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Low plasma volume in the pathophysiology of preeclampsia

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Low plasma volume in the pathophysiology of preeclampsia

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Chapter 1

General introduction

General Introduction

Hypertensive complications of pregnancy, such as preeclampsia and HELLP syndrome (Hemolysis, Elevated Liver-enzymes, Low Platelets) are associated with significant perinatal-[1] and maternal [2 3] morbidity and mortality. Hypertensive complications occur in 6-8% of pregnancies [4]. Although the pathophysiology of preeclampsia is incompletely understood, it is clear that endothelial dysfunction [5] is a common endpoint in maternal organ dysfunction and placental insufficiency. In the last decade underlying disorders such as thrombophilia [6 7], hyperhomocysteinemia [8], and angiotensinogen polymorphisms [9] have been identified as risk factors for preeclampsia. More recently a strong association between metabolic syndrome (obesity and insuline resistance) and hypertensive disorders in pregnancy have been observed [10].

Formerly preeclamptic women constitute a heterogeneous population of different subgroups, with one of these subgroups having low plasma volume as the common denominator [11]. The women in this subgroup tend to respond with circulatory maladaptation to a new pregnancy [12 13], a response which is not surprising when the normal hemodynamic adaptation to pregnancy is taken into account. The latter consists of a decrease in vascular resistance and an increase in plasma volume, heart rate, stroke volume and cardiac output [14]. Already by 5-6 weeks amenorrhea, the maternal hemodynamic function has changed significantly [15 16.] A rise in plasma volume is one of these early changes, which is characterized by a steady increase throughout pregnancy to reach a maximum of about 30-40% above nonpregnant values by 34 weeks [17] . Although the exact cause of these hemodynamic changes remains unclear, they appear to be triggered by a fall in vascular tone. [14 15]. Total peripheral vascular resistance is lowest at the end of the first trimester to gradually return to non-pregnant values in late pregnancy [18]. The fall in peripheral vascular resistance triggers a rise in cardiac output by way of increases in both stroke volume and heart rate, which is maximal by 25 weeks pregnancy [18 15]. The hemodynamic adaptation to pregnancy also includes a rise in vascular compliance and resetting of various cardiovascular regulatory setpoints. The 5-10 mmHg decline in arterial pressure [15] appears to be secondary to the latter adjustments.

In pregnancies complicated by fetal growth restriction (IUGR), the rise in cardiac output in early pregnancy is often subnormal [12]. In these complicated pregnancies also plasma volume expansion is often subnormal [13] and/or paralleled by an abnormal early rise in α-atrial natriuretic peptide [30]. Postpartum, low

plasma volume tends to persist in a large fraction of women with a history of preeclampsia [11 19]. These observations support the view that low plasma volume is involved in inadequate hemodynamic and volume adaptation to pregnancy.

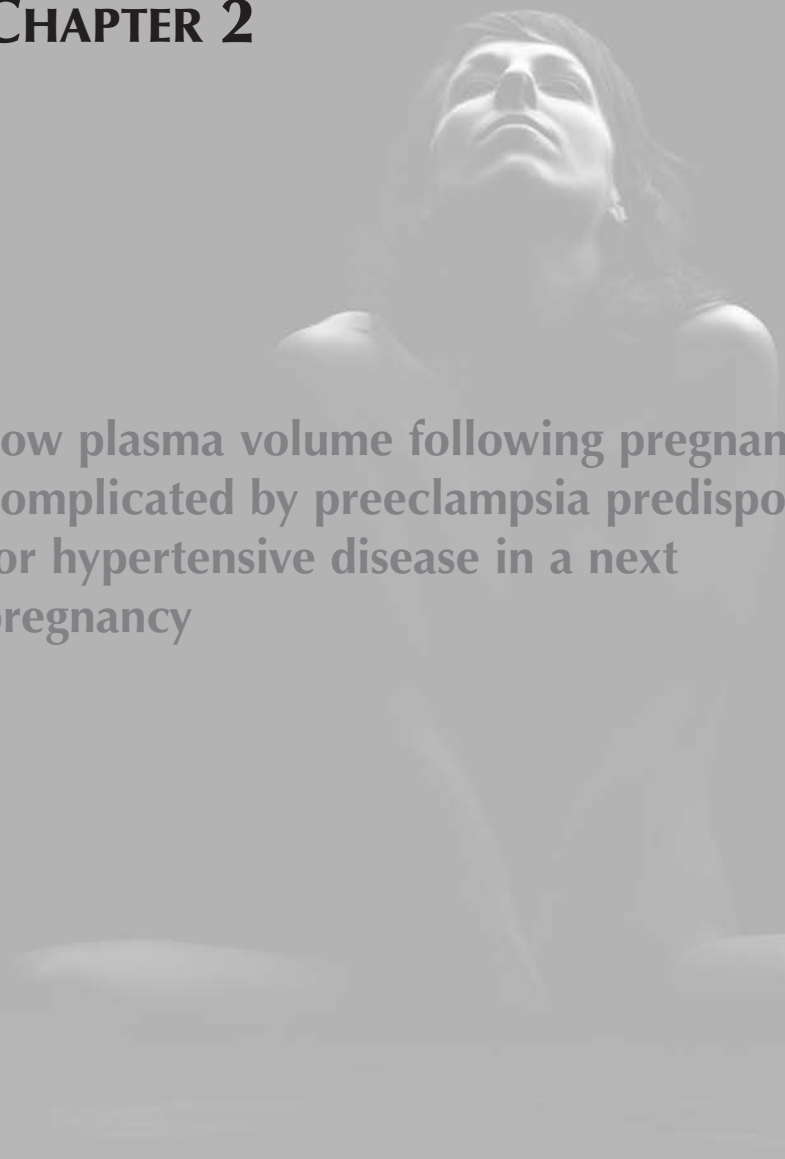
We hypothesize that a low plasma volume in formerly preeclamptic women predisposes them to an inadequate hemodynamic response to pregnancy leading to an increased risk for a recurrent hypertensive complication. When the primary hemodynamic response to pregnancy is inadequate, a back-up response consisting of a rise in cardiovascular sympathetic drive is activated to meet the higher circulatory demands of pregnancy. However, the purpose of this back-up response is only to adjust a **short-term** imbalance between cardiac pre- and afterload, so as to allow their long-term rebalancing through volume retention. If the afterload reduction induced by pregnancy can only be compensated by rising cardiovascular sympathetic drive, sustaining the circulatory function will become increasingly problematic with advancing pregnancy. At some point the latter will become inadequate, triggering the development of a hypersympathetic state and with it, a fall in heart rate and cardiac output, and a rise in total peripheral vascular resistance, mainly due to centralisation of the circulation at the cost of kidneys, intra-abdominal organs, skeletal muscle, skin and - last but not least - the uteroplacental bed. Obviously, this is accompanied by the development of the clinical signs of preeclampsia, and/or the HELLP syndrome, usually in concert with uteroplacental insufficiency [20].

This thesis consists of five studies focusing on various aspects of plasma volume in relation to preeclamptic pregnancy, divided over the following five consecutive topics:

1. The incidence of low plasma volume in women with a history of a preeclampsia;
2. The impact of low plasma volume on venous capacitance in formerly preeclamptic women as compared to parous controls;
3. The impact of low plasma volume on the response to exercise in formerly preeclamptic women as compared to parous controls;
4. The importance of low plasma volume on a next pregnancy in formerly preeclamptic women with a low plasma volume;
5. The relationship between low plasma volume and endothelium-dependent vasodilatation in formerly preeclamptic women.

In the general discussion of this thesis, the functional consequences of a low plasma volume for the adaptation to pregnancy and possible implications for clinical practice are discussed using the results of these five studies as starting point.

CHAPTER 2



Low plasma volume following pregnancy complicated by preeclampsia predisposes for hypertensive disease in a next pregnancy

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Abstract

Objective: A large number of women with a history of preeclampsia/HELLP have a low plasma volume at least six months postpartum. The objective of this study was to determine whether a low plasma volume in formerly pre-eclamptic women and HELLP patients is associated with an increased risk for recurrent hypertensive complications in a next pregnancy.

Design: Prospective observational study.

Setting: Tertiary obstetric centre.

Sample: Formerly pre-eclamptic women and controls.

Methods: In 316 women with a history of preeclampsia and/or HELLP, we measured, plasma volume along with haemodynamic, metabolic and haemostatic variables at least six months postpartum. A group of 22 healthy parous controls was used as a reference. After standardising plasma volume for body mass index, women were subdivided into normotensive and normal plasma volume (n = 199), normotensive and low plasma volume (n = 76) and hypertensive (n = 41) subgroups, which were compared for demography, clinical parameters and course of a next pregnancy.

Main outcome measures: Recurrent hypertensive disease of pregnancy.

Results: Relative to the normal plasma volume subgroup, normotensive women in the low plasma volume subgroup have a higher body mass index, a lower total vascular compliance and a shorter estimated systemic circulation time. They have a higher HOMA index and higher fasting triglyceride levels. In normotensive and hypertensive former patients alike, low plasma volume is associated with a higher recurrence of hypertensive complications in a next pregnancy compared with normotensive women with normal plasma volume.

Conclusion: Low plasma volume in normotensive women with a history of preeclampsia and/or HELLP is associated with overweight, reduced vascular compliance and insulin resistance and a predisposition for recurrent preeclampsia and HELLP syndrome in a next pregnancy.

Introduction

Preeclampsia and HELLP syndrome complicate up to 5% of all pregnancies [21]. Although there is evidence suggesting that maternal symptoms in these disorders develop secondary to endothelial dysfunction [5], the mechanism that triggers the latter is not fully understood. In recent years, more emphasis has been put upon identifying predisposing risk factors [22 23 11].

Low maternal plasma volume is a risk factor for low birthweight [24]. We have reported a subnormal plasma volume in almost half of the women with a history of preeclampsia or HELLP syndrome [11 25]. We also noticed that women with a subnormal plasma volume responded to subsequent pregnancy with defective plasma volume expansion [25] and lack of rise in vascular compliance [26] suggesting abnormal haemodynamic adaptation. However, it is unknown, whether pre-pregnant low plasma volume is a risk factor for hypertensive complications in the next pregnancy.

The objectives of this study were: 1. To determine to what extent plasma volume in women with a history of preeclampsia and HELLP syndrome is reduced compared with healthy parous women of comparable age and with a history of uneventful pregnancies only; 2. To find out whether women with a reduced plasma volume differ from women with a normal plasma volume by phenotype; and 3. To determine, whether a reduced plasma volume is a risk factor for recurrence of a hypertensive complication in a next pregnancy.

At least six months postpartum, we determined plasma volume along with haemodynamic, renal, metabolic and haemostatic variables in 316 formerly pre-eclamptic women and 22 healthy parous women. In the 95 patients and 12 controls, that conceived and delivered during the study period, we determined the value of a low pre-pregnant plasma volume to predict hypertensive complications in a next pregnancy.

Methods

We collected the data for this study from 316 women with an early onset (before 34 weeks) preeclampsia and/or HELLP syndrome, and 22 healthy parous women. We recruited a control group to determine reference values and cutoff levels for normality in women of the same age, but with a history of uneventful pregnancies only. After having accumulated sufficient data points, we discontinued recruitment of controls.

We used the definition of preeclampsia and HELLP as detailed elsewhere [4]. We only included women for whom we could verify in their hospital records that they met the diagnostic criteria for preeclampsia and HELLP in their preceding pregnancy. All former pre-eclamptics included in this study had an elevated diastolic blood pressure along with **de novo** proteinuria (>0.3 g/24 hours).

In our reference group, plasma volume corrected for body weight and height was 267 (19) mL kg^{-1} m^{-1}. Plasma volume in both former pre-eclamptics (n = 113) and former HELLP patients (n = 207) was about 10% lower [241 (37) and 243 (28) mL kg^{-1} m^{-1}, respectively]. Because of this resemblance and their—at least in part—common pathogenesis, we decided to pool the data from former pre-eclamptics and HELLP patients in the statistical analysis of plasma volume.

Measurements were performed at least six months postpartum. The choice for this interval was based upon a study that provided evidence for reaching cardiovascular steady state approximately three to four months postpartum [27], together with a study that provided evidence for lack of appreciable change in plasma volume over two consecutive ovulatory cycles in non-pregnant women [19].

None of the participants had used oral contraceptives or vitamin supplements. Breastfeeding, if applicable, had stopped at least two months before the study. Women on antihypertensive medication discontinued their medication at least four weeks before the study. Participants refrained from eating and drinking (except water), from 22:00 h the evening before the study until after blood sampling at 9:00 h. Participants used a standard low methionin breakfast during the study session as well as lunch without coffee or tea. The hospital's medical ethical committee approved the study protocol.

We determined the following variables in peripheral blood using methodology detailed previously [11]: anticardiolipin antibodies, lupus anticoagulant, the clotting factors protein S (total and free), protein C and antithrombin. In addition, we determined the presence of Factor V Leiden and prothrombin mutation G20210A. Thrombophilia was defined in line with others [28,] as the condition characterised by either the presence of one of the latter two mutations, or by circulating levels of one of the clotting factors/functions mentioned above, deviating by more than two standard deviations from the values observed in a reference population.

Hyperhomocysteinaemia was diagnosed on the basis of the abnormal outcome of a methionin loading test. Besides these more specialised measurements, we also determined by standard hospital laboratory procedures in serum or plasma, fasting glucose, HbA1c, insulin, creatinine, cholesterol, low density lipoprotein, high density lipoprotein and triglycerides. The ratio of fasting glucose and insulin (HOMA index) was used as an estimate for insulin resistance as detailed elsewhere [29].

In a 24-hour urine collection, we measured the concentrations of sodium, albumin and creatinine. We defined (micro) albuminuria as >2.5 g albumin/mol creatinine and the creatinine clearance as the ratio of 24 hours creatinine excretion and plasma creatinine level. Blood sampling was followed by 30 minutes blood pressure monitoring in standardised conditions using a semi-automatic oscillometric device (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, Florida, USA) as described previously [11]. An estimate for plasma volume was obtained using the Iodine ^{125}-albumin (^{125}I-HSA) indicator dilution method [11]. Cardiac function was assessed by echocardiography in semi-left lateral position, using a cross sectional phased array echocardiographic Doppler system (Agilent Sonos 5500, Philips Medical System, Eindhoven, The Netherlands). Cardiac output (L minute $^{-1}$) was calculated by multiplying stroke volume (mL) and heart rate (bpm) as specified previously [11 30]. Total vascular compliance (mL mmHg $^{-1}$) was calculated by taking the ratio of stroke volume and pulse pressure. The estimated circulation time (seconds) for the pulmonary and systemic circulation combined was obtained by dividing (blood volume ? 60) by (2 x cardiac output). A short estimated circulation time reflects a more hyperdynamic circulation.

Data are presented as means (SD) unless stated otherwise. Plasma volume varies as a function of body mass index, which may differ for different populations [31]. In our study population, these variables were responsible for 37% of the plasma volume variation. Therefore, we standardised plasma volume for weight and height by calculating their weighed contribution using multiple linear regression analysis on the log–log transformed variables. The 'standardised plasma volume' (PVst), was obtained by taking the ratio of plasma volume and the product of weight 0.460 and height 0.605. Similarly, we also standardised plasma volume for height only by taking the ratio of plasma volume and height 0.450.

The PV_{st} in parous controls was 266.7 (19.1) mL kg $^{-1}$ m $^{-1}$. We subdivided the formerly pre-eclamptic women into a low plasma volume subgroup (PV_{st}< 228.4 mL kg $^{-1}$ m $^{-1}$, corresponding with the mean PV_{st} in parous controls minus 2 SD; n = 76), a normal plasma volume subgroup (PV_{st}> 228.4 mL kg $^{-1}$ m $^{-1}$; n = 199) and a subgroup of hypertensive women irrespective of plasma volume (n = 41). We compared relevant clinical variables between these three subgroups and the control group by the Mann–Whitney U test (continuous data) or the χ^2 test (dichotomous data). A P value below 0.05, after Bonferroni correction, was considered statistically significant.

To test the relation between plasma volume and haemodynamic variables and plasma volume and obstetric outcome, a linear regression analysis was performed.

To test the hypothesis that plasma volume is related to the recurrence of preeclampsia, a stepwise binary logistic regression analysis of body mass index, essential hypertension, nicotine use, cholesterol, thrombophilia, hyperhomocysteinaemia, antiphospholipid disorder and plasma volume was performed.

Results

Table 1. *Demographic and obstetric variables of the first pregnancy. Values are expressed as mean (SD) or median [range] unless otherwise noted.*

	Controls (*n* = 22)	Normal plasma volume (*n* = 199)	Low plasma volume (*n* = 76)	Hypertensive (*n* = 41)
Age (year)	32.4 (3.1)	31.2 (4.0)	30.2 (5.2)	31.2 (3.7)
Primiparity (%)	86.4	81.4	88.2	86.7
Body mass index (kg m $^{-2}$)	21.8 (2.8)	24.9 (4.6)*	26.6 (5.2)*,†	27.7 (5.6)*
Family history of cardiovascular disease (%)	27.3	39.7	59.2*,†	53.3
Smoking (%)	13.6	17.6	28.9 ,†	11.1
Gestational age at birth (weeks)	$39^{5/7}$ ($1^{5/7}$)	$31^{5/7}$ ($3^{4/7}$)*	$30^{5/7}$ ($3^{5/7}$)*,†	$31^{2/7}$ ($4^{0/7}$)*
Preterm birth (<32 weeks) (%)	0	56.3*	63.2*,†	71.1*
IUGR <5th centile (%)	0	15.1	19.7*	26.7*
Birthweight (g)	3261 (549)	1391 (656)*	1328 (725)*	1141 (583)*
Birthweight centile (%)	40 [10–98]	[1–90]*	13 [1–95]*	9 [1–80]*
Interval pregnancy to day of study (years)	1.6 [0.5–6.3]	0.9 [0.5–8.9]	0.8 [0.5–7.3]*	1.4 [0.5–4.2]
Interval day of study and next pregnancy (years)	1.3 [0.9–3.3]	1.5 [0.8–4.9]	1.9 [0.9–3.9]	1.4 [0.8–2.3]

** Significant difference (P< 0.05) between patient subgroups and the reference group (after Bonferroni correction).*

† Significant difference (P< 0.05) between the low plasma volume and normal plasma volume subgroups.

