induces vascular damage by the formation of free oxygen radicals, ultimately resulting in proliferation of smooth-muscle cells and alterations in endothelial function and structure[47]. Screening and treatment for hyperhomo-cysteinemia could be considered for four reasons. First, hyperhomocysteinemia increases the risk of future cardiovascular disease, however supplementation has not been proven effective in reducing that risk[48]. Second, two multivitamin supplementation containing folic acid in women with hyperhomocysteinemia reduces the risk of preeclampsia[49, 50]. Third, hyperhomo-cysteinemia increases the risk of venous and arterial thrombosis that can be reduced by vitamin supplementation[15, 16, 51]. Fourth, hyperhomocysteinemia is associated with fetal closure defects that can be reduced by vitamin B supplementation[52]. For these reasons it seems warranted to screen formerly preeclamptic women for hyperhomo-cysteinemia. Formerly preeclamptic women are likely to benefit from screening and tailored treatment for their risk profile(s)[53].

Our study may have some methodological limitations. First, our study population represents that of a tertiary clinic. This may limit generalization of the results to the general population of formerly preeclamptic women because of possible overrepresentation of women with a history of more severe preeclampsia. It is conceivable that this may have resulted in overestimation of the prevalence of risk profiles. This effect is probably small, because adjustments for co-occurrence of HELLP and the delivery of IUGR, as indicators of severity of preeclampsia, did not affect the results. Second, the formerly preeclamptic women in our study were evaluated 6 to 12 months post partum. We cannot rule out a possible overestimation of the prevalence of cardio-vascular risk factors because improvement may still continue thereafter. Although

further recovery has been reported up to two years postpartum[57], the literature suggests that abnormal hemodynamic variables in formerly preeclamptic women largely (>80%) resolve within 6 months after giving birth. Third, our study includes only Caucasian women. This may limit generalization, since other ethnic groups may display different profiles.

In conclusion, 77.6% of women with a history of preeclampsia have one or more cardiovascular or prothrombotic risk factors. The circulatory risk profile was most prevalent. The prevalence of the circulatory risk profile and also components of the metabolic syndrome other than obesity and hyperhomocysteinemia relate inversely with the gestational age at delivery in preceding pregnancy. Although these trends were statistically significant the differences were small. The prevalence of thrombophilia is unaffected by the gestational age at delivery. Apart from the highly prevalent circulatory risk profile we found minimal overlap between the metabolic syndrome, hyperhomocysteinemia and thrombophilia. Early identification and management of cardiovascular risk factors have the potential to favorably influence the incidence of recurrent hypertensive disease in future pregnancies and also the long-term cardiovascular morbidity and mortality in this specific population of women at high risk.

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CHAPTER 3

Impaired fetal growth and low plasma volume in adult life

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Abstract

Low plasma volume precedes chronic hypertension. In line with the fetal origins of adult disease theory, our aim was to estimate whether normotensive women who were born small for gestational age have low plasma volume in adult life.

In 280 normotensive women with a history of hypertension in pregnancy we recorded recalled gestational age and weight at birth and measured plasma volume (I^{125}-HSA indicator dilution method). To correct for possible confounders, we recorded recent obstetric history and measured in each individual all constituents of the metabolic syndrome (World Health Organization criteria), sex hormones (progesterone and estradiol), renal function, and cardiac performance at rest (echocardiography). We estimated daily activity level with validated questionnaire (Short Questionnaire to Asses Health-enhancing physical activity). We studied the relation between women's own birth weight centile and her adult plasma volume (mL) and adjusted for the effects of confounding variables using multiple linear regression analysis.

Birth weight correlated positively with adult plasma volume ($P<0.001$). Linear regression analysis demonstrated that each 10-centile change in birth weight is associated with an average change of 46.6 mL [95%CI: 30.8-62.3] in adult plasma volume. This association persisted after adjustment for confounding factors (current body surface area, mean arterial pressure, total vascular resistance, glomerular filtration rate, and total 24 hours of sodium output). After adjustment, each 10-centile change in birth weight was associated with an average change of 32.1 mL [95%CI: 19.6-44.6] in adult plasma volume. Birth centile contributes 14% to the variation in total adult plasma volume.

Impaired fetal growth is associated with low plasma volume in adult life.

Introduction

The association between low birth weight and increased blood pressure in adult life and mortality from cardiovascular disease has been well established[1,2]. So far, possible mechanisms linking fetal growth restriction to higher blood pressures in adulthood may be found in persisting changes in vascular structure, including loss of elasticity in vessel walls[3] or increased insulin resistance[4,5], altered glucocorticoid balance[6] or abnormal cholesterol metabolism[7].

Low plasma volume in the absence of hypertension is related to the early, often latent, phase of chronic hypertension[8-10]. At rest, two-third of the human blood volume is localized in the venous system[11]. Quantitatively, most of the total venous return (47%) comes from veins within splanchnic organs and kidneys[11]. Therefore the splanchnic vascular bed is often regarded as the quantitatively most important system to 'store' blood volume. Theoretically, a structural small venous abdominal compartment negatively affects the total plasma volume.

Asymmetrical fetal growth restriction is characterized by a disproportionately small abdominal circumference as a consequence of loss in liver, kidney and intestinal volume[12]. This is thought to originate from selective growth failure of splanchnic organs as a consequence of circulatory redistribution away from this abdominal region[13]. Because only arteries have regenerative capacity[11], limited venous intra-uterine development may have long-lasting effects on total plasma volume.

In line with the fetal origins of adult disease theory, we hypothesized that women born small for gestational age (SGA) have lower plasma volume levels in adult life, compared to women born appropriate or large for gestational age (LGA).

Materials and methods

We conducted a retrospective observational study in 347 consecutive healthy women with a history of hypertension in pregnancy in whom plasma volume was measured between January 2008 and December 2010. We used a database of women who were extensively evaluated in our tertiary hospital after hypertensive disorders in pregnancy. We recorded recalled birth weight and gestational age at birth. If women were unsure of these characteristics, women were excluded from this analysis. Women with pre-existing medical conditions or who used any medication were also excluded.

All measurements were performed, in the non-pregnant state, 6 to 12 months after a pregnancy complicated by preeclampsia and/or HELLP syndrome. The diagnoses of gestational hypertension, preeclampsia and HELLP were according to the criteria of the American College of Obstetricians and Gynecologists (ACOG)[15]. Except for their history of complicated pregnancy all women were healthy.

Birth weight centiles of women included in this analysis were calculated according to the national reference population of women born in the same era[14]. Study group was stratified into four birth weight groups. Small for Gestational Age (SGA) was defined as birth centile less than 10%. Women born Appropriate for Gestational Age (AGA) were subdivided in women born 10th or more and less than 50th centile (AGA-1) and women born between 50th centile and 90th centile (AGA-2). Large for Gestational Age (LGA) was defined as belonging to the more than 90% birth centile.

At the time of measurements none of the women used hormonal contraceptives, nor were they breastfeeding. All women were white. The study was approved by the institutional review board of the Radboud University Nijmegen Medical Centre.

Experimental procedures

Plasma volume (PV, ml) was measured using the ^{125}I-Human Serum Albumin indicator dilution technique (^{125}I-HSA). During the measurement, subjects stayed in a semi-supine position on a comfortable bed. An intravenous catheter was inserted in the left antecubital vein for the repetitive blood sampling. A standardized dose (0.2 MBq) of ^{125}I-HSA was injected intravenously in the right antecubital vein. Every 10 minutes a venous blood sample was taken from the contra lateral intravenous catheter until 40 minutes after administration of the ^{125}I-HSA. Blood samples were analyzed using a gamma counter. Plasma volume was calculated by dividing the total injected radioactivity by the virtual volume-specific radioactivity at time zero, as described elsewhere[16]. Plasma volume was indexed for body surface area (PV/BSA, mL/m^2). The technician measuring plasma volume was unaware of the birth weight and medical history of participants.

To explore possible confounding effects of hemodynamic, metabolic, endocrine, or lifestyle factors we systematically determined these profiles in each participant on the same day as the plasma volume measurement.

All participants collected urine in the 24 hours preceding the measurements. The 24-hour urine sample was assayed for sodium output (mmol/24 hour), micro-albumine corrected for creatinine output (g/mol creatinine) and creatinine (mmol/L).

Height and body mass were measured. Body surface area (BSA) for the normalization of the plasma volume, was calculated using the Dubois and Dubois formula[17]. Subsequent measurements were performed after an acclimatization period of at least 20 minutes in the supine position in a temperature controlled room (20-22 ^{o}C). All measurements were performed in the morning, in the fasting state (overnight).

Blood pressure and heart rate were measured oscillometrically at 3-minute intervals for 30 minutes at the right upper arm. We used the median values of 9 consecutive measurements. Measurements were done with the cuff size recommended for the arm circumference. We recorded systolic (BPsys) and diastolic (BPdia) blood pressures (mmHg) and heart rate (HR; bpm). Pulse pressure (dP; mmHg) was calculated as the difference between median systolic and diastolic blood pressures. We calculated mean arterial pressure (MAP; mmHg) using the formula: (2*BPdia + BPsys)/3.

Echocardiographic measurements were obtained by an experienced cardiology technician. Measurements were performed in left lateral position, using a cross-sectional phased array echocardiographic Doppler system (Vivid 7, General Electric, Horten, Norway). Left atrial diameter was measured in the four-chamber view. Subsequently left ventricular outflow traject velocity integral, and left ventricular outflow diameter were measured. Heart rate (HR) was determined by the reciprocal of the RR interval of the ECG measured during the echo Doppler measurements. This heart rate was used only for the calculation of cardiac output. Stroke volume (SV) was calculated by multiplying the left ventricular outflow tract velocity integral and the left ventricular outflow tract diameter. Cardiac Output (CO) was calculated as SV*HR. Total peripheral vascular resistance (TPVR) was calculated as eighty times the mean arterial pressure (MAP), divided by the cardiac output (80*MAP/CO). Global Vascular Compliance (GVC) was calculated from stroke volume and pulse pressure using the equation: GVC= SV/ dP (mL/mmHg).

Venous blood samples were taken from the in situ intravenous catheter and analyzed for metabolic parameters: glucose (mmol/L), insulin (mU/L), total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides (mmol/L), sex hormone levels (progesterone (nmol/l), and estradiol (pmol/L)) and renal function (serum creatinine level (μmol/L)). HOMA-IR was calculated using the formula: [fasting serum insulin (mU/L) x fasting plasma glucose (mmol/L)/22.5]. Creatinine clearance was calculated from the 24-hour urine creatinine concentration and the plasma creatinine concentration, corrected for BSA and expressed in mL/min/1.73m^2.

Daily activity was assessed with a validated questionnaire (Short Qestionaire to Asses Health enhancing physical activity; SQUASH)[18]. Participants were asked to describe an average week. Intensity was expressed as metabolic equivalent of task (MET), a physiological concept of expressing the energy of physical activities as multiples of resting metabolic rate (RMR), set by convention to 3.5 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$. Activities were subdivided into three intensity categories 2.0 to < 4.0 MET (light), 4.0 to < 6.5 MET (moderate), and ≥ 6.5 MET (vigorous), using Ainsworth's compendium of physical activities[19]. Activities below 2 MET value were not included, since they are considered to contribute negligibly to habitual activity levels. The SQUASH questionnaire quantifies commuting and work related activities, leisure activities (including sports) and household activities. Physical activity is presented as minutes/day (median [inter quartile range]) for each intensity category (light, moderate and vigorous). For each individual, the sum of the physical activity in each intensity category (total daily activity level; min/day) was used in the multivariable analysis.

Statistical analysis

Data were expressed as means ± standard deviations or as medians with interquartile range for normally and non-normally distributed data, respectively. Normality of each variable was evaluated using Kolmogorov-Smirnov tests. Trends were analyzed by linear regression of the adult characteristics on birth centile as a continuous variable and not categorical.

We calculated the change in adult plasma volume (mL) associated with a 10 centile increase in birth centile by linear regression analysis. The effects of possible confounding variables based on their known potential association with adult plasma volume included: current age, body mass index, smoking, daily activity level, progesterone level, estradiol level, mean arterial pressure, global vascular compliance, insulin level, glomerular filtration rate, 24-hour sodium output and interval between delivery and measurement. In the end, adjustments were made by multiple linear regressions for those factors that also related to birth centile (body surface area, mean arterial pressure, total vascular resistance, creatinine clearance and total 24-hours' sodium output).

To identify factors contributing to the adult plasma volume status, we first tested univariably (Pearson's) which variables correlated with total adult plasma volume (mL). Subsequently, factors correlating with plasma volume ($P<0.05$) were introduced in a backward stepwise linear regression analysis to weigh the potential determinants of the adult total plasma volume (mL). To quantify the independent contribution of each variable in our final model for adult plasma volume, we consecutively removed factors from our model, starting with the quantitatively most important factor based on the largest change in the explained variance (R^2) after removal. We present the

explained variance (R^2) of the final model, and the R^2 of the subsequent models after consecutive removal of a variable. Statistical significance (2-sided *P*-value) was set at ≤.05. The statistical analyses were performed using the standard statistical software package SPSS 16.0.

Results

Two hundred eighty women were available for analysis after exclusion of 67 women. Exclusion followed after inability to recall their own birth characteristics (n=51); 14 women had to be excluded because of pre-existing medical conditions or use of medication. Two women in whom plasma volume could not be adequately determined due to technical reasons were also excluded (Fig. 1). Women who were excluded for not being able to recall their own birth weight (n=51) did not differ from the women included in our study (n=280) in plasma volume (2775 vs. 2761 mL, *P*=.58), current body surface area (1.81 vs.1.81 m^2, *P*= 0.71), mean arterial pressure (85 vs. 84 mmHg, *P*=.62), total vascular resistance (1297 vs. 1329 dynes·s/cm^5, P=.42), glomerular filtration rate (109 vs. 110 mL/min/1.73m^2, *P*=.81) and total 24-hour sodium output (134 vs. 135 mmol/24 hour, *P*=.75).

Table 1 demonstrates the general adult characteristics and the characteristics at birth of the women included in the analysis stratified by the four birth centile groups. With

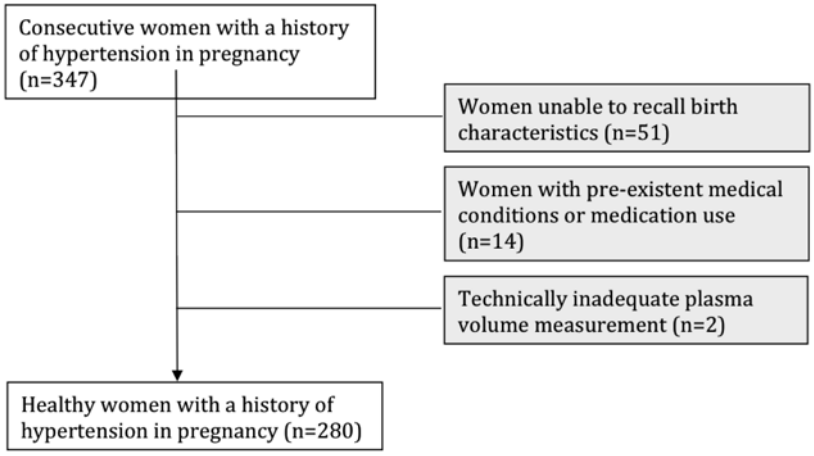

Figure 1 Overview of the in- and excluded women. 280 women were eligible for final analysis.

Table 1 General characteristics and characteristics at birth of included women, stratified by birth weight centile group (N=280).

	SGA (<p10) (n=40)	AGA-1 (p10-p50) (n=112)	AGA-2 (p50-p90) (n=84)	LGA (>p90) (n=44)	*P* for trend
General adult characteristics					
Age (years)	31 ± 5	32± 4	32 ± 5	32 ± 5	.88
Height (cm)	166 ± 6	169 ± 7	170 ± 6	174 ± 7	**.001**
Weight (kg)	70 ± 14	70 ± 14	72 ± 17	75 ± 16	**.02**
BSA (m^2)	1.76 ± 0.16	1.79 ± 0.18	1.82 ± 0.21	1.91 ± 0.18	**.004**
BMI (kg/m^2)	25 [22-27]	23 [21-26]	24 [21-28]	24 [21-28]	.22
Smoking (%)	21	12	13	18	.74
Characteristics at birth					
Birth weight (grams)	2457 ± 288	2932 ± 336	3413 ± 362	3883 ± 563	**<.001**
Gestational age at birth (days)	281 ± 10	277± 13	276 ± 14	276 ± 15	.17
Birth weight centile	5 ± 2	26 ± 11	71 ± 11	96 ± 3	

SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; BSA, body surface area; BMI, body mass index. Data are mean (±standard deviation) or median [range] unless otherwise noted. *P*-value indicates linear trend based on regression analysis with birth weight centile used as a continuous variable.

increasing birth weight centile, both adult height and weight increased, this pattern in body composition was also reflected in the body surface area (*P* for trend =.004). The gestational age at birth did not differ between birth centile groups.

Linear regression analysis demonstrated that each 10-centile change in birth weight relates to an associated with an average change of 46.6 mL [95%CI: 30.8-62.3] in adult plasma volume (Fig. 2). When plasma volume was normalized for adult body surface area, the positive correlation between birth centile and adult plasma volume persisted (*P* for trend: <.001) (Table 2). Women born SGA had 11% lower plasma volume (mean: 1349 ± 200 mL/m^2) compared to women born AGA-1 (mean: 1518 ± 156 mL/m^2). Furthermore, women born LGA had 10% higher plasma volume (mean: 1663 ± 229 mL/m^2) compared to women born AGA-1. Plasma volume in women born AGA-2 (mean: 1544 ± 137 mL/m^2) did not differ from AGA-1.

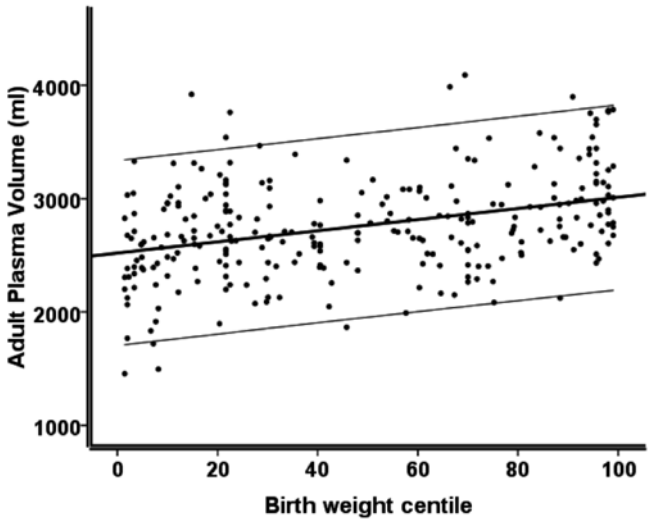

Figure 2 Adult plasma volume (mL) plotted against women's own birth weight centile, with regression line and corresponding 95% prediction interval.

Hemodynamically, birth centile related inversely to the mean arterial blood pressure (*P* for trend =.04) (Table 2). This trend was reflected in both systolic and diastolic blood pressures. Total vascular resistance in adulthood tended to decrease with increasing birth centile (*P* for trend=.09). We found no differences in cardiac performance between birth centile groups. Also physical activity levels did not differ between birth centile groups.

Insulin level, HOMA-ir and triglyceride level were higher in women born SGA compared to women born AGA-1 (Table 3). Metabolically we found no linear trends between birth centile and glucose metabolism or lipid concentrations. In contrast, glomerular filtration rate increased with birth weight centile (*P* for trend = .05). The 24-hour sodium output tended to increase with birth centile (*P* for trend = .08). When the specific cardiovascular risk factors were clustered according to WHO criteria for the metabolic syndrome, women born SGA had the highest prevalence of Metabolic Syndrome (23 %). Estrogen and progesterone levels did not differ between groups.

To explore possible confounding effects of the recent pregnancy, Table 4 summarizes the recent obstetric history of the included women. By inclusion, all women met the criteria for pregnancy-induced hypertension in previous pregnancy. In our study population 176 (63%) women additionally met the criteria for preeclampsia. As indicated

Table 2 Hemodynamic variables and current daily activity level at the time of plasma volume measurement of included women, stratified by birth weight centile group (N=280).

	SGA (<p10) (n=40)	AGA-1 (p10-p50) (n=112)	AGA-2 (p50-p90) (n=84)	LGA (>p90) (n=44)	*P* for trend
Hemodynamic profile					
Systolic blood pressure (mmHg)	119 ±11	116 ± 12	114 ± 9	113 ± 10	**.04**
Diastolic blood pressure (mmHg)	72 ± 8	71 ± 9	70 ± 6	68 ± 8	**.04**
Mean arterial pressure (mmHg)	86 ± 8	84 ± 9	83 ± 7	82 ± 8	**.04**
Resting heart rate (bpm)	71 ± 12	71 ± 12	71 ± 10	71 ± 10	.95
Stroke volume (mL)	77 ± 17	78 ± 20	75 ± 19	82 ± 20	.70
Cardiac output (L/min)	5.2 ± 1.3	5.5 ± 1.5	5.4 ± 1.6	5.7 ± 1.4	.18
Cardiac index (L/min/m^2)	2.9 ± 0.6	3.1 ± 0.8	3.0 ± 0.8	3.1 ± 0.7	.26
Total vascular resistance (dynes·s/cm^5)	1331 [1093-1655]	1242 [1058-1553]	1239 [1057-1526]	1149 [915-1374]	.09
Global vascular compliance (mL/mmHg)	1.7 ± 0.4	1.8 ± 0.5	1.7 ± 0.5	1.8 ± 0.5	.40
Total plasma volume (mL)	2389 ± 421	2714 ± 383	2796 ± 403	3096 ± 400	**<.001**
Normalized plasma volume (mL/m^2)	1349 ± 200	1518 ± 156	1544 ± 137	1663 ± 229	**<.001**
Current daily activity level					
2-4 MET, light (min/day)	362 ± 54	355 ± 61	364 ± 68	355 ± 59	.81
4-6.5 MET, moderate (min/day)	58 ± 22	58 ± 34	62 ± 39	55 ± 30	.62
≥ 6.5 MET, vigorous (min/day)	4 [1-10]	5 [1-9]	3 [0-9]	4 [1-10]	.77
Total daily activity level (min/day)	336 ± 84	354 ± 100	349 ± 102	363 ± 96	.40

SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; MET, metabolic equivalent of task. Data are mean (±standard deviation) or median [range] unless otherwise noted. *P*-value indicates linear trend based on regression analysis with birth weight centile used as a continuous variable.

Table 3 Metabolic profile, kidney function and sex hormone status at the time of plasma volume measurement, stratified by birth weight centile group (N=280).

	SGA (<p10) (n=40)	AGA-1 (p10-p50) (n=112)	AGA-2 (p50-p90) (n=84)	LGA (>p90) (n=44)	*P* for trend
Metabolic profile					
Glucose (mmol/L)	5.0 ± 0.5	4.9 ± 0.5	5.0 ± 0.5	4.8 ± 0.5	.52
Insulin (Umol/L)	13.3 [10.0-18.8]	9.9 [7.2-13.0]	11.0 [7.9-14.8]	10.0 [8.0-14.4]	.60
HOMA-ir	2.8 [1.8-3.9]	2.1 [1.5-2.9]	2.3 [1.7-3.6]	2.2 [1.6-3.1]	.62
Total cholesterol (mmol/L)	4.8 ± 0.8	4.7 ± 0.8	4.8 ± 0.9	4.5 ± 0.8	.67
LDL-c (mmol/L)	2.8 ± 0.8	2.9 ± 0.7	3.0 ± 0.8	2.8 ± 0.6	.59
HDL-c (mmol/L)	1.2 ± 0.3	1.3 ± 0.3	1.4 ± 0.3	1.2 ± 0.3	.85
Triglycerides (mmol/L)	1.1 [0.8-2.0]	0.9 [0.7-1.2]	0.9 [0.7-1.3]	0.9 [0.7-1.3]	.12
Kidney function					
Creatinine (μmol/L)	68 ± 12	65 ± 9	65 ± 10	64 ± 10	.35
GFR (mL/min/1.73m^2)	106 ± 17	109 ± 24	114 ± 30	116 ± 24	**.05**
24-hours sodium output (mmol/24 hour)	129 ± 55	130 ± 55	140 ± 56	147 ± 57	.08
Microalbuminuria (gram/mol creatinine)	0.9 [0.4-2.0]	0.8 [0.5-1.4]	0.6 [0.4-1.3]	0.7 [0.4-1.4]	.10
Metabolic Syndrome					
According WHO criteria (%)	23	10	11	14	.91
Hormonal status					
Progesterone (nmol/L)	2.4 [1.3-7.4]	2.1 [1.3-18]	2.1 [1.3-14]	2.5 [1.3-16}	.56
Oestradiol (pmol/L)	250 [144-530]	300 [160-510]	255 [133-478]	290 [130-590]	.93

SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; HOMA: Homeostasis Model of Assessment- Insulin Resistance (HOMA-ir); LDL, low-density lipoprotein; HDL, high-density lipoprotein; GFR; Glomerular Filtration Rate; WHO, World Health Organization.Data are mean (±standard deviation) or median [range] unless otherwise noted. *P*-value indicates linear trend based on regression analysis with birth weight centile used as a continuous variable.

Table 4 Outcomes of recent pregnancy of the included women stratified by birth weight centile group (N=280).

	SGA (<p10) (n=40)	AGA-1 (p10-p50) (n=112)	AGA-2 (p50-p90) (n=84)	LGA (>p90) (n=44)	*P* for trend
Recent obstetric history					
Birth weight (grams)	1860 [977-2978]	2350 [1350-2985]	1907 [1139-2987]	1797 [1019-2953]	.73
Gestational age at delivery (days)	239 [207-267]	252 [223-270]	241 [206-266]	234 [209-267]	.29
Birth centile	25 [7-50]	23 [9-50]	25 [8-64]	26 [9-52]	.75
Interval delivery and measurement (months)	9 [6-11]	8 [6-11]	9 [7-12]	8 [6-11]	.72
Preeclampsia (%)	67	63	66	60	.73
HELLP (%)	58	51	57	44	.52
IUGR (%)	40	30	36	20	.50
Eclampsia (%)	2	2	3	2	.51
Placental abruption (%)	2	2	3	2	.69
Fetal death (%)	9	11	14	2	.75

SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; HELLP, hemolysis, elevated liver enzymes, low platelets; IUGR, intrauterine growth restriction. Data are mean (±standard deviation) or median [range] unless otherwise noted. *P*-value indicates linear trend based on regression analysis with birth weight centile used as a continuous variable.

in Table 4, the obstetric outcomes did not differ between birth centile groups. Also, time interval between delivery and plasma volume measurements was the same in all birth centile groups.

When the relation between birth centile and total adult plasma volume (mL) was adjusted for current body surface area, mean arterial pressure, total vascular resistance, glomerular filtration rate, and total 24-hour sodium output, each 10 centile change in birth weight is associated with an average change of 32.1 mL [95%CI: 19.6-44.6] in adult plasma volume.

To identify predictors of adult plasma volume, we firstly explored univariably, which factors correlated with adult plasma volume (mL). Plasma volume correlated with BMI (r=0.44; *P*<.01), birth weight centile (r=0.36; *P*<.01), glomerular filtration rate (r=0.29; *P*<.01), total vascular resistance (r=-0.23; *P*<.01), daily activity level (r=0.23; *P*<.01),

global vascular compliance (r=0.23; *P*<.01), 24-hour sodium output (r=0.21; *P*=.01) and mean arterial pressure (r=-0.13; *P*<.01). Subsequent multivariable backward regression analysis revealed that only BMI, birth weight centile, daily activity level and glomerular filtration rate contributed independently to the adult plasma volume (R^2 final model=0.48). Quantitatively, BMI was the most important determinant in our model for total adult plasma volume, as indicated by the largest drop in explained variance (-0.17) after removal of the factor BMI from our final model (Table 5). Subsequent removal of birth centile indicates that the birth centile contributes for an additional 14% of the explained variance in total adult plasma volume. Similarly, daily exercise level and glomerular filtration rate accounted for 10% and 7% of the variance, respectively. Fifty two percent of the variance in total plasma volume remains unexplained by our model.

Table 5 Predictors of the adult plasma volume (mL) (N=280).

	Explained variance (R^2)	Change in R^2 after removal
Final model (BMI, birth centile, total daily exercise level, glomerular filtration rate)	0.48	
- **Final model minus BMI**	0.31	-0.17
- **Final model minus BMI and birth weight centile**	0.17	- 0.14
- **Final model minus BMI, birth weight centile, and total daily exercise level**	0.07	- 0.10

BMI: body mass index

Discussion

Our study demonstrates that women with a history of hypertension in pregnancy and who were born small for gestational age are predisposed for lower plasma volumes in adult life compared to counterparts who were born appropriate or large for gestational age. Low plasma volume is a condition known to precede chronic hypertension[20,21]. The association between birth weight centile and subsequent adult plasma volume is independent of current body composition, smoking, blood pressure, physical activity level, cardiac performance, glucose metabolism, lipid levels, kidney function, and levels of sex hormones.

Formerly pre-eclamptic women have an increased risk of developing cardiovascular disease, suggesting a common risk profile[22]. Women who had gestational hypertension have a similar, but lower, risk of future hypertension and cardiovascular disease as those who had preeclampsia[22]. Previous studies also demonstrated associations between low plasma volume and preeclamptic pregnancies[16,23,23,24]. Etiology of this low plasma volume was largely unknown. This study identifies four independent determinants of the total adult plasma volume: body composition (BMI), birth weight centile, daily exercise level and the glomerular filtration rate.

Reduced plasma volume could be an interesting factor linking, at least partially, intra-uterine development with hypertension in adulthood and remote cardiovascular disease. One process associated with fetal disproportionate growth is the redistribution of blood flow in favor of the brain, heart and adrenal glands, but away from other organs including splanchnic organs and the kidneys[12,25,26]. Interestingly, the size of splanchnic organs and kidneys is reduced in children born small for gestational age[12]. Our data suggest that reduced size of the splanchnic vascular bed may have long lasting impact on adult plasma volume status.

Kidneys of individuals born small for gestational age are known to contain fewer nephrons, a condition that is thought to initiate hypertension[27,28] Nephrogenesis is completed relatively late in gestation by 34-36 weeks, increasing the impact of fetal compromise on renal development[27,28]. People with fewer nephrons are likely to have a relatively high glomerular filtration rate in each available nephron. Over time, hyper-filtration may cause glomerular injury and ultimately glomerular loss and the development of hypertension[27]. In our study the glomerular filtration rate correlated with adult plasma volume. However the relationship between birth weight centile and subsequent adult plasma volume was independent of glomerular filtration rate. This does not rule out single nephron hyperfiltration.

Our data concerning hemodynamic and metabolic variables are consistent with earlier studies[4,7,30]. We confirm an inverse relationship between blood pressure and birth weight centile. Demonstrating this association in a young (younger than 40 years) female population that is considered to be healthy is remarkable. Our data indicates that women who were born small for gestational age and who have a history of hypertension in pregnancy, have lower cardiovascular reserve capacity (i.e. lower plasma volume) at comparable arterial demands (i.e. cardiac index) when compared with women born normal or large for gestational age. Insulin levels were evidently higher in women born small for gestational age. We observed that women born small for gestational age and with a history of hypertension in pregnancy have an almost 23% risk of metabolic syndrome compared to 10% in women born appropriate for gestational age with a similar obstetric history.

Our data are applicable to selected population of women with a history of hypertensive complications in pregnancy. This could potentially amplify certain associations between birth weight centile and current metabolic and hemodynamic variables. However, this does not per se limit generalization of our results to the common female population of comparable age without a history of hypertension in pregnancy, as a recent cohort study of formerly preeclamptic women indicates that at least hemodynamic variables resolve largely within 6 months after giving birth, although further recovery may be possible up to 2 years post partum[35]. Furthermore differences in recent obstetric outcome did not affect the relation between birth centile and adult plasma volume.

In this study we used self-reported birth weight that could have resulted in a recall bias. Nonetheless, numerous studies in women with a comparable age (30-40 years) demonstrated a high accuracy of self-reported of birth weight (Spearman's correlation with state birth records varied between: $r=0.74$ and $r=0.85$)[36,37]. Therefore, we feel that recall bias has not substantially affected our observations. Because we did not observe differences between those who were able to recall their own birth weight and those who were not, we do not expect exclusion of this latter group affected our results. This study was unable to explore all potential mechanisms explaining the relationship between birth centile and subsequent adult plasma volume. We have no data directly evaluating the sympathetic system, the renin-aldosterone-angiotensin pathway, or potential effects of the glucocorticoid axis. Effects of these systems are considered to relate inversely to the plasma volume[38]. Because we excluded women using medication against chronic hypertension, a condition likely to accompany alterations in these axes, we assume no substantial bias from these systems on our findings. Nevertheless, we were unable to quantify their contribution to the adult plasma volume status in this study. Unfortunately we had no data concerning

abdominal circumference at birth, which might have reflected size of the splanchnic organs even better.

