VASCULAR DILATORY FUNCTION AND CARDIOVASCULAR RISK FACTORS IN WOMEN WITH A HISTORY OF PRE-ECLAMPSIA

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ACADEMIC DISSERTATION

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To all my families
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ORIGINAL PUBLICATIONS


INTRODUCTION: Women with a history of pre-eclampsia have an increased risk of cardiovascular disease in later life. The mechanisms which mediate this heightened risk are poorly understood; it was long believed that pre-eclampsia was a separate disease without connection to other pathologies. The present study was undertaken to investigate the cardiovascular risk milieu, vascular dilatory function and cardiovascular risk factors, in women with pre-eclampsia, 5–6 years after index pregnancy. The aim was to understand better the cardiovascular risks associated with pre-eclampsia and add tools to the evaluation of cardiovascular risk in women.

SUBJECTS AND METHODS: The study involved 30 women with previous severe pre-eclampsia and 21 controls. The 2-day study protocol included venous occlusion plethysmography and pulse wave analysis for assessment of vascular dilatory function and central pulse wave reflection, respectively, office and ambulatory blood pressure measurements, assessment of insulin sensitivity, using a minimal model technique, and tests regarding renal function, lipid metabolism, sympathetic activity and inflammation.

RESULTS: Vasodilatory function was impaired in women with a history of pre-eclampsia; this was seen in both endothelium-dependent and endothelium-independent vasodilatation. Proteinuria during pre-eclampsia did not predict changes in vasodilatation, and renal function was similar in the two groups. Insulin sensitivity was related to vasodilatation and features of metabolic syndrome, but only in the patient group, despite similar insulin sensitivity in the control group. Arterial pressure was higher in the patient group than in the controls and correlated with endothelin-1 levels in the patient group, whilst the overall difference between the groups was diminished in 24–hour arterial pressure measurements. Additionally, women with previous pre-eclampsia were characterized by increased sympathetic activity.

CONCLUSIONS: Impaired vasodilatory function at the vascular smooth muscle level seems to characterize clinically healthy women with a history of pre-eclampsia. These vascular changes and the features of metabolic syndrome may be related to the increased risk of cardiovascular disease. Furthermore, increased blood pressure in combination with enhanced sympathetic activity may be additive as regards this risk. These women should be informed about their potential cardiovascular risk profile and the possibilities to minimize it via their own actions. Medical cardiovascular risk assessment in women should include obstetric history.
ABBREVIATIONS

ACh  acetylcholine
A/E  adrenaline=epinephrine
AIx  augmentation index
Ang II  angiotensin II
ANOVA  analysis of variance
APOA1  apolipoprotein A
APOB  apolipoprotein B
BMI  body mass index
BP  blood pressure
cAMP  cyclic adenosine mono phosphate
cGMP  cyclic guanosine mono phosphate
CI  confidence interval
CO  cardiac output
COX  cyclooxygenase
CRP  C-reactive protein
CVD  cardiovascular disease
EDHF  endothelium-derived hyperpolarizing factor
eNOS  endothelial nitric oxide synthase
ET-1  endothelin-1
ET_{A/B}  endothelin A/B receptors
fB  fasting blood
FFA  free fatty acid
FMD  flow- mediated dilatation
FSIVGTT  frequently sampled intra-venous glucose tolerance test
GFR  glomerular filtration rate
HDL  high density lipoprotein
HOMA  homeostasis assessment model
HRV  heart rate variability
ICAM  inter-cellular adhesion molecule
IL  interleukin
LDL  low density lipoprotein
Lp(a)  lipoprotein (a)
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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>MSNA</td>
<td>muscle sympathetic nerve activity</td>
</tr>
<tr>
<td>na</td>
<td>non-applicable</td>
</tr>
<tr>
<td>NA/NE</td>
<td>noradrenaline=norepinephrine</td>
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<td>NO</td>
<td>nitric oxide</td>
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<td>OGGT</td>
<td>oral glucose tolerance test</td>
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<tr>
<td>PAI-1</td>
<td>plasminogen activator inhibitor-1</td>
</tr>
<tr>
<td>PE</td>
<td>pre-eclampsia</td>
</tr>
<tr>
<td>PGH$_2$</td>
<td>prostaglandin H$_2$</td>
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<tr>
<td>PGI$_2$</td>
<td>prostacyclin</td>
</tr>
<tr>
<td>PWA</td>
<td>pulse wave analysis</td>
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<tr>
<td>RAS</td>
<td>renin-angiotensin-system</td>
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<td>ROS</td>
<td>reactive oxygen species</td>
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<tr>
<td>RR</td>
<td>relative risk</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>SE</td>
<td>standard error</td>
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<tr>
<td>sFlt-1</td>
<td>soluble fms-like tyrosine kinase-1</td>
</tr>
<tr>
<td>SHBG</td>
<td>sex hormone-binding globulin</td>
</tr>
<tr>
<td>SI</td>
<td>sensitivity index</td>
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<tr>
<td>SNP</td>
<td>sodium nitroprusside</td>
</tr>
<tr>
<td>TNF$\alpha$</td>
<td>tumour necrosis factor $\alpha$</td>
</tr>
<tr>
<td>t-PA</td>
<td>tissue-plasminogen activator</td>
</tr>
<tr>
<td>TXA$_2$</td>
<td>thromboxane A$_2$</td>
</tr>
<tr>
<td>VCAM</td>
<td>vascular cellular adhesion molecule</td>
</tr>
<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
</tr>
<tr>
<td>VLDL</td>
<td>very low density lipoprotein</td>
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<tr>
<td>VSMC</td>
<td>vascular smooth muscle cell</td>
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<tr>
<td>vWF</td>
<td>von Willebrand factor</td>
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<tr>
<td>WHR</td>
<td>waist-hip ratio</td>
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1. INTRODUCTION

Pre-eclampsia is characterized by hypertension and proteinuria by definition, and also by enhanced coagulation, and metabolic changes such as hyperlipidaemia and insulin resistance. This phenotype strikingly resembles that in metabolic syndrome, closely bound to the pathophysiology of atherosclerosis. Pre-eclampsia also shares the most common and important risk factors associated with atherosclerosis.

There is mounting data showing an increased risk of cardiovascular disease (CVD) and mortality, especially coronary artery disease, in women with a history of pre-eclamptic pregnancy. This risk seems to be even more increased when associated with early onset of pre-eclampsia and fetal growth restriction. Studies performed after pre-eclamptic pregnancy have revealed that signs of insulin resistance, hyperlipidaemia and increased testosterone levels characterize women with prior pre-eclampsia compared with women with uncomplicated pregnancies. The results of these studies are suggestive of some pathophysiological mechanisms connecting pre-eclampsia and atherosclerosis, but nevertheless, the mechanisms are still poorly understood. Of note is the fact that despite the decreasing overall prevalence of CVD, the age-adjusted cardiovascular mortality rate in women has remained stable or has even increased, in contrast to men, and is a major cause of death in women in Europe.

This study was undertaken to explore the cardiovascular risk environment in women with a history of pre-eclampsia 5–6 years earlier. We wanted to know if vascular dilatory function, a central feature of a healthy vascular bed, is impaired in these women compared with control women with uncomplicated pregnancies and in addition, if known cardiovascular risk factors are present and/or are associated with vasodilatation in women with prior pre-eclampsia. The gained information would let us better understand the cardiovascular risk associated with pre-eclampsia and add tools to the evaluation of cardiovascular risk in women.
2. REVIEW OF THE LITERATURE

2.1. Vascular endothelium and its functions

The inner surface of the vascular system is lined by a monolayer of vascular endothelial cells, the endothelium. Owing to its anatomical location, the endothelium is exposed to blood on the one side and to local tissues on the other side. Endothelial cell phenotypes can vary in regard to morphology and function between different organs according to the requirements of the individual organ and vascular section.\textsuperscript{16}

The endothelium is an active organ. Its functions are directed to maintaining vascular homeostasis and they result from the actions of blood-, endothelium- and underlying tissue-derived signals and mechanical stimuli from blood flow. The subsequent responses and actions of endothelium are mediated by release of locally active substances and/or expressing molecules/receptors on the endothelial inner or outer surface.\textsuperscript{17} The normal relatively dilated state of the vascular wall is maintained mainly by nitric oxide (NO) via inhibition of inflammation and vascular proliferation, antithrombotic actions and preservation of vascular tonus.\textsuperscript{18} The major factor maintaining NO production is pulsatile blood flow and shear stress.\textsuperscript{19} The main functions of endothelium are listed in Table 1.

**Table 1. The main functions of endothelium**

1. Anatomical barrier towards the local tissues
2. Regulation of vascular tone (vasodilation/vasoconstriction)
3. Regulation of circulating cell function
4. Regulation of coagulation and fibrinolysis
5. Control of vascular permeability
6. Central role in angiogenesis and vascular smooth muscle cell (VSMC) growth

Under normal pulsatile blood flow leucocytes do not adhere to the endothelium. Activation of endothelium results in recruitment, adhesion and migration of neutrophils and monocytes, each step mediated by specific endothelial molecules. This activation enables normal inflammatory processes in the surrounding tissues. The balance in coagulation and fibrinolysis is mediated by the endothelium; in normal physiological conditions it is antiaggregatory, anticoagulant and fibrinolytic, as a result of the action of released substances and the withholding of potentially thrombogenic factors.\textsuperscript{20}
A primary haemostatic response in the endothelium initiates activation of platelets and the coagulation cascade, which is further regulated by endothelial enzymes. The reduction of clot formation (fibrinolysis) is endothelium–mediated by balance in the production of tissue plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1). Furthermore, the endothelium regulates the transport of liquid and various solutes via both transcellular and paracellular pathways. The transcellular system provides the means of controlling tissue oncotic–pressure and host-defence mechanisms by transporting albumin and immunoglobulins, whereas the intercellular mechanisms are mostly induced during inflammation. The endothelium also plays a fundamental role in angiogenesis, vessel growth from pre-existing vessels and vessel remodelling, in concert with various tissue- and endothelium–derived angiogenic factors such as vascular endothelial growth factor (VEGF). In addition, VSMC growth is highly affected by endothelial function.

2.2. Regulation of vascular tone

Regulation of vascular tone is needed to supply adequate tissue perfusion. Changes in vascular tone, VSMC relaxation and contraction, are a result from changes in Ca$^{2+}$ concentration in VSMC, determining the level of myosin phosphorylation. Stimulation of VSMC is mediated in several ways. First, reactions of the endothelium to several local chemical and mechanical stimuli cause production and release of vasodilating and/or vasoconstrictive substances acting on VSMC. Secondly, tonus is influenced by sympathetic perivascular nerves releasing neurotransmitters, resulting in a postsynaptic Ca$^{2+}$ signal with associated contraction. The fundamental vasodilating agent is NO, but NO-independent endothelial pathways are also present. Various mediators derived from the endothelium may cause vascular relaxation by way of VSMC hyperpolarisation. Furthermore, hyperpolarisation of the endothelium by different stimuli can be followed by hyperpolarisation of VSMC leading again to relaxation of the VSMC. One of the endothelium-derived vasodilators is prostacyclin (PGI$_2$), but its role in vasodilatation is minor. The role of hyperpolarisation is not yet elucidated in detail but it seems to have an integral role in several endothelium–mediated relaxations brought about by different mediators. Vasoconstriction is mediated by withdrawal of NO and by several vasoconstrictive mediators, of which the most important are endothelin-I (ET-1), thromboxane (TXA$_2$) and angiotensin II (Ang II).
2.2.1. Nitric oxide

Nitric oxide is a freely diffusible gas that has its actions inside and on both the luminal and tissue sides of the endothelium. It is produced from the amino acid L-arginine by endothelial nitric oxide synthase (eNOS) located on the plasma membrane. Several stimuli on the endothelium, physical, like shear stress, or humoral, via activation of receptors on endothelial cell membranes, increase intracellular calcium concentrations, which results in activation of eNOS and subsequent release of NO. In VSMC NO increases the activity of guanylate cyclase, resulting in increased concentrations of cyclic 3’5’ guanosine monophosphate (cGMP). An intracellular decrease of calcium due to a direct cellular effect of NO and an increase in cGMP result subsequently in vasodilatation.

The functions of eNOS are fundamental to the bioavailability of NO. In the absence of enzyme cofactor, or deficiency of L-arginine, the actions of eNOS can be switched to increased production of superoxide anion (O$_2^-$), an oxygen radical. Deficient antioxidant defence mechanisms lead superoxide anion to react preferentially with NO to form peroxynitrate (ONOO-) or hydrogen peroxide (H$_2$O$_2$) diminishing the bioavailability of NO. The family of these reactive oxygen species (ROS), especially peroxynitrate, is able to damage cell membranes, oxidize lipids and alter endothelial protein production to become more activated/dysfunctional. The production of ROS is a component of oxidative stress, a term that is used for imbalance between antioxidant defence mechanisms and excessive formation of ROS.

2.2.2. Prostacyclin and hyperpolarisation

Prostacyclin is produced in non-activated endothelium from arachidonic acid by cyclooxygenase-1 (COX-1) and prostacyclin synthase and is the main product of this pathway. It binds to specific receptors on VSMC and activates adenylate cyclase, increasing the levels of cyclic adenosine monophosphate (cAMP) in VSMC, which results in relaxation and vasodilatation synergistically with NO. Its actions are often associated with concomitant hyperpolarisation of VSMC.