Table 1 lists demographic variables in the subgroups. The mean body mass index in controls is 21.8, which is lower than in all patient subgroups. The low plasma volume subgroup had a higher prevalence of cardiovascular disease in their family, a higher body mass index and, on the average, a one week shorter duration of the preceding complicated pregnancy, a higher percentage of preterm delivery (before 32 weeks), a higher percentage of smokers and a shorter

interval between pregnancy and participation at this study than the normal plasma volume subgroup. Other parameters did not differ appreciably between these subgroups.

Table 2. *Central haemodynamic and renal variables. Values are expressed as mean (SD).*

	Controls (*n* = 22)	Normal plasma volume (*n* = 199)	Low plasma volume (*n* = 76)	Hypertensive (*n* = 41)
Plasma volume (mL)	2423 (207)	2454 (261)	2015 (336)*,†	2422 (391)
Plasma volume corrected for weight and height	267 (19)	256 (18)*	207 (24)*,†	239 (36)*
Plasma volume corrected for height only	1917 (146)	1944 (192)	1604 (258)*,†	1913 (302)
Heart rate (bpm)	67 (11)	69 (9)	71 (11)	73 (13)
Mean arterial pressure (mmHg)	88 (12)	91 (11)	90 (12)	104 (12)*
Stroke volume (mL)	74 (11)	79 (13)	74 (10) †	80 (16)
Cardiac index (L minute $^{-1}$ m $^{-2}$)	2.8 (0.3)	3.1 (0.6)	2.9 (0.5)†	3.0 (0.6)
Total vascular compliance (mL mmHg $^{-1}$)	1.7 (0.3)	1.8 (0.4)	1.6 (0.4)†	1.6 (0.5)
Estimated systemic circulation time (seconds)	25 (3)	23 (4)	20 (4)*,†	22 (5)
(Micro) albuminuria (%)	0	12.6	18.4	15.6
Creatinin clearance (mL minute $^{-1}$)	96 (29)	124 (81)*	112 (40)*	130 (40)*

** Significant difference (P< 0.05) between patient subgroups and the reference group (after Bonferroni correction).*

† Significant difference (P< 0.05) between the low plasma volume and normal plasma volume subgroups.

Central haemodynamic and renal variables in the two patient subgroups are listed in table 2. The low plasma volume subgroup differed from the normal plasma volume subgroup by lower values for stroke volume, cardiac index, total vascular compliance and a shorter estimated systemic circulation time. MAP, heart rate and creatinine clearance were comparable between the low and normal plasma volume subgroups.

Table 3. *Linear regression analysis of plasma volume and haemodynamic parameters.*

	P	R^2
Cardiac index	0.003	0.03
Stroke volume	0.003	0.03
Total vascular compliance	0.02	0.02
Estimated circulation time	<0.0001	0.16

Table 3 lists the correlation of each of these haemodynamic variables that differed between the low and normal plasma volume group with the plasma volume corrected for body weight and height, as determined by linear regression analysis.

Table 4. *Metabolic and thrombophilic variables. Values are expressed as mean (SD) unless otherwise noted.*

	Controls (*n* = 22)	Normal plasma volume (*n* = 199)	Low plasma volume (*n* = 76)	Hypertensive (*n* = 41)
Insulin (mU L^{-1})	5.8 (1.7)	11.3 (5.6)*	13.7 (6.5)*,†	14.1 (6.9)*
Insulin resistance (HOMA)	1.2 (0.5)	2.8 (1.9)*	3.2 (1.7)*,†	3.4 (1.9)*
Total cholesterol (mmol L^{-1})	4.4 (0.9)	5.0 (1.0)	5.3 (1.3)*	5.2 (0.8)*
Triglyceride (mmol L^{-1})	0.8 (0.2)	1.0 (0.5)	1.3 (0.9)*,†	1.3 (0.8)
Hyperhomocysteinaemia (%)	0	19.1*	25.0*	24.4*
Antiphospholipid syndrome(%)	0	16.6	9.2	17.8
Thrombophilia (%)	13.6	12.1	9.2	11.1
Factor V Leiden mutation (%)	13.6	5.1	3.9	8.9
Factor II mutation (%)	0	3.0	3.9	0
Protein S deficiency (%)	4.5	2.0	0	0
Protein C deficiency (%)	0	3.5	0	2.2
Antithrombin deficiency (%)	0	1.0	1.3	3.0

** Significant difference (P< 0.05) between patient subgroups and the reference group (after Bonferroni correction).*

† Significant difference (P< 0.05) between the low plasma volume and normal plasma volume subgroups.

Table 4 summarises the metabolic and haemostatic data from all participants. The low plasma volume subgroup differed from the normal plasma volume subgroup by a higher HOMA index and higher fasting levels of insulin

and triglycerides. The incidence of thrombophilia, hyperhomocysteinaemia and the antiphospholipid syndrome did not differ between patient subgroups.

Table 5. *Obstetric outcome variables in a subsequent pregnancy. Data are expressed as mean (SD) or median [range] unless otherwise noted.*

	Controls (n = 12)	Normal plasma volume (n = 60)	Low plasma volume (n = 23)	Hypertensive (n = 12)
Gestational age at birth (weeks)	396/7 (14/7)	382/7 (24/7)*	376/7 (16/7)*	381/7 (30/7)
Birthweight (g)	3533 (590)	3180 (688)	2849 (598)*,†	3000 (809)
Birthweight centile (%)	44 [10–99]	50 [2–98]	30 [1–90]	35 [2–98]
Incidence of gestational hypertension (%)	0	50.0*	56.5*	–
Incidence of preeclampsia (%)	0	13.3	34.8*,†	25.0
Incidence of HELLP syndrome (%)	0	1.7	17.4†	8.3
IUGR <5th centile (%)	0	5.0	13.0	8.3
Delivery <32 weeks (%)	0	3.3	0	8.3

** Significant difference (P< 0.05) between patient subgroups and the reference group (after Bonferroni correction).*

† Significant difference (P< 0.05) between the low plasma volume and normal plasma volume subgroups.

Table 5 presents the data on outcome of the next pregnancy. At the time of the data analysis for this study, 95 former pre-eclamptic women had completed a next pregnancy. The low plasma volume subgroup differed from the normal plasma volume subgroup by a lower birthweight and a clearly higher recurrence rate of preeclampsia and HELLP syndrome.

Table 6. *Linear regression analysis with pregnancy outcome as dependent variable and plasma volume as independent variable.*

	P	R2
Gestational age at birth	0.118	0.02
Birthweight	0.031	0.04
Birthweight centile	0.065	0.03
Incidence of gestational hypertension	0.037	0.06
Incidence of preeclampsia	0.007	0.13
Incidence of HELLP syndrome	0.019	0.16
IUGR <5th centile	0.310	0.02
Delivery <32 weeks	0.249	0.05

Table 6 shows the results of a linear regression analysis to assess the correlation between plasma volume and various relevant clinical outcome variables of the next pregnancy, in 95 formerly pre-eclamptic women and/or HELLP patients. The recurrence of gestational hypertension, preeclampsia and HELLP syndrome are inversely correlated to plasma volume, and birthweight is positively correlated to plasma volume. However, plasma volume variation explains only a small fraction of the variation in these outcome variables, as suggested by the relatively low values of R^2. Plasma volume was the only factor that significantly (P = 0.048) contributed to the recurrence of preeclampsia in a stepwise binary logistic regression analysis. The other variables that were entered (body mass index, essential hypertension, smoking, cholesterol, hyperhomocysteinaemia, thrombophilia, antiphospholipid disorder) did not reach statistical significance.

Discussion

In this study, we explored the clinical value of plasma volume measured in formerly pre-eclamptic women to predict recurrent disease in a next pregnancy. We incorporated plasma volume in this program for the following reasons. Firstly, subnormal plasma volume is one of the abnormalities seen in the early phase of essential hypertension, possibly reflecting an increased responsiveness to vasoconstrictive stimuli [32]. Secondly, there is evidence for a link between hypertensive complications in pregnancy and a subnormal size of the plasma volume compartment [21 19 33 34]. Thirdly, in a previous study, we observed defective haemodynamic adaptation to pregnancy in women identified with subnormal plasma volume before pregnancy [25]. To secure reliable and reproducible estimates, we chose to measure plasma volume using the gold standard technique, which is by I^{125}-labelled albumin dilution [11].

The present study in formerly pre-eclamptic women and HELLP syndrome patients provides evidence for an association between plasma volume on the one hand and cardiac index, stroke volume, vascular compliance and estimated systemic circulation time on the other hand (table 3). Therefore, these data provide indirect evidence for an association between subnormal plasma volume and both a reduced venous return and a hyperdynamic circulation suggesting a lower cardiovascular efficiency. That is to say, the subnormal plasma volume is likely to reflect a lower venous capacity, which necessitates more sympathetic activity in the venous compartment to preserve venous return [35], obviously, at the cost of cardiovascular reserves and thus, at the cost of the ability to accommodate the extra circulatory demands of pregnancy.

The concept that subnormal plasma volume covaries with the dynamic state of the circulation offers some interesting inferences. We did not only observe a higher insulin resistance in former patients, but also an even higher insulin resistance in the low plasma volume subgroup. The concomitantly higher incidence of women with overweight and higher peripheral preprandial levels of triglycerides in the latter subgroup provides additional support for the concept that insulin resistance and overweight are associated conditions. Hyperinsulinaemia is a well-known cardiovascular stress condition [36 37] and therefore, may amplify, in concert with other oxidative stress factors [38] (e.g. low density lipoprotein cholesterol, homocystein, lipid peroxides, nicotine use) the cardiovascular stress, particularly in the low plasma volume subgroup [25].

The association between smoking and cardiovascular disease is well accepted. In this study, we noted a higher percentage of smokers among former patients with a low plasma volume compared with former patients with a normal plasma volume. A recent study provides experimental evidence for a central role of oxidative stress in smoking-mediated dysfunction of NO biosynthesis in endothelial cells [39]. Therefore, it is conceivable that smoking contributed to the reduced plasma volume in these patients.

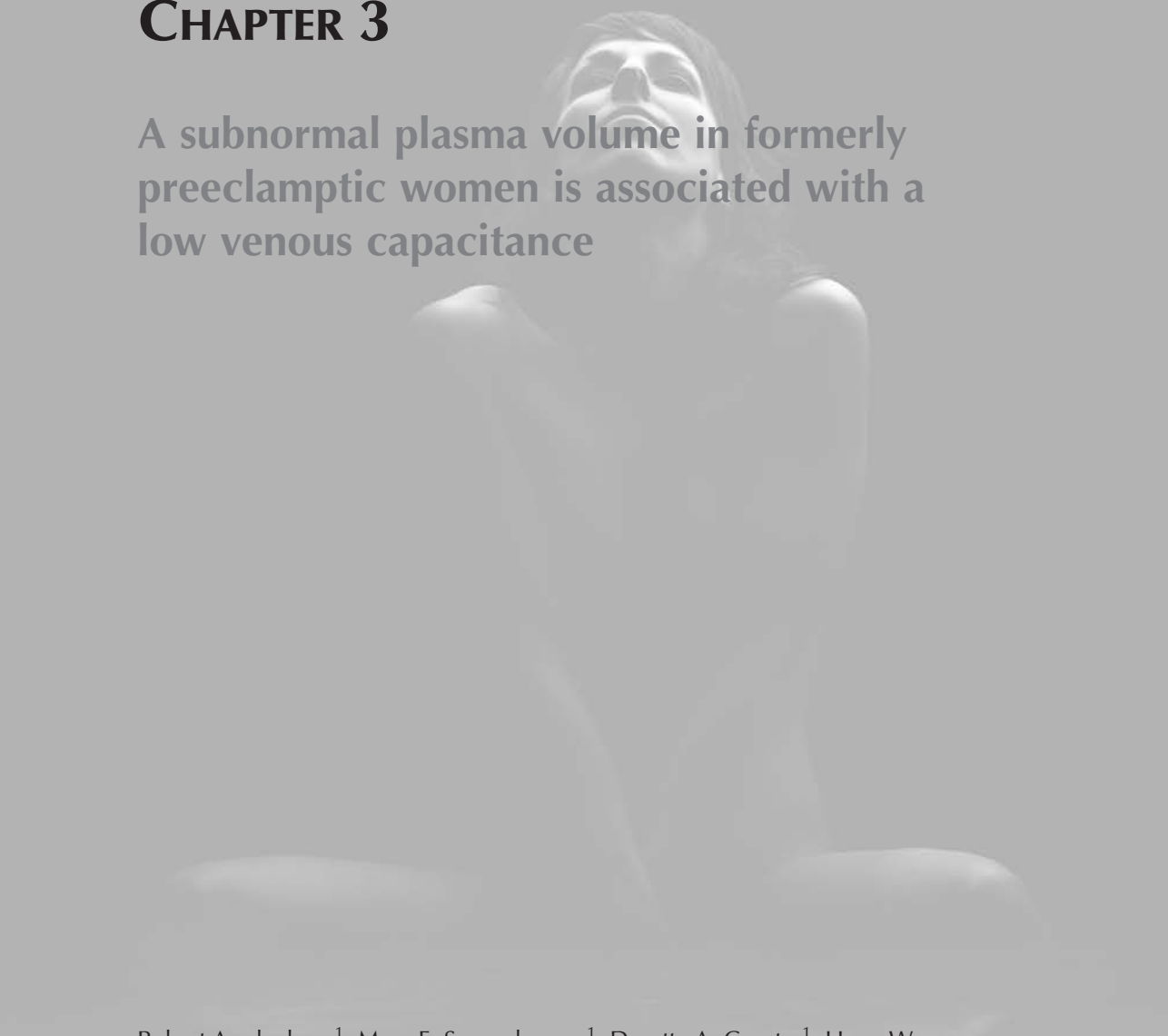
In this study, we found in the low plasma volume subgroup relative to the normal plasma volume subgroup, an approx. 3 times and approx. 10 times higher recurrence rate of preeclampsia and the HELLP syndrome, respectively. We performed a stepwise binary logistic regression analysis to determine the independent contribution of body weight, body height, nicotine use, circulating cholesterol level, and the presence of essential hypertension, thrombophilia, hyperhomocysteinaemia, antiphospholipid disorder along with plasma volume to the recurrence rate of preeclampsia and HELLP. From these parameters, only plasma volume contributed significantly ($P = 0.048$) to the recurrence of preeclampsia and HELLP. The sensitivity and specificity of low plasma volume to predict recurrent preeclampsia or HELLP syndrome were 50% and 77%, respectively. The positive and negative predictive values of a low plasma volume were 37% and 85%, respectively. To the best of our knowledge, the predictive value of (pre-pregnant) plasma volume has never been evaluated in a prospective study. Compared with the predictive value of a wide variety of other markers [40 41], these figures are clearly better. This may be partly related to the high prevalence of recurrent disease (±27%) in our patient group. However, it is also inherent to plasma volume, which is likely to become compromised in response to a wide range of different cardiovascular stressors.

The heterogeneity of other variables precludes that it is unlikely that one single marker can be identified that has a really high predictive value with respect to recurrent disease in all patients.

In none of our patients, we noticed adverse effects of the plasma volume measurement. Nevertheless, the introduction of plasma volume measurements for screening purposes requires balancing the yield of having a superior test against the need to perform an intravenous injection with a radioactive tracer. The use of a non-radioactive indicator such as dextran [70] may provide a more acceptable alternative, which can also be used in pregnancy [7].
In summary, in this study we found that subnormal plasma volume persisting for at least six months postpartum after a pre-eclamptic pregnancy identifies women at risk for recurrent disease in their next pregnancy. Subnormal plasma volume appears to be an indirect sign of cardiovascular stress and with it, of reduced cardiovascular reserves.

CHAPTER 3

A subnormal plasma volume in formerly preeclamptic women is associated with a low venous capacitance



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Abstract

Introduction: Pregnancy induces a smaller rise in plasma volume in formerly preeclamptic women with a pre-existent subnormal plasma volume than in their counterparts with a normal plasma volume. These women also have a 3 times higher recurrence rate of pregnancy-induced hypertensive disorders. In this study we tested the hypothesis that a subnormal plasma volume in these women is related to a lower capacitance of their venous compartment.

Patients and Methods:In 31 non-pregnant formerly preeclamptic women with a subnormal plasma volume and 8 parous controls, we infused intravenously 500 mL of a modified gelatin solution in 30 min. Before and after infusion we measured the circulating levels of α-Atrial Natriuretic Peptide (α-ANP) and Active Plasma Renin Concentration (APRC). During volume loading, we recorded the change in heart rate, stroke volume, and cardiac output using pulse contour analysis. We measured the ratio of % change in blood volume and % change in cardiac output during volume loading as a marker for venous capacitance.

Results: During volume loading patients differed from controls by a larger rise in α-ANP, pulse rate and cardiac output and a lower estimated venous capacitance. The concomitant response of stroke volume and APRC did not differ appreciably between groups.

Conclusion: Formerly preeclamptic women with a subnormal plasma volume differ from controls with a normal plasma volume by a reduced venous capacitance. These results support our hypothesis that in these women, a subnormal plasma volume indicates the presence of a subnormal venous capacitance.

Introduction

About two-third of non-pregnant normotensive formerly-preeclamptic women have a subnormal plasma volume [11], which is accompanied by both a reduced venous compliance [26] and a higher sympathetic activity in the autonomic control of the circulation [42 43]. In their next pregnancy, they differ from their counterparts with a normal plasma volume by a three times higher recurrence rate of hypertensive disorders [44], an observation, indirectly supported by data from several other groups [33 34 45 46]. Previously, we reported that in a next pregnancy, these women also differ from their counterparts with a normal plasma volume by a lack of plasma volume expansion and a rise instead of no change in α-atrial natriuretic peptide in the first 8 weeks [25].

Under physiological conditions, approximately 70% of the blood volume is located in the venous compartment, where it serves as a buffer to raise venous return in response to higher demands for systemic blood flow [47]. The latter is mainly achieved by venoconstriction, which raises cardiac preload by reducing venous capacitance [48]. The latter is defined as the relationship between contained volume and distending pressure in the venous compartment [49 50] in contrast to venous compliance that is the ratio of a **change** in volume to the concomitant **change** in transmural distending pressure in the venous compartment [50]. Compliance and capacitance are clearly interrelated but capacitance describes the capacity to contain volume whereas compliance describes the elastic properties of the vascular wall. Although normotensive formerly preeclamptic women with a subnormal plasma volume have a reduced venous compliance [25], it is unclear whether the subnormal plasma volume is also accompanied by a lower venous capacitance.