A particular strength of this the study is the measurement of plasma volume in a large cohort of women in combination with the many important covariates that might determine adult plasma volume. Resultingly, we were able to analyze or exclude the potential confounding effects of these covariates and eventually weigh the contribution of predictors of adult plasma volume.

In conclusion, birth weight centile relates linearly to adult plasma volume in women with a history of hypertension in pregnancy. Future studies will have to elucidate the effects of interventions aimed at modifying plasma volume on the recurrence of hypertension in pregnancy and future cardiovascular disease.

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CHAPTER 4

Prepregnancy low plasma volume and predisposition to preeclampsia and fetal growth restriction

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Abstract

The objective of this study was to estimate whether recurrence risks of preeclampsia, preterm birth and fetal growth restriction relate to prepregnancy plasma volume.

We conducted a retrospective cohort study in 580 formerly preeclamptic women and controls. In all women we measured plasma volume (^{125}Iodine-human serum albumin indicator dilution method) in the nonpregnant state. 178 normotensive formerly preeclamptic women had a subsequent pregnancy within study period (1996-2008). Odds (OR's) for recurrent preeclampsia, preterm birth and small for gestational age infant (SGA) were estimated, using multivariable logistic regression with adjustment for confounders.

Plasma volumes were lower in women who developed recurrent preeclampsia (1241 $\pm$ 158 mL/m^2, 17% lower compared with women in control group) than in women without recurrent preeclampsia (1335 $\pm$ 167 mL/m^2, 11% lower compared with women in control group). Logistic regression analysis demonstrated that each 100 mL/m^2 difference in plasma volume was associated with an odds ratio of 0.6 [95%CI: 0.5-0.8] to develop recurrent preeclampsia in subsequent pregnancy. Risk of preterm delivery (before 37 weeks of gestation) depended on preeclampsia in subsequent pregnancy; the adjusted hazard ratio for preterm birth was 0.9 [95%CI: 0.7-1.1] for each 100-mL/m^2 change in plasma volume. Risk of delivering an SGA neonate was independent of recurrent preeclampsia. Each 100-mL/m^2 change in plasma volume was associated with an adjusted odds ratio of 0.8 [95%CI: 0.5-0.9] to deliver a SGA neonate in subsequent pregnancy.

The risk of recurrent preeclampsia and fetal growth restriction in subsequent pregnancy relates inversely and linearly to prepregnancy plasma volume.

Introduction

Preeclampsia, preterm birth and fetal growth restriction contribute to substantial maternal and perinatal morbidity and mortality. Strategies to identify those women at increased risk for recurrence of these conditions will be helpful not only to assess the individual risk but also to develop tailored preventative approaches.

The recurrence rate of preeclampsia is approximately 15%[1-5], but figures range from 11% up to 65% depending on the population studied[1]. The risk of developing gestational hypertension in the pregnancy after a preeclamptic pregnancy is 1 in 3[6]. In normotensive apparently healthy formerly preeclamptic women, no *single* biomarker has been found clinically useful to predict recurrent preeclampsia, leaving preeclampsia in the first pregnancy the strongest predictor for preeclampsia in a subsequent pregnancy[1,4]. In comparison with hypertensive women, normotensive formerly preeclamptic women are considered to be at low risk for recurrent preeclampsia[1].

Low plasma volume relates both to the early phase of essential hypertension and to hypertensive complications in pregnancy[7-10]. Subnormal plasma volume before pregnancy predisposes to abnormal hemodynamic adaptation during pregnancy[9]. Whether prepregnancy plasma volume contributes in a *volume-dependent manner* to the risk of recurrent preeclampsia is currently unknown. Moreover, it is unknown whether low prepregnancy plasma volume additionally relates specifically to the fetal sequellae of preterm birth and fetal growth restriction. In this study we tested the hypothesis that the risk of preeclampsia, preterm birth and fetal growth restriction relate proportionately and independently to prepregnancy plasma volume. To this end, we studied the maternal and neonatal outcomes of pregnancy as a function of prepregnancy plasma volume in normotensive formerly preeclamptic women and healthy parous controls.

Materials and methods

We conducted a hospital-based retrospective cohort study among primiparous women. Patients were recruited from the postpartum outpatient clinic as well as from referrals to the tertiary center for preconceptional counseling (1996-2008). Healthy primiparous controls with an uneventful previous pregnancy were recruited by advertisement and measured within the timeframe of all measurements in patients (1998-2007). Women in the control group were included when previous pregnancy had no hypertensive complications, when women delivered at term and gave birth to a normal weight neonate. The same exclusion criteria were used in both the control

and case groups. Participants did not use hormonal contraceptives and had discontinued breastfeeding. In the analysis we included women who were normotensive and non-diabetic at the interpregnancy evaluation and whose next pregnancy was singleton. We excluded women with chronic hypertension, when either using antihypertensive medication or having blood pressures exceeding 140 mmHg systolic or 90 mmHg diastolic or both at the interpregnancy evaluation[11]. We excluded women with diabetes based on a nonpregnant fasting glucose level above 6.1 mmol/L at two separate occasions or the use of antidiabetic medication[12]. Finally, women who did not complete a next pregnancy within the study period were excluded (1996-2008). The study was approved by the hospital medical ethical committee (CMO2007/252).

All measurements in both patients and women in the control group were performed 6 to 12 months after the first pregnancy in the nonpregnant state. Blood pressure was measured oscillometrically (Dinamap, Vital Signs Monitor) at the right-upper arm. We used the median values of 9 consecutive recordings at 3-minute intervals starting after 5 minutes at rest in supine position. Thereafter Plasma Volume (PV) was measured in the nonpregnant state using the Iodine^{125}-human serum albumin (^{125}I-HSA) indicator dilution method[9], normalized for body surface area (mL/m^2). During this measurement, participants lay in a semi-supine position on a comfortable bed. Subsequently a standardized dose 0.2 MBq of ^{125}I-HSA was injected intravenously in the right antecubital vein. Each 10 minutes a venous blood sample was taken from the contralateral intravenous catheter until 40 minutes after administration of the ^{125}I-HSA. The person drawing blood for the plasma volume measurement was blinded to the blood pressures obtained in advance of the procedure. Blood samples were analyzed in specialized laboratory. Plasma volume was obtained by dividing the total injected radioactivity by the virtual volume-specific radioactivity at time zero. Data of previous and first subsequent pregnancies were extracted from the medical records. All formerly preeclamptic patients received acetylsalicylic acid (80mg/day) in their next pregnancy (12-36 weeks). We defined (gestational) hypertension and preeclampsia according to ACOG criteria[11]. Fetal growth restriction was defined as a birth weight below the 10th centile of the national birth weight chart[13]. We defined the interbirth interval as the time between the two delivery dates.

Statistical analysis

Data are expressed as means ± SD or as medians with interquartile ranges for normally and nonnormally distributed data, respectively. Normality of each variable was evaluated using Kolmogorov-Smirnov tests. Comparisons between groups were assessed using unpaired Student t-test for normally distributed data, Mann-Whitney U test for nonnormally distributed variables and Chi-square test for categorical parameters. *P*-value < 0.05 (two-sided) is considered statistically significant.

Pre-pregnancy plasma volumes (mL/m^2) in women with recurrent preeclampsia (RECUR) and those without recurrent preeclampsia (NON-RECUR) were compared with the control group (CONTR) using analysis of variance along with post-hoc Bonferroni correction for multiple testing.

To present our raw data, we categorized the formerly preeclamptic normotensive women into quintiles according to their prepregnancy normalized plasma volume. This illustrates in a simple way the risks of recurrent preeclampsia across the full range of prepregnancy plasma volumes. We used logistic regression to evaluate the relation between prepregnancy plasma volume and recurrent preeclampsia. Independent variables were plasma volume (mL/m^2), history of recurrent miscarriage, history of the birth of a growth restricted neonate, the gestational age at previous delivery, the mean arterial blood pressure (at the interpregnancy evaluation), smoking and body mass index (BMI) at the inter-pregnancy measurement, maternal age at subsequent delivery and the interbirth interval. Independent variables were selected based on their known association with preeclampsia in parous women. Odds ratios are presented with their 95% confidence intervals [CIs].

Based on this analysis we calculated the odds ratio [±95%CI] of recurrent preeclampsia associated with each 100 mL/m^2 change in prepregnancy plasma volume. Because all data were obtained over a long period (13 years), changes in medical management during pregnancy or otherwise could have had an effect on the outcome. Hence, we repeated the analysis with date of delivery in subsequent pregnancy as additional covariate and we evaluated whether the relation between the incidence of preeclampsia and the independent variables changed over time, that is, we evaluated the interaction between independent variables in the logistic regression model and year of delivery in subsequent pregnancy.

Analogous to the evaluation of the relation between plasma volume and subsequent recurrent preeclampsia we analyzed the risk of delivering a SGA neonate in subsequent pregnancy. The relation between pre-pregnancy plasma volume and the gestational age of delivery in subsequent pregnancy is illustrated with a Kaplan-Meier curve for each normalized plasma volume quintile. We used Cox regression analysis to analyze the duration of the subsequent pregnancy and calculated the hazard ratio, with 95% confidence intervals for preterm birth associated with each 100mL/m^2 change in plasma volume. The independent variables were the same as in the logistic regression model for recurrent preeclampsia. To explore whether the risks of delivering a SGA neonate or preterm birth or both were independent of preeclampsia in subsequent pregnancy, we introduced preeclampsia in subsequent pregnancy as an additional independent covariate into our models for SGA neonates and preterm

birth. The statistical analyses were performed using the statistical software package SPSS 16.0.

Results

We measured plasma volume in 580 primiparous women with a history of preeclampsia during their first pregnancy. For the analysis we excluded 102 hypertensive patients (17.6%) and 4 women (0.7%) with diabetes mellitus. Sixteen patients (2.7%) could not be further analyzed as a result of missing values or uncertain diagnosis. Finally, we excluded 280 women (48%) who did not have a subsequent pregnancy within the study period or whose outcome for the next pregnancy was unknown at the time of analysis. A total of 178 normotensive, nondiabetic, formerly preeclamptic women (30.6%) were available for analysis. (Fig 1)

The demographic and obstetric data of normotensive formerly preeclamptic women with a subsequent pregnancy (n=178) compared with women without a subsequent pregnancy (n=280) are shown in Table 1. Compared with those without subsequent pregnancy, women with subsequent pregnancy were younger (30 ± 4 vs. 32 ± 5 years) and previously had delivered at a lower gestational age (median: 32 [29-35] vs. 34 [31-36] weeks) of neonates with lower birth weight (1566 ± 855 vs. 1836 ± 872 g.). For all other variables, the groups were comparable.

Table 2 lists the demographic and obstetric variables in the normotensive formerly preeclamptic women with a subsequent pregnancy (FORMER PE) and healthy controls (CONTR). Compared with women in the control group, normotensive formerly preeclamptic women delivered in the previous pregnancy at a lower gestational age (32 ± 4 vs. 39 ± 1 weeks) of neonates with lower birth weight (1566 ± 855 vs. 3420 ±277 g.). At the interpregnancy measurements formerly preeclamptic women had higher body mass index (median: 24 [19-29] vs. 22 [18-26] kg/m^2) and lower plasma volume (1307 ± 175 vs. 1503 ± 130 mL/m^2). In the subsequent pregnancy, formerly preeclamptic women gave birth to neonates with lower birth weight compared with controls (3063 ± 730 vs. 3722 ± 600 g) and a higher rate of SGA neonates (12 vs. 0 %).

Thirty-four (19%; 95%CI: 13-25%) of the 178 normotensive formerly preeclamptic women developed recurrent preeclampsia (RECUR). The group without recurrent preeclampsia (NON-RECUR: 144 women, 81%) includes 55 women (31%; 95%CI: 23-40%) who developed gestational hypertension without progressing to preeclampsia and 89 women (50%; 95%CI: 43-58%) who remained normotensive during their subsequent pregnancy. RECUR neither differed from NON-RECUR with respect

to gestational age of delivery and birth weight in previous pregnancy nor the interpregnancy body mass index, smoking and mean arterial pressure. RECUR only differed from NON-RECUR by lower plasma volume (1241 ± 158 mL/m^2 versus 1335 ± 167 mL/m^2) at the interpregnancy measurement. (Figure 2) Women in the RECUR group delivered in their subsequent pregnancy at an earlier gestational age (37 ± 3 vs. 39 ± 2 weeks) and had a higher rate of SGA neonates (36% vs. 6 %). (Table 2)

Mean plasma volume is 17% lower in RECUR and 11% lower in NON-RECUR compared to controls. (1241 ± 158 vs. 1335 ± 167 vs. 1503 ± 130 mL/m^2, respectively), as shown in Figure 2. Mean plasma volume in RECUR was significantly lower compared to NON-RECUR. Within the NON-RECUR group, plasma volume was lower in women who developed gestational hypertension without progressing to preeclampsia compared with women who remained normotensive in subsequent pregnancy (1283± 154 vs. 1371 ± 170 mL/m^2; P=0.002).

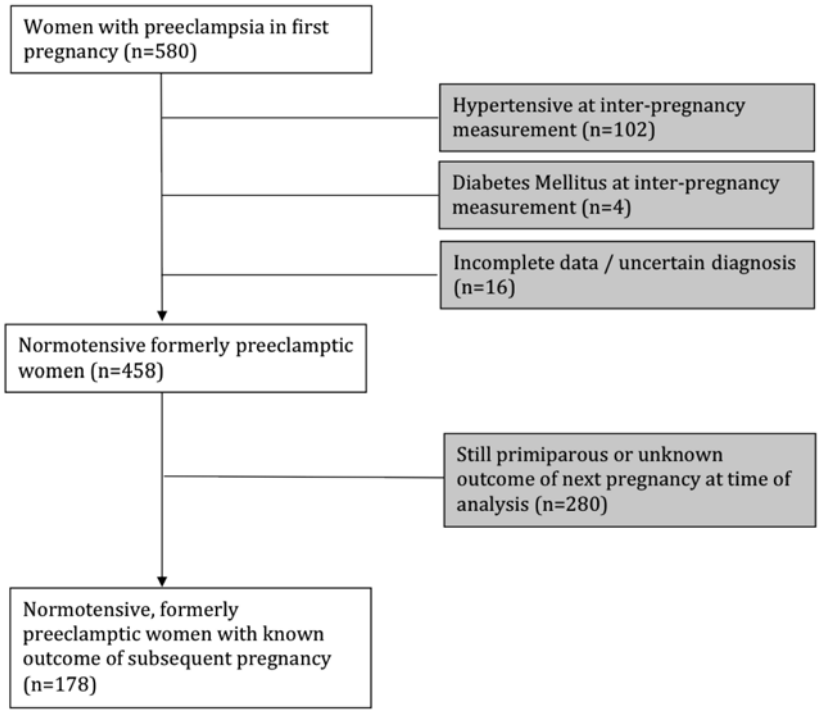

Figure 1 Schematic presentation of study population and excluded patients.

Table 1 Demographic and clinical variables of the normotensive formerly preeclamptic women.

	Normotensive formerly preeclamptic women *with* subsequent pregnancy (n=178)	Normotensive formerly preeclamptic women *without* subsequent pregnancy (n=280)	*P*-value
Obstetric history			
Miscarriage ≥2 times (%)	4	5	.59
Gestational age at delivery (weeks)	32 [29-35]	34 [31-36]	<.01*
Birth weight (g)	1566 ± 855	1836 ± 872	<.01*
Small for gestational age (%)	29	28	.78
General characteristics			
Age (years)	30 ± 4	32 ± 5	<.01*
Body Mass Index (kg/m^2)	24 [19-29]	24.9 [18-32]	.06
Smoking (%)	22	23	.76
Fasting glucose levels (mmol/L)	5.1 ± 0.5	5.2 ± 0.8	.20
Non-pregnant hemodynamic variables			
Systolic blood pressure (mmHg)	117 ± 10	116 ± 11	.15
Diastolic blood pressure (mmHg)	72 ± 8	71 ± 9	.42
Mean arterial pressure (mmHg)	89 ± 8	88 ± 9	.38
Heart rate (bpm)	70 ± 10	71 ± 9	.36
Plasma volume (mL/m^2)	1317 ± 175	1338 ± 169	.19

Data are women *with subsequent pregnancy* (n=178) compared with women *without subsequent pregnancy* (n=280) within the study period. Normally distributed data is expressed as mean (± SD), non-normally distributed is indicated as medians [inter-quartile range]. Comparisons between groups based on unpaired Student t-test for normally distributed data, Mann-Whitney U test for non-normally distributed variables and Chi-square test for categorical parameters. (*$P < 0.05$).

Figure 3 presents the relation between the interpregnancy plasma volume and the risk of recurrent preeclampsia. Logistic regression analysis demonstrated that each 100-mL/m^2 change in plasma volume was associated with an odds ratio of 0.6 [95%CI: 0.5-0.8] to develop recurrent preeclampsia in subsequent pregnancy. This indicates that as plasma volume increases, the recurrence risk of preeclampsia decreases proportionately (P<0.01). Inclusion of the date of delivery in the logistic regression model did not change the odds ratio. Neither did the interactions between

Table 2 Demographic and clinical variables for controls (CONTR) and normotensive formerly preeclamptic women (FORMER PE). PE is sub grouped into women without recurrent preeclampsia (NON-RECUR) vs. women with recurrent preeclampsia (RECUR)

		FORMER PE (n=178)		*P-value*	
	CONTR (n=17)	**NON-RECUR (n=144)**	**RECUR (n=34)**	**FORMER PE vs. CONTR**	**RECUR vs. NON-RECUR**
Obstetric History					
Miscarriage ≥2 times (%)	0	4	6	.28	.52
Gestational age at delivery (weeks)	40[38-41]	32[29-36]	31[29-36]	<.001*	.17
Birth weight (g)	3420±277	1598±886	1441±718	.003*	.33
Small for gestational age (%)	0	24	31	.009*	.41
Maternal Characteristics					
Age (years)	32±2	30±4	30±4	.20	.82
Body Mass Index (kg/m$^{2)}$	22[18-26]	24[19-29]	25[17-32]	.003*	.07
Smoking (%)	13	24	15	.44	.25
Non-pregnant hemodynamic variables					
Systolic blood pressure (mmHg)	114±10	117±10	117±10	.28	.58
Diastolic blood pressure (mmHg)	70±7	72±8	74±8	.54	.09
Mean arterial pressure (mmHg)	84±8	88±8	91±8	.07	.06
Heart rate (bpm)	68[54-82]	71[57-85]	72[62-82]	.46	.26
Plasma volume (mL/m^2)	1503±130	1335±167	1241±158	<0.001*	.003*
Characteristics of subsequent pregnancy					
Interbirth interval (years)	2[1-3]	3[1-5]	3[1-5]	.41	.32
Gestational age (weeks)	41±2	39±2	37±3	.1	.002*
Birth weight (g)	3722±600	3147±684	2736±821	.004*	.002*
Small for gestational age (%)	0	6	36	.04*	<.001*

Normally distributed data is expressed as mean (± SD), non-normally distributed is indicated as medians [inter-quartile range]. Comparisons between groups based on unpaired Student t-test for normally distributed data, Mann-Whitney U test for non-normally distributed variables and Chi-square test for categorical parameters. (*$P < 0.05$).

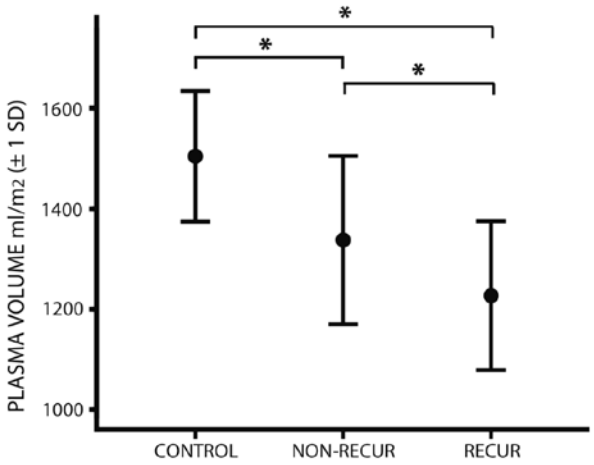

Figure 2 Plasma volume (PV, mL/m^2, ± 1SD) for nonpregnant women in the control group (CONTR) and normotensive formerly preeclamptic women who develop recurrent preeclampsia (RECUR) or those who do not develop recurrent preeclampsia (NON-RECUR). ANOVA with Bonferoni: $^*P<0.01$

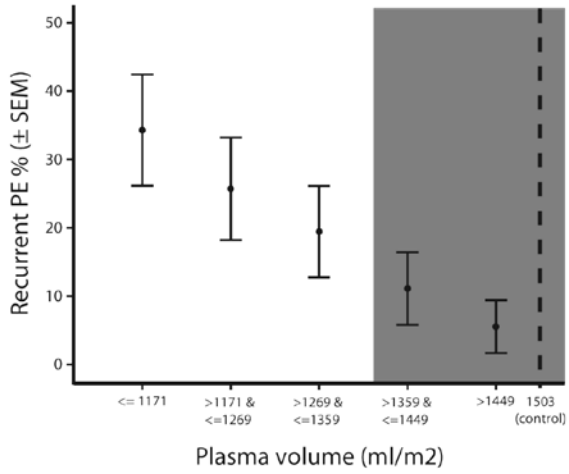

Figure 3 Risk of recurrent preeclampsia (percentage ± 1SEM) for each quintile of nonpregnant normalized plasma volume (mL/m^2). The dashed line represents mean plasma volume in healthy controls (1503 mL/m^2). The shaded area corresponds with one standard deviation of mean plasma volume in controls (130 mL/m^2).

the date of delivery in subsequent pregnancy and the risk factors in the model show an association with the incidence of recurrent preeclampsia. The decrease in the log likelihood that resulted from including the nine interactions in the model was 3.9 (p=0.92).

In our study, 111 normotensive formerly preeclamptic women (62.4%) had normal weight (BMI 25 or lower) at the interpregnancy evaluation, whereas 50 women (28.1 %) were overweight (BMI: 25-30), 13 women (7.3%) were obese (BMI 30-35) and 4 women (2.2%) morbidly obese (BMI more than 35). Recurrence rates of preeclampsia were 15.3 % in normal weight, normotensive formerly preeclamptic women, 26% in overweight women (OR1.9; 95%CI 0.9-4.4) and 30.8% in obese women (OR2.5; 95%CI 0.8-8.9). There was no recurrent preeclampsia in the 4 women who were morbidly obese at the interpregnancy evaluation.

Kaplan Meier curves illustrate the relation between plasma volume quintiles and gestational age of delivery in next pregnancy (Figure 4). Cox regression analysis demonstrated that each 100-mL/m^2 change in plasma volume is associated with a hazard ratio of 0.9 (95%CI: 0.7-1.1) of preterm delivery (<37 weeks gestation) in subsequent pregnancy. Regression analysis demonstrated a confounding effect of

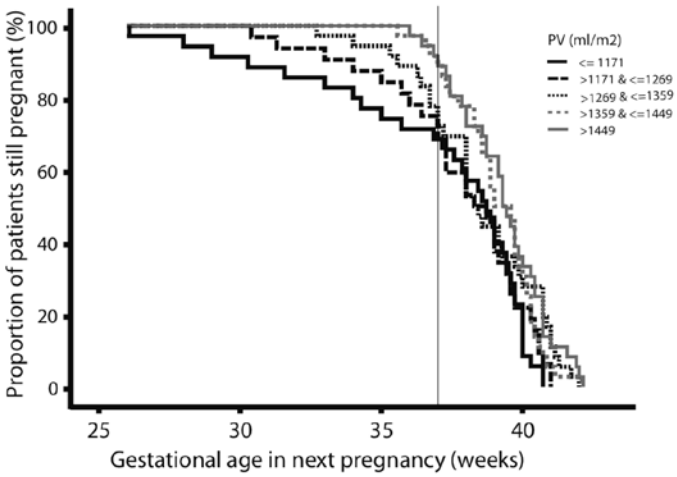

Figure 4 Kaplan-Meier curves illustrating the relation between quintiles of pre-pregnancy plasma volume (mL/m^2) and gestational age of delivery in subsequent pregnancy. The vertical line represents the gestational age of 37 weeks.

preeclampsia in subsequent pregnancy on the risk of preterm birth. Casewise analysis of the nature of preterm delivery in the subsequent pregnancy in formerly preeclamptic women demonstrated that only 8.6% of the preterm births were spontaneous. The remaining 91.4% were all indicated preterm births due to either gestational hypertensive disease or fetal growth restriction or both, explaining the large confounding effect of preeclampsia in subsequent pregnancy on the relation between prepregnancy plasma volume and risk of preterm birth.

Logistic regression analysis demonstrates that each 100-mL/m^2 change in plasma volume is associated with an adjusted OR of 0.8 (95%CI: 0.5-0.9) to deliver a neonate small for gestational age in subsequent pregnancy. This indicates that as plasma volume increases, the risk of a growth restricted neonate decreases proportionately ($P<0.01$) independent of preeclampsia in subsequent pregnancy.

Discussion

This study indicates that in apparently healthy normotensive formerly preeclamptic women, the risk of recurrent preeclampsia and fetal growth restriction in the subsequent pregnancy inversely and linearly relates to prepregnancy plasma volume.

In our study, 1 out of 5 normotensive women developed recurrent disease, whereas preeclampsia is reported to recur in approximately 1 out of 7 pregnancies[1-5]. The magnitude of recurrence risk is considered to be dependent on prior gestational age at time of previous disease onset, severity of disease, previous fetal growth restriction, and presence or absence of pre-existing hypertension and other medical disorders. This makes individual counseling difficult because the recurrence rate may vary between 1 of 9 and 2 of 3[1,3-6]. Numerous factors can explain the relatively higher recurrence rate in our study group, despite the fact that all women were normotensive. The concept of a higher recurrence risk in women who delivered at an earlier gestational age in a previous pregnancy complicated by preeclampsia is well established[4,5]. Reported recurrence rates decrease from 39% in those who deliver before 28 weeks gestation to 13% in women delivering at term[5]. Also previous birth of an SGA neonate is associated with an increased recurrence risk of preeclampsia (aOR1.7; 95%CI 1.4-2.1) compared witht women who gave birth to an appropriate sized neonate for gestational age[4]. Because we included primarily early-onset preeclamptic women, with 29% of the women giving birth to an SGA neonate in an earlier pregnancy, recurrence rate of preeclampsia is likely to be high in our studied group.

Our study demonstrates a gradient in plasma volume per body surface area between NON-RECUR who remain normotensive in subsequent pregnancy, NON-RECUR who develop gestational hypertension without progressing to preeclampsia in subsequent pregnancy and women who develop recurrent preeclampsia (RECUR). This suggests a certain continuum in the pathophysiological development of hypertensive disease during pregnancy depending on plasma volume status.

In healthy persons, plasma volume remains relatively constant as a result of tight regulation by the complex interaction between neurohormonal systems involved in sodium and water homeostasis. Important mechanisms involved in the plasma volume regulation are the sodium- and water-retaining effects of the renin angiotensin aldosterone system (RAAS), the diuretic effects of natriuretic peptides in response to atrial and ventricular wall stretch and actions of the sympathetic nervous system. The sympathetic system acts on plasma volume through a range of effects on both kidneys and vasculature,[14] the latter in particular by acting on the functional venous capacitance. In our study, the plasma volumes measured in the healthy controls (PV: 1503 ± 130 mL/m^2) compares well with plasma volumes (PV:1521 ± 180 mL/m^2) measured by Bernstein et al in healthy women of comparable age and BMI[15].

Plasma volume relates to BMI[16]. Although recurrence risk of preeclampsia may vary with higher BMI, we were unable to demonstrate such correlation[4]. This could be the result of a type 2 error. Others proposed a pathophysiologic connection between obesity, insulin resistance and preeclampsia, with a central role of increased activity of the sympathetic nervous system[17]. Increased sympathetic tone decreases plasma volume by reducing the vascular capacitance[15]. In turn, this may link low plasma volume to obesity and in particular the metabolic syndrome. In our study, plasma volume related to recurrent preeclampsia even after adjustment of prepregnancy BMI, indicating that the relationship between plasma volume and recurrent preeclampsia is independent of prepregnant BMI. There are several possible explanations for this observation. First, increased sympathetic tone is particularly associated with central obesity in contrast to peripheral obesity[18]. This is not always well reflected in the BMI. Unfortunately, we did not record data that could discriminate between central and peripheral obesity. Second, also other factors, not necessarily linked to the metabolic syndrome, may relate to low plasma volume. A fundamentally small vascular system, in line with the Barker hypothesis[19], might also account for low plasma volume. Future studies will have to elucidate the exact mechanisms resulting in low plasma volume.

Few studies have investigated the pathophysiologic connection between plasma volume and preeclampsia. At normal cardiac functioning, the plasma volume reflects

the cardiovascular reserve capacity. Healthy maternal vascular adaptation in the first trimester of pregnancy includes adequate plasma volume expansion to meet the increased demands of advanced pregnancy[20,21]. Early pregnancy plasma volume expansion is triggered by a primary fall in systemic vascular tone[9, 21]. Women with low prepregnancy plasma volume show, as a consequence of venous overfill, little rise in plasma volume during the early phases of pregnancy compared with women with normal prepregnancy plasma volume[9,22]. Inherent to this low-volume condition is the necessity for more sympathetic activity to preserve venous return, restricting the reserve capacities even more and leading to arterial vasoconstriction, circulatory redistribution, increased vascular shear and with it endothelial dysfunction[2,8,9,15,23]. Low plasma volume therefore precedes vascular complicated pregnancy. This circulatory condition extents even to the nonpregnant state[7,9], making plasma volume a potentially valuable tool in assessing individual risk in advance of the next pregnancy. This pathophysiologic link is strengthened by the observation of circulatory redistribution in nonpregnant women with reduced plasma volume at the expense of uterine perfusion[24]. This circulatory redistribution, in turn, may negatively affect fetal growth. Our study demonstrates both maternal and fetal consequences of low pre-pregnant plasma volume.

In contrast to many other factors associated with increased recurrence risk of preeclampsia, prepregnancy plasma volume is particularly interesting since it is potentially modifiable in advance of the next pregnancy. Aerobic exercise is well known for its plasma volume expanding effect. This effect is initially established mainly through activation of the Renin-Angiotensin-Aldosterone system resulting in sodium retention[25,26], and if training is continued through de novo protein synthesis, increasing oncotic intravascular pressure[25,26], and at sustained training also by lowering sympathetic tone[27] and increasing venous compliance[28]. Individuals who perform regular physical activity can increase their plasma volume approximately 10%[25,26]. In our study group the difference in prepregnancy plasma volume between RECUR and NON-RECUR is about 8%. Future research may demonstrate whether a training program with regular aerobic exercise, aimed at increasing plasma volume, reduces recurrence risk of preeclampsia in formerly preeclamptic women with low plasma volume.

Besides the observational hospital based design, this study has several limitations. First, the study is restricted to a specific group of normotensive formerly preeclamptic women with two successive pregnancies within our study period. The largest excluded group (women without a subsequent pregnancy within the study period) only differed from the included group by the maternal age, the gestational age of delivery and neonatal birth weight in previous pregnancy. Therefore, our study

population forms a reasonable representation of the initial population of normotensive formerly preeclamptic women. Second, the control group is small, therefore discrete differences between controls and other groups could have been missed. The controls however, are only used to illustrate the normal spectrum of plasma volume, and were not used for the main study outcome describing the relation between prepregnant plasma volume and recurrence risk of preeclampsia. Third, we have no data concerning change of paternity. Change of paternity may be a modifier of the risk of preeclampsia in parous women, but also relates to larger interpregnancy interval[1,3-6]. Since the mean interbirth interval in our study is only 3 years, we don't expect that possible change of paternity has contributed to our observations. Last, in this study we included women who were referred to a tertiary center. This may have led to over-representation of more severely complicated pregnancies. However, the strongest denominator of severity, gestational age at delivery in previous preeclamptic pregnancy, did not affect the relation between plasma volume and subsequent pregnancy outcomes. Therefore, we assume our data represent formerly preeclamptic women in general, independent of onset of disease in previous pregnancy.