The endothelium-dependent hyperpolarisation pathway is not a single factor but rather a mixture of mechanisms that start with hyperpolarisation of the endothelial cell or release of endothelium-derived mediators, resulting eventually in hyperpolarisation of VSMC and vasorelaxation. This pathway is not well defined; the several mediators involved and their actions are not, however, influenced by the L-arginine pathway or
inhibitors of COX.\textsuperscript{27, 28} The physiological relevance of this mechanism is somewhat open and differs depending on the vascular bed studied.\textsuperscript{27, 28} However, hyperpolarisation seems to contribute to the regulation of basal blood flow in humans and might be important when NO bioavailability is reduced.\textsuperscript{20, 27, 28, 30}

2.2.3. Endothelin-1
Endothelin-1 is one of the most potent endogenous vasoconstrictors. It is a peptide hormone that is synthesized mainly in the endothelium and once released, it binds to endothelin receptors ET\textsubscript{A} and ET\textsubscript{B}.\textsuperscript{29, 31} The production of ET-1 in vascular endothelium is increased as a result of stimuli from shear stress, hypoxia and several pro-inflammatory mediators.\textsuperscript{29} Binding to ET\textsubscript{A} and ET\textsubscript{B} receptors in VSMC results in vasoconstriction supposedly via the protein kinase–C pathway, increasing the Ca\textsuperscript{2+} sensitivity of VSMC, whereas binding to ET\textsubscript{B} receptors in the endothelium connects the complex to NO and PGI\textsubscript{2} pathways and vasodilatation.\textsuperscript{29, 32} Under normal physiological conditions it participates in regulation of arterial pressure.\textsuperscript{29, 33} Endothelin-1 also has several other actions antagonizing the effects of NO, such as induction of vascular and myocardial hypertrophy.\textsuperscript{29, 31} The causal role of ET-1 in the pathogenesis of hypertension remains controversial, but it has pro-inflammatory and pro-fibrotic effects in heart, kidney and blood vessels.\textsuperscript{29}

2.2.4. Thromboxane A\textsubscript{2} and prostaglandin H\textsubscript{2}
Thromboxane A\textsubscript{2} and prostaglandin H\textsubscript{2} (PGH\textsubscript{2}) are both vasoconstrictive products using the same receptor on VSMC leading to intracellular alterations in Ca\textsuperscript{2+} sensitivity.\textsuperscript{32} PGH\textsubscript{2} is produced from arachidonic acid by COX activity and later via cell-specific actions modulated to vasoactive substances such as PGI\textsubscript{2} or TXA\textsubscript{2}. The expression and activity of COX is modulated by different stimuli. Cyclooxygenase-2, considered to be an inducible isoform of COX, is regarded as predominantly produce vasoconstrictors, such as TXA\textsubscript{2}.\textsuperscript{34} The expression of COX-2 is proposed to be enhanced in states related to the pathophysiology of atherosclerosis, such as hyperlipidaemia, hypoxia, obesity and oxidative stress. It needs to be remembered however, that TXA\textsubscript{2} are mainly released from platelets and its actions are targeted to platelet activation and clot formation.\textsuperscript{35}

2.2.5. Angiotensin II
Activation of the renin-angiotensin-system (RAS) leads to formation of Ang II, which
is a major regulator of human haemodynamics, fluid balance and the cardiovascular system. It acts mainly via angiotensin-1 (AT-1) and angiotensin-2 receptors. The clinical actions via angiotensin-2 receptors are not established yet but are suggested to mediate protective actions.\textsuperscript{36} In contrast, AT-1 receptor-mediated actions of Ang II include vasoconstriction, generation of oxidative radicals, fibrosis and cellular migration and growth.\textsuperscript{36, 37} Angiotensin II also has metabolic actions and has been suggested to play a role in metabolic syndrome.\textsuperscript{38} These potentially harmful effects on the cardiovascular system can increase oxidative stress and reduce NO-mediated vasodilatation and increase the production of ET-1 in the endothelium.\textsuperscript{29}

\textbf{2.2.6. Vascular smooth muscle cell}

Vascular smooth muscle tone is regulated by several intracellular mechanisms but it is primarily the free Ca\textsuperscript{2+} concentration which determines the level of myosin phosphorylation; an increase in Ca\textsuperscript{2+} enhances phosphorylation leading to contraction, and restoration of Ca\textsuperscript{2+} levels leads to dephosphorylation and smooth muscle relaxation.\textsuperscript{24} Intracellular highly regulated signalling cascades are activated depending on the stimulus on VSMC.\textsuperscript{26, 39, 40}

One of the receptor-mediated actions leading to vasomotor changes begins with activation of receptor/G-protein/enzyme-complex. The receptor contains a recognition site for the substance and after binding, the guanine nucleotide regulatory protein complex (G-protein) couples occupancy by the substance to further enzymatic effects in VSMC. There are both stimulatory and inhibitory G-proteins.\textsuperscript{39} Regarding VSMC relaxation and contraction, G-protein activation is followed by activation or inhibition of adenylyl cyclase, respectively. Activation leads to synthesis of cAMP, leading to vasodilatation.\textsuperscript{39} Another intracellular second messenger is cGMP. CyclicGMP is produced after enzymatic activation by guanylate cyclase and it is the main mediator of NO activated endothelial mechanisms.\textsuperscript{40} Another pathway resulting in cGMP increase is activated by natriuretic peptides which bind to transmembrane receptors on VSMC containing guanylate cyclase. CyclicGMP activates cGMP–dependent protein kinase C, which eventually leads to a decrease in intracellular Ca\textsuperscript{2+} concentration and vasodilatation.\textsuperscript{40}

Transmitters of the perivascular nerves include adenosine-tri-phosphate (ATP), noradrenaline (NA) and neuropeptide Y (NPY). Release of these transmitters results in activation of post-synaptic receptors on VSMC, subsequent changes in intracellular
Ca^{2+} levels and vasoconstriction. Noradrenaline acts on $\alpha$- and $\beta$-receptors on the vascular bed where its $\alpha_{1}$-receptor mediated actions on VSMC play an important role in vasoconstriction, especially in resistance arteries. There are several receptor subtypes but their final contribution to vasoregulation is not clear. Actions via NPY and its receptors on VSMC are thought to enhance the effects of both ATP and NA mediated actions in VSMC. The interplay of several regulatory elements leads to flexibility and adaptability required for the effective regulation of blood pressure.

2.3. Assessment of vascular dilatory function

The role of endothelial function in the pathology of several common diseases has led to the development of methods to assess its functions. The ability of vessels to react to different vasodilating/vasoconstricting stimuli is of central interest, being one of the main functions of the endothelium and supposedly related to other functions via production of NO. Typically, testing involves physiological and/or pharmacological stimulation of endothelial release of NO and endothelium-independent pharmacological stimulation of VSMC. Subsequent changes in blood flow or vessel diameter reflect altered smooth muscle tone in resistance-/conduit arteries.

For now, the currently available tests are mostly too expensive, time-consuming and complex to be used in routine clinical decision making. Several methods can be used, however, for assessment of endothelial function in research. The methods may all involve assessment of endothelial function, but it needs to be remembered that vascular properties are dependent on the studied part of the body and that the activating stimuli and the measure indicators may differ. Therefore, the various methods can be regarded as additive in their information.

2.3.1. Venous occlusion plethysmography

Venous occlusion plethysmography is a widely used method to study blood flow in forearm resistance vessels. The principle is as follows; venous return from the forearm is obstructed and arterial flow into forearm is allowed; subsequently the forearm swells in proportion to arterial inflow. Stimulation of the artery with vasoactive substances at the same time changes the flow, reflecting the change in smooth muscle tone, provided that arterial blood pressure (BP) remains constant.

In practice, venous return is occluded by a cuff round the upper arm inflated to above 40mmHg. Blood flow in the hand is excluded from the measurement area in the
forearm because of different vascular physiology. This is accomplished by cuffs placed on the wrists inflated to above systolic blood pressure. Forearm swelling is calculated as ml per minute by a strain gauge, placed around the forearm one third of the way from the elbow to the wrist. The strain gauge is connected to the plethysmograph and computer, which subsequently estimate the total flow in the forearm. The brachial artery is cannulated with a thin needle for the infusions. Basal forearm blood flow is measured, followed by infusions of saline and vasoactive drugs at different concentrations. Inflation of the cuffs, measurement and deflation are repeated at certain intervals. To exclude confounding factors, arterial pressure is controlled and the environment is held comfortable, relaxed and warm. Measurements of blood flow are carried out in both arms simultaneously so as to have an internal control for substance effect and also to minimize the confounding effects and/or possible differences in blood pressure. The results can be expressed as absolute forearm blood flow (ml/min/100ml) and/or as change in percentage adjusted for flow in the non-infused arm and during previous saline infusion.

Venous occlusion plethysmography is regarded as the “gold standard” for assessment of vasodilatation. It is safe, reproducible and reflects vascular biology. However, it is time consuming, relatively invasive and needs substantial cooperation from the subject which makes it less repeatable and limits its use in larger studies. Impaired vasodilatation in plethysmography has been shown in resistance arteries in the presence of CVD risk factors: hypertension, hypercholesterolaemia, diabetes, insulin resistance and ageing.

2.3.2. Flow-mediated dilatation

The same idea of measuring vasodilatation after stimulation is used in an ultrasound-based method to assess conduit artery diameter in the forearm. In this method brachial artery diameter is measured before and after flow-mediated dilatation (FMD) caused by shear stress on the endothelium and subsequent release of NO. This method is non-invasive and can be used in larger studies, but it is technically demanding and it requires highly trained operators.

2.3.3. Endo-PAT

A new non-invasive system has been developed to assess endothelial dysfunction, EndoPAT 2000. The method involves the use of pressure fluctuations induced by
volume changes of the pulsating digital arteries, digital reactive hyperaemia, in the analysis of endothelial function. In primary evaluations it is suggested to have potential for assessment of peripheral endothelial dysfunction in daily clinical practice. The method has been tested against invasive intra-coronary challenge procedures and the first results seem promising.55

2.3.4. Pulse wave analysis
Arterial stiffness reflects arterial compliance in response to systolic pulse waves. It is a result of structural elements within the arterial wall and vascular tonus. Changes in vascular tone have been shown to be able to alter stiffness and could be a result of activation of the endothelium and increased vasoconstriction and/or a withdrawal of the antiproliferating effect of NO on VSMC.56,23 Increasing stiffness of the arteries leads to increased pulse wave velocity and earlier, augmented pressure wave reflected back to the heart. When in normal conditions this reflection would enter the heart in diastole, serving coronary artery perfusion, it now returns to the aorta during systole, adding to the central systolic pressure.56 Therefore, pulse wave velocity is a direct measure of arterial stiffness and augmented central pressure wave reflection a consequence, a variable, highly dependent on pulse wave velocity. Arterial stiffness is associated with pathogenesis and traditional risk factors of cardiovascular disease such as hypertension, hypercholesterolaemia and diabetes.56

Pulse wave analysis (PWA) is a non-invasive technique that allows information on a waveform recorded from a peripheral artery to be transformed into a corresponding central aortic waveform. These waveforms are then analysed and the variable “augmentation index” (AIx) can be measured. This is defined as percentage of the difference in amplitude of the first and second (augmented as a result of the reflected wave) systolic peaks (ΔP) of total pulse pressure.56,57 It is dependent on age, sex, height and heart rate and these should be taken into account when analysing the results.58 As a method, PWA is regarded as being reproducible, and easily applicable and variability in the results is small.56,59

2.3.5. Circulating markers
Products expressed by endothelial cells when the endothelium is activated can be measured from blood. These include measures of NO biology, inflammation, regulators of thrombosis and markers of endothelial damage and repair.44,45
2.3.5.1. Nitric oxide-derived and related markers

The biology of NO can be measured by way of its metabolites, nitrites and nitrosylated proteins.\textsuperscript{42, 44, 45} However, the tests are vulnerable to other sources of NO metabolism and are dependent on nutritional uptake.\textsuperscript{42, 44, 45} An increase in the levels of asymmetric dimethylarginine (ADMA), an endogenous competitive antagonist of eNOS, is associated with disease states related to increased risk of atherosclerosis and a decrease in NO bioavailability and it may contribute to endothelial vasodilatory dysfunction.\textsuperscript{42, 44, 45}

2.3.5.2. Indicators related to inflammation

Endothelial cell activation leads to increased expression of inflammatory cytokines and adhesion molecules that are able to trigger leukocyte rolling, adhesion and migration, amplifying local inflammation.\textsuperscript{60} Adhesion molecules expressed by the endothelium include selectins (E- and P-selectin), vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). Activation of the endothelium to produce these adhesion molecules involves several cytokines produced by leukocytes and the endothelium itself, and other cells and tissues.\textsuperscript{61} The soluble forms of these adhesion molecules can be detected in the blood.\textsuperscript{44}

Inflammatory cells, acute phase proteins and pro-inflammatory cytokines play central roles in activation of the endothelium and maintenance of the inflammatory stimulus. Of pro-inflammatory cytokines, interleukin-1 (IL-1) and IL-6 are markers of increased inflammatory responses whereas IL-4 and IL-10 mediate anti-inflammatory processes and are regarded as being involved in endothelial defence mechanisms.\textsuperscript{60, 62} Interleukin-6 is central in mediating acute phase responses and is known to induce synthesis of all acute phase proteins.\textsuperscript{63} Tumour necrosis factor \(\alpha\) (TNF\(\alpha\)) is in turn a pro-inflammatory cytokine produced by leukocytes and the endothelium and also by several other sources including adipose tissue. It is able to induce inflammatory activation in endothelial cells and it also regulates the production of other inflammatory signals, induces insulin resistance and stimulates the production of acute phase protein such as C-reactive protein (CRP) in liver, together with IL-6.\textsuperscript{64} C-reactive protein is a non-specific acute-phase reactant.\textsuperscript{65} Apart from the CRP values measured during acute “traditional” infection, the normal mean concentration of CRP in healthy volunteers is 0.8 mg/l. These low levels of CRP can be measured with highly sensitive techniques.
and are considered to be related to sub-clinical inflammatory states such as cardiovascular disease and its risk factors. There is accumulating data indicating that CRP may also directly promote atherosclerotic and inflammatory processes in the endothelium. In disease states including vascular pathophysiology, inflammatory status can be used in risk evaluation, but it is not, however, a direct marker of endothelial dysfunction.

2.3.5.3. Coagulation factors
Von Willebrand factor (vWF) is released from endothelial cells into the blood stream after endothelial activation/damage. It activates platelet adhesion and aggregation enhancing thrombus formation; increased levels are regarded as reflecting endothelial dysfunction and early atherogenesis. Endothelial activation also leads to procoagulant imbalance between t-PA and its inhibitor PAI-1.

2.3.5.4. Endothelial remnants
Endothelial damage can also be reflected by the presence of mature detached endothelial cells or microparticles of endothelial origin in the blood. Endothelial microparticles are vesicles formed by the cell membrane resulting from endothelial activation and they are suggested to be associated with the extent of endothelial injury. However, the pathophysiology of this group of markers is not quite clear yet.

2.4. Cardiovascular risk factors
Atherosclerosis is a complex process and far from being elucidated in detail. The pathophysiology of CVD involves several interrelated mechanisms and their contributions to the atherosclerotic process have been extensively investigated. The endothelium and its functions represent the first line mechanisms to be involved. The widely accepted prevailing theory associates atherosclerosis with leukocyte accumulation in the vascular wall, alterations in endothelial homeostatic mechanisms and subsequent effects on VSMC, and an accumulating inflammatory response, which all together are called endothelial dysfunction. The effect of traditional cardiovascular risk factors on vascular wall is tightly related to inflammatory mechanisms on endothelium.

2.4.1. Inflammation
Inflammation has a well established role in the stages of the atherosclerotic process from initiation to the complications of the plaque. The main risk factors of atherosclerosis
are those that give rise to injury, pro-inflammatory stimuli, that elicit inflammatory activation of the endothelium. This leads eventually to migration of monocytes and their transformation to macrophages enabling fatty streak formation via uptake of lipoproteins.\textsuperscript{69} The functional changes in activated endothelium do not only include inflammatory changes but also disturbance in vessel tone and development of a procoagulant vascular state, most likely as a result of decreased bioavailability of NO.\textsuperscript{60} Inflammatory markers for consideration as predictors of cardiovascular risk and of potential clinical use are listed in Table 2.\textsuperscript{69}

Table 2. Potential inflammatory markers in regard to risk evaluation of cardiovascular disease.