The present study was designed to test the hypothesis that normotensive formerly-preeclamptic women with a subnormal plasma volume have a lower venous capacitance than controls. To test this hypothesis we administered a volume load to a group of formerly-preeclamptic women with a subnormal plasma volume and compared the responses to those obtained in a group of healthy parous controls with a normal plasma volume.

Patients and Methods

Patients: We performed this study in 8 healthy parous controls and 31 non-pregnant normotensive women with both a recent history of early onset (< 34 weeks) preeclampsia and/or HELLP syndrome, and a plasma volume of at least

2 standard deviations below the mean of the control group determined at least 6 months postpartum (<48 ml/kg lean body mass). Hypertension, preeclampsia and HELLP syndrome were defined according to the criteria of the Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy [4]. We recruited the controls by advertisement. We only enrolled women in the control group who were normotensive and had a history of uneventful pregnancy. Women in both groups had singleton pregnancies only. We started recruitment after approval of the study protocol by the university hospital's medical-ethical committee. After careful explanation of the protocol all participants gave written informed consent.

Methods: Measurements were performed in the follicular phase of the menstrual cycle (cycle day 5 ± 3). Participants refrained from smoking and consuming caffeine - and/or alcohol-containing substances from the evening prior to study. During the measurements, the participants were comfortably laying in supine position under standardized conditions in a temperature-controlled room (23±1 °C).

Measurements were started after at least 30 minutes of acclimatization to the experimental conditions. Fore arm venous compliance was measured by plethysmography as detailed elsewhere [26]. Before and after infusion, we sampled blood for the measurement of the hematocrit and the circulating levels of alfa-Atrial Natriuretic Peptide (α-ANP, $ng.L^{-1}$) and of Active Plasma Renin Concentration (APRC, $mU.L^{-1}$). Blood samples were collected in chilled tubes, put on ice during transport and processed within minutes after collection to be stored at -70^0C until analysis. α-ANP and APRC were measured as detailed previously [25]. We corrected the post-infusion levels of α-ANP and APRC for dilution by multiplying the measured levels by the ratio of pre- and post-infusion hematocrit. Prior to volume loading, plasma volume was determined using dextran-70 indicator method as detailed previously [51]. Plasma volume is expressed ml/kg calculated lean body mass [11]

After blood sampling, intravenous administration of a modified gelatin solution was started using an infusion pump at a constant rate of 16.7 $mL.\ min^{-1}$. The infusion was discontinued after 30 minutes and thus after a total volume load of 500 mL. We chose for a volume load of 500 mL to be infused in 30 minutes on the basis of a small pilot study aimed to assess optimal infusion rate and total volume load. We recorded (relative) changes in stroke volume (ml), heart rate ($beats.min^{-1}$) and cardiac output ($L.min^{-1}$) in response to volume loading, using continuous beat-to-beat pulse contour analysis with the Portapress device (TNO-biomedical instrumentation, Amsterdam, The Netherlands) [52]. This technique has

been validated for estimating intra-individual changes in stroke volume over time [53 54, 55]. We assessed the hemodynamic responses to volume loading on the basis of Portapress recordings at 5-minutes intervals throughout the infusion period and the subsequent 10 minutes afterwards.

Venous capacitance is an estimate of venous filling in steady state [50]. Depending on size and compliance of the venous bed, volume loading will raise unstressed volume until cardiac preload and with it cardiac output, begins to increase. The maximum volume load, accommodated without rise in preload can be expected to vary as a function of venous capacitance. It follows that an estimate of venous capacitance can be obtained by subjecting the vascular bed to a standardized procedure of volume loading. We adopted a method to reflect venous capacitance by calculating the slope of the linear regression line of the relative rise in cardiac output for a relative rise in plasma volume. The relative change in plasma volume was obtained by taking the ratio of pre- and post infusion hematocrit.

Statistical methods: The intergroup differences and those observed in the response to volume loading in α-ANP and APRC and venous capacitance were tested by Mann-Whitney-U Test. For the intergroup difference in changes in pulse rate, stroke volume and cardiac output in response to volume loading, the area under the curve was calculated and differences were tested by Mann-Whitney-U Test. Intra group changes in α-ANP and APRC as a result of volume loading were tested using with the Wilcoxon signed rank test. A p-value below 0.05 was considered statistically significant.

RESULTS

Table 1. *Demographic variables*

	low-PV	Controls	p-value
Age (years)	31.1 ± 0.7	35.2 ± 1.0	<0.05
Body Mass Index ($kg.m^{-2}$)	25.5 ± 0.7	22.1 ± 1.1	<0.05
Primiparity (%)	80.0 %	74.0 %	NS
Smokers (%)	10.0 %	19.4	NS
Interval Pregnancy to study (years)	1.6 ± 0.2	3.5 ± 0.8	<0.05
Plasma volume (ml/kl lbm)	44.2 ± 1.6	54.7 ± 3.3	<0.05
Plasma volume (ml)	2576 ± 67	3015 ± 176	<0.05
Fore arm venous compliance ($ml.dl^{-1}.mmHg^{-1}$)	4.3 ± 0.2	4.4 ± 0.3	NS

NS = not significant; LBM = lean body mass

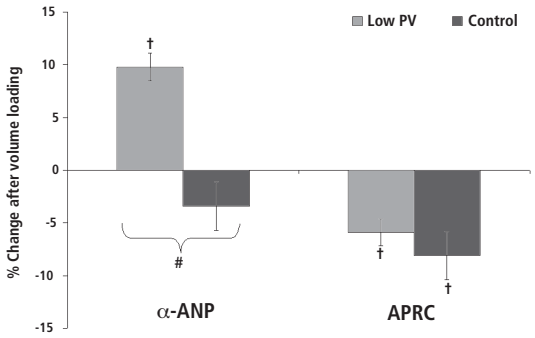
Table 1 lists the demographic characteristics of both study groups. The patient group (low-PV) had a higher body mass index, lower age and, obviously, a lower plasma volume, but comparable parity and a shorter interval between pregnancy and the time of study relative to the control group. Amongst the multiparous women 50% had 2 pregnancies and 50% had 3 pregnancies in both patients and control group.

Table 2. *Responses to volume loading*

	low-PV	Controls	p-value
α-ANP (basal value, ng.L^{-1})	66.6 ± 3.6	62.8 ± 5.4	NS
α-ANP (post loading, ng.L^{-1})	87.5 ± 4.2	61.1 ± 6.0	<0.05
APRC (basal value, mU.L^{-1})	21.0 ± 2.0	19.4 ± 6.3	NS
APRC (post loading, mU.L^{-1})	18.4 ± 1.5	16.8 ± 4.8	NS
Heart rate (basal value, beats/min)	75 ± 1	76 ± 3	NS
Heart rate (post loading, beats/min)	86 ± 1	79 ± 4	NS
Stroke Volume (basal value, mL)	89 ± 3	86 ± 6	NS
Stroke Volume (post loading, mL)	97 ± 3	92 ± 10	NS
Cardiac Output (basal value, L/min)	6.7 ± 0.2	6.6 ± 0.5	NS
Cardiac Output (post loading, L/min)	7.9 ± 0.3	7.0 ± 0.5	<0.05

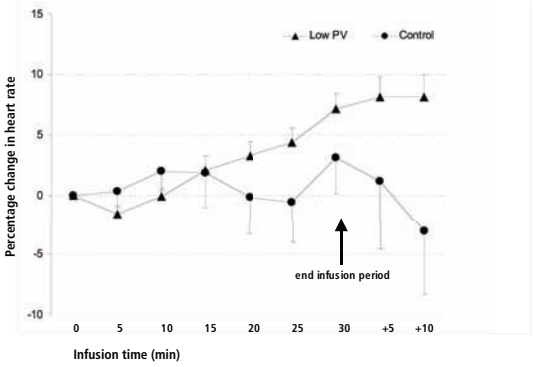
Table 2 list the absolute values of responses to volume loading. Women in the low-PV group have a significant increase in α-ANP and cardiac output in response to volume loading.

Figure 1. *Percentage change (± SEM) a-ANP and APRC in response to a volume load of 500 mL. # significant inter-group difference (p<0.05). † significant intra-group difference (p<0.05).*



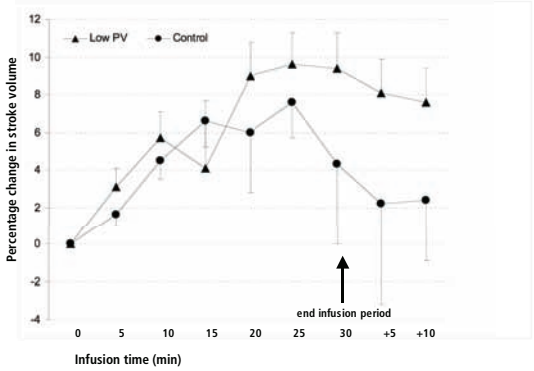
The relative changes in response to volume loading in α-ANP and APRC are displayed in figure 1. Women in the low-PV group differed from controls by an increase α-ANP. Responses of APRC to volume loading were similar in both groups.

Figure 2. *Percentage change (± SEM) in heart rate in response to a volume load of 500 mL in 30 minutes and the 10 minutes following the infusion period. The area under the curve differed between the 2 groups (Mann-Whitney U test, p<0.05)*



Relative hemodynamic changes in response to volume loading are depicted in figures 2, 3 and 4. While the response in heart rate (fig. 2) was higher in the low-PV group, the response in stroke volume did not differ between the two groups (fig. 3).

Figure 3. *Percentage change (± SEM) in stroke volume in response to a volume load of 500 mL during a 30 minutes period of infusion and the subsequent 10 minutes after discontinuation of the infusion. The area under the curve does not differ between groups.*



The latter lack of difference might be power related. Figure 4 illustrates the response in cardiac output to volume loading. Volume loading induced an initial rise in both groups, however, only in the low-PV group, cardiac output continued to increase after the first 15 minutes of volume loading, an effect which persisted after discontinuation of volume loading.

Figure 4. *Percentage change (± SEM) in cardiac output in response to a volume load of 500 mL in a 30-minutes' infusion period and the 10 minutes after its discontinuation. The area under the curve differed between the 2 groups (Mann-Whitney U test, p<0.01)*

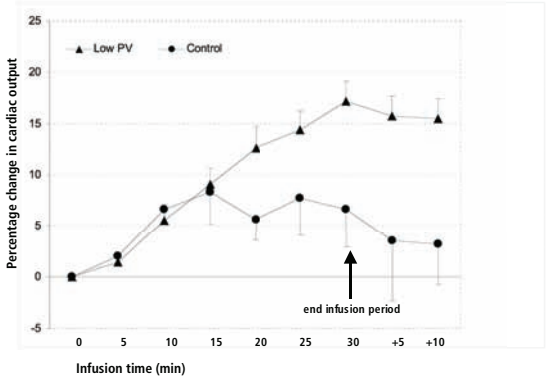
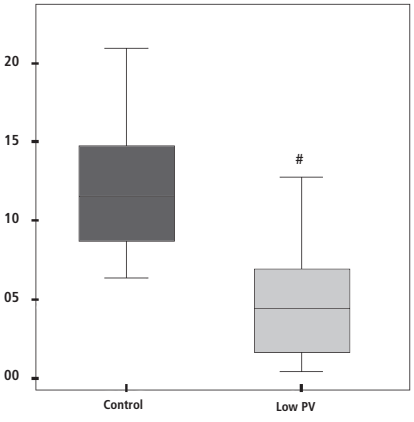


Figure 5. *The slope of the linear regression line of the relative rise in cardiac output for a relative rise in plasma volume in the low-PV and control groups. The box plots show medians, inter-quartiles and ranges. # indicates a significant difference between the 2 groups (p<0.05).*



The slope of the linear regression line of the relative rise in cardiac output for a relative rise in plasma volume is shown in figure 5. Relative to controls, this slope was 70% lower in the low-PV group.

Discussion

The response to acute volume loading in formerly-preeclamptic women with a subnormal plasma volume differs from that in parous controls by both a larger rise in cardiac output and a rise instead of no change in α-ANP. Both effects are consistent with a reduced capacity to accommodate a relatively modest volume load in the vascular bed. The observed rise in cardiac output probably indicates a volume shift from the venous- to the arterial bed, whereas the observed higher circulating levels of α-ANP will cause fluid loss from the vascular bed by accelerated natriuresis and leakage of fluid to the interstitium in conjunction with a increased capillary permeability [56, 57]. Venous capacitance can be expected to vary with the amount of extra blood that the venous bed is able to accommodate. The higher slope of the linear regression line of the relative rise in cardiac output for a relative rise in plasma volume in the low-pv group suggests that a subnormal plasma volume in formerly-preeclamptic women indicates a reduced venous capacitance.

The venous capacitance consists of a static - (size of the venous compartment) and a dynamic component (total venous compliance). Since cardiac output and venous return are closely correlated, we used changes in cardiac output as an estimate for the concomitant changes in venous return. As our estimate for venous capacitance varied independently from that in the concomitantly measured forearm venous compliance, the latter didn't seem to be a confounder of venous capacitance.

In a previous study we reported that formerly-preeclamptic women with a subnormal plasma volume respond to a new pregnancy with absent or less plasma volume expansion than parous controls with a normal plasma volume [26]. The concomitant rise in α-ANP as opposed to no change in uneventful pregnancy [25] supports the concept that also during pregnancy, subnormal plasma volume expansion results from a limited capacity to expand the unstressed volume in the venous bed, and thus venous capacitance. From these inferences we conclude that these women have a subnormal venous capacitance, which limits their ability to expand plasma volume in pregnancy.

The capacitance is an important characteristic of the venous bed as it represents a buffer, which can be mobilized in response to a higher demand for

venous return [49]. Particularly in conditions of prolonged increases in cardiac output as seen in pregnancy, this buffer is important, because the alternative option to raise cardiac output, would be by increasing the sympathetic drive in the autonomic control of the cardiovascular system. The latter is not only less efficient and associated with extra strain exerted upon heart and arteries due to increased shear stress, it also induces a redistribution of systemic blood flow at the expense of non-vital tissues, which includes the uterus.

The latter may cause the development of a hyperdynamic circulation. Although there is limited consensus about the hemodynamics of preeclampsia, several studies seem to confirm that the early stage of preeclampsia is characterised by a hyperdynamic circulation [20, 58-60]. Furthermore a hyperdynamic circulation interferes with the circulatory adaptation to pregnancy and the growth of the uteroplacental perfusion, and thus, is associated with a greater risk to develop pregnancy-induced hypertensive disorders.

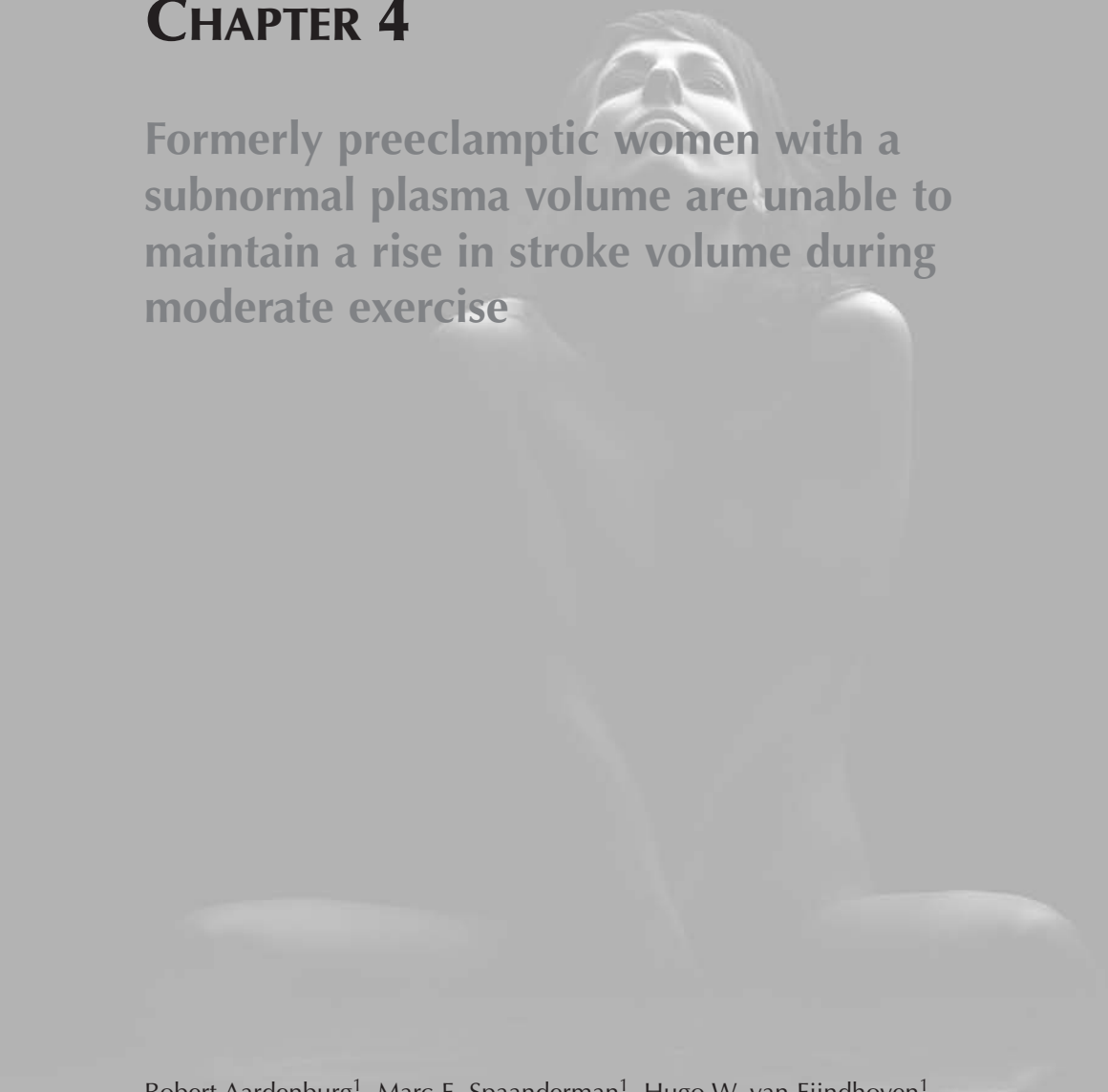
Since we did not collect data about natriuresis during volume loading we can only draw limited conclusion about volume regulatory function in these women. It remains unclear, why in some individuals plasma volume may be reduced.
In this study we did not find any differences in basal heart rate. But hyperactivity of the sympathetic nervous system [42, 43] may be associated with an increase in venous tone and consequently a subnormal venous capacitance. Angiotensinogen polymorphisms [61] may also have an influence on venous capacitance. On the other hand, in line with the Barker hypothesis, it is also possible that some of these women have an environmentally induced inborn reduction of venous capacitance [62].

