Circulatory response to pregnancy in formerly preeclamptic women and healthy parous controls

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CIRCULATORY RESPONSE TO PREGNANCY IN FORMERLY PREECLAMPTIC WOMEN
AND HEALTHY PAROUS CONTROLS

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CIRCULATORY RESPONSE TO PREGNANCY IN FORMERLY PREECLAMPTIC WOMEN AND HEALTHY PAROUS CONTROLS

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit Maastricht,
op gezag van de Rector Magnificus,
Prof. dr. A.C. Nieuwenhuijzen Kruseman,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op vrijdag 16 februari 2001 om 14:00 uur

door

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CHAPTER 1

GENERAL INTRODUCTION

CHAPTER 1

General introduction

High blood pressure complicates almost 10% of all pregnancies ¹. It is, together with obstetric hemorrhage, embolism and obstetric infection, the most common cause of maternal mortality. In the western world, an incidence of 1.7-1.8 maternal deaths per 100,000 live births has been reported ^{2, 3}. Since maternal hypertension predisposes for fetal growth restriction and iatrogenic preterm delivery, it is also an important cause of perinatal morbidity and mortality.

Elevated blood pressure during pregnancy can be subdivided into a form that antedates pregnancy (chronic hypertension) and a form that is confined to the period of pregnancy (pregnancy-induced hypertension). Pregnancy-induced hypertension complicated by proteinuria is called preeclampsia while preeclampsia developing in women with preexistent hypertension is called 'superimposed preeclampsia' ¹. In preeclampsia, generalized vasospasm, increased capillary permeability and enhanced platelet aggregation hamper the cardiovascular function. These functional defects appear to be triggered by endothelial dysfunction ⁴. They may initiate a vicious circle, which leads to further jeopardy of the endothelial function and with it, deterioration of the hypertension, enhanced activation of the clotting system, hemolysis, and finally, besides progressive placental insufficiency, compromised function of several maternal organs such as the kidneys, liver and in case of eclampsia, the brain.

Since the cause of preeclampsia is still obscure, current management is limited to symptomatic relief and delivery. Unfortunately, only the latter can be considered causal as it involves the removal of the placenta, the presumed initiator of the pathogenic cascade. For a more appropriate causal treatment of preeclampsia, a better insight in its pathogenesis is mandatory.

Morphologic evidence for endothelial-cell injury may be obtained by fundoscopic inspection of the retina. However, the typical renal lesion known as glomerular endotheliosis is pathognomonic ⁵. It is characterized by diffusely enlarged, bloodless glomeruli with, in the endothelial cells, hypertrophy of the cytoplasmatic organelles, particularly the lysosomes. Occasionally, sub-endothelial and mesangial electron-dense deposits, most likely fibrin, parallel these histologic features ⁵. Also biochemical markers for endothelial damage have been reported. These include elevated plasma levels of the following compounds: Von Willebrand factor, plasminogen activator (tPA) and plasminogen activator-inhibitor-1 (PAI-

1) endothelin and cellular fibronectin ⁶. Endothelial dysfunction may also be responsible for the observed imbalance between vasoconstrictor and vasodilator stimuli, such as between prostacyclin (PGI₂) and thromboxane (TXA₂), but also between plasminogen activator (tPA) and plasminogen activator-inhibitor-1 (PAI-1) ^{6,7}.

Epidemiological studies indicate that endothelial dysfunction may either have a more biochemical (i.e. thrombophilia, diabetes mellitus, hypercholesterolemia) or a more mechanical (i.e. hypertension, renovascular disease) background ⁸. This supports the view that preeclampsia could be a disease state superimposed upon a preexisting, often subclinical disorder. This concept is in line with the findings that, firstly, fetal growth restriction and preeclampsia in advanced pregnancy are preceded by defective maternal hemodynamic adaptation in the first weeks of pregnancy ^{9,10}. Secondly, a substantial part of apparently-healthy normotensive formerly-preeclamptic women had at least a thrombophilic disorder or persistent abnormalities in renal hemodynamics and volume status ^{11,12,13}. Whether these abnormalities were already present prior to pregnancy or to what extent they predispose for recurrent preeclampsia, remains to be elucidated.

In healthy women, hemodynamics and volume homeostasis vary with the menstrual cycle ^{14,15}. The reported data provide indirect evidence for mild vasorelaxation in the luteal phase relative to the follicular phase of the menstrual cycle. In this respect, although much less profound, the luteal phase mimics the first weeks of pregnancy ^{16,17}. Neither the mechanism responsible for these hemodynamic changes nor its functional meaning is fully understood.

The pathogenesis of hypertensive complications in pregnancy may begin as early as during implantation as indicated by shallow trophoblast invasion, different trophoblast maturation and abnormal hemodynamic adaptation ^{9,10,18}. This implies that most of the pathogenic cascade evolves sub-clinically over a period of at least 5 months. It is obscure, whether the heterogeneity in predisposing conditions as mentioned above, gives rise to a similar cascade of events in the period between implantation and endothelial dysfunction. Most early-pregnancy changes in maternal hemodynamics are compensations in response to systemic vasorelaxation ^{16,17,19,20}. That is to say, cardiac output and the activity of the renin-angiotensin-aldosterone system (RAAS) increase, while plasma volume expands. Theoretically, formerly preeclamptic women with a thrombophilic phenotype ('biochemical' risk-group) and normal cardiovascular function should have a normal menstrual and early-pregnancy hemodynamic compensatory response. Conversely, formerly preeclamptic women with diminished cardiovascular reserves in conjunction with hemodynamic abnormalities such as

hypertension ('mechanical' risk-group), are more likely to respond to pregnancy either with attenuated initial vasorelaxation or to the latter with abnormal hemodynamic compensations. If the so-called 'biochemical' and 'mechanical' risk groups do indeed differ with respect to hemodynamic cyclicity with the menstrual cycle and/or the adaptive response to pregnancy, it not only would explain the disappointing outcome of a number of recently reported prophylactic strategies in unselected groups of women at risk for preeclampsia ²¹. It would also improve our understanding of the pathogenesis of preeclampsia. The latter again is a prerequisite for the development of an effective 'tailor-made' prophylaxis for selected risk groups.

This thesis compiles six separate studies, which were designed to test the following hypotheses;

- 1. Formerly preeclamptic women have at least one underlying disorder either with a phenotype being thrombophilia or reduced (reno)vascular function (chapter 2).*
- 2. Cyclic changes in hemodynamic and renal function in formerly preeclamptic women differ from those with a history of a normal pregnancy (chapter 3).*
- 3. The early-pregnancy development of a high flow/low resistance circulation is not induced by a rise in metabolic demands (chapter 5).*
- 4. Only formerly preeclamptic women with reduced (reno)vascular function respond to pregnancy with initial cardiovascular maladaptation, while those with a thrombophilic phenotype show a normal early-pregnancy adaptive response (chapter 4 and 6).*
- 5. Pre-pregnant cardiovascular and clotting variables in formerly preeclamptic women predict hypertensive complications and fetal growth restriction in the subsequent pregnancy (chapter 7).*

To test these hypotheses, we studied once, the clotting function, immune and homocysteine status and serially, the central and renal hemodynamic function, vascular compliance and volume homeostasis and its changes throughout the menstrual cycle and in early pregnancy, in both formerly preeclamptic women and normal parous controls.

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CHAPTER 2

ASYMPTOMATIC FORMERLY PREECLAMPTIC WOMEN HAVE LATENT HEMODYNAMIC ABNORMALITIES

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Abstract

Background The majority of women with pregnancy complicated by preeclampsia have hypertension and/or a disorder with a thrombophilic phenotype. In this study we evaluated whether hemodynamics and volume homeostasis in a subgroup of normotensive formerly preeclamptic women with a normal clotting function (defined as “asymptomatic subgroup”) are comparable to that in normal parous controls.

Methods In 58 formerly preeclamptic subjects and 11 normal parous controls we measured at least 5 months postpartum, at day 5 ($\pm$ 2) of the menstrual cycle the following variables: body weight and length, mean arterial pressure, heart rate, cardiac output (CO), plasma volume (PV), glomerular filtration rate, effective renal plasma flow, and in plasma, volume regulatory hormones, clotting factors, antiphospholipid antibodies and homocysteine levels before and after a methionine load. From the measured data, we calculated body mass index, body surface area, cardiac index, left ventricular work (LVW), total peripheral - and renal vascular resistance's, effective renal blood flow and renal filtration fraction.

Results Among formerly preeclamptic women 26 were normotensive thrombophilic (45%), 14 hypertensive (24%) and 18 normotensive non-thrombophilic (31%). The asymptomatic formerly preeclamptic subjects were more obese than controls. They also had a higher cardiac output and left ventricular work, and a lower plasma volume than the normal parous controls. Therefore, they resemble the second subgroup of formerly preeclamptic subjects with hypertension rather than the controls. The third subgroup of formerly preeclamptic subjects consisted of normotensive women with thrombophilia. The hemodynamic and renal function in this subgroup was similar to that in normal parous controls.

Conclusion On the basis of this study we conclude that hemodynamics and volume homeostasis in the asymptomatic subgroup of formerly preeclamptic women are different from those in normal parous controls. Hemodynamics and volume homeostasis in this subgroup resembles that in of hypertensive

formerly preeclamptic women. Therefore, we propose to classify the asymptomatic formerly preeclamptic subjects as 'latent' hypertensives.

CHAPTER 2

Introduction

Increasing evidence supports the concept that systemic arterial vasodilation represents the first detectable systemic hemodynamic change in pregnancy. The latter then initiates a cascade of compensations in the circulation and volume homeostasis^{1,2} that include, among others, a rise in cardiac output (CO), hemodilution and activation of the renin-angiotensin-aldosterone system. Often, preeclampsia is preceded by abnormalities in these early-pregnancy changes³.

Most symptoms in preeclampsia develop secondary to endothelial dysfunction⁴. Conditions such as thrombophilia and hypertension are known to interfere with endothelial function. This may explain why women with these disorders are more prone to develop preeclampsia in pregnancy⁵. In the latter study, the authors reported an approximately 50% incidence of thrombophilia and about 25% incidence of chronic hypertension among formerly preeclamptic (ex-PE) women. Whether hemodynamic status and volume homeostasis in the remaining approx. 25% seemingly healthy, formerly preeclamptic women is indeed entirely normal and thus indistinguishable from that in normal parous controls, is still unsettled. Evidence for latent abnormalities in their hemodynamic - and/or volume regulatory function could mean that preeclampsia is in the majority of cases superimposed upon a pre-existing disorder. This would contribute importantly both to our understanding of the pathogenesis of preeclampsia and to the development of prophylactic strategies.

The objective of the present study was to test the hypothesis that hemodynamics and volume homeostasis in formerly preeclamptic individuals without thrombophilia and without hypertension differs from those in normal parous controls. To this end, we studied 58 formerly preeclamptic women subdivided over a thrombophilic -, a hypertensive - and a seemingly healthy ("asymptomatic") subgroup. These subgroups were compared with one another and with a group of normal parous controls.

Patients and Methods

Patients

In this study 58 formerly preeclamptic non-diabetic women and 11 normal parous controls were enrolled. Participants were at least 5 months post partum. All were Caucasian. None were smoking during pregnancy. Formerly preeclamptic women were recruited at the outpatient clinic at the postpartum follow-up. Most of them had a severe form of preeclampsia, either early in pregnancy or with a rapid onset later on. Controls were recruited by advertisement. All participants gave written informed consent. The hospital's medical-ethical committee approved the study. Preeclampsia and hypertension were defined according to the criteria of the International Society for the Study of Hypertension in Pregnancy (ISSHP) ⁶. All experiments were preceded by one week of standardized sodium intake (100 mmol sodium per day), representing the mean sodium intake in our population. The latter required that all participants consulted the hospital's dietitian prior to study. Women's compliance with the diet was evaluated by quantifying 24-hour sodium output on the day before the experiment. None of the women used vitamins, oral contraceptives, non-steroid anti inflammatory drugs or glucocorticosteroids in the two weeks prior to measurement. Two women used antihypertensive drugs. This medication was discontinued at least one week prior to measurement. On separate days and after an overnight fast, participants were tested for thrombophilia and hemodynamic function.

Methods

Thrombophilic screening: Anticardiolipin antibodies (ACA) for IgM and IgG were determined spectrophotometrically by enzyme-linked immunoassay ⁷. Lupus anticoagulant (LA) was determined by mixing 1:1 patient and pooled normal plasma after finding a prolonged partial thromboplastin time. Subsequently, a thromboplastin dilution test was performed by measuring prothrombin time with progressive diluted thromboplastin ⁸. Lupus anticoagulant was considered to be present when all assays were positive. The presence of anticardiolipin antibodies and/or lupus anticoagulant was, when higher than 10 GPL for IgG anticardiolipin antibodies and 6 MPL for IgM anticardiolipin antibodies, considered to be indicative of the antiphospholipid syndrome (APS).

Coagulation tests were performed after a flawless venapuncture. Blood was collected in sodium citrate containing glass tubes. Plasma was prepared by two consecutive centrifugation steps, with the second one performed in an Eppendorf centrifuge to eliminate

platelets. Samples were stored at -80 °C prior to assay. Protein C activity was determined with Coamate protein C (Chromogenix, Mölndal, Sweden) ⁹. The normal value of protein C according to the laboratory control group is 70% to 130% and has an intra-assay and inter-assay coefficient of variation of 3.3% and 4.7%, respectively. Total protein S antigen and free protein S were measured by an enzyme-linked immunosorbent assay (ELISA) (DAKO, Glostrup, Denmark) ¹⁰. The normal range of both total protein S antigen (intra-assay and inter-assay coefficient of variation of 5.1% and 5.7%) and free protein S (intra-assay and inter-assay coefficient of variation 3.4% and 3.6%) is 70% to 130% and 65% to 130%, respectively. Activated protein C resistance (APCR) was quantified by a chromogenic APC-resistance assay ¹¹. The chromogenic test fully matches DNA analysis for the factor V Leiden mutation and has a normal range between 0% and 20% ¹¹. Antithrombin activity (AT) was assayed with chromogenic substrate S-2765 (Chromogenix, Mölndal, Sweden) ¹². The normal range of AT is 80% to 120%.

A methionine-loading test was used to diagnose hyperhomocysteinemia (HHC). Homocysteine was measured by high-performance liquid chromatography with fluorescence detection ¹³. In our hands, this test has an intra- and inter-assay coefficient of variation of 3.9% and 5.9%, respectively. At 8:30 AM, the fasting homocysteine concentration was determined. Six hours after an oral dose of L-methionine (0.1 g/kg body weight), the post-load homocysteine concentration was assessed. During the test participants used a standard low-methionine breakfast and lunch. Individuals were considered to have hyperhomocysteinemia when fasting and/or post-load plasma homocysteine levels were more than 2 standard deviations higher than the mean of the laboratory control group.

Measurement of blood pressure and renal hemodynamics: In the mid-follicular period (5 ± 2) of the menstrual cycle measurements were performed under standardized environmental conditions in a temperature-controlled room (25-26 °C) and as little as possible external disturbances. The measurement session started at 08:00 AM, with the insertion of a 20-gauge catheter into a vein of the right forearm, to enable determination of renal function as detailed below. A second catheter was inserted into a vein of the contralateral forearm so as to collect blood samples. Participants did not drink caffeine- or alcohol-containing beverages and refrained from smoking and eating for at least 10 hours before the experiment. Throughout the measurement session, subjects were in supine position on a comfortable bed. Arterial blood pressure and heart rate (HR) were recorded continuously by a semi-automatic

oscillometric device (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, FL, USA), only interrupted during blood sampling and during the echocardiographic measurements (see below). Effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) were measured by continuous infusion of PAH (PAH: para-aminohippurate sodium; MSD, West Point, PA, USA) and inulin (Inutest, Laevosan Gesellschaft, Linz, Austria), respectively ¹⁴, ¹⁵. PAH and inulin, measured spectrophotometrically, had intra-assay- and inter-assay coefficient of variation of 4.2% and 7.3%, and 4.5% and 8.1%, respectively. Both variables were corrected for body surface area (BSA) and expressed in $\text{ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$. ERBF, renal vascular resistance (RVR) and filtration fraction (FF) were calculated as follows:

$$ERBF = ERPF / 1\text{-hematocrit}$$

$$RVR = 80.000 \times MAP / ERBF$$

$$FF = GFR / ERPF$$

At least two hours after the initiation of the PAH/inulin infusion, blood samples were collected for the later determination of the circulating levels of inulin and PAH and those of the hormones specified below. Active plasma renin concentration (APRC) was determined by an immunoradiometric assay technique (Nichols Institute Diagnostics, Wychen, The Netherlands) with an intra-assay coefficient of variation of 2.9% and an inter-assay coefficient of variation of 7.6% ¹⁶. Angiotensin II (AngII) was assayed by radio-immunoassay (RIA) following Phenyl column extraction (Amersham Int, Amersham, UK) with an intra-assay coefficient of variation of 4.2% and an inter-assay coefficient of variation of 7.7% ¹⁷. Aldosterone was assessed by a solid-phase protein binding RIA (Diagnostic Products Corporation, LA, USA) with an intra-assay coefficient of variation of 4.3% and an inter-assay coefficient of variation of 6.7% ¹⁸. α -Atrial natriuretic peptide (α ANP) was analyzed by radioimmunoassay (Nichols Institute Diagnostics, Wychen, The Netherlands) with an intra-assay coefficient of variation of 9% and inter-assay coefficient of variation of 12% ¹⁹. Serum 17- β -estradiol and progesterone were determined with a commercially available solid phase radioimmunoassay kit (DPC, Los Angeles, Cal, USA). Sensitivity of the assays are 0.035 and 0.15 nmol/l, respectively. Samples were collected on ice and centrifuged at 4 °C for 10 min at 1500 g immediately after sampling. All samples were stored deep-frozen (- 80 °C) prior to assay.

Measurement of plasma volume (PV). After the procedure to quantify renal function, plasma volume was measured. Plasma volume (PV, intra-assay coefficient of variation of 4.2%) was

determined with the Iodine¹²⁵ -Albumin (¹²⁵I-HSA) indicator dilution method and expressed in ml per kilogram (kg) lean body mass (LBM). The lean body mass was calculated as specified elsewhere ²⁰. Plasma volume was obtained by dividing the total injected radioactivity by the virtual volume-specific radioactivity at time zero, which corresponds with the time of bolus injection. The latter was obtained by extrapolation using the specific activity per ml of plasma at 10, 20 and 30 minutes after bolus injection.

Echocardiography to assess cardiac function was performed in semi-left lateral position, after completion of the plasma volume measurement and after approximately 5 minutes of rest, using a cross-sectional, phased array echocardiographic Doppler system (Hewlett-Packard Sonos 2000 and 2500) ¹. CO and cardiac index (CI) were calculated according to the formulas:

$$CO = stroke\ volume \times HR$$

$$CI = CO / BSA$$

In this formula, heart rate was obtained by taking the mean of the reciprocal of five consecutive R-R intervals on the ECG. Stroke volume (SV) was calculated by multiplying the aortic velocity integral and the aortic area. Aortic flow was measured across the aortic valves from an apical approach. The average area under the aortic velocity curve (aortic velocity integral) of five consecutive ejections was used to calculate stroke volume. Aortic valve diameter necessary for the calculation of the aortic area was measured off-line at the orifice during systole using M-mode. Total peripheral vascular resistance (TPVR) and total peripheral vascular resistance normalized for body surface area (TPVRi) were calculated as follows:

$$TPVR = 80 \times MAP / CO$$

$$TPVRi = 80 \times MAP / CI$$

Left ventricular work (LVW), defined as the energetic expense on blood imposed by the left ventricle per unit of time was calculated using the formula:

$$LVW = 80 \times MAP \times CO$$

The value used for MAP was obtained during the CO measurement and was calculated as the mean of three consecutive recordings.

Statistical analysis: Data are presented as means $\pm$ standard deviation (SD) unless otherwise stated. Differences between groups were tested with the Mann-Whitney-U Test along with Bonferroni correction. A p-value less than 0.05 relative to the control group, was considered statistically significant.

Results

The allocation of a formerly preeclamptic woman to either the hypertensive - or the thrombophilic subgroup was based on whether or not she had hypertension and if not, whether she had a thrombophilic disorder. Hypertension in this context was defined as a mean systolic - and/or a mean diastolic arterial blood pressure higher than 140 mmHg or higher than 90 mmHg, respectively, determined using all of the Dinamap recordings accumulated at the day of the experiment. On the basis of these criteria 14 women were assigned to the hypertensive subgroup. Only two women in this subgroup had been diagnosed before to have hypertension. Among these subjects, 5 were found to have a thrombophilic disorder as well, all but one in conjunction with hyperhomocysteinemia. Of the remaining 44 (normotensive) formerly preeclamptic subjects, 26 (59%) had one (n=22) - or more than one (n=4) thrombophilic disorder. They were allocated to the thrombophilic subgroup. The remaining 18 formerly preeclamptic subjects had neither hypertension nor thrombophilia. They were allocated to the asymptomatic subgroup. The demographic characteristics of all participants subdivided over various subgroups are listed in table I.

The three subgroups were comparable with the control group with respect to age, height, parity and urinary sodium excretion. The asymptomatic subgroup differed from controls by a higher body mass index. Obviously, the incidence of - usually iatrogenic - preterm birth was elevated in all formerly preeclamptic women. Prematurity was most obvious in hypertensives, followed by normotensive thrombophilic women and the asymptomatic subgroup.

Table II lists the subtypes of thrombophilia observed in the formerly preeclamptic participants. Antiphospholipid syndrome, protein S deficiency (prot S def) and hyperhomocysteinemia were most common. All formerly preeclamptic women, which were diagnosed as having antiphospholipid syndrome, were IgG serotypes. In our control group, one woman was diagnosed activated protein C resistant, based on factor V Leiden mutation (9%).

Table III lists the hemodynamic variables in the various subgroups. The hemodynamic status in the thrombophilic subgroup was almost identical to that in the control group. In contrast, women in the hypertensive subgroup had a higher cardiac output even when normalized for body surface area (cardiac index). In addition, left ventricular work was higher and a plasma volume per kg lean body mass lower relative to the control group. Hemodynamically, the asymptomatic subgroup resembled closely the hypertensive subgroup, except for cardiac index. In this group, the plasma volume per kg lean body mass was lower as compared to controls. After correlation analysis, this phenomenon was found to be independent of the body mass index in this subgroup.

Neurohormones, reproductive hormones and renal hemodynamics are listed in table IV. Only the hypertensives differed from controls by a higher atrial natriuretic peptide, angiotensin II and renal vascular resistance on the one hand, and a lower glomerular filtration rate and effective renal plasma flow, on the other hand. The asymptomatic formerly preeclamptic subjects did not differ significantly from the control group. Plasma levels of progesterone and 17- β -estradiol were comparable in all groups.

Table I: Demographic data from the 3 subgroups of formerly preeclampsics (ex-PE) and the group of normal parous controls. Data are presented as means $\pm$ SD. If a value is followed by an arrow, this particular variable is significantly higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group.

	controls	ex-PE		
		thrombophilic	asymptomatic	hypertensive
	(n=11)	(n=26)	(n=18)	(n=14)
Age (years)	31 $\pm$ 2	30 $\pm$ 4	30 $\pm$ 3	33 $\pm$ 4
Height (cm)	168 $\pm$ 4	167 $\pm$ 7	169 $\pm$ 5	167 $\pm$ 4
BMI (kg.m ⁻²)	21 $\pm$ 2	24 $\pm$ 4	27 $\pm$ 6 $\uparrow$	24 $\pm$ 4
Parity (median with range)	1 (1-2)	1 (1-3)	1 (1-2)	1 (1-4)
Amenorrhea at birth (wks)	39 $\pm$ 1	29 $\pm$ 4 $\downarrow$	32 $\pm$ 5 $\downarrow$	28 $\pm$ 5 $\downarrow$
Preterm birth < 32 wks. (%)	0 (0%)	21 (81%)	10 (56%)	11 (79%)
MAP (mmHg)	84 $\pm$ 8	86 $\pm$ 8	87 $\pm$ 7	106 $\pm$ 7 $\uparrow$
Natriuresis (mmol.24 h ⁻¹)	109 $\pm$ 29	99 $\pm$ 33	89 $\pm$ 31	84 $\pm$ 31

BMI = body mass index, MAP = mean arterial blood pressure.

Table II: Occurrence of the various subtypes of thrombophilic disorders in the thrombophilic - and hypertensive subgroups of formerly preeclamptic women.

Type of thrombophilic disorder	thrombophilic (n=26)	hypertensive (n=14)
ACA/LA (Antiphospholipid syndrome)	13 (50%)	1 (7%)
Hyperhomocysteinemia	5 (19%)	5 (36%)
Protein C deficiency	2 (8%)	-
Activated Protein C Resistance	3 (12%)	-
Protein S deficiency	7 (27%)	-
Anti thrombin deficiency	-	-

ACA= anticardiolipin antibodies, LA = lupus anticoagulant

Table III: Hemodynamic data in the 3 subgroups of formerly preeclamptics (ex-PE) and the group of normal parous controls. Data are presented as means $\pm$ SD. If a value is followed by an arrow, this particular variable is significantly higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group.

	controls	ex-PE		
	(n=11)	thrombophilic (n=26)	asymptomatic (n=18)	hypertensive (n=14)
MAP (mmHg)	84 $\pm$ 8	86 $\pm$ 8	87 $\pm$ 7	106 $\pm$ 7 $\uparrow$
HR (bpm)	62 $\pm$ 10	63 $\pm$ 9	66 $\pm$ 8	68 $\pm$ 12
CO (L.min ⁻¹)	4.7 $\pm$ 0.4	5.2 $\pm$ 0.7	5.4 $\pm$ 0.8 $\uparrow$	5.9 $\pm$ 0.7 $\uparrow$
CI (L.min ⁻¹ .m ⁻²)	2.8 $\pm$ 0.3	3.0 $\pm$ 0.4	2.9 $\pm$ 0.6	3.4 $\pm$ 0.4 $\uparrow$
TPVR (x10 ³)(dyne.s.cm ⁻⁵)	1.4 $\pm$ 0.2	1.3 $\pm$ 0.2	1.3 $\pm$ 0.2	1.4 $\pm$ 0.2
TPVRi (x10 ³)(dyne.s.cm ⁻⁵)	2.4 $\pm$ 0.4	2.3 $\pm$ 0.5	2.4 $\pm$ 0.4	2.5 $\pm$ 0.3
LVW (x10 ³)(mmHg.L.min ⁻¹)	31 $\pm$ 4	35 $\pm$ 6	38 $\pm$ 10 $\uparrow$	50 $\pm$ 9 $\uparrow$
PV (L)	2.5 $\pm$ 0.2	2.4 $\pm$ 0.2	2.4 $\pm$ 0.3	2.4 $\pm$ 0.3
PV/LBM (ml.kg ⁻¹)	56 $\pm$ 5	52 $\pm$ 4	47 $\pm$ 5 $\downarrow$	51 $\pm$ 5 $\downarrow$

MAP = mean arterial blood pressure, HR = heart rate, CO = cardiac output, CI = cardiac index, TPVR = total peripheral vascular resistance, TPVRi = total peripheral vascular resistance index, LVW = left ventricular work, PV = plasma volume, LBM = lean body mass.

Table IV: Endocrine - and renal variables in the 3 subgroups of formerly preeclampsics (ex-PE) and the group of normal parous controls. Data are presented as means $\pm$ SD. If a value is followed by an arrow, this particular variable is significantly higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group.

	controls	ex-PE		
	(n=11)	thrombophilic (n=26)	asymptomatic (n=18)	hypertensive (n=14)
APRC (mU.L ⁻¹)	13.5 $\pm$ 4.5	13.0 $\pm$ 7.8	13.9 $\pm$ 5.9	19.2 $\pm$ 9.9
AngII (pmol.L ⁻¹)	8.5 $\pm$ 2.4	10.6 $\pm$ 3.3	10.4 $\pm$ 2.7	12.1 $\pm$ 4.5 $\uparrow$
Aldo (nmol.L ⁻¹)	83 $\pm$ 30	89 $\pm$ 26	106 $\pm$ 41	120 $\pm$ 70
α -ANP (ng.L ⁻¹)	88 $\pm$ 31	98 $\pm$ 40	97 $\pm$ 46	160 $\pm$ 48 $\uparrow$
GFR (ml.min ⁻¹ .1.73 m ²)	125 $\pm$ 8	117 $\pm$ 16	113 $\pm$ 23	105 $\pm$ 19 $\downarrow$
ERPF (ml.min ⁻¹ .1.73 m ²)	562 $\pm$ 78	479 $\pm$ 104	490 $\pm$ 101	444 $\pm$ 123 $\downarrow$
ERBF (ml.min ⁻¹ .1.73 m ²)	877 $\pm$ 127	762 $\pm$ 179	770 $\pm$ 152	726 $\pm$ 208
RVR (x 10 ³) (dyne.s.cm ⁻⁵)	7.2 $\pm$ 1.3	9.1 $\pm$ 2.4	8.8 $\pm$ 1.9	12.0 $\pm$ 4.3 $\uparrow$
FF	0.23 $\pm$ 0.02	0.25 $\pm$ 0.03	0.24 $\pm$ 0.04	0.24 $\pm$ 0.04

APRC = active plasma renin concentration, AngII = angiotensin II, Aldo = aldosterone, α -ANP = atrial natriuretic peptide, GFR = glomerular filtration rate, ERPF = effective renal plasma flow, ERBF = effective renal blood flow, RVR = renal vascular resistance, FF = filtration fraction.

Discussion

Endothelial dysfunction plays a central role in the development of preeclampsia. Women with essential hypertension, renovascular disease, diabetes mellitus and some thrombophilic disorders are prone to develop preeclampsia in pregnancy⁵. Interestingly, in all these conditions the primary abnormality puts extra strain upon the endothelium, either mechanically (shear stress) or (bio)chemically (thrombophilia). The latter suggests that the higher incidence of preeclampsia in these conditions may result from a reduced endothelial tolerance to cope with the pregnancy-related increase in shear stress. Whether the latter is a prerequisite for preeclampsia to develop, is unknown. If so, it is difficult to explain why a large proportion of preeclamptic patients recover without apparent residual symptoms or abnormalities as detailed above. It is still unsettled whether this subgroup of apparently healthy formerly preeclamptic patients is indeed entirely comparable with normal parous controls or else, has some not yet identified subclinical abnormality that interferes with the vasculature's capacity to adapt to pregnancy. Therefore, we studied the hemodynamic -,

renal - and clotting functions in 58 formerly preeclamptic women and compared the results with those obtained in 11 normal parous controls.

Almost onethird (18 of 58) of the formerly preeclamptic women in this study had neither hypertension nor thrombophilia. In these seemingly healthy formerly preeclamptic subjects renal function and circulating levels of volume regulatory hormones were not different from controls. Although the cardiac index did not differ between the control and a-symptomatic formerly preeclamptic subgroup, the latter cardiac index was achieved at the expense of a higher cardiac load reflected in the higher left ventricular work. In the asymptomatic subgroup of formerly preeclamptic patients, plasma volume per kg lean body mass was lower as compared to controls. A low plasma volume per kg lean body mass was also observed in the hypertensive subgroup. Finally, the asymptomatic formerly preeclamptic subgroup was found to be more obese than the control group.