<table>
<thead>
<tr>
<th>Inflammatory markers</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesion molecules</td>
<td>E-selectin, P-selectin</td>
</tr>
<tr>
<td></td>
<td>ICAM-1, VCAM-1</td>
</tr>
<tr>
<td>Cytokines</td>
<td>IL-1, IL-6, IL-8, IL-10, TNF\textsubscript{a}</td>
</tr>
<tr>
<td>Acute-phase reactants</td>
<td>Fibrinogen, serum amyloid A, CRP</td>
</tr>
<tr>
<td>White blood count</td>
<td></td>
</tr>
</tbody>
</table>

All the listed inflammatory variables have been shown to be associated with cardiovascular events independent of the general risk factors.\textsuperscript{63,69} A large body of evidence shows that CRP especially, measured by means of high-sensitivity methods, has a graded, dose-response relationship to clinical CVD and adds to the predictive value of established risk factors.\textsuperscript{65,69} However, the precise role of inflammation in the atherosclerotic process is still unsolved; is the inflammatory process a step in causality or a marker of the ongoing atherosclerotic process?\textsuperscript{69,70}

2.4.2. Hypertension

Hypertension is a major cardiovascular risk factor affecting approximately 25% of the population worldwide and rising.\textsuperscript{71} Hypertension results in increased thickness and stiffness of the vascular tree related to VSMC hypertrophy and hyperplasia and increased intimal thickness.\textsuperscript{72} However, it is not only hypertension (systolic blood pressure >140 and/or diastolic BP >90 mmHg) that is predictive of cardiovascular disease. Blood pressure at pre-hypertensive levels (120–139 mmHg systolic and/or 80–89 mmHg
diastolic) seems to be related to other established cardiovascular risk factors, to be associated with higher levels of inflammatory markers and, most importantly, it appears to add to the risk of hypertension and cardiovascular events in later life. It has been suggested that every small increase in BP over 115/75 mmHg adds to the risk of fatal stroke and fatal coronary heart disease. 

Endothelial dysfunction plays a role in the pathogenesis of hypertension and is supposedly a mediator of its atherogenic effects. The results of several studies have shown impaired NO-mediated vasodilatation and reduced excretion of urinary NO metabolites in hypertensive patients, even though this has not been a universal finding. The underlying mechanisms include inhibition of NO synthesis and scavenging of NO through oxidative stress, and also an activated RAS followed by increased levels of Ang II and water retention. Furthermore, ET-1 seems to play a role in hypertension, although the mechanism is not clear.

2.4.3. Renal disease

End stage renal disease is strongly associated with increased mortality and cardiovascular event rate. Even milder forms of chronic kidney disease, classified by glomerular filtration rate (GFR), increase the risk of cardiovascular events independently of classical risk factors of CVD and other confounding factors. The lower the GFR, the higher the risk of a cardiovascular event. Even the mildest forms of renal dysfunction seem to be related to cardiovascular events; microalbuminuria, defined as persistent elevation of urinary albumin levels (>30 to <300mg/day), is a predictor of increased cardiovascular risk. According to the latest studies microalbuminuria is regarded as resulting from vascular endothelial deterioration and dysfunction, supposedly partly as a result of subclinical inflammation and oxidative stress, even though the association with CVD is not fully defined.

2.4.4. Hyperlipidaemia

Abnormal lipid levels are powerful predictors of cardiovascular disease. Individual lipid fractions, and the atherogenic phenotype, namely increased low density lipoprotein (LDL), decreased high density lipoprotein (HDL) and increased triglycerides levels are independent risk factors of CVD and the blood biomarkers recommended for cardiovascular risk prediction.
Low density lipoprotein
Low density lipoprotein represents a major risk factor as regards coronary heart disease. Particles of LDL vary in size and density following that smaller and denser particles are regarded as being the most atherogenic; with similar plasma levels, smaller LDL particles have been shown to be associated with decreased endothelial vasodilation in the forearms of healthy volunteers.\textsuperscript{53} The atherogenic effect of LDL is thought to result from low binding affinity to receptors, prolonged plasma life and low resistance to oxidative stress.\textsuperscript{82} Production of oxidized LDL seems to be an early event in the atherosclerotic process and it has several atherogenic, pro-inflammatory effects on the vascular wall and endothelium participating in atherosclerosis initiation, progression and complications.\textsuperscript{61,83}

High density lipoprotein
High density lipoprotein is believed to be involved in retardation of the atherosclerotic process, subsequently slowing down the progression of cardiovascular disease independently of other risk factors. The cellular mechanisms are not yet resolved in detail. However, these are likely to include relationships with antioxidant enzymes associated with reduced oxidation of LDL, decreased expression of adhesion molecules on endothelial cells and actions in atherosclerotic plaques, by improving their stability and reducing their susceptibility to rupture by mediating reverse cholesterol transport.\textsuperscript{84}

Triglycerides
An elevated plasma triglyceride concentration is a risk factor of CVD and the risk is more pronounced in women than in men.\textsuperscript{85} Triglycerides are involved in central processes of atherosclerosis. First, they contribute to triglyceride-rich atherogenic lipoproteins like very low density lipoprotein (VLDL), they are involved in modification of LDL and HDL towards the more atherogenic forms and thirdly, they are associated with non-lipid conditions of metabolic syndrome (insulin resistance and hypertension).\textsuperscript{86,87} The studies that have been done have shown variable effects of hypertriglyceridaemia on vasodilatation.\textsuperscript{53,88}

Apolipoproteins and lipoprotein (a)
Apolipoprotein A1 (APOA1) is the main component of HDL in plasma and is inversely associated with the risk of coronary heart disease. It may have a positive influence on
pre-existing atherosclerosis. Apolipoprotein B (APOB) is the primary apolipoprotein for LDL. It is required for LDL formation and acts as a ligand for LDL receptors. Higher levels of APOB are correlated with increased risks of cardiovascular event including myocardial infarction, ischaemic heart disease and ischaemic cerebrovascular disease. In a recent study it was shown that the non-fasting APOA1/APOB ratio is superior to any lipid ratios in predicting the risk of acute myocardial infarction. Lipoprotein (a) (Lp(a)) in turn, is bound to APOB in LDL. Owing to a structure similar to those of plasminogen and tPA and a stimulating effect on PAI-1, it is regarded as being thrombogenic. High levels of Lp(a) are associated with atherosclerosis and coronary heart disease, but, it is not yet regarded as a conventional risk factor of cardiovascular disease.

2.4.5. Insulin resistance and metabolic syndrome

Insulin resistance or alternatively called decreased insulin sensitivity can be defined as a reduced/altered biological effect for any given concentration of insulin. Insulin exerts its actions on skeletal muscle by increasing uptake of glucose from the blood and secondly, by affecting the various intracellular pathways of glucose metabolism. This physiological ability of insulin to stimulate glucose disposal is a continuum without any cut-off point separating normal subjects from those who are insulin resistant. A decrease in insulin sensitivity is compensated for by increased production of insulin to maintain normal glucose levels. However, even if near-normal glucose levels are achieved, the ensuing hyperinsulinaemia is metabolically related to some degree of glucose intolerance, atherogenic lipid changes and hypertension. These changes are not only due to impaired glucose disposal but also to impaired insulin action in the liver and in adipose tissue. This cluster of metabolic changes was first related to an increased risk of CVD in non-diabetic individuals. Now, there are several definitions for this metabolic syndrome linked to abdominal obesity, impaired glucose tolerance, dyslipidaemia and hypertension, which together lead to a considerably increased risk of CVD. Visceral obesity and insulin resistance are believed to be the main forces mediating the adverse atherogenic profile in metabolic syndrome. In the classification of metabolic syndrome by the International Diabetes Federation (IDF) 2005 central obesity is the only mandatory criterion, Table 3.
The pathophysiology of insulin resistance and its relationship to cardiovascular risk factors at a cellular level is complex, and not completely understood. Enzymatic changes in adipose tissue lead to increased levels of free fatty acids (FFAs) that in turn are able to modify the lipoprotein constellation via the liver, increasing the levels of atherogenic VLDL, small dense LDL, and triglycerides and decreasing the levels of HDL. In addition, pro-inflammatory cytokines produced by adipose tissue most likely contribute to the tissue effects of insulin resistance. Impaired intracellular metabolism in insulin resistance might eliminate the vasodilatory effect of insulin, enhancing hypertension together with increased sympathetic activity.

2.4.5.1. Assessment of insulin sensitivity

In practice, insulin resistance equates with reduced insulin-mediated glucose disposal in skeletal muscle. It can be measured by way of several well-established tests, depending on the aim of the examination and the population of interest.

A direct, “gold standard” for measurement of insulin sensitivity is the so-called euglycaemic clamp technique. In this technique, by way of fixed insulin infusion and variable glucose infusion, plasma glucose is “clamped” to a constant level. The glucose infusion rate necessary to maintain the desired plasma level is inversely correlated to insulin resistance. This method is however time-, cost-, and labour- consuming and other methods are mostly used. Nevertheless, the overall results from each method are often compared with those obtained using the clamp technique.
The frequently sampled intra-venous glucose tolerance test (FSIVGTT) - minimal model measurement of insulin sensitivity

This technique is based on computer analysis of the glucose and insulin profiles obtained after a rapid intravenous injection of glucose and a small bolus of intravenous insulin, given 20 minutes after initial glucose injection. Blood samples are obtained at frequent intervals during the following 180 minutes. The data is then used to estimate the parameters of insulin sensitivity using the MINMOD computer program (MINMOD Millennium, Minmod, Inc., USA). Insulin sensitivity is expressed as insulin sensitivity index (SI) obtained from the disappearance curves of glucose and insulin and is defined as fractional glucose disappearance per insulin concentration unit. The minimal model FSIVGTT is simpler to perform compared with the clamp technique, although it is still time-consuming and labour-intensive. The minimal model estimates and variation coefficients correlate well with those from the euglycaemic clamp technique. However, the methodology may oversimplify the physiology of glucose homeostasis and with considerably decreased insulin sensitivity the results may not be as reliable as in healthy subjects.

Other methods

Non-dynamic measurements of insulin sensitivity include HOMA (homeostasis assessment model), QUICKI (quantitative insulin sensitivity check index) and the Matsuda-index, which offer surrogate indexes of insulin sensitivity. HOMA is the most appropriate to use in larger population studies and the results correlate well with estimates from euglycaemic clamp studies and the minimal model. QUICKI is another mathematical model using fasting insulin and glucose to produce a surrogate index for insulin sensitivity. It gives a good estimate over a wide range of insulin sensitivity and the results correlate well with those from the clamp technique, and it is used in large epidemiological studies. A so-called insulin sensitivity index – the Matsuda index – involves use of values from the oral glucose tolerance test (OGTT) and gives an estimate of insulin sensitivity reflecting both hepatic and skeletal muscle insulin sensitivity. The results correlate relatively well with those from the clamp technique.

2.4.6. Obesity

Obesity, especially visceral obesity, increases the risk of cardiovascular disease and
mortality.\textsuperscript{112-114} The effect is mainly thought to be mediated by accompanied increased insulin resistance and enhanced effect of other risk factors.\textsuperscript{3,115,116} However, obesity-related increased cardiovascular mortality can also be seen independently of conventional risk factors.\textsuperscript{116} Adipose tissue is an active endocrine organ secreting several substances whose actions together may lead to increased oxidative stress in the endothelium and promote atherosclerosis.\textsuperscript{116} These substances include adipokines (leptin and adiponectin), CRP and pro-inflammatory cytokines such as TNF\textsubscript{\alpha} and IL-6. Release of PAI-1 from visceral adipose tissue could enhance the prothrombotic state of atherogenesis.\textsuperscript{3,116} Weight loss has been shown to diminish the levels of inflammatory cytokines and to improve endothelial function.\textsuperscript{117}

\textbf{2.4.7. Sympathetic over-activity}

Sympathetic activation plays a complex role in promoting atherosclerosis. For the most part, it seems to exert its actions by modifying the mechanisms of BP regulation and contributing to insulin resistance and metabolic syndrome.\textsuperscript{118}

The potential consequences of adrenergic drive regarding BP include sodium retention and increased heart rate, cardiac output (CO) and vascular resistance.\textsuperscript{104} The resulting changes in mechanical forces in the vasculature, increased pressure and changes in arterial flow pattern, could play a part in activating the endothelium and contribute to the anatomical changes in vascular walls, and the atherogenic process.\textsuperscript{72} Sympathetic activation has also been suggested to play a part in early BP changes, followed later by a reduced contribution with advancing hypertension.\textsuperscript{119,120} This neurogenic type of hypertension does not, however, portray the the situation in the whole hypertensive population and other mechanisms are apparent.\textsuperscript{104,120}

Increased sympathetic drive is associated with obesity and metabolic syndrome.\textsuperscript{104,115} Increased muscle sympathetic nerve activity (MSNA) has been shown to be increased in obesity with additive effects from hypertension.\textsuperscript{121} The association is further strengthened by studies where weight loss has been accompanied by decreased sympathetic activity.\textsuperscript{122,123} Sympathetic activity is related to decreased insulin sensitivity and levels of FFAs and the dyslipidaemia of metabolic syndrome.\textsuperscript{104} However, it is unclear if insulin resistance is the primary change; systemic infusion of insulin can cause an increase in sympathetic outflow and on the other hand, an increase in sympathetic activity can diminish insulin actions in skeletal muscle.\textsuperscript{124,125} The latter would be in
line with the results of recent follow-up studies showing increased sympathetic activity before the onset of hyperinsulinaemia.126, 127

2.4.7.1. Assessment of sympathetic activity

There is no method superior to others in quantifying sympathetic activity. The physiologic variety in regional sympathetic flow makes comparison and interpretation of the results difficult. However, the various methods are complementary to each other and when used in combination increase the strength of analyses.128 Measurement of catecholamines in plasma is the most commonly used method. It is easy and patient-friendly to apply. The employment of more sensitive techniques of analysis has also increased the precision of this method. However, the limitations include the inability to extrapolate the results to different target organs, dependence on the clearance and re-uptake rate of noradrenaline (NA) and poorer reproducibility and sensitivity compared with MSNA. Nevertheless, with careful thought concerning the potential external confounding factors and repeated samples, the accuracy of the results can be increased.128, 129 The effect of clearance rate on the results can be overcome by using isotope dilution methodology, the so-called NA spillover rate, which can be used for either whole body or regional spillover measurements.128 The most commonly used method to assess sympathetic activity at heart level is to measure heart rate variability, but it gives more information on relative sympathetic activity combining the effects of sympathetic and parasympathetic activity.128 By using MSNA recording it is possible to assess the efferent muscle sympathetic nerve activity as regards amplitude and number of bursts. This method is highly reproducible and used increasingly, but its use is limited by its invasiveness and need for special equipment and expertise.128

2.5. Physiological adaptation to normal pregnancy

Adaptation to pregnancy consists of changes in anatomy and physiology to ensure the crucial blood and nutrient supply to the growing fetus via the placenta. Maternal hormonal changes result in adjustments, especially in the physiology of cardiovascular, haemodynamic, haematological and metabolic systems.130-132 These changes begin after fertilization and continue throughout pregnancy. Thus, pregnancy can be regarded as a time period of general physiological stress in a woman’s life.130, 131, 133
2.5.1. Placentation
During normal placental development cytotrophoblasts invade and penetrate the maternal uterine spiral arterioles. The ensuing remodelling transforms the normal arterial structures into distended, low-resistance and high-capacity vessels. This includes disappearance of the arterial muscle layer and replacement of endothelial cells by endovascular trophoblasts preventing arterial constriction to vasoactive stimuli and guaranteeing high uteroplacental flow volume crucial to fetal development.

2.5.2. Inflammatory response
Normal pregnancy is characterized by a mild systemic inflammatory response. Many acute phase inflammatory variables are increased in normal pregnancy. Leucocytosis can be seen from the early stages of pregnancy, increasing towards the third trimester and there is evidence for leukocyte activation. Increased levels of pro-inflammatory cytokines, such as TNFα and IL-6 are also present in normal pregnancy.