Conclusion

The capacitance of the venous compartment in normotensive formerly preeclamptic women with a subnormal plasma volume is substantially lower than that in healthy parous controls. The latter may predispose for circulatory maladaptation to pregnancy, and with it for pregnancy-induced hypertensive disorders.

CHAPTER 4

Formerly preeclamptic women with a subnormal plasma volume are unable to maintain a rise in stroke volume during moderate exercise



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Abstract

Introduction:In formerly preeclamptic women with a low plasma volume, the recurrence rate of preeclampsia is higher than in women with a normal prepregnant plasma volume. In a recent study, we demonstrated that the low plasma volume subgroup also had a subnormal venous capacitance. In the present study, we determined the impact of subnormal plasma volume on the hemodynamic response to moderate exercise.

Patients and Methods:We performed this study in the follicular phase of the menstrual cycle, in 31 formerly-preeclamptic women with a subnormal plasma volume (low-PV) and 8 parous controls. The exercise consisted of 60 minutes cycling in supine position at 35 % of the individualized maximum capacity. Before, during and after cycling, we measured the percentage change in heart rate, stroke volume and cardiac output. Before and after exercise, we measured the effective renal plasma flow (ERPF, para-amino-hippurate clearance), glomerular filtration rate (GFR, inulin clearance) the circulating levels of alpha-atrial natriuretic peptide (α-ANP) and active plasma renin concentration (APRC).

Results: The response to exercise of formerly-preeclamptic women with a subnormal plasma volume differed from that in controls by a lack of rise in stroke volume, a smaller rise in cardiac output and α-ANP, and a greater fall in GFR. The response in heart rate, ERPF and APRC did not differ between the two groups.

Conclusion: The response to moderate exercise of formerly-preeclamptic women with a subnormal plasma volume differs from that in healthy parous controls with a normal plasma volume and suggests a lower capacity to raise venous return in conditions of a higher demand for systemic flow. The lower capacity to raise venous return in these conditions is associated with more cardiovascular drift. The physiological consequence is a lower aerobic endurance performance during moderate exercise.

Introduction:

In formerly-preeclamptic women, the prevalence of a subnormal plasma volume is approx. 50% [11]. We and others observed that a subnormal plasma volume predisposes to recurrent hypertensive complications in a next pregnancy [33, 34, 44-47]. In the nonpregnant state, these women with a subnormal plasma volume differed from their counterparts with a normal plasma volume by a lower stroke volume, lower cardiac index and shorter estimated systemic circulation time [44]. Although their health state in resting conditions appears to be good, it is not clear, whether these women have less cardiovascular reserves in conjunction with a lower capacity to raise and maintain cardiac preload. If so, their ability to deal with conditions of chronically elevated cardiac output may be compromised and cardiovascular drift might be increased.

A decline in circulating volume due to dehydration reduces the aerobic endurance performance during exercise [63]. Furthermore, an experimentally induced acute reduction in total blood volume by withdrawal of approx 0.5 L of blood attenuates the normal rise in stroke volume in response to exercise [64]. These observations suggest that a (sub)acute decrease in blood volume diminishes the exercise-induced rise in both stroke volume and total body oxygen uptake. The negative impact of a reduced plasma volume on aerobic endurance performance causes "cardiovascular drift", defined as a decline in stroke volume that may occur in conditions of prolonged moderately-intensive exercise [65] .

The present study was designed to test the hypothesis that the response to exercise of formerly-preeclamptic women with a subnormal plasma volume differs from that in parous controls with a normal plasma volume by more cardiovascular drift. To this end, we compared the response in heart rate, stroke volume, cardiac output, renal perfusion (ERPF & GFR) and that in 2 important hormones involved in volume regulation (α-ANP & APRC), to 60-minutes moderate-intensive exercise in 31 formerly-preeclamptic women with a subnormal plasma volume, with that in 8 parous controls with a normal plasma volume.

Patients and Methods:

Patients: This study was performed in 8 healthy parous controls and in 31 non-pregnant seemingly-healthy normotensive women with a recent history of early-onset (< 34 weeks) preeclampsia / HELLP as well as a plasma volume

of at least 2 standard deviations below the mean of the control group (<48 ml/kg lean body mass) [11] determined at least 6 months postpartum at the time of postpartum counseling for recurrent hypertensive complications in a next pregnancy. Former patients were invited to participate in this study when they seemed to be healthy, when their plasma volume turned out to be subnormal and finally, when no other abnormalities could be detected. We used the definitions for hypertensive complications in pregnancy as detailed elsewhere [4]. Controls, recruited by advertisement, were all normotensive and had a history of uneventful pregnancies only. We started recruitment after approval of the study protocol by the University hospital's medical-ethical committee. After careful explanation of the protocol all participants gave written informed consent.

Methods: All exercise experiments were performed in the follicular phase of the menstrual cycle (cycle day 5 ± 3) and were preceded by a "max-test" on the preceding day. The latter implied the assessment of maximum cycling capacity in all participants, defined as the maximum power at which a subject was able to cycle in our experimental set-up. To this end, all women cycled on the same ergometer at a starting power of 60 watts, with power being raised by increments of 30-20-10-10 etc. watts, every 2 minutes. Women were instructed to continue cycling until unable to comply. The maximum power was defined as the highest power level, during which they were able to keep cycling for at least one minute.

Participants refrained from smoking and consuming caffeine - and/or alcohol-containing substances after the max-test until the exercise experiment on the subsequent day. During that experiment, the participants were quietly laying in supine position in a room standardized for temperature (23±1 °C), humidity and noise level. Before and during exercise we sampled blood for the measurement of circulating levels of alpha-Atrial Natriuretic Peptide (α-ANP, $ng.L^{-1}$) and Active Plasma Renin Concentration (APRC, $mU.L^{-1}$). For this purpose, we collected blood samples in chilled tubes, which were put on ice during transport and processed within minutes after collection. Processed samples were stored at -700C until analysis. α-ANP and APRC were measured as detailed previously [25]. 120 minutes prior to the exercise experiment, we determined plasma volume by the dextran-70 indicator dilution method [51]. Plasma volume is expressed in mL per kg calculated lean body mass [11]

We measured Effective Renal Plasma Flow (ERPF) and Glomerular Filtration Rate (GFR) on the basis of a continuous i.v. infusion of para-amino-hippurate sodium (PAH) and Inulin [11]. After at least 120 minutes of PAH/Inulin

infusion, blood samples were collected to measure basal α-ANP, APRC, ERPF and GFR. After blood sampling each participant was carefully positioned on a supine cycle ergometer (Echo Cardiac Stress Table, Lode Medical Technology, Groningen, The Netherlands) and allowed to acclimatize to this position for 30 minutes. Than they started exercise for a period of 60 minutes at a power (watt), corresponding with 35% of each individual's own maximum power as determined on the previous day.

We recorded the percentage change in stroke volume, heart rate and cardiac output during exercise, using the mean values of 3 minutes of beat-to beat analysis by the Portapress device for continuous pulse contour analysis (TNO-biomedical instrumentation, Amsterdam, The Netherlands) [52]. This technique has been validated for estimating intra-individual changes in stroke volume over time, without providing information on absolute values [53 54, 55].

Statistical methods: We compared patient and control groups with respect to basal levels of α-ANP and APRC and the response to exercise in α-ANP, APRC, ERPF and GFR using the Mann-Whitney-U Test. We quantified for both groups the response to exercise in heart rate, stroke volume and cardiac output, by calculating the area-under-the-curve (AUC). The AUC for % change in stroke volume relative to baseline throughout 60 minutes of exercise provides an estimate for "cumulative" rise in stroke volume. The latter varies as a function of the decline in stroke volume throughout the exercise period, and therefore, was used in this study as an estimate for relative cardiovascular drift. Differences between the 2 groups in the 3 AUC's were tested using the Mann-Whitney-U Test. The Wilcoxon Signed Rank Test was used to compare in each group, the pre-and post-exercise values for α-ANP, APRC, ERPF and GFR. A p-value below 0.05 was considered statistically significant.

To determine whether plasma volume was an independent predictor of stroke volume changes or whether these changes were confounded by differences in BMI, we performed a logistic regression analysis with stroke volume change as dependent, and plasma volume and BMI as independent variables.

Results

Table 1 lists the (demographic) characteristics of the 2 study groups. As compared to the control group, the patient group (low-PV) was younger, had a higher

body mass index (BMI), comparable parity and by definition, a lower plasma volume and were comparable regarding microalbuminuria.

Table 1. *Demography of the participants in each of the 2 study groups.*

	low-PV	Controls	p-value
Age (years)	31.1 ± 0.7	35.2 ± 1.0	<0.05
Body Mass Index (kg/m^{-2})	25.5 ± 0.7	22.1 ± 1.1	<0.05
Primiparity (%)	80.0 %	75.0 %	ns
Plasma volume (ml/kg lbm)	44 ± 2	55 ± 3	<0.05
Mean Arterial Pressure (mmHg)	91 ± 13	87 ± 11	ns
Plasma volume (ml)	2576 ± 67	3015 ± 176	<0.05
Microalbuminuria			
(g albumin/mol creatinin) 0.8 ±0.5	0.7 ± 0.8	ns	

NS = Not significant

Table 2 indicates that maximal power, basal heart rate, heart rate at maximal power were comparable in patient and control groups. The same holds for absolute values of ERPF and GFR before and after 60 minutes of exercise. The coefficient of variation (CV) in ERPF and GFR was clearly higher in controls (CV = 9-10 %) than in the patient groups (CV = 3-4 %), most likely in conjunction with the limited size of the control group.

Table 2. *Exercise variables as obtained in the 2 study groups*

	low-PV	Controls	p-value
Maximal Power (watt)	124 ± 19	142 ± 17	ns
Heart Rate (basal, beats/min)	76 ± 9	76 ± 9	ns
Heart Rate (maximal, beats/min)	173 ± 14	174 ± 12	ns
ERPF (basal, ml.min-1.1.73-2)	506 ± 18	550 ± 50	ns
ERPF (exercise, ml.min-1.1.73-2)	407 ± 13	447 ± 43	ns
GFR (basal, ml.min-1.1.73-2)	121 ± 3	120 ± 10	ns
GFR (exercise, ml.min-1.1.73-2)	112 ± 3	116 ± 9	ns

NS = not significant

Figures 1, 2 and 3 illustrate the relative changes in response to exercise, in heart rate, stroke volume and cardiac output, respectively. In both groups,

Figure 1. *heart rate response to exercise. The area under the curve is not different between groups*

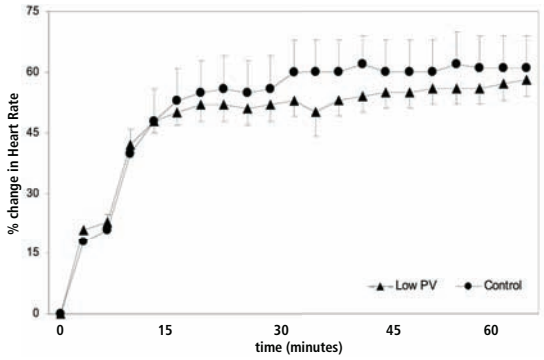


Figure 2. *stroke volume response to exercise. The area under the curve differs between groups (p<0.05)*

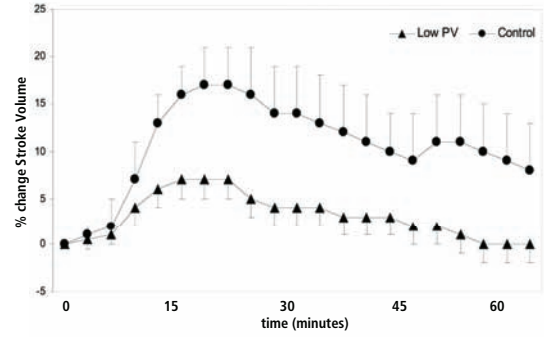
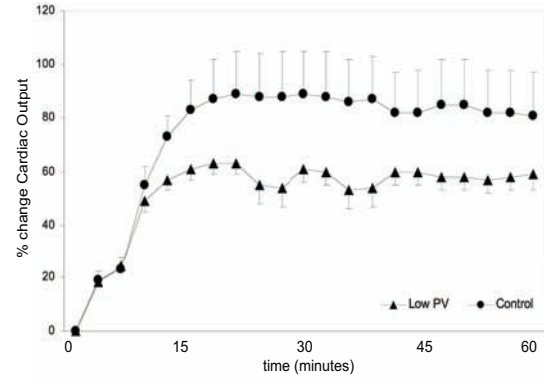


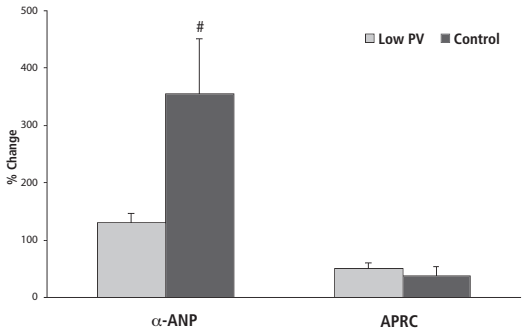
Figure 3. *cardiac output response to exercise. The area under the curve differs between groups (p<0.05)*



exercise induced a rapid rise in heart rate, which reached a comparable and stable plateau of about 50-60% above baseline, 173 beats/min ± 14 in low PV versus 174 beats/min ± 12 in controls, after about 15 minutes (fig. 1). In contrast, the response of stroke volume to exercise differed between the low-PV and control groups. Although the pattern of response - transient initial rise in stroke volume followed by cardiovascular drift after 15 minutes - was comparable in the two groups, the average percentage rise in stroke volume was clearly higher in the control group (fig. 2; 17 ± 3 vs. 7 ± 2 %; $p<0.05$). Figure 3 shows that exercise induced a smaller rise in cardiac output in the low-PV group then in the control group (70 ± 5 % vs. 90 ± 17 %, $p < 0.05$).

The percentage change in α-ANP and APRC are displayed in figure 4. The exercise-induced increase in absolute values in circulating α-ANP levels was smaller in women in the low-PV group than in the controls (101 ± 15 and 166 ± 44 ng.L-1, $p<0.05$). The two study groups were comparable with respect to the exercise-induced response in APRC.

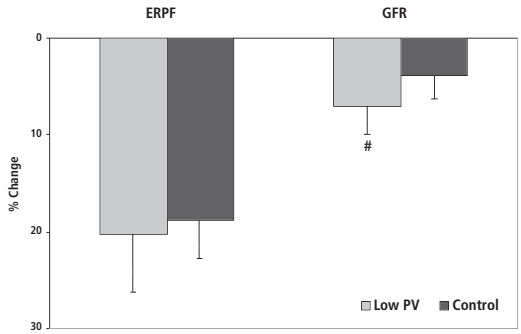
Figure 4. *responses in -ANP and APRC to exercise.*
Exercise induced values were significantly different from baseline values within each group (p<0.05). # marks a difference between groups (p<0.05)



Finally, figure 5 depicts the effect of exercise on ERPF and GFR. In both groups, ERPF and GFR decreased during exercise. Absolute values in ERPF and GFR did not differ, most likely due to the limited size of the control group, On the other hand, the % decrease in GFR was significantly larger in the low-PV group than in the control group (-7 ± 1% vs. –4 ± 1%, $p < 0.05$). The exercise-induced increase in filtration fraction was similar in the low-PV and control

groups: from 25 ± 1 to 28 ± 1 % and from 22 ± 1 to 26 ± 1, respectively. Logistic regression analysis showed that the changes in stroke volume during exercise were dependent to plasma volume (p=0.017) and not to differences in BMI (p=0.762).

Figure 5. *responses in ERPF and GFR to exercise.*
Exercise induced values were significantly different from baseline values within each group (p<0.05). # marks a difference between groups (p<0.05)



Discussion

The response to moderate exercise in formerly preeclamptic women with a subnormal plasma volume differs from that in controls by a smaller rise in α-ANP, a blunted rise in stroke volume, a smaller rise in cardiac output and finally, a comparable pattern of cardiovascular drift. Although the absolute loss in surplus stroke volume by cardiovascular drift after 60 minutes exercise was larger in the control group, the remaining extra stroke volume above baseline was still larger in the control group than in the patient group (fig.2). The observations in this study suggest that women with a subnormal plasma volume have a reduced capacity to raise cardiac preload and with it, stroke volume and cardiac output in response to exercise. As these women have a reduced venous capacitance [66], we postulate that the impact of a certain degree of venoconstriction on cardiac preload is smaller in these women than in their counterparts with a normal plasma volume. Although one would expect a greater rise in heart rate in the low PV-group, to compensate for the lack of rise in stroke volume, we observed the opposite, i.e. a tendency towards a lower

heart rate (fig. 1). We speculate that the inadequate rise in heart rate to compensate for the relatively small increase in stroke volume may be explained by a blunted rise in sympathetic nervous activity in the low PV-group, possibly in conjunction with an already elevated basal sympathetic activity in these women [67].

In both the low-PV group and the controls, exercise triggered a comparable fall in ERPF, an observation that is in line with observations by others [68] and which probably results from a higher sympathetic nervous activity. Interestingly, our low-PV group differed from controls by responding to exercise with a significant fall in GFR, similarly as previously reported for subjects with impaired renal function [69]. However, it was not possible to deduce from our data whether the larger fall in GFR in our low-PV patients was related to latent renal dysfunction or to the subnormal vascular filling state. Microalbuminuria in basal conditions did not differ between the 2 groups (0.8 ± 0.5 g albumin/mol creatinin in low-PV vs. 0.7 ± 0.8 g albumin/mol creatinin in controls). And macroalbuminuria did not occur in the low-PV group. In addition, all renal PAH/inulin clearances were within the normal range for all participants. Therefore, the abnormal hemodynamic response to exercise in the low-PV group is most likely a direct consequence of the subnormal filling state. The women in the low PV-group had a slightly higher BMI. The latter may be of some influence on the hemodynamic response to exercise. However we think that the difference in BMI between the 2 groups is too small to contribute to substantial differences in hemodynamic responses to exercise.