Obesity, chronic hypertension and preeclampsia are related conditions ^{21, 24}. Obesity and preeclampsia could have been related because of the associated hypertension. However, chronic hypertension and overweight contribute independently to the development of preeclampsia ²⁴. Obesity and total plasma volume are also positively related ²¹. Therefore, indexation for body mass is necessary to enable comparison of plasma volume. We observed in both obese and non-obese asymptomatic formerly preeclamptic women and hypertensives a lower plasma volume per kg lean body mass as compared to controls. It is known that in the early stages of essential hypertension plasma volume is reduced ²². Therefore asymptomatic formerly preeclamptic women seem to have a condition that can be considered latent - or pre-hypertension. This is supported by the observation that formerly preeclamptic women have an increased likelihood to develop hypertension in the next decades ^{4, 23}.

Thrombophilia not only predisposes for preeclampsia, but also for miscarriage, placental infarcts, placental abruption and stillbirth ²⁵. Especially women with more than one clotting disorder are at risk. In the present study, about 46% of the formerly preeclamptic participants had thrombophilia. Similar percentages have been reported earlier for the Dutch population ⁵. In thrombophilic formerly preeclamptic women, antiphospholipid syndrome, protein S deficiency, hyperhomocysteinemia and activated protein C resistance are most common. We diagnosed hyperhomocysteinemia in 36% of the hypertensive formerly preeclamptic subjects. It is tempting to conclude from this observation that hyperhomocysteinemia accelerates the development of vascular disease.

Gestational age at birth is the lowest in hypertensive formerly preeclamptics, followed by the normotensive thrombophilic- and latent hypertensive subgroup. Therefore, hemodynamic and hemostatic subdivision of formerly preeclamptic women seems to be related with the onset and severity of preeclampsia.

In summary, formerly preeclamptic women can be subdivided into a hypertensive -, a thrombophilic and an asymptomatic subgroup. The latter subgroup resembles hemodynamically the hypertensive subgroup. Therefore, we propose to categorize the women in the latter group as latent hypertensives. These observations not only confirm the central role of the endothelium in the development of preeclampsia. They also provide evidence for the view that in the majority of cases preeclampsia is superimposed upon a pre-existing vascular - or clotting disorder. This offers attractive possibilities to develop prophylactic strategies in women identified with one of these risk conditions.

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CHAPTER 3

CHANGES IN HEMODYNAMICS AND VOLUME HOMEOSTASIS WITH THE MENSTRUAL CYCLE IN WOMEN WITH A HISTORY OF PREECLAMPSIA

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Abstract

Background

Among formerly preeclamptic women the prevalence of hemodynamic - and clotting disorders is raised. In this study we tested the hypothesis that the normal cyclicity in hemodynamics and renal function with the menstrual cycle, as seen in healthy women, is preserved in formerly preeclamptic subjects irrespective of whether or not they had an underlying hemodynamic or clotting disorder.

Methods

We compared the hemodynamic and volume cyclicity during the menstrual cycle in formerly preeclamptic women (n=39) with that in normal parous controls (CONTROLS, n=10). The formerly preeclamptic participants were subdivided into women with hypertension (HYPERT, n=10), women with a thrombophilic disorder (THROMB, n=17) and women without these abnormalities (NONTROMB, n=12). We measured at least 5 months postpartum, once during the follicular phase (day 5 ± 2) and once during the luteal phase (day 22 ± 2) of the menstrual cycle, the following variables: body weight and length, mean arterial pressure, heart rate, cardiac output, plasma volume, glomerular filtration rate, effective renal plasma flow, renal volume homeostatic hormones, reproductive hormones and catecholamines. From the measured data, we calculated body mass index, cardiac index, left ventricular work, total peripheral - and renal vascular resistance, effective renal blood flow and renal filtration fraction.

Results

HYPERT differed from CONTROLS by a higher basal (follicular phase) value for cardiac output, cardiac output, left ventricular work, renal vascular resistance, atrial natriuretic peptide and norepinephrine. NONTROMB differed from CONTROLS by a lower basal plasma volume and a higher basal cardiac output and left ventricular work. Women in the THROMB group were comparable with respect to basal hemodynamics and - renal function, except for a higher renal vascular work. In CONTROLS, heart rate, plasma volume, effective renal plasma volume, effective renal blood flow, renin-angiotensin-aldosterone system hormones and norepinephrine in the follicular phase had increased in the luteal phase while the renal vascular resistance and atrial natriuretic

peptide had decreased. In the three formerly preeclamptic subgroups, this cyclic pattern with the menstrual cycle was preserved for most of these parameters.

Conclusion

Although basal hemodynamic - and volume status in formerly preeclamptic women differs from that in normal parous controls, its cyclic pattern with the menstrual cycle is largely preserved.

CHAPTER 3

Introduction

In healthy women, hemodynamics and volume homeostasis vary with the menstrual cycle ^{1, 2}. The reported data provide indirect evidence for mild vasorelaxation in the luteal phase relative to the follicular phase of the menstrual cycle. In this respect, although much less profound, the luteal phase mimics the early weeks of pregnancy when systemic vasorelaxation is extensive enough to induce a compensatory rise in cardiac output together with mild hemodilution as a sign of volume expansion and activation of volume regulatory hormones ³. In contrast, pregnancies complicated by severe fetal growth restriction and/or preeclampsia, are often preceded by defective development of these early pregnancy changes ^{4, 5}.

Epidemiological studies indicate that disorders with a thrombophilic phenotype or hypertension predispose for preeclampsia ^{6, 7}. Recently another associated subgroup has been described in which the plasma volume was found to be reduced as compared to normal parous controls ⁸. These findings might explain the different clinical phenotypes observed before preeclampsia occurs and could be indicative for separate pathophysiological entities which finally all can lead to endothelial damage and the development of preeclampsia.

The defective hemodynamic adaptation in early preeclamptic pregnancy together with the occurrence of vasorelaxation both in the luteal phase and in early pregnancy, raises the question whether the hemodynamic and volume cyclicity during the menstrual cycle in ex-preeclamptic women is different as compared to that in women with past uneventful pregnancies.

The objective of the present study was to test the hypothesis that the cyclicity in hemodynamic - and volume parameters during the menstrual cycle are different in formerly preeclamptic women as compared to normal parous controls. To this end, we compared in both phases of the menstrual period blood pressure, cardiac function, renal hemodynamics, plasma volume, volume regulatory hormones and catecholamines, between formerly preeclamptic women and normal parous controls.

Methods

Selection and patient characteristics.

In this study 58 formerly preeclamptic women and 14 normal parous controls were enrolled. Only those with a biochemically-confirmed ovulation in the luteal phase measurement session (as detailed below), were included for further analysis. As a consequence, in this paper 39 ovulating formerly preeclamptic non-diabetic Caucasian women and 10 controls were analyzed. Participants were at least 5 months postpartum. Formerly preeclamptic women were recruited at the outpatient clinic at the postpartum follow-up, whereas controls were recruited by advertisement. Preeclampsia and hypertension were defined according to the criteria of the International Society for the Study of Hypertension in Pregnancy ⁹. Therefore, hypertension was defined as a mean systolic - and/or a mean diastolic arterial blood pressure higher than 140 mmHg or higher than 90 mmHg, respectively, using all of the Dinamap recordings accumulated at the day of the experiment. All experiments were preceded by one week of standardized sodium intake (100 mmol sodium per day), representing the mean sodium intake in our population. This required that all participants consulted the hospital's dietitian, prior to study. Measuring 24-hour sodium output on the day before the experiment assessed women's compliance with the diet. None of the women used vitamins, oral contraceptives, antihypertensive drugs, non-steroid anti-inflammatory drugs or glucocorticosteroids in the two weeks prior to the measurements. On separate days and after an overnight fast, participants were tested for thrombophilia and hemodynamic/renal function. Thrombophilia was defined as the condition characterized by the presence of at least one clotting disorders known to be associated with an increased risk to develop venous thrombo-embolism. All participants gave written informed consent. The hospital's medical-ethical committee approved the study.

Experimental procedure

Methods

The methodology of the measurements and calculations on the thrombophilic screening and hemodynamic function were described previously ²³.

Thrombophilic screening: In short, anticardiolipin antibodies were determined spectrophotometrically by enzyme-linked immunoassay. Lupus anticoagulant was determined by mixing 1:1 patient and pooled normal plasma after finding a prolonged partial

thromboplastin time. Subsequently, a thromboplastin dilution test was performed by measuring prothrombin time with progressive diluted thromboplastin. Lupus anticoagulant was considered to be present when all assays were positive. We defined the antiphospholipid syndrome as the condition where anti-cardiolipin antibodies and/or lupus anticoagulant, when higher than 10 GPL for IgG antiphospholipid antibodies and 6 MPL for IgM antiphospholipid antibodies, could be detected in peripheral blood in women with a history of preeclampsia.

Coagulation tests were performed after a flawless venapuncture. Plasma samples were stored at -80 °C prior to assay. Protein C activity was determined with Coamate protein C (Chromogenix, Mölndal, Sweden). Total protein S antigen and free protein S were measured by an enzyme-linked immunosorbent assay (ELISA) (DAKO, Glostrup, Denmark). A chromogenic activated protein C-resistance assay quantified activated protein C resistance. Antithrombin activity was assayed with chromogenic substrate S-2765 (Chromogenix, Mölndal, Sweden).

A methionine-loading test was used to diagnose hyperhomocysteinemia. Homocysteine was measured by high-performance liquid chromatography with fluorescence detection. At 8:30 AM, the fasting homocysteine concentration was determined. Six hours after an oral dose of L-methionine (0.1 g/kg body weight), the post-load homocysteine concentration was assessed. During the test participants used a standard low-methionine breakfast and lunch. Individuals were considered to have hyperhomocysteinemia when fasting and/or post-load plasma homocysteine levels were more than 2 standard deviations (SD) higher than the mean of the laboratory control group.

Measurement of blood pressure and renal hemodynamics: Both in the follicular phase (day 5 $\pm$ 2) and luteal phase (day 22 $\pm$ 2) of the menstrual cycle (random order), measurements were performed under standardized environmental conditions in a temperature-controlled room (25-26 °C) and with as minimal external disturbances as possible. Participants did not drink caffeine- or alcohol-containing beverages and refrained from smoking and eating for at least 10 hours before the experiment. The measurement session started at 8:00 AM, with the insertion of a 20-gauge catheter into a vein of the right forearm, to enable determination of renal function as detailed below²³. A second catheter was inserted into a vein of the contralateral forearm for sampling of blood. Throughout each measurement session, subjects were in supine position on a comfortable bed. Arterial blood pressure and heart rate were recorded

repeatedly at two minutes interval by a semi-automatic oscillometric device (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, FL, USA), throughout the measurement session. Effective renal plasma flow and glomerular filtration rate were measured by continuous infusion of PAH (PAH: para-aminohippurate sodium; MSD, West Point, PA, USA) and inulin (Inutest, Laevosan Gesellschaft, Linz, Austria), respectively. PAH and inulin were measured spectrophotometrically. Renal hemodynamics were corrected for body surface area (BSA) and expressed in $\text{ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$. Effective renal blood flow (ERBF), renal vascular resistance (RVR) and filtration fraction (FF) were calculated as follows:

$$ERBF = ERPF / 1\text{-hematocrit}$$

$$RVR = 80.000 \times MAP / ERBF$$

$$FF = GFR / ERPF$$

At least two hours after the initiation of the PAH/inulin infusion, three blood samples were taken for determination of inulin and PAH. In addition, blood was collected for assay of active plasma renin concentration, angiotensin II, aldosterone, α atrial natriuretic peptide, sex-hormones and catecholamines. Active plasma renin concentration was determined by an immunoradiometric assay technique (Nichols Institute Diagnostics, Wychen, The Netherlands). Angiotensin II was assayed by radioimmunoassay (RIA) following Phenyl column extraction (Amersham Int, Amersham, UK). A solid-phase protein binding RIA (Diagnostic Products Corporation, LA, USA) assessed aldosterone. α -Atrial natriuretic peptide was analyzed by radioimmunoassay (Nichols Institute Diagnostics, Wychen, The Netherlands). Norepinephrine (intra-assay coefficient of variation (CV) 4.5%, inter-assay CV 8.1%), epinephrine (intra-assay CV 4.1%, inter-assay CV 8.6%) and dopamine (intra-assay CV 3.9%, inter-assay CV 7.1%) were assessed by chromatograph after liquid-liquid extraction and derivatization with a selective fluorescent agent ¹⁰. Serum 17- β -estradiol and progesterone were determined with a commercially available solid phase radioimmunoassay kit (DPC, Los Angeles, Cal, USA). Sensitivity of the latter assays are 0.035 and 0.15 nmol/l, respectively. Ovulation was confirmed when mid-luteal serum progesterone exceeded 15 pg/l ²². Samples were collected on ice and centrifuged at 4 °C for 10 min at 1500 g immediately after sampling. All samples were stored deep-frozen (- 80 °C) prior to assay.

Measurement of plasma volume. After the procedure to quantify renal function, plasma volume was measured. Plasma volume was determined with the Iodine¹²⁵ -Albumin (¹²⁵I-

HSA) indicator dilution method and expressed in ml per kilogram (kg) lean body mass (LBM)²³.

Echocardiography to assess cardiac function was performed in semi-left lateral position, after completion of the PV measurement and after 5 minutes of rest, using a cross-sectional, phased array echocardiographic Doppler system (Hewlett-Packard Sonos 2000 and 2500)²³.

Cardiac output (CO) and cardiac index (CI) were calculated according to the formulas:

$$CO = \text{stroke volume} \times HR$$

$$CI = CO / BSA$$

In this formula, heart rate (HR) was obtained by taking the mean of five consecutive R-R intervals on the ECG. Stroke volume was calculated by multiplying the aortic velocity integral and the aortic area. Aortic flow was measured across the aortic valves from an apical approach. The average area under the aortic velocity curve (aortic velocity integral) of five consecutive ejections was used to calculate stroke volume. Aortic valve diameter necessary for the calculation of the aortic area, was measured off-line at the orifice during systole using M-mode. Total peripheral vascular resistance (TPVR) and total peripheral vascular resistance index (TPVRi) were calculated as follows:

$$TPVR = 80 \times MAP / CO$$

$$TPVRi = 80 \times MAP / CI$$

Left ventricular work (LVW), defined as the energetic expense on blood imposed by the left ventricle per unit of time was calculated using the formula:

$$LVW = 80 \times MAP \times CO$$

The value used for mean arterial blood pressure (MAP) was obtained during the cardiac output measurement and was calculated as the mean of three consecutive recordings.

Statistical analysis: Data are presented as means $\pm$ SD unless otherwise stated. Differences between groups were tested with the Mann-Whitney-U Test along with Bonferroni correction.

Differences within groups between the follicular phase and luteal phase were evaluated by Wilcoxon Matched-Pairs Signed-Ranks test. Correlations, when calculated, between concomitantly measured potentially related variables, were tested by Spearman's Rank correlation analysis. In the comparisons a p-value less than 0.05 was considered statistically

significant. The calculated minimum detectable changes of the mean of variables within controls ($n = 10$, $\alpha = 0.05$, $\beta = 0.10$), assuming a normal distribution of the data were 10% (mean arterial pressure), 9% (heart rate), 14% (stroke volume), 11% (cardiac output), 11% (cardiac index), 14% (total peripheral vascular resistance), 9% (plasma volume), 8% (glomerular filtration rate), 4% (effective renal plasma flow), 7% (effective renal blood flow), and 15% (renal vascular resistance), respectively.

Results

The allocation of a formerly preeclamptic woman to either the hypertensive (HYPERT) - or the normotensive subgroup was based on whether or not she had hypertension. If not, then she was allocated to the normotensive thrombophilic group (THROMB) or the normotensive nonthrombophilic group (NONTROMB) based on whether she had a thrombophilic disorder or not. On the basis of these criteria 10 of 39 women were assigned to the HYPERT subgroup. Two women in this subgroup had been diagnosed as having hypertension prior to the study. Among the hypertensive subjects, three were found to have a thrombophilic disorder as well, all in conjunction with hyperhomocysteinemia (30%). Of the remaining 29 (normotensive) formerly preeclamptic subjects, 17 (44%) had one ($n=16$) - or more than one ($n=1$) thrombophilic disorder(s). They were allocated to the THROMB subgroup. The remaining 12 formerly preeclamptic subjects had neither hypertension nor thrombophilia. They were allocated to the NONTROMB subgroup. The demographic characteristics of all participants subdivided over the various subgroups are listed in table I. As compared to controls, the formerly preeclamptic subgroups did not differ with respect to age, height, parity and urinary sodium excretion. Formerly preeclamptic females in the NONTROMB subgroup had a higher body mass index than the controls. Obviously, mean arterial blood pressure was higher in the HYPERT subgroup as compared to controls.

Among the 39 formerly preeclamptic participants 51% (20/39) had at least one disorder associated with thrombophilia. In the subjects allocated to the THROMB subgroup, antiphospholipid syndrome (7/17, 41%) and protein S deficiency (6/17, 35%) were most common, followed by hyperhomocysteinemia (3/17, 18%), activated protein C resistance (1/17, 6%) and protein C deficiency (1/17, 6%). All formerly preeclamptic women, which were diagnosed as having antiphospholipid syndrome, were IgG serotypes. We did not identify women with antithrombin deficiency.

Table II lists the systemic hemodynamic data measured in this study, with those from the follicular phase being defined as basal values. Baseline systemic hemodynamics in the THROMB subgroup were comparable to those in the control group. Basal mean arterial blood pressure, cardiac output, cardiac index and left ventricular work were higher in the HYPERT subgroup as compared to controls. In the NONTHROMB subgroup basal values of cardiac output and left ventricular work were higher and plasma volume lower as compared to the control group. After correlation analysis, plasma volume was found to be independent of the body mass index in this subgroup, but also for the other subgroups. With respect to the cyclicity, luteal plasma volume was found to have increased relative to value in the follicular phase, both in controls and in all 3 formerly preeclamptic subgroups. After analysis, the mean rise in plasma volume between the control group and the THROMB -, NONTHROMB - and HYPERT subgroups, respectively, was found to be comparable. Luteal heart rate was consistently higher in the controls and in the THROMB subgroup, but not in the NONTHROMB - and HYPERT subgroups. In neither group did any of the other hemodynamic variables listed in table II vary with the menstrual cycle.

Table I: Demographic data (follicular phase) from the 3 subgroups of formerly preeclamptic women (formerly PE) and the group of normal parous controls. If a value is followed by an arrow, this particular variable is higher (↑) or lower (↓) than the one in the control group.

	controls	formerly PE		
	(n=10)	THROMB (n=17)	NONTHROMB (n=12)	HYPERT (n=10)
Age (years)	31 ± 2	31 ± 4	29 ± 3	33 ± 4
Height (cm)	168 ± 3	166 ± 7	167 ± 5	166 ± 4
BMI (kg.m ⁻²)	22 ± 2	24 ± 4	28 ± 5 ↑	22 ± 3
Parity (median with range)	1 (1-2)	1 (1-3)	1 (1-2)	1 (1-4)
MAP (mmHg)	83 ± 8	84 ± 8	88 ± 6	107 ± 6 ↑
Natriuresis (mmol.24 h ⁻¹)	108 ± 26	97 ± 37	97 ± 33	81 ± 23

BMI = body mass index, MAP = mean arterial blood pressure.

Table II: Hemodynamic data obtained in the follicular phase (FP) and luteal phase (LP) of the menstrual period (MP). An arrow that follows a particular value indicates this variable to be higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group in the corresponding menstrual period phase. The symbol * indicates the luteal phase value in a particular subgroup to be different from that in the follicular phase.

		controls	formerly preeclamptic		
		(n = 10)	THROMB (n = 17)	NONTHROMB (n = 12)	HYPERT (n = 10)
MP					
MAP (mmHg)	FP	83 $\pm$ 8	84 $\pm$ 8	88 $\pm$ 6	107 $\pm$ 6 $\uparrow$
	LP	82 $\pm$ 5	84 $\pm$ 7	85 $\pm$ 8	106 $\pm$ 11 $\uparrow$
HR (bpm)	FP	60 $\pm$ 9	62 $\pm$ 10	67 $\pm$ 8	70 $\pm$ 10
	LP	65 $\pm$ 10*	65 $\pm$ 8*	67 $\pm$ 8	70 $\pm$ 12
SV (ml.beat ⁻¹)	FP	74 $\pm$ 8	78 $\pm$ 10	72 $\pm$ 12	79 $\pm$ 11
	LP	69 $\pm$ 6	80 $\pm$ 11 $\uparrow$	76 $\pm$ 12	70 $\pm$ 12
CO (L.m ⁻²)	FP	4.6 $\pm$ 0.4	5.3 $\pm$ 0.7	5.5 $\pm$ 0.9 $\uparrow$	5.9 $\pm$ 0.6 $\uparrow$
	LP	4.6 $\pm$ 0.7	5.5 $\pm$ 1.1 $\uparrow$	5.6 $\pm$ 1.1	6.0 $\pm$ 0.5 $\uparrow$
CI (L.min ⁻¹ .m ⁻²)	FP	2.7 $\pm$ 0.2	3.1 $\pm$ 0.4	3.0 $\pm$ 0.5	3.5 $\pm$ 0.4 $\uparrow$
	LP	2.7 $\pm$ 0.3	3.2 $\pm$ 0.5 $\uparrow$	3.1 $\pm$ 0.6	3.6 $\pm$ 0.4 $\uparrow$
TPVR ($\times 10^3$) (dyne.s.cm ⁻⁵)	FP	1.4 $\pm$ 0.2	1.3 $\pm$ 0.2	1.3 $\pm$ 0.2	1.5 $\pm$ 0.2
	LP	1.5 $\pm$ 0.2	1.2 $\pm$ 0.2 $\downarrow$	1.3 $\pm$ 0.2	1.4 $\pm$ 0.2
TPVRi ($\times 10^3$) (dyne.s.cm ⁻⁵ .m ⁻²)	FP	2.4 $\pm$ 0.4	2.2 $\pm$ 0.4	2.4 $\pm$ 0.4	2.5 $\pm$ 0.3
	LP	2.5 $\pm$ 0.2	2.1 $\pm$ 0.4 $\downarrow$	2.3 $\pm$ 0.3	2.4 $\pm$ 0.3
LVW ($\times 10^3$) (mmHg.L.min ⁻¹)	FP	30 $\pm$ 4	35 $\pm$ 7	39 $\pm$ 10 $\uparrow$	49 $\pm$ 7 $\uparrow$
	LP	31 $\pm$ 6	35 $\pm$ 9	40 $\pm$ 12 $\uparrow$	50 $\pm$ 6 $\uparrow$
PV (ml.kg ⁻¹)	FP	55 $\pm$ 4	52 $\pm$ 3	45 $\pm$ 4 $\downarrow$	53 $\pm$ 5
	LP	62 $\pm$ 8*	57 $\pm$ 7*	48 $\pm$ 6* $\downarrow$	59 $\pm$ 8*

MAP = mean arterial blood pressure, HR = heart rate, SV = stroke volume, CO = cardiac output, CI = cardiac index, TPVR = total peripheral vascular resistance, TPVRi = total peripheral vascular resistance index, LVW = left ventricular work, PV = plasma volume.

Table III: Renal hemodynamics as determined in the follicular phase (FP) and luteal phase (LP) of the menstrual period (MP). An arrow that follows a particular value indicates this variable to be higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group in the corresponding menstrual period phase. The symbol * indicates the luteal phase value in a particular subgroup to be different from that in the follicular phase.

	MP	controls (n = 10)	formerly preeclamptic		
			THROMB (n = 17)	NONTHROMB (n = 12)	HYPERT (n = 10)
GFR (ml.min ⁻¹ .1.73 m ⁻²)	FP	125 $\pm$ 8	115 $\pm$ 13	114 $\pm$ 24	111 $\pm$ 10
	LP	130 $\pm$ 12	119 $\pm$ 18	122 $\pm$ 27*	120 $\pm$ 18*
ERPF (ml.min ⁻¹ .1.73 m ⁻²)	FP	565 $\pm$ 77	495 $\pm$ 71	500 $\pm$ 98	480 $\pm$ 105
	LP	596 $\pm$ 64*	516 $\pm$ 77 $\downarrow$	519 $\pm$ 111	557 $\pm$ 133*
ERBF (ml.min ⁻¹ .1.73 m ⁻²)	FP	881 $\pm$ 127	777 $\pm$ 129	791 $\pm$ 144	770 $\pm$ 169
	LP	935 $\pm$ 105*	809 $\pm$ 132	820 $\pm$ 163	897 $\pm$ 205*
RVR ($\times 10^3$) (dyne.s.cm ⁻⁵ .1.73m ⁻²)	FP	7.2 $\pm$ 1.3	8.4 $\pm$ 1.6 $\uparrow$	8.6 $\pm$ 1.5 $\uparrow$	10.9 $\pm$ 2.6 $\uparrow$
	LP	6.7 $\pm$ 0.9*	7.9 $\pm$ 1.5	8.2 $\pm$ 1.7	9.2 $\pm$ 2.6 $\uparrow$
FF (GFR/ERPF)	FP	0.22 $\pm$ 0.03	0.23 $\pm$ 0.03	0.23 $\pm$ 0.02	0.24 $\pm$ 0.04
	LP	0.22 $\pm$ 0.03	0.23 $\pm$ 0.03	0.24 $\pm$ 0.03	0.22 $\pm$ 0.05*
Sodium excretion (mmol.24h ⁻¹)	FP	108 $\pm$ 26	97 $\pm$ 37	97 $\pm$ 33	81 $\pm$ 23
	LP	123 $\pm$ 32	89 $\pm$ 39 $\downarrow$	92 $\pm$ 25	84 $\pm$ 30 $\downarrow$

GFR = glomerular filtration rate, ERPF = effective renal plasma flow, ERBF = effective renal blood flow, RVR = renal vascular resistance, FF = filtration fraction.

The renal hemodynamic data are listed in table III. All basal data points were comparable between groups except for a higher renal vascular resistance in all formerly preeclamptic subgroups relative to the controls. The cyclicity in renal hemodynamics differed between groups. In the control group luteal effective renal plasma flow and effective renal blood flow had increased and the renal vascular resistance had decreased relative to the follicular phase. In the HYPERT subgroup these changes were supplemented with a luteal rise in glomerular filtration rate and fall in filtration fraction relative to the follicular phase. In the NONTHROMB subgroup only the luteal rise in glomerular filtration rate reached significance whereas in the THROMB subgroup none of the renal variables had changed significantly between the follicular phase and luteal phase. In the HYPERT and THROMB subgroups, the luteal natriuresis was lower than that in the follicular phase in spite of a presumed similar sodium intake.

Table IV: Endocrine variables as determined in the follicular phase (FP) and luteal phase (LP) of the menstrual period (MP). An arrow that follows a particular value indicates this variable to be higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group in the corresponding menstrual period phase. The symbol * indicates the luteal phase value in a particular subgroup to be different from that in the follicular phase.

	MP	controls (n = 10)	formerly preeclamptic		
			THROMB (n = 17)	NONTHROMB (n = 12)	HYPERT (n = 10)
APRC (mU.L ⁻¹)	FP	13.7 $\pm$ 4.5	12.1 $\pm$ 6.9	14.0 $\pm$ 5.3	18.3 $\pm$ 9.4
	LP	21.7 $\pm$ 7.3*	23.7 $\pm$ 11.6*	21.2 $\pm$ 5.5*	25.9 $\pm$ 10.4*
Angiotensin II (pmol.L ⁻¹)	FP	8.7 $\pm$ 2.4	9.9 $\pm$ 3.7	10.2 $\pm$ 2.4	10.7 $\pm$ 3.7
	LP	13.7 $\pm$ 10.4*	12.9 $\pm$ 5.1*	13.0 $\pm$ 4.3*	15.7 $\pm$ 6.0*
Aldosterone (nmol.L ⁻¹)	FP	85 $\pm$ 30	82 $\pm$ 23	107 $\pm$ 39	109 $\pm$ 68
	LP	203 $\pm$ 141*	233 $\pm$ 106*	285 $\pm$ 175*	183 $\pm$ 105*
α ANP (ng.L ⁻¹)	FP	93 $\pm$ 26	97 $\pm$ 34	105 $\pm$ 50	141 $\pm$ 46 $\uparrow$
	LP	84 $\pm$ 21*	77 $\pm$ 29*	98 $\pm$ 31	126 $\pm$ 73*
Norepinephrine (nmol.L ⁻¹)	FP	0.63 $\pm$ 0.20	0.84 $\pm$ 0.47	0.86 $\pm$ 0.19	1.28 $\pm$ 0.64 $\uparrow$
	LP	1.02 $\pm$ 0.20*	1.25 $\pm$ 0.68*	1.01 $\pm$ 0.24*	1.27 $\pm$ 0.85
Epinephrine (nmol.L ⁻¹)	FP	0.06 $\pm$ 0.04	0.06 $\pm$ 0.03	0.06 $\pm$ 0.03	0.10 $\pm$ 0.09
	LP	0.05 $\pm$ 0.03	0.06 $\pm$ 0.03	0.07 $\pm$ 0.07	0.06 $\pm$ 0.05
Dopamine (nmol.L ⁻¹)	FP	0.21 $\pm$ 0.12	0.27 $\pm$ 0.13	0.18 $\pm$ 0.07	0.18 $\pm$ 0.09
	LP	0.21 $\pm$ 0.10	0.22 $\pm$ 0.11	0.23 $\pm$ 0.10	0.19 $\pm$ 0.10
17 β -Estradiol (nmol.L ⁻¹)	FP	0.13 $\pm$ 0.09	0.14 $\pm$ 0.05	0.15 $\pm$ 0.04	0.16 $\pm$ 0.07
	LP	0.25 $\pm$ 0.11*	0.34 $\pm$ 0.12*	0.31 $\pm$ 0.10*	0.35 $\pm$ 0.11*
Progesterone (nmol.L ⁻¹)	FP	1.3 $\pm$ 0.5	2.2 $\pm$ 1.1	2.3 $\pm$ 1.4	1.7 $\pm$ 0.8
	LP	30.1 $\pm$ 14.2*	29.7 $\pm$ 9.2*	26.5 $\pm$ 9.6*	29.6 $\pm$ 10.9*

APRC = active plasma renin concentration, α ANP = α atrial natriuretic peptide.

The endocrine data are listed in table IV. Basal α atrial natriuretic peptide and norepinephrine were higher in the HYPERT subgroup than in the control group. With respect to the cyclicity we noted a luteal rise in the hormones of the renin-angiotensin-aldosterone system and a fall in α atrial natriuretic peptide in all groups except for NONTHROMB women. Except for the HYPERT subgroup, in all other groups luteal norepinephrine was found to have increased relative to the follicular phase. The difference between the luteal and follicular value for the other neurohormones was inconsistent.

Finally, baseline levels and cyclic change in 17- β -estradiol and progesterone were comparable in all groups.