2.5.3. Haemodynamic changes
Normal pregnancy is characterized by hyperdynamic circulation; increased heart rate, cardiac output (CO) and plasma volume, decreased vascular resistance and decreased BP. A drop in systemic vascular resistance is a major maternal physiological adjustment to pregnancy; beginning in early pregnancy, reaching a maximum in mid-pregnancy, followed by a slow rise until term. Blood volume expansion follows the fall in vascular resistance reaching at plateau at 28–30 weeks of gestation - a total 40–50% increase over the non-pregnant state. These changes are reflected in enhanced resting vascular blood flow, with a tendency to increase towards the third trimester. Mean BP changes only a little, reaching a minimum in mid-pregnancy and returning towards the non-pregnant level near term. Renal adaptive haemodynamic alterations include renal vasodilatation and an increase in GFR as a result of enhanced renal plasma flow. Vasodilatation in the kidney is mainly mediated by relaxin, an ovarian hormone, via ET-1 and ETB-receptors in the endothelium, causing an increase in NO synthesis. Low urinary protein excretion is a normal finding in uncomplicated pregnancy, not exceeding 300 mg/24 hours. Owing to these adaptive changes the maternal cardiovascular status resembles the response to physical training, with related anatomical and functional changes.
2.5.4. Metabolic changes
During the first two trimesters of pregnancy the mother is in anabolic condition. As a result of hyperphagia and enhanced lipogenesis, maternal fat deposits increase to guarantee the energy supplies for accelerated fetal growth in the third trimester.\textsuperscript{131} Advancing pregnancy is characterized by increasing levels of total cholesterol, hypertriglyceridaemia and modification of lipoproteins towards smaller and denser subfractions.\textsuperscript{158-160} From the end of the second trimester enhanced mobilisation of FFAs takes place as a result of accelerated lipolysis and gives rise to a supplemental fetal energy source. This phenomenon is supposedly facilitated by the decrease in insulin sensitivity.\textsuperscript{161}

Early pregnancy is characterized by an enhanced insulin response to glucose. Insulin sensitivity remains the same or is slightly improved.\textsuperscript{133, 162} Insulin resistance develops towards the third trimester, increasing the availability of glucose, the main energy source for the growing fetus.\textsuperscript{162-164} Several maternal hormones have been associated with the genesis of insulin resistance.\textsuperscript{133} The results of the latest studies have suggested an impact of the inflammatory milieu and placenta/adipose tissue-derived peptides on the development of insulin resistance in normal pregnancy.\textsuperscript{165, 166}

2.5.5. Sympathetic activity
Increased sympathetic activity is present in normal pregnancy compared with the non-pregnant state, as measured by MSNA.\textsuperscript{167} It could be associated with activation of the renin-angiotensin-system, increased insulin levels or increased levels of estradiol and progesterone during pregnancy, or alternatively, with the hemodynamic changes during pregnancy.\textsuperscript{104, 167-169}

2.6. Pre-eclampsia and related pathophysiological changes

2.6.1. Definition
Pre-eclampsia (PE) is a pregnancy-specific syndrome, characterized by de novo appearance of hypertension (systolic blood pressure $\geq 140$ mmHg or diastolic blood pressure $\geq 90$ mmHg) and new-onset proteinuria ($\geq 300$ mg/24 hours) after 20 weeks of gestation.\textsuperscript{157} It can be distinguished from other hypertensive disorders of pregnancy, listed in Table 4.
Table 4. Classification of hypertensive disorders in pregnancy according to the Working Group on Research in Hypertension in Pregnancy. 

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Definition criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-eclampsia</td>
<td>De novo hypertension of pregnancy with new-onset proteinuria &gt; 20 gestational weeks</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>De novo hypertension of pregnancy Absence of proteinuria/other clinical finding Arising after 20 gestational weeks Normalizing within 3 months postpartum</td>
</tr>
<tr>
<td>Transient hypertension of pregnancy</td>
<td>Gestational hypertension Normalized blood pressure after delivery</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>Hypertension predating pregnancy /&lt; 20 gestational weeks without proteinuria /hypertension &gt;12 weeks postpartum</td>
</tr>
<tr>
<td>Superimposed pre-eclampsia</td>
<td>Hypertension predating pregnancy with new-onset proteinuria &gt; 20 gestational weeks</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>Convulsive complication of pre-eclampsia during pregnancy or in puerperium</td>
</tr>
</tbody>
</table>

2.6.2. Epidemiology and risk factors
Pre-eclampsia affects 2–10% of all pregnancies, depending on the measured population and methods. It is a major cause of maternal and neonatal morbidity and mortality worldwide. Epidemiological studies have revealed numerous factors that increase the risk of PE. In a systematic review the highest risk of PE was found in women with antiphospholipid antibodies or with a history of PE. A modified list of risk factors is presented in Table 5.
Table 5. Risk factors of pre-eclampsia, modified from Duckitt et al., Sibai and Kaaja.\textsuperscript{170, 174, 175}

<table>
<thead>
<tr>
<th>Maternal</th>
<th>Pregnancy-related</th>
<th>Couple-related</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous pre-eclampsia</td>
<td>Multiple gestation</td>
<td>Limited sperm exposure</td>
</tr>
<tr>
<td>Maternal age</td>
<td>Nulliparity</td>
<td>Primipaternity</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>Chromosome aberrations</td>
<td>Pregnancies with</td>
</tr>
<tr>
<td>Renal disease</td>
<td>in the fetus</td>
<td>donated gametes/embryos</td>
</tr>
<tr>
<td>Rheumatic disease</td>
<td></td>
<td></td>
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<tr>
<td>Maternal low birth weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
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<tr>
<td>Insulin resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregestational diabetes mellitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombophilias</td>
<td></td>
<td></td>
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<tr>
<td>Maternal susceptibility genes</td>
<td></td>
<td></td>
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<tr>
<td>Family history of pre-eclampsia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black race</td>
<td></td>
<td></td>
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<tr>
<td>Malnutrition</td>
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</tbody>
</table>

The couple-related and pregnancy-related risk factors of PE can be considered to be mainly associated with poor placentation. Of the maternal risk factors of PE, many share the common feature of pre-existing vascular endothelial dysfunction present as impaired vasodilatation; diabetes\textsuperscript{176}, obesity,\textsuperscript{3, 116} insulin resistance\textsuperscript{103, 177}, chronic hypertension\textsuperscript{33, 75}, renal disease\textsuperscript{178}, maternal infections\textsuperscript{179, 180}, increasing age\textsuperscript{181, 182} and maternal low birth weight\textsuperscript{183, 184}. It is interesting that the maternal risk factors of PE in particular resemble the list of risk factors of CVD and atherosclerosis, the most central of them presented in Figure 1. However, mixed presentations of these risk factors are supposedly common and a multifactorial aetiology is likely.
2.6.3. Aetiology and pathophysiology

The true causes of PE remain unknown. It is clear, however, that PE cannot develop without the presence of a placenta; a fetus and/or intra-uterine pregnancy are not obligatory. It is likewise generally thought that the clinical symptoms of PE mainly result from vascular insult and endothelial dysfunction. With today’s knowledge, PE has been proposed to be based in two main aetiological categories; placental and maternal.

2.6.3.1. Placental disease

In placental pre-eclampsia the problem is considered to arise from maternal–fetal immune maladaptation, leading to restricted spiral artery invasion of the cytotrophoblasts and subsequent inadequate arterial remodelling. Later in pregnancy, the compromised perfusion would lead to placental hypoxia and oxidative stress along with the demands of the growing fetus. The transition to maternal disease occurs via mediators released from the increasingly oxidatively stressed placenta. Several candidate factors have been proposed as mediators. Soluble fms-like tyrosine kinase 1 (sFlt1), which is an unbound, circulating variant of VEGF receptor, is one of the strongest candidates at
present. It is produced in large amounts in pre-eclampsia by the placenta before the onset of the disease and is able to induce hypertension, proteinuria and glomerular endotheliosis. Other proposed candidates contributing to the clinical syndrome include anti-angiogenic factor soluble endoglin, neurokinin-B and placental debris.

2.6.3.2. Maternal disease
According to the hypothesis of a maternal origin of pre-eclampsia, the endothelium is suspected to be already compromised before pregnancy. A low-grade systemic inflammatory environment and endothelial dysfunction characterize women with pre-eclamptic risk factors. With the physiological stress, caused by an increased inflammatory burden and the physiological changes associated with normal pregnancy, the endothelium would become further dysfunctional, causing the clinical syndrome of pre-eclampsia. Obviously, these categorial aetiologies, maternal and placental, are not exclusive and combined presentations with contributions from both are supposedly common.

2.6.3.3. Role of inflammation and oxidative stress
Regardless of the origin of endothelial damage, it is widely thought that dysfunctional vascular endothelium mediates the clinical symptoms of pre-eclampsia. Compared with the inflammatory milieu of normal pregnancy, higher levels of pro-inflammatory cytokines such as TNFα, IL-6 and IL-1 are seen in pre-eclamptic pregnancy. These can stimulate the endothelium to express adhesion molecules, increase permeability and induce leukocyte infiltration, all features of pre-eclamptic women. Even if the primary origin of increased cytokine production is not known, a continuous inflammatory stimulus could generate changes in endothelial intracellular mechanisms and gene expression, promoting oxidative stress and endothelial dysfunction, in a tightly interrelated manner. In pre-eclampsia there is evidence of increased oxidative stress, including high levels of metabolic products of oxidative processes and decreased levels of anti-oxidants. There is also strong evidence of oxidative stress in the pre-eclamptic poorly perfused placenta. In the endothelium, changes in enzymatic activity and in gene transcription might lead to further enhancement of inflammation by way of increased expression of adhesion molecules, decreased synthesis of vasodilators, increased levels of vasoconstrictive agents and impairment of the bioavailability of NO.
2.6.4. The clinical syndrome of pre-eclampsia

The clinical syndrome of PE is very heterogeneous. This might be a result of the nature of its aetiology: an exaggerated inflammatory response to pregnancy of placental, maternal or combined origin, or possibly differences in the level of damage in different organs and the vascular bed. The clinical spectrum is everything from mild to severe and it can even result in fatal complications. Furthermore, the clinical picture does not always fulfil all the diagnostic criteria and PE should be suspected even when primary criteria are lacking, if additional findings are present. As mentioned above, the clinical features and symptoms of PE supposedly have their origins mainly at the endothelial level.

2.6.4.1. Hypertension

Hypertension during PE could be a result of imbalance between endothelium-derived vasodilating and vasoconstrictive mediators, an increased endothelial vasopressor response and sympathetic activity. As mentioned previously, the bioavailability of NO is probably diminished, the balance between vasodilating PGI₂ and vasoconstrictive TXA₂ favours the latter and in addition the compensatory endothelium-derived hyperpolarizing factor (EDHF) might be impaired. Accordingly, impaired vasodilatation is characteristic of PE.

2.6.4.2. Proteinuria

Glomerular endothelial changes result in alterations in renal filtration and permeability, seen as pre-eclamptic proteinuria. Swollen capillary endothelial cells creating “bloodless” glomeruli, (endotheliosis), is characteristic of PE and seem to be responsible for the decreased GFR. The degree of endotheliosis can vary and have a focal appearance and it is not entirely pathognomonic to PE. As mentioned above, sFlt-1 is able to induce pre-eclamptic changes in the glomeruli, but the mechanism is still unknown. Findings from mouse models support the idea of a sFlt-1 mediated insult on the endothelium; VEGF seems to be central to endothelial health and maintenance of the filtration barrier.

2.6.4.3. Hypercoagulability

The inflammatory process in PE is related to activation of the coagulation system. Activated platelets can promote vascular damage and obstruction, leading to tissue
ischaemia, facilitated by parallel vasoconstriction.\textsuperscript{227, 228} A reduced platelet count and lifespan is associated with low-grade disseminated intravascular coagulation activated in parallel with down-regulation of the fibrinolytic system.\textsuperscript{212,213,228} The coagulation disorder seems to be even more exaggerated in early-onset disease and in the presence of hereditary and acquired thrombophilies.\textsuperscript{229}

2.6.4.4. Hyperlipidaemia
Alterations in the lipid profile seem to be more atherogenic in PE. Changes include increased levels of small dense LDL particles, VLDLs and triglycerides and decreased levels of HDL.\textsuperscript{158, 230-232} Small, dense LDL particles are more susceptible to oxidative modification and represent a potent theoretical mediator of endothelial dysfunction in pre-eclampsia.\textsuperscript{188} Furthermore, FFAs, also increased in PE, have been shown to increase oxidative stress and pro-inflammatory signalling and have a possible direct damaging effect on endothelial cells.\textsuperscript{233, 234}

2.6.4.5. Insulin resistance
Insulin resistance seems to be exaggerated in PE compared with normal pregnancy.\textsuperscript{232, 235-237} There is evidence of decreased insulin sensitivity even before the established disease, suggesting an impact on the pathophysiology.\textsuperscript{237, 238} Endothelial dysfunction related to inflammatory stimulus and/or altered lipid metabolism associated with insulin resistance (increased FFAs, triglycerides and VLDL) could contribute to the diminished vasodilatory actions of insulin.\textsuperscript{231, 233-235} Furthermore, insulin resistance could induce sympathetic over-activity, and vice versa.\textsuperscript{102, 164}

2.6.4.6. Severity of pre-eclampsia
The outcome of PE is dependent on gestational age at onset of disease and at the time of delivery, the availability of medical services, severity of the disease and pre-existing medical conditions.\textsuperscript{239} Pre-eclampsia, which most often occurs in the mid/late third trimester in mild to moderate form, can be considered to be severe according to the criteria presented in Table 6.\textsuperscript{174,240}
Table 6. Criteria of severe pre-eclampsia.

<table>
<thead>
<tr>
<th>Severe pre-eclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure ≥ 160 mmHg</td>
</tr>
<tr>
<td>Diastolic blood pressure ≥ 110 mmHg</td>
</tr>
<tr>
<td>Proteinuria ≥ 5g / 24 hours</td>
</tr>
<tr>
<td>Pulmonary oedema</td>
</tr>
<tr>
<td>Central nervous system disturbances</td>
</tr>
<tr>
<td>Oliguria (&lt;500ml / 24 hours)</td>
</tr>
<tr>
<td>Thrombocytopenia &lt; 100</td>
</tr>
<tr>
<td>Abnormal liver enzymes</td>
</tr>
<tr>
<td>Epigastric/right upper-quadrant pain</td>
</tr>
<tr>
<td>Early onset &lt; 34 gestational weeks</td>
</tr>
<tr>
<td>Fetal growth restriction</td>
</tr>
</tbody>
</table>

2.7. Early and late “consequences” of pre-eclampsia

In a small percentage of women the disease worsens or even appears during the first days after delivery, but in general, PE is cured by delivery of the placenta. Several studies have been conducted to examine the reversing process of PE with the pre-eclamptic phenotype in mind (hypertension, proteinuria, distinct lipid profiles and insulin resistance, Table 7). Since the first large epidemiological studies on previously pre-eclamptic women were published, showing an increased risk of cardiovascular disease, interest and data on existing/persisting alterations after pre-eclampsia have increased.