Exercise triggers a rise in cardiac output, which in turn, requires a higher venous return. The latter is achieved by venoconstriction, which reduces venous capacitance and with it, increases cardiac preload [70, 71 72 49]. In a recent study, we provided experimental evidence for an association between plasma volume and venous capacitance [73]. From these data and the results in the present study, we conclude that women with a subnormal plasma volume have a reduced capacity to raise venous return during exercise as a result of a subnormal venous capacitance, which limits their capacity to increase preload.

Venous return is related to plasma volume. In trained athletes an increased plasma volume acts as a volume buffer during exercise, enabling a sustained elevation of stroke volume [74 75]. During exercise, mildly hypovolemic subjects have a lower increase in stroke volume and a compensatory higher rise in heart rate than their normovolemic counterparts [64 65]. Interestingly, the latter resembles the smaller rise in stroke volume in response to exercise in the low-PV group of this study, although the latter group fails to compensate for this effect by an appropriate rise in heart rate. At any rate, these inferences suggest that size of the plasma volume compartment and stroke volume are causally related [76].

In the present study, we evaluated the cardiovascular reserves of formerly preeclamptic women with a subnormal plasma volume. It is difficult to extrapolate these results obtained during an one-hour period of moderate exercise to pregnancy, when cardiac output remains elevated for a period of months. Previous data on the adaptation of women with a subnormal plasma volume to pregnancy provide evidence for a blunted plasma volume expansion [25], accompanied by signs of increased cardiovascular sympathetic tone [26]. A low pre-pregnant plasma volume and a lack of plasma volume expansion can be expected to hamper the institution of a high flow/low resistance circulation. Such an adaptive response predisposes to the development of preeclampsia [20] .

Conclusion

Normotensive formerly preeclamptic women with a subnormal plasma volume are unable to keep stroke volume elevated during moderate exercise. We speculate that this response also applies to pregnancy, when cardiac output is elevated for a prolonged period. Their inability to preserve an elevated cardiac output raises their chance to develop a hypertensive disorder in pregnancy.

CHAPTER 5

A low plasma volume in formerly preeclamptic women predisposes to the recurrence of hypertensive complications in the next pregnancy

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Abstract

Introduction: Formerly preeclamptic women with a subnormal plasma volume (PV) have an increased risk to develop a hypertensive disorder in a subsequent pregnancy as compared to women with normal PV. In this study we tested the hypothesis that formerly preeclamptic women who develop recurrent disease in their next pregnancy differ from their counterparts with an uneventful next pregnancy by a lower pre-pregnant PV, a lower venous capacitance, smaller rises in these indices in early pregnancy, a lower renal adaptive response and a lower response to mild exercise.

Patients and Methods: We enrolled 33 formerly preeclamptic women in this study. Only 14 conceived within the study period with seven of developing a recurrent hypertensive disorder in their next pregnancy (RECUR), while seven had an uneventful next pregnancy (NORM). Before pregnancy and at 12 weeks of gestational age, we compared the following variables between these subgroups: PV, venous capacitance, effective renal plasma flow, glomerular filtration rate, and the responses in stroke volume and heart rate to mild exercise. To estimate venous capacitance, we infused 500 mL of a modified gelatine solution in 30 min, while recording the change in cardiac output (pulse contour analysis). The ratio of % change in blood volume to % change in cardiac output in response to a standardized small volume load provides an estimate for venous capacitance.

Results: RECUR differed from NORM by a 20% lower pre-pregnant PV ($P<0.02$) and venous capacitance (0.29 [0.11-0.55] vs 0.86 [0.64-2.03] $P=0.002$). NORM and RECUR were comparable with respect to pregnancy-induced rise in PV, renal hemodynamics and function, and response to mild exercise at 12 weeks. Newborn weight correlated positively with pre-pregnancy PV ($R^2= 0.53$ and $p=0.04$).

Conclusion: Formerly preeclamptic women with a recurrent hypertensive disorder in their next pregnancy differed from their counterparts with an uneventful next pregnancy by a lower pre-pregnant PV and a lower venous capacitance, the latter two indices correlating also inversely with the incidence of fetal growth restriction. The preserved acute response to volume related stimuli in women with a low pre-pregnant PV supports the view that the predisposition of low pre-pregnant PV to adverse pregnancy outcome may result from a concomitant, PV dependent change setpoint and/or gain in the stimulus/response interrelation of the volume regulatory system.

Introduction

Approximately 50% of women with a history of severe preeclampsia turn out to have a subnormal plasma volume (PV, mL), 6-12 months post delivery [11]. Clinically these women differ from their counterparts with a normal PV by a 3 times higher chance of developing recurrent disease in their next pregnancy [44]. Functionally, a low PV coincides with a lower venous capacitance in the non-pregnant state [66] and a reduced capacity to respond to mild exercise [77].

The cardiovascular adaptation to pregnancy is dominated by secondary adjustments to accommodate an initial systemic vasorelaxation [25]. Indirect evidence suggests that already in the 5th week of pregnancy, both arterial and venous compliance have increased in response to a still unknown pregnancy-specific factor [25]. These effects tend to lower cardiac pre- and afterload and therefore trigger a compensatory rise in the cardiovascular sympathetic drive and activation of the volume-retaining mechanisms. The latter results in plasma volume expansion, thus restoring pre-load and normalizing sympathetic tone. Meanwhile, the afterload reduction triggers a rise in cardiac output, which serves to maintain blood pressure. It follows that these compensations lead to the institution of a high flow/low resistance circulation [14]. The concomitant PV expansion can be considered a response needed to sustain the latter cardiovascular adaptation for a prolonged period [14 74 76]. In several studies, a reduced pre-pregnant PV has been found to predispose to either fetal growth restriction or hypertensive complications in advanced pregnancy [44, 45, 78, 79]. However, it is still obscure, whether or not the negative impact of a low pre-pregnant PV on pregnancy outcome is mediated by defective cardiovascular and volume adaptation to pregnancy.

This study was designed to test the hypothesis that formerly preeclamptic women developing a recurrent hypertensive disorder in their next pregnancy differ from their counterparts with an uneventful next pregnancy, by 1. a lower pre-pregnant PV and venous capacitance, 2. a blunted pregnancy-dependent rise in these indices as well as renal function and 3. poorer performance during mild exercise in the 12th week. To this end, we measured in 7 formerly preeclamptic women, who developed recurrent hypertensive disorder in their next pregnancy, the following variables: Pre-pregnant PV, venous capacitance, renal blood flow, glomerular filtration rate and the cardiovascular response to mild exercise at 12 weeks pregnancy. We compared the results in these 7 complicated pregnancies with those in 7 formerly preeclamptic women who had an uneventful course and outcome of their next pregnancy.

Patients and Methods

Patients

Primiparous normotensive women with a history of early-onset (< 34 weeks) preeclampsia and/or HELLP syndrome and who intended to conceive again, were invited to participate in this study at least 6 months after the index pregnancy. Pregnancy-induced hypertension (PIH), preeclampsia and the HELLP syndrome were defined according to the criteria of the Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy [4]. We started recruitment after approval of the study protocol by the University hospital's medical-ethical committee. After explanation of the protocol, a total of 33 participants gave written informed consent. All of these women had a prior preeclampsia that was comparable in severity with respect to gestational age at delivery and birthweight centiles (table I). Women, who failed to conceive within 6 months after their pre-pregnant measurement, were excluded from further participation (n=19). 14 Women completed both study sessions and had an ongoing (no miscarriage) next pregnancy. From these women, 7 developed a recurrent hypertensive disorder in late pregnancy, such as PIH, preeclampsia and/or the HELLP syndrome (RECUR), whereas pregnancy course was uneventful in 7 women (NORM). Women who did not conceive within 6 months did not differ with respect to age, BMI, PV and obstetrical outcome in the first pregnancy from those who did conceive.

Methods

Measurements were performed in the follicular phase of the menstrual cycle (cycle day 5 ± 3 days) and again at 12 weeks amenorrhea (± 3 days). Participants refrained from smoking and consuming caffeine- and/or alcohol-containing substances from the evening prior to study. During measurements participants were comfortably laying in supine position under standardized conditions in a temperature-controlled room (23±1 °C).

We determined PV by the dextran-70 indicator dilution technique [51], before pregnancy and again at 12 weeks gestation. We expressed PV in mL per kg calculated lean body mass [11] and estimated Effective Renal Plasma Flow (ERPF, mL.min-1) and Glomerular Filtration Rate (GFR, mL.min-1) on the basis of a continuous intravenous infusion of para-amino-hippurate sodium (PAH, MSD, West Point, PA, USA) and Inulin (Inutest, Laevosan Gesellschaft, Linz, Austria), respectively, as detailed previously [11]. After at least 120 minutes infusion, blood samples were collected to measure basal ERPF and GFR. After blood sampling, each participant was positioned on a supine cycle ergometer (Echo Cardiac Stress

Table, Lode Medical Technology, Groningen, The Netherlands) and allowed to acclimatize to this position for 30 minutes. Than they started cycling for a period of 60 minutes at a power (watt), corresponding with 35% of each individual's own maximum power as determined on the previous day [77]. We recorded stroke volume and heart rate during exercise using the means of 3 minutes of beat-to-beat analysis by pulse contour analysis (Portapress, TNO-biomedical instrumentation, Amsterdam, The Netherlands) [52]. From the 20 equidistant measurement points we calculated the area under the curve (AUC) for % change in stroke volume and in heart rate relative to baseline throughout 60 minutes of exercise. This figure provides an estimate for "cumulative" rise in stroke volume and heart rate [77]. After the cycling experiment the participants had a standardized one-hour lunch break.

Intravenous administration of a modified gelatine solution was started after at least 30 minutes of acclimatization to the experimental conditions, using an infusion pump at a constant rate of 16.7 $mL.min^{-1}$. Infusion was discontinued after 30 minutes, when 500 mL volume had been administered. We recorded the relative rise in cardiac output in response to volume loading using continuous beat-to-beat pulse contour analysis with the Portapress device and estimated venous capacitance from the ratio of % relative rise in cardiac output and % induced rise in PV, as detailed previously [66]. For this purpose, we estimated the relative change in PV from the ratio of pre- and post-infusion hematocrit.

The clinicians taking care of the clinical management of the participants during pregnancy and labour were unaware of their patient's test results obtained from the study protocol. Post delivery we carefully checked the chart of each participant to retrieve the data on clinical course and outcome of pregnancy (gestational age at delivery, birth weight, birth weight centile and any other clinically relevant information) to determine whether or not the preceding pregnancy had been complicated by a hypertensive complication to enable post-hoc categorization of the participants into the two subgroups. To meet the criteria for recurrent disease, women had to develop at least de novo gestational hypertension (diastolic blood pressure > 90 mmHg or a systolic blood pressure > 140 mmHg after 20 weeks of gestation [4]).

Statistical Analysis

Using the Mann-Whitney-U Test, we compared both subgroups with respect to demography (age, body mass index and parity), cardiovascular - and volume parameters measured before - and at 12-weeks pregnancy (PV, GFR, ERPF, venous capacitance) and clinical outcome variables (gestational age at delivery,

birth weight and birth weight centile). For both subgroups, we quantified the increase in stroke volume and in heart rate in response to exercise by calculating the area-under-the-curve (AUC) and also compared the two subgroups using the Mann-Whitney-U Test. Relevant relations were tested by Spearman's Rho correlation. Data are presented as medians with range given between parentheses. A p-value below 0.05 was considered statistically significant.

Results

Table I lists the demographic characteristics of the 2 study groups. Age, BMI, blood pressure, heart rate, obstetric outcome in the index pregnancy and interval between delivery in index pregnancy and subsequent pregnancy, did not differ between subgroups. Pre-pregnant PV was ± 20% lower in RECUR than in NORM. RECUR differed from NORM by both a lower pre-pregnant (0.29 [0.11 - 0.55] vs. 0.86 [0.64 - 2.03] P=0.002, arbitrary units) and 12-weeks venous capacitance (0.40 [0.15 - 0.98] vs. 1.50 [0.43 - 2.40] P=0.007, arbitrary units), the pregnancy-induced rise in this variable - both relative (43% [-21% - +91%] vs. 61% [-9% - +140%]) and absolute (0.18 [-0.21 - +0.87] vs. 0.44 [-0.66 to +1.40] arbitrary units) - did not differ appreciably.

Table I. *Demographics of the participants in the 2 study groups*

	NORM	RECUR	P value
Age (years)	29 [26 – 31]	31 [25 – 33]	NS
BMI (kg/m^{-2})	24.2 [17.2 - 29.4]	26.2 [20.2 - 30.9]	NS
Plasma volume (mL/ kg lean body mass)	48.8 [45.7 - 60.9]	42.4 [24.6 - 43.6]	0.019
Smokers	2/7	0/7	NS
Plasma volume (mL)	2357 [2016 - 2734]	2033 [1631 – 2375]	0.025
Pre-pregnantVenous capacitance (arbitrary units)	0.86 [0.64 - 2.03]	0.29 [0.11 - 0.55]	0.002
Pregnant Venous Capacitance (arbitrary units)	1.50 [0.43 - 2.40]	0.40 [0.15 to 0.98]	0.007
Systolic Blood Pressure (mmHg)	120 [105 – 134]	125 [104 – 133]	NS
Diastolic Blood Pressure (mmHg)	72 [58 – 81]	76 [65 – 87]	NS
Mean Arterial Pressure (mmHg)	88 [76 – 117]	97 [81 – 102]	NS
Heart Rate (beats/min)	71 [60 – 94]	69 [62 –80]	NS
Gestational Age index pregnancy (weeks)	32 6/7 [28 5/7 - 35 6/7]	34 0/7 [25 0/7 - 34 6/7]	NS
Birth Weight Centile Index pregnancy (%)	5 [2 – 65]	15 [6 – 50]	NS
Delivery Interval (months)	28 [22 – 42]	26 [18 –41]	NS

NS = not significant

Figure 1 shows the relation between pre-pregnant PV and the percentage PV expansion at 12 weeks gestation. PV expansion is not correlated to pre-pregnant PV. However, all participants with a pre-pregnant PV below 44 mL.[lean body mass]-1 had developed recurrent hypertensive disease.

Figure 1. *The relation between pre-pregnant plasma volume per lean body mass and the percentage plasma volume expansion at 12 weeks of gestation. The vertical line marks the 44 mL cut-off level. Pre-pregnant plasma volume and plasma volume expansion are not correlated (Spearman test for correlation, P>0.05).*

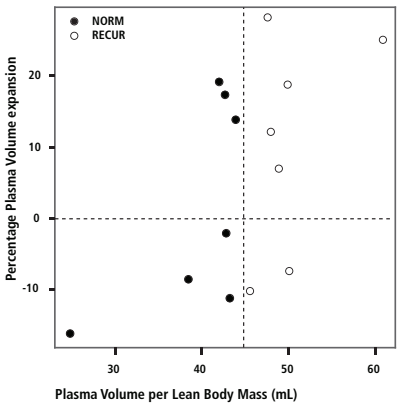

Table II lists the pre-pregnant and 12-weeks values for GFR, ERPF, filtration fraction (FF, defined as GFR/ERPF) and AUC for SV and HR during exercise in RECUR and NORM. Pre-pregnant and 12-weeks values in the two subgroups were comparable, as were the changes in response to pregnancy. The pre-pregnant and 12-weeks pregnant values for rise in SV and HR in response to exercise were comparable.

Table II. *renal hemodynamics and central hemodynamics of the participants in the 2 study groups.*

	NORM			RECUR		
	Non Pregnant	P value pregnant vs non pregnant	Pregnant	Non Pregnant	P value pregnant vs non pregnant	Pregnant
GRF ($mL.\ min^{-1}.\ 1.73^{-2}$)	112 [102 – 176]	0.01	155 [121 – 215]	129 [105 – 146] P=0.21*	0.01	171 [135 – 208] P=0.23#
ERPF ($mL.\ min\text{-}1.\ 1.73^{-2}$)	501 [461 – 938]	0.24	679 [567 – 1006]	620 [339 – 668] P=0.98*	0.02	874 [595 - 970] P=0.60#
Filtration Fraction (GFR/ERPF)	0.23 [0.11 - 0.26]	0.87	0.23 [0.19 - 0.24]	0.22 [0.21 - 0.31] P=0.97*	0.23	0.23 [0.18 - 0.27] P=0.62#
AUC SV during exercise	0.99 [0.98 - 1.19]	0.50	1.03 [0.97 - 1.14]	1.03 [0.94 - 1.06] P=0.32*	0.35	1.03 [0.96 - 1.12] P=0.84#
AUC HR during exercise	1.62 [1.32 - 1.82]	0.87	1.50 [1.44 - 1.87]	1.31 [1.20 - 1.86] P=0.62*	0.36	1.52 [1.38 - 1.74] P=0.95#

** marks the P value compared to Non Pregnant NORM*

 # marks the P value compared to Pregnant NORM

Table III lists the change in PV and venous capacitance by 12 weeks pregnancy along with the obstetric outcome in the two subgroups. The median rise in PV by 12 weeks seemed lower in RECUR (-70 [-554 - +527 mL]) than in NORM (339 [-289 - +759 mL]). However, the difference did not reach statistical significance. RECUR differed from NORM by earlier delivery, lower birth weights and lower birth weight percentiles. In RECUR, 1 participant had preterm birth ($36\ ^{6/7}$ weeks) and in NORM, none.

Finally, birth weight increased as a function of pre-pregnant PV (R^2= 0.53 and p=0.04). However, the relevance of this correlation is limited due to clustering of PV data points around the median.

Table III. *obstetric outcome of the participants in the 2 study groups*

	NORM	RECUR	P value
Plasma volume expansion at 12 weeks (mL)	339 [-289 - 759]	-70 [-554 - 527]	NS
Change in Venous Capacitance at 12 weeks	0.44 [-0.85 - 1.40]	0.18 [-0.21 - 0.87]	NS
Gestational age at delivery (weeks)	40 $^{0/7}$ [38 4/7 - 40 $^{5/7}$]	37 $^{3/7}$ [36 $^{6/7}$ - 40 $^{5/7}$]	0.05
Birthweight (grams)	3505 [3050 - 4340]	2975 [2525 - 3200]	0.01
Birthweight centile (%, median and range)	50 [40 - 95]	25 [5 - 50]	0.03
Incidence preeclampsia/HELLP (%)	-	2/7	NS
Incidence newborns < 10th centile	-	2/7	NS

Discussion

In this study, formerly preeclamptic women with recurrent hypertensive disorder in their next pregnancy only differed from their counterparts with uneventful subsequent pregnancy by a lower pre-pregnant PV and venous capacitance. Previously, we demonstrated that in these women PV varies with venous capacitance [66]. The observations in the present study support the view that a subnormal PV and venous capacitance, antedate a complicated pregnancy. This confirms an earlier observation in a different but larger group of women with a low plasma volume [44]. Others have observed plasma volume reduction in women with an angiotensinogen polymorphism [61] which resembles the degree of plasma volume reduction in RECUR. This could also have contributed to the reduced plasma volume in RECUR.