Discussion

Endothelial dysfunction plays a central role in the development of preeclampsia ¹¹. Endothelial function can be jeopardized either biochemically (i.e. thrombophilia, diabetes mellitus) or mechanically (i.e. hypertension, renovascular disease) ^{7, 12}. Preeclampsia seems to be superimposed upon a preexisting disorder and may be the clinical expression of "endothelial decompensation" which represents the common endpoint of these two different pathophysiologic pathways. We therefore subdivided our group of formerly preeclamptic subjects into a normotensive thrombophilic (THROMB) - and hypertensive (HYPERT) subgroup, thus separating them from a group of normotensive formerly preeclamptic women without thrombophilia (NONTROMB). Since in healthy females menstrual changes in hemodynamics and renal function largely mimic early pregnancy changes and preeclampsia often is preceded by hemodynamic and renal maladaptation, these finding raised the question whether cyclic menstrual changes in these functions, as seen in healthy women, would be preserved in the above mentioned formerly preeclamptic subgroups. To answer this question, we studied mid follicular and mid luteal of the menstrual cycle, clotting, metabolic, renal and cardiovascular function in formerly preeclamptic women and controls and compared the data in the various formerly preeclamptic subgroups with those found in a reference group of normal parous controls.

With respect to the general characteristics, the three subgroups were comparable with the control group, except for a higher body mass index in the NONTROMB subgroup. In most women in the THROMB subgroup, thrombophilia was caused by antiphospholipid syndrome, protein-S-deficiency and hyperhomocysteinemia, an observation in line with reports by others ⁶. Comparison of all basal hemodynamic, renal and endocrine data in the three formerly preeclamptic subgroups relative to the control group indicated that in the HYPERT subgroup, the higher mean arterial blood pressure was paralleled by a higher cardiac output and cardiac index, but a comparable total peripheral vascular resistance at the expense of a higher left ventricular work load. In this group renovascular resistance was increased and circulating levels of α atrial natriuretic peptide and norepinephrine elevated. It is unlikely that the higher cardiac index was secondary to the awareness of having hypertension as in only 2 of the 10 women in the HYPERT subgroup the diagnosis "hyper-

tension" was made at the time of the study and the raised blood pressure was seen during both measurement sessions¹³. Most baseline values for central and renal hemodynamics and endocrine status in the THROMB subgroup resembled those in the control group. This indicates that the altered endothelial function in response to thrombophilia does not seem to influence appreciably cardiovascular function. In the NONTHROMB subgroup the relative overweight and the lower plasma volume were most conspicuous.

With respect to the central and renal hemodynamic cyclicity with the menstrual cycle, we found that, in the luteal phase, most variables had changed modestly in the control group. In controls, the observed luteal fall in renal vascular resistance was paralleled by a rise in heart rate, effective renal plasma- and blood flow and plasma volume. Most of these changes are paralleled by endocrine and autonomic nervous stimuli; the cyclic changes in almost all these stimuli were significant. In the luteal phase the renin-angiotensin-aldosterone-system hormones had increased and the α atrial natriuretic peptide decreased relative to the follicular phase. These changes are in agreement with previous observations reported by others and are compatible with a state of vasorelaxation giving rise to volume expansion as a result of activation of the sympathetic system and the renin-angiotensin-aldosterone-system with adaptive suppression of α atrial natriuretic peptide^{1, 2, 14}. In contrast to controls, HYPERT not only had higher basal circulating norepinephrine levels, also its luteal rise remained absent. Otherwise, their cyclic changes in renal and endocrine variables were comparable to those in controls. In the normotensive formerly preeclamptic groups, we observed a trend towards a decrease in renal vascular resistance and a rise in renal perfusion and clearance. Hormonal changes were similar as in controls. These observations indicate that most hemodynamic, renal and hormonal changes with the menstrual cycle in normal parous women are preserved in formerly preeclamptic individuals.

Although it is known that in the early stages of essential hypertension plasma volume is reduced, we did not find such an abnormality in our hypertensive formerly preeclamptic patients¹⁵. However we did observe a reduced plasma volume in the NONTHROMB group. Therefore NONTHROMB seem to have a condition that can be considered latent - or pre-hypertension. The lower plasma volume could then be viewed upon as a compensatory mechanism to minimize a rise in blood pressure. Why plasma volume is not reduced in the HYPERT subgroup, is less easy to explain. Perhaps this group already has more advanced renal lesions which interfere with volume control.

A lower plasma volume can be secondary to an increased vascular tone. However plasma volume may also be reduced when the total volume of the venous bed is relatively small, a condition which either could be genetically or acquired during the early phases of vascular development¹⁶. About 2/3 of the plasma volume is located within the venous compartment and can be mobilized in response to a fall in arterial tone¹⁷. In order to compensate for the luteal decrease in afterload, both the renin-angiotensin-aldosterone-system and the sympathetic nerve system will be activated^{17, 18, 19}. It is conceivable that an inadequacy of the resulting volume retention to balance the increased demands for venous return will lead to a higher sympathetic tone as a second line compensation. The latter not only leads to a higher cardiac preload, but also to a higher heart rate and mean arterial blood pressure^{17, 19}. The α atrial natriuretic peptide level provides an indirect estimate for vascular filling state²⁰. The cyclic change in α atrial natriuretic peptide in all groups supports the development of a state of relative vascular underfill in the luteal phase. On the other hand, α atrial natriuretic peptide also correlates positively to right atrial pressure²¹. A rise in sympathetic tone can be expected to raise cardiac preload and with it, increase of right atrial pressure. Therefore, we postulate that a subnormal size of the venous compartment with inadequate capacity to raise venous return in response to a rise in flow demands, will induce a rise in sympathetic tone. When sustained, the latter leads to a higher atrial pressure and with it an increase in α atrial natriuretic peptide. The observation of higher, in hypertensive formerly preeclamptic basal norepinephrine levels relative to controls supports the view that in these individuals a balance between venous return and cardiac output is only possible at the expense of a higher sympathetic tone.

The type of difference between the subgroups suggests that two preexisting states appear to be associated with an increased risk to develop preeclampsia: 1. thrombophilia which is associated with chronically elevated biochemical strain put upon the endothelium and 2. marginal size of the vascular compartment in conjunction with a chronically elevated cardiac output, a condition that limits the ability of the vasculature to respond to prolonged increased demands.

In summary, although basal hemodynamics and volume status differed between formerly preeclamptic subgroups and controls, the cyclicity with the menstrual cycle in the three subgroups of formerly preeclamptic women, was preserved.

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CHAPTER 4

THE EFFECT OF PREGNANCY ON THE COMPLIANCE OF LARGE ARTERIES AND VEINS IN NORMAL PAROUS CONTROLS AND FORMERLY PREECLAMPTICS

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Abstract

Background Early pregnancy is characterized by a fall in total peripheral vascular resistance. In this study we tested the hypothesis that this phenomenon is accompanied by a rise in the compliance of the large arteries and veins.

Methods In 42 formerly preeclamptic women (ex-PE) and 10 normal parous controls (CONTR), we measured the following variables midfollicular (FP) and midluteal (LP) during the menstrual cycle and again at 5 (AM5) and at 7 (AM7) weeks amenorrhea: cardiac output (CO), mean arterial pressure (MAP), carotid (CaC) and femoral artery compliance (FeC) and venous compliance (VeC). The total peripheral vascular resistance (TPVR) was obtained as the ratio of MAP and CO. After screening for thrombophilia and hypertension, ex-PE's were subdivided into a hypertensive subgroup (HYPERT), a thrombophilic subgroup (THROMB) and a subgroup of subjects without these abnormalities (NONTROMB).

Results In parallel to the early pregnancy fall in TPVR, FeC and VeC increases in CONTR. Except for VeC, these changes were also observed in the THROMB ex-PE subjects. In the HYPERT and NONTROMB subjects arterial and venous compliance failed to increase, in spite of a drop in TPVR.

Conclusion These data support the concept that in normal pregnancy the fall in TPVR is paralleled by improved macrovascular compliance. The latter adaptive change does not occur in normotensive nonthrombophilic and hypertensive ex-PE women.

CHAPTER 4

Introduction

The current idea about the hemodynamic adaptation to pregnancy is, that systemic arterial vasodilation represents the trigger for a cascade of compensatory changes such as a rise in cardiac output (CO), activation of the renin-angiotensin-aldosterone system and resetting of the osmoreceptor^{1,2,3}. In rat pregnancy the compliance of the descending aorta was found to increase in concert with this early-pregnancy hemodynamic adaptation²³. Data obtained in human pregnancy suggest that the aortic compliance and venous distensibility only increases at the end of the first trimester^{5,6,7}. Therefore, it is still unsettled whether or not the human pregnancy-related rise in the compliance of the large vessels develops independently or in response to the institution of the high-flow/low-resistance circulation in the first weeks of pregnancy.

Pregnancies complicated by intra uterine growth retardation have been associated with absent early pregnancy vasorelaxation^{2,3}. Furthermore, various studies have shown that pregnancies of women with either a vascular disorder such as hypertension or a clotting disorder with a thrombophilic phenotype, are at risk to become complicated by preeclampsia^{4,21,22}. Whether absent vasorelaxation in early preeclamptic pregnancy occurs in both subgroups, is unknown. Theoretically, the vasorelaxation can be expected only to be blunted in the subgroup of women with reduced vascular elasticity, as is the case in those with hypertension. At any rate, exploration of potential differences in the pathogenesis of preeclampsia between these two subgroups is expected to improve our understanding of this syndrome.

The objectives of this study were (1). to evaluate whether the compliance of the large arteries and veins increases in early human pregnancy in concert with the drop in total peripheral vascular resistance (TPVR), and if so, to determine whether the magnitude of the rise in vascular compliance correlates with that of the drop in TPVR, (2). to evaluate whether the effect of pregnancy on the vascular compliance differs between women with a history of preeclampsia and normal parous controls, and between women with a preexistent vascular disorder and those with thrombophilia. To this end, we determined longitudinally during the menstrual cycle and the first weeks of subsequent pregnancy CO, arterial blood pressure, the dynamic function of the carotid and femoral artery and forearm veins, along with a number of biochemical and endocrine variables, in parous controls and in formerly preeclamptic women.

Methods

Selection and patient characteristics.

In this study 42 ex-preeclamptic non-diabetic women and 10 normal parous controls were enrolled. Participants were at least 5 months postpartum. Ex-PE women were recruited at the outpatient clinic during the postpartum follow-up, whereas controls were recruited by advertisement. All participants gave written informed consent. The hospital's medical-ethical committee approved the study. PE and hypertension were defined according to the criteria of the International Society for the Study of Hypertension in Pregnancy (ISSHP)⁸. That is to say, hypertension was defined as a mean systolic blood pressure above 140 mmHg, and/or a mean diastolic arterial blood pressure above 90 mmHg, using all of the Dinamap recordings accumulated on the day of the experiment. None of the women used vitamins, oral contraceptives, antihypertensive drugs, NSAID's or glucocorticosteroids in the two weeks prior to the measurements. On separate days and after an overnight fast, participants were tested for thrombophilia, and hemodynamic and vascular function.

Experimental procedure

Methods

Thrombophilic screening: Anticardiolipin antibodies (ACA; IgM and IgG) were determined spectrophotometrically by enzyme-linked immunoassay⁹. Lupus anticoagulant (LA) was determined by mixing 1:1 patient and pooled normal plasma after finding a prolonged partial thromboplastin time. Subsequently, a thromboplastin dilution test was performed by measuring prothrombin time with progressive diluted thromboplastin¹⁰. LA was considered to be present when all assays were positive. The presence of LA and/or an ACA titer over 10 GPL (IgG) or over 6 MPL (IgM) was considered indicative of the antiphospholipid syndrome (APS).

Coagulation tests were performed after a flawless venapuncture. Blood was collected in sodium citrate containing glass tubes. Plasma was prepared by two consecutive centrifugation steps, with the second one performed in an Eppendorf centrifuge to eliminate platelets. Samples were stored at -80 °C prior to assay. Protein C activity was determined with Coamate protein C (Chromogenix, Mölndal, Sweden)¹¹. The normal value of protein C according to the laboratory control group is 70% to 130% and has an intra-assay and inter-assay coefficient of variation (CV) of 3.3% and 4.7%, respectively. Total protein S antigen

and free protein S were measured by an enzyme-linked immunosorbent assay (ELISA) (DAKO, Glostrup, Denmark)¹². The normal range for both total protein S antigen (intra-assay and inter-assay CV of 5.1% and 5.7%, respectively) and free protein S (intra-assay and inter-assay CV 3.4% and 3.6%, respectively) is 70% to 130% and 65% to 130%, respectively. Activated protein C resistance (APCR) was quantified by a chromogenic APC-resistance assay¹³. The chromogenic test fully matches DNA analysis for the factor V Leiden mutation and has a normal range between 0% and 20%¹⁴. Antithrombin activity (AT) was assayed with chromogenic substrate S-2765 (Chromogenix, Mölndal, Sweden)¹⁵. The normal range of AT is 80% to 120%.

A methionine-loading test was used to diagnose hyperhomocysteinemia (HHC). Homocysteine was measured by high-performance liquid chromatography with fluorescence detection¹⁶. In our hands, this test has an intra- and inter-assay coefficient of variation (CV) of 3.9% and 5.9%, respectively. At 8:30 AM, the fasting homocysteine concentration was determined. Six hours after an oral dose of L-methionine (0.1 g/kg body weight), the post-load homocysteine concentration was assessed. During the test participants used a standard low-methionine breakfast and lunch. Individuals were considered to have hyperhomocysteinemia when fasting and/or post-load plasma homocysteine levels were more than 2 standard deviations (SD) higher than the mean of the laboratory control group.

Measurement of hemodynamics and vascular compliance: Both in the FP (day 5 ± 2) and LP (day 22 ± 2) of the menstrual cycle (random order) and again in early pregnancy at 5 and 7 weeks amenorrhea, measurements were performed under standardized environmental conditions in a temperature-controlled room (25-26 °C) and with external disturbances as minimized as possible. Participants did not drink caffeine- or alcohol-containing beverages and refrained from smoking and eating for at least 10 hours before the experiment. The measurement session started at 8:00 AM. Throughout each measurement session, subjects were in supine position on a comfortable bed. Arterial blood pressure and heart rate (HR, beats.min⁻¹) were recorded at two minutes interval by a semi-automatic oscillometric device (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, FL, USA), throughout the measurement session. At 8:15 AM, after a 15-minute rest, carotid and femoral artery compliance was assessed non-invasively. Arterial wall displacement was visualized by a two-dimensional real time B-mode imager attached to a vessel wall moving detector system (Pie Medical, Maastricht, The Netherlands). Based on the B mode image, an M-line

perpendicular to the artery is selected. The received radio frequency (RF) signals covering the artery of interest are stored in a personal computer system for off-line analysis. Analysis of the RF signals from sample windows covering the anterior and posterior walls provides the artery distension waveform. Three recordings of five consecutive heartbeats were used to establish the changes in arterial diameter during the cardiac cycle as a function of time. From the recorded data the mean absolute distension (Δd) from diastole to systole and relative distension ($\Delta d \cdot d^{-1}$) were calculated. The measurement of the carotid and femoral artery $\Delta d \cdot d^{-1}$ and d is associated with an intra- and interobserver CV of 8 and 12%, respectively, for $\Delta d \cdot d^{-1}$ and 2.8-4.5 % and 2.0-3.0 %, respectively, for d ¹⁷.

The distensibility coefficient (DC, in kPa^{-1}) and compliance (CC, $\text{mm}^2 \cdot \text{kPa}^{-1}$) were calculated as follows:

$$DC = 2 \cdot \Delta d \cdot d^{-1} \cdot \Delta p^{-1}$$

$$CC = \pi \cdot d \cdot \Delta d \cdot 2 \cdot \Delta p^{-1}$$

The brachial pulse pressure (systolic minus diastolic pressure) is taken as Δp and was calculated by taking the mean of three sets of Dinamap recordings measured during establishment of the vascular images.

Venous compliance (VeC), representing venous visco-elastic properties, was defined as the plethysmographically determined slope of the relationship of intravenous volume- and pressure changes (Periflow, JSI, Beerse, Belgium)²⁰. Intravenous pressure was measured by means of a 20-gauge catheter in the left forearm fossa cubiti. A pressure transducer was placed 5 cm below the sternal angle (HP, Böblingen, Germany). Forearm volume changes were measured using a silastic strain gauge filled with mercury, which is connected to a venous occlusion plethysmograph. Measurement started 30 minutes after venapuncture at 09:30 AM. During the first pressure step, the upper arm cuff was inflated to a cuff pressure of 25 mmHg. It was kept inflated for three minutes. This interval was chosen to reach steady-state in arm volume and pressure. During the subsequent two minutes, the cuff was deflated in order to minimize accumulation of interstitial fluid due to capillary filtration. Changes in volume and intravenous pressure during each subsequent inflational pressure step (starting at 25 mmHg, followed by 30, 35, 40, 45 and ending at 50 mmHg) were taken at the end of the three minutes of cuff-inflation and after deflation of the cuff. Venous compliance was defined as the slope of the relationship between the changes in forearm

volume (δV (ml . 100 ml⁻¹ forearm volume)) on the ordinate and corresponding venous pressure (δP (mmHg)) on the abscissa calculated by linear regression analysis and has a CV of 11.4%²⁰.

Finally, blood was collected for assay of sex-hormones. Serum 17- β -estradiol and progesterone were determined with a commercially available solid phase radioimmunoassay kit (DPC, Los Angeles, Cal, USA). Sensitivity of the latter assays are 0.035 and 0.15 nmol/l, respectively. Samples were collected on ice and centrifuged at 4 °C for 10 min at 1500 g immediately after sampling. All samples were stored deep-frozen (-80 °C) prior to assay.

Measurement of plasma volume (PV): After the procedure to quantify vascular function, PV was measured. During the FP, plasma volume (PV, intra-assay CV of 4.2%) was determined with the Iodine¹²⁵-Albumin (¹²⁵I-HSA) indicator dilution method and expressed in ml per kilogram (kg) lean body mass (LBM). The LBM was calculated as specified elsewhere¹⁸. PV was obtained by dividing the total injected radioactivity by the virtual volume-specific radioactivity at time zero¹⁹. The latter, which corresponds with the time of bolus injection, was obtained by extrapolation using the specific activity per ml of plasma at 10, 20 and 30 minutes after bolus injection.

Echocardiography to assess cardiac function was performed in semi-left lateral position, after completion of the PV measurement and after 5 minutes of rest, using a cross-sectional, phased array echocardiographic Doppler system (Hewlett-Packard Sonos 2000 and 2500)². CO and cardiac index (CI) defined as CO per m² body surface area (BSA), were calculated according to the formulas⁴:

$$CO = \text{stroke volume} \cdot HR$$

$$CI = CO \cdot BSA^{-1} \quad (BSA = 0.007184 \cdot \text{height}^{0.725} \cdot \text{weight}^{0.425})$$

In this formula, HR was obtained by taking the mean of the reciprocal of five consecutive R-R intervals on the ECG. Stroke volume (SV, ml) was calculated by multiplying the aortic velocity integral and the aortic area. Aortic flow was measured across the aortic valves from an apical approach. The average area under the aortic velocity curve (aortic velocity integral) of five consecutive ejections was used to calculate SV. Aortic valve diameter necessary for the calculation of the aortic area, was measured off-line at the orifice during systole using M-mode. TPVR (dyne . s⁻¹ . cm⁻⁵) was calculated as follows:

$$TPVR = 80 \cdot MAP \cdot CO^{-1}$$

The value used for mean arterial pressure (MAP, mmHg) was obtained during the CO measurement using the Dinamap apparatus and was calculated as the mean of three consecutive recordings.

Statistical analysis: Data are presented as means with SD unless stated otherwise. Intra-group differences were tested with Wilcoxon Signed-Ranks Matched-Pairs test along with Bonferroni correction, using the value observed in the follicular phase as the reference. Differences between groups were tested with the Mann-Whitney-U Test along with Bonferroni correction.

Correlation, when calculated, between concomitantly measured potentially related variables, was tested by Spearman's Rank Correlation Analysis. The calculated minimum detectable changes of the mean of variables within controls ($n = 10$, $\alpha = 0.05$, $\beta = 0.10$), assuming a normal distribution of the data were 7% (mean arterial pressure), 15% (heart rate), 10% (stroke volume), 11% (cardiac output), 11% (total peripheral vascular resistance), 23% (carotis compliance coefficient), 16% (carotis distensibility coefficient), 46% (femoral compliance coefficient), 59% (femoral distensibility coefficient) and 16% (venous compliance), respectively. In all analyses, a p-value less than 0.05, when appropriate relative to the control group, was considered statistically significant.

Results

The demographic characteristics of all participants divided over various subgroups are listed in table I. From the 42 ex-PE subjects, eleven women were assigned to the HYPERT subgroup. Among them, three had also a thrombophilic disorder (see table II). Eighteen of the remaining 31 (normotensive) ex-PE subjects had at least one thrombophilic disorder(s) and, therefore, were allocated to the THROMB subgroup. The rest group of eleven ex-PE subjects had neither hypertension nor thrombophilia. They were allocated to the NONTHROMB subgroup. The 3 ex-PE subgroups and the controls were comparable with respect to age, height, BMI, parity and the time elapsed between delivery of the previous pregnancy and first measurement session. Ex-PE females in the NONTHROMB subgroup had a lower PV than the controls. By definition, MAP was higher in the HYPERT subgroup than in the controls.

Table II lists the distribution of thrombophilic disorders over the THROMB and HYPERT subgroups. The incidence of at least one thrombophilic disorder in the total group of ex-PE subjects was 50 % (21/42). In the THROMB subgroup, APS and protein S deficiency (prot S

def) were most common. All formerly preeclamptic women, which were diagnosed as having anti-phospholipid syndrome, were IgG serotypes. One subject in this subgroup had more than one thrombophilic disorder.

Central hemodynamic data are listed in table III. During the menstrual cycle HYPERT subjects had a higher CO and CI as compared to controls. In all subgroups except for the NONTHROMB, MAP had decreased and HR increased in early pregnancy relative to the FP reference value. The concomitantly determined TPVR had decreased - and SV increased in all groups. The latter was associated with an increase in CO and CI in all groups except for the HYPERT.

Tables IV and V list the results on vascular dynamics in all participants. Only the HYPERT subgroup had a higher pulse pressure during carotid artery parameter assessment (carotid PP) at 5 and 7 weeks and a higher pulse pressure during femoral artery parameter assessment (femoral PP) in the FP and at 5 weeks. Intergroup differences in carotid and femoral artery diameter and the intra-group changes with pregnancy were in general small. Only in the HYPERT subgroup in the FP (only carotid) and at 5 and 7 weeks, the carotid and femoral CC and DC were smaller than in the control group. Only in controls, carotid DC had changed with pregnancy, while femoral CC and DC had increased in controls and THROMB subjects, but not in NONTHROMB and HYPERT women. Only in the controls, the VeC (Table V) had increased in early pregnancy.

In all groups absolute values in the FP and changes in early pregnancy in 17β -estradiol and progesterone were comparable (Table VI).

Only in controls, the absolute change in TPVR correlated with femoral compliance ($r = 0.81$, $r^2 = 0.66$, $p = 0.03$). Only in HYPERT, we noticed a correlation between the rise in progesterone and the fall in TPVR ($r = 0.85$, $r^2 = 0.72$, $p = 0.004$). In the FP of normotensive formerly preeclamptic groups PV correlated with venous compliance ($r = 0.63$, $r^2 = 0.39$, $p = 0.0004$).

Table I: Demographic data (FP) from the 3 subgroups of ex-preeclampsics and the group of normal parous controls. If a value is followed by an arrow, this particular variable is higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group ($p < 0.05$).

	controls	ex-PE		
		THROMB	NONTHROMB	HYPERT
	(n=10)	(n=18)	(n=13)	(n=11)
Age (years)	31 $\pm$ 2	29 $\pm$ 4	29 $\pm$ 3	33 $\pm$ 4
Height (cm)	168 $\pm$ 4	169 $\pm$ 6	168 $\pm$ 5	165 $\pm$ 4
BMI (kg.m ⁻²)	22 $\pm$ 2	24 $\pm$ 4	27 $\pm$ 6	25 $\pm$ 6
Parity (median with range)	1 (1-2)	1 (1-3)	1 (1-2)	1 (1-4)
Interval (months) (median with range)	18 (6-48)	12 (6-46)	20 (6-46)	11 (6-29)
MAP (mmHg)	82 $\pm$ 7	85 $\pm$ 8	87 $\pm$ 6	109 $\pm$ 8 $\uparrow$
PV (ml.kg ⁻¹ .LBM ⁻¹)	56 $\pm$ 4	51 $\pm$ 4	46 $\pm$ 3 $\downarrow$	50 $\pm$ 5

Interval = time elapsed between delivery previous pregnancy and first measurement session

Table II: Occurrence of the various subtypes of thrombophilic disorders in the thrombophilic - and hypertensive subgroups of ex-PE- women.

Type of thrombophilic disorder	THROMB (n = 18)	HYPERT (n = 11)
ACA/LA (Antiphospholipid syndrome)	9 (50%)	
Hyperhomocysteinemia	2 (11%)	3 (27%)
Protein C deficiency		
Activated Protein C Resistance	2 (11%)	
Protein S deficiency	6 (33%)	
Anti thrombin deficiency		

Table III: Hemodynamic data obtained in the FP and LP of the menstrual cycle (MC) and at 5 and 7 weeks amenorrhea (AM5 and AM7, respectively). An arrow indicates higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group in the corresponding MC phase ($p < 0.05$). The symbol * indicates the value in a particular subgroup to be different from that in the FP ($p < 0.05$).

		controls	ex-preeclamptic		
		(n = 10)	THROMB (n = 18)	NONTHROMB (n = 13)	HYPERT (n = 11)
MC					
MAP (mmHg)	FP	82 $\pm$ 7	86 $\pm$ 8	87 $\pm$ 6	109 $\pm$ 8 $\uparrow$
	LP	82 $\pm$ 4	86 $\pm$ 6	86 $\pm$ 7	108 $\pm$ 8 $\uparrow$
	AM5	74 $\pm$ 8*	81 $\pm$ 8	83 $\pm$ 6*	98 $\pm$ 10 $\uparrow$
	AM7	76 $\pm$ 5*	80 $\pm$ 7*	82 $\pm$ 5*	95 $\pm$ 7* $\uparrow$
HR (bpm)	FP	63 $\pm$ 8	63 $\pm$ 8	67 $\pm$ 7	67 $\pm$ 12
	LP	68 $\pm$ 7*	66 $\pm$ 9	66 $\pm$ 5	72 $\pm$ 12
	AM5	68 $\pm$ 7*	69 $\pm$ 7*	69 $\pm$ 6	72 $\pm$ 9*
	AM7	69 $\pm$ 9*	64 $\pm$ 6	66 $\pm$ 6	74 $\pm$ 9*
SV (ml.beat ⁻¹)	FP	71 $\pm$ 6	78 $\pm$ 11	74 $\pm$ 9	78 $\pm$ 10
	LP	68 $\pm$ 6	79 $\pm$ 11 $\uparrow$	77 $\pm$ 11	77 $\pm$ 13
	AM5	79 $\pm$ 9*	81 $\pm$ 12	78 $\pm$ 9*	81 $\pm$ 11
	AM7	77 $\pm$ 7*	86 $\pm$ 14*	82 $\pm$ 9*	84 $\pm$ 14*
CO (L.min ⁻¹)	FP	4.8 $\pm$ 0.4	5.2 $\pm$ 0.6	5.3 $\pm$ 0.7	6.0 $\pm$ 0.5 $\uparrow$
	LP	4.9 $\pm$ 0.6	5.6 $\pm$ 1.0	5.4 $\pm$ 0.8	6.1 $\pm$ 0.5 $\uparrow$
	AM5	5.6 $\pm$ 0.7*	6.0 $\pm$ 0.8*	6.1 $\pm$ 0.8*	6.1 $\pm$ 0.6
	AM7	5.7 $\pm$ 0.6*	6.1 $\pm$ 1.0*	6.1 $\pm$ 0.8*	6.3 $\pm$ 0.8
CI (L.min ⁻¹ .m ⁻²)	FP	2.7 $\pm$ 0.2	3.0 $\pm$ 0.4	3.0 $\pm$ 0.5	3.5 $\pm$ 0.4 $\uparrow$
	LP	2.9 $\pm$ 0.4	3.2 $\pm$ 0.5	3.0 $\pm$ 0.4	3.6 $\pm$ 0.5 $\uparrow$
	AM5	3.3 $\pm$ 0.4*	3.4 $\pm$ 0.3*	3.3 $\pm$ 0.4*	3.6 $\pm$ 0.4
	AM7	3.3 $\pm$ 0.3*	3.4 $\pm$ 0.4*	3.3 $\pm$ 0.3*	3.6 $\pm$ 0.5
TPVR.(x10 ³) (dyne.s.cm ⁻⁵)	FP	1.4 $\pm$ 0.2	1.3 $\pm$ 0.2	1.4 $\pm$ 0.2	1.5 $\pm$ 0.2
	LP	1.4 $\pm$ 0.2	1.2 $\pm$ 0.2	1.3 $\pm$ 0.2	1.4 $\pm$ 0.2
	AM5	1.1 $\pm$ 0.1*	1.1 $\pm$ 0.2*	1.1 $\pm$ 0.1*	1.3 $\pm$ 0.2*
	AM7	1.1 $\pm$ 0.1*	1.1 $\pm$ 0.2*	1.1 $\pm$ 0.2*	1.2 $\pm$ 0.2*

Table IV: Carotid vascular data obtained in the FP and LP of the menstrual cycle (MC) and at 5 and 7 weeks amenorrhea (AM5 and AM7, respectively). An arrow indicates higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group in the corresponding MC phase ($p < 0.05$). The symbol * indicates the value in a particular subgroup to be different from that in the FP ($p < 0.05$).