Table 7. Studies on women with previous pre-eclampsia in regard to risk factors of cardiovascular disease. (Next pages)
<table>
<thead>
<tr>
<th>Study</th>
<th>Studied variables</th>
<th>Time period from index pregnancy</th>
<th>Number of studied women</th>
<th>Results describing the pre-eclamptic group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiovascular system</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adams et al. 1961(^{241})</td>
<td>Blood pressure</td>
<td>17 yrs</td>
<td>149 (185)</td>
<td>Increased BP</td>
</tr>
<tr>
<td>Sibai et al. 1986(^{242})</td>
<td>Blood pressure</td>
<td>2-24 yrs</td>
<td>406 (409)</td>
<td>Increased BP</td>
</tr>
<tr>
<td>Spaanderman et al. 2000(^{243})</td>
<td>Hemodynamics, renal function</td>
<td>&gt;5 mo</td>
<td>58 (11)</td>
<td>Increased CO, decreased plasma volume</td>
</tr>
<tr>
<td>Chambers et al. 2001(^{244})</td>
<td>FMD; intra-arterial stimulation</td>
<td>min 3 mo-median 3 yrs</td>
<td>113 (48)</td>
<td>Impaired vasodilatation</td>
</tr>
<tr>
<td>Ramsay et al. 2003(^{245})</td>
<td>Small vessel function</td>
<td>15-25 yrs</td>
<td>10 (10)</td>
<td>Impaired vasodilatation</td>
</tr>
<tr>
<td>Agatia et al. 2004(^{246})</td>
<td>FMD; mental stress</td>
<td>6-12 mo</td>
<td>16 (14) NP 20</td>
<td>Impaired vasodilatation</td>
</tr>
<tr>
<td>Blaauw et al. 2006(^{247})</td>
<td>Vascular intima-media thickness</td>
<td>3 mo-6 wk after breastf</td>
<td>22 (22) NP 22</td>
<td>Increased IMT</td>
</tr>
<tr>
<td>Hamad et al. 2007(^{248})</td>
<td>FMD; reactive hyperemia</td>
<td>12-18 mo</td>
<td>18 (17)</td>
<td>Impaired vasodilatation, increased BP</td>
</tr>
<tr>
<td>Nisell et al. 1995(^{249})</td>
<td>Renal function, blood pressure</td>
<td>7 yrs</td>
<td>45 (44) PIH 49</td>
<td>Microalbuminuria, increased BP</td>
</tr>
<tr>
<td>Bar et al. 1999(^{250})</td>
<td>Renal function, blood pressure</td>
<td>2-4 mo and 3-5 yrs</td>
<td>48 (48)</td>
<td>Microalbuminuria, increased BP</td>
</tr>
<tr>
<td><strong>Carbohydrate metabolism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jacober et al. 1994(^{251})</td>
<td>Insulin sensitivity (clamp)</td>
<td>3-6 mo</td>
<td>10 (7)</td>
<td>Normal</td>
</tr>
<tr>
<td>Fuh et al. 1995(^{252})</td>
<td>Glucose tolerance (OGTT + SSP/PG)</td>
<td>2 mo</td>
<td>26 (13)</td>
<td>Hyperinsulinemia</td>
</tr>
<tr>
<td>Kaaja et al. 1999(^{235})</td>
<td>Insulin sensitivity (minimal model)</td>
<td>3 mo</td>
<td>22 (16)</td>
<td>Decreased</td>
</tr>
<tr>
<td>Laivuori et al. 1996(^{10})</td>
<td>Glucose tolerance (OGTT+IRI)</td>
<td>17 yrs</td>
<td>22 (22)</td>
<td>Hyperinsulinemia</td>
</tr>
<tr>
<td>Authors</td>
<td>Parameter</td>
<td>Age Range</td>
<td>Participants</td>
<td>Data</td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------------------</td>
<td>-----------</td>
<td>--------------</td>
<td>----------</td>
</tr>
<tr>
<td>Nisell et al. 1999</td>
<td>Insulin sensitivity (FIRI)</td>
<td>6 mo - 2 yrs</td>
<td>21 (22)</td>
<td>Increased; insulin and glucose and FIRI</td>
</tr>
<tr>
<td>Wolf et al. 2004</td>
<td>Insulin sensitivity (HOMA)</td>
<td>9 mo - 2.5 yrs</td>
<td>29 (32)</td>
<td>Increased HOMA</td>
</tr>
<tr>
<td><strong>Lipid metabolism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>He et al. 1999</td>
<td>Lipids, lipoproteins</td>
<td>3.7 - 5.3 yrs</td>
<td>25 (24)</td>
<td>Increased; cholesterol, VLDL, triglycerides</td>
</tr>
<tr>
<td>Hubel et al. 1996</td>
<td>Cholesterol, triglycerides, FFA</td>
<td>48 hrs</td>
<td>8 (9)</td>
<td>Increased triglycerides, FFA</td>
</tr>
<tr>
<td>Hubel et al. 2000</td>
<td>Lipids, lipoproteins</td>
<td>postmenopausal</td>
<td>30 (30)</td>
<td>Smaller LDL size, increased APOB</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laivuori et al. 1998</td>
<td>Sex hormones, endothelial markers</td>
<td>17 yrs</td>
<td>22 (22)</td>
<td>Higher circulating testosterone</td>
</tr>
<tr>
<td>Courtar et al. 2006</td>
<td>Sympathetic activity (HRV)</td>
<td>6-12 mo</td>
<td>11 (7)</td>
<td>Symp hyperactivity and decreased baroreflex sensitivity related to low plasma volume</td>
</tr>
<tr>
<td>Schobel et al. 1996</td>
<td>Sympathetic activity (MSNA)</td>
<td>1-3 mo</td>
<td>6 (8)</td>
<td>Normal</td>
</tr>
<tr>
<td>Portelinha A. 2008</td>
<td>Hemostatic factors; t-PA, PAI-1, D-dimer</td>
<td>4-8 yrs</td>
<td>65 (54)</td>
<td>Increased D-dimer.</td>
</tr>
<tr>
<td>Forest et al. 2005</td>
<td>Metabolic syndrome parameters</td>
<td>5-13 yrs</td>
<td>63 (168)</td>
<td>Increased APOB and insulin and BP</td>
</tr>
</tbody>
</table>

SSPI/PG=steady-state plasma insulin/plasma glucose, IRI=immunoreactive insulin, FIRI=fasting insulin resistance index, IMT=intima-media thickness, NP=non pregnant, PIH=pregnancy induced hypertension
According to the meta-analyses, women with previous PE have an increased future risk of hypertension, stroke, venous thromboembolism and peripheral arterial disease. Furthermore, the risk of CVD seems to be positively correlated to the severity of PE. The risk of cardiac disease showed a stepwise increase with increasing severity of PE (mild; uncomplicated PE, with a relative risk (RR) of 2.0, moderate;
PE + eclampsia or intrauterine growth restriction (IUGR), with RR 2.99, severe; PE + preterm delivery and/or fetal death with RR 5.36). A relationship between prematurity and later risk of ischaemic heart disease was supported by findings from two studies, where preterm delivery (before 37 gestational weeks) combined with PE increased the risk to 7.71 compared with normotensive pregnant women who delivered after 37 weeks of gestation. Parity did not have any significant effect on the risk of cardiac disease.
3. AIMS OF THE STUDY

The aim of the study was to map out the cardiovascular risk profile in women with previous severe pre-eclampsia 5–6 years after index pregnancy. This analysis was broken down into the following areas.

1. To investigate if vasodilatation, endothelium-dependent or endothelium-independent, or central wave reflections as measures of vascular health would be altered in women with previous pre-eclampsia in comparison with women with previous uncomplicated pregnancy.

2. To see if renal function in women with previous pre-eclampsia would be impaired in comparison with controls with uncomplicated pregnancy. Secondly, to investigate if the amount of proteinuria during pre-eclamptic pregnancy would have any correlation to later vasodilatation or renal function.

3. To investigate the presence of metabolic syndrome and insulin sensitivity in particular, in women with a history of pre-eclampsia, and secondly, to see if insulin sensitivity has an impact on vasodilatory capacity and/or characteristics of the index pre-eclamptic pregnancy.

4. To see if there are any signs of sympathetic activation in women with a history of pre-eclampsia and if so, if it is associated with insulin sensitivity, arterial pressure and/or vascular dilatory function.
4. MATERIAL AND METHODS

4.1. Material
The material for the study was collected from the hospital records of women treated for pre-eclampsia at Helsinki University Central Hospital during the time period 1.1.1996 to 31.12.1998. The total number of deliveries during that time was 14,432 and the number of women with pre-eclampsia was 634, which gives an incidence of 4.4%. The patient group was selected from the moderate/severe pre-eclampsia group (International Classification of Diseases, tenth revision, diagnosis O14.1) of 134 pregnancies. The medical charts were personally reviewed to confirm the accuracy of the diagnosis of pre-eclampsia (blood pressure at least 140 mmHg systolic or 90 mmHg diastolic on at least two occasions after the 20th week of gestation and proteinuria ≥ 300 mg/24 hours or ≥ 1+ on dipstick) and other medical conditions. Only women with registered 24-hour urine sampling were included. Women with the following diseases were excluded: diabetes, history of gestational diabetes, chronic hypertension, kidney disease, vascular disease/thrombosis or coagulation disorder. The patient group consisted of 30 women selected from the remaining sample of 83 and were reachable, Caucasian and living in the Helsinki area. The control women (n=21) were selected at random from the hospital records of healthy women with normal, uncomplicated pregnancies during the same time period. The women were first contacted by letter and then called by phone personally to give more information about the study and to request the interest of their participation in the study.

In paper no II the patient group of 30 women with previous pre-eclampsia was divided into two groups according to maximal total diurnal proteinuria; the “low” group < 5 g/24 hours and the “high” group ≥ 5 g/24 hours. In papers III and IV the material was reduced by 3 women (two in the patient group and one in the control group) who were excluded as a result of technical problems during the FSIVGTT. This resulted in group sizes of 28 in the previously pre-eclamptic group and 20 in the control group.
All women were studied 5–6 years after the index pregnancy. The studies were accomplished in the non-menstrual phase of the cycle and none of the studied women had reached menopause. The experimental protocol was approved by the Ethics Committee of Helsinki University Central Hospital. Before entering, after written and oral information about the aim and the protocol of the study, every woman gave written consent to participate in the study. All of the used procedures adhered to the principles of the Declaration of Helsinki.
Non-steroidal anti-inflammatory drugs and other drugs were withheld for 7 days before the study. None of the studied women were on hormone replacement therapy. Two women with hypertension, diagnosed years after pre-eclamptic pregnancy withheld their hypertensive medication for three days before the study.

4.2. Methods

4.2.1. Study protocol—first day of the study

The studies were conducted in a quiet, temperature-controlled (20–21°C) room after an overnight fast and accomplished in two successive days for each woman, beginning each day at 8 a.m. First, the demographic variables were recorded and BP measured sitting and lying, by a trained midwife. Blood samples were collected for the analyses and the women emptied their bladders. Twenty-four hour urine sampling was started.

Vasodilatation was assessed in vivo by measurement of forearm resistance artery blood flow, using venous occlusion plethysmography (EC 4 Strain Gauge Plethysmograph; D.E. Hokanson, Inc., Bellevue, Washington, USA). The women lay supine with elbows resting on foam pads, allowing the forearms to be positioned above heart level. The non-dominant arm was used for drug infusions while the dominant arm served as a control. The cuffs of the plethysmograph were placed around both arms and wrists. Patient introduction of the practical measurement technique was performed. After a minimum of 10 minutes' rest the basal level of blood flow was measured before brachial artery needle insertion. A 27 gauge needle (Portex) was inserted in the brachial artery of the non-dominant hand and connected to the infusion system (saline at 1ml/min). Blood flow was measured after rest and once it had returned to the basal level measured previously (a rest of 12 minutes minimum), the study infusions were started. The drugs were infused for 6 minutes each at an infusion rate of 1 ml/min in the following sequence: sodium nitroprusside (SNP; Nitropress; Abbott Laboratories, North Chicago, Illinois, USA) as an endothelium-independent vasodilator at 3 µg/min and 10 µg/min, followed by an infusion of saline for 18 minutes during which blood flow returned to the initial basal level. This was followed by infusion of the endothelium-dependent vasodilator acetylcholine (ACh; Miochol-E; Laboratories CibaVision Faure, Annonay Cedex, France) at concentrations of 7.5 µg/min and 15 µg/min. Forearm blood flow was measured during the last 3 minutes of the 6-minute infusion period and the mean of the last five recordings was used in the analyses. The arterial needle was removed.
Change in blood flow was expressed as a percentage adjusted for flow in the non-infused control arm and for flow during the previous saline infusion in the infused arm to minimize the possible systemic effects of the drugs used (Figure 3). The endothelial function index (EFI; change in flow ratio (ACh)/change in flow ratio (SNP)) was calculated to compare endothelium-independent and endothelium-dependent vasodilatation. Within-subject reproducibility has been reported to be 31–39% at rest and 19% when the ratios of unilateral flows are used.

**Figure 3.** The equation used for expression of the results in venous occlusion plethysmography, adjusted for confounding factors, and expressed as change in flow ratio.

\[
\frac{[\text{Flow (drug) exp / ctrl arm}] - [\text{Flow (previous saline) exp / ctrl arm}]}{\text{Flow (previous saline) exp / ctrl arm}}
\]

The women were then allowed to go to the toilet. After 30 minutes of rest in a supine position the orthostatic test was performed. Samples for adrenaline (A) and noradrenaline (NA) were drawn from the antecubital vein in the forearm. Blood pressure was measured (OMRON M4, HEM-722C1-E; OMRON Healthcare Europe B.V., Hoofddorp, the Netherlands) and after 5 minutes in an upright position blood samples were collected again for A and NA and BP was measured. The blood samples were immediately placed in ice, centrifuged (4 °C, and 7000 rpm for 10 min) and the plasma was stored at -70 °C. The volume of the forearm was measured by means of the water displacement method. Samples for catecholamine analyses were later sent by courier in dry ice to Christchurch, New Zealand, and assayed by means of high-performance liquid chromatography (HPLC) at the Endocrine Laboratory of Christchurch Hospital. All samples from each woman were measured in a single assay. Intra-assay variability in HPLC for A was 2.7–5.8% and it was 2.1–4.0% for NA and inter-assay variability was 2.2–9.0% for A and 2.1–5.2% for NA. Ambulatory measurement of 24-hour blood pressure (ABP) was started after a light meal offered to the subjects. We used 24-hour BP equipment (Model 90217-1B, Spacelabs Medical Inc., Redmont, WA, USA) in which BP is measured every 20 minutes in the daytime (6 a.m. to 10 p.m.) and every hour at night (10 p.m. to 6 a.m.). The equipment was attached to the subject’s left
upper arm by a trained midwife. The women were encouraged to live normal lives and not to take the equipment away at any time. No formal diary was kept, but the women were asked to report any peculiarities during the evaluation time.