We also observed for both subgroups that by 12 weeks, the subnormal pre-pregnant PV has affected neither the renal adaptation to pregnancy, nor the cardiovascular response to mild exercise. The concomitant PV expansion in both subgroups combined appeared to be small (median PV rise 9%), relative to the previously observed 20% PV expansion by 8 weeks normal pregnancy [25].

Inter-group differences in PV change (coefficient of variation of 5% [51]) carries a relatively large measurement error. That is to say, this method will only enable the detection of a PV difference larger than 10% of total PV. In normal pregnancy, PV increases by 17-47% [17, 80, 81]. The median rise in PV by 12 weeks pregnancy in all 14 formerly preeclamptics combined was only 9% which is clearly lower than the figure reported for normal pregnancy, but consistent with our previous observations [25]. Probably, because of the small sample size in this study, together with the large measurement error, the difference in PV expansion between the two subgroups did not reach statistical significance, even though they differed markedly in pre-pregnant PV. The difference in early-pregnancy adaptation, between RECUR and NORM may consist of subtle abnormalities in the response of cardiovascular and/or volume regulatory systems, which we were unable to identify with the methods employed in this study. The lack of difference between both subgroups in the pregnancy-induced change in renal function and the cardiovascular response to mild exercise suggests limited inter group difference regulatory response to acute volume stimuli. However these observations do not exclude the possibility that subnormal pre-pregnant PV predisposes to defective PV expansion in pregnancy. Previously, we reported a reduced venous compliance in formerly preeclamptic women with a subnormal PV [26]. This particular feature implies a higher venous resistance to stretch and probably with it, a reduced responsiveness of the stretch receptors located in the venous wall. Meanwhile, the abberant rise in ANP in response to pregnancy in these women [25] can be considered a consequence of the lower venous capacitance and thus lower ability to buffer orthostatic stress. It is tempting to speculate that these two features contribute to the abnormal response to the chronic stimulus to retain volume in early pregnancy. Obviously, experimental prove of this concept requires quantification of PV expansion either in response to pregnancy or to some chronic standardized volume challenge e.g. by extra salt intake [82] in a much larger cohort of women with their basal PV varying over a wide range.

The venous system is a volume rather than a pressure compartment, with, as mentioned above, stretch providing the most important input for volume regulation. About 75% of the venous blood resides in small veins and venules [50]. The smaller the absolute size of the venous compartment, the larger will be the

impact on venous resistance for a given venoconstriction, as (according to Poisseuile's law) a given reduction in radius (r) causes a resistance increment equal to r^4. Meanwhile, also the venous return generated by venoconstriction will become proportionally smaller with a decrease in the size of the venous compartment [83]. These effects can be expected to further increase an already increased sympathetic drive [84] in the autonomic control of a relatively small cardiovascular system adapting to pregnancy [85]. In addition, these effects may cause the venular pressure to increase giving rise to compromise of the microcirculation.

On the basis of these inferences, we postulate that recurrence risk in formerly preeclamptics varies as a function of pre-pregnant size of a furthermore normally functioning cardiovascular bed. It follows that the cardiovascular reserves to accommodate the increased circulatory demands of pregnancy are directly correlated with the pre-pregnant size of the vascular bed [85 26]. The latter together with the observed positive correlation between pre-pregnant PV and birth weight centile supports a relationship between pre-pregnant PV and the magnitude of the abnormality that triggers uteroplacental insufficiency and/or a hypertensive disorder later in pregnancy.

In this study all participants with a PV below 44 mL.[lean body $mass^{-1}$] had recurrent disease, which is in line with our previous observation in a different population [79]. The cut-off value of a PV below 44 mL.lean body $mass^{-1}$ might prove to be a valuable screening tool for assessing the risk for recurrent hypertensive complications in formerly preeclamptic women.

On the basis of our data, we speculate that sympathetic hyperactivity will be inadequate to accommodate the arterial demands of pregnancy. The latter will induce redistribution of cardiac output in favor of vital organs and away from non-vital tissues such as the implantation site in the uterus.

In conclusion, subnormal PV and venous capacitance in formerly preeclamptic women predispose to recurrent hypertensive disorder in a next pregnancy. Although the latter does not seem to interfere with both the renal adaptation to pregnancy and the cardiovascular response to mild exercise by 12 weeks, the abnormal pre-pregnant volume status seems to reflect a vascular condition, which interferes in a dose-dependent fashion with normal cardiovascular adaptation to pregnancy, in spite of seemingly normally functioning volume regulatory mechanisms.

Chapter 6

Endothelium-dependent vasodilatation in formerly preeclamptics correlates inversely with body mass index and varies independently of plasma volume

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Abstract

Introduction: Disorders associated with endothelial dysfunction and conditions associated with subnormal plasma volume (PV) predispose to preeclampsia. Whether subnormal PV and endothelial dysfunction are causally related, is unclear. The aims of this study were 1. to determine whether endothelium-dependent vasodilatation (as reflected by a change in Forearm Blood Flow = FBF) is indeed lower in formerly preeclamptic women compared to parous controls, 2. to explore whether in former patients low Δ FBF coincides with low PV and 3. to find out, whether Δ FBF correlates with a number of relevant circulating endothelial stressors.

Patients and Methods: In 32 formerly preeclamptic women and 10 parous controls, we estimated Δ FBF from the increase in forearm blood flow after a standardized period of arterial occlusion. In addition, we measured PV, arterial blood pressure and a wide range of circulating variables. We also performed an echocardiography. Using non-parametric tests we compared former patients with controls. To identify factors associated with Δ FBF (including PV), we used Spearman correlation - and multiple linear regression analysis.

Results: Δ FBF did neither differ between former patients and controls, nor did it correlate with PV in the patient group. However, Δ FBF did correlate negatively with body mass index (BMI) and positively with global arterial compliance. Additional analysis suggested that the inverse correlation between Δ FBF and BMI reflected the negative impact of the metabolic syndrome on Δ FBF.

Conclusion: In formerly preeclamptic women, endothelium-dependent vasodilatation is comparable to that in normal parous controls and varies independently of plasma volume. However, in these former patients endothelium-dependent vasodilatation does vary inversely with features of the metabolic syndrome.

Introduction

Disorders characterized by endothelial dysfunction such as hyperhomocysteinemia, autoimmune diseases and diabetes mellitus, predispose to hypertensive disorders in pregnancy [86]. An important consequence of endothelial dysfunction is a reduced capacity to release vasodilator agents such as nitric oxide (NO) and prostacyclin [87]. It is still unknown, whether this feature of endothelial dysfuncton is also responsible for inadequate circulatory adaptation to pregnancy and the associated higher risk to develop preeclampsia.

In previous studies in formerly preeclamptic women, we found an inverse relation between the recurrence rate of hypertensive disorders in a next pregnancy and prepregnant plasma volume (PV) [88 44]. From the available information, it was not possible to determine the mechanism that led to subnormal PV. The following possibilities can be considered but since we did not prove these assumptions to be true, they remain speculative. Subnormal PV may be a feature of a normally functioning, but nevertheless underdeveloped cardiovascular bed. Alternatively, PV could be low due to a limited renal capacity to retain salt giving rise to a compensatory rise in the sympathetic contribution to the autonomic control of the circulation. Finally, the PV could be low due to a subnormal capacity to induce endothelium-dependent vasodilation (Δ FBF). This last option is supported by the apparent important role of endothelial NO in the cardiovascular adaptation to pregnancy [89, 90] and by the observation that maternal NO deficiency due to endothelial dysfunction, appears to be involved in the development of preeclampsia [5, 90] .

This study was designed, firstly to determine whether formerly preeclamptic women differ from parous controls by a lower Δ FBF, secondly, to test the hypothesis that - in former patients - PV varies as a function of Δ FBF, and thirdly, to explore whether in ex-patients, Δ FBF correlates with a number of relevant circulating endothelial stressors. To this end, we measured - after an overnight fast - circulating levels of glucose and insulin, HDL- and LDL-cholesterol, triglycerides. We also quantified PV, performed an echocardiography and measured Δ FBF in 32 former patients, with the Δ FBF also being measured in 10 parous controls.

Patients and Methods

Participants

Women with a history of preeclampsia and/or HELLP syndrome were invited to participate in this study. Preeclampsia and the HELLP syndrome were defined according to the criteria of the Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy [4]. By advertisement we recruited parous women to serve as controls. They were considered eligible for participation, when their obstetrical history consisted of normal pregnancies only, their current health state was good and their blood pressure had been normal during pregnancy. Recruitment was started after approval of the study protocol by the University hospital medical ethical committee. After explanation of the protocol, a total of 32 patients and 10 controls gave informed consent. After inclusion all participants appeared to be Caucasian women.

Measurements were performed at least 6 months after delivery. The choice for this interval was based on a study that provided evidence for reaching cardiovascular steady state approximately three to four months postpartum [19]. None of the participants had used oral contraceptives or vitamin supplements during the 2 months preceding the measurement and breastfeeding was discontinued at least two months before study in order to avoid influence by endocrinological state on volume homeostasis and of vitamin suppletion on the methionin loading test. Participants discontinued any antihypertensive medication at least four weeks before entering the study. Patients refrained from eating and drinking (except water), from 22:00 hours the evening before the study until after blood sampling at 9:00 hours.

Methods

We performed all measurements on a single day between 9 am and 4 pm, starting with the withdrawal of a blood sample for the determination of fasting glucose, insulin, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, triglycerides and creatinin, by standard hospital laboratory procedures. A methionin loading test was performed to diagnose Hyperhomocysteinaemia. We estimated insulin resistance by dividing the product of fasting glucose and - insulin, by 22.5 (HOMA-index) [29], and glomerular filtration rate (GFR, ml.min-1) using the Cockcroft formula [91].

After the first blood sampling, we determined PV using the Iodine^{125} - albumin (I^{125}-HSA) indicator dilution method as detailed previously [11]. Plasma volume is expressed as plasma volume per lean body mass (PV ml/kg LBW).

We estimated lean body mass using an impedance analyzer (Bia 101/S, RJL systems, Detroit, MI, USA) and applying the formula of van Deurenberg [92].

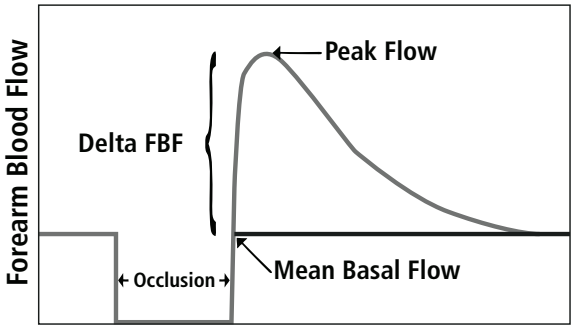
Than, we performed an echocardiography in semi-left lateral position by using a cross-sectional phased-array echocardiographic Doppler system (Agilent Sonos 5500, Philips Medical System, Eindhoven, the Netherlands) to estimate left ventricular mass (LV-mass) and to calculate cardiac output by multiplying stroke volume with heart rate as specified previously [11]. Next, we measured blood pressure in a standardized steady-state condition using a semi-automatic oscillometric device (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, FL, USA). Pulse pressure (PP) was calculated by subtracting diastolic blood pressure from systolic blood pressure. We calculated global arterial compliance using the ratio of stroke volume (SV) to pulse pressure.

We ended the data acquisition with the measurement of the forearm blood flow (FBF) in a quiet, temperature-controlled room (23 ± 1 °C) using electrocardiography (ECG)-triggered strain gauge venous occlusion plethysmography (ID-plethysmograph, Maastricht University, the Netherlands), as described previously [93, 94, 95, 96.]

During most of the measurements, participants were comfortably laying in supine position, from at least 10 minutes before the first blood sampling until completion of the measurement session.

All measurements were performed on the left arm. As the hand circulation is highly variable due to shunt-flow, we excluded this during the FBF measurement by inflating a wrist cuff to suprasystolic pressure, starting 1 minute before each FBF measurement [97]. Venous occlusion (50 mmHg) was applied with an upper arm cuff for 3 heartbeats and released for 2 heartbeats. Thus, an average of 12 inflow curves per minute were recorded[93]. [93]Baseline FBF was measured during 4 minutes. The mean value of the last three minutes of this period was used for calculations. Next, arterial occlusion (200 mmHg) was applied for 5 minutes by the upper arm cuff. Immediately following the arterial occlusion, reactive hyperemic forearm blood flow was measured for a period of 4 minutes. Shear stress and alterations in hydrostatic pressure during this period result in the local release of NO and endothelium-derived hyperpolarizing factor (EDHP) [98], which induced smooth muscle cell relaxation. The FBF rise during reactive hyperemia relative to baseline provides an estimate for endothelium-induced dilating capacity [99]. That is to say, a lower Δ FBF is consistent with less endothelial-dependent vasodilatation. From the recorded FBF curve (fig. 1), we derived the following parameters: mean basal flow, peak flow during post-occlusion hyperemia and the difference between these two parameters (Δ FBF).

Figure 1. *Forearm Blood Flow (FBF) curve*



Statistical analysis

We compared the patient - and control groups using the non-parametric Mann-Whitney-U Test. To test the hypothesis that PV and Δ FBF in former patients are interrelated, we used a Spearman correlation test. Also for the exploration of correlations between Δ FBF on the one hand and factors that may induce endothelial dysfunction, we performed a Spearman correlation analysis. To determine the effect of an independent variable adjusted for the effect of other variables, we performed multiple linear regression analysis. This analysis was preceded by the verification of absence of collinearity between the variables tested. A p-value below 0.05 was considered statistically significant. All data are presented as median (Inter Quartile Range), unless stated otherwise.

RESULTS

Table I lists some demographic characteristics of the patient - and control groups. The two groups differed only in a higher parity and a more advanced gestation at birth in the control group.

Table I. *demographic and flow parameters in patients and controls.*

	PATIENTS (N=32)	CONTROLS (N=10)	P-VALUE
Age (years)	30.5 (27.0-33.8)	33 (31-36)	0.076
Body Mass Index ($kg.m^{-2}$)	24.0 (21.7-31.0)	23.4 (21.7-24.1)	0.298
Parity	1(1-1)	2 (1-2)	0.005
Delivery-study Interval (years)	0.78 (0.64-1.6)	1.7 (0.83-3.4)	0.174
Number of smokers (%)	4 (12.5%)	1 (10%)	0.675
Gestational age at birth (weeks)	34.6 (31.0-37.0)	39.8 (38.9-40.5)	< 0.001
Basal FBF ($ml.min^{-1}.100ml^{-1}$)	3.14 (2.66-3.76	2.80 (2.11-3.85)	0.637
Max FBF ($ml.min^{-1}.100ml^{-1}$)	17.3 (12.2-21.0)	18.1 (13.3-24.9)	0.679
Δ FBF ($ml.min^{-1}.100ml^{-1}$)	19.6 (15.8-24.7)	21.6 (17.1-27.3)	0.535

The Δ FBF in the former patients (21.0 ± 7.5 $ml.min^{-1}.100\ ml^{-1}$) did not differ ($p = 0.54$) from that in the controls (22.2 ± 6.8 $ml.min^{-1}.100\ ml^{-1}$), nor did the baseline FBF or maximal FBF.

We first analyzed whether the Δ FBF in the patient group correlated with some relevant demographic, hemodynamic and biochemical variables. There were no differences in delivery-to-study Interval, systolic blood pressure, pulse pressure, LV-mass, fasting insulin and HOMA index HDL-cholesterol and glomerular filtration rate between the two study groups. However the data listed in tables IIa and IIb illustrate that delivery-to-study Interval, body mass index (BMI, $kg.m^{-2}$), systolic blood pressure, pulse pressure, LV-mass, fasting insulin and HOMA index correlated negatively - and global arterial compliance, HDL-cholesterol and glomerular filtration rate (GFR) correlated positively with Δ FBF.

Table IIa. *Correlations in the patient group between ΔFBF on the one hand, and demographic - and cardiovascular variables on the other hand as calculated by Spearman Correlation Analysis. (D-S-interval: delivery to study interval; GA: Gestational Age; MAP: Mean Arterial Blood Pressure; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; PP: Pulse Pressure; Global compliance: ratio of stroke volume and pulse pressure; PV: Plasma Volume; LV-mass: left ventricle mass; GFR: Glomerular Filtration Rate)*

	MEDIAN (IQR)	SPEARMAN'S RHO	P-VALUE
Age (years)	31 (27-34)	-0.03	0.870
Parity	1 (1-1)	0.042	0.820
D-S Interval (years)	0.8 (0.6-1.6)	-0.357	0.040
GA at birth (weeks)	35(31-37)	0.089	0.630
PE severity score	4 (3-5)	-0.26	0.160
BMI ($kg.m^{-2}$)	24 (22-31)	-0.638	<0.001
MAP (mmHg)	86 (79-94)	-0.290	0.121
SBP (mmHg)	112 (106-128)	-0.486	0.007
DBP (mmHg)	69 (64-76)	-0.208	0.271
PP (mmHg)	45 (37-76)	-0.596	0.001
Global compliance (ml.mmHg-1)	1.6(1.4-1.8)	0.394	0.031
PV (ml/kg LBW)	47 (43-52)	0.302	0.092
LV-mass ($g.m^{-2}$)	129 (113-147)	-0.518	0.002
GFR ($ml.min^{-1}$)	116 (93-156)	0.516	0.002

Than we performed a stepwise backward multiple linear regression analysis with Δ FBF as the dependent - and the previously identified correlating parameters as independent variables: delivery-to-study interval, BMI, systolic blood pressure, pulse pressure, LV-mass, arterial compliance, GFR, glucose, insulin, HOMA index, LDL- and HDL-cholesterol, and homocysteine. Only BMI and pulse pressure contributed significantly to the regression resulting in the following equation: Δ FBF = 46.9 - 0.49 [BMI] - 0.28 [pulse pressure] ($r2=0.48$ and $p < 0.001$). The graphical representation of the relation between Δ FBF and BMI and pulse pressure is shown in figures 2 and 3.
In our patient group, PV and Δ FBF varied independently from one another.