In summary, our results demonstrate that in normotensive formerly preeclamptic women, the risk of recurrent preeclampsia and fetal growth restriction in subsequent pregnancy independently increase with lower prepregnant plasma volumes.

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CHAPTER 5

Low plasma volume in normotensive formerly preeclamptic women predisposes to hypertension

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Abstract

Formerly preeclamptic women are at risk for cardiovascular disease. Low plasma volume may reflect latent hypertension and potentially links preeclampsia with chronic cardiovascular disease. We hypothesized that low plasma volume in normotensive formerly preeclamptic women predisposes to hypertension.

We longitudinally studied n=104 formerly preeclamptic women in whom plasma volume was measured 3 to 30 months after the preeclamptic pregnancy. Cardiovascular variables were assessed at two points in time (3 to 30 months postpartum and 2-5 years thereafter). Study population was divided into low plasma volume (≤1373mL/m^2) and normal plasma volume (>1373mL/m^2). Primary endpoint was hypertension at the second visit; defined as ≥140 mmHg systolic or ≥90 mmHg diastolic. Secondary outcome of this study was change in traditional cardiovascular risk profile between visits. Variables correlating univariately with change in blood pressure between visits were introduced in regression analysis.

Eighteen of 104(17%) formerly preeclamptic women who were normotensive at first visit had hypertension at second evaluation 2 to 5 years later. Hypertension developed more often in women with low plasma volume (10/35(29%)) than in women with normal plasma volume (8/69(12%); OR: 3.2 [95%CI: 1.4-8.6]). After adjustments, relationship between plasma volume status and subsequent hypertension persisted (aOR: 3.0 [95%CI: 1.1-8.5]). Mean arterial pressure at second visit correlated inverse linearly with plasma volume (r=-0.49, $P<.01$).

Initially normotensive formerly preeclamptic women have 17% chance to develop hypertension within five years. Women with low plasma volume have higher chance to develop hypertension than women with normal plasma volume. Clinically, follow-up of blood pressure seems warranted in women with history of preeclampsia, even when initially normotensive.

Introduction

Preeclampsia (PE) is a serious hypertensive pregnancy disorder complicating 2 to 5% of all pregnancies. Preeclampsia is characterized by hypertension and proteinuria after 20 weeks gestation. Women with a history of preeclampsia are at increased risk to develop early onset cardiovascular disease (CVD) later in life compared to women who had an uneventful pregnancy[1]. Therefore, in 2011, the American Heart Association added preeclampsia to the list of risk factors for developing CVD[2]. The mechanistic explanation for the link between preeclampsia and later CVD remains to be elucidated[3].

Numerous studies demonstrated increased prevalence of cardiovascular risk factors after a pregnancy complicated by preeclampsia[4-6]. Most formerly preeclamptic women, however, do not demonstrate a traditional cardiovascular risk profile within the first years following preeclampsia[6]. Yet, when compared to healthy parous controls, the relative risk of developing chronic hypertension within 15 years after preeclampsia is estimated 3.7 [95% CI: 2.7-5.5][1]. This observation indicates that a subset of normotensive formerly preeclamptic women is at risk to develop hypertension within the first years following the affected pregnancy. It is conceivable that these formerly preeclamptic women have subtle or latent abnormalities that could explain the increased cardiovascular risk after pregnancy.

Low plasma volume (PV) is considered to reflect latent hypertension[7] and normotensive formerly preeclamptic women with low PV (LPV) are prone to develop recurrent hypertensive disease in subsequent pregnancy[8]. Whether PV status also relates to chronic hypertension after a pregnancy complicated by preeclampsia has never been studied before. We hypothesized that LPV status in normotensive formerly preeclamptic women predisposes to the development of hypertension. In this longitudinal study, we additionally studied how the traditional cardiovascular and metabolic risk profiles evolve over time after a preeclamptic pregnancy.

Marterials and methods

In this study we used a database of women with a history of preeclampsia (n=386) who had been tested twice in the years following their preeclamptic pregnancy. We selected women who were normotensive and apparently healthy at their first assessment 3 to 30 months post partum. Reassessment of the cardiovascular and metabolic risk profile took place prospectively 2 to 5 years after the initial assessment.

Preeclampsia was defined according the criteria set by the International Society for the Study of Hypertension in Pregnancy (ISSHP)[9] (systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, measured twice, > 6 hours apart, plus consistent proteinuria of ≥ 300 mg in 24 hours or protein to creatinine ratio > 30 g/mol after gestational week 20 in previously normotensive women. All formerly preeclamptic women included in the database were of Northern European Ancestry and completed a cardiovascular evaluation 3 to 30 months after their complicated pregnancy (visit 1) in the period January 2008 - December 2010. A follow-up program was started in 2011 and all women were invited for reassessment of their cardiovascular risk profile (visit 2) at 2 to 5 years after the first visit. For this study we compared data from both assessments.

Both cardiovascular evaluations were performed in the nonpregnant state at least three months postpartum and all women had discontinued breastfeeding for at least 4 weeks. Women who were hypertensive at the first assessment were excluded. Hypertension was defined as SBP ≥ 140 mmHg and/ DBP ≥ 90 mmHg, or when taking antihypertensive drugs. Also excluded from analysis were women diagnosed with diabetes mellitus (type 1 or 2), or autoimmune diseases at the first visit. Women were only invited for the second cardiovascular assessment when at least 2 years after the initial evaluation.

The study was approved by the Medical Ethics Committee of the Radboud University Medical Centre Nijmegen (CMO 2010/245) All women gave written informed consent. This study adhered to the principles of the declaration of Helsinki.

Experimental procedures

PV was measured only during visit 1, and not during visit 2. All other measurements were performed at both visits according identical protocol.

The plasma volume (PV, mL) indicates the total blood volume minus the cellular volume and was measured using the golden standard method: the iodine^{125} albumin indicator dilution technique (^{125}I-HSA). During the measurement, women were in semi-supine position on a comfortable bed. An 18-gauge intravenous catheter was inserted in the left antecubital vein for repetitive blood sampling. A standardized dose (0.2 MBq) of ^{125}I-HSA was injected intravenously in the right antecubital vein. Every 10 minutes a venous blood sample was taken from the contra lateral intravenous catheter until 40 minutes after administration of the ^{125}I-HSA. Blood samples were analyzed using a gamma counter. PV was calculated by dividing the total injected radioactivity by the virtual volume specific radioactivity at time zero, as described elsewhere[8]. PV was normalized for body surface area (mL/m^2). Plasma volume was categorized into

2 groups based on measurements in control group: low plasma volume (LPV): ≤ 1373 mL/m^2 corresponding with ≤ -1SD below mean plasma volume in control subjects; and normal plasma volume (NPV): >1373 mL/m^2 equivalent to > -1SD of controls.

At each visit a detailed history of complaints including family history was taken. A positive family history of CVD, hypertension or diabetes was defined as having at least one first-degree relative who was diagnosed with any of these conditions before the age of 65 years. All measurements were performed after an overnight fast and scheduled between day 3 and 11 of the menstrual cycle to minimize possible endocrine influences of the sex hormones on the cardiovascular system.[10] The measurements started at 8.00 AM to prevent diurnal variations between measurements in a temperature controlled room (≈22 °C) with external disturbances kept to a minimum. Participants were instructed to abstain from strenuous physical activity in the 24 hours prior to testing. Participants collected urine for 24 hours preceding the measurements. The 24 hours urine sample was assayed for albumin, protein and creatinine to establish (micro-) albuminuria corrected for creatinine output (g/mol creatinine) and total protein concentration (g/24hrs) (Aeroset, Abbot Laboratories, Illinois USA). Body weight and height were measured. Body mass index (BMI, kg/m^2) was calculated as body weight (kg) divided by squared height (m^2), body surface area (BSA, m^2) as 0.007184·height (cm)$^{0.725}$·weight (kg)$^{0.425}$.[11]

After 30 minutes of rest in semisupine position, blood pressure and HR were measured oscillometrically (Dinamap, Vital Signs Monitor 1846, Critikon, Tampa, Florida) in this half-sitting position, with the cuff at the right upper arm at heart level at 3-minute intervals for a period 30 minutes with a cuff size recommended for the arm circumference (13.5x30.7cm if arm circumference ranged between 27.5 and 36.5cm or 17x38.6cm if arm circumference ranged between 35.5 and 46cm). We recorded systolic (SBP,mmHg), diastolic (DBP,mmHg) and mean blood pressure (MBP, mmHg) and heart rate (HR,bpm), and used the median values of 9 consecutive measurements for analysis. Hypertension was defined as SBP ≥140mmHg and DBP ≥ 90mmHg.

Venous blood samples were taken from an antecubital vein and analyzed for glucose, insulin, lipid profile, and creatinine concentration (Aeroset, Abbot Laboratories, Illinois USA). The Homeostatic Model Assessment (HOMA) score was used to calculate the level of insulin resistance with the following equation: (fasting glucose x fasting insulin)/22.5.

Statistical analysis

Normally distributed data are presented as means ± SD, non-normally distributed data as medians with interquartile range. Differences between groups were tested using ANOVA statistics, Wilcoxon ranks sum or Chi-square tests where appropriate.

Associations of variables with change in mean blood pressure between assessments were explored using univariate analysis (Spearman-Rho). All variables with significant association ($p<0.05$) with change in blood pressure between two assessments were subsequently included in a multivariate regression model. For the multivariate analyses, we used stepwise backward elimination on the basis of Wald's test, using a p-value of 0.05 for elimination of variables. The NPV group was used as statistical reference group. Group-size calculation was estimated based on combining the observed occurrence of hypertension within 12 months (25%)[9] and within 15 years (52%)[1] after preeclampsia. It was estimated that 12% of initially normotensive formerly preeclamptic women develop de novo hypertension within 5 years. To compensate for possible errors in the estimation we based our sample size calculation on a prevalence of only 10% chronic hypertension in women 2 to 5 years after a pregnancy complicated by preeclampsia. In the general Dutch population the prevalence of hypertension in women of similar age is: 3.7%. Using this percentage as a reference, than the study population would need at least 98 subjects to detect 10% chronic hypertension with a power of 80% and alpha of .05. All computations were performed using SPSS version 20.0.

Results

From 386 formerly preeclamptic women who completed a cardiovascular evaluation after a preeclamptic pregnancy (visit 1), 104 (27%) women were eventually included in the final analysis. As shown in Figure 1, 183 women met the exclusion criteria (Figure 1). The remaining 203 normotensive apparently healthy formerly preeclamptic women were eligible and therefore invited for the second assessment (visit 2). At time of analysis, 99 out of the 203 (49%) eligible women were lost to follow-up.

The first cardiovascular and metabolic assessment was at a median of 7 months after the index pregnancy (interquartile range: 6-11 months) and the median interval between visits was 47 months (interquartile range: 42-53 months). Consequently, the median interval between delivery and second assessment was 55 months (interquartile range: 50-65).

The characteristics of the 104 women included in the final analysis are shown in Table 1. Women who were eligible, but not available at follow-up (n=99) were less often primiparous (79 vs. 94%), delivered at a later gestational age (35 vs. 33 weeks) of heavier infants (2149 vs. 1945 g) and had less often a positive first-degree family history for hypertension (54 vs. 63%) and/or cardiovascular disease (25 vs. 42 %). Thirty-five of the 104 included women (34%) had LPV at the first visit.

Table 2 shows the characteristics of the 104 formerly preeclamptic women who were normotensive at the first visit and either stayed normotensive (n=86; 83%) or developed hypertension (n=18; 17%) 2 to 5 years later. Women who developed hypertension when compared with women who remained normotensive were comparable for all measured variables at the first visit except for a significantly lower mean plasma volume (1322 vs. 1509 mL/m^2). In women who stayed normotensive 25/86 (29%) had LPV, in contrast to 10/18 (56%) women who developed hypertension (*P*=.03). Average systolic, mean and diastolic blood pressures were slightly, but not significantly, higher in women destined to develop hypertension (123 vs. 119; 92 vs. 88 and 75 vs. 72 mmHg, respectively).

The majority of women in both groups had a second pregnancy between the two visits. Outcomes of these pregnancies were comparable, although gestational hypertension in the next pregnancy tended to occur more often in women who subsequently developed de novo hypertension (73 vs. 45%).

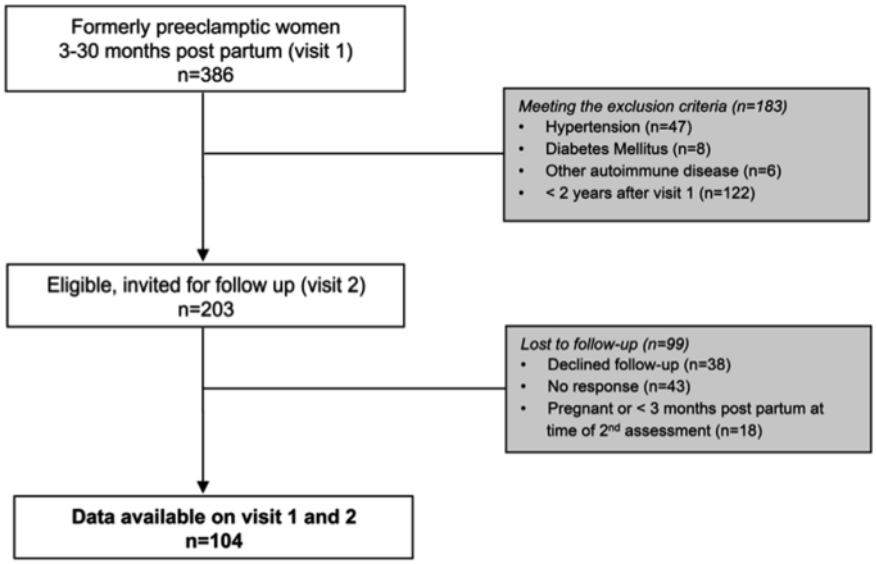

Figure 1 Flowchart of women included and excluded from retrospective analysis. Exclusions are shown in gray boxes.

Table 1 Characteristics of normotensive formerly preeclamptic women at 3 to 30 months following a preeclamptic pregnancy (visit 1).

Characteristics	Included in analysis (n=104)	Lost to follow-up (n=99)	*P*-value
Obstetric history			
Primiparous (n/N (%))	97/104 (94)	78/99 (79)	<.01
Gestational age at delivery index pregnancy (weeks)	33 (30-38)	35 (32-40)	<.01
Birth weight at index pregnancy (g)	1945 (1090-2795)	2149 (1602-3265)	.01
Birth weight centile at index pregnancy	22 (5-37)	27 (9-44)	.18
Baseline characteristics			
Interval index pregnancy to visit 1(months)	7 (6-11)	8 (6-12)	.67
Age (years)	32±4	33±5	.49
Smoking (n/N (%))	18/104 (17)	18/99 (18)	.42
BMI (kg/m^2)	24±6	25±6	.12
Systolic blood pressure (mmHg)	120±10	119±10	.18
Diastolic blood pressure (mmHg)	72±7	70±9	.28
Mean blood pressure (mmHg)	88±7	87±8	.32
Heart rate (bpm)	69±9	72±10	.10
Plasma volume (mL/m^2)	1476±200	1492±171	.28
Low plasma volume (n/N (%))	35/104 (34%)	32/99 (32%)	.68
Cholesterol (mmol/L)	4.6±0.9	4.7±0.7	.44
HDL (mmol/L)	1.4±0.3	1.4±0.3	.68
LDL (mmol/L)	2.8±0.7	2.9±0.7	.57
Triglycerides (mmol/L)	1.0±0.5	1.1±0.3	.38
Creatinine (micromol/L)	67±7	67±7	.72
Microalbuminuria (gmol/creat)	1.6 (0.6-5.8)	1.5 (0.6-5.2)	.37
Proteinuria (g/24 hrs)	0.09 (0.06-0.13)	0.09 (0.05-0.14)	.46
Family history (1st degree)†			
Hypertension (n/N (%))	64/101 (63)	53/98(54)	<.01
Cardiovascular disease (n/N (%))	42/101 (42)	34/96 (35)	.02
Diabetes (n/N (%))	45/101 (45)	40/97 (41)	.30

Data are n/N, mean ± standard deviation, or median (interquartile range). Statistics χ^2, Student *t,* or Wilcoxon ranks sum test. BMI indicates body mass index, HDL: High density lipoprotein, LDL: Low density lipoprotein, PV: plasma volume. *Low Plasma Volume: PV≤1373 mL/m^2. †In 6 subjects, family history was missing or incomplete.

Table 2 Characteristics of formerly preeclamptic women at 3 to 30 months post-partum (visit 1) and 2 to 5 years after the initial visit (visit 2), divided between persistently normotensive women and women who developed hypertension during follow-up.

Characteristics	Women persistently normotensive (n=86)	Women developing hypertension (n=18)	*P*-value
Obstetric history			
Primiparous (n/N (%))	82/86 (95%)	17/18 (94%)	.76
Gestational age at delivery index pregnancy (weeks)	34 (30-39)	33 (29-38)	.80
Birth weight at index pregnancy (g)	1845 (1570-2500)	1420 (1030-2775)	.27
Birth weight centile at index pregnancy	20 (7-42)	12 (5-33)	.12
Characteristics at first follow-up visit			
Interval index pregnancy and visit 1 (months)	7 (6-11)	9(7-15)	.34
Age (years)	32±4	33±3	.48
Smoking (n/N (%))	14/86 (16)	3/18 (17)	.62
BMI (kg/m^2)	24±6	24±4	.90
Systolic blood pressure (mmHg)	119±10	123±10	.13
Diastolic blood pressure (mmHg)	72±7	75±6	.03
Mean blood pressure (mmHg)	88±7	92±5	.02
Heart rate (bpm)	68±9	69±11	.72
Plasma volume (mL/m^2)	1509±191	1322±176	<.01
Low plasma volume (n/N (%)	25/86 (29)	10/18 (56)	.03
Fasting insulin (mU/L)	9.2±3.9	10.7±3.8	.16
HOMA-IR	2.0±0.9	2.4±1.0	.07
Cholesterol (mmol/L)	4.7±0.9	4.6±0.8	.79
HDL (mmol/L)	1.4±0.3	1.4±0.3	.87
LDL (mmol/L)	2.9±0.7	2.6±0.5	.22
Triglycerides (mmol/L)	1.0±0.5	1.1±0.4	.41
Creatinine (micromol/L)	67±8	66±6	.64
Microalbuminuria (gmol/creat)	1.5 (0.6-4.1)	1.8 (0.8-5.4)	.14
Proteinuria (g/24 hrs)	0.09 (0.06-0.12)	0.09 (0.07-0.14)	.22
Family history (1st degree)†			
Hypertension (n/N (%))	63/84 (62)	12/17 (71)	.43
Cardiovascular disease (n/N (%))	44/84 (43)	7/17 (41)	.77

Table 2 Continued.

Characteristics	Women persistently normotensive (n=86)	Women developing hypertension (n=18)	*P*-value
Family history (1st degree)†			
Diabetes (n/N (%))	45/84 (44)	8/17 (47)	.45
Pregnancy after first follow-up visit?			
No pregnancy (n/N (%))	20/86 (23)	3/18 (17)	.33
Normotensive pregnancy (n/N (%))	36/66 (55)	4/15 (27)	.08
Gestational hypertension (n/N (%))	30/66 (45)	11/15 (73)	.08
Preeclampsia (n/N (%))	13/66(20)	5/15 (33)	.30
Characteristics at second follow-up visit			
Interval index pregnancy to visit 2 (months)	54 (48-64)	60 (55-73)	.02
Interval between visits (months)	46 (42-52)	50(48-55)	.02
Age (years)	35±3	36±3	.27
BMI (kg/m^2)	25±6	26±6	.48
Systolic blood pressure (mmHg)	115±11	141±5	<.01
Diastolic blood pressure (mmHg)	71±8	89±8	<.01
Mean blood pressure (mmHg)	85±7	106±6	<.01
Heart rate (bpm)	67±11	68±10	.63
Fasting glucose (mmol/L)	4.7±0.4	5.1±0.8	<.01
Fasting insulin (mU/L)	9.8±5.3	11.3±4.7	.26
HOMA-IR	2.1±1.2	2.6±1.3	.07
Cholesterol (mmol/L)	4.6±0.7	4.5±0.7	.50
HDL (mmol/L)	1.3±0.3	1.3±0.2	.29
LDL (mmol/L)	2.9±0.7	3.0±0.5	.73
Triglycerides (mmol/L)	0.9±0.5	1.0±0.4	.65
Creatinine (micromol/L)	65±9	67±6	.62
Microalbuminuria (gmol/creat)	1.5 (0.4-5.2)	1.9 (0.7-6.6)	.87
Proteinuria (g/24 hrs)	0.07 (0.04-0.15)	0.11 (0.07-0.18)	.80

Data are n/N, mean ± standard deviation, or median (interquartile range). Statistics χ^2, Student *t,* or Wilcoxon ranks sum test. BMI indicates body mass index, BP: blood pressure, HDL: High density lipoprotein, LDL: Low density lipoprotein, PV: plasma volume. *Low Plasma Volume: PV≤1373 mL/m^2. †In 3 subjects, family history was unavailable.

At the second cardiovascular evaluation most women who qualified as hypertensive had blood pressures just above the cutoff values for hypertension. Average systolic blood pressure in the hypertensive group was 141±4 mmHg, mean blood pressure 106±6mmHg and diastolic blood pressure 89±8 mmHg. In the persistently normotensive women average values were systolic 115±11mmHg, mean 85±7 and diastolic 71±8 mmHg. Women who developed hypertension had higher mean blood glucose levels at the second visit than women who remained normotensive (5.1±0.8 vs. 4.7±0.4 mmol/L; $P<.01$). Otherwise groups were metabolically comparable.

Mean arterial blood pressure at the second cardiovascular visit related inverse linearly to normalized plasma volume measured at the first visit (mL/m^2) (r=-0.49, $P<.01$). Women with lowest PV at the first follow-up visit had highest mean arterial blood pressure at the second follow-up 2 to 5 years thereafter (Figure 2). Change in mean arterial blood pressure between the two visits did not only correlate with PV, but also with positive first-degree family history for hypertension (r=0.23; $P=0.02$) and diastolic blood pressure at the first visit (r=0.21; $P=0.03$) (Table 3).

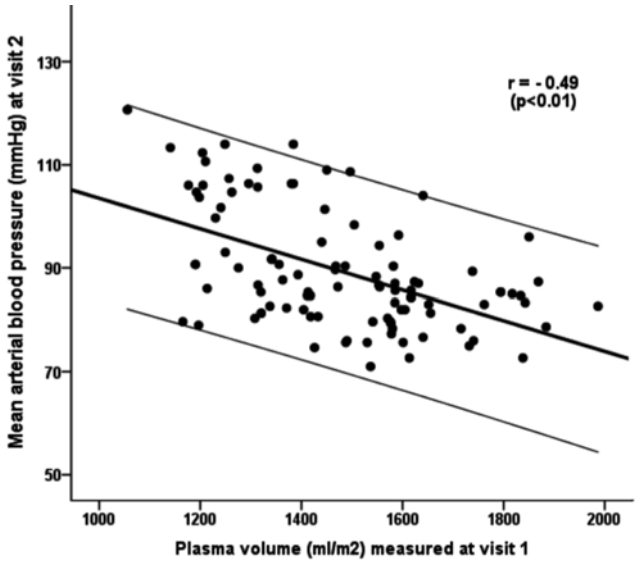

Figure 2 Inverse linear relationship of mean arterial pressure at visit 2 as a function of normalized plasma volume at visit 1 in formerly preeclamptic women. All women by selection were normotensive at the time of plasma volume measurement.

Table 3 Correlation coefficients of co-variables with change in mean blood pressure between the two assessments in initially normotensive formerly preeclamptic women.

Characteristics	Correlation coefficient (r)	*P*-value
General characteristics at first visit		
Age (years)	0.10	.33
Smoking (yes/no)	0.13	.14
BMI (kg/m^2)	0.07	.45
Change in weight (kg) between visits	0.14	.10
First degree relative with hypertension (yes/no)	0.23	.02
Hemodynamic characteristics at first visit		
Systolic blood pressure (mmHg)	0.03	.75
Diastolic blood pressure (mmHg)	0.21	.03
Mean blood pressure (mmHg)	0.12	.21
Heart rate (bpm)	0.05	.60
Plasma volume (mL)	-0.26	$<$.01
Plasma volume (mL/m^2)	-0.32	$<$.01
Metabolic characteristics at first visit		
Fasting glucose (mmol/L)	0.10	.31
Fasting insulin (mU/L)	0.12	.24
Cholesterol (mmol/L)	-0.02	.84
HDL (mmol/L)	0.04	.70
LDL (mmol/L)	-0.11	.25
Triglycerides (mmol/L)	0.03	.80
Time interval		
Interval index-pregnancy to visit 1 (months)	0.13	.18
Interval between visits (months)	0.11	.27
Interval index-pregnancy to visit 2 (months)	0.11	.25

BMI indicates body mass index, BP: blood pressure, HDL: High density lipoprotein, LDL: Low density lipoprotein

When study group was divided based on PV status, chance of developing hypertension after preeclampsia was 10 of 35 (29%) in women with LPV status and 8 of 96 (12%) in women with NPV status (OR: 3.2 [95%CI: 1.4-8.6]) (Table 4). When adjusted for possible confounders like family history for hypertension and diastolic blood pressure at first visit the relationship between PV and developing hypertension after preeclamptic pregnancy persisted (aOR: 3.0 [95%CI: 1.1-8.5]).

Table 4 Absolute and relative risk and odds ratios of developing hypertension in initially normotensive formerly preeclamptic women.

Characteristics	Low plasma volume (n=35)	Normal plasma volume (n=69)
Prevalence (n/N (%))	10/35 (29)	8/69 (12)
Crude OR [95%CI]	3.2 [1.4-8.6]	*Reference*
Adjusted OR [95%CI] *	3.0 [1.1-8.5]	*Reference*

Prevalence (n/N (%)) and odds ratios of de novo hypertension at the second visit in women with a history of preeclampsia. Study population classified in 2 groups according plasma volume**.** Low plasma volume defined as $<$ 1373 mL/m^2; normal plasma volume as $\geq$ 1373 mL/m^2. OR indicates Odds Ratio, CI: confidence interval. *Adjustments for family history of hypertension (yes/no) and diastolic blood pressure (mmHg) at the first visit were performed using backward multivariate regression analysis.

Discussion

This study demonstrates that 1 out of 6 (17%) formerly PE women who are normotensive at 3 to 30 months post-partum develop overt hypertension within the subsequent 2 to 5 years. Women with low plasma volume were more prone to develop hypertension than women with normal plasma volume. Low plasma volume may therefore reflect a latent hypertensive profile that can become manifest within a few years after the preeclamptic pregnancy.

It has been well established that formerly preeclamptic women are at increased risk of developing cardiovascular disease later in life[1]. It is hypothesized that preeclampsia and CVD share several common risk factors that may lead to expression of these disease entities at different times in a woman's life[5,6,12-14]. An alternative hypothesis is that preeclampsia itself may induce subclinical vascular and metabolic changes that in time increase the risk for overt hypertension and CVD later in life. Our study does not differentiate between these possible mechanisms. Irrespective of the underlying mechanism, a history of preeclampsia may identify women at risk for CVD early in life, thus offering a unique opportunity for timely screening and preventative strategies[15].

The prevalence of 17% hypertension in our study is markedly higher than the 3.7% prevalence of hypertension in the general population of Dutch women between 30 and 39 years[16]. Adding the excluded women who remained hypertensive after the preeclamptic index pregnancy (47/386 (12%)), the overall prevalence of hypertension after preeclampsia is comparable with the mean prevalence of 30 to 40% found in other studies in formerly preeclamptic women[6,17-20]. Our data show that a substantial proportion of women progressed within a few years from normotension to hypertension.

The exact cause of the phenotype low plasma volume is currently unknown, but several explanations have been hypothesized. With twothirds of resting blood volume in the venous compartment, an actively constricted venous system might chronically reduces venous dimensions and (resting) elastic properties of the venous wall and thereby reducing its capacitance. An intrinsically small venous system, in line with the Barker hypothesis might also account for the observed plasma volume. Lastly, plasma volume may be functionally reduced as a result of hormonal misbalance. Recent studies have suggested a pathophysiological role of increased vasopressin levels in preeclampsia[21]. If vasopressin increases one would expect higher plasma volume levels unless the vasopressin is increased secondary to low plasma volume status. In pregnancy however women who develop preeclampsia are not chronically underfilled, since compensatory neuro-humoral changes such as elevated renin, angiotensin and aldosterone levels are lacking[23]. Whether neurohumoral changes contribute, either by cause or effect, to low plasma volume status in the nonpregnant situation of formerly preeclamptic women is currently unknown. Therefore neurohumoral explanations for low plasma volume cannot be completely excluded.

The mechanistic explanation for the relationship between low plasma volume and the development of hypertension is hypothetical. One possible pathophysiological explanation could be that low plasma volume status negatively affects cardiac preload, driving the sympathetic nervous system to compensate the reduced venous reserve. The resultant reduction in circulation time of the blood to pass the heart at each rotation will increase shear forces on the vascular endothelium, setting the stage for chronic hypertension[7,14,22].

Formerly preeclamptic women who developed hypertension gained more weight between the two visits and had higher fasting glucose levels at the second visit. This suggests a less healthy lifestyle in formerly preeclamptic women destined to develop hypertension. Lipid profile and microalbuminuria remained relatively stable in the first 5 years following the preeclamptic pregnancy, independent if women developed hypertension or not. This may indicate that short-term cardiovascular follow-up in

normotensive formerly preeclamptic women should focus on aberrations in blood pressure, body weight and glucose metabolism and less on dyslipidemia or albuminuria.

Generalizability of the study is limited because of several reasons. First, women with a positive family history of hypertension or cardiovascular disease may have had an incentive to participate in our follow-up study. This may have resulted in an overrepresentation of women who are genetically more susceptible to develop hypertension at a young age. Also women lost to follow-up were more often multiparous and delivered at a later gestational age. Therefore women with earlier onset preeclampsia, an indicator of more severe forms of preeclampsia, might be overrepresented in our study group. Second, there was a considerable loss to follow-up, mainly because interval after the first assessment was not yet more than 2 years. Because group characteristics of included women were generally comparable with women lost to follow-up, we do not expect a large selection bias. Third, women who developed hypertension had more often a hypertensive pregnancy between the two assessments compared to women who remained normotensive. Although the second assessment was at least 3 months post partum to allow for hemodynamic recovery, we cannot rule out a possible overestimation of the blood pressure as recovery may still continue thereafter. Pregnancy outcome however did not correlate with change in blood pressure between visits, suggesting a nonsignificant impact on outcome. Follow-up period of maximally 5 years is relatively short for developing hypertension. Longer follow-up would likely influence the reported occurrence of hypertension in formerly preeclamptic women.

Perspectives

Monitoring of blood pressure seems warranted in women with a history of preeclampsia, even in absence of hypertension in the first two years after pregnancy. It should be noted that the effects of structural cardiovascular and metabolic follow-up on clinical cardiovascular outcomes in this specific population are unknown. Nevertheless, based on the strong association between preeclampsia and future cardiovascular disease, structured follow-up is often recommended. This may vary from follow-up in primary care or in more specialized cardiovascular risk management collaborations of care takers including perinatology, cardiology and vascular internal medicine specialists[7]. Structured follow-up more likely secures regular surveillance of CVD risk factors, and hypertension in particular, with treatment according to national guidelines for CVD prevention in these women. Ideally this should be coupled to primary prevention strategies focusing at lifestyle modifications (physical exercise, healthy diet, weight loss and smoking cessation) in order to optimize maternal cardiovascular health. Lifestyle interventions have demonstrated to reduce cardio-

vascular risks substantially in various populations at risk for cardiovascular disease[24,25]. Aerobic exercise and diet prevent hypertension, excessive weight gain and reduce insulin resistance[24]. Interestingly, women are particularly motivated to change their lifestyle habits during pregnancy or the postpartum period in order to optimize outcomes of mother and child and it is therefore considered an ideal timing to encourage primary prevention strategies[26,27].

Conclusion

One in six initially normotensive formerly preeclamptic women develop hypertension within the next 2 to 5 years. In these apparently healthy women low plasma volume correlates with increased risk to develop hypertension. Clinically, follow-up of blood pressure seems warranted after a pregnancy complicated by preeclampsia, even in women who are initially normotensive.