### 4.2.2. Study protocol–second day of the study

The next morning, after an overnight fast, insulin sensitivity was assessed by means of the **insulin-enhanced frequently sampled intra-venous glucose tolerance test (FSIVGTT)** with minimal model analysis (MINMOD Millennium, Minmod, Inc., USA). We used a 12-sample protocol; the results are shown to correlate well with the original 30-sample test and results from the clamp technique. During the FSIVGTT the women were lying supine and were asked not to move about in the room. The cubital vein was cannulated. FSIVGTT was started with baseline samples for assays of insulin and glucose drawn twice; at -5 min and 0 min in regard to glucose injection. The glucose injection (0.3 g/kg body weight) was given after the later basal sample and blood samples for assay of insulin and glucose were drawn at 4, 6, 8, 10, 19, 22, 29, 37, 67, 90 and 180 minutes after administration of the glucose bolus dose. At 20 minutes the subjects were given an intravenous injection of insulin (0.03 U/kg) (Velosulin Human 100 IU/ml, Novo Nordisk, Denmark). The glucose and insulin levels were then used to estimate the variable for insulin sensitivity, which is obtained from the disappearance curves of glucose and insulin. Blood glucose was measured by the hexokinase method (Glucoquant, Roche Diagnostics) and insulin by using AutoDelfia Insulin kits (Automatic Immunoassay System [B080-101], Perkin Elmer Life and Analytic Sciences, Inc.). Three women were excluded from the analyses of insulin sensitivity because of technical problems during the FSIVGTT (2 in the study group and 1 in the control group). This resulted in sizes of 28 in the study group and 20 in the control group in analyses of insulin sensitivity.

Subsequently, **pulse wave analysis** was performed, starting with BP measurement. Peripheral pulse waves were recorded from the radial artery with an applanation tonometer (SPT-301B; Millar Instruments, Houston, Texas, USA), stored in a personal computer and processed to generate a corresponding aortic waveform (SCOR–2000 software, version 6.31; AtCor Medical Pty, Ltd., Sydney, Australia). Aortic BP, augmentation (ΔP – pressure difference between the reflected second and first systolic peaks, mmHg) and augmentation index were calculated using the Sphygmocor PWA System (AtCor Medical Pty, Ltd). The AIx is regarded as augmentation of aortic systolic
pressure (ΔP percentage of aortic pulse pressure). It was adjusted at the heart rate of 75 beats/min, since the timing of the wave reflection in regard to the cardiac cycle is influenced by heart rate. The blood samples were analysed by using the methods listed in Table 9.

### Table 9. Methods used in analysing the blood samples, and their characteristics.

<table>
<thead>
<tr>
<th>Measured variable</th>
<th>Method/Technique</th>
<th>Equipment</th>
<th>Interassay Variability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dU-Albumin-Mi</td>
<td>Immunoturbidimetric “in-house”</td>
<td>Hitachi Ltd, Japan</td>
<td>4.1</td>
</tr>
<tr>
<td>U-creatinine</td>
<td>Enzymatic colorimetric</td>
<td>Modular, Hitachi Ltd, Japan</td>
<td>2.4</td>
</tr>
<tr>
<td>U-protein</td>
<td>Turbidimetric (bentsethonium chloride)</td>
<td>Roche Professional</td>
<td>2.7</td>
</tr>
<tr>
<td>S-Urate</td>
<td>Enzymatic colorimetric</td>
<td>Modular, Hitachi Ltd, Japan</td>
<td>3.8</td>
</tr>
<tr>
<td>S-testosterone- free</td>
<td>Coated tube radioimmunoassay</td>
<td>Orion Diagnostica, Finland</td>
<td>5</td>
</tr>
<tr>
<td>SHBG</td>
<td>Immunofluorometric assay</td>
<td>Wallac, Finland</td>
<td>5</td>
</tr>
<tr>
<td>Fs-cholesterol</td>
<td>Enzymatic colorimetric</td>
<td>ABX Diagnostics, France</td>
<td>1.5 - 2.3</td>
</tr>
<tr>
<td>HDL</td>
<td>Heparin-Mn-CL precipitation</td>
<td>PerkinElmer, USA</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Enzymatic colorimetric</td>
<td>ABX Diagnostics, France</td>
<td>1.9 - 3.7</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>Immunoturbidimetric</td>
<td>Mira Instruments</td>
<td>na</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>Immunoturbidimetric</td>
<td>Mira Instruments</td>
<td>na</td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>Immunoturbidimetric</td>
<td>Mira Instruments</td>
<td>na</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>ELISA immunoassay</td>
<td>R &amp; D Systems Inc, USA</td>
<td>7.8</td>
</tr>
<tr>
<td>CRP</td>
<td>Immunoenzymometric assay</td>
<td>Medix Biochemica, Finland</td>
<td>na</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>Immunoturbidimetric</td>
<td>Roche Professional</td>
<td>4.3</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>ELISA immunoassay</td>
<td>R &amp; D Systems Inc, USA</td>
<td>5.7</td>
</tr>
</tbody>
</table>

### 4.2.3. Statistics

Normally distributed data is given as mean ± SD/SE and non-normally distributed data as median with interquartile range (25th to 75th percentiles). Student’s t-test was used for normally distributed data and the Mann–Whitney U test for non-normally distributed data.
distributed data when evaluating differences between two groups. For categorial data we applied Fisher’s exact probability test or the $\chi^2$–test. For comparisons in three groups, one-way analysis of variance was performed, followed by the Bonferroni post–hoc test for normally distributed data and Kruskall-Wallis analysis of variance by ranks for non-normally distributed data. Repeated measures analysis of variance (ANOVA) was used to analyse measurements taken on several occasions. Logarithmic transformation was carried out to correct for skewness in the material. Univariate and multiple linear regression analyses were performed. Correlations between vasodilatation and various parameters were analysed by means of Spearman’s rank correlation ($r_s$) or Pearson’s correlation. Calculations were performed by using NCSS 2000 or NCSS 2007 software (Number Cruncher Statistical Systems, Kaysville, Utah, USA). The power to detect a 1.5-fold difference in vasodilatation at a $p$-level of 0.05 was 80%. Values of $p \leq 0.05$ were considered statistically significant.
5. RESULTS

5.1. Characteristics during the index pregnancy

The characteristics of the study groups during their index pregnancies can be seen in Table 10. Pre-eclamptic women were slightly older and more often primiparous compared with the controls. They delivered earlier, and the newborns were more often small-for-gestational age (SGA), and had lower Apgar scores at 1 minute, but there were no significant differences in umbilical artery pH values or Apgar scores of < 7 at 1 minute.

Table 10. Clinical characteristics of the patient and control groups during index pregnancy. Values are expressed as mean ± SD, or median with interquartile range (25–75th percentiles).

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 30)</th>
<th>Controls (n = 21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>33 ± 6</td>
<td>30 ± 4</td>
<td>0.046</td>
</tr>
<tr>
<td>Primiparous in index pregnancy (%)</td>
<td>23/30 (77)</td>
<td>7/21 (33)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI, kg/m²&lt;10 weeks of pregnancy</td>
<td>24 ± 3</td>
<td>24 ± 2</td>
<td>0.6</td>
</tr>
<tr>
<td>Proteinuria in pregnancy, g/day</td>
<td>6.3 (5–13)</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>Systolic BP maximum, mmHg</td>
<td>174 ± 18</td>
<td>121 ± 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP maximum, mmHg</td>
<td>109 ± 8</td>
<td>72 ± 7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Delivery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>33 ± 4</td>
<td>40 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apgar points,1 min</td>
<td>8 (7–9)</td>
<td>9 (9–9)</td>
<td>0.018</td>
</tr>
<tr>
<td>Apgar points &lt;7, (%)</td>
<td>5/30 (17)</td>
<td>1/21 (5)</td>
<td>0.2</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>1795 ± 782</td>
<td>3537 ± 419</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Small for gestational age, (%)</td>
<td>12/30 (40)</td>
<td>1/21 (5)</td>
<td>0.004</td>
</tr>
<tr>
<td>pH, umbilical artery</td>
<td>7.27 (7.24 – 7.29)</td>
<td>7.27 (7.19 – 7.29)</td>
<td>0.97</td>
</tr>
</tbody>
</table>
5.2. Characteristics during the study
At the time of the study the groups were similar with regard to age and weight. The time elapsed from the index pregnancy was longer in the control group. The women in the pre-eclamptic group were more often still primiparae and they had higher systolic and diastolic blood pressure levels. Various characteristics are shown in Table 11.

Table 11. Clinical characteristics of the groups during the study. Values are expressed as mean ± SD or median with interquartile range (25–75th percentiles) * Values obtained from pulse wave analysis. ** > 1 cigarette per day.

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 30)</th>
<th>Controls (n = 21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>38 ± 6</td>
<td>36 ± 4</td>
<td>0.3</td>
</tr>
<tr>
<td>Own birth weight, g</td>
<td>3364 ± 474</td>
<td>3387 ± 564</td>
<td>0.9</td>
</tr>
<tr>
<td>Time since the delivery, years</td>
<td>5 (4.2 – 5.6)</td>
<td>6 (5.2 – 6.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>Primipara during study, (%)</td>
<td>14/30 (47)</td>
<td>3/21 (14)</td>
<td>0.02</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25 ± 4</td>
<td>25 ± 4</td>
<td>0.4</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.80 ± 0.05</td>
<td>0.81 ± 0.04</td>
<td>0.2</td>
</tr>
<tr>
<td>Brachial systolic BP, mmHg</td>
<td>123 (113 – 136)</td>
<td>112 (102 – 123)</td>
<td>0.01</td>
</tr>
<tr>
<td>Brachial diastolic BP, mmHg</td>
<td>80 (76 – 91)</td>
<td>78 (68 – 81)</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>93 (89 – 103)</td>
<td>89 (81 – 95)</td>
<td>0.02</td>
</tr>
<tr>
<td>Aortic systolic BP, mmHg *</td>
<td>112 (105 – 127)</td>
<td>104 (94 – 112)</td>
<td>0.02</td>
</tr>
<tr>
<td>Aortic diastolic BP, mmHg *</td>
<td>77 ± 11</td>
<td>70 ± 10</td>
<td>0.03</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>64 ± 9</td>
<td>68 ± 9</td>
<td>0.2</td>
</tr>
<tr>
<td>Antihypertensive medication, (%)</td>
<td>2/30 (7)</td>
<td>0/21 (0)</td>
<td>0.5</td>
</tr>
<tr>
<td>Smoking, (%) **</td>
<td>5/30 (17)</td>
<td>7/21 (33)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

5.3. Vascular dilatory function and pulse wave analysis (I)
Basal forearm blood flow in the patient and control groups was similar and averaged 1.98 ± 0.6 (SD) vs. 2.2 ± 0.7 (SD) ml/100ml per minute (p=0.31), respectively. Both vasoactive drugs caused dose-dependent vasodilatation in both groups. In the previously pre-eclamptic group, both endothelium-dependent and endothelium-independent
vasodilatations were impaired compared with the controls (Figure 4). An adjusting calculation was carried out to minimize the effect of confounding factors (Table 12).

**Figure 4.** Absolute forearm blood flow in response to the endothelium-independent vasodilator sodium nitroprusside (SNP) and the endothelium-dependent vasodilator acetylcholine (ACh).

■ — previous pre-eclampsia and ▲ — control group. Values are expressed as mean ± SE and the concentration of doses is in µg/ml. Reproduced with permission.
Table 12. Change in forearm blood flow calculated as a percentage adjusted for flow during the previous saline infusion and flow in the contra-lateral arm. Values are presented as median and interquartile range (25–75th percentiles).

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 30)</th>
<th>Controls (n = 21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low dose SNP</td>
<td>277 (198–352)</td>
<td>397 (325–450)</td>
<td>0.004</td>
</tr>
<tr>
<td>High dose SNP</td>
<td>494 (284–596)</td>
<td>581 (386–581)</td>
<td>0.057</td>
</tr>
<tr>
<td>Low dose ACh</td>
<td>313 (219–456)</td>
<td>443 (340–575)</td>
<td>0.045</td>
</tr>
<tr>
<td>High dose ACh</td>
<td>395 (233–538)</td>
<td>547 (424–678)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Impaired vasodilatation was associated with a history of pre-eclamptic pregnancy and with parity in multiple regression analysis as regards both endothelium-independent ($p = 0.007$, $p = 0.047$, respectively) and endothelium-dependent vasodilatation ($p = 0.032$, $p = 0.003$, respectively).

Augmentation index obtained from PWA did not differ between the groups, being 15 ± 9% (SD) in previously pre-eclamptic women and 13 ± 9% (SD) in the control women ($p = 0.46$).

5.4. Severity of proteinuria during pre-eclampsia—impact on later renal function and vascular dilatory function (II).

The pre-eclamptic group was divided into proteinuria groups $dU$-$prot < 5g/24$ hrs (8 women) and $dU$-$prot \geq 5$ g/24 hours (22 women). There were no significant differences in the demographic parameters or clinical parameters of pre-eclampsia between the proteinuric groups apart from proteinuria itself by definition. Blood pressure was higher, gestational weeks at delivery were lower and birth weights of the children were lower in both pre-eclamptic groups compared with the controls.

During the study there were no differences in renal function test results between the proteinuric groups or in comparison with the control group. Systolic and mean blood pressures were higher in the high proteinuric group, but only when compared with the controls. The data is shown in Table 13.
Table 13. Clinical characteristics and kidney function data in the previously preeclamptic proteinuria groups and the control group. Values are expressed as mean ± SD or median with interquartile range (25–75th percentiles). * p<0.05 vs control group.

<table>
<thead>
<tr>
<th></th>
<th>Patients (dU-prot &lt; 5g) (n = 8)</th>
<th>Patients (dU-prot ≥ 5g) (n = 22)</th>
<th>Controls (n = 21)</th>
<th>Overall ANOVA P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37 ± 7</td>
<td>38 ± 5</td>
<td>36 ± 4</td>
<td>0.4</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>27 ± 5</td>
<td>25 ± 3</td>
<td>25 ± 4</td>
<td>0.3</td>
</tr>
<tr>
<td>Blood pressure systolic (mmHg)</td>
<td>124 (115–139)</td>
<td>123 (113–136)*</td>
<td>112 (102–123)</td>
<td>0.03</td>
</tr>
<tr>
<td>Blood pressure diastolic (mmHg)</td>
<td>84 ± 8</td>
<td>82 ± 13</td>
<td>75 ± 7</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>95 (92–108)</td>
<td>93 (86–103)*</td>
<td>89 (81–95)</td>
<td>0.048</td>
</tr>
<tr>
<td>Smoking &gt;1/day (%)</td>
<td>1/8 (13%)</td>
<td>4/22 (18%)</td>
<td>7/21 (30%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Urine volume / 24hours (mL)</td>
<td>1675 (1075–2350)</td>
<td>1625 (1100–2275)</td>
<td>1750 (1000–2400)</td>
<td>0.9</td>
</tr>
<tr>
<td>dU-alb-Mi (mg/day)</td>
<td>5.5 (4–10)</td>
<td>7 (6–13)</td>
<td>6 (4.5–9)</td>
<td>0.3</td>
</tr>
<tr>
<td>U-alb (mg/L)</td>
<td>3.5 (3.4–5)</td>
<td>7.4 (3–6.3)</td>
<td>4 (3–5)</td>
<td>0.8</td>
</tr>
<tr>
<td>S-Urate (µmol/L)</td>
<td>283 ± 57</td>
<td>245 ± 64</td>
<td>240 ± 49</td>
<td>0.2</td>
</tr>
<tr>
<td>U-Crea (mmol/L)</td>
<td>7.0 ± 2.4</td>
<td>7.6 ± 3.5</td>
<td>7.5 ± 3.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Crea-cl (ml/s/1.73m²)</td>
<td>1.8 (1.3–2.1)</td>
<td>2.1 (2.0–2.2)</td>
<td>2.1 (2.0–2.5)</td>
<td>0.08</td>
</tr>
<tr>
<td>fB-gluc (mmol/L)</td>
<td>4.3 (3.9–4.7)</td>
<td>4.4 (4.2–4.8)</td>
<td>4.4 (4.2–4.6)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

The increase in vasodilatation was dose-dependent in each group. The increases (%) in vasodilatation in response to each vasodilator and dose are listed in Table 14.
There were no significant differences between the three groups in post hoc tests. The amount of proteinuria during pre-eclampsia was not correlated to later, (5–6 years after PE) endothelium-independent or endothelium-dependent vasodilatation. In the three women with recurrent episodes of PE, vasodilatation was reduced compared with that in single-episode pre-eclamptic women by 10–26% as regards endothelium-independent vasodilatation and by 24–26% as regards endothelium-dependent vasodilatation. However, this was not statistically significant.