Table IIb. *Correlations in the patient group between ΔFBF and biochemical variables calculated by Spearman Correlation Analysis. (Cholesterol, triglycerides and fasting glucose in mmol.L-1; fasting insulin in mU.L-1; Basal - and postchallenge homocystein in Ìmol.L-1) HOMA index= fasting serum insulin * fasting glucose/ 22.5*

	MEDIAN (IQR)	SPEARMAN'S RHO	P-VALUE
Total cholesterol (mmol.l^{-1})	4.7 (4.2-5.1)	-0.006	0.976
HDL-cholesterol (mmol.l^{-1})	1.2 (1.0-1.4)	0.377	0.033
LDL-cholesterol (mmol.l^{-1})	3.1 (2.5-3.4)	-0.038	0.837
HDL / LDL ratio (mmol.l^{-1})	0.43 (0.29-0.50)	0.347	0.051
Triglycerides (mmol.l^{-1})	0.88 (0.68-1.2)	-0.243	0.181
Fasting insulin (mU.l^{-1})	11 (5.4-16)	-0.488	0.005
Fasting glucose (mmol.l^{-1})	5.0 (4.7-5.4)	-0.208	0.253
Hba1c (%)	5.5 (5.4-5.7)	-0.094	0.610
HOMA-index	2.3 (1.2-3.4)	-0.514	0.003
Basal homocystein (umol.l^{-1})	9.4 (8.3-12.5)	0211	0.245
Postchallenge homocystein (umol.l^{-1})	36.2 (30.5-46.6)	0.004	0.981

Figure 2. *relation between BMI and FBF*

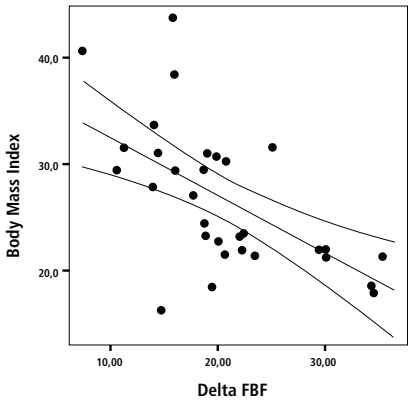
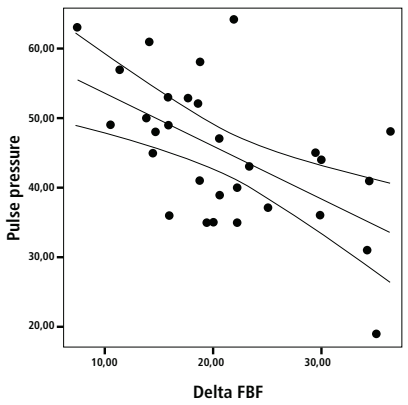


Figure 3. *relation between pulse pressure and FBF*



Discussion

Vascular endothelial dysfunction is one of the factors involved in the pathophysiology of preeclampsia [5, 100, 101]. In the present study performed at least six months postpartum, we did not find a difference in the endothelium-dependent vasodilator function between formerly preeclamptic women and normal parous controls. Multiple linear regression analysis indicated that BMI together with pulse pressure explained almost half (48%) of the variability in Δ FBF in the patient group. The preceding analysis on single variables suggested that the BMI in this correlation combined a number of features of the metabolic syndrome indicating that in our study population of former patients, the metabolic syndrome, that is associated with increased levels of circulating endothelial stressors, interferes most with endothelium-dependent vasodilatation. On the other hand, the contribution of pulse pressure, which is one of the factors used to estimate global arterial compliance, emphasizes the importance of arterial elastic properties to enable a normal response to vasodilator stimuli.

More than half of normotensive formerly preeclamptic women have a reduced PV [11, 19]. These women have a three times higher risk to develop recurrent hypertensive disease in a next pregnancy as compared to their counterparts with a normal PV [44, 88]. In addition, low PV in these women is consistent with a reduced venous capacitance [66] indicating that cardiovascular reserves are to be smaller [77]. Therefore, we were surprised that Δ FBF and PV varied independently from one another. This finding supports the view that the endothelial capacity to release vasodilator agents is probably not involved in the mechanism that leads to a subnormal PV. Interestingly, we noticed a higher incidence of low PV in former patients, which seemed at least in part due to overrepresentation of obese women. Therefore, it is conceivable that obesity and overweight, contribute independently to the recurrence risk of a hypertensive complications in pregnancy, by their negative impact on endothelial vasodilator capacity. This observation is in line with the accumulating evidence in the literature that obesity interferes with endothelial dysfunction 102 103 and therefore, can be expected to be associated with a higher risk to develop hypertensive disorders of pregnancy [104 105 10].

In conclusion, the present study provides evidence that 6 months after delivery, endothelium-dependent vasodilatation does not differ between formerly preeclamptic women and healthy parous controls. Decreased endothelium-dependent vasodilatation is an independent risk factor for hypertensive

complications in pregnancy. As this risk factor is associated with the metabolic syndrome, we speculate that it explains at least part of the extra recurrence risk in obese formerly preeclamptic women with a subnormal PV.

Chapter 7

General discussion

General Discussion

In this thesis we have studied the role of the plasma volume compartment in its relation to hypertensive disorders of pregnancy, because:

1. Plasma volume is subnormal in the early phase of essential hypertension [32]
2. In hypertensive complications of pregnancy, plasma volume is usually reduced [21 19, 33 34].
3. Plasma volume in women with a recent history of preeclampsia, is often low [11] and the presence of this characteristic predisposes to defective hemodynamic adaptation to pregnancy and subsequent recurrent hypertensive complications [25].

Chapter 2 describes our observations in formerly preeclamptic women with a low plasma volume. These women not only have a higher risk to develop recurrent hypertensive disorders in their next pregnancy. Relative to controls, they are also more obese, have less cardiovascular reserves, a lower global arterial compliance and more often raised circulating levels of endothelial stressors (triglycerides and insulin). Finally, their 1st and 2nd degree family members more often have cardiovascular disorders, particularly chronic hypertension.
Chapter 3 outlines our results in these former patients, when subjected to acute volume loading. Relative to parous controls, they respond to volume loading with a larger rise in α-atrial natriuretic peptide (α-ANP), pulse rate and cardiac output. In addition, they have a lower venous capacitance.
Chapter 4 presents our results regarding the response to mild exercise of former patients with a subnormal plasma volume relative to controls. In former patients exercise induces a smaller rise in stroke volume and cardiac output, a larger rise in α-ANP, and more cardiovascular drift. They are also unable to sustain the elevated stroke volume for the entire exercise period of 60 minutes, which suggests less cardiovascular reserves as compared to controls.
Chapter 5 describes our study, in which we demonstrate that former patients with the lowest plasma volume and venous capacitance have the highest chance to develop a recurrent hypertensive disorder in a next pregnancy.
Chapter 6 presents our data on the interrelation between low plasma volume and endothelial vasodilator capacity. Although these phenomena do not seem to be causally related, they are both more common in obese women. The data from this study provide evidence for low endothelial vasodilator function to be directly related to the metabolic syndrome, with low plasma volume only being a confounder.

From the observations in the previous chapters we hypothesize that formerly preeclamptic women with a low plasma volume have a condition of reduced cardiovascular reserves. The latter is characterized by a low venous capacitance (which limits the venous return to the heart), a higher sympathetic tone [26 85] giving rise to a higher heart rate and a higher average vascular tone and with it, lower global arterial compliance. In basal conditions the cardiovascular function will be normal, although also slightly hyperdynamic. The circulatory response to exercise (normal daily physical activities) will be normal, provided intensity and duration of the exercise is limited. However, these women are unable to sustain an elevated stroke volume and with it, cardiac output, when the exercise duration and/or intensity exceed a certain threshold, as a consequence of their reduced cardiovascular reserves [70, 71 72 49]. We postulate that the same constrains limit their ability to maintain an elevated stroke volume and cardiac output throughout pregnancy.

In human pregnancy systemic vasodilatation and the compensatory institution of a high flow and low resistance circulation is an essential adaptive response to pregnancy, even though its functional meaning is still incompletely understood [80]. In conjunction with the institution of a high flow and low resistance circulation, water and salt will be retained in order to raise plasma volume [14] and with it, cardiac preload. Women with a subnormal plasma volume have a reduced venous capacitance, which limits their ability to raise plasma volume in response to conditions of chronically elevated cardiac output. By volume expansion cardiac preload can be raised for a prolonged period, as opposed to the short-lasting rises in cardiac output, which are generated by a higher sympathetic tone [49 50]. Early sensing of venous fullness in women with a limited venous capacitance triggers ANP release and with it, volume dissipation (chapter 3). Previously, we demonstrated that these women respond to pregnancy with an aberrant rise in α-ANP, which can be expected to interfere with the expansion of the venous compartment [25]. We postulate that these women do not respond to pregnancy with plasma volume expansion, a defect, which limits their ability to sustain the elevated cardiac output for the entire length of pregnancy [14 74 76] and with it, creates the need for a compensatory higher sympathetic drive in the autonomic control of the circulation. As a consequence and also confirmed by us, the chance to develop a hypertensive complication is greater in these women than in their counterparts with a normally functioning volume homeostasis. Moreover, in the subclinical period preceding these complications, the circulation in these women is more hyperdynamic than in uneventful pregnancies [20, 60], suggesting that sympathetic dominance in the autonomic control of the circulation may

be accompanied by extra mechanical strain exerted upon the endothelium.

In women with a subnormal plasma volume, global arterial compliance is reduced. Arterial compliance is a property of the vascular wall that serves to protect the endothelium against excessive shear stress during systole. Therefore, the stiffer arteries in these women makes them prone to mechanical damage of their endothelium after the vasorelaxing stimulus has become operative at approximately 5 weeks of gestation. The systemic vasorelaxation in early pregnancy is accompanied by a complex series of events at the implantation site, which includes vascular remodeling of the spiral arteries. The sympathetic hyperactivity in women with a low plasma volume may be paralleled by a higher sympathetic tone in the spiral arteries with unfavorable effects on the downstream oxygen and nutrient supply. We speculate that the latter interferes with trophoblast invasion and differentiation resulting in defective placentation [106, 107].

Subnormal trophoblast invasion results in defective placentation. In the 1st half of pregnancy, the fetal and placental demands for oxygen and nutrients are relatively small and therefore, easily met by the uteroplacental supply. Afterwards, fetal size and growth become increasingly relevant requiring a rapidly growing supply of oxygen and nutrients. It follows that the chance that defective placentation causes a mismatch between uteroplacental supply and demand increases with advancing pregnancy. When that happens, intervillous hypoxia develops giving rise to both increased placental release of free oxygen radicals and microvillous debris into the maternal circulation. These compounds are known to damage the vascular endothelium triggering its dysfunction, which plays a key role in the pathogenesis of preeclampsia [5, 100, 101 108.]

Endothelial damage is associated with enhanced capillary leakage, which further reduces the already marginal size of the intravascular compartment. As a matter of fact, the latter represents an extra challenge for the already maladapted cardiovascular function, which required sympathetic dominance in the autonomic control of the circulation to compensate for the inadequate plasma volume expansion. The higher tone of the vasculature increases its stiffness causing more mechanical strain exerted upon the endothelium. The onset of the cascade of events eventually leading to the typical clinical symptoms of e.g. preeclampsia, is likely to start as early as in the first half of pregnancy as suggested by reports on abnormal findings in midtrimester in pregnancies becoming complicated in the 3rd trimester [109]. Although this cascade may be interrupted by compensatory mechanisms in some pregnancies, it is likely that in a significant number of these pregnancies, this cascade evolves into a negative spiral that eventually culminates into a manifest hypertensive complication. If so, the cardiovascular function begins to show the typical signs

of a hypersympathetic state. This combines a low output/high resistance circulation with a low plasma volume and also with hypertension and proteinuria [20]. The natural course of this condition is further deterioration of the maternal cardiovascular function, evidenced by severe hypertension, additional constriction of the plasma volume compartment secondary to increasing protein loss and accelerated edema formation, and finally hypoperfusion with variable dysfunction of nonvital organs (kidneys, liver, uteroplacental bed).

We postulate that in severe preeclampsia the reduced intravascular volume is the resultant of a cascade of events starting with a preexistent subnormal venous capacitance. The latter interferes with the normal response to the initial systemic vasorelaxation. That is to say, plasma volume retention remains inadequate for raising cardiac preload, so as to sustain the approximately 35% elevation in cardiac output for the entire length of pregnancy. As a consequence, the maternal cardiovascular system reacts with a so-called "backup" response consisting of sympathetic dominance in the autonomic control of the circulation. Although this backup enables normal cardiovascular function for at least the first half of pregnancy, it has various drawbacks. Firstly, it can be expected to impact placentation negatively by the higher tone in the arterial supply to the implantation site. Secondly, it leads to a less compliant vascular bed, thus raising the mechanical strain exerted upon the endothelial cells. Thirdly, it affects the metabolism by its insulin-antagonizing properties. Obviously, the degree of sympathetic overactivity may vary, as may its impact on each of these three effects, which may also act in concert to initiate the cascade that leads to a hypertensive complication of pregnancy.

This thesis answers several questions regarding the implications of a low plasma volume for a subsequent pregnancy. But it remains unclear why these normotensive formerly preeclamptic women have a reduced plasma volume. In these women we did not observe an increased prevalence of renal disorders, such as microalbuminuria or abnormalities in basal ERPF, GFR, α-ANP levels or renin activity, when compared to normal controls [44, 66]. Therefore, it seems highly unlikely that the subnormal plasma volume results from defects in the renal function or the volume regulatory system.

We have observed that post-occlusion forearm blood flow in these women is comparable to healthy parous controls, suggesting a normally functioning endothelium [110]. Therefore persisting endothelial dysfunction after preeclampsia (or even pre-existent endothelial dysfunction) is not likely to be the causally related to a subnormal plasma volume, despite the presence of more endothelial stress in these women [44, 110].

Potential candidates responsible for a subnormal plasma volume are:

1) Sympathetic dominance in the autonomic control of the circulation irrespective primary cause. This condition increases the tone of the venous compartment thus reducing venous capacitance and plasma volume [42, 43 85.]
2) Angiotensinogen polymorphisms, which can be associated with a reduced venous capacitance and plasma volume [61 9, 111].
3) A congenital smaller venous compartment and with it, venous capacitance in e.g. growth-restricted infants, which would be consistent with the Barker hypothesis [62].

With our current knowledge we cannot point out one of these factors as the sole cause of a subnormal plasma volume in our study group. It seems likely that the presence of a subnormal plasma volume may result of a combination of the above-mentioned factors.

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Summary

SUMMARY

Hypertensive complications of pregnancy are associated with perinatal- and maternal morbidity and mortality and occur in 6-8% of pregnancies. Underlying disorders such as thrombophilia, hyperhomocysteinemia and angiotensinogen polymorphisms are risk factors for preeclampsia. Formerly preeclamptic women constitute a heterogeneous population, but a large number of them have a low plasma volume. These women respond to pregnancy with circulatory maladaptation instead of the normal response, which consists of a decrease in vascular resistance and an increase in plasma volume and cardiac output. In complicated pregnancies plasma volume expansion is often subnormal. These observations support the view that low plasma volume is involved in inadequate adaptation to pregnancy.

Chapter 2 describes our observations in formerly preeclamptic women with a low plasma volume. These women have a higher risk to develop recurrent hypertensive disorders in a subsequent pregnancy and compared to controls they have a higher body mass index, less cardiovascular reserves, a lower global arterial compliance and raised circulating levels of endothelial stressors. A positive family history for cardiovascular disorders is more frequent in women with a low plasma volume.

In **Chapter 3** formerly preeclamptic women and healthy controls were subjected to acute volume loading. Relative to controls, women with a low plasma volume respond to volume loading with a larger rise in α-atrial natriuretic peptide, pulse rate and cardiac output. These observations support the presence of a reduced venous capacitance.

Chapter 4 presents the response to exercise of women with a low plasma volume compared to controls. Exercise induces a smaller rise in stroke volume and cardiac output, a larger rise in α-ANP and more cardiovascular drift in women with a low plasma volume. These women are unable to sustain an elevation in stroke volume for a period of 60 minutes, which suggests less cardiovascular reserves as compared to controls.

In **Chapter 5** we demonstrate that among formerly preeclamptic women those with the lowest plasma volume and lowest venous capacitance have the highest chance to develop a recurrent hypertensive disorder in a next pregnancy.

Chapter 6 presents our data on the relation between plasma volume and forearm blood flow in response to arterial occlusion as an estimate for endothelial function. Although a low plasma volume does not seem to be causally related to endothelial dysfunction, they are both more common in

obese women. This study provides evidence a relation between endothelial function and the metabolic syndrome.

We hypothesize that formerly preeclamptic women with a low plasma volume have reduced cardiovascular reserves. In these women cardiovascular function is normal but slightly hyperdynamic. The response to exercise of limited intensity and duration is normal.

Systemic vasodilatation and the institution of a high flow/low resistance circulation is an essential response to pregnancy. In pregnancy volume expansion raises cardiac preload for a prolonged period. Women with a limited venous capacitance have a high α-ANP release in response to plasma volume expansion and do not respond to pregnancy with adequate plasma volume expansion. These women are unable to sustain the elevated cardiac output throughout pregnancy. A compensatory rise in cardiovascular sympathetic drive is achieved in order to meet the higher circulatory demands of pregnancy. But this results in extra mechanical strain upon the endothelium. Systemic vasorelaxation is accompanied by vascular remodeling of the spiral arteries. A higher sympathetic tone in the spiral arteries results in defective placentation and a smaller placenta.

After 20 weeks of gestation fetal growth may cause a mismatch between uteroplacental supply and fetal demand. As a result intervillous hypoxia leads to release of free oxygen radicals and microvillous debris into the maternal circulation. This causes endothelial damage, capillary leakage and reduction of the intravascular compartment. This cascade evolves into a negative spiral that results in a hypersympathetic state with a low output/high resistance circulation and hypertension. Additional constriction of the plasma volume secondary to increasing protein loss and accelerated edema formation, and finally hypoperfusion of nonvital organs will occur.