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CHAPTER 6

Aerobic exercise training in formerly preeclamptic women: effects on venous reserve

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Abstract

In women with a history of preeclampsia, low plasma volume (≤1373 mL/m^2) is associated with recurrent preeclampsia and chronic hypertension. Interventions that improve volume reserve may reduce these risks in formerly preeclamptic women. In this study we examined the effects of aerobic exercise training on venous reserves in 24 normotensive formerly preeclamptic women and 20 controls.

Before and after 12-week aerobic exercise training, we measured plasma volume with albumin indicator dilution technique and venous compliance with venous occlusion plethysmography. Venous compliance and hemodynamic responses were assessed dynamically during graded head-up tilt (HUT). Formerly preeclamptic women had lower pretraining plasma volume and venous compliance than controls (1348±78 vs.1529±112mL/m^2; (P<.01) and 0.04±0.02 vs. 0.07±0.01 mL/dL/mmHg; (P<.01) respectively). Blood pressure decreased comparably between groups in response to HUT (P=0.11); the increase in heart rate in response to HUT was however more pronounced in formerly preeclamptic women than in controls (P=0.01). Training increased plasma volume comparably in both groups (+180 vs.+135mL/m^2; P=0.22; and similarly physical fitness +3.4 and +3.7mL/min/kg; P=.43). Venous compliance increased twice as much in formerly preeclamptic women than in controls (supine +0.02 vs. +0.01mL/dL/mmHg; P<.01). After training, HUT decreased mean blood pressure comparable with pre-training responses in both groups, whereas both groups fulfilled the HUT testing at a persistently lower heart rate.

These results demonstrate that 12 weeks of aerobic exercise training improves venous reserve in postpartum women. Training normalized plasma volume and venous compliance in formerly preeclamptic women to pretraining levels of controls.

Introduction

Women with a history of preeclampsia are at increased risk for recurrent gestational hypertensive disease and cardiovascular disease later in life[1,2]. Fifty percent of formerly preeclamptic women have low plasma volume (PV)[3] and the recurrence risk of preeclampsia varies inversely with PV[4]. In pregnancy, a marked PV expansion (+30 to 60%) is required to meet the physiological demands of advanced pregnancy[5,6]. Formerly preeclamptic women with low PV show reduced PV expansion in pregnancy, which suggests that the adaptive capacity of the venous system in these women is limited[7]. When PV expansion cannot meet the physiological demands, the circulation may compensate for it by an increase in sympathetic activity[7]. The resulting low-volume high-output circulation will exert extra shear stress upon the endothelium[3,7]. This may set the stage for endothelial dysfunction, vascular damage, and hypertensive disease in pregnancy and later in life.

The venous compartment is the most prominent blood volume reservoir of the body. Allthough at rest two-thirds of PV is localized in the venous system, it can be rapidly mobilized by sympathetic mediated venoconstriction in times of increased arterial demands, including pregnancy and exercise[8,9]. Venous capacity is largely determined by venous compliance (VeC), and varies dynamically with sympathetic tone predominantly controlled by the baroreflex system[8]. Dynamic changes in venous compliance can be tested through orthostatic stress testing. Previous studies demonstrated that formerly preeclamptic women at rest have low venous reserve, characterized by low PV, low venous compliance and high sympathetic tone[10].

Aerobic exercise is perhaps the best intervention to improve venous reserve. It increases PV and reduces sympathetic tone in healthy individuals[11,12]. In formerly preeclamptic women with low PV, aerobic exercise training could help to normalize venous reserve and thereby reduce the risk of hypertensive disease in future pregnancy and later in life. The extent to which formerly preeclamptic women are able to improve their venous reserve by exercise training is unknown. Our study addresses the question to what extent an aerobic exercise training program (12 weeks of cycling at 70-80% VO_2max, 2-3 times per week) can improve venous reserve in formerly preeclamptic women compared to parous controls. Based on the observation that formerly preeclamptic women demonstrate reduced PV expansion in pregnancy, we hypothesized that formerly preeclamptic women may be less able to increase venous reserve in response to exercise training compared with parous controls.

Materials and methods

We recruited 25 normotensive formerly PE women and 22 controls. Primiparous formerly preeclamptic women were recruited during their postpartum checkup at the Radboudumc, controls from the community by advertisement. Preeclampsia in prior pregnancy was defined by the combination of gestational hypertension (≥140/90 mmHg, measured twice, six hours or more apart), and proteinuria (consistently ≥300 mg/24 hours) after 20 weeks of pregnancy in previously normotensive women[13]. Controls were healthy primiparous women; their pregnancy charts were checked to ensure that the preceding pregnancy had been normal.

All participants were white women, healthy and normotensive at the time of measurements. None had diabetes mellitus, autoimmune disease or overt cardiovascular disease. None of the women smoked or used medication or supplements that might affect the cardiovascular system and none of the women included were pregnant, breastfeeding, or using hormonal contraceptives. Excluded from analysis were women who became pregnant during the course of training and women who did not finish the exercise protocol. The study was approved by the Medical Ethics Committee of the Radboudumc (CMO: 2008/226) All participants gave written informed consent before entering the study. The study was registered at clinicaltrials.gov (id: NCT00900458).

Experimental design

Measurements and training were performed in the non-pregnant state, 6 to 12 months after pregnancy to allow for cardiovascular recovery after pregnancy[14]. Subjects were tested before and after 12 weeks of the exercise training. All measurements, except VO_2max testing, were performed during the same visit. VO_2max was tested 1 to 5 days from the other visit. In sequential order, we measured body characteristics, resting blood pressure, cardiac output, PV and venous and hemodynamic responsiveness during head-up tilt (HUT). This study protocol is part of a more extensive protocol that also involves functional arterial measurements. Results of the arterial measurements are published elsewhere[15,16].

Experimental procedures

Tests and measurements were performed between day 3 and 11 of the menstrual cycle to minimize possible endocrine influences of the sex hormones on the cardiovascular- and autonomic nervous system[17]. All measurements except VO_2max were performed following an overnight fast. Participants were instructed to abstain from strenuous physical activity in the 24 hours prior to testing. Participants collected urine in the 24 hours preceding the measurements. The 24-hour urine sample was

assayed for albumin, protein and creatinine to define the (micro) albuminuria corrected for creatinine output (g/mol creatinine) and total protein level (g/24 hrs) (Aeroset, Abbot Laboratories, Illinois USA).

Tests were performed under standardized conditions in a temperature-controlled room (22±0.5°C). Measurements were performed at the same time in the morning to prevent diurnal variation. After 30-minute rest in supine position, blood pressure and heart rate (HR) were measured oscillometrically (Dinamap, Vital Signs Monitor 1846, Critikon, Tampa, Florida) in the upright sitting position, at the right upper arm, with the cuff size recommended for the arm circumference, at 3-minute intervals for 30 minutes.[18] We recorded systolic (SAP, mmHg), diastolic (DAP, mmHg) and mean (MAP, mmHg) arterial pressures and heart rate (HR; bpm) and used the median values of 9 consecutive measurements for analysis. Cardiac output (CO; L/min) was measured in the left-lateral position, using a validated, non-invasive, inert gas rebreathing method (Innocor, Innovision, Copenhagen) as detailed elsewhere[19]. Stroke volume (SV, mL) was calculated as CO/HR. Venous blood samples were taken from the antecubital vein and analyzed for creatinine concentration (Aeroset, Abbot Laboratories, Illinois USA).

Plasma volume

Plasma volume (PV, mL) was measured using the iodine^{125} albumin indicator dilution technique (^{125}I-HSA). During the measurement, women were in semi-supine position on a comfortable bed. An 18-gauge intravenous catheter was inserted in the left antecubital vein for repetitive blood sampling. A standardized dose (0.2 MBq) of ^{125}I-human serum albumin was injected intravenously in the right antecubital vein. Every 10 minutes a venous blood sample was taken from the contra lateral intravenous catheter until 40 minutes after administration of the ^{125}I-HSA. Blood samples were analyzed using a gamma counter. PV was calculated by dividing the total injected radioactivity by the virtual volume specific radioactivity at time zero, as described elsewhere[20]. PV was normalized for body surface area (PV, mL/m^2). Low plasma volume was defined as PV ≤1373 mL/m^2 [4]. The technician who measured PV was unaware of the medical history of participants.

Head-up tilt test

Head-up tilt (HUT) was performed after voiding the bladder, and executed under standardized environmental conditions in a quiet and partially darkened room. Subjects were positioned on the tilt table on a comfortable mattress to minimize muscular activity and both arms were positioned at heart level. Participants remained supine for 20 minutes; thereafter head-up tilt was imposed by passively changing the body posture from 20 degrees head-down tilt (-20°) to 60 degrees head-up tilt (+60°),

in steps of 20° at 8-minute interval. Some women experienced presyncope and could not complete the whole test. Presyncope was defined by a precipitous fall in systolic arterial pressure > 15 mmHg concurrent with symptoms such as bradycardia, light-headedness, blurred vision, sweating and/or nausea. Women who experienced pre-syncope were returned to supine position.

Venous compliance during HUT

Venous compliance (VeC, mL/dL/mmHg) was measured on the forearm using strain gauge venous occlusion plethysmography with direct intravenous pressure measurement[21]. Venous pressure was directly measured through a catheter in the left antecubital vein connected to a pressure transducer system at atrial height. Changes in forearm volume were measured by a mercury-in-silastic strain gauge 5 cm distal to the antecubital crease. A venous collective cuff was placed 5 cm proximal to the antecubital crease. The pressure cuff was connected to a rapid cuff inflator (Hokanson E20, Denmark) to ensure rapid and accurate filling and deflation of the cuff. Data signals were recorded by computer at a sampling rate of 100Hz and stored for further analysis (MIDAC, Biomedical Engineering Department, Radboudumc, Nijmegen, The Netherlands). Cuff pressure was gradually increased from 0 to 40 mmHg in 60 seconds. Changes in forearm volume and intravenous pressure were recorded at the end of each rotational step. VeC was defined as the ratio of the slope of the volume-time curve and the slope of the pressure-time curve:

$$\text{VeC} = \frac{\Delta \text{ volume} / \Delta \text{ time}}{\Delta \text{ pressure} / \Delta \text{ time}}$$

Only the data of the linear part of the relationship were used.

Hemodynamic responses during HUT

During the HUT, HR and arterial blood pressure were measured continuously using a monitoring device attached to the 3rd finger of the right hand and a sampling rate of 100Hz (Finometer, Finapres BV, the Netherlands). We used the hemodynamic data only to study heart rate and relative changes of blood pressure in response to orthostatic stress. It is known that absolute values of blood pressure acquired from Finometer are not reliable enough as these values are reconstructed from waveform transformation. Changes in blood pressure can however be measured accurately.[22] We excluded data of the first minute after postural change because we have shown in previous experiments that a new steady state is reached within 60 seconds after postural change[23]. Head-down tilt was performed to test the responses with maximized venous return. Post hoc, the recordings were analyzed by calculating mean heart rate and mean blood pressure over five minutes starting 60 seconds after postural changes at each rotational step.

Physical Fitness

Physical fitness was measured before and after a 12-week aerobic training program. Fitness was defined as the peak oxygen uptake (VO_2max, mL/min/kg) during a maximal test on a cycle ergometer (Excalibur Sport, Lode BV, Groningen NL). Tests were performed in the afternoon after a light lunch ad libitum. The initial workload was set at 10W for 1 minute and followed by 10W increments every minute until exhaustion. Breath by breath oxygen uptake was measured using spiro-ergometric equipment (Quark CPET, Cosmed, Italy). A 3-lead ECG continuously recorded HR and rhythm. Maximal workload (Workmax) was defined as the last completed workload before exhaustion. Test performance was considered to be adequate when (1) The increase in VO_2 during Workmax was <150mL compared to the previous workload, indicating plateau formation in oxygen uptake (2) HR at Workmax was <10 bpm from estimated maximal HR (220-age) (3) Respiratory Exchange Ratio (RER, CO2/O2) during Workmax was consistently >1.1 and (4) Capillary lactate level was >8mmol/L, 90 seconds after exhaustion. If the test failed to achieve these 4 qualifications the test was repeated 2-3 days later. In three cases (2 formerly PE women, 1 control) the test had to be repeated, all tests eventually fulfilled the criteria.

Exercise training

Exercise training consisted of 12 weeks of HR controlled, supervised cycle training (cycle ergometer, Corival, Lode BV, Groningen Netherlands) at 70-80% of VO_2max. Participants trained twice a week during the first 6 weeks and 3 times a week during the last 6 weeks. Each training session was supervised and executed in the gymnasium of our laboratory. Care was taken that each training session had at least one day in between to allow adequate recovery. Participants were instructed not to exercise in addition to the exercise protocol given. During each training session, HR was continuously monitored and recorded (RS800CX, Polar Electro Inc, NY USA). Each training session started with 10 minutes warming-up at 50% of the HR reserve (HRR) above resting HR. HRR was calculated as $HRR=HR_{max}-HR_{rest}$ in which HR_{max} is maximal HR during the fitness test at study entry and HR_{rest} is the HR determined at rest. Training consisted of 40 minutes of cycling at 70-80% of the individual HRR. Within their target HR zone, participants were free to choose the number of revolutions per minute (RPM). Cooling down for 5 minutes at warming-up workload completed the training.

Statistical analysis

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL) software. All normally distributed data are reported as mean ± SD; other data are presented as median [interquartile range]. Normality of data was tested with a Kolmogorov-Smirnov test. Two-way repeated-measures ANOVA was used to examine differences

between formerly PE women and controls groups, to assess the effects of training (intervention) and to determine if training effects differed between formerly PE women and controls (training x group). A mixed linear model for repeated measures was performed to test differences between groups and effects of training on the response curves of venous and hemodynamic variables to HUT. When significant effects were found, Bonferroni posthoc comparison tests were used. Statistical significance was assumed at $P<.05$. We based our group-size calculation for the intervention part of our study on an anticipated difference in exercise induced PV expansion (primary outcome measure) of 4% between groups, a power of 90% and an alpha of .05. Assumed standard deviation of the exercise induced plasma volume expansion was based on a pilot study in 9 formerly PE women (4%)[24]. Based on these assumptions, 18 subjects were required per group. To anticipate possible dropout we decided to include at least 20 subjects per group.

Results

Included in the analysis were 24 formerly preeclamptic women and 20 controls, after exclusion of 1 formerly preeclamptic woman (who became pregnant) and 2 controls (one who became pregnant and one who did not finish the training protocol). Mean age and interval from delivery to test were comparable between groups (32±4 vs. 32±4 years, $P=.62$ and 7±2 vs.7±1 months, $P=.88$, respectively). Formerly preeclamptic women had delivered at an earlier gestational age compared with controls (32 [29-37] vs. 40 [38-41] weeks, $P<0.01$), of children with a lower birth weight (1571±675 vs. 3532±311 grams, $P<.01$).

Prior to training

Physical and hemodynamic characteristics of formerly preeclamptic women and controls are shown in Table 1. Prior to training, mean physical fitness was comparable between both groups (VO_2max: 27.0±4.0 and 28.2±3.7 mL/min/kg, $P=.32$) as were body mass index, stroke volume, cardiac output and serum creatinine concentration. All subjects were normotensive (by inclusion). In formerly preeclamptic women, average values of HR, systolic arterial pressure, diastolic arterial pressure, MAP, creatinine clearance and albuminuria were higher than in controls. PV was 13% lower in formerly preeclamptic women than in controls (1348±78 vs. 1529±112 mL/m^2, $P<.01$), as shown in Figure 1.

The venous response curves to graded HUT are shown in Figure 2. Before training, VeC was lower in formerly preeclamptic women than in controls (supine: 0.04±0.02 vs. 0.07±0.01 mL/dL/mmHg, $P<.01$). Formerly preeclamptic women had lower VeC

Table 1 Characteristics of controls and formerly preeclamptic women, before and after exercise training

Characteristics	Controls (n=20)		Formerly preeclamptic women (n=24)		2-way ANOVA (*P*-value)		
	Before training	After training	Before training	After training	Group	Training	Group x Training
BMI, kg/m²	26.8±3.4	25.1±6.8	25.9±4.9	24.4±7.0	.63	.04	.88
Hemodynamic parameters (in rest)							
Systolic arterial pressure, mmHg	109±6	104±6	119±10*	112±8	<.01	<.01	.39
Diastolic arterial pressure, mmHg	66±6	63±6	74±7*	69±7	<.01	<.01	.19
Mean arterial pressure, mmHg	78±5	74±5	86±8*	80±7	<.01	<.01	.26
Heart rate, bpm	63±9.8	57±7.6	77±8.7*	59±8.9	.15	<.01	.03
Cardiac output, L/min[1]	5.4±0.5	5.5±0.8	5.5±1.6	5.5±0.8	.81	0.44	.60
Cardiac index, L/min/m²	2.8±0.3	2.9±0.4	3.0±0.6	3.0±0.5	.35	.73	.68
Stroke volume, mL	76±17	82±15	66±16	80±14	.19	<.01	.07
Renal function							
Creatinine, μmol/L	65.2±9.3	63.8±9.8	66.8±14.5	67.1±13.7	.52	.35	.14
Creatinine clearance, mL/min/1.73m²	83±23	86±15	116±35*	97±32	.03	.08	<.01
Albuminuria, mg/mmol creatinine	0.3 [0.1-0.6]	0.3 [0.1-0.6]	1.3 [0.6-2.1]*	0.7 [0.3-2.1]*	.04	.07	.08
Maximal exercise test							
VO_{2max}, mL O_2/kg/min	28.2±3.7	31.9±3.5	27.0±4.0	30.4±5	.32	<.01	.52
Maximal heart rate, bpm	186±9	185±7	189±7	189±6	.08	.57	.32
Maximal work load, Watt	195±27	210±22	188±25	204±27	.27	<.01	.53

Values are mean±SD. * $P<0.05$ compared with controls before training (t-test). Results 2-way ANOVA: Group: patients vs. controls. Intervention: main effect exercise program. Group x intervention: interaction between groups and exercise training

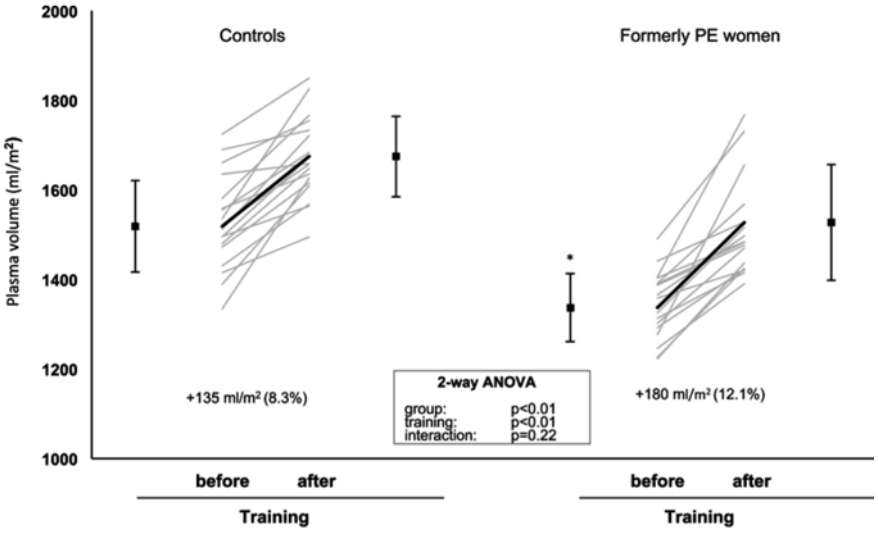

Figure 1 Effects of 12 weeks aerobic exercise training on plasma volume (mL/m^2) in formerly preeclamptic women (n=24) and controls (n=20). Grey lines represent individual data. Black lines represent means, error bars standard deviation, * indicates significant difference from healthy parous control women *before* training ($P<0.05$).

at all levels of HUT and a more gradual slope of the VeC response to progressive HUT than controls (P=.004), indicating a relatively poor venous response to orthostatic stress.

Blood pressure decreases significantly secondary to orthostatic stress (P<.01). The response curves of mean blood pressure to graded HUT show that prior to exercise training blood pressure drops comparably between formerly preeclamptic women and controls in response to HUT ($P=.11$)(Figure 3). The increase in heart rate in response to HUT was however more pronounced in preeclamptic women than in controls ($P=.01$). With progressive HUT mean heart rate increased. From 20 degrees HUT onward, the slope of the orthostatically induced increase in heart rate was steeper in formerly preeclamptic women than in controls ($P=.01$) (Figure 4). Presyncope at 60 degrees HUT tended to occur more frequently in formerly preeclamptic women than in controls (6/24 (25%) vs. 1/20 (5%), $P=.07$).

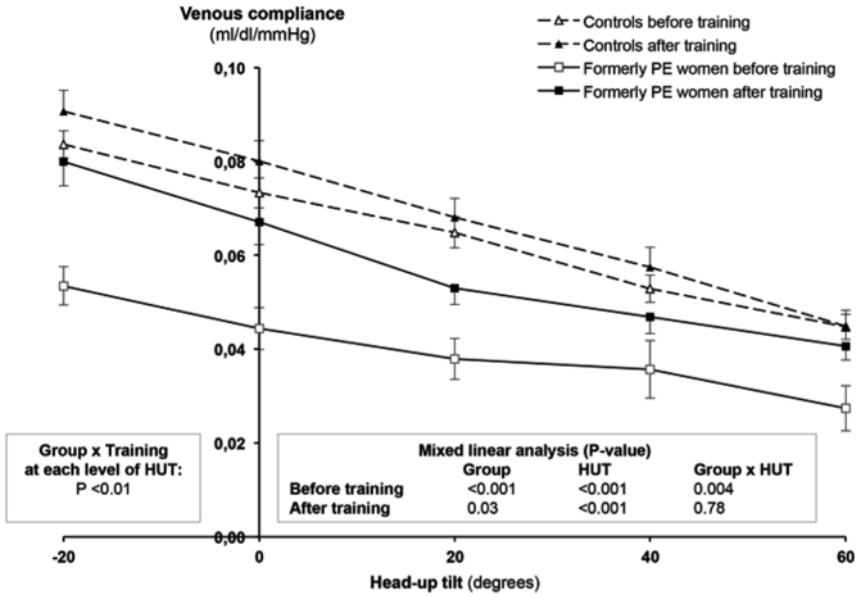

Figure 2 Effects of orthostatic stress testing on venous compliance in formerly preeclamptic women and controls before and after 12 weeks aerobic exercise training. On x-axis degrees of head-up tilt, on y-axis venous compliance (mL/dL/mmHg). Error bars represent standard error.

Effects of training

Twelve weeks of training improved physical fitness comparably in both groups, as mean VO_2max increased by +3.4 and +3.7 mL/min/kg respectively. Training increased PV in both groups (+180 vs.+135 mL/m^2; *P*=.22) (Figure 1) and it increased PV in formerly preeclamptic women up to pretraining values of controls (*P*=.84).

As shown in Table 1, training reduced BMI, systolic arterial pressure, diastolic arterial pressure, and MAP comparably in both groups. Although training increased stroke volume, cardiac output was unaffected as the reduction in HR was more pronounced in formerly preeclamptic women than in controls (interaction: *P*=.03). Training abolished the differences in blood pressures between formerly preeclamptic women and controls, but did not affect serum creatinine levels and albuminuria, so that albuminuria remained higher in formerly preeclamptic women than in controls.

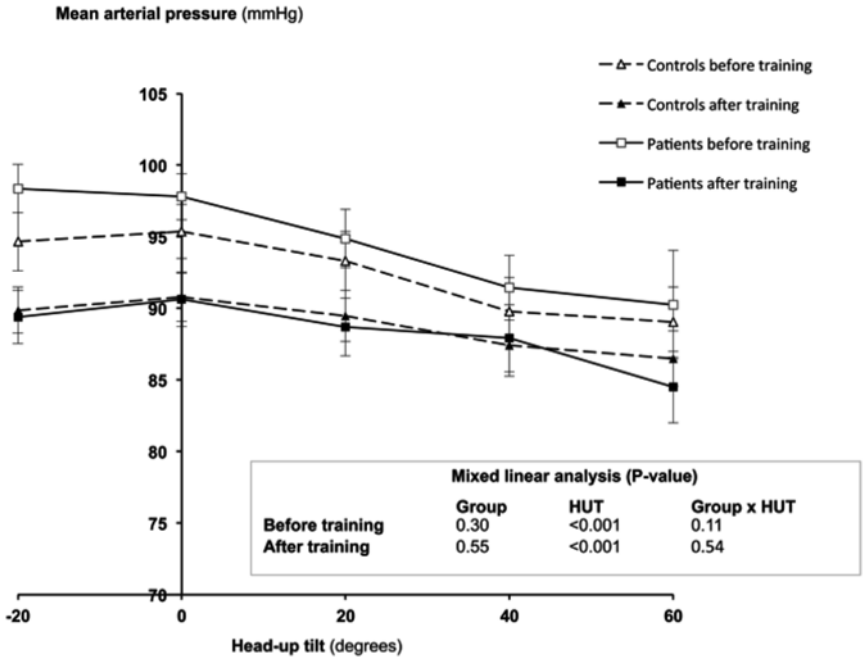

Figure 3 Effects of orthostatic stress testing on mean arterial pressure (MAP) in formerly preeclamptic women and controls before and after 12 weeks aerobic exercise training. On x-axis degrees of head-up tilt, on y-axis mean arterial pressure (mmHg). Error bars represent standard error.

Training improved VeC (Figure 2). The improvement was significantly more pronounced in formerly preeclamptic women than in controls (supine VeC +0.02 vs. +0.01 mL/dL/mmHg respectively; training x group: $P<.01$), and supine VeC in trained formerly preeclamptic women became comparable with that of untrained controls ($P=.30$). The slope of the VeC curve in response to HUT increased with training (HUT x training: $p<.01$), so that it became comparable for formerly preeclamptic women and controls (group x HUT: $P=.78$).

After 12 weeks of training the average MAP over the 5 rotational steps ($P=.55$) and the slopes of the MAP curves in response to HUT ($P=.54$) were similar in formerly preeclamptic women and controls. Training shifted the heart rate response curve to HUT downwards in both groups. After 12-week exercise training, the slopes of the response curves of heart rate were comparable between formerly preeclamptic women and controls ($P=.14$) although formerly preeclamptic women still performed at a higher heart rate at each level of HUT ($P=.05$).

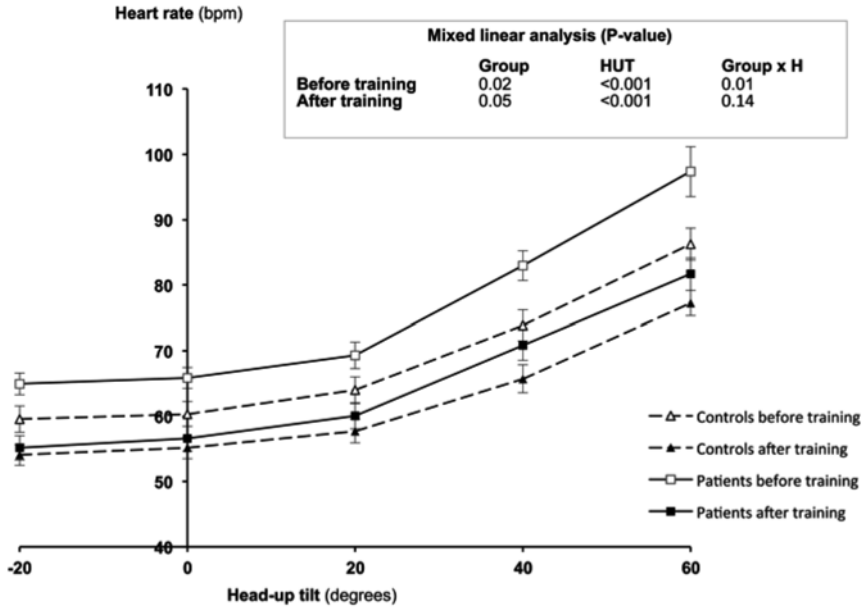

Figure 4 Effects of orthostatic stress testing on heart rate in formerly preeclamptic women and controls before and after 12 weeks aerobic exercise training. On x-axis degrees of head-up tilt, on y-axis heart rate (bpm). Error bars represent standard error.

Training reduced the rate of pre-syncope in formerly preeclamptic women, albeit not significantly (from 6/24 (25%) to 3/24 (12.5%), P=.27). After training, none of the control women showed symptoms of presyncope.

Discussion

The major finding in this study is that a 12-week aerobic exercise program increases plasma volume and venous compliance in formerly preeclamptic women to pretraining levels of parous women without a history of preeclampsia.

Preeclampsia is a multifactorial disorder. In this study, we focused on the link between preeclampsia and reduced venous reserve. Although it has been repeatedly shown that formerly preeclamptic women have low PV[3,4,7,20], the exact cause for low PV is unknown. Formerly PE women had higher creatinine clearance and microalbuminuria, both characteristics of borderline hypertension[25-26]. Therefore, one could argue that hyperfiltration may have contributed to reduced PV in formerly preeclamptic

women. However in pregnancy women with prepregnant low plasma volume demonstrate reduced plasma volume expansion with concurrent increased levels of atrial natriuretic peptide suggesting a restricted volume compartment rather than hyperfiltration[7]. Therefore, other possible pathogenic factors for low PV may be more likely such as a constitutionally small venous compartment[18] or a constricted venous system[27,28] Both glomerular hyperfiltration and a constricted venous system are linked to sympathetic overactivity[26,27]. Our results are consistent with a constricted, but not necessarily constitutionally small, venous compartment[29,30]. A constitutionally small venous compartment most likely would have resulted in a small increase in PV with training in formerly preeclamptic women, yet our data show that PV increased at least as much in formerly preeclamptic women as in controls. The normal increase in PV and the larger increase in VeC in formerly preeclamptic women than in controls in response to training are consistent with a venous compartment that was initially constricted but was able to relax secondary to exercise-induced reduction of sympathetic tone. The magnitude of the exercise-induced plasma volume expansion in our study is comparable with first trimester PV expansion in normal pregnancy (~10%). If pregnancy proceeds normally, PV expands further to more than ±40%. It is conceivable that in pregnancy venous capacitance problems arise when >10% plasma volume expansion needs to be accommodated. The effects of the exercise-induced PV expansion in formerly preeclamptic women on the subsequent recurrence risk of preeclampsia have not yet been studied.

Formerly preeclamptic women apparently have an increased venous tone and reduced venous compliance, similar to hypertensive subjects[31-33]. The VeC at rest was lower in formerly preeclamptic women than in controls prior to training and improved markedly by exercise training. VeC modulation during HUT was initially blunted in formerly preeclamptic women compared with controls, but training shifted the curve upward and increased the slope. This implies that the ability of the veins to mobilize venous volume through venoconstriction was initially limited but improved markedly with exercise training. Twelve weeks of aerobic training allowed formerly preeclamptic women to effectively improve VeC modulation up to the pretraining level of controls. We presume that an increase in venous compliance before a new pregnancy will likely allow better accommodation of the necessary PV expansion in pregnancy at a lower sympathetic activity level.

We would like to address several methodological issues. First, a time control group could have strengthened our results to rule out effects of post-partum recovery resulting in an overestimation of our training effects. We studied our participants 6 to 12 months postpartum, which may not have been enough for full recovery. Yet, our data probably approach full recovery as venous functions have been reported to

normalize within 3 months after delivery[34], and hemodynamic and metabolic recovery is at least 80% complete within 6 months[14]. The postpartum interval was similar between groups; it is therefore unlikely that this has significantly impacted our comparisons. Moreover, the fact that formerly preeclamptic women only improved up to a level that is comparable with that of untrained controls suggests that the observed training effects are not only reversion to the mean. Second, we measured venous compliance in a limb as a proxy for the whole body. One could argue that the splanchnic venous bed would have been more relevant because it contains the largest volume of venous blood and therefore plays a more important role in restoring venous return. However measuring splanchnic venous compliance is technically too challenging, especially during dynamic testing. Measuring limb venous compliance is a well-accepted method for studying the venous vascular bed. Finally, controls were recruited from the community; therefore women with subjectively poor fitness may have had an incentive to participate in the study, knowing that their cardiovascular health would be assessed and improved by training.