5.5. Insulin sensitivity and vascular dilatory function after pre-eclampsia (III)

The demographic data concerning the index pregnancy and at the time of the study of is presented in Table 15. It should be noted that two women in the patient group and one woman in the control group were excluded from the analyses in the Studies III and IV (see Methods). Statistical analysis of the data in Table 15 was comparable to that in Studies I and II.

Table 14. Change in forearm blood flow calculated as percentage adjusted for flow during the previous saline infusion and flow in the contra-lateral arm. Values are presented as median and interquartile range (25–75th percentiles).

<table>
<thead>
<tr>
<th>Patients (dU-prot&lt;5g) (n = 8)</th>
<th>Patients (dU-prot ≥5g) (n = 22)</th>
<th>Controls (n = 21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low dose SNP 340 (267–368)</td>
<td>270 (190–336)</td>
<td>397 (325–451)</td>
<td>0.009</td>
</tr>
<tr>
<td>High dose SNP 522 (494–540)</td>
<td>338 (276–729)</td>
<td>581 (386–687)</td>
<td>0.148</td>
</tr>
<tr>
<td>Low dose ACh 447 (189–672)</td>
<td>313 (219–377)</td>
<td>443 (340–575)</td>
<td>0.090</td>
</tr>
<tr>
<td>High dose ACh 462 (261–851)</td>
<td>365 (233–508)</td>
<td>547 (424–678)</td>
<td>0.60</td>
</tr>
</tbody>
</table>
Table 15. Characteristics of the two groups at the time of the index pregnancy and at the time of the study. Values are expressed as mean ± SD or median with interquartile range (25–75th percentiles).

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 28)</th>
<th>Controls (n = 20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Data at index pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity; primipara (%)</td>
<td>22/28 (79)</td>
<td>7/20 (35)</td>
<td>0.002</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33±5</td>
<td>30±4</td>
<td>0.02</td>
</tr>
<tr>
<td>Body Mass Index (kg/m², &lt;10 gw)</td>
<td>24±4</td>
<td>24±3</td>
<td>0.59</td>
</tr>
<tr>
<td>Proteinuria, max (g)</td>
<td>8.2 (5.0–13.8)</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>Mean Arterial Pressure, max (mmHg)</td>
<td>129 (123–139)</td>
<td>88 (83–94)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational weeks at delivery</td>
<td>33 (29–36)</td>
<td>40 (40–41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1765 (1010–2392)</td>
<td>3553 (3264–3700)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Data at the study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>38±6</td>
<td>36±4</td>
<td>0.16</td>
</tr>
<tr>
<td>Time from the delivery (years)</td>
<td>5(4.3–5.4)</td>
<td>6(5.0–6.2)</td>
<td>0.005</td>
</tr>
<tr>
<td>Family history of CVD (%)</td>
<td>20/28 (71)</td>
<td>13/20 (65)</td>
<td>0.64</td>
</tr>
<tr>
<td>Metabolic syndrome (IDF 2005) (%)</td>
<td>2/28 (7)</td>
<td>0/20 (0)</td>
<td>0.22</td>
</tr>
<tr>
<td>Parity; primipara (%)</td>
<td>14/28 (50)</td>
<td>3/20 (15)</td>
<td>0.012</td>
</tr>
<tr>
<td>Own birth weight (g)</td>
<td>3355±490</td>
<td>3431±540</td>
<td>0.62</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>25±4</td>
<td>25±4</td>
<td>0.44</td>
</tr>
<tr>
<td>Waist–Hip Ratio</td>
<td>0.80±0.05</td>
<td>0.81±0.04</td>
<td>0.20</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>80 (75–92)</td>
<td>77 (67–81)</td>
<td>0.02</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>124 (113–138)</td>
<td>112 (102–122)</td>
<td>0.006</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>94 (88–104)</td>
<td>88 (81–94)</td>
<td>0.01</td>
</tr>
<tr>
<td>Heart rate (per minute)</td>
<td>64±8</td>
<td>67±10</td>
<td>0.21</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>5/28 (18)</td>
<td>7/20 (35)</td>
<td>0.17</td>
</tr>
</tbody>
</table>
There were no differences between the two groups in carbohydrate, androgen or lipid metabolism; insulin sensitivity was similar in the two groups. Neither were any any significant differences in IL-6, sensitive CRP or vWF as marker of endothelial activation. The results are listed in Table 16.

Table 16. Laboratory values at the time of the study.

<table>
<thead>
<tr>
<th>Laboratory values</th>
<th>Patients (n = 28)</th>
<th>Controls (n = 20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin sensitivity index SI</td>
<td>4.62 (3.3–7.6)</td>
<td>5.97 (3.72–8.15)</td>
<td>0.24</td>
</tr>
<tr>
<td>fB-insulin (mmol/L)</td>
<td>2.9 (2.0–5.8)</td>
<td>2.6 (2.35–3.7)</td>
<td>0.9</td>
</tr>
<tr>
<td>fB-glucose (mU/L)</td>
<td>4.5 (4.2–4.7)</td>
<td>4.5 (4.2–4.6)</td>
<td>0.68</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.2 ± 0.3</td>
<td>5.1 ± 0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>S-Urate (µmol/L)</td>
<td>254 ± 64</td>
<td>242 ± 50</td>
<td>0.47</td>
</tr>
<tr>
<td>S-testosterone-free (pmol/L)</td>
<td>17 (14–21)</td>
<td>20 (13–25)</td>
<td>0.67</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>57 (41–70)</td>
<td>53 (41–76)</td>
<td>0.78</td>
</tr>
<tr>
<td>fS-cholesterol (mmol/L)</td>
<td>4.66 (4.33–5.17)</td>
<td>4.79 (4.12–5.43)</td>
<td>0.89</td>
</tr>
<tr>
<td>HDL, total (mmol/L)</td>
<td>1.58 (1.43–1.75)</td>
<td>1.71 (1.49–1.9)</td>
<td>0.13</td>
</tr>
<tr>
<td>HDL 2 (mmol/L)</td>
<td>0.44 (0.34–0.55)</td>
<td>0.52 (0.41–0.79)</td>
<td>0.10</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.65 (0.51–0.95)</td>
<td>0.65 (0.58–0.77)</td>
<td>0.89</td>
</tr>
<tr>
<td>Apolipoprotein A1 (g/L)</td>
<td>1.4 ± 0.17</td>
<td>1.39 ± 0.22</td>
<td>0.81</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>0.9 (0.81–1.01)</td>
<td>0.95 (0.81–1.05)</td>
<td>0.97</td>
</tr>
<tr>
<td>Lipoprotein(a) (mg/dL)</td>
<td>12.85 (6.4–16.45)</td>
<td>7.65 (6.7–11.85)</td>
<td>0.06</td>
</tr>
<tr>
<td>Interleukin-6 (pg/mL)</td>
<td>1.56 (0.8–2.92)</td>
<td>1.73 (1.0–2.44)</td>
<td>0.83</td>
</tr>
<tr>
<td>Sensitive C-reactive protein (mg/L)</td>
<td>0.59 (0.33–1.63)</td>
<td>0.51 (0.25–1.18)</td>
<td>0.54</td>
</tr>
<tr>
<td>von Willebrand factor (IU/mL)</td>
<td>87.5 (68–101.5)</td>
<td>81 (70.3–104)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

fB=fasting blood, S=serum, fS=fasting serum, IU=international units
Univariate analyses, with insulin SI as dependent variable, were performed separately in the previously pre-eclamptic group and in the control group. In the control group only BMI was associated significantly to SI. In the pre-eclamptic group several features of metabolic syndrome were associated with insulin sensitivity; weight (first trimester BMI (p = 0.008), BMI at the time of the study (p = 0.01) and WHR at the time of the study (p = 0.0001), BP (diastolic and mean BP, p = 0.004 and p = 0.02, respectively), lipids (total cholesterol (p = 0.02), triglycerides (p = 0.0001) and APOB (p = 0.002) and inflammation (CRP, p = 0.009). Further, SI was associated with variables of androgen status; negatively with free testosterone (p = 0.006) and positively with sex hormone-binding globulin (SHBG) (p = 0.048). When SI was correlated to time of onset of the disease i.e. onset of hypertension and proteinuria, there was a positive correlation to both, p = 0.02 and p = 0.02, respectively and to the birth weight of the child. Maximum diastolic blood pressure during pregnancy had a significant negative association with later SI (p = 0.005).

The association between insulin sensitivity and vasodilatation was tested in both vasodilators and concentrations. A positive correlation was seen with both concentrations of ACh and with the high concentration of SNP, but only in the patient group (Figures 5 and 6). This association was independent of WHR, CRP or lipids, with the exception of the high concentration of ACh in multiple regression analysis.

Figure 5. Correlation of insulin sensitivity to increase (%) in forearm blood flow in response to the endothelium-independent vasodilator SNP; low concentration (3µg/ml) (upper) and high concentration (10µg/ml) (lower). (p. 59)

Figure 6. Correlation of insulin sensitivity to increase (%) in forearm blood flow in response to the endothelium-independent vasodilator ACh; low concentration (7.5µg/ml) (upper) and high concentration (15µg/ml) (lower). (p.60)
Increase (%) in forearm blood flow (log)

Insulin Sensitivity Index SI (log)

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>b</th>
<th>r²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>b = 0.58</td>
<td></td>
<td>0.12</td>
<td>0.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control Group</th>
<th>b</th>
<th>r²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>b = -0.28</td>
<td></td>
<td>0.05</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Increase (%) in forearm blood flow (log)

Insulin Sensitivity Index SI (log)

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>b</th>
<th>r²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>b = 0.0007</td>
<td></td>
<td>0.26</td>
<td>0.006</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control Group</th>
<th>b</th>
<th>r²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>b = 0.0001</td>
<td></td>
<td>0.03</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Increase (%) in forearm blood flow (log)

Insulin Sensitivity Index SI (log)

Patient group: $b = 0.59$, $r^2 = 0.15$, $p = 0.04$

Control group: $b = -0.13$, $r^2 = 0.01$, $p = 0.65$
Variables of the highest explanatory value ($r^2$) in univariate analysis were chosen for multivariate analysis: WHR ($r^2 = 0.45$), triglycerides ($r^2 = 0.44$) and diastolic BP during the study ($r^2 = 0.28$). This model explained 60% of the variation in insulin sensitivity in the patient group; WHR and serum triglycerides being significant. In the whole group, when triglycerides, WHR, diastolic BP, CRP and vasodilatation after higher dose of SNP were tested in multivariate analysis, the explanatory strength was 51% and SI was associated significantly only with diastolic BP.

5.6. Sympathetic activity and vascular dilatory function after pre-eclamptic pregnancy (IV)

The demographic data was similar in the previously pre-eclamptic and control groups with the exception of increased blood pressure, as mentioned above. Furthermore, there were no differences between groups in self-reported amounts of physical exercise per week, volume of the forearm or central pressures.

Venous plasma levels of NA were higher in the pre-eclamptic group in both the supine position ($p = 0.016$) and after standing for 5 minutes ($p = 0.006$) while the increase in NA levels with an upright posture (ANOVA factor position, $p=0.0001$) was similar in the two groups (ANOVA, group*position interaction, $p = 0.6$) (Figure 7). Plasma levels of A were similar in two studied groups in both supine and upright positions ($p = 0.4$ and $p = 0.6$, respectively), increasing significantly with upright posture (ANOVA factor position, $p = 0.002$) and this change was similar in the two groups (ANOVA group*position interaction, $p = 0.2$).
Figure 7. Levels of noradrenaline and adrenaline in the patient and control groups in the orthostatic test, after rest, in a supine position and at 5 minutes after standing up. The values are presented as mean ± SE. * p < 0.05 and ** p < 0.01.

In the whole group, resting levels of plasma NA correlated positively and significantly with the increase in systolic BP during the orthostatic test (r = 0.28, p = 0.048,) and in the two groups separately an association was noted in the previously pre-eclamptic group but not in the controls (r = 0.36, p = 0.06, vs. r = 0.15, p = 0.5, respectively). There was no significant association between change in NA levels and parallel changes
in systolic arterial pressure during the orthostatic test (data not shown). Resting, supine NA levels in the patient group had a significant positive association with vasodilatation induced by the higher dose of SNP (10µg/min), but not with BP, heart rate, body composition or indices of carbohydrate metabolism.

Blood pressures measured sitting and supine before plethysmography, supine after plethysmography at the beginning of the orthostatic test, and after being in an upright position for 5 minutes were all increased in the patient group versus the controls (Figure 8).

**Figure 8.** Systolic and diastolic blood pressures in the previously pre-eclamptic women and in the control group. Values are presented as mean ± SE. * p < 0.05, ** p < 0.01.

In the 24-hour BP measurements the peak systolic and diastolic BP values were significantly higher during the whole registered time period in the patient group, whilst the overall difference in mean BP levels was small and did not reach significance (Figure 9). There were no statistically significant associations between plasma catecholamine concentrations or the levels of ET-1 versus BP in the 24-hour arterial pressure recordings.
Levels of ET-1 were similar in the two groups at rest ($p = 0.18$) and in an upright posture ($p = 0.5$). There was a positive correlation between supine and upright values ($p < 0.0001$) and the change to an upright posture was similar in the two groups ($p = 0.9$). In the resting state ET-1 levels did not correlate significantly with BP measurements or AIx. Levels of ET-1 in the upright position, for the two groups combined, were associated positively and significantly with diastolic BP ($r = 0.3$, $p = 0.04$), but not with systolic BP ($r = 0.26$, $p = 0.08$) or AIx ($r = 0.27$, $p = 0.07$). When the groups were analysed separately, a significant positive associations between ET-1 levels and diastolic BP ($r = 0.42$, $p = 0.026$), systolic BP ($r = 0.39$, $p = 0.04$) and AIx ($r = 0.42$, $p = 0.02$) were seen in the patient group only, the difference being significant between the groups as regards systolic ($p = 0.04$) and diastolic BP ($p = 0.05$), but not AIx ($p = 0.19$). There were no significant correlations between ET-1 levels in an upright position and vasodilatation or levels of NA.
6. DISCUSSION

Impairment in vasodilatation is regarded as a pathophysiological feature of atherosclerosis and it also seems to characterize pre-eclampsia.\textsuperscript{220-222, 267} These two diseases share several of their risk factors and previous pre-eclampsia has been shown to increase the risk of cardiovascular disease in later life.\textsuperscript{9, 261} The present study was undertaken to see if vascular dilatory function is impaired in women with a history of pre-eclampsia 5–6 years earlier and if the known cardiovascular risk factors would be present and/or be associated with vasodilatation. There were several findings.