It remains unclear why these formerly preeclamptic women have a reduced plasma volume. We did not observe renal disorders and it seems unlikely that the subnormal plasma volume results from defects in the renal function.

We have observed that endothelial function is comparable to healthy controls. Therefore persisting endothelial dysfunction is not likely to be related to a subnormal plasma volume.

Potential candidates responsible for a subnormal plasma volume are: 1) Sympathetic hyperactivity. 2) Angiotensinogen polymorphisms. 3) A congenital reduction of the venous compartment in accordance with the Barker hypothesis. It is likely that a subnormal plasma volume is caused by a combination of these factors.

Samenvatting

SAMENVATTING

Hypertensieve aandoeningen van de zwangerschap gaan gepaard met aanzienlijke perinatale– en maternale morbiditeit en mortaliteit. Deze aandoeningen komen voor in 6-8% van alle zwangerschappen. Onderliggende aandoeningen zoals stollingsafwijkingen, hyperhomo-cysteinemie en angiotensinogeen polymorphismen, zijn risicofactoren voor preeclampsie.

Hoewel vrouwen met een voorgeschiedenis van preeclampsie een heterogene groep vormen heeft een groot deel van deze vrouwen een laag plasma volume. Deze vrouwen vertonen een gestoorde circulatoire aanpassing aan de zwangerschap. In plaats van de normale aanpassing waarbij de vaatweerstand daalt en het plasma volume en het hart minuut volume stijgt.

Bij gecompliceerde zwangerschappen is er vaker sprake van een onvoldoende toename van het plasma volume. Dit ondersteunt de gedachte dat een laag plasma volume betrokken is bij een inadequate aanpassing aan de zwangerschap.

Hoofdstuk 2 beschrijft de waarnemingen bij een groep vrouwen met een laag plasma volume en preeclampsie in de voorgeschiedenis. Deze vrouwen hebben een verhoogd risico op het opnieuw doormaken van een hypertensieve aandoening in een volgende zwangerschap en hebben, in vergelijking met gezonde vrouwen, een hogere body mass index, een verminderde cardiovasculaire reserve, verminderde globale arteriële compliantie en verhoogde serumspiegels van endotheliale stressoren. Daarnaast hebben zij vaker een positieve familie anamnese voor cardiovasculaire aandoeningen.

In **Hoofdstuk 3** worden zowel vrouwen met een voorgeschiedenis van preeclampsie als gezonde proefpersonen blootgesteld aan een acute volumebelasting. In vergelijking met de gezonde proefpersonen vertonen vrouwen met een laag plasma volume een sterkere stijging van het α-atrium natriuretisch peptide, hart frequentie en hart minuut volume. Deze waarnemingen ondersteunen de aanwezigheid van een verlaagde veneuze capacitantie

Hoofdstuk 4 toont de respons van vrouwen met een verlaagd plasma volume op inspanning in vergelijking met gezonde proefpersonen. Gedurende inspanning vertonen vrouwen met een laag plasma volume een geringere stijging van het slagvolume, een sterkere stijging in α-ANP en meer cardiovasculaire drift. Daarnaast blijken zij niet in staat om een stijging in het slagvolume gedurende 60 minuten vol te houden, hetgeen een verminderde cardiovasculaire reserve suggereert.

In Hoofdstuk 5 tonen we aan dat onder vrouwen met een voorgeschiedenis van preeclampsie diegene met het laagste plasma volume en de laagste veneuze capacitantie het meeste kans hebben op het opnieuw doormaken van een hypertensieve aandoening in een volgende zwangerschap.

In Hoofdstuk 6 presenteren we onze gegevens met betrekking tot de relatie tussen plasma volume en post occlusie onderarms doorbloeding als een maat voor endotheel functie. Hoewel er geen verband blijkt te bestaan tussen plasma volume en endotheel dysfunctie, komen beide vaker voor bij obese vrouwen. Deze studie toont aan dat er een verband is tussen endotheel functie en het metabole syndroom.

Onze hypothese is dat vrouwen met een voorgeschiedenis van preeclampsie en een laag plasma volume een verminderde cardiovasculaire reserves hebben. Cardiovasculair gezien functioneren deze vrouwen normaal, zij het dat er sprake is van een lichte mate van hyperdynamische circulatie. Daarbij is hun respons op lichte inspanning van een korte duur ongestoord.

Een systemische vaatverwijding en het tot stand komen van een hoge flow/lage weerstand circulatie is een essentiele aanpassing aan de zwangerschap. Bij zwangerschap ondersteunt een plasma volume expansie een langdurige toename van de cardiale pre-load. Vrouwen met een verminderde veneuze capacitantie vertonen bij volume expansie een sterke stijging van α-ANP en onvoldoende toename van het plasma volume tijdens de zwangerschap. Daardoor zijn deze vrouwen niet in staat om een verhoging van het hart minuut volume gedurende de gehele zwangerschap in stand te houden. Om toch aan de verhoogde circulatoire behoefte van de zwangerschap te voldoen ontstaat een compensatoire toename van de sympathische activiteit. Dit resulteert echter in een toename van de mechanische stress op het endotheel.

Systemische vaatverwijding in de zwangerschap gaat gepaard met vasculaire remodelering van de spiraal arterieën. Een hoge sympatische tonus in de spiraal arterieën resulteert in een verstoring van de placentatie en daardoor ook in een kleinere placenta.

Na de 20e week van de zwangerschap zal door foetale groei de balans tussen uteroplacentair aanbod en foetale behoefte verstoord raken. Hierdoor ontstaat intervilleuze hypoxie waarbij vrije zuurstofradicalen en microvilleuze fragmenten in de maternale circulatie vrijkomen. Dit veroorzaakt endotheel schade met een toename van capillaire permeabiliteit en een afname van het intra vasculaire compartiment. Dit resulteert in een negatieve spiraal met een sterk hypersympathische toestand en een bloedsomloop die gekenmerkt wordt door een lage flow/hoge weerstand en een forse toename van de bloeddruk. Dit

zorgt voor verdere afname van het plasma volume als gevolg van eiwit verlies en oedeemvorming en uiteindelijk tot verminderde doorbloeding van niet vitale organen.

Het is nog niet duidelijk waardoor vrouwen met een voorgeschiedenis van preeclampsie een verlaagd plasma volume hebben. Aangezien we bij hen geen evidente nieraandoeningen hebben waargenomen is het onwaarschijnlijk dat het lage plasma volume het resultaat van nierlijden is. De endotheelfunctie is vergelijkbaar met die van gezonde proefpersonen waardoor het ook onwaarschijnlijk is dat het verlaagde plasma volume gerelateerd is aan endotheel dysfunctie.

Mogelijke oorzaken voor het verlaagd plasma volume kunnen worden gezicht in: 1) Sympathische hyperactiviteit. 2) Angiotensinogeen polymorphismen. 3) Een aangeboren klein veneuze compartiment overeenkomstig de Barker hypothese. Meest waarschijnlijk is het verlaagde plasma volume het gevolg van een combinatie van deze factoren.

Dankwoord

Het verrichten van klinisch wetenschappelijk onderzoek is slechts mogelijk met de medewerking van velen. Tijdens en voorafgaand aan het schrijven van dit proefschrift ben ik niet alleen op wetenschappelijk gebied geschoold, maar ook in de kliniek gevormd. Middels dit dankwoord wil ik ieder die daaraan heeft bijgedragen danken en enkelen, zonder anderen te kort te willen doen, in het bijzonder noemen.

Allereerst wil ik Inez Schreij en Timo Ekhart noemen. Beste Inez en Timo, in de artikelen heet onze onderzoekskamer heel mooi een **temperature controlled room**. In de praktijk is het een constant warm en donker hok, waar wij vele uren in opperste concentratie hebben doorgebracht om metingen bij proefpersonen te doen en daarna de verzamelde data en monsters te bewerken. Bepaald niet altijd een makkelijke opgave. Zonder jullie inzet, kennis en kunde was er niet één proefpersoon gemeten. Daarvoor, en voor alle gezelligheid binnen en buiten de onderzoekssetting, wil ik jullie beiden hartelijk bedanken.

Dr. L.L.H. Peeters, beste Louis, de door jouw geleide onderzoeksgroep is een bijzonder prettige thuisbasis voor het doen van onderzoek. Jouw manier van aansturen van deze groep wordt gekenmerkt door betrokkenheid, enthousiasme, inventiviteit, creativiteit, vrijheid (in de goede zin van het woord dus zonder vrijblijvendheid) en een enorme kennis op het gebied van (patho)fysiologie van de zwangerschap. Ik heb me er als een vis in het water gevoeld. Aan onze brainstormsessies, statistische bijeenkomsten, voorbespreken van presentaties en artikelen, onderzoeksgroep barbeques en diners denk ik met veel plezier terug. De keren dat je, liefst een kwartier voor het begin van een voordracht, op bijvoorbeeld de SGI, nog met enkele welgemeende suggesties kwam ter verbetering van dia's zijn in je in het licht van het voorgaande zeker vergeven. Ik ben je dankbaar dat je hebt aangedurfd om met mij het AGIKO traject in te gaan en ben blij met de manier waarop we dit traject bewandeld hebben.

Prof. dr J. de Haan, professor, tijdens een congres verzocht U mij een sollicitatie brief naar Maastricht te sturen. Heel snel kreeg ik desondanks antwoord dat er vele andere sollicitanten waren en dat de kans op het verwerven van een opleidingsplaats niet erg groot was. Gelukkig werd ik niet lang daarna door U toch aangenomen om in Maastricht tot gynaecoloog en klinisch onderzoeker te worden opgeleid. Op enige afstand bleef U al die jaren een constante factor die de wetenschappelijk voortgang bewaakte. Daarvoor hartelijk dank.

Prof. dr. P.W. de Leeuw, Uw praktische inbreng en suggesties ter verbetering bij de bespreking van het onderzoek en de artikelen die daar uit voortvloeiden hebben zonder meer geleid tot meer kwaliteit in de publicaties. De manier waarop dat gebeurde, zakelijk, maar toch aangenaam en met veel kennis van zaken, heeft mijn waardering.

Dr. M.E.A. Spaanderman, beste Marc, in de onderzoekslijn ben jij mijn directe voorganger en dus mijn voorbeeld. Hoewel op vele gebieden tegenpolen, zijn we echte maatjes geworden. Dat jij co-promotor bent, is niet alleen heel prettig maar ook volkomen logisch. In het begin van mijn onderzoeksperiode hielp jij mij op weg met praktische en theoretische adviezen en bracht jij me bij wat ik nog niet wist. Terwijl je later juist met kritische vragen en stellingen het onderzoek de goede weg op stuurde. Niet alleen in de wetenschap, maar juist ook daarbuiten ben ik je dankbaar voor je vriendschap en steun. Ik ga er vanuit dat we in wetenschap en daarbuiten elkaars maatje blijven.

De leden van de beoordelingscommissie: Prof. dr. J.G. Nijhuis, Prof. dr. H.W. Bruinse, Prof. dr. H. Kuipers, Prof. dr. E.A.P. Steegers en Dr. J. Kooman wil ik bedanken voor de tijd en de moeite die zij besteed hebben aan het beoordelen van dit proefschrift.

Het laatste hoofdstuk van dit proefschrift was niet mogelijk geweest zonder het werk van Tessa Lommerse en Boy Houben, hartelijk dank.

Dank ook aan de opleiders tijdens mijn opleiding tot gynaecoloog: Dr. M.P.M.L. Snijders, Prof. dr. J. de Haan, Prof. dr. J.L.H. Evers en Dr. H.J. van Geuns die ieder op verschillende wijze hebben bijgedragen aan de bagage waarmee ik nu als gynaecoloog op pad ben. Daarnaast wil ik uiteraard ook de overige stafleden, assistenten en verpleegkundigen van het VieCuri Medisch Centrum en het academisch ziekenhuis Maastricht danken voor hun bijdrage aan mijn opleiding.

Voordat ik in opleiding kwam, was ik erg lang Agnio (met de N van Nooit dacht ik weleens). In deze periode heb ik van een groot aantal specialisten, mede-assistenten en verpleegkundigen veel geleerd. Helaas zijn het er te veel om allemaal te noemen.

In het bijzonder wil ik toch bedanken Dr. J. Van Eyck en Dr. B. Arabin, beste Birgit en Jim, met jullie passie voor kliniek, wetenschap, opleiding, kunst en cultuur, hebben jullie me de eerste stappen in de wetenschap laten zetten. Jullie maakten het voor mij mogelijk om posters en voordrachten te presenteren

op tal van internationale congressen. Dankzij die ervaring en de Zwolle-Maastricht connectie kwam er een vervolg als AGIKO in Maastricht.

De Perim onderzoeksgroep: Louis Peeters, Mariane Curfs, Marc Spaanderman, Timo Ekhart, Inez Schreij, Hugo van Eijndhoven, Olivier van der Heijden, Dorette Courtar, Audrey Coumans en Michael Kars. We vormden tijdens onze onderzoeksperiode een band die zo stevig was als beton. Naast de wetenschap, is vooral ook het sociale aspect een belangrijk en aangenaam onderdeel van deze groep. Afgezien van de vaste leden van de groep, zijn we langzamerhand allemaal uit het Perim-nest gevlogen om onze eigen weg te gaan. Dat het jullie allen goed moge gaan.

Mijn maten van de maatschap gynaecologie van het Maaslandziekenhuis te Sittard wil ik bedanken voor het feit dat wij ook echt een Maatschap van Maten vormen. In de afgelopen tijd hebben jullie me de ruimte gegeven om aan het afronden van dit proefschrift te werken, ik ben jullie daar dankbaar voor.

Ik mag mij gelukkig prijzen dat ik bij het verdedigen van dit proefschrift terzijde zal worden gestaan door de paranimfen Nathalie van Breugel en Olivier van der Heijden.

Beste Olivier, de weg van overbuurman en co-assistent in Zwolle, via mede onderzoeker in de Perim-family, reisgenoot op SGI trips (waar we vele onvergetelijke momenten hebben meegemaakt), mijn paranimf zijn bij jouw promotie en jouw paranimf zijn bij het verdedigen van mijn proefschrift is lang. In de meer dan tien jaar die achter ons ligt hebben we dan ook veel meegemaakt, was je mij vaak tot steun en waren we aanwezig bij bijzondere momenten in elkaars leven. Daarom is het passend dat ik jou als paranimf heb gevraagd en ben ik blij dat je dat ook daadwerkelijk wil doen. Nog even bikkelen en dan zit jouw opleiding er ook op, je zult zien dat het sneller gaat dan je denkt.

Beste Nathalie, als de dag van gisteren herinner ik me onze eerste ontmoeting in het rookhol van de Universiteit aan de UNS 50. Jij vertelde iets te willen doen met een keuzeblok perinatologie of neonatologie. Jij werd student-assistent bij dit promotieonderzoek en was ook nog nauw betrokken bij het ontwikkelen van een ander onderzoeksprotocol dat nog steeds loopt. In de loop der jaren ben je een trouwe vriendin van zowel Suzanne als mij geworden. Het enthousiasme waarmee je accepteerde om paranimf te worden heeft me blij verast. Van ons drieën (jij, Olivier en ik) heb je qua promotie en opleiding nog de langste weg te gaan. Ik ben er echter van overtuigd dat je die weg zonder veel hobbels zult afleggen en wens je daarbij veel succes.

Dankwoord

Mijn ouders zijn beiden niet meer in leven. Echter, zij hebben mij in staat gesteld te worden wie ik ben en hebben daardoor een wezenlijke bijdrage geleverd aan dit proefschrift.

Met name mijn moeder had heel graag bij de verdediging van dit proefschrift aanwezig willen zijn. Helaas is dat haar niet gegund. Gelukkig heb ik aan haar ziekbed de laatste versie van dit manuscript met haar door kunnen nemen. Uit dankbaarheid naar mijn beide ouders, draag ik dit proefschrift speciaal op aan mijn moeder.

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Als laatste, Suzanne. Natuurlijk kom jij voor mij juist in elk opzicht op de eerste plaats.

Wij hebben in het recente verleden indrukwekkende hoogte- en dieptepunten meegemaakt. Het feit dat jij bij deze gebeurtenissen aan mijn zijde stond maakt mij een gelukkig mens. Ik ben je dankbaar voor jouw steun door dik en dun.

Het voltooien van dit proefschrift valt, overigens geheel toevallig, samen met een heel nieuwe fase in jouw leven. Ik wens jou en ons toe dat we in elke fase van de rest van ons leven samen gelukkig zullen zijn.

Curriculum Vitae

Robert Aardenburg werd op 4 oktober 1962 geboren te Ruinen in Drenthe, zoon van een Antwerpse moeder en een Beverwijkse vader. Hij bracht zijn jeugd tot aan het einde van zijn middelbare schooltijd door op de Veluwe met name in Wageningen.
In 1982 begon hij de studie geneeskunde aan de Universiteit Maastricht (destijds Rijksuniversiteit Limburg). Na het behalen van het artsexamen in november 1988 vervulde hij de militaire dienstplicht van 1990 tot 1991 als reserve officier-arts.

Daarna begon een periode van assistentschappen in de Sint Elisabethkliniek te Heerlen (Dr. F.J.M.E. Roumen & Dr. P.X.J.M. Bouckaert), Academisch Ziekenhuis Maastricht (Prof. Dr. G. Kootstra), Elkerliek Ziekenhuis te Helmond (Dr. R. Barentsen) en Sophia Ziekenhuis te Zwolle (Dr. J. van Eyck).

In 1997 starte hij als AGIKO met de opleiding tot gynaecoloog en klinisch onderzoeker. Het niet academisch deel van de opleiding vond plaats in het VieCuri Medisch Centrum te Venlo (opleiders Dr. M.P.M.L. Snijders en later Dr. H.J. van Geuns). In het academisch ziekenhuis Maastricht werd het academisch- en onderzoeksdeel van de opleiding gevolgd (opleiders: Prof. dr. J. de Haan en later Prof. dr. J.L.H. Evers).

In 1996 werd hij genomineerd voor the young investigator award tijdens het 6th World Congress on Ultrasound in Obstetrics and Gynecology te Rotterdam en in 2004 won hij tijdens de 51st annual scientific meeting van de Society for Gynecological Investigation te Houston (TX) de Burroughs Wellcome travel award.

Sinds September 2005 is hij als gynaecoloog werkzaam in de maatschap gynaecologie van het Maaslandziekenhuis te Sittard met als aandachtsgebied de verloskunde.
Hij woont samen met Suzanne Meursinge in Einighausen.