		controls	ex-preeclamptic		
MC		(n = 10)	THROMB (n = 18)	NONTHROMB (n = 13)	HYPERT (n = 11)
Carotis MAP (mmHg)	FP	82 $\pm$ 6	88 $\pm$ 5	89 $\pm$ 6	103 $\pm$ 7 $\uparrow$
	LP	83 $\pm$ 6	86 $\pm$ 7	89 $\pm$ 6	106 $\pm$ 6 $\uparrow$
	AM5	75 $\pm$ 4*	85 $\pm$ 8	83 $\pm$ 5	97 $\pm$ 7 $\uparrow$
	AM7	78 $\pm$ 6*	81 $\pm$ 4*	82 $\pm$ 6*	95 $\pm$ 7* $\uparrow$
Carotis PP (Δ mmHg)	FP	41 $\pm$ 2	46 $\pm$ 6	45 $\pm$ 4	48 $\pm$ 10
	LP	44 $\pm$ 4	48 $\pm$ 8	48 $\pm$ 6	50 $\pm$ 7
	AM5	45 $\pm$ 5	45 $\pm$ 7	49 $\pm$ 8	54 $\pm$ 7 $\uparrow$
	AM7	42 $\pm$ 6	48 $\pm$ 6	51 $\pm$ 9	52 $\pm$ 12 $\uparrow$
Carotis $\varnothing$ (mm)	FP	6.7 $\pm$ 0.3	6.7 $\pm$ 0.3	6.8 $\pm$ 0.5	6.8 $\pm$ 0.2
	LP	6.5 $\pm$ 0.3	6.7 $\pm$ 0.2	6.8 $\pm$ 0.3 $\uparrow$	6.9 $\pm$ 0.2 $\uparrow$
	AM5	6.5 $\pm$ 0.3	6.6 $\pm$ 0.3	6.7 $\pm$ 0.2	6.6 $\pm$ 0.3
	AM7	6.6 $\pm$ 0.3	6.6 $\pm$ 0.2	6.7 $\pm$ 0.3	6.5 $\pm$ 0.4
Carotis CC (mm ² kPa ⁻¹)	FP	1.08 $\pm$ 0.26	1.04 $\pm$ 0.23	0.98 $\pm$ 0.27	0.80 $\pm$ 0.05 $\downarrow$
	LP	0.97 $\pm$ 0.24	1.00 $\pm$ 0.19	1.05 $\pm$ 0.27	0.85 $\pm$ 0.21
	AM5	1.07 $\pm$ 0.22	1.12 $\pm$ 0.10	1.10 $\pm$ 0.27	0.71 $\pm$ 0.10 $\downarrow$
	AM7	1.18 $\pm$ 0.35	1.06 $\pm$ 0.16	1.05 $\pm$ 0.18	0.79 $\pm$ 0.19 $\downarrow$
Carotis DC (kPa ⁻¹)	FP	30.5 $\pm$ 7.9	29.5 $\pm$ 5.9	28.0 $\pm$ 9.1	22.6 $\pm$ 1.3 $\downarrow$
	LP	28.8 $\pm$ 6.3	28.3 $\pm$ 6.1	28.9 $\pm$ 8.1	23.1 $\pm$ 4.2
	AM5	32.0 $\pm$ 6.4	33.0 $\pm$ 4.5	31.9 $\pm$ 8.4	21.1 $\pm$ 4.2 $\downarrow$
	AM7	36.0 $\pm$ 9.4*	30.8 $\pm$ 4.4	29.9 $\pm$ 6.5	23.2 $\pm$ 4.6 $\downarrow$

Table V: Femoral and venous vascular data obtained in the FP and LP of the menstrual cycle (MC) and at 5 and 7 weeks amenorrhea (AM5 and AM7, respectively). An arrow indicates higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group in the corresponding MC phase ($p < 0.05$). The symbol * indicates the value in a particular subgroup to be different from that in the FP ($p < 0.05$).

		controls	ex-preeclamptic		
	MC	(n = 10)	THROMB (n = 18)	NONTHROMB (n = 13)	HYPERT (n = 11)
Femoral MAP (mmHg)	FP	83 $\pm$ 6	88 $\pm$ 6	89 $\pm$ 5	105 $\pm$ 5 $\uparrow$
	LP	84 $\pm$ 5	86 $\pm$ 5	89 $\pm$ 7	106 $\pm$ 5 $\uparrow$
	AM5	76 $\pm$ 8*	86 $\pm$ 8	83 $\pm$ 6	98 $\pm$ 8 $\uparrow$
	AM7	78 $\pm$ 7*	82 $\pm$ 3*	82 $\pm$ 5*	97 $\pm$ 6* $\uparrow$
Femoral PP (Δ mmHg)	FP	42 $\pm$ 3	45 $\pm$ 5	46 $\pm$ 7	49 $\pm$ 8 $\uparrow$
	LP	44 $\pm$ 4	49 $\pm$ 9	48 $\pm$ 8	52 $\pm$ 8
	AM5	46 $\pm$ 6	47 $\pm$ 6	50 $\pm$ 9	55 $\pm$ 9 $\uparrow$
	AM7	42 $\pm$ 6	49 $\pm$ 6	50 $\pm$ 10	50 $\pm$ 7
Femoral $\varnothing$ (mm)	FP	8.7 $\pm$ 0.8	8.5 $\pm$ 0.7	8.1 $\pm$ 0.4	8.3 $\pm$ 0.8
	LP	8.6 $\pm$ 0.6	8.3 $\pm$ 0.9	8.4 $\pm$ 0.8	8.2 $\pm$ 0.8
	AM5	8.3 $\pm$ 0.7	8.6 $\pm$ 0.6	8.2 $\pm$ 0.6	8.3 $\pm$ 1.0
	AM7	8.6 $\pm$ 0.8	8.3 $\pm$ 0.6	8.2 $\pm$ 0.8	8.3 $\pm$ 1.1
Fem CC ($\text{mm}^2 \cdot \text{kPa}^{-1}$)	FP	0.32 $\pm$ 0.10	0.45 $\pm$ 0.19	0.38 $\pm$ 0.13	0.37 $\pm$ 0.09
	LP	0.40 $\pm$ 0.15	0.43 $\pm$ 0.16	0.39 $\pm$ 0.16	0.26 $\pm$ 0.12
	AM5	0.51 $\pm$ 0.22	0.62 $\pm$ 0.24*	0.40 $\pm$ 0.14	0.27 $\pm$ 0.13 $\downarrow$
	AM7	0.62 $\pm$ 0.20*	0.54 $\pm$ 0.23	0.35 $\pm$ 0.15 $\downarrow$	0.33 $\pm$ 0.14 $\downarrow$
Femoral DC (kPa^{-1})	FP	5.5 $\pm$ 1.6	8.0 $\pm$ 3.6	7.2 $\pm$ 2.1	7.1 $\pm$ 1.2
	LP	6.6 $\pm$ 1.9	7.7 $\pm$ 2.6	7.0 $\pm$ 2.0	4.8 $\pm$ 1.8
	AM5	7.8 $\pm$ 2.1	10.4 $\pm$ 3.5*	7.2 $\pm$ 2.6	4.7 $\pm$ 1.9 $\downarrow$
	AM7	10.6 $\pm$ 4.1*	9.9 $\pm$ 3.9	6.1 $\pm$ 2.0 $\downarrow$	6.2 $\pm$ 3.5 $\downarrow$
VeC ($\text{ml} \cdot \text{dl}^{-1} \cdot \text{mmHg}^{-1}$)	FP	5.8 $\pm$ 0.9	5.6 $\pm$ 1.3	4.6 $\pm$ 1.3	5.6 $\pm$ 1.2
	LP	5.7 $\pm$ 1.1	5.6 $\pm$ 1.5	4.6 $\pm$ 1.1	6.3 $\pm$ 1.5
	AM5	5.5 $\pm$ 1.0	5.3 $\pm$ 1.4	4.8 $\pm$ 1.9	5.6 $\pm$ 0.8
	AM7	6.7 $\pm$ 1.0*	5.2 $\pm$ 1.6 $\downarrow$	4.5 $\pm$ 0.7 $\downarrow$	6.3 $\pm$ 0.8

Table VI: Sex-hormones data obtained in the FP and LP of the menstrual cycle (MC) and at 5 and 7 weeks amenorrhea (AM5 and AM7, respectively). An arrow indicates higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group in the corresponding MC phase ($p < 0.05$). The symbol * indicates the value in a particular subgroup to be different from that in the FP ($p < 0.05$).

	MC	controls	ex-preeclamptic		
		(n = 10)	THROMB (n = 18)	NONTHROMB (n = 13)	HYPERT (n = 11)
17 β Estradiol (nmol.L ⁻¹)	FP	0.12 $\pm$ 0.07	0.12 $\pm$ 0.05	0.14 $\pm$ 0.05	0.14 $\pm$ 0.05
	LP	0.25 $\pm$ 0.10*	0.32 $\pm$ 0.09*	0.28 $\pm$ 0.09*	0.35 $\pm$ 0.09*
	AM5	0.86 $\pm$ 0.62*	1.10 $\pm$ 0.55*	1.03 $\pm$ 0.30*	0.99 $\pm$ 0.32*
	AM7	1.81 $\pm$ 0.87*	2.37 $\pm$ 1.05*	2.40 $\pm$ 0.66*	1.85 $\pm$ 0.92*
Progesterone (nmol.L ⁻¹)	FP	1.3 $\pm$ 0.5	2.0 $\pm$ 0.9	1.9 $\pm$ 0.7	1.5 $\pm$ 0.8
	LP	27.0 $\pm$ 11.0*	29.0 $\pm$ 9.7*	30.0 $\pm$ 9.0*	27.4 $\pm$ 6.6*
	AM5	48.0 $\pm$ 12.5*	64.2 $\pm$ 22.6*	59.3 $\pm$ 24.9*	55.7 $\pm$ 33.4*
	AM7	54.0 $\pm$ 8.4*	60.0 $\pm$ 24.4*	61.6 $\pm$ 22.0*	62.6 $\pm$ 27.9*

Discussion

This study was designed to determine whether the institution of a high-flow/low resistance circulation in the first weeks of pregnancy is accompanied by a rise in the compliance of the large vessels. In addition, we wanted to find out whether the pregnancy-induced change in this macrovascular compliance differs between women with and those without an increased risk to develop a hypertensive complication during pregnancy. To this end, we studied longitudinally in 42 high-risk women and 10 low-risk controls in the menstrual cycle and subsequent early pregnancy total peripheral vascular resistance and the compliance of both capacitive (predominantly elastic wall properties; carotid artery) and conductive arteries (predominantly muscular wall properties; femoral artery) and forearm veins. We chose the two functionally different arteries as vasorelaxation was expected to affect the compliance of a muscular artery more than that of an elastic artery. Measurements were performed in the follicular-and luteal phases of the menstrual cycle and again at 5 and 7 weeks pregnancy. The high-risk participants were subdivided into three subgroups, depending on the type of preexistent disorder^{4, 21, 22}.

The general characteristics in the three subgroups selected were comparable with those in the control group, except for a lower plasma volume in the NONTHROMB subgroup and

obviously, a higher blood pressure in the HYPERT group. The incidence of the various thrombophilic disorders in the THROMB subgroup was in line with reports by others^{21,22}. In all groups, total peripheral vascular resistance had decreased by 20 to 25% in early pregnancy, an abrupt decline already reached by 5 weeks, which is consistent with previous studies². However, only in controls, this phenomenon was paralleled by a gradual increase in carotid distension coefficient, femoral artery and forearm venous compliance. Except for venous compliance and carotid distension coefficient, these changes were also discernible in THROMB ex-PE subjects. Interestingly, both NONTHROMB and HYPERT ex-PE subgroup failed to respond to pregnancy with a rise in vascular compliance. Previous reports showed a rise in aortic compliance in the first trimester of human pregnancy, which was maintained until term^{5,7}. In the present study, this adaptive change was already noticeable in the early part of the first trimester of pregnancy. Our observation that this phenomenon develops very early in pregnancy, is supported by similar observations in the conscious pregnant rat²³. The magnitude in response to pregnancy of the femoral and carotid arterial compliance suggests that the arterial compliance increases as a result of relaxation of the muscular layer of the vessel wall. Theoretically, the impact on vascular impedance of a given rise in vascular compliance increases progressively moving downstream in the vascular tree from the aorta to the microcirculation. This may explain why the early-pregnancy fall in total peripheral vascular resistance seemed to occur earlier and to be larger than the rise in femoral artery compliance. It should be emphasized that other phenomena are also influencing the vascular compliance in early pregnancy. The vascular filling state, the decreased mean arterial blood pressure and the rise in heart rate tend to lower vascular compliance. It is likely that both decrement in vascular resistance and increment in compliance share a common, still obscure, mechanism triggered by the endocrine environment of pregnancy. In-vitro studies support the involvement of a lower myogenic vascular tone and reactivity²⁴. However, the exact pathway is still to be unraveled.

The rise in both arterial and venous compliance, as occurring in controls, supports the view that a systemically acting vascular relaxant can be considered a strong candidate initiating these changes. This relaxant initiates a cascade of responses. Centrally, both cardiac preload and afterload will fall. These changes trigger volume retention and the institution of a high-flow/low-resistance circulation, respectively. The rise in heart rate indicates that the sympathetic tone in the circulation is elevated in this period, in order to warrant an adequate rise in cardiac output to meet the peripheral demands. Possibly, when the venous

compartment is relatively small and contracted, as indicated by a lower plasma volume and also its correlation with venous compliance, autonomic responses can be expected to interfere with a normal rise arterial and venous vascular compliance.

Interestingly, NONTHROMB and HYPERT subjects did not respond to pregnancy with a rise in femoral compliance. The defective response may be caused by a weak pregnancy-related stimulus or by blunted responsiveness of the target tissue, for instance by a mechanism mentioned above. The changes in circulating steroid levels were comparable in controls and all ex-PE subgroups. Therefore, in both NONTHROMB and HYPERT subjects, reduced responsiveness of the vascular smooth muscle appears to be the most plausible explanation for the lack of increase in arterial compliance.

In summary, these data support the concept that in normal pregnancy the fall in total peripheral vascular resistance is paralleled by improved macrovascular compliance. The latter adaptive change does not occur in normotensive nonthrombophilic and hypertensive formerly preeclamptic women.

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CHAPTER 5

THE CARDIAC OUTPUT INCREASES INDEPENDENT OF BASAL METABOLIC RATE IN EARLY HUMAN PREGNANCY

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Abstract

- Background** Early pregnancy is characterized by the institution of a high-flow low-resistance circulation. In this study we tested the hypothesis that these hemodynamic changes develop independent of changes in basal metabolic rate.
- Methods** In 12 healthy women, we determined and calculated once during the follicular phase (FP, day 5 ± 2) and at 6, 8, 10 and 12 weeks of pregnancy, the following variables: body weight and length, body mass index (BMI), fat free mass (ffm), mean arterial pressure (MAP), heart rate (HR), stroke volume (SV), cardiac output (CO), total peripheral vascular resistance (TRVR), resting energy expenditure (REE), REE/ffm (REE_{ffm}) and respiratory quotient (RQ).
- Results** At 6 weeks gestational age HR and CO had increased while MAP and TPVR had decreased. These changes persisted throughout the study period. Meanwhile, REE, REE_{ffm}, RQ, ffm and body weight did not change consistently. The changes with pregnancy in hemodynamics did not correlate with those in basal metabolic rate.
- Conclusion** In early pregnancy the institution of a high-flow low-resistance circulation develops without a concomitant rise in basal metabolic rate. These findings support the concept that the hemodynamic changes in early pregnancy develop independent of concomitant changes in basal metabolic rate.

CHAPTER 5

Introduction

In pregnancy, systemic arterial vasodilation represents one of the first detectable changes in systemic hemodynamics. This vasodilation then initiates a cascade of compensations in the circulation and volume homeostasis that include, among others, a rise in cardiac output (CO), hemodilution and activation of the renin-angiotensin-aldosterone (RAA) system^{6, 8, 11, 12, 13, 21}. Neither the mechanism responsible for the initial hemodynamic change in early pregnancy nor its functional meaning in this period of pregnancy is understood. Insight in the interrelation between the early pregnancy increase in CO and a concomitant change in metabolic rate would improve our understanding of the mechanism and functional meaning of the early pregnancy vasorelaxation. Although in late pregnancy basal metabolism is elevated, the available data on change in metabolic rate in early pregnancy are scarce and inconclusive^{4, 9, 19, 20}. Moreover, to the best of our knowledge, the metabolic rate in early pregnancy has never been measured simultaneously with CO in a longitudinally study. Therefore, it is still obscure whether or not CO increased before or after a rise in metabolic rate in the first trimester of pregnancy.

The objective of the present study was to test the hypothesis that in early pregnancy the CO increases independent of concomitant changes in metabolic rate. To this end, we measured serially in 12 women during the follicular phase of the menstrual cycle and in subsequent early pregnancy, blood pressure, cardiac output and resting energy expenditure.

Methods

Selection and patient characteristics

In this study 12 non-smoking healthy women were enrolled. Participants were recruited by advertisement. None of the women used oral contraceptives or other medication. Measurements were performed in the FP (follicular phase; day 5 ± 2) and at 6, 8, 10 and 12 weeks of pregnancy. The gestational age at the time of measurement was verified by ultrasound biometry of the embryo (series 150, Pie Medical, Maastricht, NL) prior to the first measurement session in pregnancy. Gestational age was blinded for the individuals that determined metabolic and hemodynamic parameters.

Experimental procedure

Metabolism

After an overnight fast, each experimental session was started with the measurement of fat free mass (FFM, kg) and resting energy expenditure (REE, kcal. kcal.24 h^{-1}). Mean arterial blood pressure (MAP, mmHg) and heart rate (HR, bpm) were recorded intermittently throughout the measurement session by a semi-automatic oscillometric device (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, FL, USA). FFM was determined by bioelectrical impedance (BIA-101, RJL Systems, Detroit, MI, USA), a method associated with 0.3 kg variation between consecutive days²². REE, which comprises the sleeping metabolic rate supplemented with the energy cost of being awake, was determined by indirect calorimetry using a computerized open-circuit ventilated hood system (Oxycon B, Jaeger, Breda, NL). In a horizontal position, each participant breathes inside a canopy that forms a gastight barrier between the room air and the respirable air. Using a continuous inflation and suction pump, a constant flow of exhaled air and room air is drawn from and to the canopy. The gas composition and the velocity of the air flow are measured at the outlet of the air suction pump. In steady state, differences in gas composition between the canopy and room air varies as a function of the oxygen consumption (VO_2 , L.min^{-1}) and carbon dioxide production (VCO_2 , L.min^{-1})²³. From these values, the respiratory quotient (RQ) was calculated, according to the formula:

$$RQ = \text{VCO}_2 \text{ production} / \text{VO}_2 \text{ consumption}$$

Twenty-four hour resting energy expenditure is calculated using the abbreviated Weir formula ²⁵ and expressed in kcal.24h⁻¹ and kcal.24h⁻¹.kg fat free mass⁻¹:

$$REE \text{ (kcal.24h}^{-1}\text{)} = (3.9 + 1.1RQ) \times VO_2 \times 1440$$

$$REE_{ffm} \text{ (kcal.24h}^{-1}\text{.kg}^{-1}\text{)} = (3.9 + 1.1RQ) \times VO_2 \times 1440 / FFM$$

In our laboratory, the determination of RQ and that of REE has an intra-individual CV of 6% and 1.2%, respectively ²³.

Hemodynamics

Echocardiography to assess cardiac output was performed in semi-left lateral position, immediately after completion of the REE measurement after approximately 5 minutes of rest, using a cross-sectional, phased array echocardiographic Doppler system (Hewlett-Packard Sonos 2000 and 2500) ¹².

CO was calculated according to the formula:

$$CO \text{ (L.min}^{-1}\text{)} = \text{stroke volume} \times HR$$

In this formula, HR was obtained by taking reciprocal of the mean of five consecutive R-R intervals on the ECG. Stroke volume (SV, ml) was calculated by multiplying the aortic velocity integral and the aortic area. Aortic flow was measured across the aortic valves from an apical approach. SV was calculated using the average area under the aortic velocity curve (aortic velocity integral) of five consecutive ejections. Aortic valve diameter necessary for the calculation of the aortic area, was measured during systole by M-mode, off-line and at the orifice. Total peripheral vascular resistance (TPVR) was calculated as follows:

$$TPVR \text{ (dyne.s.cm}^{-5}\text{)} = 80 \times MAP / CO$$

The value used for MAP was obtained during the CO measurement and was calculated as the mean of three consecutive recordings.

Statistical analysis: Data are presented as means $\pm$ SD unless otherwise stated. Differences relative to the value obtained during the follicular phase were evaluated with ANOVA for repeated measures. Correlations between concomitantly measured variables in the different phases were tested by Spearman's rank correlation analysis. A p-value less than 0.05 was considered statistically significant.

Results

The demographic characteristics of the participants are listed in table I. In all subjects, the course of pregnancy was uneventful.

REE, REE_{ffm} and RQ did not change consistently in the study period between the FP and the 12th week of pregnancy (Table II). Body weight and FFM varied little and inconsistently between the 5 measurement sessions. Meanwhile, CO and HR had increased, and TPVR and MAP had decreased consistently in the study period, a change already noticed by the 6th week (Table III, Figure I).

The CO per unit REE and per unit REE_{ffm} increased significantly over the study period ($p = 0.04$, $r = 0.90$, $r^2 = 0.80$ and $p = 0.02$, $r = 0.94$, $r^2 = 0.89$, respectively). Within one measurement session, HR did not change appreciably between the period of REE measurement and the subsequent period of CO determination.

Neither the change in REE, nor that in REE_{ffm} in the course of the five measurement sessions correlated with the observed concomitant changes in CO ($r = -0.29$, $r^2 = 0.09$ and $r = -0.44$, $r^2 = 0.19$, respectively).

Table I: Demographic data (follicular phase).

Age (years)	29 ± 3
Height (cm)	168 ± 7
BMI (kg.m ⁻²)	23 ± 3
Parity (% nulliparous)	50
MAP (mmHg)	91 ± 12

Table II: Metabolic data and body- and fat free mass are from the follicular phase (FP) of the menstrual cycle and at 6, 8, 10 and 12 weeks of pregnancy. An asterisk indicates a significant difference as compared to the FP.

	Follicular	6 wks	8 wks	10 wks	12 wks
Weight	65 ± 10	65 ± 10	65 ± 11	65 ± 11	65 ± 12
FFM	45 ± 5	46 ± 4	44 ± 5	45 ± 5	45 ± 5
REE	1496 ± 165	1536 ± 141	1451 ± 215	1486 ± 201	1426 ± 117
REE _{ffm}	33.5 ± 3.4	33.2 ± 2.9	31.5 ± 4.0	32.8 ± 3.4	32.8 ± 1.5
RQ	0.88 ± 0.04	0.88 ± 0.06	0.87 ± 0.04	0.92 ± 0.06	0.89 ± 0.04

REE = resting energy expenditure (kcal.24h⁻¹), FFM = fat free mass (kg), RQ = respiratory quotient

Table III: Hemodynamic data are from the follicular phase (FP) of the menstrual cycle and at 6, 8, 10 and 12 weeks of pregnancy. An asterisk indicates a significant difference as compared to the FP.

	Follicular	6 wks	8 wks	10 wks	12 wks
HR	66 ± 5	$72 \pm 5^*$	$70 \pm 6^*$	67 ± 8	$71 \pm 7^*$
SV	79 ± 7	85 ± 12	85 ± 12	84 ± 11	80 ± 8
CO	5.2 ± 0.6	$6.0 \pm 0.7^*$	$5.9 \pm 1.0^*$	$5.8 \pm 0.8^*$	$6.1 \pm 0.4^*$
MAP	92 ± 11	$87 \pm 7^*$	$87 \pm 7^*$	$88 \pm 6^*$	$85 \pm 6^*$
TPVR	1434 ± 284	$1188 \pm 242^*$	$1213 \pm 272^*$	$1192 \pm 228^*$	$1174 \pm 111^*$

HR = heart rate (BPM), SV = stroke volume (ml), CO = cardiac output ($\text{L} \cdot \text{min}^{-1}$), MAP = mean arterial pressure (mmHg), TPVR = total peripheral vascular resistance ($\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5}$).

Figure I: Percent change, relative to the follicular phase (FP), in cardiac output (CO, solid bars) and resting energy expenditure (REE, open bars). Data are presented in means and SE and originate from the FP of the menstrual cycle and at 6, 8, 10 and 12 weeks of pregnancy. An asterisk indicates a significant difference as compared to the FP.

Discussion

Early pregnancy is characterized by the development of a high-flow low-resistance circulation^{6, 7, 9, 12, 13, 21}. However, at present the exact mechanism responsible for this effect is still obscure. A rise in metabolic rate could be one of the possible triggers for these circulatory adjustments. Conversely, when the metabolic rate does not increase, the high-flow low resistance circulation is associated with a CO rise in excess of metabolic demands. In this study, we explored the possible interrelationship between systemic vasorelaxation and metabolic rate in twelve healthy women, by measuring CO together with REE during five consecutive sessions in the first weeks of pregnancy when hemodynamic changes are the largest. In our study, the institution of a high-flow low-resistance circulation in early pregnancy develops without a concomitant rise in basal metabolic rate.

Reports on REE in early pregnancy are rather conflicting with some studies reporting an increment, others a fall in basal metabolic rate in early pregnancy^{9, 20}. Some of these differences in can be explained by the methods employed, the procedure to standardize measurement conditions and the role of fat mass with REE. In fact, suppression of REE in early pregnancy is mainly observed in women with a low body mass index and also, in advanced pregnancy, the largest increase in REE occurs in women with the highest body mass indices^{4, 20}. During the first 12 weeks of pregnancy we did not find an appreciable change in REE and RQ. Conversely, the concomitantly measured CO had already increased by the 6th week of pregnancy. Meanwhile, the time-dependent change in CO and that in REE did not correlate. Also the CO per unit REE and REE_{ffm} was found to increase with advancing amenorrhea. All these phenomena support the development of a hyperdynamic circulation in the first weeks of pregnancy. To maintain the balance in Starling forces within the capillary bed, the institution of a hyperdynamic circulation should be paralleled by the opening of protective arteriovenous shunts and with it a fall in arteriovenous oxygen difference over the circulation, an effect which has been observed by others in both human and animal pregnancy^{2, 5, 14, 18}. Opening of these arteriovenous fistulae is considered to be primarily systemic rather than placental, since intervillous circulation has not been established until the end of the first trimester¹⁶.

Obviously, these results should be interpreted in the context of the methods employed, particularly with respect to potential pitfalls. We performed the CO measurement immediately after the REE determination, a choice made for logistic reasons. These observations can only be considered 'simultaneously made', if the participants were really in

a steady state. The latter was highly probable, since REE is a continuously measured variable that can only be determined reliable when RQ had been stable for at least 15 minutes. The steady-state conditions during the REE measurement was maintained during the subsequent CO measurement, a condition verified on the basis of maintained steady-state in HR and MAP values throughout the latter period until completion of data acquisition.

These findings indicate that the early pregnancy systemic vasorelaxation develops independent of changes in basal metabolism. How this initial change in pregnancy is induced remains to be established. Prolonged changes in steroid environment giving rise to an altered balance between vasoconstrictive and vasodilatory stimuli, are thought to be responsible for the initial arterial relaxation^{15, 17}. The support for this concept comes from observations during the menstrual cycle and in the (pseudo)pregnant rat. During the luteal phase of the menstrual cycle, the hemodynamics and renal function changes slightly in the direction similar to that observed in early pregnancy⁸. In rat pseudopregnancy, initial adaptations in hemodynamic and volume homeostasis are similar to those observed in normal rat pregnancy^{1, 3, 24}. Moreover, although not found by all investigators, suppletion of sex steroid hormones in ovariectomized rats to a level observed in pregnancy can alter vascular sensitivity to vasodilatory stimuli comparable to that seen in normal pregnancy^{10, 15}. This indicates that neither trophoblastic hormones, nor the placenta itself, are needed to induce these hemodynamic changes.

In conclusion, early pregnancy is characterized by the development of a hyperdynamic circulation. This adaptive change seems to be the result of a primary relaxation of the arterial vasculature together with the opening of protective arteriovenous shunts.

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CHAPTER 6

MALADAPTATION TO PREGNANCY: A ROLE FOR ATRIAL NATRIURETIC PEPTIDE?

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Submitted

Abstract

Background: The majority of women with a history of preeclampsia have either an underlying thrombophilic or vascular disorder. In this study we tested the hypothesis that only the latter condition predisposes for abnormal hemodynamic adaptation to pregnancy.

Methods: 37 formerly preeclamptic subjects were subdivided into a hypertensive (HYPERT, n=10), a normotensive thrombophilic (THROMB, n=13) and a normotensive nonthrombophilic subgroup (NONTHTROMB, n=14). In these women and in ten normal parous controls, we measured the following variables at least 5 months postpartum at day 5 (± 2) of the menstrual cycle, and again at 5 and 7 weeks amenorrhea in the next pregnancy: Mean arterial pressure, heart rate, cardiac output, central cardiovascular dimensions, plasma volume, glomerular filtration rate, effective renal plasma flow, 17- β estradiol, progesterone, the hormones of the renin-angiotensin-aldosterone (RAAS) axis, catecholamines and α -atrial natriuretic peptide.

Results: The early-pregnancy rise in cardiac output, renal variables, RAAS activity and plasma volume was comparable in all groups. However, the HYPERT and NONTHTROMB subgroups differed from both THROMB and controls, by a higher cardiac output, lower renal hemodynamics and a lower plasma volume in the pre-pregnant state. In addition, only the women in these two subgroups responded to pregnancy by a rise in circulating α -atrial natriuretic peptide. In addition, at 7 weeks, in the subjects belonging to the HYPERT and NONTHTROMB subgroups plasma volume was the lowest and correlated inversely with the concomitant circulating level of α -atrial natriuretic peptide.

Conclusion: The hemodynamic adaptation to pregnancy in the HYPERT and NONTHTROMB subgroups differs from that in THROMB and controls by an early-pregnancy rise in α -atrial natriuretic peptide. As a consequence, the early-pregnancy plasma volume expansion in the NONTHTROMB and HYPERT subgroups is less than in normal parous controls.

CHAPTER 6

Introduction

In the last decade evidence is accumulating supporting the concept that preeclampsia and HELLP syndrome (Hemolysis, Elevated Liver enzymes, Low Platelets) are pregnancy-related syndromes that develop in women with a pre-existent vascular, metabolic or clotting disorder ^{1, 2}. The pathogenesis of these syndromes may begin as early as at the time of implantation as indicated by poor trophoblast invasion, and abnormal hemodynamic adaptation with common endpoint endothelial dysfunction ^{3, 4}. It follows that most of the pathogenetic cascade evolves subclinically over a period of at least 5 months. It is obscure, whether the heterogeneity in predisposing conditions as mentioned above, gives rise to a similar cascade of events in the period between defective implantation and the appearance of clinical symptoms caused by endothelial dysfunction. Most early-pregnancy changes in maternal hemodynamics are compensations triggered by systemic vasorelaxation ^{5, 6, 7}. These changes include the rise in cardiac output, renin-angiotensin system (RAAS) activity and plasma volume. Theoretically, formerly preeclamptic women with a thrombophilic phenotype and normal cardiovascular function can be expected to have a normal early-pregnancy hemodynamic compensatory response. Alternatively, formerly preeclamptic women with diminished cardiovascular reserves in conjunction with hypertension or “latent” hypertension, are more likely to show abnormal early-pregnancy changes ⁸. If the pathogenetic cascade between these two most important risk groups is indeed different in early pregnancy, it not only explains the modest yield of various recently reported prevention strategies executed in unselected groups of women at risk for preeclampsia ⁹. It also increases our understanding of the pathogenesis of preeclampsia and may provide valuable information about possibilities for preventive measures in selected risk groups.

The objective of this study was to test the hypothesis that the adaptation to pregnancy in women with a history of preeclampsia depends upon the predisposing condition. That is to say, the adaptation to pregnancy differs between women likely to have a pre-existent cardiovascular disorder and women with a thrombophilic phenotype. To this end, we divided a group of formerly preeclamptic women into subgroups with or without hypertension. The normotensive group was further subdivided into females with or without thrombophilia. These three groups were compared with a group of normal parous controls.

Methods

Selection and patient characteristics.