Perspectives

Pregnancy represent a unique screening test in which preeclampsia indicates women at high risk for future gestational hypertension and/or cardiovascular disease in later life[2,35]. Women at risk for cardiovascular disease are likely to be detected at a young age, early enough to benefit from subsequent preventative strategies. Although the reported effect of exercise before and during pregnancy on the reduction of recurrence risk of preeclampsia is conflicting[36,37], aerobic exercise training is a powerful nonpharmacological and low cost strategy to reduce blood pressure[38]. The protective effects of exercise are complex and go beyond the effects on traditional cardiovascular risk factors alone[39]. Our study has shown that formerly PE women can improve their venous reserve capacity through aerobic training to the level of that of untrained healthy control women who carried their pregnancy without developing hypertension. It is likely that if the active lifestyle is not maintained many of the exercise induced venous and autonomic changes will reverse. Further studies are needed to demonstrate the extent to which aerobic training prior to pregnancy is of clinical benefit to reduce recurrent hypertensive disease in pregnancy and cardiovascular disease later in life.

Conclusion

Normotensive formerly preeclamptic women have reduced plasma volume and lower venous compliance indicating reduced venous reserve capacity. Twelve weeks of aerobic training at 70-80% VO_2max 2 to 3 times per week significantly improves the venous reserve capacity in postpartum women. In formerly preeclamptic women it restores these functions to the level of untrained healthy parous controls.

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CHAPTER 7

Cardiovascular effects of aerobic exercise training in formerly preeclamptic women and healthy parous control subjects

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Abstract

Formerly preeclamptic women demonstrate higher prevalence of metabolic syndrome (MetS), impaired vascular function and increased sympathetic activity, and are at increased risk of cardiovascular disease. The aim of this study was to assess the effects of 12 weeks exercise training (70-80%VO_2max) in formerly preeclamptic women on components of metabolic syndrome, vasculature and autonomic functions compared with healthy controls.

Our prospective case-control study included 24 normotensive formerly preeclamptic women and 20 controls who were matched for age and postpartum interval (all 6 to 12 months after delivery). Before and after training, we measured all components of MetS (i.e. blood pressure, lipids, glucose/insulin and albuminuria), carotid intima-media thickness (IMT) and brachial (BA) and superficial femoral artery (SFA) endothelial function using flow mediated dilation (FMD). Autonomic activity was quantified using power spectral analysis (Low Frequency/High Frequency power (LF/HF)-ratio).

At baseline, formerly preeclamptic women demonstrated higher values of most components of MetS. Compared with control subjects, formerly preeclamptic women had increased IMT (580±92μm vs. 477±65 μm), impaired endothelial function (FMD BA: 5.3±2.2% vs. 10.8±3.5% and FMD SFA: 4.9±2.1% vs. 8.7±3.2%) and increased LF/HF-ratio (2.2±1.0 vs. 1.3±0.4, all $p<0.05$). In both groups exercise training decreased values of most components of MetS and IMT, improved FMD and concurrently reduced LF/HF. Despite these improvements, vascular and autonomic variables did not normalize by 12 weeks training in women with a history of preeclampsia.

This study demonstrates that exercise training in formerly preeclamptic women and control subjects improves components of MetS, endothelial function, vascular wall thickness and autonomic control. Nonetheless, trained women with a history of preeclampsia only reached a cardiovascular status that is comparable to sedentary healthy control subjects.

Introduction

Women who have experienced preeclampsia during pregnancy are prone to cardiovascular disease later in life[1]. The cardiovascular stress test of pregnancy therefore may unmask a previously unrecognized cardiovascular risk profile in young women. Most likely the development of preeclampsia during pregnancy reflects their preexisting, often latent, cardiovascular risk condition[2,3]. Alternatively, the increased risk can be attributed to factors that originate from the complicated pregnancy itself[4]. In either case, formerly preeclamptic women may benefit from selective screening for cardiovascular risk markers[5], assuming that targeted treatment alter these risk factors, and consequently effectively alter the risk for pregnancy-related vascular complications and future cardiovascular disease.

Women with a history of preeclampsia demonstrate higher prevalence of traditional cardiovascular risk factors[3,6-8] (e.g. hypertension, dyslipidemia and reduced insulin sensitivity), endothelial dysfunction[9-11], increased arterial wall thickness[12] and augmented sympathetic nervous system activity[13-15] compared with healthy control subjects, which likely relates to the increased cardiovascular risks in these women. These dysfunctions are potentially amenable for improvement by exercise training[16-18].

Aerobic exercise training is a well-established potent physiological stimulus that reduces primary and secondary cardiovascular events[19]. The beneficial effects of exercise on cardiovascular risk are not only mediated through improvement of traditional cardiovascular risk factors[20,21], but also through up-regulation of nitric oxide (NO)-derived endothelium mediated vasodilation[16,17,22], reduction of arterial wall thickness[23], and reduction of sympathetic tone[24,25]. Although suggested in many articles[26], no previous study examined the effects of exercise training in formerly preeclamptic women that could provide strong rationale for advising exercise training in women with increased cardiovascular risk.

This study therefore addressed the question to what extent an exercise training program (12 weeks of cycling at 70-80% VO_2max, 2 to 3 times per week) in formerly preeclamptic women affects physical fitness, components of the metabolic syndrome, endothelial function, arterial wall thickness and autonomic function, and how these changes compare with control subjects. The primary outcome of this study was endothelial function, which was measured by flow-mediated dilation (FMD). Endothelial dysfunction is not only a key feature in preeclampsia, but also it has proven to be associated with cardiovascular disease burden and outcome. We hypothesize that exercise training improves vascular function and structure as well as autonomic control, we expect these vascular and autonomic changes to be related closely. The question

is, however, whether formerly preeclamptic women will respond adequately to the exercise stimulus, because these women maladapted to the cardiovascular stimulus of previous pregnancy. Based on the circulatory maladaptation to previous pregnancy, we hypothesized that formerly preeclamptic women are less able to improve vascular and autonomic characteristics with exercise training compared with healthy control subjects. We therefore performed an experimental intervention study in 24 formerly preeclamptic women and 20 control subjects.

Materials and methods

We recruited 25 normotensive formerly preeclamptic women and 22 control subjects. Primiparous women with a history of preeclampsia were recruited from the Radboud University Nijmegen Medical Centre, and control subjects were recruited from the community by advertisement at day care centers. Preeclampsia in previous pregnancy was defined by the combination of gestational hypertension (≥140/90 mmHg, measured twice, six or more hours apart), and proteinuria (consistently ≥300 mg/24 hours) after 20 weeks of pregnancy in previously normotensive women[27]. In our hospital, women with a history of preeclampsia are invited for cardiovascular follow-up evaluation 6 to 12 months after pregnancy that was complicated by preeclampsia. At this follow-up visit, women who were eligible for our study were given written information about this study, and if interested these women contacted our laboratory for further information and inclusion. Control subjects contacted our laboratory after reading the advertisement. If interested, they received the same written information as formerly preeclamptic women. Control subjects were healthy primiparous women whose pregnancy charts were checked to ensure a normal pregnancy course that resulted in a term delivery. Participants were not compensated in this study.

All participants were white women, who were healthy and normotensive at the time of measurements. None of the women had diabetes mellitus, autoimmune disease or overt cardiovascular disease. None of the women smoked or used medication or supplements that might affect the cardiovascular system. None of the women who were included were pregnant, breastfeeding, or using hormonal contraceptives. Excluded from analysis were women who became pregnant during the course of the study and women who were unable to cope with physical exercise training. The study was approved by the Medical Ethics Committee of the Radboud University Nijmegen Medical Centre (CMO: 2008/226) All participants gave written informed consent before entering the study. The study adhered to the principles of the Declaration of Helsinki. The study was registered at clinicaltrials.gov (id: NCT00900458).

Experimental design

Measurements and training were performed in the nonpregnant state, 6 to 12 months after pregnancy. Subjects were tested before and after 12 weeks of exercise training. All measurements, except VO_2max, were performed during the same visit. VO_2max was tested 1 to 5 days from the other visit. In sequential order, we measured: body characteristics (weight, height, waist circumference), metabolic components (lipids, glucose and insulin concentrations), blood pressure (BP), heart rate (HR), autonomic function by spectral analysis of spontaneous fluctuations in heart rate and blood pressure and vascular ultrasound measurements: intima media thickness (IMT), flow-mediated dilatation (FMD) and vasodilation response to glyceryl trinitrate (GTN).

Experimental procedures

Tests and measurements were performed between day 3 and 11 of the menstrual cycle to minimize possible endocrine influences of the sex hormones on the cardiovascular- and autonomic nervous system. All measurements, except VO_2max were performed following an overnight fast. Participants were instructed to abstain from strenuous physical activity in the 24 hours before testing. Participants collected urine in the 24 hours preceding the measurements. The 24-hour urine sample was assayed for albumin, protein and creatinine to define the microalbuminuria corrected for creatinine output (g/mol creatinine) and total protein level (g/24 hours) (Aeroset, Abbot Laboratories, Illinois USA).

Height and body mass (Seca 888 scale, Hamburg, Germany), and waist circumference were measured. Venous blood samples were taken from the antecubital vein and analyzed for metabolic parameters: glucose, insulin, total cholesterol, high and low density lipoproteins and triglycerides (Aeroset, Abbot Laboratories, Illinois USA). The homeostasis model assessment index (HOMA) was calculated as fasting insulin [mU/L] x fasting glucose [mmol/L]/22.5 to estimate insulin resistance[28].

Tests were performed under standardized conditions in a temperature-controlled room (22±0.5ºC). Measurements were performed at the same time in the morning to prevent diurnal variation in the vascular and autonomic responses.[29] After 30 minutes rest in supine position, blood pressure and heart rate were measured oscillometrically (Dinamap, Vital Signs Monitor 1846, Critikon, Tampa, Florida), at the right upper arm, with the cuff size recommended for the arm circumference, at 3-minute intervals for 30 minutes. We recorded systolic (SAP) diastolic (DAP) and mean (MAP) arterial pressures and heart rate (HR; bpm) and used the median values of 9 consecutive measurements for analysis. This resting heart rate was used for the calculation of the training intensity.

Autonomic function

Autonomic function was measured with subjects comfortably lying in supine position in a quiet, partially darkened room. Autonomic activity and baroreflex sensitivity were quantified by spectral analysis, from a 5 minute recording of spontaneous fluctuations in HR and BP.[30] Heart rate and arterial blood pressure (ABP) were measured continuously using a finger arterial blood pressure monitoring device attached to the 3rd digit of the right hand at a sampling rate of 100Hz (Finometer, Finapres BV, the Netherlands). Post hoc, these recordings were subdivided into data segments of 100s, overlapping for 50% and resampled at 5.12 Hz. Each segment was then analyzed with Fast Fourier Transformation to search for rhythmic fluctuations in systolic arterial pressure (SAP) and pulse interval with a frequency range between 0 and 2.56 Hz. The amplitude of each fluctuation determines the power at each frequency. The ratio (LF/HF) of absolute low frequency (LF) and high frequency (HF) powers of the pulse interval was used to represent the autonomic balance between the sympathetic and vagal system. The LF component is regarded a marker of mainly sympathetic modulation, HF components mainly reflect vagal activity. Therefore, a higher LF/HF can be interpreted as a sign of increased sympathetic dominance. Baroreflex sensitivity (BRS, ms/mmHg), was defined as the (low frequency) transfer gain from SAP to pulse interval, which provides information about the changes in the heart rate (output) in response to fluctuation in SAP (input).

Vascular measurements

All measurements were performed by the same sonographer (RS) according to recent guidelines for assessment of the Flow Mediated Dilation (FMD)[29] using a 10-MHz multifrequency linear array probe attached to a high resolution ultrasound machine (T3000, Terason, Burlington, MA). Ultrasound parameters were set to optimize longitudinal B-mode images of the lumen/arterial wall interface. Continuous Doppler velocity was assessed simultaneously using the lowest possible insonation angle (always$<60^{o}$). For assessment of the FMD responses, a rapid inflation/deflation pneumatic cuff (Hokanson, Bellevue, WA) was positioned distal to the imaged artery and inflated for 5 minutes (220mmHg) to provide the stimulus for reactive hyperemia. FMD represents a predominantly nitric-oxide (NO)-mediated, endothelium dependent vasodilation.

Carotid artery IMT. The intima-media thickness of the left common carotid artery (CA) was measured 2cm proximal to the bulbus. Perpendicular incidence of the B-mode imaging ultrasound beam in relation to the orientation of the vessel provided clearly demarcated intima medial boundaries, which were optimized using contrast controls. Wall thickness and diameter were measured continuously for 1 minute. The measurement was repeated once, from a different perpendicular plane ($\pm 90^{o}$

compared with previous measurement). From the 2 measurements, mean diameter and wall thickness were calculated and used for analysis[31].

Brachial artery FMD. Brachial artery FMD was measured with subjects positioned supine with the right arm extended and immobilized with foam, supported at an angel of approximately 80 degrees from the torso. The pneumatic cuff was placed distal to the olecranon. The brachial artery (BA) was scanned 2-5 cm above the antecubital fossa. Before inflation, resting baseline diameter and blood flow velocity were recorded continuously for at least 1 minute. After cuff deflation, diameter and blood flow velocity were recorded continuously for 3 minutes.

Superficial femoral artery FMD. Superficial femoral artery FMD was measured with subjects positioned supine with the lower leg slightly elevated (approximately 10 degrees). The pneumatic cuff was placed 15 cm below the inguinal ligament. The superficial femoral artery (SFA) was scanned in the proximal one-third of the thigh, at least 5 cm distal from the bifurcation and above the cuff position. Before inflation, resting baseline diameter and blood flow velocity were recorded continuously for at least 1 minute. After cuff deflation, diameter and blood flow velocity were recorded continuously for 5 minutes.

Vasodilation after glyceryl trinitrate (GTN). GTN responses were measured after a resting period of 30 minutes to allow arterial diameter to return to baseline, another 1-minute baseline recording of the superficial femoral artery was made. Endothelium independent dilation was examined using a single sublingual dose of GTN (400μgr; G-Pohl-Boskamp GmbH and Co KG, Hohenlockstedt, Germany), a NO donor, followed by ≥10 minutes recording of the arterial diameter. The maximal dilatation after administration of GTN represents the endothelium independent vasodilation.

Conduit artery diameter, wall thickness and blood flow analysis

Posttest analysis of diameter, velocity and wall thickness of the 3 conduit arteries (CA, BA and SFA) was performed using edge-detection and wall-tracking DICOM based software[32] to minimize investigator bias[29,32]. The post-hoc analysis of the diameter, velocity and wall thickness was performed blinded (to subject and moment of assessment). FMD was calculated as the percentage of rise in peak diameter from the preceding baseline diameter. The post deflation shear rate data, derived from simultaneously acquired velocity and diameter measures at 30Hz, was used to calculate the area under the shear rate curve for data up to the point of maximal post-deflation diameter (FMD) for each measurement. Reproducibility of the brachial artery FMD using this semi-automated software has a coefficient of variance of 6.7%[32], the superficial femoral artery FMD has a coefficient of variance of 15%[29].

Physical Fitness

Physical fitness was measured before and after a 12-week training program. Fitness was defined as the peak oxygen uptake (VO_2max, mL min^{-1} kg^{-1}) during a maximal cycling test on a cycle ergometer (Excalibur Sport, Lode BV, Groningen NL). Tests were performed in the afternoon after a light lunch. The initial workload set at 10 Watt for 1 minute followed by 10-Watts increments every minute until complete exhaustion. Breath-by-breath oxygen uptake was measured using spiro-ergometric equipment (Quark CPET, Cosmed, Italy). Heart rate and rhythm were continuously recorded by 3-lead ECG. Maximal workload (Workmax) was defined as the last completed workload before exhaustion. Test performance was considered to be adequate when: 1. The increase in VO_2 (ml) during Workmax was <150mL compared to the previous workload, indicating plateau formation in oxygen uptake 2. HR at Workmax was <10 bpm from estimated maximal heart rate (220-age) 3. Respiratory Exchange Ratio (RER, CO2/O2) was consistently >1.1 during Workmax and 4. Capillary lactate level was >8mmol/L, 90 seconds after exhaustion. If the test failed to achieve these 4 qualifications the test was repeated 2-3 days later. In three cases (2 formerly preeclamptic women, 1 control subject) the test had to be repeated, all tests were eventually considered adequate.

Exercise training

Exercise training consisted of 12 weeks of HR-controlled cycle training (cycle ergometer, Corival, Lode BV, Groningen Netherlands) at 70-80% of VO_2max for 2 to 3 times per week (according most recent American College of Sports Medicine (ACSM) guideline)[33]. Participants trained twice a week during the first 6 weeks and 3 times a week during the last 6 weeks. Each training session was supervised and was executed in the gymnasium of our laboratory. Participants were instructed not to exercise in addition to the exercise protocol that they were given. During each training session, heart rate was continuously monitored and recorded (RS800CX, Polar Electro Inc, NY USA). Each training session started with 10 minutes warming-up at 50% of the heart rate reserve (HRR) above the resting heart rate. HRR was calculated as: $HRR=HR_{max}-HR_{rest}$ in which HR_{max} is the maximal heart rate measured during the fitness test at study entry and HR_{rest} is the heart rate determined at rest. Training consisted of 40 minutes of cycling between 70 to 80% of the individual HRR above HRrest. Within their target heart rate zone, participants were free to choose the number of revolutions per minute (RPM). The training was completed by cooling down for 5 minutes at warming-up workload.

Statistical analysis

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL) software. All data are reported as mean± SD unless stated otherwise. Normality of data was

tested using Kolmogorov-Smirnov tests. Statistical significance was assumed at $p<0.05$. Two-way repeated-measures ANOVA was used to examine differences between formerly preeclamptic women and control subjects (groups), to assess the effects of training (intervention) and to determine if the training effects differed between patients and control subjects (groups*intervention-interaction). Post-hoc Bonferroni analysis was used to correct for multiple comparisons. Pearson correlation coefficient was used to examine the relation between exercise-induced change in autonomic function (LF/HF ratio) and endothelial function (FMD) in both groups. Based on an anticipated difference in %FMD, which has a coefficient of variation (CV) of 6.7-10.5%[32], between formerly preeclamptic women and control subjects of 4%, a power of the study of 90%, and an alpha level of .05, we needed 17 subjects per individual group. We based our group-size calculation for the intervention part of our study on an anticipated change in %FMD (primary outcome measure) of 2%, a power of 90% and an alpha of .05. Based on these assumptions, we required 18 subjects per group. To anticipate possible dropouts, we decided to include at least 20 subjects per group.

Results

Included in the analysis were 24 women with a history of preeclampsia and 20 control subjects, after exclusion of 1 formerly preeclamptic woman (who became pregnant) and 2 control subjects (1 woman who became pregnant, 1 woman who did not finish the training). Mean age (32±4 vs. 32±4 years, $P=.62$) and post-partum interval (7±2 vs.7±1 months. $P=.88$) were comparable between groups. Formerly preeclamptic women delivered at an earlier gestational age compared with control subjects (32 [29-37] vs. 40 [38-41] weeks, $P<.01$) and delivered children with a lower birth weight compared with control subjects (1571±675 vs. 3532±311 grams).

Baseline values

Prior to training, physical fitness was similar in both groups (VO_2max: controls: 28.2±3.7; patients: 27.0±4.0 mL min^{-1} kg^{-1}, $P=.32$), as were BMI, resting HR, fasting glucose and cholesterol concentrations (Table 1). In formerly preeclamptic women, group average values of SAP, DAP, MAP, fasting insulin and triglyceride concentrations and albuminuria were higher than in control subjects. Vascular diameters (CA, BA and SFA) were not different between groups. In formerly preeclamptic women, CA-IMT was larger and BA- and SFA-FMD responses were smaller than in controls (Table 2). Mean shear rate AUC, time-to-peak of FMD and vasodilation in response to GTN was not different between groups. In formerly preeclamptic women, the average LF/HF ratio was higher and BRS lower than in control subjects (Table 3).

Table 1 Characteristics of healthy controls and formerly preeclamptic women, before and after exercise training

	Healthy controls (n=20)		Formerly preeclamptic women (n=24)		2-way ANOVA (*P*-values)		
	Before training	After training	Before training	After training	Group	Intervention	Group X Intervention
BMI, kg/m²	26.8±3.4	25.1±6.8	25.9±4.9	24.4±7.0	.63	.04	.88
Waist circumference, cm	93±10	91±10	92±12	89±11	.84	<.01	.25
Arterial pressure							
Systolic, mmHg	109±6	104±6	119±10*	112±8	<.01	<.01	.39
Diastolic, mmHg	66±6	63±6	74±7*	69±7	<.01	<.01	.19
Mean, mmHg	78±5	74±5	86±8*	80±7	<.01	<.01	.26
Resting heart rate, bpm	63±9.8	57±7.6	77±8.7*	59±8.9	.15	<.01	.03
Biochemical parameters							
Fasting glucose, mmol/L	4.7±0.5	4.6±0.5	4.8±0.5	4.7±0.5	.51	.01	.32
Fasting Insulin, mmol/L	7.7±6.9	5.9±4.3	13.3±9.3*	8.4±5.0	.04	<.01	.04
HOMA-IR index	1.7±1.8	1.3±1.1	3.0±2.3*	1.8±1.1	.04	<.01	.04
Total Cholesterol, mmol/L	4.5±0.9	4.1± 0.8	4.6±1.0	4.5±1.1	.45	<.01	.14
HDL Cholesterol, mmol/L	1.2±0.3	1.3±0.3	1.1±0.2	1.2±0.2	.19	<.01	.88
LDL Cholesterol, mmol/L	2.8±0.8	2.5±0.7	2.8±0.8	2.7±0.8	.58	<.01	.58
Triglycerides, mmol/L	0.8±0.4	0.8±0.3	1.4±0.9*	1.1±0.5*	.02	.18	.19
Albuminuria, mg/mmol creat	0.4±0.3	0.4±0.3	2.4±4.4*	1.7±3.0*	.04	.07	.08
Physical fitness							
VO_{2max}, mL min^{-1} kg^{-1}	28.2±3.7	31.9±3.5	27.0±4.0	30.4±5	.32	<.01	.52
Maximal heart rate, bpm	186±9	185±7	189±7	189±6	.08	.57	.32
Maximal VE, L/min	96±20	108±10	88±19	99±18	.07	<.01	.74
Maximal work load, Watt	195±27	210±22	188±25	204±27	.27	<.01	.53

Values are mean±SD. * $P<0.05$ compared with controls before training (t-test). BMI: body mass index. HOMA-IR: Homeostatic Model Assessment of Insulin Resistance. HDL: High Density Lipoprotein. LDL: Low Density Lipoprotein. Results 2-way ANOVA: Group: patients vs. controls. Intervention: main effect exercise program. Group x intervention: interaction between groups and exercise training

Table 2 Vascular characteristics of healthy controls and formerly preeclamptic women, before and after exercise training

	Healthy control subjects (n=20)		Formerly preeclamptic women (n=24)		2-way ANOVA (*P*-values)		
	Before training	After training	Before training	After training	Group	Intervention	Group X Intervention
Carotid Artery (CA)							
Resting diameter, mm	5.6±0.5	5.6±0.5	5.7±0.6	5.6±0.4	.98	.99	.74
IMT, μm	477±65	423±65	580±92*	530±138	<.01	<.01	.86
Wall to lumen ratio	0.086±0.012	0.076±0.012	0.104±0.02*	0.095±0.025	<.01	<.01	.86
Brachial Artery (BA)							
Diameter at baseline, mm	2.8±0.3	3.0±0.3	3.0±0.3	3.1±0.3	.15	<.01	.10
FMD, %	11.8±3.5	13.3±3.6	5.3±2.2*	8.1±2.7*	<.01	<.01	.58
Shear rate AUC, $\cdot 10^3$ sec^{-1}	27.9±10.4	27.1±12.7	26.3± 11.2	24.3±13.1	.44	.41	.93
Time to peak diameter, sec	48±15	51±13	50 ±14	59±20	.26	.08	.43
Superficial Femoral Artery (SFA)							
Diameter at baseline, mm	5.6±0.7	5.8±0.6	5.7±0.7	5.7± 0.7	.90	.81	.84
FMD, %	8.7±3.2	10.5±2.8	4.9±2.1*	6.1±2.2*	<.01	<.01	.98
Shear Rate AUC, $\cdot 10^3$ s^{-1}	14.5±9.1	14.7±5.5	15.8±10.5	14.4±9.1	.82	.53	.69
Time to peak diameter, sec	83±36	74±27	85±36	78±38	.62	.08	.88
GTN, %	11.9±4.0	13.0±3	10.9±3.3	12.1±2.1	.24	.04	.75

Values are mean±SD. * $P<0.05$ compared with controls before training (t-test). IMT: intima media thickness, FMD: flow mediated dilation, AUC: area under the curve, GTN: glyceryl trinitrate Results 2-way ANOVA: Group: patients vs controls. Intervention: main effect exercise program. Group x intervention: interaction between groups and exercise training.

Table 3 Autonomic characteristics of healthy controls and formerly preeclamptic women, before and after exercise training

	Healthy control subjects (n=20)		Formerly preeclamptic women (n=24)		2-way ANOVA (*P*-values)		
	Before training	After training	Before training	After training	Group	Intervention	Group X Intervention
Heart Rate Variability analysis							
LF power, ms^2	1005±329	911±315	1455±526*	1106±442	<.01	.07	.27
HF power, ms^2	806±259	965±369	768±276	789±294	.04	.19	.41
LF/HF ratio	1.3±0.4	1.0±0.3	2.2±1.0 *	1.5±0.6*	<.01	<.01	.51
BRS, $ms \cdot mmHg^{-1}$	17.6±5.7	22.1±7.9	8.5±3.6*	12.5±5.1*	<.01	<.01	.86

Values are mean±SD. * $P<0.05$ compared with controls before training (t-test). LF Low Frequency, HF: High Frequency, BRS baroreceptor sensitivity .Results 2-way ANOVA: Group: patients vs controls. Intervention: main effect exercise program. Group x intervention: interaction between groups and exercise training.

Effects of training

Twelve weeks of training improved physical fitness in both groups to the same extent, because VO_2max increased by 14±8% in control subjects and 13±7% in patients (Table 1). Training reduced HRrest, SAP, DAP, MAP, BMI, waist circumference, fasting glucose, insulin, total cholesterol and LDL cholesterol concentrations in formerly preeclamptic women and control sunjects, while it increased HDL cholesterol. The reduction in HRrest and fasting insulin concentration was 14% and 13% respectively, which was more in formerly preeclamptic women than in control subjects. Training abolished differences in blood pressure and insulin resistance between formerly preeclamptic women and control subjects. Albuminuria and triglyceride concentration remained higher in formerly preeclamptic women than in control subjects after training (Table 1). Training reduced CA-IMT and increased BA- and SFA-FMD responses in formerly preeclamptic women and control subjects to a similar extent (Fig. 1) indicating improved endothelial function. Yet, values did not normalize in formerly preeclamptic women. Training did not affect shear rate AUC, time-to-peak of FMD or vasodilatation in response to GTN in either group (Table 2). Training reduced LF/HF ratios and increased BRS to a similar extent in both groups. Sympathetic dominance and the reduced BRS did not normalize in formerly preeclamptic women, and remained significantly elevated compared to trained control subjects (Table 3).

Correlations. Training similarly improved autonomic functioning (delta LF/HF) and endothelial function (delta FMD) in women with a history of preeclampsia and control subjects. Training induced changes in sympathetic dominance and endothelial function correlate inversely in both BA and SFA. (Fig 2)

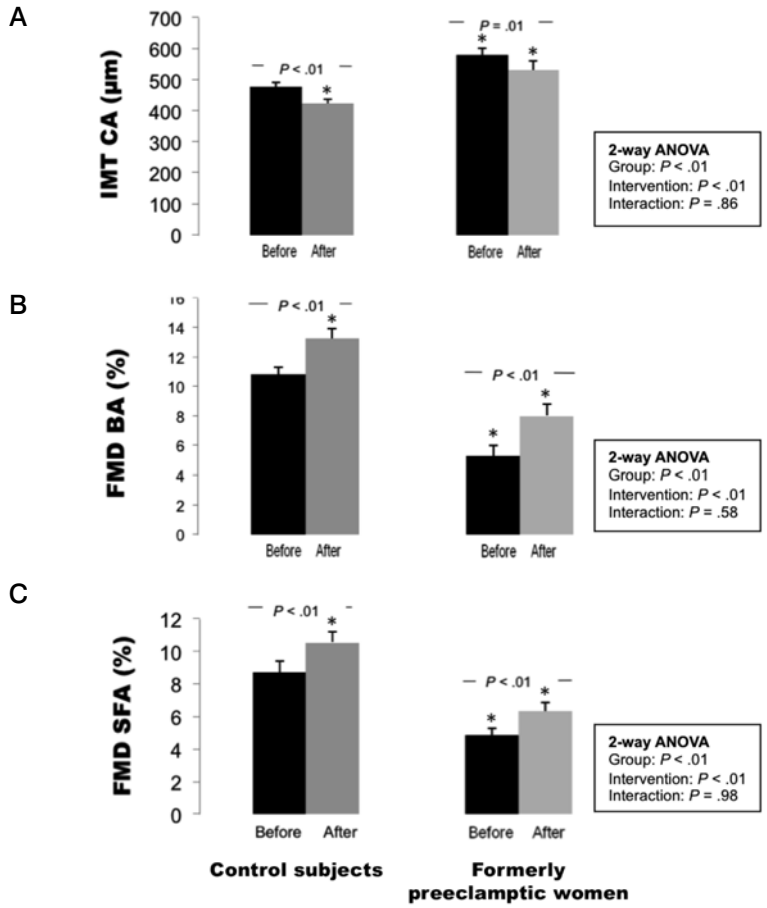

Figure 1 Effect of exercise training on **A**. carotid artery intima-media thickness (CA, IMT) **B**. Brachial artery flow mediated dilation (BA, FMD) and **C**. Superficial femoral artery flow mediated dilation (SFA, FMD) in healthy control subjects (n=20) and formerly preeclamptic women (n=24). Before (black bars) and after (grey bars) 12 weeks exercise training. Error bars represent SE; * t-test formerly preeclamptic women compared with control subjects *before* training ($P<.05$).

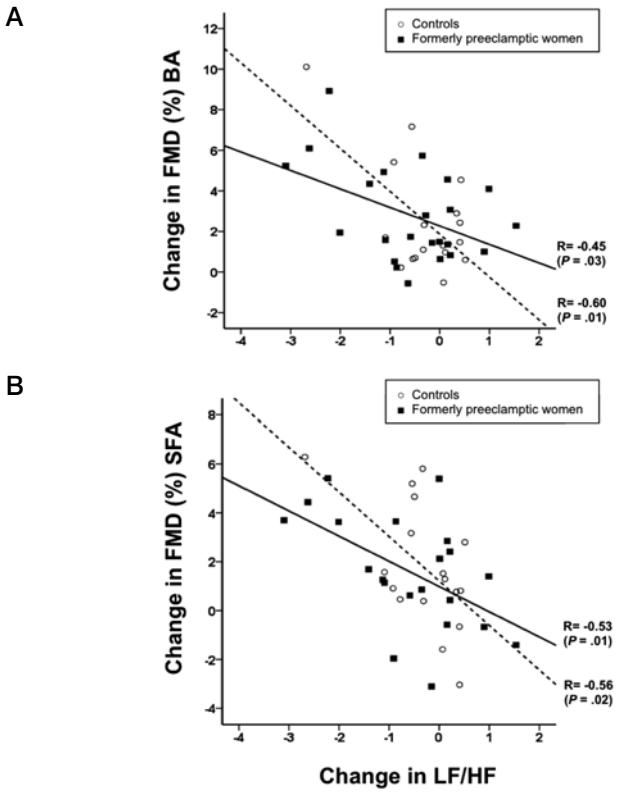

Figure 2 Correlation between exercise induced changes in LF/HF ratio and changes in flow mediated dilation (FMD%) in **A**. Brachial artery (BA) and **B**. superficial femoral artery (SFA) in response to 12 weeks of exercise training in healthy control subjects (n=20) and formerly preeclamptic women (n=24). The dashed line represents the regression line of controls, and the thick black line represents the regression line of formerly preeclamptic women. R represents the Pearson correlation coefficient.

Discussion

The cardiovascular and autonomic effects of aerobic training in formerly preeclamptic women have not been studied previously. This study demonstrates that formerly preeclamptic women and healthy parous control subjects can improve their cardiovascular risk profile with 12 weeks of exercise training. The improvement of components of the metabolic syndrome in both groups suggests reduced biochemical vascular stress. The cardioprotective effects of training in formerly preeclamptic women and

healthy post partum women are further supported by the improved endothelial function (BA- & SFA- FMD), vascular structure (CA-IMT) and autonomic function (LF/HF and BRS). Despite these improvements, exercise training did not normalize vascular and autonomic variables in formerly preeclamptic women. Interestingly, we found that exercise induced vascular improvements correlated with training induced changes in autonomic functioning in both healthy control subjects and formerly preeclamptic women.