First, according to our results, both endothelium-independent and endothelium-dependent vasodilatations were impaired 5–6 years after pre-eclamptic pregnancy. The finding of equally impaired endothelium-independent vasodilatation was not expected. Most of the studies on vascular dilatation, carried out with various methods from 3 months to 25 years after index pregnancy suggest alterations in the NO-mediated vasodilatory pathway.\textsuperscript{244-246} Nevertheless, there is evidence of impaired endothelium-independent vasodilatation characterizing subjects with cardiovascular risk factors and women with previous PE.\textsuperscript{51, 248} One year after pre-eclampsia, endothelium independent vasodilatation was shown to be impaired in conduit arteries accompanied by increased daytime blood pressure. Vasodilatation associated negatively with systolic blood pressure during the index pregnancy but was not studied as regards present blood pressure.\textsuperscript{248} In hypercholesterolaemia impairment was found in metacholine- and SNP-mediated vasodilatation in resistance arteries, but not when stimulated with phenylephrine.\textsuperscript{51} This finding suggests alterations in guanylate cyclase – cGMP mediated intercellular mechanisms, but not in perivascular innervation and related Ca\textsuperscript{2+} increase. Our finding is in agreement with these studies; impaired SNP-and endothelium-mediated NO-releasing mechanisms suggest altered cGMP-mediated vasodilatation. Additionally, impaired endothelium-independent vasodilatation has been shown to be associated with traditional risk factors of atherosclerosis and endothelium-dependent vasodilatation in a cohort of asymptomatic subjects, in other words early in the pathogenesis of atherosclerosis.\textsuperscript{268} Our finding therefore strengthens the suggestion that changes in the vessel wall may not be limited to the endothelium; reduction in vasodilatation in response to both endothelium-dependent and endothelium-independent vasodilatation may be mediated by changes in VSMC and be related to the increased risk of CVD in women with previous pre-eclampsia.
Secondly, the impaired vasodilatation related to a history of PE did not seem to depend on age, body composition, actions of vasoconstrictive agents such as catecholamines or ET-1 or the increased BP characterizing these women. Interestingly, regardless of similar insulin sensitivity index values and comparable, non-obese levels of BMI and WHR, insulin sensitivity was associated positively with vasodilatation only in the patient group. This effect could be related to features of metabolic syndrome; these were shown to be associated with insulin sensitivity, and furthermore, 60% of the insulin sensitivity was explained by WHR and triglycerides, but only in the patient group. Since the association between insulin sensitivity and SNP-and low concentration of ACh-mediated vasodilatation was independent of WHR, CRP and triglycerides, we cannot rule out factors other than those associated with metabolic syndrome, playing a role in this process. Additionally, since the length of gestation at the onset of pre-eclamptic proteinuria and hypertension and the birth weight of the child were positively associated with later insulin sensitivity and the risk of CVD is additionally increased in women with early-onset PE, it could be assumed that reduced insulin sensitivity might play a role in the pathogenesis of CVD in such cases.8, 261

The third observation was that vascular dilatation could not be predicted by the degree of proteinuria during PE. Despite the strong association between later risk of CVD and early-onset PE, the latter characterized often by higher proteinuria, vascular dilatation 5–6 years after pregnancy was not affected by the amount of protein excreted in urine per 24 hours during the disease. This indicates that the severe proteinuria during pre-eclampsia would be a phenomenon associated with the disease and merely an indicator of the early-onset pattern of the disease, with no predictive medical value as regards the risk of later CVD risk, at least 5–6 years after PE. However, even though 24-hour sampling of urine is the golden standard in the assessment of proteinuria during pregnancy, some observations should be made.240, 269, 270 Twenty four-hour measurement of urinary protein in seriously ill pre-eclamptic patients is not always possible, the sampling might be interrupted and not always started for clinical reasons and this may lead to a reduced representative value of 24 hour sampling in research. Additionally, the division of proteinuria into mild and severe using the value of 5g/day is somewhat artificial, having no particular association with the clinical picture of PE. However, 24-hour sampling is still the basis for diagnosis of PE and proteinuria of ≥5g/day is the defined amount as regards severity; these criteria were used to create a
well defined group of women.

Fourth, renal function in the study group was similar to that in the controls. The degree of proteinuria during PE, despite the massive amounts in some cases, did not have an effect on renal function tests in the patient group. Our findings are in accordance with those of a study showing no evidence of residual renal dysfunction 3 months after delivery in cases of PE. However, these findings are somewhat different compared with those of other studies. Tests on renal haemodynamics have shown alterations >5 months after PE, but only when hypertension was present. There is also evidence of increased levels of microalbuminuria 3–7 years after pre-eclamptic pregnancy, but again with parallel hypertension supposedly affecting renal excretion. In these two studies the results of renal function tests were otherwise normal. Furthermore, the results of a recent epidemiological study showed an increased risk of end-stage renal disease in women with previous PE. The absence of renal findings in our study could be related to the absence of hypertension in general in our patient group and the possibility in small studies of excluding superimposed pre-eclamptic cases from the material. However, we cannot exclude the possibility that the levels of BP, which were slightly increased, or the same factors that predispose women to pre-eclampsia and cardiovascular disease, could have an effect on renal function, but only later on.

The fifth finding was repeatedly higher BP levels in the patient group than in the controls. Previous investigators have suspected or shown hypertension to be the first cardiovascular event to characterize women after pre-eclamptic pregnancy. Despite the close relationship between BP and vasodilatation, BP could not statistically explain the whole difference in vasodilatation between the groups. Furthermore, central wave reflection was similar in the two groups and not correlated to vasodilatation or BP. These negative findings could be related to the size of the study group. Secondly, the lack of association could be dependent on increased, but not hypertensive, levels of BP in previously pre-eclamptic women, also shown in 24-hour BP measurement. Thirdly, not all studies have confirmed an association between BP and impaired vasodilatation. The negative association between BP and insulin sensitivity in the patient group, absent in the control group, could be related to changes associated with metabolic syndrome, to some degree at least.

Sixth, circulating plasma levels of NA were higher in the patient group than in
the control group, suggesting heightened sympathetic activity, possibly maintained after index pregnancy. It is possible that sympathetic over-activity could contribute to the increased risk of CVD via increased arterial pressure and/or associated metabolic changes, in particular, closely inter-related obesity, insulin resistance and dyslipidaemia. Since insulin sensitivity and WHR were negatively correlated only in our patient group, it could be hypothesized that increasing WHR, together with increased sympathetic activity, could increase the risk of insulin resistance. This would be in accordance with the results of follow-up studies showing sympathetic activity to precede hyperinsulinaemia and elevated arterial pressure. Increased sympathetic activity could also be associated with or caused by decreased plasma volume, which has been demonstrated in women with previous pre-eclamptic pregnancy and chronic hypertension, but which was not measured in our study.

Despite the lack of robust correlation between catecholamine concentrations versus arterial pressure and heart rate, we cannot definitely rule out an effect in our cohort. We observed a significant positive correlation between resting NA plasma levels and a rise in systolic BP when the two groups were combined. Furthermore, sympathetic outflow has a differentiated pattern among organs as regards time and intensity; enhanced in one organ while not so in another, which may affect findings concerning arterial pressure and plasma NA levels. The measured levels of NA seemed not to have an influence on impaired vasodilatation. This finding is in accordance with the results of the study by Fischer et al. which showed increased MSNA that was not in correlation with peripheral vasoconstriction or the level of BP during pre-eclampsia. In addition, impaired vasodilatation was not explained by local forearm NE spillover, even if the total NE spillover and venous and arterial NA levels were higher in cases of borderline hypertension when compared with controls. Finally, in the present study, ET-1 levels were similar in the tested groups. This was not unexpected, since plasma ET-1 levels seem to be similar even in hypertensive and normal subjects. However, the positive association between ET-1 and arterial pressure only in the patient group can be speculated upon; it could be associated with altered ET$_{A}$-mediated vasodilatation, increased sensitivity/production of ET-1 in the vasculature or alternatively, an altered relationship with the renin-angiotensin-system, as is considered to occur in hypertension.

Increased parity in pre-eclamptic pregnancy was an independent determinant of
decreased vasodilatation in later life. Given that Jonsdottir et al have shown women with increased parity in pre-eclamptic pregnancy to be later at an increased risk of death from ischaemic heart disease, our result can be regarded as indicative of impaired vasodilatation being related to an increased risk of CVD in later life. However, the results of a meta-analysis did not support the effect of parity as regards the risk of CVD.

Finally, our study material consisted of a well defined, relatively homogeneous but small group of women. Since calculation as regards the power of the study concerned vasodilatation, comparisons and associations with other variables are somewhat limited by the number of subjects and this needs to be taken into consideration when drawing conclusions. Nevertheless, the observations made are suggestive of possible pathophysiological mechanisms between PE and CVD risk and these can later be assessed in larger studies. It needs to be kept in mind, however, that the nature of the study was time-consuming (2 days for each woman), relatively invasive and needed considerable cooperation from the recruited women, who received no financial reward. The methods chosen for the study are generally well defined and accepted; venous occlusion plethysmography is regarded as the gold standard by which to measure vascular dilatation in resistance arteries and the results of minimal model FSIVGTs correlate well with those of the clamp technique. Compromises were inevitable regarding measurements of sympathetic activity, keeping in mind the already heavy study protocol.
7. SUMMARY AND CONCLUSIONS

Pre-eclampsia and cardiovascular disease can affect a woman’s health and life tremendously; the former at fertile age and the latter in the postmenopausal period. These two disease entities seem at first to be far from each other, but in fact they share several risk factors, are characterized by similar findings and finally, have been shown to be connected; a history of pre-eclampsia increases the risk of cardiovascular disease.

The present study shows that clinically healthy women with a history of pre-eclampsia are indeed characterized by impaired ability of resistance arteries to dilate in response to two vasodilators. Our finding is indicative of altered functions in the vascular wall and suggestive of changes at the vascular smooth muscle cell level. According to our findings this change could be associated with insulin sensitivity and related features of metabolic syndrome, especially waist-hip-ratio. The presence of increased arterial pressure in combination with enhanced sympathetic activity in women with previous pre-eclampsia could mean that these two factors act synergistically with insulin resistance. These observations of subclinical changes in women with a history of pre-eclampsia may be related to their well known risk of cardiovascular disease in later life.

These women should be informed about their potentially harmful cardiovascular risk profile. Several of the cardiovascular risk factors are in fact modifiable and these women should be given the possibility to minimize their cardiovascular risks through their own actions. It is of great importance for the medical profession to include obstetric history in cardiovascular risk assessment in women.
Raskausmyrkytys, preeklampsia, on raskausajan komplikaatio, johon sairastuu noin 5% raskaana olevista naisista. Taudin aiheuttaja ei vielä tiedetä, mutta sen syntyyn vaikuttanevat genettiset syyt, immunologiset tekijät sekä äidin terveydentila raskauden alkaessa. Taudille tyypillisä piirteitä ovat kohonnut verenpaine, valkuiaisineiden erittyminen virtsaan sekä aineenvaihdon alla sekaisin muutokset; insuliinherkkyyden vähentyminen kudoksissa, veren rasvojen muutokset sekä veren hyytymismekanismin aktivoituminen. Tavallisimmassa muodossaan raskausmyrkytys on lievä ja esiintyy loppuraskaudessa. Tauti voi kuitenkin alkaa jo keskiraskauden aikana ja johtaa tällöin useammin vakaviin verenpaineen hallintavaikeuksiin, veren hyytymishäiriöihin sekä maksan ja pahimmillaan keskusherrmoston säätelyhäiriöihin. Aikaiseen taudin alkanemiseen usein liittyvät toiminnottyörentäminen häiriö saattaa johtaa sikiön kasvun hidastumiseen ja heikentoikkeisiin stressin sietokykyyn. Koska taudin tämähoito on synnytys, johtaa aikainen raskausmyrkytys usein ennen kännykkä naisen sydän- ja verisuonitautien syntyyn. Äidin taudin vaikeusasteeseen ja ennenkaikuisuuteen liittyen, raskausmyrkytys onkin suurin äidin sekä vastasyntyneiden sairastavuuden ja kuolleisuuden syvyinä syinä länsimaissa.

Raskausmyrkytyksen sairastaneisuus lisää naisen sydän- ja verisuonitautien kuolleisuusriskein noin kaksinkertaiseksi. Emme kuitenkaan tiedä, miksi näin on ja mitkä patofysiologiset mekanismit siihen liittyvät. Tiedämme, että aterioskleroosilla ja raskausmyrkytyksellä on monia yhteisiä riskitekijöitä ja raskausmyrkytyksen kliininen kuva muistuttaa metabolista oireyhtymää, joka on sydän- ja verisuonitautien yksi tärkeimmistä altistavista tekijöistä. Lisäksi, verisuonen sisäpinnan, endoteelin, toiminnan häiriöt, jotka ovat keskeinen osa aterioskleroosin kehittymisessä, ovat nykykäsitteen mukaan myös raskausmyrkytyksen kliinisten oireiden taustalla.

Tutkimuksessamme selvitimme, onko yksi verisuonen tärkeimmistä tehtävistä, sen laajenemiskyky, heikentynyt naisilla, jotka ovat sairastaneet raskausmyrkytyksen 5–6 vuotta aiemmin verrattuna normaaliraskauden läpi tämä ala naisiin. Lisäksi tutkimme sydän- ja verisuonitautiriskitekijöitä naisilla ja niiden yhteyttä verisuonen laajenemiskykyyn. Tavoitteenamme oli hankkia tietoa niistä aterioskleroosin riskitekijöistä ja mekanismeista, jotka liittyvät raskausmyrkytyksen jälkeiseen aikaan ja mahdollistaa yksityiskohtaisempia naisen sydän- ja verisuonitautien preventio.

Tulostemme mukaan raskausmyrkytyksen sairastaneet naiset ovat klinisesti terveitä.

8. YLEISTIETEELLINEN LYHENNELMÄ SUOMEKSI
5–6 vuotta raskausmyrkytyksen jälkeen. Tästä huolimatta, heidän verisuontensa laajemiskyky on heikentynyt verrattuna normaalin raskauden läpikäyneisiin naisiin. Raskauden aikaisella proteiinin erittymisen määrällä virtsaan ei ollut ennustavaa vaikutusta suomen laajenemisen heikentymiseen ja munuaisten toiminta oli normaalia. Insuliiniherkkyyys oli samanlainen testatuissa ryhmissä, mutta se oli vastaavuussuhteessa suonien laajenemiskykyn ja metabolisen oireyhtymän piirteisiin vain potilasryhmässä. Lisäksi, raskausmyrkytyksen sairastaneilla naisilla oli korkeampi verenpaine, vaikkakin vielä normaalin verenpaineen rajoissa, ja heillä todettiin merkkejä lisääntyneestä sympaattisen hermoston toiminnasta.

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