Sixty-three formerly preeclamptic, non-diabetic women and 15 normal parous controls were enrolled in this study. Data acquisition was initiated at least 5 months postpartum. Inclusion for further analysis required a pregnancy occurring within one year after the pre-pregnant measurement session. Furthermore, only ongoing singleton pregnancies (gestational age > 16 weeks) were included. As a consequence, from the group of 63 formerly preeclamptics and 15 controls having completed the non-pregnant measurement sessions, only 37 formerly preeclamptic women and 10 controls could be included for final analysis. All subjects were Caucasian and the ones who smoked (n=9), refrained from smoking during pregnancy. Formerly preeclamptic women were recruited from our outpatient clinic at the time of postpartum follow-up. Controls were recruited by advertisement. Preeclampsia and (pregnancy-induced) hypertension were defined according to the criteria of the National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy¹⁰. All experiments were preceded by one week of standardized sodium intake (100 mmol sodium per day), representing the mean sodium intake in our population. To this end, all participants consulted the hospital's dietitian, prior to study. Measuring 24-hour sodium output on the day before the experiment assessed women's compliance with the diet. None of the participants used vitamin supplements or any other medication in the two weeks prior to measurement. Women on antihypertensive drugs (n=2, labetalol) discontinued this medication at least one week prior to the study session but not in early pregnancy. On separate days and after an overnight fast, participants were tested for thrombophilia and hemodynamic function. Thrombophilia was defined as the condition characterized by the presence of at least one clotting disorder known to be associated with an increased risk to develop venous thrombo-embolism. All participants gave written informed consent. The hospital's medical-ethical committee approved the study.

Experimental procedure

Methods

The methodology of the measurements and calculations on the thrombophilic screening and hemodynamic function were described previously⁸.

Thrombophilic screening: In short, anticardiolipin antibodies were determined spectrophotometrically by enzyme-linked immunoassay. Lupus anticoagulant was determined by mixing 1:1 patient and pooled normal plasma after finding a prolonged partial thromboplastin time. Subsequently, a thromboplastin dilution test was performed by measuring prothrombin time with progressively diluted thromboplastin. Lupus anticoagulant was considered to be present when all assays were positive. We defined the antiphospholipid syndrome as the condition where anti-cardiolipin antibodies, when higher than 10 GPL for IgG and 6 MPL for IgM antiphospholipid antibodies, respectively, could be detected in peripheral blood in women with a history of preeclampsia/HELLP syndrome. Coagulation tests were performed after a venous puncture. Plasma samples were stored at -80 °C prior to assay. Protein C activity was determined with Coamate protein C (Chromogenix, Mölndal, Sweden). Total protein S antigen and free protein S were measured by an enzyme-linked immunosorbent assay (ELISA) (DAKO, Glostrup, Denmark). Activated protein C resistance was quantified by a chromogenic activated protein C-resistance assay. Antithrombin activity was assayed with chromogenic substrate S-2765 (Chromogenix, Mölndal, Sweden). A methionine-loading test was used to diagnose hyperhomocysteinemia. Homocysteine was measured by high-performance liquid chromatography with fluorescence detection. Individuals were considered to have hyperhomocysteinemia when fasting and/or post-load plasma homocysteine levels were more than 2 standard deviations higher than the mean of the laboratory control group.

Measurement of blood pressure and renal hemodynamics: In the mid-follicular phase (day 5 $\pm$ 2) of the menstrual period and at 5 and 7 weeks in the subsequent pregnancy, measurements were performed under standardized environmental conditions in a temperature-controlled room (25-26 °C) and with as minimal external disturbances as possible. Participants did not drink caffeine- or alcohol-containing beverages and refrained from smoking and eating for at least 10 hours before the experiment. The measurement session started at 8:00 AM, with the insertion of a 20-gauge catheter into a vein of the right forearm, to enable determination of renal function as detailed below ⁸. A second catheter was inserted into a vein of the contralateral forearm for sampling of blood. Throughout each measurement session, subjects were in supine position on a comfortable bed. Mean arterial blood pressure (MAP) and heart rate (HR) were recorded repeatedly at two minutes interval by a semi-automatic oscillometric device (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, FL, USA), throughout the measurement session. Effective renal plasma flow (ERPF)

and glomerular filtration rate (GFR) were measured by continuous infusion of PAH (PAH: para-aminohippurate sodium; MSD, West Point, PA, USA) and inulin (Inutest, Laevosan Gesellschaft, Linz, Austria), respectively. PAH and inulin were measured spectrophotometrically. Renal hemodynamics were corrected for body surface area (BSA) and expressed in $\text{ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$. Effective renal blood flow (ERBF) was obtained by dividing the effective renal plasma flow by (1-hematocrit). Renal vascular resistance (RVR, $\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5} \cdot 1.73^{-2}$) was obtained by dividing mean arterial blood pressure by the effective renal blood flow. The ratio of glomerular filtration rate and effective renal plasma flow was taken to obtain the filtration fraction (FF).

At least two hours after the initiation of the PAH/inulin infusion, three blood samples were taken for determination of inulin and PAH. In addition, blood was collected for assay of active plasma renin concentration, angiotensin II, aldosterone, α -atrial natriuretic peptide, 17- β estradiol, progesterone and catecholamines. Active plasma renin concentration was determined by an immunoradiometric assay technique (Nichols Institute Diagnostics, Wychen, The Netherlands). Angiotensin II was assayed by radioimmunoassay (RIA) following Phenyl column extraction (Amersham Int, Amersham, UK). A solid-phase protein binding RIA (Diagnostic Products Corporation, LA, USA) assessed aldosterone. In our laboratory, by the methods employed, the tests do not cross-react with progesterone. α -Atrial natriuretic peptide was analyzed by radioimmunoassay (Nichols Institute Diagnostics, Wychen, The Netherlands). Norepinephrine, epinephrine and dopamine were assessed by chromatograph after liquid-liquid extraction and derivatization with a selective fluorescent agent. Finally, serum 17- β -estradiol and progesterone were determined with a commercially available solid phase radioimmunoassay kit (DPC, Los Angeles, Cal, USA). Samples were collected on ice and centrifuged at 4 °C for 10 min at 1500 g immediately after sampling. All samples were stored deep-frozen (- 80 °C) prior to assay. Urinary sodium concentration was measured by standard chemical procedures in a urine sample collected over the 24 hours preceding the measurement session.

Venous compliance, representing venous visco-elastic properties, was defined as the plethysmographically determined slope of the relationship of intravenous volume- and pressure changes (Periflow, JSI, Beerse, Belgium)^{11, 12}. The measurement procedure has been detailed previously and has a coefficient of variation of 11.4%¹¹.

Measurement of plasma volume. Since 2 of the 3 measurements took place in early pregnancy, plasma volume was determined with the dextran-70 indicator dilution method and expressed in ml per kilogram (kg) lean body mass (LBM)¹³. Plasma volume was obtained by dividing the total injected dextran-70 by the virtual volume-specific dextran-70 concentration at time zero. The latter was obtained by extrapolation using the dextran-70 concentration determined at 10, 20 and 30 minutes after bolus injection^{8, 13}.

Echocardiography to assess cardiac function was performed in semi-left lateral position, after completion of the plasma volume measurement and after 5 minutes of rest, using a cross-sectional, phased array echocardiographic Doppler system (Hewlett-Packard Sonos 2000 and 2500)⁸. Cardiac output (CO, L.min⁻¹) was calculated by multiplying stroke volume (ml) and heart rate (HR, beats per minute). In this formula, heart rate (HR) was obtained by taking the reciprocal of the mean of five consecutive R-R intervals on the ECG. Stroke volume was calculated by multiplying the aortic velocity integral and the aortic area. Aortic flow was measured across the aortic valves from an apical approach. The average area under the aortic velocity curve (aortic velocity integral) of five consecutive ejections was used to calculate stroke volume. Aortic valve diameter necessary for the calculation of the aortic area, was measured off-line at the orifice during systole using M-mode. Cardiac index (CI, L.min⁻¹.m⁻²) was calculated by dividing cardiac output by body surface area. Total peripheral vascular resistance (TPVR, dyne.s.cm⁻⁵) was calculated by dividing mean arterial blood pressure by cardiac output. The value used for mean arterial blood pressure (MAP) was obtained during the cardiac output measurement and was calculated as the mean of three consecutive recordings. Left atrial diameter was measured by 2-D echocardiography. Finally, we determined the ultrasonic diameter of the inferior vena cava⁷.

Statistical analysis: Data are presented as means $\pm$ standard deviation unless otherwise stated. Differences between groups were tested with the Mann-Whitney-U Test, differences within groups between the follicular phase and other measurement sessions by Wilcoxon Matched-Pairs Signed-Ranks test, both tests with Bonferroni correction. Correlations, when calculated, between concomitantly measured potentially related variables, were tested by Spearman's Rank correlation analysis. The minimum detectable changes in the variables studied, between consecutive observations was calculated for the control group ($n = 10$, $\alpha = 0.05$, $\beta = 0.10$), assuming a normal distribution. The obtained values were 5% for mean arterial pressure, 10% for heart rate, 8% for stroke volume, 15% for cardiac output, 15% for

total peripheral vascular resistance, 9% for plasma volume, 12% for glomerular filtration rate, 16% for effective renal plasma flow, 15% for effective renal blood flow, 18% for renal vascular resistance and 38% for atrial natriuretic peptide. Similarly, we calculated the minimum detectable difference in these variables between the control group and the subgroups ($n = 10$, $\alpha = 0.05$, $\beta = 0.10$), also assuming a normal distribution of the data. This led to the following percentages; 19% for mean arterial pressure, 22% for heart rate, 12% for stroke volume, 12% for cardiac output, 19% for total peripheral vascular resistance, 10% for plasma volume, 8% for glomerular filtration rate, 20% for effective renal plasma flow, 21% for effective renal blood flow, 27% for renal vascular resistance and 49% for atrial natriuretic peptide. In all analyses, we considered a p-value of less than 0.05 statistically significant.

Results

Among the 37 formerly preeclamptics, 10 subjects had hypertension and were assigned to the HYPERT subgroup. Among these hypertensive subjects, three were found to have a thrombophilic disorder as well, all in conjunction with hyperhomocysteinemia (30%). Of the remaining 27 normotensive formerly preeclamptic subjects, 13 had one ($n=12$) or more ($n=1$) thrombophilic disorder(s). They were assigned to the THROMB subgroup. The other 14 had neither hypertension nor thrombophilia. They were assigned to the NONTHROMB subgroup. The demographic characteristics of all participants are listed in Table I. The 3 subgroups of formerly preeclamptics and the controls were comparable with respect to age, height, parity and urinary sodium output. The subjects in the NONTHROMB subgroup had a higher body mass index. Obviously, mean arterial blood pressure was higher in the HYPERT subgroup as compared to controls.

Among the 37 formerly preeclamptic participants, 43% (16/37) had at least one thrombophilic disorder. In the subjects allocated to the THROMB subgroup, the anti-phospholipid syndrome (5/13, 38%) and protein S deficiency (4/13, 31%) were most common, followed by hyperhomocysteinemia (2/13, 15%), APC resistance (2/13, 15%) and protein C deficiency (1/13, 8%). All formerly preeclamptic women, which were diagnosed as having the anti-phospholipid syndrome, were IgG serotypes. We did not identify women with antithrombin deficiency.

Table I: Demographic data (follicular phase) from the 3 subgroups of formerly preeclamptic women and the group of normal parous controls. If a value is followed by an arrow, this particular variable is higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group ($p < 0.05$).

	controls	formerly preeclamptic		
		THROMB	NONTHROMB	HYPERT
	(n=10)	(n = 13)	(n = 14)	(n = 10)
Age (years)	31 $\pm$ 2	29 $\pm$ 4	29 $\pm$ 3	33 $\pm$ 4
Height (cm)	168 $\pm$ 4	168 $\pm$ 5	168 $\pm$ 6	165 $\pm$ 5
Body mass index (kg.m ⁻²)	22 $\pm$ 2	22 $\pm$ 3	27 $\pm$ 6 $\uparrow$	26 $\pm$ 7
Parity (median with range)	1 (1-2)	1 (1-3)	1 (1-2)	1 (1-4)
Mean arterial pressure (mmHg)	79 $\pm$ 6	82 $\pm$ 6	84 $\pm$ 7	104 $\pm$ 9 $\uparrow$
Natriuresis (mmol.24h ⁻¹)	115 $\pm$ 25	94 $\pm$ 32	89 $\pm$ 33	99 $\pm$ 22

The data regarding systemic hemodynamics are listed in Table II. Data collected during the follicular phase were defined as 'baseline'. Baseline systemic hemodynamics in the THROMB subgroup did not differ from those in the control group. On the other hand, in both the NONTHROMB and HYPERT subgroups, baseline cardiac output was higher and baseline plasma volume lower than in the control group. Baseline cardiovascular dimensions and venous compliance did not differ between groups. In early pregnancy, all groups responded with a rise in stroke volume, cardiac output, cardiac index and plasma volume, and a fall in total peripheral vascular resistance and mean arterial blood pressure. Heart rate had increased in all groups except HYPERT. Venous compliance had only increased in the HYPERT and control groups. At 7 weeks, venous compliance was lower in the NONTHROMB subgroup as compared to controls. Except for an increase in left atrial diameter in the NONTHROMB subgroup at 7 weeks gestational age, none of the central cardiovascular dimensions differed between groups or had changed consistently with pregnancy in the various groups.

The renal hemodynamic data are listed in Table III. Baseline renal data in THROMB and controls were comparable. In contrast, the NONTHROMB and HYPERT subgroup differed from controls by a lower glomerular filtration rate (only in NONTHROMB), effective renal plasma flow and effective renal blood flow, and a higher renal vascular resistance.

Table II: Hemodynamic and vascular data obtained in the follicular phase (FP) of the menstrual period (MP) and at 5 and 7 weeks amenorrhea (AM5 and AM7). An arrow indicates higher (↑) or lower (↓) than the one in the control group in the corresponding phase ($p < 0.05$). The symbol * indicates the value in a particular subgroup to be different from that in the follicular phase ($p < 0.05$).

		controls	formerly preeclamptic		
		(n = 10)	THROMB (n = 13)	NONTHROMB (n = 14)	HYPERT (n = 10)
MP					
Mean arterial pressure (mmHg)	FP	79 ± 6	82 ± 6	84 ± 7	104 ± 9 ↑
	AM5	74 ± 7*	78 ± 6*	81 ± 6*	93 ± 7* ↑
	AM7	74 ± 10*	79 ± 7*	79 ± 7*	94 ± 7* ↑
Heart rate (bpm)	FP	60 ± 9	60 ± 7	63 ± 7	69 ± 11
	AM5	68 ± 7*	68 ± 6*	70 ± 7*	73 ± 9
	AM7	68 ± 9*	64 ± 7*	67 ± 6*	73 ± 8
Stroke volume (ml.beat ⁻¹)	FP	71 ± 6	76 ± 11	77 ± 10	76 ± 9
	AM5	79 ± 9*	79 ± 11*	81 ± 11*	81 ± 11
	AM7	77 ± 7*	83 ± 13*	83 ± 11*	86 ± 14*
Cardiac output (L.min ⁻¹)	FP	4.8 ± 0.4	5.2 ± 0.4	5.3 ± 0.7 ↑	5.8 ± 0.5 ↑
	AM5	5.6 ± 0.7*	5.7 ± 0.7*	6.3 ± 0.9*	6.1 ± 0.6*
	AM7	5.7 ± 0.6*	5.7 ± 0.6*	6.2 ± 0.9*	6.5 ± 1.0*
Cardiac index (L.min ⁻¹ .m ⁻²)	FP	2.8 ± 0.3	3.0 ± 0.3	2.9 ± 0.4	3.3 ± 0.5 ↑
	AM5	3.3 ± 0.4*	3.4 ± 0.3*	3.3 ± 0.4*	3.5 ± 0.4*
	AM7	3.3 ± 0.3*	3.4 ± 0.4*	3.3 ± 0.3*	3.7 ± 0.5*
Total peripheral vascular Resistance (x10 ³)(dync.s.cm ⁻⁵)	FP	1.4 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.5 ± 0.2
	AM5	1.1 ± 0.1*	1.1 ± 0.2*	1.1 ± 0.1*	1.3 ± 0.2*
	AM7	1.1 ± 0.1*	1.1 ± 0.2*	1.1 ± 0.2*	1.2 ± 0.2*
Venous compliance (ml.100ml ⁻¹ .mmHg ⁻¹)	FP	5.8 ± 0.9	5.9 ± 1.0	4.7 ± 1.3	5.2 ± 1.4
	AM5	5.5 ± 1.0	5.6 ± 1.4	4.7 ± 1.6	5.6 ± 0.8
	AM7	6.7 ± 1.0*	5.7 ± 1.7	4.5 ± 1.0 ↓	6.0 ± 1.1*
Plasma volume (ml.kg ⁻¹ .LBM ⁻¹)	FP	60 ± 4	55 ± 4	50 ± 5 ↓	50 ± 6 ↓
	AM5	63 ± 7*	57 ± 5*	49 ± 6 ↓	53 ± 5* ↓
	AM7	71 ± 7*	67 ± 6*	55 ± 8* ↓	54 ± 8* ↓

In early pregnancy, glomerular filtration rate, effective renal plasma and blood flow had increased, while renal vascular resistance and filtration fraction (all groups except NONTHROMB) had decreased. Throughout the measurement period, renal vascular resistance was consistently higher and effective renal blood flow was consistently lower in the NONTHROMB and HYPERT subgroups as compared to the control group.

Table III: Renal hemodynamics obtained in the follicular phase (FP) of the menstrual period (MP) and at 5 and 7 weeks amenorrhea (AM5 and AM7). An arrow indicates higher ($\uparrow$) or lower ($\downarrow$) than the one in the control group in the corresponding phase ($p < 0.05$). The symbol * indicates the value in a particular subgroup to be different from that in the follicular phase ($p < 0.05$).

	MP	controls	formerly preeclamptic		
		(n = 10)	THROMB (n = 13)	NONTHROMB (n = 14)	HYPERT (n = 10)
Glomerular filtration rate ($\text{ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$)	FP	124 $\pm$ 7	115 $\pm$ 20	111 $\pm$ 11 $\downarrow$	113 $\pm$ 23
	AM5	145 $\pm$ 11*	137 $\pm$ 24*	130 $\pm$ 7* $\downarrow$	136 $\pm$ 21*
	AM7	157 $\pm$ 14*	156 $\pm$ 27*	141 $\pm$ 11* $\downarrow$	146 $\pm$ 28*
Effective renal plasma flow ($\text{ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$)	FP	563 $\pm$ 78	502 $\pm$ 107	474 $\pm$ 70 $\downarrow$	421 $\pm$ 92 $\downarrow$
	AM5	758 $\pm$ 72*	676 $\pm$ 131*	645 $\pm$ 113* $\downarrow$	651 $\pm$ 154*
	AM7	797 $\pm$ 92*	777 $\pm$ 169*	665 $\pm$ 113* $\downarrow$	671 $\pm$ 125* $\downarrow$
Effective renal blood flow ($\text{ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$)	FP	878 $\pm$ 127	796 $\pm$ 190	753 $\pm$ 110 $\downarrow$	691 $\pm$ 157 $\downarrow$
	AM5	1160 $\pm$ 120*	1035 $\pm$ 230*	1013 $\pm$ 192*	1026 $\pm$ 251*
	AM7	1217 $\pm$ 162*	1167 $\pm$ 256*	1028 $\pm$ 170* $\downarrow$	1033 $\pm$ 190*
Renal vascular resistance ($\times 10^3$)($\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5} \cdot 1.73 \text{ m}^{-2}$)	FP	7.1 $\pm$ 1.3	8.7 $\pm$ 2.3	8.9 $\pm$ 1.8 $\uparrow$	12.5 $\pm$ 3.6 $\uparrow$
	AM5	5.0 $\pm$ 0.7*	6.1 $\pm$ 1.2*	6.4 $\pm$ 1.2* $\uparrow$	7.7 $\pm$ 2.4* $\uparrow$
	AM7	4.8 $\pm$ 0.6*	5.4 $\pm$ 1.3*	6.1 $\pm$ 1.1* $\uparrow$	7.2 $\pm$ 1.5* $\uparrow$
Filtration fraction ($\text{GFR} \cdot \text{ERPF}^{-1}$)	FP	0.22 $\pm$ 0.02	0.23 $\pm$ 0.03	0.24 $\pm$ 0.03	0.27 $\pm$ 0.04 $\uparrow$
	AM5	0.19 $\pm$ 0.02*	0.21 $\pm$ 0.03	0.21 $\pm$ 0.04	0.21 $\pm$ 0.04*
	AM7	0.20 $\pm$ 0.03*	0.20 $\pm$ 0.02*	0.22 $\pm$ 0.03	0.22 $\pm$ 0.04*
Sodium excretion ($\text{mmol} \cdot 24 \text{ h}^{-1}$)	FP	115 $\pm$ 25	94 $\pm$ 32	89 $\pm$ 33	99 $\pm$ 22
	AM5	134 $\pm$ 49	104 $\pm$ 40	108 $\pm$ 57	116 $\pm$ 42
	AM7	157 $\pm$ 77*	95 $\pm$ 34	117 $\pm$ 49	99 $\pm$ 23

The endocrine data are listed in Table IV. The baseline values for the volume-regulatory hormones, catecholamines and sex steroids were comparable in the 4 groups except for a higher norepinephrine in the HYPERT subgroup as compared to the control group. All groups responded to pregnancy with a similar rise in renin-angiotensin-aldosterone system activity and sex steroids. Only in HYPERT and NONTHROMB subjects, these changes were accompanied by a rise in α -atrial natriuretic peptide. In these latter 2 groups the norepinephrine level in the 7th week of gestation was lower than that in the follicular phase and lower than that in 7 weeks pregnant controls. Changes in epinephrine and dopamine were inconsistent.

Table IV: Endocrine variables as determined in the follicular phase (FP) of the menstrual period (MP) and at 5 and 7 weeks amenorrhea (AM5 and AM7). An arrow indicates higher (↑) or lower (↓) than the one in the control group in the corresponding phase ($p < 0.05$). The symbol * indicates the value in a particular subgroup to be different from that in the follicular phase ($p < 0.05$).

	MP	controls	formerly preeclamptic		
		n = 10)	THROMB (n = 13)	NONTHROMB (n = 14)	HYPERT (n = 10)
Active plasma renin Concentration (mU.L ⁻¹)	FP	14.2 ± 3.9	14.1 ± 6.9	16.7 ± 8.6	17.0 ± 8.6
	AM5	41.8 ± 13.0*	39.8 ± 10.2*	51.4 ± 13.4*	40.1 ± 19.0*
	AM7	40.7 ± 12.4*	37.9 ± 11.2*	41.9 ± 13.4*	34.8 ± 14.0*
Angiotensin II (pmol.L ⁻¹)	FP	8.9 ± 2.0	9.8 ± 3.2	10.6 ± 2.5	12.8 ± 4.1
	AM5	11.2 ± 3.2*	15.9 ± 9.1*	15.9 ± 5.4*	13.0 ± 7.7*
	AM7	13.0 ± 3.9*	14.3 ± 6.3*	18.4 ± 7.1*	17.8 ± 10.6*
Aldosterone (nmol.L ⁻¹)	FP	75 ± 16	82 ± 22	106 ± 39	108 ± 43
	AM5	258 ± 165*	265 ± 83*	288 ± 118*	187 ± 149*
	AM7	207 ± 75*	223 ± 105*	236 ± 108*	206 ± 73*
αatrial-natriuretic peptide (ng .L ⁻¹)	FP	85 ± 29	105 ± 41	104 ± 50	109 ± 42
	AM5	79 ± 18	83 ± 32	142 ± 44*↑	136 ± 68*↑
	AM7	80 ± 20	103 ± 29	155 ± 73*↑	168 ± 119*↑
Norepinephrine (nmol.L ⁻¹)	FP	0.71 ± 0.30	0.80 ± 0.34	0.76 ± 0.26	1.10 ± 0.36↑
	AM5	0.91 ± 0.25*	1.23 ± 0.46*	0.98 ± 0.37	1.07 ± 0.37
	AM7	0.96 ± 0.40*	0.97 ± 0.38*	0.64 ± 0.18*↓	0.70 ± 0.19*↓
17β-Estradiol (nmol.L ⁻¹)	FP	0.12 ± 0.09	0.11 ± 0.04	0.17 ± 0.08	0.14 ± 0.05
	AM5	0.86 ± 0.62*	1.02 ± 0.54*	1.15 ± 0.65*	0.99 ± 0.32*
	AM7	1.81 ± 0.87*	2.21 ± 0.79*	2.38 ± 0.86*	2.11 ± 1.16*
Progesterone (nmol.L ⁻¹)	FP	1.3 ± 0.5	2.0 ± 1.0	2.0 ± 0.7	1.4 ± 0.7
	AM5	48.0 ± 12.5*	65.1 ± 17.0*	51.0 ± 25.9*	55.7 ± 33.4*
	AM7	54.0 ± 8.4*	59.2 ± 20.7*	63.2 ± 25.2*	60.0 ± 27.6*

The change in α-atrial natriuretic peptide in early pregnancy correlated with various changes in hemodynamic and renal adaptation. We noticed that the larger the *fall* in α-atrial natriuretic in early-pregnancy, the greater were the concomitant rises in effective renal plasma flow ($r = -0.32$, $p < 0.05$), and heart rate ($r = -0.32$; $p < 0.05$) but also the higher were plasma volume ($r = -0.35$; $p < 0.05$), venous compliance ($r = -0.33$; $p < 0.05$) and renal hemodynamics in the 7th week of pregnancy (effective renal plasma flow: $r = -0.42$, $p < 0.01$; effective renal blood flow: $r = -0.36$, $p < 0.05$; and glomerular filtration rate: $r = -0.31$, $p < 0.05$).

In addition, at 7 weeks pregnancy, the circulating level of α -atrial natriuretic peptide inversely correlated with the 7 weeks' plasma volume ($r = -0.49$, $p < 0.001$) (Figure 1a), renal hemodynamics (effective renal plasma flow: $r = -0.49$ (Figure 1b), $p < 0.001$; effective renal blood flow: $r = -0.40$, $p < 0.01$; glomerular filtration rate: $r = -0.40$, $p < 0.01$) and total peripheral vascular resistance ($r = -0.29$, $p < 0.05$). On the other hand, at this gestational age, the α -atrial natriuretic peptide correlated positively with left atrial diameter ($r = 0.51$, $p < 0.0001$), stroke volume ($r = 0.52$, $p < 0.0001$) (Figure 1c), cardiac output ($r = 0.30$, $r < 0.05$) and left ventricular work ($r = 0.32$, $p < 0.05$).

Figure 1a: Linear regression analysis of plasma volume (ml.kg^{-1} lean body mass) as a function of α -atrial natriuretic peptide (nmol.l^{-1}) at 7 weeks of gestation. ($y = -0.11x + 74$, $r = 0.49$, $p < 0.001$).

Figure 1b: Linear regression analysis of effective renal plasma flow ($\text{ml} \cdot \text{min}^{-1} \cdot 1.73^{-2}$) as a function of α -atrial natriuretic peptide ($\text{nmol} \cdot \text{l}^{-1}$) at 7 weeks of gestation. ($y = -1.5x + 903$, $r = -0.49$, $p < 0.001$).

Figure 1c: Linear regression analysis of stroke volume (ml) as a function of α -atrial natriuretic peptide ($\text{nmol} \cdot \text{l}^{-1}$) at 7 weeks of gestation. ($y = 0.11x + 70$, $r = 0.52$, $p < 0.0001$).

Discussion

It is generally accepted that the clinical expression of preeclampsia arises from endothelial dysfunction, a condition which can be provoked by excessive exposure to biochemical (i.e. thrombophilia, diabetes mellitus, hypercholesterolemia) and/or mechanical stressors (i.e. hypertension, renovascular disease)¹⁴. Therefore it is plausible that preeclampsia is a disease state superimposed upon a pre-existing disorder, with "endothelial decompensation" representing the common endpoint of different pathophysiologic pathways. With this concept as a starting point, we subdivided our group of formerly preeclamptic subjects into a HYPERT - and normotensive subgroup, further subdivided into a group of normotensive formerly preeclamptic women either with or without thrombophilia (THROMB and NONTHROMB, respectively). Since preeclampsia often is preceded by early-pregnancy hemodynamic and renal maladaptation as compared to uncomplicated pregnancies, these findings raised the question whether this adaptation, as seen in healthy women, would be preserved in the above mentioned formerly preeclamptic subgroups. To elucidate this pathogenetic problem, we performed 3 consecutive hemodynamic and renal measurements, once in the pre-pregnant state and twice in early pregnancy, in women at risk for preeclampsia on the basis of their medical history.

The inter-group differences in demographic variables were small and inconsistent except for a higher body mass index in the NONTHROMB subgroup. Most women in the THROMB subgroup had thrombophilia in conjunction with the antiphospholipid syndrome, protein-S-deficiency or hyperhomocysteinemia, an observation in line with previous reports^{1, 2}. An important finding in the present study was the higher cardiac output and renovascular resistance, and lower plasma volume in the non-pregnant state, in HYPERT and NONTHROMB subgroups as compared to controls. Nevertheless, at first sight these differences did not seem to have affected the early-pregnancy adaptation which also have been reported by others^{7, 15, 16}: Both controls and THROMB participants as well as HYPERT and NONTHROMB subjects responded to pregnancy by a rise in cardiac output, renal hemodynamics, RAAS activity and plasma volume expansion, together with a fall in mean arterial blood pressure and total peripheral vascular resistance. However, the response to pregnancy in the latter two groups differed in one important aspect from that in controls and THROMB: The early-pregnancy rise in circulating level of α -atrial natriuretic peptide was only observed in HYPERT and NONTHROMB, a phenomenon, at 7 weeks pregnancy accompanied by an inverse correlation between the achieved plasma volume and the

circulating level of α -atrial natriuretic peptide. These data support the concept that in these subgroups elevated α -atrial natriuretic peptide levels could have interfered with the initial plasma volume expansion. The absence of an increase in α -atrial natriuretic peptide in early normal pregnancy has been observed previously ¹⁵. In NONTHROMB the rise in α -atrial natriuretic peptide was paralleled not only by a rise in left atrial diameter, but also by a lack of improvement in venous compliance. These phenomena suggest that α -atrial natriuretic peptide increases in response to a relative venous overfill. In this reasoning, early-pregnancy plasma volume expansion may be inhibited by a lack of concomitant increase in venous storage capacity as a consequence of either the absent rise in venous compliance or of an adverse response of the autonomic control of the cardiovascular system. As a consequence, the rise in plasma volume is inadequately accommodated in the venous system causing the venous pressure to increase and with it the α -atrial natriuretic peptide release.

It is known that in the early stages of essential hypertension plasma volume is reduced ¹⁷. Plasma volume was indeed reduced in the subjects in the HYPERT subgroup, possibly as an early compensation to keep arterial blood pressure in the normal range in spite of the presence of blood-pressure raising stimuli. It is unlikely that the lower plasma volume was secondary to the awareness of having hypertension as in only 2 of the 10 women in the HYPERT subgroup the diagnosis "hypertension" was made prior to the study ¹⁸. Most data on baseline hemodynamic and renal variables, plasma volume and hormones in the NONTHROMB subgroup were intermediate between HYPERT and controls. In addition, the NONTHROMB subjects resembled the HYPERT subjects with respect to the abnormal rise in circulating α -atrial natriuretic peptide. The resemblance in laboratory findings with the HYPERT subgroup led us to classify these normotensive women as "latent" hypertensives ⁸.