In our study, baseline physical fitness was not different between formerly preeclamptic women and controls. Our baseline data were similar to those of previous cross-sectional studies that reported increased prevalence of traditional cardiovascular risk factors[6,8,33], vascular dysfunction[9-11,35], increased vascular wall thickness[12] and increased sympathetic activity[13] in formerly preeclamptic women compared to control subjects. The comparable GTN responses between groups are in agreement with previous studies suggesting endothelial dysfunction, rather than vascular smooth muscle cell dysfunction in formerly preeclamptic women[9,29]. Our study adds the novel observation that endothelial dysfunction in formerly preeclamptic women represents a systemic process, because endothelial function was impaired in both BA an SFA and arterial wall was thickened in yet another vascular bed (CA). Another novel observation was the correlation between endothelial dysfunction and sympathetic dominance. High sympathetic outflow in conjunction with endothelial dysfunction is thought to have synergistic and detrimental effects on cardiovascular risk[36]. Particularly interesting is the potential of detecting these risk profiles in women at an early enough stage to benefit from low-cost interventions such as modification of lifestyle.

The training program (12 weeks of cycling at 70-80% VO_2max for 2 to 3 times per week) increased VO_2max to the same extent in both groups. The improvement of physical fitness was within the expected range[37]. Training improved most traditional cardiovascular risk factors to the same extent in formerly preeclamptic women and control subjects. HRrest and HOMA reductions were more pronounced in formerly preeclamptic women than in control subjects. Most likely this reflects the higher baseline values in formerly preeclamptic women. The beneficial effects of exercise training cannot be explained by changes in traditional cardiovascular risk factors alone[17], but also by direct effects on vasculature and autonomic nervous system. In our study, vascular and autonomic variables improved similarly in both groups. The effects in all studied arteries suggest a systemic effect of exercise training on the vasculature. Studies have demonstrated that exercise induced changes in shear stress provide the principal physiological stimulus to adaptation in vascular function and structure, likely by enhancing nitric oxide bioavailability[38], but also by effects on

other angiogenic factors (e.g. endothelin-1[39], angiotensin-2, VEGF and oxidative stress)[40]. The change in FMD with training correlated inversely with the change in LF/HF in both groups (Figure 2). This is in line with cross-sectional studies in healthy men that have shown that tonic elevation in sympathetic nerve activity is associated with increased IMT[41] and reduced FMD[42]. These data suggest that FMD not only reflects NO mediated endothelial vasodilation but that FMD is also affected by alterations in sympathetic activity.

We did not observe a difference in baseline VO_2max between formerly preeclamptic women and controls. This may originate from unintended selection of controls with relatively poor VO_2max recruited by newspaper advertisement. Women with subjectively poor fitness may have had an incentive to participate in the study, knowing that their cardiovascular health would be assessed as well as improved by training. However, despite the similar VO_2max, age and BMI between formerly preeclamptic women and control subjects, comparisons between groups indicate significant differences in metabolic, circulatory and autonomic profiles between groups. This observation suggests that these differences in circulatory and metabolic profiles do not originate from differences in a priori physical fitness levels. The formerly preeclamptic women included in our study were referred to our tertiary center. This may have led to overrepresentation of more severely complicated pregnancies, explaining the median gestational age of 32 weeks. Care should therefore be taken to extrapolate our findings to the general formerly preeclamptic population (including women who developed preeclampsia at term). In a previous study, we demonstrated that the prevalence of cardiovascular risk factors increase with a earlier onset of preeclampsia[43]. It is conceivable that the baseline differences between formerly preeclamptic women and controls are limited to a particularly high risk set of women.

Another potential limitation is that participants were studied 6 to 12 months postpartum. Even though >80% of recovery takes place within 6 months, recovery may have been incomplete[44]. If one would assume that recovery in our study was only 80%, later testing at higher baseline fitness, using the same training program, most likely would have resulted in smaller training effects. Because the post-partum interval was similar for both groups, it is unlikely that the postpartum interval has affected the comparisons between formerly preeclamptic women and controls in our study.

Perspectives

Formerly preeclamptic women are at increased cardiovascular risk. The risk of a cardiovascular event after preeclampsia is estimated to be 2-fold compared to

women who had normotensive pregnancies. Because the risk of a cardiovascular event increases with age, absolute risk at 60-69 years would be 14.2% and 30.7% for a woman without and with a history of preeclampsia, suggesting that a woman who had preeclampsia might become eligible for primary prevention at an earlier age[1]. We observed that training markedly improved indicators of cardiovascular health in formerly preeclamptic women, suggesting reduction in the risk of recurrent preeclampsia and cardiovascular disease in later life. Our vigourous and supervised training program (12 weeks of cycling at 70% VO_2max, 2 to 3 times per week) was unable to normalize vascular and autonomic variables in formerly preeclamptic women. One might argue that 12 weeks training is not long enough for formerly preeclamptic women to achieve full normalization of cardiovascular and metabolic functions. Our study is at least the proof of principle that the cardiovascular risk profile in formerly preeclamptic women is amenable to lifestyle interventions. At any rate, our data support the recommendation of exercise training in post partum women, especially in women with a history of preeclampsia. Future studies will have to evaluate whether longer duration of exercise training, less supervised or different types of exercise training (resistance training, combined aerobic + resistance training) equally or even more effectively reduce cardiovascular risks and whether exercise training may improve future pregnancy outcomes in women with a history of preeclampsia. Based on observations in other populations, but with longer training programs, it is likely that formerly preeclamptic women can continue to improve their cardiovascular risk profile with continued training[45]. This idea is supported by the general lack of interaction effects in our study, suggesting a similar response to the exercise stimulus between formerly preeclamptic women and controls.

Conclusions

Exercise training in women with a history of preeclampsia improves traditional cardiovascular risk factors, endothelial function, vascular wall thickness and autonomic balance. Nonetheless after 12 weeks training, formerly preeclamptic women only reached a vascular and autonomic status that is comparable to sedentary control subjects, stressing the recommendation of prolonged exercise training in young women with a latent cardiovascular risk profile that is unmasked by their hypertensive pregnancy course.

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CHAPTER 8

Retrograde shear rate in formerly preeclamptic and healthy women before and after exercise training: relationship with endothelial function

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Abstract

Blood flow patterns in conduit arteries characterised by high levels of retrograde shear stress can be detrimental for vascular health. In this study we examined whether retrograde shear rate and endothelial function are related in healthy control subjects and formerly preeclamptic women and whether this relationship is altered by exercise training.

Formerly preeclamptic women (32±4 years, n=20) and control subjects (32±4 years, n=20), all 6 to 12 months post-partum, performed 12 weeks aerobic exercise training. We measured brachial artery shear rate (SR) and endothelial function by flow-mediated dilation (FMD, echo-Doppler). We additionally performed power spectral analysis of heart rate variability and calculated low-frequency/high-frequency (LF/HF) ratio.

Antegrade SR was not different between groups, while retrograde SR was significantly higher and FMD% lower in formerly preeclamptic women compared with control subjects (both $P<.05$). Retrograde shear correlated strongly with FMD% in formerly preeclamptic women and controls ($P<.05$). LF/HF ratio inversely correlated with brachial artery retrograde SR and FMD% (both $P<.05$) in formerly preeclamptic women and controls. Exercise training reduced retrograde shear, improved FMD%, and reduced LF/HF-ratios similarly in both groups (all $P<.05$). Training-induced changes in retrograde SR correlated with changes in FMD% and LF/HF ratio.

A higher brachial artery retrograde SR relates to lower brachial artery endothelial function, in both controls and formerly preeclamptic women. Exercise training improves retrograde SR, whilst the magnitude of this change correlated strongly with improvements in FMD and reductions in LF/HF ratio. Therefore, the impact of preeclampsia and exercise training on endothelial health may, at least partly, be related to retrograde shear rate.

Introduction

Shear stress, i.e. the frictional force of blood on the arterial wall, represents a key stimulus for adaptation in artery function and structure[1,2]. Changes in shear stress directly influence the endothelium of arteries, which plays a crucial role in the regulation of blood flow and maintanance of the quaility of blood vessels. Across the cardiac cycle, shear stress demonstrates a typical pattern, flowing toward the periphery during systole (antegrade shear), and back to the heart during diastole (retrograde shear). Increases in shear stress are beneficial for arterial function, while in vitro and animals studies have demonstrated that shear stress patterns characterised by high levels of retrograde shear can increase the expression of pro-atherogenic genes (see reviews[3-5]) with consequent detrimental impacts for vascular health[6]. We recently observed within subjects, that an increase in retrograde shear rate is followed by an immediate "dose"-dependent decrease in endothelial function[6]. To date, no study has examined whether retrograde shear rate relates to endothelial dysfunction between humans and whether such relationships differ between healthy subjects and those with a priori endothelial dysfunction. We therefore measured endothelial function and resting shear pattern in healthy women and formerly preeclamptic women, the latter possessing endothelial dysfunction[7]. In the current study we hypothesised that increased levels of retrograde shear rate would be associated with lower endothelial function in healthy subjects as well as in those with endothelial dysfunction. The mechanisms that account for an increased risk of cardiovascular disease in women with a history of preeclampsia are not well understood, but endothelial dysfunction, which has been linked to atherosclerosis, persists in formerly preeclamptic women many years after an hypertensive pregnancy. Endothelial dysfunction and cardiovascular disease after preeclampsia may be attributable to preexisting risk factors[8]. A recent study of cardiovascular risk factors present before and after pregnancy suggest that nearly half of the elevated risk for future hypertension after preeclampsia can be explained by prepregnancy risk factors. However, further investigation will be necessary to determine whether preeclampsia itself may injure the endothelium and thereby increase the risk of atherosclerosis and cardiovascular disease.

Exercise training is a stimulus which improves endothelial function in healthy subjects as well as in groups with a priori endothelial dysfunction[9,10]. To date, no study has specifically examined whether exercise training affects shear rate patterns, or also whether exercise training-induced changes in shear pattern relate to adaptations in endothelial function. Therefore, we examined the effects of 12 week exercise training on the relationships between retrograde shear and endothelial function. We hypothesized that exercise training would reduce retrograde shear and improve

endothelial function, without altering the reciprocal relationships between these factors. Previous data demonstrated that (changes in) retrograde shear is related to muscle sympathetic nerve activity[11]. To further explore this relation with activity of the autonomic nerve system, we examined autonomic modulation by power spectral analysis of resting heart rate variability and related this outcome to retrograde shear rate levels and endothelial function (before and after training).

Materials and methods

Subjects

Twenty healthy female participants and 20 women with a history of preeclampsia participated in our study (Table 1). Preeclampsia was diagnosed in the previous pregnancy, if women had blood pressure of ≥140/90 mmHg, measured twice, six or more hours apart, and consistent proteinuria of ≥300 mg/24 hours after gestational week 20 in previously normotensive women. All subjects were 6 to 12 post-partum to control for the interval between delivery and cardiovascular evaluation, all women were primiparous. None reported having been diagnosed with cardiovascular disease, diabetes mellitus, insulin resistance or cardiovascular risk factors (such as hypercholesterolemia or hypertension). Subjects who smoked or were on medication of any type were excluded from participation. Informed consent was gained from all participants prior to the experimental procedures. The study procedures were approved by the local ethics committee of the Radboud University Nijmegen Medical Centre (CMO: 2008/226) and adhered to the Declaration of Helsinki. The study was registered at clinicaltrials.gov under id: NCT00900458.

Experimental design

Measurements and training were performed in the nonpregnant state, 6 to 12 months after pregnancy. Subjects were tested before and after 12 weeks of exercise training according to a standardized protocol. All measurements, except for VO_2max, were performed during the same visit. VO_2max was tested 1 to 5 days from the other visit.

Measurements

Subjects reported to the laboratory after an overnight fast and were instructed to abstain from alcohol and caffeine for 16 hours, and not to perform any exercise in the 24 hours preceding the measurements[12]. To minimize possible endocrine influences of the sex hormones on the cardiovascular and autonomic nervous systems, all measurements were performed in the follicular phase of the menstrual cycle, between day 3 and 11[13,14]. Upon arrival at the laboratory, body characteristics were measured. A venous blood sample was taken for fasting glucose and lipid profile and kidney

function (Aeroset, Abbot Laboratories, Illinois USA). All tests were performed under standardized conditions in a temperature-controlled room (22±0.5ºC). Subjects then rested in supine position for 20 minutes, followed by assessment of blood pressure. Blood pressure and heart rate were measured oscillometrically (Dinamap, Vital Signs Monitor 1846, Critikon, Tampa, Florida) at 3-minute intervals for 30 minutes at the right upper arm. We used the median values of 9 consecutive measurements. Measurements were done with the cuff size recommended for the arm circumference[15]. This was followed by assessment of autonomic balance and, subsequently, brachial artery blood flow and shear rate patterns and endothelial function via the flow-mediated dilation technique (FMD).

Brachial artery shear rate pattern and endothelium dependent, nitric oxide-mediated dilation (FMD). Vascular assessments were conducted in a quiet, temperature-controlled environment and in accordance with recent guidelines[12]. To examine brachial artery shear patterns and flow-mediated dilation (FMD), the arm was extended and positioned at an angle of ~80° from the torso. A rapid inflation and deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was positioned around the forearm to provide a stimulus to forearm ischemia. A 10-MHz multi- frequency linear array probe, attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA) was then used to image the brachial artery in the distal of the upper arm. Continuous Doppler velocity assessments were also obtained using the ultrasound, and were collected using the lowest possible insonation angle (always <60°). First, we performed a 1 minute baseline recording of brachial artery diameter and velocity to calculate the mean, antegrade and retrograde shear rate. Shear rate was defined as 4 · velocity/diameter[16]. Oscillatory shear index, an indicator of the magnitude of shear oscillation, was defined as area-under-the-curve (retrograde shear)/(antegrade shear + retrograde shear)[11,17]. The mean brachial artery blood flow was calculated based on continuous measurement of blood velocity and diameter of brachial artery (Flow= π·radius^2 · velocity). Mean resting brachial blood flow of the one minute recording was taken. Brachial vascular resistance was subsequently estimated by dividing the mean arterial pressure by brachial artery blood flow.

Subsequently, the forearm cuff was inflated (>200 mmHg) for 5 minutes. Diameter and flow recordings resumed 30 sec prior to cuff deflation and continued for 3 minutes thereafter[12,16]. Post-test analysis of brachial artery diameter and velocity was performed using custom-designed edge-detection and wall-tracking software, which is independent of investigator bias. Recent papers contain detailed descriptions of our analysis approach[18]. Peak diameter was automatically detected according to an algorithm and is described in detail elsewhere[19]. With this technique data could be analyzed with a temporal resolution of 30 Hz, and a spatial resolution of ~0.0065 cm

for diameter and ~1cm/s for velocity. Reproducibility of FMD using this semi-automated software possesses a CV of 6.7-10.5%[6]. The post-deflation shear rate data, derived from simultaneously acquired velocity and diameter measures, was used to calculate the area under the shear rate curve (SR_{AUC}) for data up to the point of maximal post-deflation diameter for each individual[20].

Physical Fitness. We examined physical fitness before and after a 12 weeks training program. Fitness was defined as the peak oxygen uptake (VO_2max, mL min^{-1} kg^{-1}) during a maximal cycling test on a cycle ergometer (Excalibur Sport, Lode BV, Groningen NL). Tests were performed in the afternoon, after a light lunch ad libitum. The initial workload was set at 10W for 1 minute, followed by 10W increments every minute until complete exhaustion. Breath by breath oxygen uptake was measured using spiroergometric equipment (Quark CPET, Cosmed, Italy). Heart rate and rhythm were continuously recorded by 3 lead ECG. Maximal workload (Workmax) was defined as the last completed workload before exhaustion. Test performance was considered to be adequate when: 1. The increase in VO_2 (mL) during Workmax was <150ml compared to the previous workload, indicating plateau formation in oxygen uptake, 2. HR at Workmax was <10 bpm from estimated maximal heart rate (220-age), 3. Respiratory Exchange Ratio (RER, CO2/O2) was consistently >1.1 during Workmax, and 4. Capillary lactate level was >8mmol/L, 90 seconds after exhaustion. If the test failed to achieve these 4 qualifications the test was repeated 2-3 days later. In three cases (2 formerly preeclamptic women, 1 control) the test had to be repeated, all tests were eventually considered adequate.

Autonomic function. We quantified autonomic modulation by spectral analysis from a 5-minute recording of spontaneous fluctuations in HR and BP[21]. Heart-rate and arterial blood pressure (ABP) were measured continuously at a sampling rate of 100Hz using a finger ABP-monitoring device attached to the 3rd digit of the right hand (Finometer, Finapres BV, the Netherlands). Measurements were performed in a quiet, partially darkened room with subjects comfortably lying in the supine position. Posttest, these recordings were subdivided into data segments of 100s, overlapping for 50% and resampled at 5.12 Hz. Each segment was then analyzed with fast Fourier transformation that searches for rhythmic fluctuations in systolic blood pressure (BPsys) and pulse interval with a frequency range between 0 and 2.56 Hz. The amplitude of each fluctuation determines the power at each frequency. As an indicator of autonomic modulation we used the ratio of absolute low frequency (LF) and high frequency (HF) powers of the pulse interval representing the cardiac autonomic balance between the sympathetic and vagal system respectively (LF/HF)[21].

Exercise training

Exercise training consisted of 12 weeks of HR controlled cycle training (cycle ergometer, Corival, Lode BV, Groningen Netherlands) at 70-80% of VO_2max for 2 to 3 times per week. Participants trained twice a week during the first 6 weeks and 3 times a week during the last 6 weeks. During each training session, heart rate was continuously monitored and recorded (RS800CX, Polar Electro Inc, NY USA). Each training session started with 10 minutes warming-up at 50% of the heart rate reserve (HRR) above the resting heart rate. HRR was calculated as: $HRR=HR_{max}-HR_{rest}$ in which HR_{max} is the maximal heart rate measured during the fitness test at study entry and HR_{rest} is the heart rate determined at rest. Training consisted of 40 minutes of cycling between 70-80% of the individual HRR above HRrest. Within their target heart rate zone, participants were free to choose the number of revolutions per minute (RPM). The training was completed by cooling down for 5 minutes at warming-up workload.

Statistics

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, Illinois) software. All data were reported as mean±SD unless stated otherwise, while statistical significance was assumed at $P<.05$. Two-way repeated-measures ANOVA were used to examine differences between groups and to assess the effects of training. Pearson's correlation coefficient was used to examine the relation between retrograde shear rate, FMD and LF/HF ratio's in both groups before and after exercise training.

Results

Baseline differences between healthy controls versus formerly preeclamptic women

Clinical and biochemical characteristics. Formerly PE-women demonstrated higher systolic and diastolic blood pressure and higher triglycerides, but all were within the normal range. Although fasting blood glucose levels were comparable between groups, formerly preeclamptic women had higher fasting insulin levels. Physical fitness at baseline was comparable between groups (Table 1).

Shear pattern and endothelial function. Mean brachial artery blood flow was not different between formerly preeclamptic women and controls (31±6 mL/min *versus* 29±5 mL/min, respectively, $P=.09$). However, when correcting for differences in blood pressure, we found a significantly higher brachial artery vascular resistance in formerly preeclamptic women compared to control subjects (3.1±0.5 mL/min/mmHg

Table 1 Clinical and biochemical characteristics of controls (n=20) and formerly preeclamptic women (formerly PE, n=20). Both groups are 6-12 months postpartum.

	Control subjects (n=20)		Formerly preeclamptic women (n=20)		2-way ANOVA (*P*-value)		
	Pre- training	Post- training	Pre- training	Post- training	group	time	group* time
Age, y	32±4		32±4		.92		
Time postpartum, months	7.5±1.0		7.6±1.1		.74		
BMI, kg/m^2	26.8±3.4	25.1±6.6	24.7±3.9	23.2±6.6	.17	.06	.88
Waist to hip ratio	0.89±0.06	0.88±0.07	0.89±0.07	0.87±0.08	.68	.11	.19
Systolic BP, mmHg	109±6	104±6	118±10	112±8	.001	<.001	.57
Diastolic BP, mmHg	66±6	63±6	74±7	69±7	.002	<.001	.33
Glucose, mmol/L	4.7±0.5	4.7±0.4	4.7±0.4	4.7±0.3	.84	.22	.27
Insulin, mmol/L	7.7±6.9	6.2±4.3	12.9±7.2	8.1±4.7	.04	<.001	.12
Total cholesterol, mmol/L	4.5±0.9	4.1±0.8	4.7±1.1	4.6±1.1	.30	.10	.07
HDL cholesterol, mmol/L	1.2±0.3	1.3±0.3	1.1±0.2	1.2±0.2	.33	.22	.42
LDL cholesterol, mmol/L	2.8±0.8	2.6±0.7	2.9±0.8	2.7±0.8	.45	.09	.44
Triglycerides, mmol/L	0.8±0.4	0.8±0.3	1.4±0.8	1.2±0.5	.01	.15	.16
Creatinine, μmol/L	66±9	65±9	65±14	66±14	.81	.61	.10
VO_2max, mL min^{-1} kg^{-1}	27.8±3.9	31.9±3.7	27.6±3.8	31.1±5.5	.63	<.001	.60

BMI: body mass index; BP: blood pressure, HDL: high-density lipoproteins; LDL: low-density lipoproteins.

versus 2.6±0.5 mL/min/mmHg, *P*=.01). Antegrade and mean shear rate were not different between groups (102±21 s^{-1} *versus* 90±32 s^{-1}, *P*= .17 and 68±20 s^{-1} *versus* 73±28 s^{-1}, *P*=.48 respectively), while retrograde shear rate was significantly higher in formerly PE-women compared to healthy controls (-33±13 s^{-1} *versus* -17±10 s^{-1} *P*<.001) (Figure 1). Oscillatory shear index was significantly higher in formerly preeclamptic women compared to controls (0.6±0.3 *versus* 0.3±0.2, *P*=.002). A lower brachial artery FMD% was found in formerly preeclamptic women compared to control subjects (Table 2). A strong and significant correlation was found between brachial artery retrograde shear rate and FMD% in controls and formerly preeclamptic women, as well as for the pooled data set of controls and formerly preeclamptic women (Figure 2).

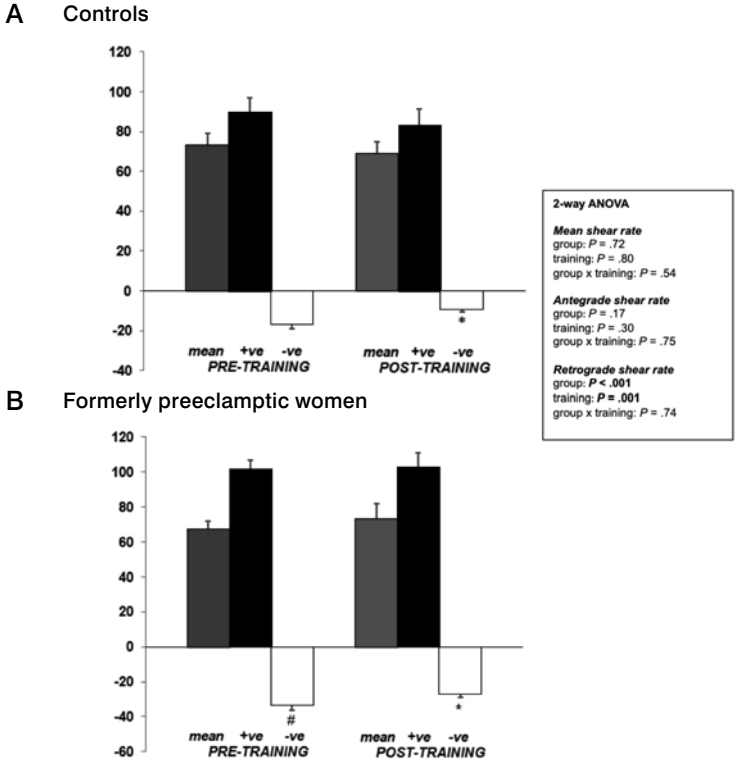

Figure 1 Baseline brachial artery mean (grey bars), antegrade (black bars) and retrograde shear (white bars) rate in healthy women (controls, n=20) and women with history of preeclampsia (formerly PE, n=20) before and after 12-weeks exercise training. All women were 6-12 months post-partum. Error bars represent SE. * $P < .05$ compared to baseline. # $P<.05$ compared to healthy controls at baseline.

Table 2 Brachial artery flow-mediated dilation (FMD) of controls (n=20) and formerly preeclamptic women (formerly PE, n=20). Both groups are 6-12 months postpartum.

	Controls (n=20)		Formerly preeclamptic women (n=20)		2-way ANOVA (*P*-value)		
	Pre- training	Post- training	Pre- training	Post- training	group	time	group*time
Baseline diameter, mm	2.8±0.3	3.0±0.3	2.9±0.3	3.0±0.4	.26	<.01	.42
Flow-mediated dilation, %	11.8±3.5	13.3±3.4	5.5±2.2	7.7±2.0	<.01	<.01	.18

Table 3 Autonomic balance based on heart rate variability analysis of controls (n=20) and formerly preeclamptic women (formerly PE, n=20). Both groups are 6-12 months postpartum

	Controls (n=20)		Formerly preeclamptic women (n=20)		2-way ANOVA (*P-value)*		
	Pre-training	Post- training	Pre- training	Post- training	group	time	group*time
Low Frequency power, ms^2	1005±329	911±315	1387±563	1173±396	.01	.12	.37
High Frequency power, ms^2	806±259	965±369	729±245	795±285	.03	.16	.54
LF/HF ratio	1.3±0.4	0.9±0.3	2.1±0.9	1.5±0.7	<.001	.001	.36

Autonomic function. Absolute low-frequency and high-frequency power and LF/HF ratio were higher in formerly preeclamptic women compared to healthy control subjects (P=.01, P=.03 and P<.001 respectively) (Table 3). A correlation existed between the LF/HF ratio and brachial artery FMD% in the pooled data set (r=-0.62, P<.001), but also when examined in controls (r=-0.69, P=.001) and formerly preeclamptic women only (r=-0.42, P=.04) (Figure 3A). In addition, a strong and significant correlation was present between the LF/HF ratio and the retrograde shear rate for the pooled data set (r=-0.79, P<.001) as well as in both controls (r=-0.69, P=.001) and formerly preeclamptic women (r=-0.73, P<.001) (Figure 3B).

Impact of 12-week exercise training

Healthy controls and formerly preeclamptic women. Exercise training decreased blood pressure and tended to lower BMI in both groups (Table 1). Exercise training lowered fasting insulin levels and improved VO_2max similarly in both groups (Table 1). Waist-to-hip-ratio, glucose, cholesterol, HDL, LDL, triglycerides and creatinine did not change after training (Table 1). Exercise training had no effect on resting mean and antegrade shear rate in both controls and formerly preeclamptic women (Figure 1). We observed a significantly reduced retrograde shear rate in controls after exercise training, but also in formerly preeclamptic women (Figure 1). In both controls and formerly preeclamptic women, twelve weeks exercise training improved brachial FMD% and

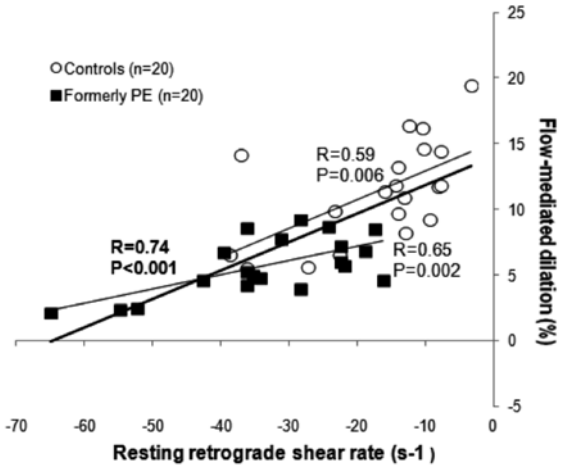

Figure 2 Correlation between brachial artery retrograde shear rate and brachial artery flow-mediated dilation (FMD%, presented as a relative change from baseline diameter) in healthy women (controls, n=20) and women with preeclampsia during their pregnancy (formerly PE, n=20). All women were 6-12 months post-partum.

lowered LF/HF (Table 2 and 3 respectively). The 2-way ANOVA interaction effect revealed that the magnitude of change in retrograde shear rate, FMD% or LF/HF ratio did not differ between controls and formerly preeclamptic women.

Correlations. The pooled data set revealed that the magnitude of the reduction in retrograde shear rate correlated with the improvement in FMD% (r=-0.61, *P*<.001, Figure 4A), but also with reduction in LF/HF ratio (R=0.60 *P*<.001, Figure 4B). When analysed per group both controls (r= -0.66 *P*=.002) and formerly preeclamptic

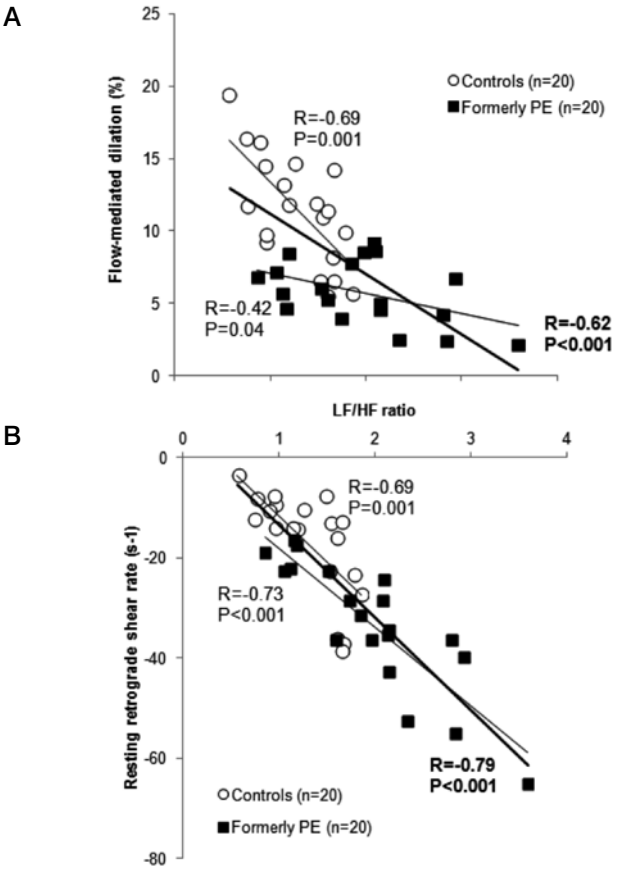

Figure 3 Correlation between resting autonomic balance (LF/HF ratio) and brachial artery flow-mediated dilation (**A**, FMD%, presented as a relative change from baseline diameter) and brachial artery retrograde shear rate (**B**, s^{-1}) in healthy women (controls, n=20) and women with preeclampsia during their pregnancy (formerly PE, n=20). All women were 6-12 months post-partum.

women (r=-0.62, *P*=.004) showed a significant correlation between the change in retrograde shear and the training induced improvement of FMD%. In turn, the change in retrograde shear correlated strongly with the exercise-induced change in LF/HF ratio in patients (r=0.82, *P*<.001), but not in controls (r=0.37, *P*=.10) (Figure 4B).