On the basis of the results of the present study we postulate the following sequence of events in normal and complicated pregnancy. The early-pregnancy endocrine environment triggers by some still unknown mechanism, a generalized fall in systemic vascular tone. This is accompanied by a higher compliance of the arteries, veins and possibly also the myocardium, together with vasodilation in the microcirculation. As a consequence, the relative filling state of the vascular bed decreases giving rise to a fall in both cardiac preload and afterload. The cardiovascular regulatory systems are activated in the arteries (baroreceptors), the kidneys (renin-angiotensin-aldosterone system) and the central veins and right atrium (stretch receptors). Furthermore, concomitantly with the fall in vascular tone,

resetting and alteration of these blood pressure regulating systems occurs. These latter effects include firstly an altered baroreceptor response to blood pressure variation, secondly a reduction in angiotensin-sensitivity and renal sensitivity to α -atrial natriuretic peptide, and thirdly resetting of the osmoreceptor without concomitant change in its sensitivity^{6, 19, 20, 21}. The rise in baroreceptor activity, triggered by the afterload reduction, will raise heart rate, cardiac contractility and mobilization of venous blood towards the arterial vascular compartment. Meanwhile, increased renin-angiotensin-aldosterone system activity and non-osmotic vasopressin release accelerates volume retention and restoration of the cardiac preload. A concomitant pregnancy-related decrease in atrial responsiveness to stretch suppresses the release of α -atrial natriuretic peptide during the gradual restoration of the central venous filling state in conjunction with the ongoing plasma volume expansion²². In HYPERT and NONTHROMB subjects the effects on these systems seems to be insufficient and paradoxical, as evidenced by the increase in α -atrial natriuretic peptide, a decrease in nor-epinephrine and a lower plasma volume at 7 weeks as compared to controls and THROMB. The α -atrial natriuretic peptide level not only provides an indirect estimate for vascular filling state, it also correlates positively with changes in right atrial pressure^{22, 23, 24}. A baroreceptor-mediated rise in sympathetic tone can be expected to raise cardiac preload and with it right atrial pressure. The latter effect can be counterbalanced by an increased cardiac contractility and heart rate to prevent atrial overflow and increased release of α -atrial natriuretic peptide. Since an isovolumetric load induces a lower pressure rise at increased compliance, rise in arterial compliance can be expected to elevate baroreceptor activity when stroke volume remains unaltered. In contrast, reduced arterial compliance can be expected to lower baroreceptor activity. Therefore, a reduction in vascular resistance, compensated by elevation in stroke volume without elevation of arterial compliance, can be expected to lower baroreceptor activity when arterial filling is restored. In that case, to compensate for a reduction in afterload, the required higher cardiac output can only be maintained at the expense of an elevated stroke volume and atrial diameter, leading to the paradoxical enhanced release of α -atrial natriuretic peptide and a reduced release in norepinephrine as observed in NONTHROMB and HYPERT women. Even though pregnancy is associated with an attenuated renal response to α -atrial natriuretic peptide¹⁹, its rise can be expected to alter volume regulation, which is supported by the correlation's between atrial natriuretic peptide and renal, cardiac, volume and vascular adaptation. The rise in venous compliance,

as observed in HYPERT subjects, may be the result of the anti-hypertensive treatment given after the non-pregnant measurement session.

In summary, the pattern of hemodynamic and volume response to pregnancy is similar in formerly preeclamptic subjects and controls. However, HYPERT and NONTHROMB subjects differed from controls and THROMB by a paradoxical early-pregnancy rise in α -atrial natriuretic peptide, together with a lower plasma volume at 7 weeks.

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CHAPTER 7

PRE-PREGNANT PREDICTION OF RECURRENT PREECLAMPSIA AND/OR FETAL GROWTH RESTRICTION IN FORMERLY PREECLAMPTIC WOMEN

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Abstract

Background In the last decade evidence is accumulating that preeclampsia is superimposed upon a preexisting hemodynamic, hemostatic, autoimmune or metabolic disorder. In this study we tested the hypothesis that in formerly preeclamptic subjects certain abnormalities in hemodynamic and hemostatic function as measured under non-pregnant conditions predict the development of hypertensive disorders and/or fetal growth restriction in the subsequent pregnancy.

Methods In 250 non-diabetic formerly preeclamptic women and 15 normal parous controls, we measured the following variables at least 5 months postpartum, at day 5 (± 2) of the menstrual cycle: Mean arterial pressure, heart rate, cardiac output, central cardiovascular dimensions, venous compliance, plasma volume, glomerular filtration rate, effective renal plasma flow, 17β -estradiol and progesterone, the hormones of the renin-angiotensin-aldosterone (RAAS) axis and α -atrial natriuretic peptide. In the subsequent pregnancy we determined, birth weight and birth weight centile, recurrence rate of prematurity, of fetal growth restriction and of incidence of pregnancy-induced hypertension, preeclampsia and HELLP-syndrome. Inclusion for final analysis required a subsequent singleton pregnancy, established within one year following the pre-pregnant evaluation and ongoing beyond 16 weeks gestation. As a consequence, 57 formerly preeclamptic women and 12 controls could be included for final analysis. All formerly preeclamptic participants received aspirin throughout pregnancy. Furthermore, those with thrombophilia, hyperhomocysteinemia and preexistent hypertension were treated with low molecular-weight heparin, with pyridoxine and folic acid supplementation and with antihypertensives, respectively.

Results Among 250 formerly preeclamptic women, 202 (81%) were normotensive and 48 (19%) hypertensive. Furthermore, 151/250 (60%) formerly preeclamptic women had a thrombophilic disorder, whereas 71/250 (28%) had neither thrombophilia nor hypertension. In controls neither hypertension nor thrombophilia was observed. Fifty-seven (23%) formerly preeclamptic participants and 12 (80%) controls succeeded in establishing an ongoing

pregnancy within one year. From this subset of formerly preeclamptic women, 12 (21%) were hypertensive (HYPERT), 23 (20%) normotensive thrombophilic (THROMB) and 22 (39%) normotensive nonthrombophilic (NONTROMB). The latter subgroup was more obese than controls. The HYPERT and NONTROMB subgroups differed from controls, by a higher non-pregnant cardiac output, lower renal hemodynamic function, a lower plasma volume and a lower venous compliance. The THROMB subgroup did not differ from controls for all measured variables. With respect to pregnancy outcome, the women in all formerly preeclamptic subgroups gave birth to an infant with a lower birth-weight centile relative to controls. The incidence of pregnancy-induced hypertension was the highest in the NONTROMB subgroup (73 %) and the lowest in the THROMB participants (44%). Preeclampsia with or without the HELLP-syndrome recurred in 13%, 23% and 33% of the participants in the THROMB, the NONTROMB and the HYPERT subgroup, respectively. Predisposing factors differed between these three risk groups. In THROMB, a low pre-pregnant plasma volume predisposed for pregnancy-induced hypertension, preeclampsia and HELLP syndrome. In NONTROMB women, an elevated cardiac output and cardiac index prior to pregnancy were associated with a higher incidence of pregnancy-induced hypertension. Finally, in HYPERT participants, we noticed that a higher pre-pregnant blood pressure was associated with a higher recurrence rate of preeclampsia.

Conclusion Pre-pregnant hemodynamic, renal, metabolic and clotting variables in formerly preeclamptic women can predict hypertensive complications and fetal growth restriction in the subsequent pregnancy.

CHAPTER 7

Introduction

High blood pressure complicates almost 10 percent of all pregnancies ¹. Together with obstetric hemorrhage, embolism and obstetric infection, it is the most common cause of maternal mortality ². It is also an important cause of perinatal mortality and morbidity, because it predisposes for fetal growth restriction and iatrogenic preterm birth.

In recent years, evidence is accumulating that particularly women with a preexisting vascular, metabolic or clotting disorder are prone to pregnancy-related hypertensive syndromes ^{3, 4, 5, 6, 7}. The latter suggests that in these cases the preexistent disorder predisposes for “endothelial decompensation” in pregnancy, the common endpoint of different pathophysiologic pathways in pregnancy-related hypertensive syndromes ⁸. However, in most studies, subgroups of patients at risk were identified only after their complicated pregnancy. It is not clear whether the risk conditions identified, do indeed predispose for *recurrent* vascular complications in a next pregnancy, and if so, whether the recurrence rate differs between different disorders. Such information would be essential to develop a predictive test for formerly preeclamptic women seeking counseling with respect to a next pregnancy. In addition, it would contribute to our understanding of the pathogenesis of the vascular complications of pregnancy.

Taking a preexisting risk condition as a starting point ⁶, the following questions emerge: Firstly, what is the recurrence rate of hypertensive complications in a subsequent pregnancy for certain identifiable risk conditions? Secondly, does the presence of more than one disorder influence this recurrence rate? And thirdly, does the severity of the vascular complications in the preceding pregnancy predict the development of recurrent disease in a subsequent pregnancy?

The objective of the present study was to test the hypothesis that in formerly preeclamptic women, hemodynamic - hemostatic, metabolic and volume variables determined in the follicular phase of the menstrual cycle can be used as predictors for recurrent hypertensive disorders and/or fetal growth restriction in a subsequent pregnancy. To this end, we determined in formerly preeclamptic women and normal parous controls clotting function, central and renal hemodynamics, venous compliance, plasma volume and a number of neurohumoral factors in the follicular phase of one of the menstrual periods that preceded the subsequent pregnancy. Subsequently, we recorded the recurrence rate of hypertensive complications and/or fetal growth restriction in the next pregnancy.

Methods

Selection of patients.

250 formerly preeclamptic, non-diabetic women and 15 normal parous controls participated in this study. Data acquisition was initiated at least 5 months postpartum. Formerly preeclamptic women were recruited from our outpatient clinic at the postpartum follow-up. Controls were recruited by advertisement. Preeclampsia and (pregnancy-induced) hypertension were defined according to the criteria of the National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy¹. Therefore, hypertension was defined as a mean systolic - and/or a mean diastolic arterial blood pressure higher than 140 mmHg or higher than 90 mmHg, respectively, using all of the Dinamap recordings accumulated at the day of the experiment. We defined fetal growth restriction on the basis of the birth of a baby with a neonatal birth weight below the 10th centile. All experiments were preceded by one week of standardized sodium intake of 100 mmol per day, a figure that corresponds with the mean sodium intake in our population. Quantification of the 24-hour urinary sodium output on the day before the experiment was performed to estimate compliance with the diet. None of the participants used vitamin supplements or any other medication in the two weeks prior to measurement. Women on antihypertensive drugs discontinued this medication at least one week prior to the study session. After an overnight fast, participants were tested for thrombophilia, hemodynamic and renal function. Thrombophilia was defined as a condition characterized by the presence of at least one clotting disorder known to be associated with an increased risk of venous thrombo-embolism. Inclusion for final analysis required the establishment of an ongoing (>16 weeks gestational age) singleton pregnancy within one year after the pre-pregnant measurements. As a consequence, 57 formerly preeclamptic women and 12 controls could be included for final analysis. After the measurement sessions, formerly preeclamptic women were managed as illustrated in Figure 1. All subjects were Caucasian. Those who smoked refrained from smoking during pregnancy. All participants gave written informed consent. The hospital's medical-ethical committee approved the study.

Experimental procedure

Methods

The methodology of the measurements and calculations with respect to the thrombophilic screening and hemodynamic and renal function have been detailed elsewhere⁶.

Thrombophilic screening: Anticardiolipin antibodies were determined spectrophotometrically by enzyme-linked immunoassay. Lupus anticoagulant was determined by mixing 1:1 patient and pooled normal plasma after finding a prolonged partial thromboplastin time. Subsequently, a thromboplastin dilution test was performed by measuring prothrombin time with progressively diluted thromboplastin. Lupus anticoagulant was considered to be present when all assays were positive. We defined the antiphospholipid syndrome as the condition where the titer of the circulating anti-cardiolipin antibodies was higher than 10 GPL for IgG antiphospholipid antibodies and 6 MPL for IgM antiphospholipid antibodies.

Coagulation tests were performed after a venous puncture. After elimination of platelets, samples were stored at -80 °C prior to assay. Protein C activity was determined with Coamate protein C (Chromogenix, Mölndal, Sweden). Total protein S antigen and free protein S were measured by an enzyme-linked immunosorbent assay (ELISA) (DAKO, Glostrup, Denmark). Activated protein C resistance was quantified by a chromogenic APC-resistance assay. Antithrombin activity was assayed with chromogenic substrate S-2765 (Chromogenix, Mölndal, Sweden).

A methionine-loading test was used to diagnose hyperhomocysteinemia. Homocysteine was measured by high-performance liquid chromatography with fluorescence detection. During the test participants used a standard low-methionine breakfast and lunch. Individuals were considered to have hyperhomocysteinemia when fasting and/or post-load plasma homocysteine levels were more than 2 standard deviations higher than the mean of the laboratory control group.

Measurement of blood pressure and renal hemodynamics: In the mid-follicular phase (day 5 $\pm$ 2) of the menstrual period these measurements were performed under standardized environmental conditions in a temperature-controlled room (25-26 °C) with as little as possible external disturbances. The measurement session started at 08:00 AM, with the insertion of a 20-gauge catheter into a vein of the right forearm, to enable determination of renal function as detailed below. A second catheter was inserted into a vein of the contralateral forearm so as to collect blood samples. Participants did not drink caffeine- or alcohol-containing beverages and refrained from smoking and eating for at least 10 hours before the experiment. Throughout the measurement session, subjects were in supine position on a comfortable bed. Arterial blood pressure and heart rate were recorded intermittently by a semi-automatic oscillometric device (Dinamap Vital Signs Monitor 1846, Critikon, Tampa,

FL, USA), only interrupted during blood sampling and during the echocardiographic measurements (see below). Effective renal plasma flow and glomerular filtration rate were measured by continuous infusion of PAH (PAH: para-aminohippurate sodium; MSD, West Point, PA, USA) and inulin (Inutest, Laevosan Gesellschaft, Linz, Austria), respectively. Both variables were corrected for body surface area and expressed in $\text{ml} \cdot \text{min}^{-1} \cdot 1.73 \text{m}^{-2}$. Effective renal blood flow was calculated by dividing renal plasma flow by (1-hematocrit). Renal vascular resistance (RVR, $\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5} \cdot 1.73 \text{m}^{-2}$) was obtained by dividing the mean arterial blood pressure by the effective renal blood flow. From these data the filtration fraction was calculated as defined as the ratio of glomerular filtration rate and effective renal plasma flow.

At least two hours after the initiation of the PAH/inulin infusion, blood samples were collected for the later determination of circulating levels of inulin, PAH and creatinine. Creatinine was measured by standard chemical procedures. In addition, blood was collected for assay of active plasma renin concentration, angiotensin II, aldosterone, α atrial natriuretic peptide, 17β estradiol, progesterone and catecholamines. Active plasma renin concentration was determined by an immunoradiometric assay technique (Nichols Institute Diagnostics, Wychen, The Netherlands). Angiotensin II was assayed by radioimmunoassay (RIA) following Phenyl column extraction (Amersham Int, Amersham, UK). A solid-phase protein binding RIA (Diagnostic Products Corporation, LA, USA) assessed aldosterone. α -Atrial natriuretic peptide was analyzed by radioimmunoassay (Nichols Institute Diagnostics, Wychen, The Netherlands). Serum 17β -estradiol and progesterone were determined with a commercially available solid phase radioimmunoassay kit (DPC, Los Angeles, Cal, USA). Samples were collected on ice and immediately centrifuged at 4°C for 10 min at 1500 g immediately after sampling. All samples were stored deep-frozen (-80°C) prior to assay.

Urinary sodium and creatinine concentration were measured by standard chemical procedures in a urine sample collected over the 24 hours preceding the measurement session. Creatinine clearance, expressed in $\text{ml} \cdot \text{min}^{-1}$, was calculated according to the formula:

$$\text{Creatinine Clearance} = 10^3 \times \frac{\text{urinary creatinine (mmol/l)} \times \text{urinary volume (l/1440 min)}}{\text{serum creatinine (mmol/l)}}$$

Venous compliance, representing venous visco-elastic properties, was defined as the plethysmographically determined slope of the relationship of intravenous volume- and

pressure changes (Periflow, JSI, Beerse, Belgium)^{9,16}. The measurement procedure has been detailed previously¹⁶. This variable has a coefficient of variation of 11.4%⁹.

Measurement of plasma volume. Plasma volume was measured next, using the Iodine¹²⁵ - Albumin (¹²⁵I-HSA) indicator dilution method and expressed in ml per kilogram (kg) lean body mass (LBM)⁶.

Echocardiography to assess cardiac function was performed in semi-left lateral position, after completion of the PV measurement and after 5 minutes rest, using a cross-sectional, phased array echocardiographic Doppler system (Hewlett-Packard Sonos 2000 and 2500)⁶.

Cardiac output (L.min⁻¹) was calculated by multiplying stroke volume (ml) and heart rate (bpm). In this formula, heart rate was obtained by taking the reciprocal of the mean of five consecutive R-R intervals on the ECG. Stroke volume was calculated by multiplying the aortic velocity integral and the aortic area. Aortic flow was measured across the aortic valves from an apical approach⁶. Both total peripheral vascular resistance (dyne. s. cm⁻⁵) and left ventricular work (mmHg.L.min⁻¹) were obtained by taking the quotient and product, respectively, of mean arterial pressure and cardiac output. The value used for mean arterial pressure (mmHg) was obtained during the cardiac output measurement using the recordings of the Dinamap apparatus and was calculated as the mean of three consecutive recordings.

Statistical analysis: Data are presented as means with standard deviations unless stated otherwise. Intra-group differences were tested with Wilcoxon Signed-Ranks Matched-Pairs test, differences between groups with the Mann-Whitney-U Test, both tests with Bonferroni correction. Correlations, when calculated, between concomitantly measured potentially related variables, were tested by Spearman's Rank Correlation Analysis. A p-value less than 0.05 relative to the control group, was considered statistically significant.

Results

Of the 250 eligible formerly preeclamptic participants and 15 controls, 57 formerly preeclampsics and 12 controls were available for final analysis. This implies that 57/250 or 23% of the formerly preeclamptic subjects and 12/15 or 80% of the parous controls conceived and had an ongoing singleton pregnancy, within 12 months after the measurement session (p<0.005). Among the formerly preeclampsics, 12 subjects had hypertension and were assigned to the HYPERT subgroup. From these 12 hypertensive subjects, four were found to have a thrombophilic disorder as well, three in conjunction with

hyperhomocysteinemia (25%) and one in conjunction with a combination of protein S deficiency and the antiphospholipid syndrome. Of the remaining 45 normotensive formerly preeclamptic subjects, 23 had either one (n=21) or more (n=2) thrombophilic disorder(s). They were assigned to the THROMB subgroup. The remaining 22 formerly preeclamptics had neither hypertension nor thrombophilia. They were assigned to the NONTHROMB subgroup. The demographic characteristics of all participants are listed in Table I. The 3 subgroups of formerly preeclamptics and the controls were comparable with respect to age, height, parity and urinary sodium output. The subjects in the NONTHROMB subgroup had a higher body mass index relative to controls. Obviously, mean arterial blood pressure was higher in the HYPERT subgroup than in the controls.

Figure 1: Management, subdivision and treatment of formerly preeclamptic participants.

APCR = activated protein C resistance. Vitamin B6 20 mg per day, vitamin B11 5 mg per day.
LMWH = low molecular-weight heparin (5800 anti Xa units per day). GA = gestational age (weeks).

Table I: Demographic data from the 3 subgroups of formerly preeclamptics and the group of normal parous controls. If a value is followed by an arrow, this particular variable is higher (↑) or lower (↓) than the one in the control group ($p < 0.05$).

	controls	formerly preeclamptic		
		THROMB	NONTHROMB	HYPERT
	(n=12)	(n=23)	(n=22)	(n=12)
Age (years)	31 ± 3	29 ± 3	29 ± 3	32 ± 4
Height (cm)	168 ± 4	170 ± 7	170 ± 6	166 ± 6
Body mass index (kg.m ⁻²)	22 ± 2	24 ± 5	26 ± 6 ↑	26 ± 7
Parity (median with range)	1 (1-2)	1 (1-2)	1 (1-2)	1 (1-4)
Sodium excretion (mmol.24h ⁻¹)	108 ± 31	93 ± 33	91 ± 26	91 ± 15

Among the 57 formerly preeclamptic participants, 47% (27/57) had at least one thrombophilic disorder. In the subjects allocated to the THROMB subgroup, the anti-phospholipid syndrome (9/23, 39%) and hyperhomocysteinemia (7/23, 30%) were most common, followed by protein S deficiency (5/23, 22%), APC resistance (3/23, 13%) and protein C deficiency (1/23, 4%). Seven formerly preeclamptic women, which were diagnosed as having the anti-phospholipid syndrome, were IgG serotypes. We did not identify women with antithrombin deficiency.

Table II lists the cardiovascular data and plasma volume. None of the variables listed differed between the THROMB subgroup and the control group. In both HYPERT and NONTHROMB subjects, cardiac output and left ventricular work were higher, and plasma volume and venous compliance lower than in controls. When normalized for body surface area, cardiac output was elevated only in the HYPERT subgroup.

Renal hemodynamic variables, volume regulating hormones and gonadal steroids are listed in table III. Differences between the THROMB subgroup and controls were small and inconsistent. In contrast, the NONTHROMB and HYPERT subgroup differed from controls by a lower effective renal plasma and blood flow, a lower glomerular filtration rate and a higher renal vascular resistance and filtration fraction (the latter only in HYPERT). Values for 17-β-estradiol, progesterone, α-atrial natriuretic peptide and the hormones of the renin-angiotensin-aldosterone system did not differ appreciably between subgroups and controls.

Table II: Hemodynamic and vascular data. The symbol * indicates the value in a particular subgroup to be different from that in the controls ($p < 0.05$).

	controls	formerly preeclamptic		
		THROMB	NONTHROMB	HYPERT
	(n=12)	(n=23)	(n=22)	(n=12)
Mean arterial pressure (mmHg)	83 ± 7	85 ± 7	87 ± 7	$110 \pm 8^*$
Heart rate (bpm)	64 ± 9	63 ± 9	65 ± 9	68 ± 14
Stroke volume (ml. beat ⁻¹)	71 ± 6	78 ± 12	79 ± 12	77 ± 10
Cardiac output (L.min ⁻¹)	4.8 ± 0.4	5.2 ± 0.5	$5.5 \pm 0.8^*$	$5.7 \pm 0.6^*$
Cardiac index (L.min ⁻¹ . m ²)	2.8 ± 0.3	3.0 ± 0.3	2.9 ± 0.5	$3.3 \pm 0.6^*$
TPVR (.10 ³ dyne.s.cm ⁻⁵)	1.4 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.5 ± 0.2
Left ventricular work (.10 ³ mmHg.L.min ⁻¹)	30 ± 4	36 ± 4	$39 \pm 9^*$	$50 \pm 6^*$
Venous compliance (ml.100ml ⁻¹ .mmHg ⁻¹)	6.0 ± 1.2	6.0 ± 1.2	$4.6 \pm 1.5^*$	$4.9 \pm 1.0^*$
Plasma volume (ml.kg ⁻¹ .LBM ⁻¹)	54 ± 5	51 ± 5	$46 \pm 3^*$	$48 \pm 6^*$

TPVR = total peripheral vascular resistance.

Maternal and fetal data with respect to the preceding and subsequent pregnancies are listed in tables IV and V, respectively. In controls, gestational age at delivery did not differ appreciably the two pregnancies. Both birth weight (287 ± 195) and birth weight centile (12 ± 15) had increased relatively to the preceding pregnancy. Each of the outcome variables in the three subgroups had improved markedly in the subsequent- relative to the preceding pregnancy. Nevertheless, as compared to controls, irrespective subgroup, formerly preeclamptic subjects persisted in having a higher incidence of pregnancy-induced hypertension and fetal growth restriction as indicated by a lower birth weight centile. Pregnancy-induced hypertension and fetal growth restriction in the subsequent pregnancy were observed more often in the NONTHROMB subgroup as compared to controls. As compared to the previous pregnancy, NONTHROMB delivered $8 (\pm 4)$ weeks later, had a $1845 (\pm 927)$ grams heavier baby with also a $30 (\pm 29)\%$ higher birth weight centile. If preeclampsia occurred in the NONTHROMB subgroup, it developed by a median of 7 [6-8] weeks later as compared to the index pregnancy. With respect to maternal pregnancy outcome measures, in NONTHROMB, we found the recurrence rate of pregnancy-induced hypertension to increase as a function of pre-pregnant cardiac output ($r = 0.62$, $p = 0.003$), cardiac index ($r = 0.49$, $p = 0.02$), left ventricular work ($r = 0.67$, $p = 0.009$) and heart rate ($r = 0.56$, $p = 0.03$) (Table VI a). A receiver operating characteristic (ROC) curve to explore

the predictive potential of the pre-pregnant cardiac output measurements indicated that NONTHROMB participants with a cardiac output over $4.8 \text{ L}\cdot\text{min}^{-1}$ had a high chance to develop pregnancy-induced hypertension in the subsequent pregnancy, the sensitivity being 93% and the specificity 67%. The associated positive and negative predictive values were 88% and 80%, respectively (Figure 2). A pre-pregnant cardiac output above $5.6 \text{ L}\cdot\text{min}^{-1}$ in a NONTHROMB woman is associated with an increased recurrence risk of preeclampsia, the sensitivity and specificity being 60% and 75%, respectively (Figure 2). THROMB subjects developed more often pregnancy-induced hypertension and fetal growth restriction in the subsequent pregnancy than controls. As compared to the preceding complicated pregnancy, we noted an $8 (\pm 4)$ weeks prolongation of pregnancy, which contributed to an increase in neonatal weight by $1815 (\pm 771)$ gram corresponding with a $15 (\pm 22)\%$ increase in birth weight centile. If in the THROMB subgroup the subsequent pregnancy was complicated by recurrent preeclampsia, the complication developed approximately 2 [-4-8] weeks later than in the preceding index pregnancy. In THROMB subjects, pre-pregnant plasma volume correlated inversely with the recurrence rate of pregnancy-induced hypertension ($r = -0.52$, $p = 0.01$), preeclampsia ($r = -0.50$, $p = 0.01$) and HELLP syndrome ($r = -0.50$, $p = 0.01$). Also pre-pregnant venous compliance correlated inversely with the subsequent development of preeclampsia ($r = -0.59$, $p = 0.02$) and HELLP syndrome ($r = -0.59$, $p = 0.02$) (Table VI b). On the basis of a calculated ROC curve, it could be determined that in THROMB participants, a plasma volume below $49 \text{ ml}\cdot\text{kg}^{-1}_{\text{LBM}}$ was highly predictive with respect to the recurrence of preeclampsia in a subsequent pregnancy, the sensitivity and specificity being 100% and 75%, respectively. The associated positive and negative predictive values were 38% and 100%, respectively (Figure 3). Two-third of the THROMB population (15/23) had a plasma volume of at least $49 \text{ ml}\cdot\text{kg}^{-1}_{\text{LBM}}$. None of these women developed recurrent preeclampsia. Conversely, a plasma volume below $49 \text{ ml}\cdot\text{kg}^{-1}_{\text{LBM}}$, identified THROMB women destined to develop PIH with a sensitivity and specificity of 89% and 86%, and positive and negative predictive values of 80% and 92%, respectively (Figure 3). The development of fetal growth restriction in THROMB women correlated inversely with pre-pregnant venous compliance ($r = -0.52$, $p = 0.04$). Furthermore, the gestational age at delivery ($r = 0.54$, $p = 0.04$), and the achieved birth weight ($r = 0.59$, $p = 0.02$) and birth weight centile ($r = 0.57$, $p = 0.02$) in the subsequent pregnancy increased as a function of pre-pregnant venous compliance (Table VI b).

Table III: Renal hemodynamics, volume regulating hormones and gonadal steroids. The symbol * indicates the value in a particular subgroup to be different from that in the controls ($p < 0.05$).

	control: (n=12)	formerly preeclamptic		
		THROMB (n=23)	NONTHROMB (n=22)	HYPERT (n=12)
Glomerular filtration rate ($\text{ml} \cdot \text{min}^{-1} \cdot 1.73\text{m}^{-2}$)	123 ± 9	118 ± 17	$111 \pm 11^*$	$109 \pm 25^*$
Effective renal plasma flow ($\text{ml} \cdot \text{min}^{-1} \cdot 1.73\text{m}^{-2}$)	551 ± 86	522 ± 86	$462 \pm 76^*$	$400 \pm 95^*$
Effective renal blood flow ($\text{ml} \cdot \text{min}^{-1} \cdot 1.73\text{m}^{-2}$)	866 ± 133	828 ± 159	$734 \pm 114^*$	$651 \pm 158^*$
Renal vascular resistance ($10^3 \text{ dyne} \cdot \text{s} \cdot \text{cm}^{-5} \cdot 1.73\text{m}^{-2}$)	7.4 ± 1.7	8.0 ± 1.9	$9.3 \pm 1.7^*$	$13.4 \pm 3.7^*$
Filtration fraction	0.23 ± 0.03	0.23 ± 0.03	0.24 ± 0.03	$0.28 \pm 0.05^*$
Creatinine Clearance ($\text{ml} \cdot \text{min}^{-1}$)	104 ± 19	107 ± 26	112 ± 26	98 ± 30
Active plasma renin concentration ($\text{mU} \cdot \text{L}^{-1}$)	14.0 ± 3.9	15.8 ± 9.5	14.4 ± 7.2	16.3 ± 7.8
Angiotensin II ($\text{pmol} \cdot \text{L}^{-1}$)	9.4 ± 1.9	9.9 ± 3.4	10.2 ± 2.6	12.0 ± 4.5
Aldosterone ($\text{nmol} \cdot \text{L}^{-1}$)	92 ± 40	88 ± 41	87 ± 34	113 ± 47
α Atrial natriuretic peptide ($\text{ng} \cdot \text{L}^{-1}$)	100 ± 50	94 ± 41	106 ± 49	103 ± 47
17β Estradiol ($\text{nmol} \cdot \text{L}^{-1}$)	0.13 ± 0.09	0.14 ± 0.08	0.13 ± 0.05	0.14 ± 0.05
Progesterone ($\text{nmol} \cdot \text{L}^{-1}$)	1.3 ± 0.6	2.4 ± 1.7	2.3 ± 1.5	1.6 ± 0.9

Table IV: Maternal pregnancy outcome. An arrow indicates the value in a particular subgroup to be different from that in the controls, the symbol * indicates the value to be different from the previous pregnancy within the same subgroup ($p < 0.05$). Data are presented as means and SD unless ending by [], indicating median with range.

	GROUP	Previous pregnancy	Current pregnancy
		n (%)	n (%)
Pregnancy induced hypertension #	CONTROLS	0 (0%)	0 (0%)
	THROMB	23 (100%)↑	10 (44%)↑*
	NONTHROMB	22 (100%)↑	16 (73%)↑*
	HYPERT	12 (100%)↑	8 (67%)↑*
Preeclampsia	CONTROLS	0 (0%)	0 (0%)
	THROMB	23 (100%)↑	3 (13%)*
	NONTHROMB	22 (100%)↑	5 (23%)*
	HYPERT	12 (100%)↑	4 (33%)↑*
HELLP syndrome	CONTROLS	0 (0%)	0 (0%)
	THROMB	12 (52%)↑	3 (13%)*
	NONTHROMB	7 (32%)↑	3 (14%)*
	HYPERT	5 (42%)↑	2 (17%)*
Gestational age PE/HELLP (weeks)	CONTROLS	-	-
	THROMB	29 ± 4	36 [21-39] *
	NONTHROMB	28 ± 4	36 [29-37] *
	HYPERT	27 ± 5	34 [32-37] ↓*

In HYPERT, pregnancy-induced hypertension is defined as that condition in which diastolic blood pressure rose more than 15 mmHg (followed by higher dose of the instituted antihypertensive medication). HELLP syndrome = hemolysis, elevated liver enzymes, low platelets. PE = preeclampsia.

Table V: Fetal pregnancy outcome. An arrow indicates the value in a particular subgroup to be different from that in the controls, the symbol * indicates the value to be different from the previous pregnancy within the same subgroup ($p < 0.05$). Data are presented as means and SD unless ending by [], indicating median with range.

	GROUP	Previous pregnancy	Current pregnancy
		n (%)	n (%)
Gestational age at delivery (weeks)	CONTROLS	40 ± 2	40 ± 2
	THROMB	$31 \pm 4 \downarrow$	$39 \pm 3^*$
	NONTHROMB	$30 \pm 4 \downarrow$	$39 \pm 3^*$
	HYPERT	$29 \pm 5 \downarrow$	$38 \pm 2^*$
Prematurity	CONTROLS	0 (0%)	0 (0%)
	THROMB	20 (87%) $\uparrow$	2 (9%)*
	NONTHROMB	21 (96%) $\uparrow$	6 (27%)*
	HYPERT	12 (100%) $\uparrow$	5 (42%) $\uparrow$ *
Birthweight (grams)	CONTROLS	3283 ± 390	$3628 \pm 577^*$
	THROMB	$1240 \pm 810 \downarrow$	$3134 \pm 773^*$
	NONTHROMB	$1225 \pm 734 \downarrow$	$3070 \pm 752^*$
	HYPERT	$1020 \pm 790 \downarrow$	$2901 \pm 742 \downarrow^*$
Centile	CONTROLS	47 ± 20	$66 \pm 25^*$
	THROMB	$20 \pm 19 \downarrow$	$38 \pm 30 \downarrow^*$
	NONTHROMB	$15 \pm 15 \downarrow$	$45 \pm 30^*$
	HYPERT	$14 \pm 13 \downarrow$	$36 \pm 27 \downarrow^*$
Fetal growth Restriction	CONTROLS	0 (0%)	0 (0%)
	THROMB	10 (43%) $\uparrow$	7 (30%) $\uparrow$
	NONTHROMB	12 (55%) $\uparrow$	4 (18%)*
	HYPERT	6 (50%) $\uparrow$	3 (25%)*
Stillbirth	CONTROLS	0 (0%)	0 (0%)
	THROMB	5 (22%)	0 (0%)*
	NONTHROMB	4 (18%)	0 (0%)*
	HYPERT	6 (50%) $\uparrow$	0 (0%)*

Table VI a: Correlations between a *high* pre-pregnant cardiac output, cardiac index, left ventricular work and heart rate and subsequent maternal and fetal outcome among normotensive non-thrombophilic (**NONTHROMB**) women.

	cardiac output ↑↑	cardiac index ↑	left ventricular work ↑	heart rate ↑
Pregnancy induced hypertension	↑↑	↑	↑↑	↑
Preeclampsia	-	-	-	-
HELLP syndrome	-	-	-	-
Gestational age at delivery	-	-	-	-
Fetal growth restriction	-	-	-	-
Birth weight	↓	-	-	-
Birth weight centile	-	-	-	-

↑ or ↓ indicates $p < 0.05$, ↑↑ or ↓↓ indicates $p \leq 0.01$, ↑↑↑ or ↓↓↓ indicates $p \leq 0.001$.

Table VI b: Correlations between a *low* pre-pregnant plasma volume_{IBM} and venous compliance and subsequent maternal and fetal outcome among normotensive thrombophilic (**THROMB**) women.

	plasma volume ↓	venous compliance ↓
Pregnancy induced hypertension	↑↑	-
Preeclampsia	↑↑	↑
HELLP syndrome	↑↑	↑
Gestational age at delivery	-	↓
Fetal growth restriction	-	↑
Birth weight	-	↓
Birth weight centile	-	↓

↑ or ↓ indicates $p < 0.05$, ↑↑ or ↓↓ indicates $p \leq 0.01$, ↑↑↑ or ↓↓↓ indicates $p \leq 0.001$.

Table VI c: Correlations between a *high* pre-pregnant plasma volume_{TOTAL}, left ventricular work, systolic, diastolic and mean arterial blood pressure and subsequent maternal and fetal outcome among hypertensive (**HYPERT**) women.

	plasma volume ↑	left ventricular work ↑	systolic pressure ↑	diastolic pressure ↑	mean pressure ↑
Pregnancy-aggravated hypertension	-	-	-	-	-
Preeclampsia	-	-	↑	↑	↑↑
HELLP syndrome	-	-	-	↑	↑
Gestational age at delivery	-	↓	-	↓	↓
Fetal growth restriction	-	-	↑	-	↑
Birth weight	↑	-	-	↓	↓↓
Birth weight centile	↑↑	-	-	↓	↓

↑ or ↓ indicates $p < 0.05$, ↑↑ or ↓↓ indicates $p \leq 0.01$, ↑↑↑ or ↓↓↓ indicates $p \leq 0.001$.

Figure 2. Receiver operating characteristic curve for both pregnancy induced hypertension (PIH)(dotted line, open circles) and preeclampsia (PE)(dashed line, solid circles) as a function of cardiac output (CO) among normotensive nonthrombophilic formerly preeclamptic women. An asterisk* indicates the test optimum.

*PIH: $\text{CO} \geq 4.8 \text{ L.min}^{-1}$:
 sensitivity = 93%
 specificity = 67%
 positive predictive value = 88%
 negative predictive value = 80%

* PE: $\text{CO} \geq 5.6 \text{ L.min}^{-1}$:
 sensitivity = 60%
 specificity = 75%
 positive predictive value = 43%
 negative predictive value = 86%

Figure 3. Receiver operating characteristic curve for both pregnancy-induced hypertension (PIH)(dotted line, open circles) and preeclampsia (PE)(dashed line, solid circles) as a function of plasma volume among normotensive thrombophilic formerly preeclamptic women. An asterisk* indicates the test optimum.

- * PIH: $PV \leq 49 \text{ ml.kg}_{\text{LBM}}^{-1}$: sensitivity = 89%
 specificity = 86%
 positive predictive value = 80%
 negative predictive value = 92%
- * PE: $PV \leq 48 \text{ ml.kg}_{\text{LBM}}^{-1}$: sensitivity = 100%
 specificity = 75%
 positive predictive value = 38%
 negative predictive value = 100%

Figure 4. Receiver operating characteristic curve for both pregnancy-aggravated hypertension (PAH)(dotted line, open circles) and preeclampsia (PE)(dashed line, solid circles) as a function of mean arterial blood pressure (MAP) among hypertensive formerly preeclamptic women. An asterisk* indicates the test optimum.

*PAH: MAP ≥ 112 mmHg: sensitivity = 63%
 specificity = 100%
 positive predictive value = 100%
 negative predictive value = 57%

* PE: MAP ≥ 112 mmHg: sensitivity = 100%
 specificity = 88%
 positive predictive value = 80%
 negative predictive value = 100%

Finally, the worse the outcome in the preceding pregnancy, the less favorable was the outcome in the subsequent pregnancy: The chances for low birth weight centile in the subsequent pregnancy were higher with: 1. a history of earlier onset preeclampsia ($r = -0.60$, $p = 0.005$) and delivery ($r = -0.69$, $p = 0.001$), 2. a history of a lower birth weight ($r = -0.68$, $p = 0.001$) and centile ($r = -0.59$, $p = 0.008$) and 3. the previous preeclamptic pregnancy being complicated by a HELLP syndrome ($r = -0.51$, $p = 0.02$). Interestingly, a history of stillbirth was associated with a high birth weight centile in the subsequent pregnancy ($r = 0.72$, $p = 0.0001$).

As compared to the preceding pregnancy, HYPERT subjects delivered approximately $9 (\pm 6)$ weeks later in the subsequent pregnancy, giving rise to a $1957 (\pm 1139)$ grams higher neonatal weight but also a $24 (\pm 32)\%$ higher birth-weight centile. Nevertheless, gestational age, birth weight and birth-weight centile in the subsequent pregnancy in this subgroup were still significantly lower than in the controls. In 8/12 of the hypertensive participants, antihypertensive medication needed to be augmented in the course of the subsequent pregnancy. Recurrent preeclampsia developed by a median of 7 [5-13] weeks later than in the preceding pregnancy. A higher pre-pregnant systolic, diastolic and mean arterial blood pressure were associated with a higher incidence of superimposed preeclampsia ($r = 0.59$, $p = 0.04$; $r = 0.62$, $p = 0.03$; $r = 0.78$, $p = 0.003$, respectively) and HELLP-syndrome (for diastolic and mean arterial bloodpressure, $r = 0.65$, $p = 0.02$ and $r = 0.65$, $p = 0.02$, respectively)(Table VI c). Determining the ROC curve for pregnancy-aggravated hypertension and preeclampsia as a function of mean arterial blood pressure, the optimum predictive blood pressure would be above 112 mmHg, a figure associated with a sensitivity of 63% and 100% and a specificity of 100% and 88%, respectively (positive and negative predictive value of 100% and 57% for pregnancy-aggravated hypertension and 80% and 100% for preeclampsia, respectively) (Figure 4). Furthermore, lower pre-pregnant diastolic and mean arterial blood pressure were followed by a more advanced gestational age at delivery and with it a higher birth weight and corresponding birth-weight centile (for diastolic blood pressure $r = 0.61$, $p = 0.04$; $r = 0.63$, $p = 0.03$; $r = 0.63$, $p = 0.03$, for mean arterial blood pressure $r = 0.611$, $p = 0.04$; $r = 0.70$, $p = 0.01$; $r = 0.61$, $p = 0.04$, respectively) (Table VI c). Finally, pre-pregnant total plasma volume correlated with birth weight ($r = 0.66$, $p = 0.03$) and birth-weight centile ($r = 0.79$, $p = 0.004$) (Table VI c).

Discussion

Although the underlying pathogenetic mechanisms of preeclampsia are still unclear, there is general agreement about a central role for dysfunctional endothelium in triggering the typical clinical symptoms⁸. Endothelial function can be jeopardized by numerous factors, either biochemically, such as in thrombophilia or diabetes mellitus, or mechanically, such as in hypertension. Normal pregnancy is associated with a physiological rise in the strain exerted upon the endothelium. Therefore, it is not surprising that accompanying risk factors enhance the development of vascular complications during pregnancy. In this prospective study, preexistent maternal features with extra endothelial strain as common denominator, were found to predispose for recurrent hypertensive disorders and fetal growth restriction in high-risk pregnancies.

With respect to the demographic characteristics, except for a higher body mass index in the NONTHROMB, the three formerly preeclamptic subgroups did not differ appreciably from the control group. A higher body mass index in formerly preeclamptic women has also been reported by others and is a well-known risk factor for both cardiovascular and metabolic complications¹⁴. The increased incidence of thrombophilic disorders among the formerly preeclamptic individuals relative to normal parous controls is in line with previous findings in Israeli and Dutch populations^{3,4,5}.

Despite their differences in mean arterial blood pressure, both HYPERT and NONTHROMB subjects were characterized by an elevated cardiac output, reduced venous compliance, low plasma volume, reduced renal perfusion and glomerular filtration, and increased renal vascular resistance. On the basis of these observations we previously proposed to consider NONTHROMB women as being latent hypertensive⁶. This view is further supported by our observation that in the majority of NONTHROMB subjects (73 %) pregnancy is complicated by the development of hypertension. The consistently low pre-pregnant plasma volume in (latent) hypertensive women and the contribution of a low plasma volume to the risk of recurrent preeclampsia in normotensive thrombophilic formerly preeclamptic women in this study emphasize the importance of this variable. Our data indicate that reduced plasma volume is a sensitive marker to identify women at risk for recurrent hypertensive disorders in pregnancy.

Most previous studies on recurrence of hypertensive disorders in pregnancy are based on obstetrical history as a starting point rather than underlying disorders. Furthermore, most of these studies were performed retrospectively, in untreated women and in a different

geographically localized population. Nevertheless, among normotensive formerly preeclamptic women, the reported recurrence rate of preeclampsia in a subsequent pregnancy ranged from 32 to 55%^{10, 11, 15}. Normotensive women with a history of HELLP-syndrome had recurrent preeclampsia and HELLP-syndrome in 19 to 23% and 3 to 27%, respectively^{12, 13}. In hypertensive formerly preeclamptic women, preeclampsia recurred in 46 - 97% of the cases, which contrasts with the only 5% recurrence rate of HELLP-syndrome in the subsequent pregnancy^{11, 13, 15}. In addition, with respect to gestational age, subsequent HELLP-pregnancies lasted on the average 2 weeks longer than the preceding pregnancy¹². In our study, preeclampsia recurred in 18% of the normotensive- and in 33% of the hypertensive formerly preeclamptic participants. Taking the underlying disorder into consideration, we found recurrent preeclampsia to occur in about 1/8 of the THROMB women, 1/4 of the NONTHROMB and 1/3 of the HYPERT population. However, clinically it is important to consider this recurrence rate in the context of a milder course of the recurrent disease as indicated by its later onset and the substantially higher birth weight and corresponding birth-weight centile.

Most hypertensive women in our study were unaware of their elevated blood pressure. As a consequence, they were evaluated without any antihypertensive medication. Those who used antihypertensive drugs discontinued their medication for at least one week prior to measurement. After evaluation, antihypertensive medication was (re)instituted, with the aim of reducing diastolic blood pressure to below 90 mmHg. During the subsequent pregnancy, superimposed hypertension, preeclampsia and HELLP syndrome recurred in 67%, 33% and 17% of the HYPERT subgroup, respectively. Although in all patients the preexistent hypertension was adequately controlled with antihypertensive drugs, their 'pre-treatment' systolic and mean arterial blood pressure before pregnancy was still predictive for recurrent hypertensive complications in the next pregnancy. This suggests that in these preeclamptic recidivists, even though blood pressure can be normalized, vascular adaptive capacity to pregnancy is impaired, possibly as a result of structural changes in the vessel wall^{16, 17}.

In contrast to NONTHROMB and HYPERT women, pre-pregnant hemodynamic, renal and vascular function in THROMB were not different from those in controls. All these women received a prophylactic regimen as detailed in Figure 1. In spite of this, pregnancy-induced hypertension, preeclampsia and HELLP syndrome recurred in 44%, 13% and 13% of the cases. Interestingly, the recurrence rate was closely linked to a low plasma volume, the most characteristic feature of a (latent) hypertensive hemodynamic phenotype⁶. More explicitly, if

plasma volume exceeded 49 ml/kg lbm, which was the case in 65% of the THROMB population, preeclampsia and HELLP syndrome did not recur. Likewise, a reduced venous compliance was associated with recurrent preeclampsia, HELLP-syndrome and fetal growth restriction. Both plasma volume and venous compliance seem to provide an index of cardiovascular adaptive capacity. Low values for these parameters may therefore indicate a diminished capacity to meet the increased blood flow demands of pregnancy, which, in some cases, can be expected to lead to hemodynamic maladaptation and with it, the development of hypertensive complications and fetal growth restriction.

In normotensive women with a history of preeclampsia and HELLP-syndrome, the reported incidence of preterm birth, fetal growth restriction and perinatal mortality in a subsequent pregnancy were 21%, 12% and 4%, respectively ¹³. These figures are at least four times higher in hypertensive formerly preeclamptic patients ¹³. In our study, also the course of the preceding complicated pregnancy contributed to the recurrence rate of fetal growth restriction in the subsequent pregnancy. In THROMB participants, a previous late-onset of hypertensive disease and a relatively high previous birth weight and birth-weight centile were associated with a lower recurrence rate of fetal growth restriction. In contrast, a history of stillbirth seemed to 'protect' against fetal growth restriction. The latter finding supports the view that an acute intrauterine accident, such as placental thrombosis or infarction resulting in fetal mortality, is not associated with a poor prognosis with respect to the outcome of a subsequent pregnancy.

The present study was not designed to prove the efficacy of low molecular-weight heparin and/or vitamin supplementation in reducing the recurrence risk of hypertensive complications in pregnancy in formerly preeclamptic women with a thrombophilic phenotype. Nevertheless, in thrombophilic formerly preeclamptic women, recurrent preeclampsia, HELLP syndrome and growth restriction seems to be related to the latent hypertensive profile rather than the underlying thrombophilia. Therefore, our data indicate that a prospective randomized study designed to evaluate the efficacy of this prophylactic regimen, might benefit from the consideration that concomitantly reduced vascular reserves be able to interfere with the study results. The results of the present study suggest that insight into the hemodynamic and clotting functions adds an extra dimension to the counseling of women with a history of severe vascular complications in a preceding pregnancy.

In conclusion, pre-pregnant hemodynamic and hemostatic variables in formerly preeclamptic women predict recurrent hypertensive complications and fetal growth restriction in a subsequent pregnancy.

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CHAPTER 8

GENERAL DISCUSSION

CHAPTER 8

General discussion

Most of current obstetric care involving the management of vascular complications of pregnancy consists of symptomatic treatment only. This is primarily related to the limited insight in cause and pathogenesis of these problems. The present doctorate thesis is composed of six studies each focusing on different aspects of vascular adaptations and limitations in pregnancy. In this context the following clinical entities are considered vascular complications of pregnancy: placental insufficiency giving rise to fetal growth restriction, pregnancy-induced hypertension, preeclampsia, eclampsia, HELLP-syndrome (hemolysis, elevated liver enzymes, low platelets) and placental abruption.

The objectives of the 4 studies presented in chapters 2, 3, 4 and 7 were to describe the composition, characteristics and responses to a next pregnancy of the heterogeneous subset of women with a history of some severe vascular complication in pregnancy. The following aspects formed separate topics of study in this target population: demography, prevalence and type of thrombophilic and vascular disorders, fluctuations in hemodynamics, renal function and volume homeostasis with the menstrual cycle and recurrence rate of vascular complications in the next pregnancy. The two studies presented in chapters 5 and 6 were designed to explore some aspects of the initial hemodynamic adaptation (chapter 5) or maladaptation (chapter 6) to pregnancy.

The demonstration of the heterogeneity in preexistent disorders among women at risk for vascular complications in pregnancy not only provides us with a plausible explanation for the disappointing outcome of large trials in unselected risk groups designed to evaluate some promising prophylactic strategies, but it also encourages the development of tailor-made prevention for homogeneous subgroups¹.

ASYMPTOMATIC FORMERLY PREECLAMPTIC WOMEN HAVE LATENT HEMODYNAMIC ABNORMALITIES

Women with essential hypertension, renovascular disease, diabetes mellitus and some thrombophilic disorders are prone to develop preeclampsia in pregnancy^{2, 3, 4, 5}. Interestingly, in all these conditions the preexistent disorder is known to be associated with extra strain exerted upon the endothelium. It is still unsettled how the various subgroups of formerly preeclamptic women differ from each other and from normal parous controls with

respect to pathogenesis of the vascular complications in pregnancy. Therefore, we studied the hemodynamic -, renal - and clotting functions in formerly preeclamptic women and compared the results with those obtained in normal parous controls.

Among formerly preeclamptic women almost 25% had hypertension and almost 50% had some thrombophilic disorder. About one-third of these women had neither hypertension nor thrombophilia. In the latter subgroup, neither renal function and circulating levels of volume regulatory hormones, nor cardiac index differed from the values observed in controls. However, probably as a consequence of the higher body mass index in most of these women, their absolute cardiac output and with it, left ventricular work was higher. Although obesity, chronic hypertension and preeclampsia are related conditions, chronic hypertension and overweight contribute independently to the development of preeclampsia ^{6, 7}. Total plasma volume increases as a function of body mass ⁶. Therefore, indexing for body mass is necessary to enable comparisons of plasma volume. We observed in both obese and non-obese asymptomatic formerly preeclamptic women and hypertensives a lower plasma volume per kg lean body mass as compared to controls. It is known that in the early stages of essential hypertension plasma volume is reduced ⁸. Therefore, asymptomatic formerly preeclamptic women may be considered to be latent - or pre-hypertensive. This is supported by the observation that formerly preeclamptic women have an increased likelihood to develop hypertension later in life ¹⁰.

In the present study, about 46% of the formerly preeclamptic participants had thrombophilia, which agrees with the figure reported by others in a comparable population ⁴. Thrombophilia not only predisposes for preeclampsia, but also for miscarriage, placental infarcts, placental abruption and stillbirth ¹¹. In thrombophilic formerly preeclamptic women, antiphospholipid syndrome, protein S deficiency, hyperhomocysteinemia and activated protein C resistance are most common. We diagnosed hyperhomocysteinemia in 36% of the hypertensive formerly preeclamptic participants.

Based on the above inferences, formerly preeclamptic women can be subdivided into a hypertensive (HYPERT) -, a normotensive thrombophilic (THROMB)- and a normotensive non-thrombophilic subgroup. The latter subgroup resembles the hypertensive subgroup closely with respect to hemodynamic function. Therefore, we propose to categorize these women as latent hypertensives (LATHYPERT). These observations support the notion that the endothelium plays a central role in the development of preeclampsia and that this disease

is mostly superimposed upon a pre-existing disorder either with a thrombophilic- or (latent) hypertensive phenotype.

CHANGES IN HEMODYNAMICS AND VOLUME HOMEOSTASIS WITH THE MENSTRUAL CYCLE IN WOMEN WITH A HISTORY OF PREECLAMPSIA

The magnitude of the cyclic changes in central and renal hemodynamics with the menstrual cycle in healthy females was for most variables less than 5% and thus often difficult to discern with the methodology employed. Consistent changes in the luteal phase relative to the follicular phase, were a fall in renal vascular resistance, and a rise in heart rate, effective renal plasma- and blood flow and plasma volume. These changes were paralleled by activation of the renin-angiotensin-aldosterone axis, a decrease in the plasma level of α -atrial natriuretic peptide and an increase in the plasma level of norepinephrine. Similar findings have been reported by others^{12, 13}. These changes are consistent with a state of mild vasorelaxation in the luteal phase of the menstrual cycle, resembling that of early pregnancy. Preeclampsia is often preceded by hemodynamic and renal maladaptation. These findings raised the question whether the normal pattern and magnitude of the hemodynamic and neuroendocrine fluctuations differ in the above mentioned formerly preeclamptic subgroups as compared to normal parous controls. If so, this would offer interesting possibilities to identify women at risk for vascular complications in pregnancy. To answer this question, we studied in the follicular and luteal phases of the menstrual cycle, the renal and cardiovascular functions in formerly preeclamptic women and compared the results with the ones delineated above for normal parous controls. Neither pattern nor magnitude of the cyclic changes with the menstrual cycle differed appreciably between controls and the three subgroups of formerly preeclamptic women, except for an elevated and non-cycling plasma level of norepinephrine in HYPERT only. It follows that this physiologic phenomenon is not usable to screen women at risk for vascular complications in pregnancy. In this study we found in both phases of the menstrual cycle, a lower plasma volume in the HYPERT and LATHYPERT subgroups, as compared to controls and the THROMB subgroup. A lower plasma volume may be a consequence of an increased vascular tone. However, the venous compartment and with it, plasma volume, may also be reduced as a result of interference with early vascular development e.g. due to a genetic defect or some intra-uterine insult¹⁴. About 2/3 of the plasma volume is located within the venous compartment and can be

mobilized in response to a fall in arterial tone¹⁵. The luteal decrease in afterload most likely activates both the renin-angiotensin-aldosterone-system and the sympathetic nervous system^{15, 16, 17}. The latter enhances venous return by increasing venous tone. It also raises cardiac contractility and heart rate. Together, these effects enable the preservation of the arterial blood pressure at the expense of a diminished venous capacitance. Meanwhile, the higher activity of the renin-angiotensin-aldosterone axis and the lower circulating levels of α -atrial natriuretic peptide accelerate volume retention so as to restore venous filling state. In concert with the restoration of the venous filling state, sympathetic activity in the cardiovascular system returns to the level of the pre-stimulus condition. It is conceivable that inadequate volume retention leads to chronically rather than transiently increased sympathetic tone. Consequences of the latter are not only a higher heart rate, myocardial contractility and mean arterial blood pressure, but also a sustained rise in cardiac pre-load, and eventually structural changes of heart and vessels^{15, 17}.

The release of α -atrial natriuretic peptide by the atrial wall increases as a function of both vascular filling state and right atrial pressure^{18, 19}. Therefore, we postulate that a subnormal size of the venous compartment with inadequate capacity to raise venous return in response to higher arterial flow demands triggers an increase in sympathetic tone and with it, a higher atrial pressure and higher plasma levels of α -atrial natriuretic peptide. The observation of higher basal norepinephrine levels in HYPERT relative to controls may indicate that in these individuals the higher demand for venous return in the luteal phase is primarily achieved by a rise in sympathetic tone rather than by volume retention.

THE EFFECT OF PREGNANCY ON THE COMPLIANCE OF LARGE ARTERIES AND VEINS IN FORMERLY PREECLAMPTIC WOMEN AND NORMAL PAROUS CONTROLS

The early-pregnancy drop in vascular resistance is accompanied by an increase in vascular compliance. The present study was designed to determine the contribution of the latter adaptive change in large vessels, to the institution of a high-flow/low resistance circulation in the first weeks of pregnancy. In addition, we wanted to find out whether possible pregnancy-dependent changes in the compliance of large vessels differ between formerly preeclamptic women and controls. To this end, we studied longitudinally in these populations total peripheral vascular resistance and the compliance of a capacity artery

(predominantly elastic wall properties: carotid artery) a conductive artery (predominantly muscular wall properties: femoral artery) and of forearm veins. We chose two functionally different arteries as vasorelaxation was expected to affect the compliance of a muscular artery more than that of an elastic artery. Measurements were performed in the follicular- and luteal phases of the menstrual cycle and subsequently at 5 and 7 weeks of gestation.

In all groups, total peripheral vascular resistance decreased by 20 to 25% in early pregnancy, with an abrupt decline already by 5 weeks gestational age, which is consistent with previous studies^{20, 21}. However, only in controls, this phenomenon was paralleled by a gradual increase in the carotid and femoral artery distension coefficient, and forearm venous compliance. In the THROMB subgroup the rise in femoral artery distension coefficient was not accompanied by an increase in venous compliance. Interestingly, both LATHYPERT and HYPERT women failed to respond to pregnancy with a rise in vascular compliance as observed in controls. Previous reports showed a rise in aortic compliance in the first trimester of human pregnancy, which was maintained until term^{22, 23}. In the present study, this adaptive change was already noticeable in the early part of the first trimester of pregnancy. The observation that this phenomenon develops very early in pregnancy is supported by similar observations in the conscious pregnant rat²⁴. The different response to pregnancy of the femoral and carotid arterial compliance suggests that arterial compliance increases as a result of relaxation of the muscular layer of the vessel wall. Theoretically, the impact of a given rise in vascular compliance on vascular impedance increases progressively towards the microcirculation. This may explain why the early-pregnancy fall in total peripheral vascular resistance seemed to occur earlier and to be larger than the rise in femoral artery compliance. It should be emphasized that factors, such as concomitant changes in vascular filling state and sympathetic tone, may modulate the pregnancy-dependent rise in vascular compliance. It is likely that both the decrement in total peripheral vascular resistance and the increment in vascular compliance are effects resulting from the same, still obscure mechanism, possibly triggered by the endocrine environment of pregnancy. In-vitro studies support the involvement of a lower myogenic vascular tone and reactivity²⁵. However, the exact pathway is still to be unraveled. Interestingly, LATHYPERT and HYPERT subjects did not respond to pregnancy with a rise in arterial compliance. This abnormality could be the result of a faint pregnancy-related stimulus or a blunted responsiveness of the vascular bed. Since the changes in circulating steroid levels were comparable in controls and all formerly preeclamptic subgroups, reduced relaxational

responsiveness of vascular smooth muscle seems to be the most plausible explanation for the lack of increase in arterial compliance. In summary, the data in this study support the concept that in normal pregnancy the fall in total peripheral vascular resistance is paralleled by improved macrovascular compliance. The latter adaptive change is not seen in latent hypertensive and hypertensive formerly preeclamptic women.

CARDIAC OUTPUT INCREASES INDEPENDENTLY OF BASAL METABOLIC RATE IN EARLY HUMAN PREGNANCY

Early pregnancy is characterized by the institution of a high-flow / low-resistance circulation. It is unclear, whether or not the latter develops in response to a rise in metabolic rate^{20, 21, 26, 27, 28, 29}. To clarify this issue, we determined longitudinally the relationship between blood pressure, cardiac output and basal metabolic rate in healthy women, once before pregnancy and subsequently at 6, 8, 10 and 12 weeks of gestational age.

Reported results on the change in resting energy expenditure with pregnancy are conflicting, mostly due to differences in study design, methodology employed and standardization procedure^{29, 30}. With respect to standardization, particularly the body mass index is important. In early pregnancy, for instance, only slim women seem to respond to pregnancy with a fall in resting energy expenditure^{30, 31}. During the first 12 weeks of pregnancy we found neither an appreciable change in resting energy expenditure nor in respiratory quotient, while cardiac output had already increased by the 6th week of gestation. Also the cardiac output per unit resting energy expenditure, both in absolute terms and when expressed per kilogram fat free mass, increased with advancing amenorrhea. All these phenomena support the development of a hyperdynamic circulation in the first weeks of pregnancy. To balance the Starling forces in the capillary bed, the institution of a hyperdynamic circulation requires the concomitant opening of protective arteriovenous shunts. This will lead to a fall in the extraction of oxygen by the systemic circulation, an effect indeed observed by various other investigators, in both human and animal pregnancy^{32, 33, 34, 35}. Opening of these arteriovenous fistulae is considered to be limited to the non-placental tissues, as the intervillous space is deprived of perfusion by maternal blood until the end of the first trimester³⁶.

These findings indicate that the early pregnancy systemic vasorelaxation develops independently of changes in basal metabolism. How this is brought about remains to be

established. Prolonged changes in steroid environment giving rise to an altered balance between vasoconstrictor and vasodilator stimuli, are thought to be responsible for the initial vasorelaxation^{37, 39}. Support for this concept comes from observations during the menstrual cycle and from rat studies. During the luteal phase of the menstrual cycle, the pattern of change in hemodynamics and renal function, although much smaller, resembles that observed in early pregnancy¹³. Meanwhile, in the pseudopregnant rat, the initial adaptations in hemodynamic and volume homeostasis are similar to those observed in normal rat pregnancy^{38, 40, 41}. There are also reports indicating that the administration of 17 β -estradiol and progesterone to ovariectomized rats, to a level similar as observed in pregnancy, increases the vascular sensitivity to vasodilator stimuli as seen in normal rat pregnancy^{37, 42}. The latter studies indicate that, at least in the rat, the trophoblast is not involved in the mechanism that results in the institution of the high-flow low-resistance circulation of pregnancy.

In summary, early pregnancy is characterized by the development of a hyperdynamic circulation. This adaptive change seems to be the result of a primary relaxation of the arterial bed, thereby inducing the compensatory opening of protective arteriovenous shunts.