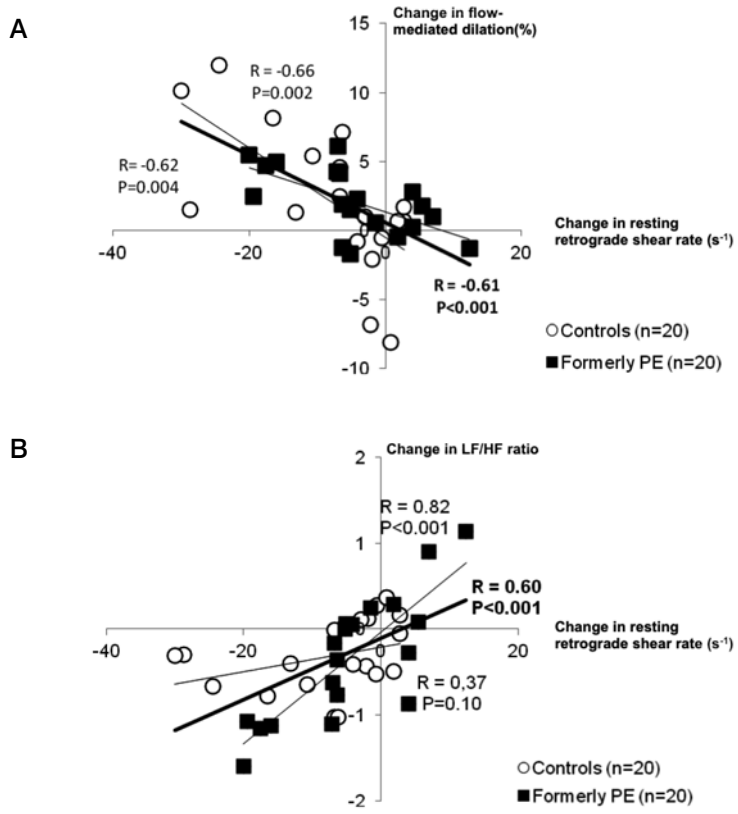

Figure 4 Correlation between the absolute change mediated by 12-weeks exercise training in brachial artery retrograde shear rate (s^{-1}, a 'negative' change relates to a smaller retrograde shear rate, i.e. more towards '0') and brachial artery flow-mediated dilation (**A**, FMD%, presented as a relative change from baseline diameter) and autonomic balance (**B**, LF/HF ratio) in healthy women (controls, n=20, open circles) and women with preeclampsia during their pregnancy (formerly PE, n=20, solid circles).

Discussion

This study has a number of novel findings. First, we demonstrated, using a between-subject approach, that brachial artery retrograde shear rate under resting conditions has a strong and inverse relationship with brachial artery endothelial function. This finding was observed in healthy subjects, but also in subjects with a history of pre-eclampsia who possessed brachial artery endothelial dysfunction. Second, in both groups, we found significant relationship between retrograde shear rate and estimated sympathetic dominance. Therefore, a higher resting retrograde shear rate correlates with the presence and magnitude of endothelial (dys)function in healthy humans as well as in formerly preeclamptic women. Third, we showed that exercise training is an effective strategy to diminish retrograde shear rate, and also to improve endothelial function and autonomic balance in healthy controls and formerly pre-eclamptic women. Finally, we demonstrated a strong correlation between the decrease in retrograde shear rate after training and the improvement after 12-weeks exercise training in endothelial function. Taken together, these findings suggest that retrograde shear relates to changes in arterial function in humans in vivo, with exercise training representing an effective strategy to improve retrograde shear rate and arterial function.

We compared two distinct groups who demonstrated differences in resting shear pattern and endothelial function. Women with a history of preeclampsia showed a higher retrograde shear rate compared to healthy controls. The higher levels of resting retrograde shear rate were accompanied by a significantly lower endothelial function, a well-established finding in women with a history of preeclampsia that is believed to contribute to their increased risk for future cardiovascular disease[7,22-24] The correlation between retrograde shear rate and endothelial function between subjects, is in line with the presence of endothelial dysfunction after an acute increase in retrograde shear using a within-subject approach[6]. Another novel observation was that the correlation between retrograde shear rate and endothelial function is present in healthy control subjects (i.e. 'normal' endothelial function) and formerly preeclamptic women (i.e. endothelial dysfunction). This suggests that the relation between retrograde shear rate and magnitude of the endothelial function is independent of a priori endothelial dysfunction in humans.

The underlying mechanisms for the higher retrograde shear rate in previously PE women may relate to increases in vascular tone in the resistance vessel beds. Indeed, formerly PE-women demonstrated a significantly higher blood pressure compared to healthy controls, a finding which may be related to higher peripheral vascular tone compared to healthy controls. A potential explanation for the higher vascular resistance, and therefore retrograde shear rate, may relate to the sympathetic nervous

system. Padilla *et al.* recently found that acute elevations in muscle sympathetic nervous activity are associated with an increase in conduit artery retrograde and oscillatory shear[11]. Increased sympathetic activity has been demonstrated in formerly preeclamptic women[25]. In our study we examined LF/HF-ratio, which provides an indirect measure of (cardiac) sympathetic activity pattern, and we found that increased LF/HF ratio was associated with higher retrograde shear rate. While previous studies have convincingly reported a strong relation between the sympathetic nervous system and endothelial function, these studies assessed changes in this relation after acute elevations in sympathetic nerve activity[26,27] rather than resting levels of activity such as in the present study. It should be noted that our measurement of LF/HF-ratio does not provide direct information about muscle sympathetic nerve activity. Although speculative, this provides evidence that higher retrograde shear rate may be related to activity of the sympathetic nervous system, and that subsequent increased retrograde shear rate may relate to changes in endothelial function.

Recent studies have demonstrated that an increased retrograde shear rate in healthy older men is related, at least partly, to a decreased contribution of nitric oxide to vascular tone and/or increased alpha-adrenergic tone[28-30]. Similarly, such explanations may relate to our study, as changes in these vasoactive substances relate to the increased retrograde shear rate (or reversal in retrograde shear rate after training) in formerly preeclamptic women. Previous studies have demonstrated that higher levels of vasoactive constrictors (e.g. endothelin-1[31] or angiotensin-II)[32] or impairment of the vasodilator pathways (e.g. NO-pathway)[33] relate to an increased peripheral vascular tone, which may result in changes in shear rate patterns (or vice versa). Interestingly, activation of the endothelin-1 pathway and/or impairment of the NO-pathway are both hypothesised to contribute to the detrimental vascular adaptations in preeclampsia[34]. In addition, insulin may contribute to changes in shear patterns based on its ability to increase sympathetic nerve activity[35]. Interestingly, we observed higher insulin levels in formerly preeclamptic women in our study. Taken together, various pathways that (in)directly alter vascular tone potentially contribute to elevated retrograde shear levels and concomitant lowering of the endothelial function.

Exercise training demonstrated a modest effect size on blood pressure and insulin, but not on any of the other parameters. This observation of a modest or even absent effect of exercise training on individual cardiovascular risk factors is reported previously. More specifically, a previous study found that only 40% of the beneficial effects of exercise training on cardiovascular risk can be explained by changes in traditional cardiovascular risk factors.

The observed effects of aerobic exercise training on blood pressure in our study are in line with a large meta-analysis that demonstrated a mean reduction of ~3 ± 2.4mmHg with a more pronounced effect in (pre-)hypertensive population[36]. Other effects such as direct effects on the vasculature, might explain a part of the remaining cardiovascular risk reduction with exercise training. To support this notion, Green et. al. demonstrated that the effects of exercise on traditional cardiovascular risk factors do not relate to exercise induced changes in endothelial function[37]. Therefore, effects of exercise on endothelial function and shear pattern are unlikely a direct resultant of the changes in traditional cardiovascular risk factors, but may represent a direct effect of exercise training on the vessels.

A potential limitation of our study is the cross-sectional nature of the assessment of the relationship between retrograde shear rate and endothelial function. However, we have extended these between subject observations by performance of a 12-week exercise training study, which allowed for within subject comparisons. To our knowledge, our study provides the first data that indicates that exercise training in humans can reverse retrograde shear rate. A potential limitation of our study is that we presented our data as shear rate rather than shear stress. However, we expect no differences in viscosity between groups and/or after training, while the length of a vessels is unlikely to change after training. As these are important assumptions in the calculation of shear rate/stress, we believe that this did not important impact upon our results. Another limitation relates to the method of examining the sympathetic nervous system, as we examined cardiac sympatho-vagal balance (LF/HF) derived from heart rate variability. Although related to peripheral (muscle) sympathetic nerve activity, care should be taken when LF/HF ratios are translated to vascular sympathetic tone because of possible differential sympathetic outflow between heart and vasculature. A fourth limitation is that our data does not allow to *directly* examine the relation between retrograde shear rate and FMD. However, our study was designed to examine the presence of such a relationship (and whether this depends on a priori endothelial dysfunction and fitness levels). Future prospective studies are warranted to further examine this relationship including causality. In this study women were all examined in the follicular phase of the menstrual cycle when estrogen levels are lowest. We expect no different responses in male subjects; however, generalization of our data to humans is speculative untill confirmed in male subjects.

Perspectives

Data from animal studies have demonstrated an important role for retrograde shear or oscillatory shear pattern in the upregulation of pro-atherogenic and downregulation of anti-atherogenic genes, leading to an atherogenic endothelial phenotype[5, 6]. The presence of elevated levels of retrograde shear rate may therefore be a central feature

in the development of atherosclerosis and its complications, such as plaque instability or rupture. Women with a history of preeclampsia have increased risk of developing cardiovascular disease later in life[24], possibly through the presence of endothelial dysfunction[7,22,38]. Elevated levels of retrograde shear in formerly preeclamptic women may provide a mechanistic link that explains the previously suggested relationship between endothelial dysfunction and development of cardiovascular morbidity. Furthermore, this study also highlights the potency of exercise training to improve retrograde shear rate, endothelial function and LF/HF-ratio, and therefore contribute to an improved cardiovascular risk after exercise training.

Conclusions

Results from the present study demonstrate that resting levels of retrograde shear rate are strongly related to endothelial function in humans. This finding was observed in healthy women and in women with a history of preeclampsia who exhibit brachial artery endothelial dysfunction. We also found that sympathetic dominance related to retrograde shear rate in healthy subjects as well as in formerly preeclamptic women. Moreover, these differences and correlations remained significant after 12-week exercise training, while the change in retrograde shear rate correlated significantly with the changes in FMD% and LF/HF-ratio. Therefore, our data provide evidence that a higher retrograde shear rate is strongly related to the presence and magnitude of endothelial dysfunction in healthy humans and those with an increased cardiovascular risk, with exercise training being able to change these parameters (without affecting the relation between them).

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CHAPTER 9

General discussion

This thesis includes seven studies with the purpose to better understand the hemodynamic profile that is characterized by low plasma volume in normotensive formerly preeclamptic women. This hemodynamic profile may contribute to the increased risk to develop recurrent hypertensive disease in future pregnancies and cardiovascular disease later in life in women with a history of preeclampsia.

This thesis is grounded on five main research questions: 1. To what extent does nonpregnant plasma volume relate to the onset of preeclampsia and to the co-occurrence of other cardiovascular risk factors in women with a history of preeclampsia? (Chapter 2) 2. To what extent is the adult plasma volume determined at birth? (Chapter 3) 3. To what extent does nonpregnant plasma volume relate to the recurrence rate of preeclampsia and to the development of chronic hypertension after a pregnancy complicated by preeclampsia? (Chapter 4 and 5) 4. To what extent can plasma volume in formerly preeclamptic women be improved by means of aerobic exercise training in comparison with healthy parous control subjects? (Chapter 6) 5. What are the hemodynamic consequences of aerobic exercise training aimed at increasing plasma volume in women with a history of preeclampsia compared with healthy parous controls? (Chapter 6, 7 and 8)

We used these five main research questions to summarize and discuss our findings systematically in this final chapter. At the end of this chapter we will discuss future perspectives based on the implications of the studies included in this thesis.

In summary, our studies show that the hemodynamic profile characterized by low plasma volume is the most prevalent risk profile in formerly preeclamptic women. The exact aetiology of low plasma volume is largely unknown, but we demonstrate that adult plasma volume is partially determined at birth depending on intra-uterine development. Prepregnant plasma volume links in a volume dependent manner with the recurrence risk of preeclampsia and it predisposes to chronic hypertension later in life in initially normotensive formerly preeclamptic women. Accordingly low plasma volume reflects latent hypertension in normotensive formerly preeclamptic women. Aerobic exercise training improves plasma volume and its' associated venous characteristics suggesting increased venous reserve capacity. We observed that 12 weeks of vigorous aerobic exercise training normalized the traditional cardiovascular risk profile in formerly preeclamptic women and with it training improved endothelial function, arterial wall thickness and autonomic balance in post partum women. The exercise induced improvement in endothelial function correlated with changes in autonomic balance. A possible explanation for this correlation is autonomic induced changes in the shear characteristics of vascular blood flow affecting endothelial functioning. Taken together, our studies provide not only rationale to warrant follow-up

of blood pressure in normotensive formerly preeclamptic women, but also support the recommendation of lifestyle interventions including aerobic exercise as part of the cardiovascular risk management in women with a history of preeclampsia.

1. The occurrence of low plasma volume in women with a history of preeclampsia

The link between preeclampsia and cardiovascular disease later in life is well established[1]. Consequently many women nowadays undergo cardiovascular and metabolic testing, sometimes even additional thrombophilia screening, after their preeclamptic pregnancy to identify risk factors for future cardiovascular disease. Formerly preeclamptic women are tested under the assumption that these risk factors are modifiable and that improvement is beneficial for the outcome in next pregnancy and beyond.

We structurally tested a large cohort of formerly preeclamptic women for the presence of cardiovascular risk factors and thombophilia 6-12 months after the complicated pregnancy (Chapter 2). So far, most studies involved only a single risk profile in relation to preeclampsia in the preceding pregnancy. Therefore any possible interrelation between separate risk profiles within women with a history of preeclampsia is currently unclear. Hence we analysed the co-occurence of circulatory abnormalities, metabolic syndrome, thrombophilia and hyperhomocysteinemia. When structurally tested for these risk profiles, 78% of formerly preeclamptic women had one or more abnormal profile. The most prevalent risk profile found in formerly preeclamptic women was the circulatory risk profile indicating latent or overt hypertension (66%). Low plasma volume suggesting latent hypertension in normotensive formerly preeclamptic women mainly accounted for the high occurrence of the circulatory risk profile. The prevalence of the traditional cardiovascular risk profile (metabolic syndrome) after preeclampsia was 15%, approximately 3 times higher than that in the general female population of comparable age. Of all metabolic factors tested, hyperinsulinemia indicating insulin resistance was the most common metabolic aberration found in formerly preeclamptic women. Apart from hyperhomocysteinemia, the prevalence of thrombophilia was comparable with those in the Dutch general population; therefore the added value of routine thrombophilia screening other than hyperhomo-cysteinemia after preeclampsia seems limited.

The prevalence of the circulatory risk profile, hyperhomocysteinemia and all components of the metabolic syndrome other than obesity relate inversely with the gestational age at delivery in preceding pregnancy. This suggests a relationship between these risk

factors and severity of disease. Other studies have debated whether early and late onset preeclampsia are two distinct disorders resulting from different pathophysiological mechanisms[2,3]. The observed continuous relationship between cardiovascular risk factors and gestational age at delivery in previous pregnancy fits best in a spectrum of severity of one and the same disease, rather than a dual aetiology of preeclampsia. Only prospective studies are however able to differentiate if aetiology differs between early vs. late onset preeclampsia. Increased body mass index is regarded as a strong independent risk factor for preeclampsia. Interestingly, we observed that constituents of the metabolic syndrome other than obesity related to the onset of disease in prior pregnancy. This implies that not obesity itself but rather the concomitant cardio-metabolic aberrations associate with severity of hypertensive disease in pregnancy. This observation is consistent with the perspective that obese women can be subdivided in either metabolically healthy or unhealthy phenotype[4,5], the latter with a higher risk to develop cardiovascular disease later in life[6].

After studying the prevalence of cardiovascular risk factors in a large cohort of preeclamptic women, it seems rational that cardiovascular risk management after a pregnancy complicated by preeclampsia should focus on controlling blood pressure and insulin resistance; especially after early onset preeclampsia (Chapter 2). It should however be noted that, although plausible, effectiveness of follow-up and altering cardiovascular risk factors in order to prevent adverse clinical outcomes after preeclampsia are yet to be proven. Meanwhile the American Heart Association, the European Society of Cardiology and the American College of Obstetrics and Gynecology now include preeclampsia as a risk factor for future cardiovascular disease with the recommendation of obtaining a history of preeclampsia and improving lifestyle behaviours for women with such a history[7-9].

Women who are hypertensive at the screening 6 to 12 months post partum clearly belong to a risk population for recurrent hypertensive disease in future pregnancies and cardiovascular disease later in life[10,11]. Much less is known about the apparently healthy, normotensive formerly preeclamptic women. Our study confirms earlier studies that approximately 50% of these normotensive formerly preeclamptic women have low plasma volume[12]. This thesis further focused at this population of normotensive formerly preeclamptic women. It is unknown if the observed low plasma volume status is pre-existent or rather a consequence of the preeclamptic pregnancy[10]. At any rate, the high prevalence of low plasma volume in normotensive formerly preeclampsia warrants further studies on the possible role of plasma volume in this population.

2. The aetiology of low plasma volume

The exact origin of the phenotype low plasma volume is currently unknown. In healthy persons, plasma volume remains relatively constant as a result of the complex interaction between neurohormonal systems involved in sodium and water homeostasis (Figure 1). The primary volume receptors monitoring volume status are stretch receptors in the cardiopulmonary circulation, the atria, the carotid sinus and aortic arch, and in the juxtaglomerular apparatus of the kidney.

The extra-renal stretch receptors govern the activity of the sympathetic nervous system, the production of atrial natriuretic peptide (ANP) and antidiuretic hormone (ADH). In times of volume depletion sympathetic neural tone and the secretion of catecholamines by the adrenal medulla are enhanced. In contrast, in response to volume expansion, ANP is released by the cardiac atria[13]. ANP subsequently increases glomerular filtration rate, and decreases sodium retention in the proximal

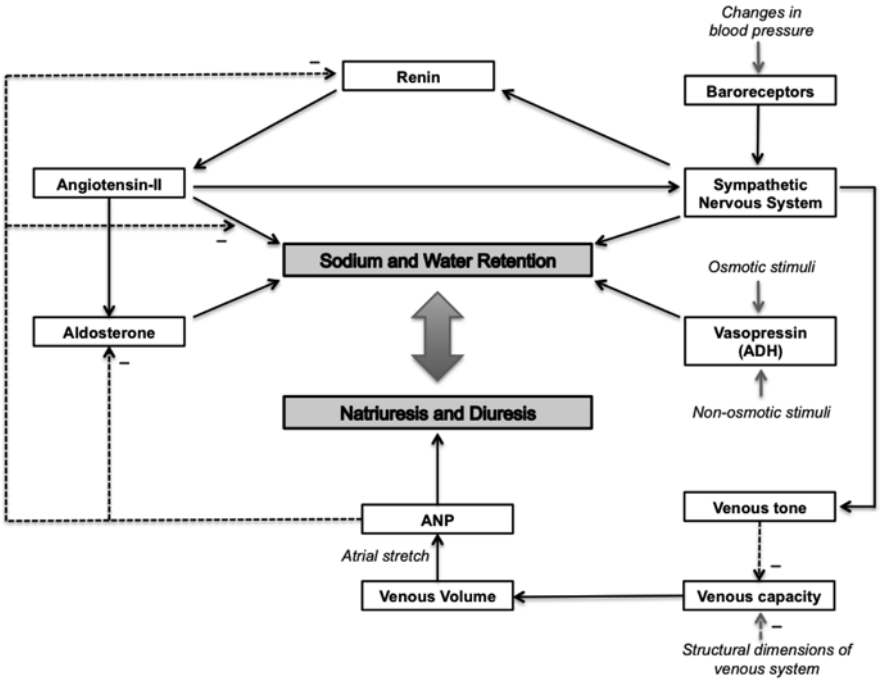

Figure 1 Schematic overview of the plasma volume regulation

ANP = Atrial Natriuretic Peptide; ADH = Anti-Diuretic Hormone

tubule and collecting ducts of the kidney. ADH or vasopressin is released by the pituitary gland predominantly in response to hyperosmolality sensed hypothalamic receptors and to a lesser extent to volume depletion sensed by receptors in the carotids[14]. ADH augments water permeability in the collecting tubeles of the kidney, hence promoting water reabsorption.

The renal receptors affect volume balance by influencing the renin-angiotensin-aldosterone system (RAAS). Renin secretion increases when circulating volume and renal perfusion pressure decreases[15]. Renin induces the conversion of angiotensinogen into angiotensin. Angiotensin has two major actions: arterial vasoconstriction and renal sodium and water retention both directly and by increasing the secretion of aldosterone from the adrenal cortex[16-18].

In addition to the stretch and osmolality receptors, pressure receptors (baroreceptors) mainly located in the carotid arteries and aortic arch, respond to variations in blood pressure by acting on the interplay between the orthosympathetic and parasympathetic nervous system affecting cardiac inotropy and chronotropy and vascular constriction[15]. Effects of the baroreceptors may indirectly influence plasma volume status by acting on the volume capacity of the vascular system, primarily the venous system[19].

With so many complex interacting neurohormonal and cardiovascular factors contributing to the plasma volume status, it is challenging to determine the exact aetiology of low plasma volume status observed in 50% of the normotensive formerly preeclamptic women. To date, there have been few studies evaluating plasma volume regulation in formerly preeclamptic women.

Non-pregnant formerly preeclamptic women with low plasma volume do not seem to be chronically vascular underfilled since compensatory neuro-hormonal changes such as elevated renin, angiotensin and aldosterone levels are lacking and baseline hemodynamic values are quite similar to their normal plasma volume counterparts[12]. In addition, reports on renal hemodynamics suggest that low plasma volume in these women is not supposed to be caused by primary renal dysfunction[12,20]. Studies demonstrate no difference between ANP levels in formerly preeclamptic women and parous controls[21]. Formerly preeclamptic women with low plasma volume however responded with increased ANP secretion upon volume loading, while in control subjects ANP levels remained unchanged[20]. In contrast, formerly preeclamptic women with low plasma volume responded to a single bout of exercise with a smaller rise in ANP compared with parous controls[22]. During an exercise period of 60 minutes women with low plasma volume showed reduced increase in stroke volume and

cardiac output and were unable to sustain this elevated stroke volume. Taken together, these observations are consistent with a reduced capacity of the vascular system to accommodate a relatively modest volume load or increase cardiac preload in times of increased arterial demands. Although resetting of the hormonal activity and/or sensitivity of their receptors cannot be ruled out as contributors to the low plasma volume levels in formerly preeclamptic women, it seems that the low plasma volume status may be best explained by a reduced volume capacity of the vascular system.

The venous system acts quantitatively as the most important volume reservoir[23] and its' dynamic capacity depends on venous tone which is under exclusive baroreceptor mediated sympathetic control[19,24,25]. Plasma volume status is therefore strongly influenced by both venous and autonomic characteristics. Reduced plasma volume may originate from a chronically higher resting sympathetic tone resulting in chronic venoconstriction and a consequent reduction of the functional capacity of the venous system[26,27]. Alternatively, plasma volume can be low as a result of structurally reduced dimension of the venous system as a consequence of intra-uterine inhibited venous development in line with the Barker hypothesis[28]. Both functional and structural reduction of the venous capacity will likely hamper the accommodation of the necessary plasma volume expansion in pregnancy.

In an effort to estimate the contribution of intra-uterine development to the adult plasma volume we have tested the hypothesis that women born small for gestational age have lower plasma volume in adult life, compared with women born appropriate or even large for gestational age. Asymmetrical fetal growth restriction is characterized by a disproportionately small abdominal circumference as consequence of loss in liver, kidney an intestinal volume[29]. This is thought to originate from selective growth failure of splanchnic organs as a consequence of fetal circulatory redistribution away from this abdominal region[30]. The splanchnic vascular bed forms quantitatively the most important volume reserve compartment. As only arteries have regenerative capacity, limited intra-uterine development of the venous splanchnic vascular bed may have long-lasting effects on total plasma volume. Our study demonstrated that women born small for gestational age are predisposed for lower plasma volume in adult life compared to counterparts who were born appropriate or large for gestational age (Chapter 3). Therefore our data suggest that reduced size of the splanchnic vascular bed at birth may indeed have impact on adult plasma volume. The contribution of intra-uterine development to the adult plasma volume is however estimated to be modest, only 14% of the variation in plasma volume between subjects could be explained by variation in birth centile, still higher than many other studied variables. It should be noted that we were unable to explain most of the variation in plasma

volume between individuals. This emphasizes the potential contribution of factors that functionally reduce the venous capacity, perhaps most notably sympathetic over activity. Some studies already demonstrated a higher sympathetic tone in formerly preeclamptic women with low plasma volume[24,26,27]. The potential role of the sympathetic nervous system including baroreceptor functions and consequent venous wall properties should therefore be further studied in formerly preeclamptic women with low plasma volume.

3. Low plasma volume and recurrent preeclampsia and chronic hypertension

Normotensive formerly preeclamptic women with low plasma volume are more likely to develop recurrent preeclampsia than their counterparts with normal plasma volume[31]. Our study shows that prepregnancy plasma volume in normotensive formerly preeclamptic women even contributes in a volume dependent manner to the risk of recurrent preeclampsia and fetal growth restriction (Chapter 4). This strongly suggests a continuum in the pathophysiological role of plasma volume in the development of hypertensive disease in pregnancy. But how does low plasma volume fit in a pathophysiological disease model of preeclampsia?

In pregnancy the maternal cardiovascular system undergoes profound changes in order to provide the growing fetus with oxygen and nutrients. In a healthy pregnancy these cardiovascular changes are characterized by a generalized reduction in systemic vascular resistance and a concomitant rise in plasma volume[32]. Together these adaptations result in a significant increase in blood flow. If the maternal circulatory systems adapts adequately, blood pressure in mid-pregnancy is about 10% lower than in non-pregnant women while the intravascular plasma volume has increased with 40-50%[33]. This extra volume can only be accommodated without a consequent rise in blood pressure if the vascular system is sufficiently compliant. With the venous system acting as the most important volume reserve, a highly compliant venous system is essential in pregnancy. Studies have demonstrated that women with pre-pregnant low plasma volume have reduced plasma volume expansion in pregnancy[34,35]. If plasma volume expansion in pregnancy is blunted, the ability to increase cardiac preload is limited. Consequently the maternal cardiovascular system can only respond to the increased demands of advanced pregnancy by a compensatory rise in sympathetic tone. This will likely further constrict the venous system and increase heart rate creating an abnormal hyperdynamic circulation. The resulting sympathetically driven low-volume/high-output circulation will exert extra shear stress upon the endothelium, and eventually increase arterial vascular

resistance; thereby setting the stage for endothelial dysfunction and hypertension in pregnancy, or in clinical terms: preeclampsia[25,34,36-38].

Our study demonstrates also fetal consequences of low pre-pregnant plasma volume. We observed that as pre-pregnant plasma volume increases, the risk of a growth restricted neonate decreases proportionally independent of preeclampsia in subsequent pregnancy. In non-pregnant women with low plasma volume circulatory redistribution at the expense of uterine perfusion has already been demonstrated. It is therefore conceivable that if plasma volume expansion is also blunted in pregnancy, continued redistribution away from the uterine circulation affects fetal growth[39]. Based on the observed volume dependent relationships observed between prepregnant plasma volume and the occurrence of recurrent preeclampsia and fetal growth restriction in the next ongoing pregnancy it is conceivable that prepregnant plasma volume expansion may have a favourable effect on these outcomes in the next pregnancy.

Because low plasma volume linearly linked with recurrent preeclampsia and fetal growth restriction, we hypothesized that low plasma volume may also provide a possible pathophysiological link between preeclampsia and the observed cardiovascular risk later in life. Early studies already linked low plasma volume with latent or borderline hypertension in other populations than formerly preeclamptic women[40]. Our prospective follow-up study demonstrates that 1 out of 6 (17%) of the normotensive formerly preeclamptic women develop chronic hypertension within the subsequent 5 years, and plasma volume linked inverse linearly with blood pressure measured 2 to 5 years later (Chapter 5). Other studies have attempted to unravel the pathophysiological pathway between low plasma volume and the gradual development of overt hypertension. These longitudinal studies in subjects with low plasma volume demonstrated a gradual change in hemodynamics from a hyperdynamic/low resistance circulation into a more hypodynamic/high resistance circulation with a consequent increase in blood pressure[41]. The hemodynamic transition resulting in chronic hypertension is thought to be secondary to a decrease in cardiac responsiveness and an increase in vascular responsiveness over the course of hypertension. In established hypertension, high vascular resistance is the hemodynamic hallmark, whereas a high cardiac output prevails in young mildly hypertensive individuals[42,43]. Combined blockade of sympathetic and parasympathetic cardiac receptors abolishes the elevation in cardiac output and the heart rate in these young borderline hypertensive patients with low plasma volume[44], and an addition of alpha-adrenergic blockade to the cardiac blockade normalizes their blood pressure[45]. Consequently, the hyperkinetic state and the eventual blood pressure elevation in these individuals are likely to be neurogenic. The sympathetic overactivity is regarded as the key characteristic of neurogenic

hypertension. It is unknown if this sympathetic overactivity is primarily responsible for the observed low plasma volume status or rather a compensatory consequence of the low plasma volume. Prospective studies in formerly preeclamptic women including hemodynamics and autonomic functions may help explain the observed hypertension in formerly preeclamptic women. Eventually long follow-up of formerly preeclamptic women beyond the age of 50 will be necessary to determine the impact of low plasma volume on actual cardiovascular events.

4. Improving plasma volume status with exercise training

Ultimately, improving the plasma volume status may reduce the risk of hypertensive disease in future pregnancy and beyond. Aerobic exercise training is known to induce plasma volume expansion, although heterogeneity in the magnitude of plasma volume expansion upon exercise training has been described[46].

The beneficial effects of exercise training on the human cardiovascular system are nowadays indisputable[47-49]. Exercise training decreases risk for cardiovascular disease in a dose-dependent way. Approximately 60% of this decreased risk can be explained by the exercise-induced improvement in traditional cardiovascular risk factors, mainly by reducing blood pressure, insulin resistance and dyslipidaemia. The remaining 40% if often referred to as the so called 'risk factor gap'[50]. Direct effects of exercise training on the vessel wall and changes in hemodynamics may explain this gap[51]; for example by improving endothelial functions, reduction of arterial wall thickness and reduction of sympathetic tone and perhaps plasma volume.

To test how modifiable the cardiovascular risk profile in formerly preeclamptic women is, we have designed a case-control exercise training study using an integrative approach. This study design enabled us to study the effects of exercise training not only on the traditional cardiovascular risk profile, but also on plasma volume and venous characteristics (Chapter 6), arterial characteristics (including arterial wall thickness, endothelial function and arterial shear stress characteristics (Chapter 7 and 8) and finally autonomic characteristics (sympathetic activity and baroreceptor sensitivity) (Chapter 7 and 8).

Our 12-weeks aerobic exercise-training program improved cardiorespiratory fitness (VO_{2max}) by ≈13% in both formerly preeclamptic women and healthy parous controls indicating a successful training stimulus. While formerly preeclamptic women show reduced plasma volume expansion in pregnancy, we demonstrate a similar plasma

volume expansion upon exercise training in formerly preeclamptic women (+12.1%) and healthy controls (+8.3%). Yet, after 12 weeks of aerobic exercise training formerly preeclamptic women had a plasma volume status that was comparable with pre-training sedentary controls. Continued training may theoretically further expand the plasma volume[46]. The key question remains if the observed exercise induced plasma volume expansion is enough to have an impact on recurrence risk of preeclampsia and fetal growth restriction and ultimately the development of chronic hypertension in formerly preeclamptic women. The mean plasma volume expansion induced by our 12-weeks exercise training in formerly preeclamptic women was 180 ml/m^2 (Chapter 6), a change that is larger than the difference in mean plasma volume between formerly preeclamptic women who developed recurrent preeclampsia and women who did not have recurrent disease in the next on-going pregnancy (Chapter 4). Therefore, theoretically, if this exercise induced plasma volume expansion is extrapolated to the plasma volume dependent recurrence risk of preeclampsia and risk of delivering a growth restricted infant, exercise training should be able to offset the contribution of low plasma volume to these risks in future pregnancies. Likewise, based on the observed linear relationship between plasma volume and blood pressure 2-5 years later in formerly preeclamptic women (Chapter 5), the exercise induced plasma volume expansion theoretically corresponds with an estimated reduction in mean arterial pressure of approximately 7 mmHg. Obviously, these estimations assume a persistent plasma volume expansion and therefore a persistent active lifestyle as detraining is associated with reduction of plasma volume. Exercise training is likely to have an impact on more risk factors associated with increased recurrence risk of preeclampsia and chronic hypertension, therefore the beneficial effects may in fact even be higher.