MALADAPTATION TO PREGNANCY: A ROLE FOR ATRIAL NATRIURETIC PEPTIDE?

In contrast to uncomplicated pregnancies, preeclampsia is often preceded by early-pregnancy hemodynamic and renal maladaptation^{43, 44}. These findings raise the question whether this adaptation, is preserved in the above mentioned formerly preeclamptic subgroups. To elucidate this pathogenetic problem, we performed 3 consecutive hemodynamic and renal measurements, once before and twice during the early-pregnancy period.

All participants showed comparable patterns in hemodynamic, renal and endocrine changes from five-weeks pregnancy onwards. That is to say, mean arterial blood pressure and total peripheral vascular resistance decreased, while heart rate, stroke volume, cardiac output, cardiac index, plasma volume and renal hemodynamics together with circulating levels of active plasma renin, angiotensin and aldosterone, increased. Similar findings have been reported by others^{20, 21, 26}. One important difference between controls and THROMB on the one hand, and the HYPERT and LATHYPERT on the other hand, was the early-pregnancy rise in circulating levels of α -atrial natriuretic peptide. Moreover, circulating levels of α -

atrial natriuretic peptide at 7 weeks were inversely correlated with the concomitant plasma volume. This data supports the concept that in these subgroups elevated α -atrial natriuretic peptide levels could have interfered with the initial plasma volume expansion. The absence of a rise in α -atrial natriuretic peptide in early normal pregnancy has been observed before²¹. In LATHYPERT, the rise in α -atrial natriuretic peptide was paralleled not only by an increase in left atrial diameter, but also by a lack of improvement in venous compliance. These findings indicate that α -atrial natriuretic peptide may have increased in response to relative venous overfill. In this reasoning, early-pregnancy plasma volume expansion may be inhibited by a lack of concomitant increase in venous storage capacity. That is to say, the venous compartment fails to increase its compliance, needed to accommodate a larger blood volume. As a consequence, venous pressure increases, which, in turn, stimulates the release of α -atrial natriuretic peptide. The latter effect may have led to a reduced plasma volume expansion. A similar explanation may underlie the fact that in the early stages of essential hypertension, plasma volume is reduced⁸. In the HYPERT subgroup, a reduction in plasma volume was indeed found, and which may be seen as an early compensation to keep blood pressure in the normal range in spite of the presence of blood-pressure raising stimuli.

On the basis of the results of this study we postulate the following sequence of events in normal and complicated pregnancy. The early-pregnancy endocrine environment triggers by some still unknown mechanism, a generalized fall in systemic vascular tone. This must be accompanied by a higher compliance of the arteries and veins, together with vasodilatation in the microcirculation. As a consequence, the relative filling state of the vascular bed decreases giving rise to a fall in both cardiac preload and afterload. The cardiovascular regulatory systems are activated in the arteries (baroreceptors), the kidneys (renin-angiotensin-aldosterone system), central veins and right atrium (stretch receptors) (figure 1).

Figure 1. Illustration of the atrial stretch receptor. An increase in volume leads to more stretch and with it enhanced release of α -atrial natriuretic peptide.

Concomitantly with the fall in vascular tone, resetting and alteration of these blood pressure regulating systems occurs. These latter effects include firstly an altered baroreceptor response to blood pressure variation, secondly a reduction in angiotensin-sensitivity and renal sensitivity to α -atrial natriuretic peptide, and thirdly resetting of the osmoreceptor without a concomitant change in its sensitivity^{45, 46, 47, 48, 49}. The rise in baroreceptor activity, triggered by the afterload reduction, will raise heart rate, cardiac contractility and mobilization of venous blood towards the arterial vascular compartment. Meanwhile, increased renin-angiotensin-aldosterone system activity and non-osmotic vasopressin release accelerate volume retention and restoration of the cardiac preload. A concomitant pregnancy-related decrease in atrial responsiveness to stretch, suppresses the release of α -atrial natriuretic peptide during the gradual restoration of the central venous filling state in conjunction with the ongoing plasma volume expansion⁵⁰. In HYPERT and LATHYPERT subjects the effects on these systems seem to be insufficient and paradoxical, as evidenced by the increase in α -atrial natriuretic peptide, a decrease in norepinephrine and a lower plasma volume at 7 weeks as compared to controls and THROMB. The α -atrial natriuretic peptide level not only provides an indirect estimate for vascular filling state, it also correlates positively with changes in right atrial pressure^{18, 19, 50}. A baroreceptor-mediated rise in sympathetic tone can be expected to raise cardiac preload and with it right atrial pressure. The latter effect can be counterbalanced by an increased cardiac contractility and heart rate to prevent atrial overflow and increased release of α -atrial natriuretic peptide. Since an isovolumetric load induces a smaller pressure rise at increased compliance, rise in arterial compliance can be expected to elevate baroreceptor activity, when stroke volume remains unaltered (figure 2a and 2b). In contrast, reduced arterial compliance can be expected to lower baroreceptor activity. Therefore, a fall in total peripheral vascular resistance triggers a compensatory rise in stroke volume. If the latter is not accompanied by a rise in arterial compliance, baroreceptor activity is not adequately raised while the vascular filling state is restored. In this case, the response to the reduction in afterload consists also of a rise in cardiac output, only achieved by a higher stroke volume and atrial diameter, giving rise to the paradoxical release of α -atrial natriuretic peptide, while circulating levels of norepinephrine are reduced, a response observed in LATHYPERT and HYPERT women (figure 3). Even though pregnancy is associated with an attenuated renal response to α -atrial natriuretic peptide, its rise can be expected to affect volume regulation, which is supported

by the correlations between α -atrial natriuretic peptide and renal, cardiac, volume and vascular adaptation ⁴⁶.

Figure 2a. The baroreceptor as it is positioned within the endothelial layer (view from endothelial lumen).

Figure 2b. The baroreceptor as it is positioned in the vascular wall (transverse view). The higher the baroreceptor activity, the more heart rate is tempered. At low transmural pressure P , the signal that is transduced corresponds with distance d_1 . At raised pressure P' , the signal transduction increases as a function of increasing d'_1 . However, when the vessel becomes more compliant, a certain pressure P' will lead to a smaller protrusion and therefore a lower nerve excitation. As a consequence, an increase in vascular compliance can be expected to decrease baroreceptor activity and to increase heart rate, even when blood pressure remains unaltered.

Therefore, in general, the pattern of hemodynamic and volume response to pregnancy is similar in formerly preeclamptic subjects and controls. However, HYPERT and LATHYPERT subjects differ from controls and THROMB by a paradoxical early-pregnancy rise in α -atrial natriuretic peptide, together with a lower plasma volume at 7 weeks. These results suggest that normal early-pregnancy volume expansion requires a sufficiently large vascular reserve capacity prior to pregnancy.

Figure 3. Volume regulatory response system. The red compartment represents the arterial system, the blue compartment the venous system. In healthy females, blood pressure falls in response to the pregnancy-related arterial relaxation, which is characterized by a decrease in vascular resistance, increment of vascular compliance and opening of arterio-venous shunts (AV shunt). As a consequence, the renin-angiotensin-aldosterone system (RAAS) will be activated. With respect to volume retention, the latter response will accelerate fluid retention by the kidney. Furthermore, both the decrease in arterial blood pressure and the increase in arterial compliance are followed by a compensatory, baroreceptor-mediated rise in cardiac output. This early baroreceptor-mediated response results in an increase in heart rate and cardiac contractility and with it venous constriction and a reduction in end-diastolic atrial filling volume. As a consequence, atrial distension will be reduced, leading to diminished release of α -atrial natriuretic peptide (ANP). Together with the activated RAAS, the latter response invigorates renal sodium- and fluid retention. In addition, the early pregnancy non-osmotic anti diuretic hormone release results in an even more pronounced volume retention. The ongoing restoration of vascular filling state is paralleled by increments in both arterial and venous compliance. The latter two effects will contribute to enhanced responsiveness of the baroreceptors and with it reduction in atrial stretch. These effects give rise to enhanced RAAS activity and reduced release of ANP, thereby reinforcing a sustained volume-retaining situation until vascular filling state is restored.

PRE-PREGNANT PREDICTION OF RECURRENT PREECLAMPSIA AND/OR FETAL GROWTH RESTRICTION IN FORMERLY PREECLAMPTIC WOMEN

A women who experienced a severe vascular complication during her pregnancy, is usually traumatized and puzzled about many aspects of her preceding pregnancy. The following questions are commonly posed, when she consults her physician: 'Why did I develop this disease in spite of my good health?', 'Is it sure that I will recover completely?' and 'What would be my recurrence risk in a next pregnancy?' These questions are difficult to answer, as reliable information on this topic is scarce. We intended to address this clinical problem by trying to answer the following questions in a large group of formerly preeclamptic patients: What is the recurrence rate of hypertensive disorders and fetal growth restriction in a subsequent pregnancy in these women? If these complications recur, are they equally severe as experienced in the preceding pregnancy? Furthermore, is it possible to predict this recurrence on the basis of the severity of the vascular complication in the preceding pregnancy and/or on the basis of abnormal hemodynamic - hemostatic, metabolic and volume parameters in the pre-pregnant state?

After extensive pre-pregnancy testing, we were able to identify a number of fairly homogeneous subgroups in our study population of formerly preeclamptic women. In their subsequent pregnancy, we offered them a prophylactic regime. In doing so, the patients were informed about the limited experimental evidence for the efficacy of some of these strategies. All formerly preeclamptic participants received aspirin throughout pregnancy. Furthermore, those with thrombophilia, hyperhomocysteinemia and preexistent hypertension were treated with low molecular weight heparin, with pyridoxine and folic acid supplementation and with antihypertensives, respectively.

The results of this prospective study supported the usefulness of pre-pregnant maternal testing for a number of constitutional factors. Particularly, plasma volume and venous compliance were found to be clinically useful predictors of the recurrence rate of hypertensive complications and fetal growth restriction in the next pregnancy. However, in spite of our attempts to minimize the recurrence rate, by instituting even preventive measures that are still under investigation in large clinical trials, we were unable to abolish the recurrence of these complications. The latter is partly attributable to the occurrence of multiple (latent) disorders in some individuals. For instance, about 1/3 of the THROMB formerly preeclamptic women also had a latent hypertensive phenotype as evidenced by a

reduced plasma volume. Recurrent vascular complications in the next pregnancy were confined to this particular subgroup. This brings up the heterogeneity of the whole group of patients and emphasizes the need for subdivision into homogeneous subgroups. It also indicates the problems that we face when we want to compare our results with data reported by others. Among normotensive formerly preeclamptic women, the reported recurrence rates of preeclampsia ranged from 32 to 55%^{7, 51, 52}. Normotensive women with a history of HELLP-syndrome had recurrent preeclampsia and HELLP-syndrome in 19 to 23% and 3 to 27%, respectively^{53, 54}. Apparently, preeclampsia recurs more often than HELLP. If a formerly preeclamptic woman (with or without HELLP-syndrome) turns out to have hypertension, her reported chance to develop recurrent preeclampsia ranges from 46 to 97%. Similarly, the recurrence rate of prematurity is higher in hypertensive formerly preeclamptics, whereas that of HELLP-syndrome is low as indicated by the reported recurrence rate of only 5%^{51, 52, 54}. With respect to gestational age, subsequent HELLP-pregnancies lasted approximately 2 weeks longer than the previous complicated pregnancy⁵³. In our study, we also found a higher recurrence rate of preeclampsia in hypertensive- (33%) as compared to normotensive formerly preeclamptic women (18 %). Considering the underlying disorder, preeclampsia recurred in about 1/8 of the THROMB population, 1/4 of the LATHYPERT women and 1/3 of the HYPERT participants. However, in counseling it is important to view these recurrence rates in the context of the milder course of the recurrent disease as indicated by its approximately 8 weeks later onset and the substantial higher birth weight and birth weight centile.

Another interesting result of this study was the association between the 'untreated' pre-pregnant blood pressure and the recurrence of vascular complications in the subsequent pregnancy among HYPERT participants. Apparently, optimal blood pressure control with antihypertensive drugs prior to pregnancy does not eliminate the recurrence of these complications in the next pregnancy. The latter indicates that in these preeclamptic recidivists, the untreated blood pressure provides indirect information about the impaired cardiovascular reserve capacity when faced with the extra demands of pregnancy. In this respect, the reduced cardiovascular reserve capacity may be related to structural changes in the arterial bed, such as diminished cardiovascular compliance⁵⁵.

Besides hemodynamic findings, also the course of the previous pregnancy had predictive value with respect to fetal growth in the subsequent pregnancy. In THROMB participants, a previous late onset of hypertensive disease and a high previous birth weight and centile were

associated with a lower incidence of fetal growth restriction. In contrast, a history of stillbirth seemed to 'protect' against fetal growth restriction. The latter finding supports the hypothesis that fetal distress and mortality result from an acute intrauterine accident, such as placental thrombosis or infarction.

Conclusions

Functionally, formerly preeclamptic women can be subdivided into four pathophysiological entities; 1. A hypertensive phenotype (1a: latent, 1b: established), 2. A thrombophilic phenotype (2a: inherited, 2b: acquired) 3. A metabolic disordered phenotype. 4. An (auto)immunological phenotype.

Pre-pregnant evaluation of the hemodynamic -, clotting - and metabolic function was found to be both feasible and suitable to subdivide the heterogeneous group of formerly preeclampsics into more homogenous subgroups. Information on pattern and amplitude of the cyclic changes in hemodynamic and renal function with the menstrual cycle did not contribute appreciably to this categorization. In contrast, the response to early pregnancy was found to facilitate the identification of the (latent) hypertensive subgroup, as women with this phenotype respond to pregnancy with a subnormal rise in plasma volume and an aberrant rise in α -atrial natriuretic peptide. The importance of the identification of the latter subgroup is emphasized by the fact that these women were found to have the poorest prognosis with respect to recurrent vascular complications during pregnancy and recurrent fetal growth restriction

The institution of preventive measures with anti-inflammatory, anti-coagulant and/or vitamin supplementary medication failed to reduce the recurrence rate of hypertensive complications in pregnancy and fetal growth restriction in formerly preeclamptic women to the level seen in normal parous controls. Nevertheless, our data enabled us to estimate this elevated recurrence rate of hypertensive disorders and fetal growth restriction in a subsequent pregnancy for the various subgroups. This information is clinically relevant not only for the obstetrician providing care to those women in the subsequent pregnancy, but also for these women, who want to know whether the risks of a subsequent pregnancy are acceptable for her. Another important finding of this study is the limited efficacy of current preventive strategies to reduce the recurrence rate of these complications, especially for women classified as having a (supplementary) hypertensive phenotype.

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CHAPTER 9

FUTURE RESEARCH

CHAPTER 9

Future research

Until now, it is still obscure why and how vascular resistance falls in early pregnancy. Likewise, it is not clear, why women with a (latent) hypertensive phenotype fail to increase the compliance of their cardiovascular system in response to pregnancy and whether this is related to structural or functional abnormalities. Current research suggests that the steroid environment of early pregnancy activate some still unknown mechanism, which results in systemic vasodilatation and with it, a fall in cardiac pre- and afterload. Ex-vivo and in-vitro (molecular) techniques may contribute to more insight in the underlying mechanisms at the subcellular level.

The studies lined up in this thesis indicate that a (latent) hypertensive phenotype represents the most common underlying disorder associated with maladaptation in pregnancy. Most likely the origin of such a phenotype is multifactorial, as is the case with essential hypertension. Currently, the information on the distribution of 'hypertensive' genetic polymorphisms in populations at risk is increasing rapidly and this may improve our understanding of the relationships between 'risk'-genes and the characteristics of the (latent) hypertensive phenotype. Hypertensive complications of pregnancy have been associated with cardiovascular morbidity at later age. Therefore, longitudinal studies of these women are headed, which will not only lead to a better understanding of the pathogenesis and management of cardiovascular disease, but may also contribute to the development of prophylactic strategies to delay or prevent the development of overt cardiovascular morbidity later in life.

Preeclampsia is a multifactorial disease. Therefore, in reviewing the efficacy of current preventive strategies in women at risk for vascular complications in pregnancy, one should be aware of the heterogeneity of underlying disorders, which frequently appear simultaneously among high-risk patients.

Both non-pregnant clotting and volume status are strong predictors for recurrent vascular disease in pregnancy among formerly preeclamptic women. A randomized controlled study evaluating these parameters among nulliparous women with a wish to become pregnant, and comparing two groups with or without a 'tailor-made' treatment regime, may provide more insight in the possibilities for preventive care. In concert with the obstetrical and/or family history, the development of simple, inexpensive and reliable tests and treatment regimes in women at risk for vascular complications in pregnancy may contribute to repel the incidence

of preeclampsia among primiparous women. In our opinion, 'tailor-made' preventive medication should be designed according to the underlying phenotypical disorder, such as detailed in chapter 7 and expanded by 'anti-hypertensive' treatment among latent hypertensives.

CHAPTER 10

SUMMARY

CHAPTER 10

Summary

The mechanism leading to maternal hypertensive complications in pregnancy and fetal growth restriction is still unsolved. Nevertheless, some important inferences can be deduced from epidemiological observations. For instance, the incidence of these complications is markedly increased in women with preexistent vascular, metabolic and clotting disorders. Interestingly, these disorders have in common that they all are associated with extra strain exerted upon the endothelial lining of the cardiovascular bed. The objective of the set of studies clustered in this thesis was to obtain detailed pre- and early-pregnant information on reno- and hemodynamic function, volume homeostasis and clotting function from women with a history of these complications to resolve, at least in part, the heterogeneity in the composition of the population of formerly preeclamptics. Furthermore we intended to explore the similarities and differences in hemodynamic changes with the menstrual cycle as well as the hemodynamic adaptation to pregnancy in formerly preeclamptics and healthy parous controls. Moreover we wanted to determine whether some elements of the pre-pregnant information on hemodynamic, renal and volume regulatory function might be useable to identify women at risk for recurrent disease in their next pregnancy.

The study in **chapter 2** was designed to categorize formerly preeclamptic women into more or less homogeneous subgroups selected on the basis of underlying disorder. For this purpose we determined in the pre-pregnant state, the hemodynamic, renal and volume functions as well as clotting functions and compared the results with those obtained in healthy parous controls. Functionally, three subgroups of formerly preeclamptic women could be identified; women with a hypertensive phenotype, either manifest or latent and women with a thrombophilic phenotype. Only women with a hypertensive phenotype differed hemodynamically from healthy parous controls by a lower plasma volume and an elevated cardiac output.

Chapter 3 describes the changes in hemodynamic and renal function with the menstrual cycle. In spite of differences in baseline values, we noticed preserved fluctuations with the menstrual cycle in all formerly preeclamptic women, similar as seen in healthy parous controls. The pattern of change in hemodynamic and renal function in the luteal phase relative to the follicular phase of the menstrual cycle resembles that observed in early pregnancy.

The study described in **chapter 4** provides evidence for an early-pregnancy increase in the compliance of large arteries and veins in concert with the fall in total peripheral vascular resistance. This rise in vascular compliance, though, was blunted only in the subgroup of (latent) hypertensive formerly preeclamptic women.

Chapter 5 describes a study, which was designed to determine whether or not the systemic vasorelaxation of early pregnancy develops in response to a rise in basal metabolism. The evidence generated supports the concept that the early-pregnancy vasorelaxation develops independent of changes in basal metabolic rate. It indicates that the opening of protective arteriovenous shunts is required to accommodate the early-pregnancy rise in cardiac output.

Chapter 6 explores the differences and similarities in central- and renal vascular adaptations to pregnancy in formerly preeclamptic women and controls. Most aspects of the adaptive responses to the fall in vascular resistance are similar in controls and formerly preeclamptics. However, the women allocated to the two hypertensive subgroups differed from those with a thrombophilic phenotype and normal parous controls by a paradoxical rise in the circulating levels of α -atrial natriuretic peptide and a subnormal plasma volume expansion achieved by 7 weeks gestation.

Chapter 7 describes our evaluation of hemodynamic, renal and volume homeostatic parameters in the pre-pregnant state, as predictors of recurrent vascular complication and /or fetal growth restriction in a subsequent pregnancy. Clinically effective predictors in the various subgroups of formerly preeclamptics were among others obstetrical history, cardiac output, plasma volume and venous compliance.

In summary, among formerly preeclamptic women, four functional phenotypes can be

- identified:
1. hypertensive (latent or established)
 2. thrombophilic (inherited or acquired)
 3. metabolic-disordered
 4. (auto)immunological

All subgroups share the potency to damage the endothelium, either mechanically (group 1) or biochemical (group 2, 3 and 4). Only the hypertensive subgroup is characterized by limited systemic and renal hemodynamic reserves. Although the central-hemodynamic and renal response to the luteal fall in vascular resistance is also preserved in this subgroup, the much larger vasodilator effects of early-pregnancy elicit a paradoxical rise in α -atrial natriuretic peptide, an effect accompanied by a subnormal plasma volume expansion. We postulate that the resulting hemodynamic maladaptation is caused by the inability to raise

arterial compliance properly in concert with the early-pregnancy fall in vascular resistance. By pre-pregnant assessment of hemodynamic, hemostatic and metabolic parameters, most formerly preeclamptic patients can be categorized and counseled about their risk for recurrent hypertensive disorders and fetal growth restriction in a future pregnancy.

Preeclampsia appears to be a fairly homogeneous derailment of the cardiovascular function in the second half of pregnancy. However, the wide variety in predisposing risk conditions supports a heterogeneous pathogenesis. The latter should be taken into account, when one intends to develop preventive measures. That is to say, it may turn out to be necessary to develop a different 'tailor-made' prophylactic strategy for each identified risk condition. It is obvious that the latter will benefit markedly from a better insight in the initial stimulus and subsequent sequence of events that lead to the normal early-pregnancy fall in vascular tone and in the exact defect in this cascade in women destined to develop preeclampsia. Together with the obstetrical and family history, these insights may lead to the development of simple and inexpensive tests to screen women at risk for vascular complications in pregnancy. Such tests should be developed with the intention to screen not only risk groups, but rather all women who wish to conceive for the first time, since the largest incidence of preeclampsia is observed among primiparous women.

Samenvatting

Het mechanisme, dat tijdens de zwangerschap leidt tot maternale hypertensieve complicaties en foetale groei vertraging, is nog steeds onopgelost. Desalniettemin kunnen sommige belangrijke conclusies worden getrokken uit epidemiologische observaties. Zo is bijvoorbeeld het vóórkomen van deze problemen verhoogd onder vrouwen met van tevoren bestaande stolling- en stofwisselingsstoornissen en hart- en vaatlijden. Deze stoornissen hebben gemeenschappelijk dat zij allen geassocieerd zijn met toegenomen belasting op de vaatwand. Dit proefschrift heeft tot doel gedetailleerde informatie te verkrijgen over de niet-zwangere en de hierop volgende vroeg-zwangere nierfunctie, bloedsomloop, vochthuishouding en stollingsfunctie van vrouwen met een belaste voorgeschiedenis ten aanzien van deze complicaties, ten einde meer inzicht te krijgen in de heterogeniteit van deze populatie vrouwen. Bovendien wilden we de overeenkomsten en verschillen in veranderingen van de bloedsomloop tijdens de menstruele cyclus en de vroege zwangerschap bij voormalige preeclampsie patiënten en gezonde controles in kaart brengen. Daarenboven wilden we bepalen, of sommige elementen uit de niet-zwangere bloedsomloop, nierfunctie en vochtregulerende functie, bruikbaar waren risicopatiënten voor herhaalde ziekte in de volgende zwangerschap te identificeren.

De studie in **hoofdstuk 2** was ontworpen om voormalige pre-eclampsie patiënten op basis van onderliggende problematiek in te delen in min of meer homogene groepen. Voor dit doel werd in een niet-zwangere situatie, de bloedsomloop, nier- en stollingsfunctie alsmede vochthuishouding bestudeerd en vergeleken met dezelfde gegevens van gezonde vrouwen met een probleemloze zwangerschap in de voorgeschiedenis. Er konden drie functionele subgroepen voormalige preeclampsie patiënten worden onderscheiden; vrouwen met een hypertensief bloedsomloopprofiel, aanwezig (1) dan wel sluimerend (2) en vrouwen met een thrombofilisch profiel (neiging tot aderlijke stolselvorming) (3). Alleen de vrouwen met een hypertensief profiel verschilden voor wat betreft bloedsomloop van gezonde controles in een kleinere hoeveelheid plasma volume en een groter hartminuut volume.

Hoofdstuk 3 beschrijft de veranderingen in bloedsomloop en nierfunctie tijdens de menstruele cyclus. Zowel bij voormalige preeclampsie patiënten alsmede bij gezonde controles bleven, ondanks verschillen in de waarden van de eerste cyclushelft (folliculaire fase), de schommelingen in functie tijdens de menstruele cyclus bestaan. De veranderingen van bloedsomloop en nier functies tijdens de tweede cyclushelft (luteale fase) ten opzichte

van de eerste cyclushelft van de menstruele cyclus gelijken op die van de vroege zwangerschap.

De studie beschreven in **hoofdstuk 4** voert bewijzen aan voor een vroeg-zwangere toename in de rekbaarheid van grote arteriën en venen tezamen met de daling in totaal perifere weerstand. De toename in rekbaarheid van de vaten was echter aanzienlijk minder uitgesproken bij de (latent) hypertensieve voormalig pre-eclampsie patiënten.

Hoofdstuk 5 beschrijft een studie waarin onderzocht wordt of de systemische vaatverslapping van de vroege zwangerschap zich ontwikkelt in reactie op een stijging in basale stofwisseling. Het gegenereerde bewijs ondersteunt het concept dat de vroeg-zwangere vaatverslapping zich onafhankelijk van veranderingen in basale stofwisseling ontwikkelt. Voor de aanpassing aan de vroeg-zwangere stijging in hartminuut volume is de opening van beschermende arterioveneuze shunts noodzakelijk.

Hoofdstuk 6 onderzoekt de overeenkomsten en verschillen in bloedsomloop- en nierfunctie-aanpassingen aan de zwangerschap in voormalig pre-eclampsie patiënten en controles. De meeste aspecten van deze aanpassende reacties op de daling in vaatweerstand zijn vergelijkbaar. Echter de twee hypertensieve subgroepen verschilden van vrouwen met een thrombofilisch profiel en gezonde controles in een paradoxale stijging in circulerend α -atriaal natriuretisch peptide en een subnormale expansie van het plasma volume bij een amenorrhoeëduur van 7 weken.

Hoofdstuk 7 beschrijft onze evaluatie van de niet-zwangere bloedsomloop, nierfunctie en vochthuishouding als voorspellers van herhaalde vaatproblemen en/of foetale groeivertraging in een daaropvolgende zwangerschap. Klinisch bruikbare voorspellers in de verscheidene subgroepen van voormalige pre-eclampsie patiënten waren onder andere obstetrische voorgeschiedenis, hartminuut volume, plasma volume en aderlijke rekbaarheid.

Samenvattend kunnen onder voormalig pre-eclampsie patiënten vier functionele profielen worden onderscheiden:

1. hypertensief (latent of manifest)
2. thrombofilisch (aangeboren of verworven)
3. metabool gestoord
4. (auto)immunologisch

Al deze groepen delen de eigenschap de vaatwand te kunnen schaden, ofwel op mechanische basis (groep 1) danwel op biochemische basis (groep 2, 3 en 4). Alleen de hypertensieve subgroep wordt gekarakteriseerd door een beperkte reservecapaciteit van de bloedsomloop en nierfunctie. Ofschoon de reactie van de bloedsomloop en nierfunctie op de luteale daling

in vaatweerstand in deze groep is behouden, ontlokt de veel grotere vaatverslapping van de vroege zwangerschap een paradoxale stijging in α -atriaal natriuretisch peptide, een effect geflankeerd door een subnormale toename in plasma volume. Wij postuleren dat de resulterende maladaptatie van de bloedsomloop veroorzaakt wordt door de onmogelijkheid de slagaderlijke rekbaarheid, in samenspraak met de vroeg-zwangere daling in vaatweerstand, te vergroten. Door niet-zwangere bepaling van het functioneren van bloedsomloop, stolling en stofwisseling, kunnen de meeste voormalig pre-eclampsie patiënten worden onderverdeeld in één van bovengenoemde subgroepen en daarmee worden voorgelicht ten aanzien van hun risico op herhaling van de hypertensieve stoornissen en foetale groei vertraging in een toekomstige zwangerschap.

Pre-eclampsie lijkt een vrij homogene ontsporing te zijn van het functioneren van het hart- en vaatstelsel in de tweede helft van de zwangerschap. Echter, de uitgebreide variatie in predisponerende risico factoren, ondersteunt een heterogene ontstaanswijze. Deze diversiteit moet in ogenschouw worden genomen als men preventieve strategieën wil ontwikkelen. Daarom lijkt het eerder noodzakelijk voor ieder risico profiel een 'op-maat-ontworpen' profylaxe te kiezen. Het moge duidelijk zijn dat een beter inzicht in de initiële stimulus leidend tot de fysiologische daling in vaatwandspanning vroeg in de zwangerschap en het specifieke tekort in de aanpassingsmogelijkheden van sommige vrouwen gedoemd pre-eclampsie te ontwikkelen, zal leiden tot betere ontwikkeling van profylactische mogelijkheden. Tezamen met de obstetrische en familiale voorgeschiedenis zouden deze inzichten kunnen leiden tot de ontwikkeling van eenvoudige en goedkope testen om vrouwen te kunnen identificeren met een verhoogd risico op vasculaire complicaties tijdens de zwangerschap. Aangezien de grootste incidentie van pre-eclampsie met name onder eerst-zwangeren wordt gezien, zouden dergelijke testen niet alleen beschikbaar moeten zijn voor risicopatiënten, maar ook voor vrouwen die voor de eerste keer zwanger willen worden.

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Curriculum vitae

Marc Spaanderman werd op 16 maart 1966 in Waldbauer, Duitsland, geboren. Als kind was hij naast een dromer ook vaak ziek, gemiddeld 1 tot 2 dagen per week. Dit was reden voor zijn ouders hem een 'vrij-tempo' middelbare school te laten doorlopen. Aansluitend kon hij starten met de studie Geneeskunde aan de Rijksuniversiteit Utrecht. Tijdens deze periode was hij onder andere werkzaam als student-assistent anatomie en als steward bij de KLM. In de tweede helft van 1993 begon hij als arts-assistent gynaecologie in het Diaconessen Ziekenhuis te Utrecht (opleider dr. M.V.A.M. Kroeks). Na enkele maanden werd deze loopbaan vervolgd in het Elisabeth Ziekenhuis te Tilburg (opleider dr. J. de Graaff). In 1994 werd gestart met de opleiding tot gynaecoloog in het Academisch Ziekenhuis Maastricht (opleider prof. dr. J. de Haan en later prof. dr. J.L.H. Evers). De start tot de studies beschreven in dit proefschrift werd gemaakt in 1996. Hiervoor ontving hij in 1998 een 'Young Investigator Award for distinguished research' ter gelegenheid van het elfde wereld congres van de ISSHP in Kobe, Japan. Thans is hij werkzaam in het Atrium Ziekenhuis te Heerlen (opleider dr. F.J.M.E. Roumen). Vanaf oktober 2001 hoopt hij geregistreerd te zijn als gynaecoloog. Hij is nog steeds vaak in gedachten, ziek echter zelden.

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