In normotensive formerly preeclamptic women we observed increased sympathetic activity and reduced venous compliance compared to parous controls, suggesting increased venous tone in formerly preeclamptic women. Our exercise study indicates that the exercise induced plasma volume expansion coincides with reduction of sympathetic activity and improvement of venous compliance. This implies an exercise-induced functional improvement of the venous reserve capacity. We further analysed the venous reserve capacity, or in other words the ability of the venous system to mobilize blood volume, using a validated dynamic orthostatic stress test. The results of the head-up tilt testing revealed that that the ability of the veins to mobilize venous volume through venoconstriction was limited prior to exercise training but improved markedly with exercise training. The effects of exercise training on venous functioning were more pronounced in formerly preeclamptic women than in parous controls (Chapter 6). Since veins are under exclusive baroreceptor mediated sympathetic control, the increased venous tone observed in formerly preeclamptic

women prior to training might originate from abnormal baroreceptor functioning. We observed that baroreceptor sensitivity was significantly lower in formerly preeclamptic women compared to parous controls (Chapter 7) and training significantly improved baroreceptor sensitivity in both formerly preeclamptic women and parous controls. Training was however unable to normalize baroreceptor sensitivity in formerly preeclamptic women. The observed abnormal baroreflex control in formerly preeclamptic women is consistent with observations in subjects with (borderline) hypertension[52,53]. Structural changes in large arteries are often considered the predominant mechanism responsible for decreased baroreflex sensitivity and baroreceptor resetting in hypertension[54]. Although more recent evidence demonstrated that "functional" mechanisms also contribute to altered baroreflex responses, for example at the level of the peripheral sensory endings and within the central nervous system, or as a result of endothelial dysfunction with altered release of certain paracrine endothelial factors. The head-up tilt protocol with intact physiological reflexes does not allow us to determine if the autonomic changes are responsible for the observed reduced venous reserve capacity in formerly preeclamptic women or vice versa. This requires intervention studies with selective blockade of autonomic transduction.

5. Other hemodynamic effects of exercise training in women with a history of preeclampsia

When studying the traditional cardiovascular risk profile we observed that before exercise training formerly preeclamptic women differed from women who had an uneventful pregnancy by having a higher blood pressure and fasting insulin level, and more micro-albuminuria. Twelve weeks exercise training abolished all differences apart from micro-albuminuria between formerly preeclamptic women and controls. The improvement of components of the metabolic syndrome suggests reduced vascular stress. The effect of aerobic exercise training on insulin resistance was remarkably large in formerly preeclamptic women, underscoring the potency of aerobic exercise on insulin sensitivity. Other studies indeed demonstrated that young age, female sex, and a priori mild level of insulin resistance predict a relatively larger improvement of insulin sensitivity with exercise training[55].

The protective effects of exercise training are further supported by the observed improved endothelial function measured in two separate conduit arteries. We confirmed other studies demonstrating persistent endothelial dysfunction in formerly preeclamptic women compared to women who had an uneventful pregnancy[56-58]. Endothelial dysfunction measured with flow mediated dilation technique has proven

to be independently associated with cardiovascular disease burden and outcome in both low and high-risk populations[59]. Although 12-weeks exercise training significantly improved endothelial function, it was unable to normalize endothelial function in formerly preeclamptic women. The comparable vasodilation responses to sublingual nitrates between formerly preeclamptic women and controls confirm endothelial dysfunction rather than vascular smooth muscle dysfunction in women with a history of preeclampsia. Some exercise studies demonstrated a time-course in vascular adaptations to exercise training; first endothelial function improves, than arterial outward remodelling occurs after which endothelial function appears to return to baseline values after 8-12 weeks of exercise training[60]. We however observed in both formerly preeclamptic women and controls that after 12 weeks exercise training improved endothelial function as well as increased arterial diameter and reduced carotid wall thickness. It is likely that subjects with a priori endothelial dysfunction do not show complete return to baseline, this could explain the improved endothelial function in formerly preeclamptic women. It is disturbing that endothelial function after 12 weeks exercise training remains significantly lower in formerly preeclamptic women compared to controls. Perhaps longer training may restore endothelial function eventually. More difficult to explain is the fact that healthy parous controls demonstrate an almost equal improvement in endothelial function and arterial structure compared to subjects with a priori endothelial dysfunction. Theoretically this may reflect endothelial recovery back to the actual baseline value in sedentary post-partum women, even after an uneventful pregnancy. Alternatively, women may have a different time course of vascular adaptation to exercise training compared to men, in line with possible gender differences in exercise training responses as suggested in other studies. At any rate endothelial functions in postpartum women appear to respond well to the exercise stimulus, irrespective of their obstetric history.

We observed that endothelial function correlated with autonomic balance in both formerly preeclamptic women and healthy controls. High sympathetic outflow in conjunction with endothelial dysfunction as we observed in formerly preeclamptic women is thought to have synergistic and detrimental effects on cardiovascular risk[50]. To study the relationship between endothelial functioning and the autonomic system we further analysed the vascular shear patterns in formerly preeclamptic women and controls before and after 12-weeks aerobic exercise training. Studies have demonstrated that exercise-induced changes in shear stress provide the principle physiologic stimulation to adaptation in vascular function and structure[61]. The blood flow-generated endothelial shear stress during repeated bouts of exercise induces altered gene expression in endothelial cells and induces the release of several factors which can influence the vascular endothelium and the underlying smooth muscle cells. One key factor is the nitric oxide release induced by shear stress via phosphor-

ylation of endothelial NO Synthase. Therefore changes in shear stress directly influence the endothelium of arteries, which plays a crucial role in the regulation of blood flow and maintenance of the quality of blood vessels. Increases in the antegrade shear stress are believed to be beneficial for arterial function, while high levels of retrograde shear increase the expression of proatherogenic genes with consequent detrimental effects for vascular health[62,63]. First we demonstrated increased levels of resting retrograde shear in formerly preeclamptic women compared to parous controls. Second, our study demonstrates a clear relationship between retrograde shear stress and endothelial function. Exercise training proved to be an effective strategy to diminish retrograde shear rate and also improve endothelial function and autonomic balance in both healthy controls and formerly preeclamptic women. The exercise induced changes in retrograde shear and endothelial function correlated with each other, underscoring the reciprocal dependency. The underlying mechanisms for the higher retrograde shear rate in formerly preeclamptic women may relate to increases in vascular tone in the resistance vessel beds. Indeed we observed higher vascular resistance and blood pressure in formerly preeclamptic women compared to parous controls. Higher insulin levels as demonstrated in formerly preeclamptic women, might (in) directly alter vascular tone via sympathetic modulation. Apart from sympathetic over-activity other pathways may explain the increased vascular tone, for example reduced contribution of nitric oxide to vascular tone or higher levels of vasoactive constrictors (endothelin-1 or Angiotensin-2).

Taken together our exercise studies demonstrate important beneficial effects of aerobic exercise training on both traditional and non-traditional cardiovascular risk factors in formerly preeclamptic women who are known to be at increased risk of developing cardiovascular disease. Our studies can be seen as a “proof of principle” that exercise training can significantly impact both the traditional and non-traditional cardiovascular risk profile in women, especially after a pregnancy complicated by preeclampsia.

Future perspectives

The studies included in this thesis provide more insight into the cardiovascular risk profile in normotensive formerly preeclamptic women. We have demonstrated that post partum women respond well to the exercise stimulus, therefore aerobic exercise training has the potential to improve outcome of future pregnancy and perhaps reduce remote cardiovascular risk in formerly preeclamptic women. Further studies are needed to optimize the care of women with a history of preeclampsia.

We still need to establish whether preeclampsia acts as a stress test to unmask women who are already at risk for cardiovascular disease, or whether pregnancy course itself induces the observed remote risk in women with a history of preeclampsia. Irrespective of causality, a key question is the extent to which pregnancy history can be used to improve the current cardiovascular risk-scoring systems for women such as the Framingham Risk score. At present these scoring systems are of debatable utility for women under the age of 70 years. If pregnancy outcome is useful for early CVD risk identification, the next question is whether early risk identification (e.g. at the post-partum evaluation) is a cost-effective way of reducing future CVD risk. The associations of preeclampsia and CVD are remarkably consistent. Although untested, the use of preeclampsia history to screen women for targeted CVD prevention has potential to improve public health, given the magnitude of the associations, the prevalence of preeclampsia and the importance of the CVD burden in women. Pregnancy complications occur early enough in a woman's life to potentially offer a significant meaningful prevention by lifestyle intervention.

We need to test the extent to which promoting exercise and healthy diet or in some cases even pharmacological prevention is effective in improving future pregnancy outcome and preventing CVD in young or middle aged women with a history of preeclampsia. It is important to examine if standard screening, prevention and therapy protocols can be optimized or tailored on the basis of a woman's particular risk profile that is identified post partum. Based on our observation, cardiovascular risk management after preeclampsia should, at least initially, focus at controlling blood pressure and insulin resistance in the first years following the preeclamptic pregnancy.

The exercise intervention used in this thesis involved a 12-weeks supervised vigorous aerobic training protocol. Effects of this specific training protocol cannot be extrapolated to expected effects of merely advising active lifestyle to women with a history of preeclampsia. The effectiveness of different modes and intensity levels of increasing physical activity level in post partum women still need to be established. Well known boundaries exist when implementing preventative lifestyle strategies. To begin, doctors and patients need to recognize the increased cardiovascular risk and the expected beneficial effects of an active lifestyle. Second, practical concerns to implement a more active lifestyle need to be addressed. Active coaching may help introducing and enduring healthy behaviour. Interestingly some studies have indicated that the postpartum period is a stage in the life course of women, when women are particularly receptive to lifestyle interventions.

Taken together future research requires large data sets that have prospectively collected accurate data on cardiovascular risk factors before, during and after pregnancy, into middle age and beyond when cardiovascular disease begins to emerge. Ultimately randomized controlled trials will be necessary to establish whether continued monitoring and early treatment of women identified at risk after a pregnancy complicated by preeclampsia are cost-effective ways to improve future pregnancy outcomes and reduce CVD risk in women. It remains a challenge in young individuals to demonstrate a clear reduction in cardiovascular disease because of the long follow-up period needed and the amount of potential confounders during this period. The use of surrogate markers of cardiovascular disease could be helpful (e.g. hypertension and endothelial dysfunction). Moreover the next pregnancy is an important relatively short-term outcome that should be taken into account in future studies.

Meanwhile we need to improve our understanding of the pathophysiological pathways that lead to the hypertensive complications in pregnancy and cardiovascular disease later in life. Studying these pathways has been proven difficult given the existing heterogeneity among women who develop preeclampsia. In this thesis we have mainly focused on the potential role of low plasma volume. We observed that low plasma volume is closely linked with activity of the sympathetic nervous system either by cause or consequence. Better understanding of the hemodynamic and autonomic changes in women with a history of preeclampsia, may improve targeted screening and management in pregnancy and beyond. Ideally, these changes can be recognized even well before hypertensive disease develops in nulliparous women and subsequent tailored management may improve clinical outcome of pregnancy.

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Summary

After a pregnancy complicated by preeclampsia, women are not only at risk to develop recurrent hypertensive disease in future pregnancies, but also have an increased risk to develop cardiovascular disease later in life. Preeclampsia and cardiovascular disease are thought to have disease mechanisms in common. The prevalence of cardiovascular risk factors is increased in women with a history of preeclampsia. Nowadays many women are tested for these risk factors for cardiovascular disease after a pregnancy complicated by preeclampsia, under the assumption that early recognition and modification is beneficial for future health. Although plausible, the effects of these early interventions are yet to be proven.

In this thesis we studied the hemodynamic profile characterized by low plasma volume in normotensive formerly preeclamptic women. Low plasma volume is thought to reflect latent hypertension and may therefore represent an interesting mechanistic link between preeclampsia and remote cardiovascular disease.

Chapter 1 introduces preeclampsia and the possible role of low plasma volume in the development of recurrent preeclampsia and future cardiovascular disease. It describes the potential of exercise training to improve plasma volume in women with a history of preeclampsia. Finally, the 5 main research questions are introduced that form the basis of this thesis.

In **Chapter 2** we studied the co-occurrence of risk factors for cardiovascular disease in a large population (n=1234) of formerly preeclamptic women. We examined the prevalence of a circulatory risk profile (hypertension or latent hypertension), metabolic syndrome, thrombophilia and hyperhomocysteinemia. We observed that the circulatory risk profile was the most prevalent risk profile (66%) present in formerly preeclamptic women. The high prevalence of this circulatory risk profile was mainly attributable to reduced plasma volume status. Apart from low plasma volume, formerly preeclamptic women often demonstrated insulin resistance. The prevalence of the circulatory risk profile, metabolic syndrome and hyperhomocysteinemia related inversely with the gestational age at delivery. This may indicate a relationship between these cardiovascular risk factors and severity of disease in pregnancy either by cause or effect. Based on the observed prevalence of cardiovascular risk factors after preeclampsia, we believe that cardiovascular follow-up in these women should focus, at least initially, on controlling blood pressure and insulin resistance.

The exact origin of the phenotype low plasma volume is currently unknown. The venous system contains quantitatively the most blood volume and consequently acts as the most important volume reserve. Theoretically low plasma volume status may therefore originate from constitutionally reduced dimension of the venous system. In **Chapter 3**

we examined, in line with the Barker hypothesis, if intra-uterine growth restriction impacts adult plasma volume status. We linked women's own birth weight centile with measured plasma volume in adulthood (n=280). We observed that birth weight correlated positively and independently with adult plasma volume. The contribution of birth centile to the adult plasma volume status is however limited; 14% of the variation in adult plasma volume could be explained with the birth centile.

Women with prepregnant low plasma volume demonstrate reduced plasma volume expansion in pregnancy. This limited plasma volume expansion likely reflects hemodynamic maladaptation to the increased demands of pregnancy that may contribute to the hypertensive deterioration in pregnancy. In **Chapter 4** we further examined the relationship between plasma volume status and recurrent preeclampsia in normotensive formerly preeclamptic women (n=178). The risk of recurrent preeclampsia and fetal growth restriction in subsequent pregnancy relates inversely and linearly to prepregnancy plasma volume in apparently healthy normotensive women. This observation suggests a certain continuum in the pathophysiological development of hypertensive disease during pregnancy depending on plasma volume status. This also implies that improving the plasma volume in advance of the next pregnancy may decrease the recurrence risk of preeclampsia.

In **Chapter 5** we hypothesized that low plasma volume not only predisposes to recurrent preeclampsia in subsequent pregnancy, but also to the development of hypertension in normotensive formerly preeclamptic women. We studied blood pressure and important covariates 2 to 5 years after the plasma volume measurement in n=104 women. We observed that 1 out of 6 (17%) formerly preeclamptic women developed de novo hypertension within 5 years. Women with low plasma volume were more prone to develop hypertension than women with normal plasma volume. Therefore, low plasma volume indeed reflects a latent hypertensive profile that can become manifest within a few years after the preeclamptic pregnancy.

Improving plasma volume may reduce risk of recurrent preeclampsia and chronic hypertension in formerly preeclamptic women. Aerobic exercise is perhaps the best intervention to improve plasma volume. Based on the observation that formerly preeclamptic women demonstrate reduced plasma volume expansion in pregnancy, we hypothesized that formerly preeclamptic women may be less able to increase their plasma volume in response to exercise training compared with parous controls. In **Chapter 6** we examined this hypothesis by studying n=25 normotensive formerly preeclamptic women and n=22 controls before and after a 12 weeks vigorous aerobic exercise training protocol. Exercise training improved plasma volume comparably in both groups. After 12 weeks aerobic training formerly preeclamptic women however

had a plasma volume status that was comparable to pretraining values of controls. Along with the plasma volume expansion, exercise training improved venous compliance and reduced sympathetic overactivity. Using a validated orthostatic stress model we demonstrated increased venous reserve capacity after exercise training.

The effects of exercise training are not expected to be confined to the venous side of the circulation. In **Chapter 7** we studied the effects of 12-weeks aerobic exercise training on important arterial characteristics: carotid intima media thickness (IMT) and brachial and superficial femoral artery endothelial function using flow-mediated dilation (FMD). Twelve weeks exercise training improved the traditional cardiovascular risk profile (e.g. components of the metabolic syndrome) in both groups suggesting reduced biochemical vascular stress. The exercise training even abolished differences in blood pressure and insulin resistance between formerly preeclamptic women and healthy parous controls. The protective effects of exercise training in post partum women were further supported by the reduced IMT and improved FMD after training. Despite these improvements, the 12-weeks exercise training did not normalize these vascular variables in women with a history of preeclampsia. Perhaps sustained aerobic training and/or other types of exercise training may induce continued improvement of vascular functions in formerly preeclamptic women. Interestingly, we observed that exercise induced improvements of endothelial functions correlated with training induced changes in autonomic functioning in both healthy control subjects and formerly preeclamptic women.

The relationship between autonomic activity and endothelial function was further explored in **Chapter 8**. Sympathetic overactivity as observed in formerly preeclamptic women increases vascular resistance that affects blood flow patterns in the upstream conduit arteries. While blood flow patterns characterized by high levels of antegrade shear stress are beneficial for arterial function, retrograde shear stress is believed to be detrimental for vascular health. We studied the relationship between shear rate patterns, endothelial functions and sympathetic balance in formerly preeclamptic women and parous controls before and after 12-weeks exercise training. This study demonstrates strong correlations between retrograde shear and endothelial function in both formerly preeclamptic women and controls. Retrograde shear rate was higher in formerly preeclamptic women compared with controls and related to sympathetic overactivity. These observations provide mechanistic insight in the relationship between autonomic activity and endothelial function. Exercise training represents an effective strategy to reduce retrograde shear, decrease sympathetic overactivity and improve endothelial function.

In the final **Chapter 9** we discussed our findings, reflect on the implications of our studies and suggest opportunities for future research. We conclude that the hemodynamic profile characterized by low plasma volume is highly prevalent in formerly preeclamptic women. Low plasma volume predisposes to recurrent preeclampsia in next pregnancy and reflects latent hypertension in normotensive formerly preeclamptic women. Our exercise studies can be seen as "the proof of principle" that post partum women respond well to the exercise stimulus. Aerobic exercise training improves plasma volume in formerly preeclamptic women and in healthy control subjects. Aerobic exercise may therefore contribute to reducing recurrence risk of preeclampsia and decrease risk to develop chronic hypertension later in life. Our studies support the advice to monitor blood pressure and recommend an active lifestyle in women with a history of preeclampsia; even if women are normotensive 6 to 12 months post partum.

Samenvatting

Vrouwen bij wie de voorgaande zwangerschap werd gecompliceerd door een pre-eclampsie hebben behalve een toegenomen risico op hypertensie in toekomstige zwangerschappen, ook een toegenomen risico op hart- en vaatziekten op latere leeftijd. Men veronderstelt dat pre-eclampsie en hart- en vaatziekten tot op zekere hoogte een vergelijkbare ontstaanswijze hebben. Bij vrouwen met pre-eclampsie in de voorgeschiedenis worden vaker bekende risicofactoren voor hart- en vaatziekten aangetoond. Tegenwoordig worden veel vrouwen dan ook getest op de aanwezigheid van deze risicofactoren voor hart- en vaatziekten na een zwangerschap gecompliceerd door pre-eclampsie. Hierbij wordt aangenomen dat vroegtijdige herkenning en aanpassing van deze risicofactoren voor hart- en vaatziekten gunstig is voor de toekomstige gezondheid van deze vrouwen. Hoewel dit aannemelijk is, zijn de effecten van vroegtijdige screening en behandeling in deze groep vrouwen nog niet bewezen.

In dit proefschrift bestuderen wij het hemodynamische profiel dat wordt gekenmerkt door een gering plasma volume bij normotensieve vrouwen met pre-eclampsie in de voorgeschiedenis. Gering plasma volume wordt gezien als een latente vorm van hypertensie en zou daarmee een interessante mechanistische link kunnen zijn tussen pre-eclampsie en cardiovasculaire aandoeningen op latere leeftijd.

Hoofdstuk 1 vormt de introductie van dit proefschrift. In dit hoofdstuk wordt het ziektebeeld pre-eclampsie geïntroduceerd alsmede de mogelijke rol van een gering plasma volume in het ontstaan van (herhaalde) pre-eclampsie en het ontwikkelen van hart- en vaatziekten in het latere leven. Dit hoofdstuk besluit met de 5 voornaamste onderzoeksvragen waarop dit proefschrift is gebaseerd.

In **Hoofdstuk 2** bestudeerden wij de prevalentie van risicofactoren voor cardiovasculaire aandoeningen in een grote groep vrouwen met pre-eclampsie in de voorgaande zwangerschap (n=1234). Wij onderzochten de prevalentie van een circulatoir risico profiel (hypertensie of latente hypertensie), metabool syndroom, thrombofilie en hyperhomocysteinemie 6 tot 12 maanden na de gecompliceerde zwangerschap. Het circulatoire risico profiel bleek het meest voorkomende risico profiel (66%) te zijn bij vrouwen met pre-eclampsie in de voorgaande zwangerschap. De hoge prevalentie van dit circulatoire profiel was vooral toe te schrijven aan een gering plasma volume in deze groep vrouwen. Naast een gering plasma volume, bleken veel voormalig pre-eclamptische vrouwen insuline resistentie te vertonen. Het circulatoire risicoprofiel, metabool syndroom en hyperhomocysteinemie kwamen vaker voor naarmate de voorgaande zwangerschap vroeger werd gecompliceerd door pre-eclampsie. Dit impliceert een relatie tussen de prevalentie van deze cardiovasculaire risicofactoren en de ernst van pre-eclampsie in de voorgaande zwangerschap. Op basis van de prevalentie van cardiovasculaire risicofactoren na preeclampsie, zijn wij van mening

dat de cardiovasculaire follow-up in deze groep vrouwen zich initieel zou moeten concentreren op het reguleren van de bloeddruk en insuline resistentie.

De etiologie van het fenotype gering plasma volume is grotendeels onbekend. Het veneuze systeem bevat in kwantitatief opzicht het meeste bloedvolume en vormt daarmee de voornaamste volume reserve in het menselijk lichaam. Theoretisch zou een gering plasma volume daarom verklaard kunnen worden door constitutioneel beperkte dimensies van het veneuze systeem. In **Hoofdstuk 3** onderzochten wij, in lijn met de Barker hypothese, of intra-uteriene groei restrictie invloed heeft op de plasma volume status op volwassen leeftijd. Wij vergeleken de geboortepercentielen van een groot aantal vrouwen met pre-eclampsie in de voorgeschiedenis (n=280) met het plasma volume dat werd gemeten op volwassen leeftijd. We toonden aan dat de geboortepercentiel positief en onafhankelijk correleerde met het plasma volume op volwassen leeftijd. De bijdrage van het geboortepercentiel aan het plasma volume op volwassen leeftijd is echter beperkt; 14% van de variatie in plasma volume kon worden verklaard met het geboortepercentiel van deze vrouwen.

Vrouwen met een preconceptioneel gering plasma volume blijken een verminderde plasma volume expansie te hebben in de zwangerschap. Deze beperkte plasma volume expansie in de zwangerschap is waarschijnlijk een uiting van hemodynamische maladaptatie aan de zwangerschap en draagt mogelijk bij aan de hypertensieve ontsporing van de zwangerschap. In **Hoofdstuk 4** onderzoeken wij de relatie tussen preconceptioneel plasma volume en de prevalentie van herhaalde pre-eclampsie in normotensieve vrouwen (n=178) met pre-eclampsie in de eerste zwangerschap. Het risico op herhaalde pre-eclampsie en foetale groeirestrictie in de volgende zwangerschap blijkt omgekeerd evenredig samen te hangen met het preconceptionele plasma volume in ogenschijnlijk gezonde, normotensieve vrouwen. Deze observatie suggereert dat er een continuüm is in de pathofysiologische ontwikkeling van hypertensieve aandoeningen tijdens de zwangerschap welke afhankelijk is van de preconceptionele plasma volume status. Dit impliceert tevens dat plasma volume expansie voorafgaand aan de zwangerschap mogelijk bijdraagt aan de reductie van het risico op herhaalde pre-eclampsie.

In **Hoofdstuk 5** testten wij de hypothese dat gering plasma volume niet alleen predisponeert voor herhaalde pre-eclampsie in de volgende zwangerschap, maar ook voor het ontwikkelen van hypertensie in normotensieve voormalig pre-eclamptische vrouwen. Hiervoor bestudeerden wij de bloeddruk en belangrijke co-variabelen 2 tot 5 jaar na de bepaling van het plasma volume in n=104 vrouwen. Een op de zes vrouwen (17%) ontwikkelde de novo hypertensie binnen 5 jaar. Vrouwen met een gering plasma volume ontwikkelden vaker hypertensie dan vrouwen met een normaal

plasma volume. Gering plasma volume reflecteert daarmee latente hypertensie bij voormalig pre-eclamptische vrouwen.

Het verbeteren van het plasma volume zou kunnen bijdragen aan de reductie van het risico op herhaalde pre-eclampsie en chronische hypertensie bij vrouwen met pre-eclampsie in de voorgeschiedenis. Aerobe inspanning is wellicht de beste interventie om het plasma volume te laten toenemen. Gezien de beperkte toename van het plasma volume tijdens de zwangerschap bij vrouwen die pre-eclampsie ontwikkelen, is het de vraag of vrouwen met een pre-eclampsie in de voorgeschiedenis überhaupt in staat zijn om het plasma volume te vergroten middels aerobe inspanning in vergelijking met gezonde controle vrouwen. In **Hoofdstuk 6** onderzochten wij het plasma volume in n=25 normotensieve voormalig pre-eclamptische vrouwen en n=22 controles voor en na een 12-weken durende intensieve aerobe training. Het plasma volume steeg in beide groepen vergelijkbaar in reactie op de training. Na 12 weken aerobe training hadden voormalig pre-eclamptische vrouwen echter een plasma volume dat vergelijkbaar was met het gemiddelde plasma volume van de controle groep voorafgaand aan de training. Behalve het plasma volume, induceerde de aerobe training ook een verbetering van de veneuze vaatfunctie en nam de sympathische overactiviteit af. Door middel van een gevalideerd orthostatisch stress model konden wij aantonen dat de training daarmee de veneuze reserve capaciteit vergroot.

De effecten van aerobe training zullen zich niet beperken tot de veneuze zijde van de circulatie. In **Hoofdstuk 7** bestudeerden wij de effecten van 12 weken aerobe inspanning op belangrijke arteriële karakteristieken: de intima media dikte (IMT) gemeten in de halsslagader en de endotheelfunctie gemeten in de grote slagaders van arm en been. De endotheelfunctie werd gemeten door middel van flow gemedieerde dilatatie (FMD). Twaalf weken aerobe training verbeterde het traditionele risicoprofiel voor hart- en vaatziekten (oftewel de componenten van het metabole syndroom) in beide groepen; dit impliceert een vermindering van de biochemische stress op de vaatwand. Na 12 weken intensieve training waren de verschillen in bloeddruk en insuline resistentie tussen voormalig pre-eclamptische vrouwen en gezonde controles zelfs verdwenen. De beschermende effecten van aerobe training in postpartum vrouwen werd verder ondersteund door een afname van de vaatwanddikte en verbetering van de endotheelfunctie na training. Echter na 12 weken intensieve training bleken deze vaatkarakteristieken in voormalig pre-eclamptische vrouwen nog niet te zijn genormaliseerd. Wellicht dat aanhoudende aerobe training en/of andere vormen van training voortgaande verbetering bewerkstelligt. De door aerobe training geïnduceerde verbetering van de endotheelfunctie correleerde met de training geïnduceerde veranderingen van de activiteit van het autonome zenuwstelsel in zowel gezonde controles als in voormalig pre-eclamptische vrouwen.

De relatie tussen de activiteit van het autonome zenuwstelsel en endotheelfunctie werd verder onderzocht in **Hoofdstuk 8.** Sympathische overactiviteit zoals werd aangetoond in voormalig pre-eclamptische vrouwen verhoogt de vasculaire weerstand; dit heeft direct weerslag op het doorstromingsprofiel in de stroomopwaarts gelegen arteriën. Bloeddoorstromingspatronen die worden gekarakteriseerd door hoge mate van voorwaartse (antegrade) frictiekrachten op de vaatwand zijn gunstig voor de arteriële vaatfunctie; overwegend achterwaartse (retrograde) frictiekrachten daarentegen worden verondersteld schadelijk te zijn voor de gezondheid van bloedvaten. Wij bestudeerden de relaties tussen arteriële doorstromingsprofielen, endotheelfunctie en de activiteit van het autonome zenuwstelsel in voormalig pre-eclamptische vrouwen en gezonde controles, voor en na een 12 weken intensieve aerobe training. Deze studie toonde aan dat er een sterke correlatie bestaat tussen retrograde frictiekrachten op het endotheel en de endotheelfunctie in zowel voormalig pre-eclamptische vrouwen als in gezonde controles. Deze retrograde frictiekrachten waren toegenomen in voormalig pre-eclamtische vrouwen in vergelijking tot gezonde controles en correleerden eveneens met sympathische overactiviteit. Deze observaties geven inzicht in de relaties tussen de activiteit van het autonome zenuwstelsel en endotheelfunctie. Intensieve aerobe training vormt een effectieve strategie om de retrograde frictiekrachten op de vaatwand te beperken, sympathische overactiviteit te reduceren en endotheel functie te verbeteren.

In het laatste **Hoofdstuk 8** bediscussiëren wij onze bevindingen, reflecteren wij op de implicaties van onze studies en geven wij tot slot suggesties voor toekomstig onderzoek. We concluderen dat het hemodynamische profiel dat wordt gekarakteriseerd door een gering plasma volume frequent voorkomt bij voormalig pre-eclamptische vrouwen. Gering plasma volume predisponeert voor herhaalde pre-eclampsie in de volgende zwangerschap en weerspiegelt latente hypertensie in normotensieve voormalig pre-eclamptische vrouwen. Onze aerobe training studies tonen aan dat postpartum vrouwen in beginsel goed responderen op een intensieve training stimulus. Aerobe training verbetert het plasma volume in zowel voormalig pre-eclamptische vrouwen als gezonde controles. Deze training geïnduceerde plasma volume expansie draagt mogelijk bij aan een reductie van de herhaalkans op pre-eclampsie en een afname van chronische hypertensie bij vrouwen met pre-eclampsie in de voorgeschiedenis. Onze studies ondersteunen het advies om de bloeddruk te blijven controleren en een actieve leefstijl te promoten bij vrouwen die een pre-eclampsie hebben doorgemaakt, zelfs wanneer vrouwen initieel normotensief zijn na de gecompliceerde zwangerschap.

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Dear Mr. Cooper, they say no man is an island; you are an island... you are bloody Ibiza!

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Curriculum Vitae

Ralph Scholten werd op 14 februari 1979 geboren te Doetinchem. Hij behaalde zijn eindexamen VWO in 1997 aan het Isala College te Silvolde. Vervolgens studeerde hij tussen 1997 en 2004 Geneeskunde aan de Radboud Universiteit in Nijmegen. Naast zijn opleiding verrichtte hij extra-curriculair onderzoek op de afdeling Klinische Neurofysiologie (Prof. M.J. Zwarts) van het Radboudumc. Voorafgaand aan zijn co-schappen heeft hij zijn studie een jaar onderbroken voor een reis door Azië en Afrika. Hij behaalde zijn arts-examen nadien Cum Laude in 2004. Na ruim 2 jaar werkzaam te zijn geweest als assistent niet in opleiding in het Rijnstate Ziekenhuis te Arnhem werd hij in 2007 aangenomen voor de opleiding tot gynaecoloog. Hij volbracht zijn perifere deel van zijn opleiding in Ziekenhuis de Gelderse Vallei te Ede (opleider: Dr. E. Scheenjes) en het academische deel in het Radboudumc te Nijmegen (opleiders: Prof. dr. D.D.M. Braat en Dr. R.L.M. Bekkers). De differentiatie Perinatologie werd voltooid zowel academisch (Radboudumc) als perifeer in het het Rijnstate Ziekenhuis te Arnhem (opleider: Dr. F.H.P.L Dijkhuizen). Bij de start van zijn opleiding ontving hij een AGIKO stipendium dat hem in staat stelde zijn opleiding te combineren met klinisch wetenschappelijk onderzoek. In 2008 startte hij met promotie-onderzoek op het gebied van de hemodynamiek bij vrouwen met een pre-eclampsie in de voorgeschiedenis, waarvan dit proefschrift het resultaat is. Hij verrichtte zijn promotie onderzoek op de afdelingen Fysiologie en Verloskunde van het Radboudumc. Op de afdeling Fysiologie was hij daarnaast betrokken bij een aantal basale onderzoeken naar de vaatfunctie onder begeleiding van Prof. dr. M.T. Hopman en Prof. dr. D.H.J. Thijssen. In 2015 voltooide hij zijn opleiding tot gynaecoloog. Momenteel is hij werkzaam als gynaecoloog in het Rijnstate ziekenhuis te Arnhem. Binnenkort vertrekt hij naar Canada om zijn opleiding te vervolgen tot subspecialist in Maternal-Fetal Medicine aan de University of Toronto.