

Department of Obstetrics and Gynaecology  
Helsinki University Hospital  
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# **Prediction and Prevention of Pre-Eclampsia**

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## **ACADEMIC DISSERTATION**

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*To my beloved ones  
Pilvi, Otso and Elmo*

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## ABSTRACT

Pre-eclampsia is a pregnancy-specific disorder affecting 2-8% of all pregnancies. Every year approximately 70 000 women die due to pre-eclampsia and its complications. It accounts for nearly one fifth of all maternal deaths. The only treatment for pre-eclampsia is delivery, which may lead to a premature birth. The aim of this study was to find ways to predict pre-eclampsia and test the performance of low dose aspirin in the prevention of pre-eclampsia in women at high risk.

This thesis includes data from two study cohorts. Participants of Study I comprise 21 nulliparous, pre-eclamptic women and 11 normotensive, non-proteinuric women recruited between January 1996 to February 1998 in the maternity clinics, the prenatal clinic and the antenatal ward of Helsinki University Central Hospital. The participants of Studies II-V consist of 947 pregnant women with and 117 without known risk factors for pre-eclampsia who were included in the PREDO project between September 2005 and December 2009 from 12<sup>+0</sup> to 13<sup>+6</sup> weeks of gestation in 1 of the 10 participating hospital maternity clinics.

To study the role of different combinations of risk factors in predicting pre-eclampsia, we studied 903 women using cluster analysis (Study IV). Of these, 86 (9.5%) developed pre-eclampsia, 10 (11.6%) had early-onset disease and 36 (41.9%) had severe disease. Women who have had pre-eclampsia, or small for gestational age (SGA) newborn in an earlier pregnancy, or who have chronic hypertension (CHT) or type 1 diabetes mellitus were at highest risk of early-onset pre-eclampsia, severe pre-eclampsia and preterm pre-eclampsia. Pre-eclampsia in a previous pregnancy and a body mass index over 30 kg/m<sup>2</sup> were the most important risk factors for term pre-eclampsia. Early-onset and late-onset pre-eclampsia had different risk profiles. Moreover, the risk of pre-eclampsia increased exponentially with respect to the number of risk factors.

To assess the role of vasoactive agents in the prediction of pre-eclampsia, we studied placental growth factor (PlGF) and soluble vascular endothelial growth factor receptor-1 (VEGF-1 or sFlt-1) and their ratio, at 12-14, 18-20 and 26-28 weeks of gestation in a total of 53 women at high risk for pre-eclampsia and healthy controls (Study III). Of these, 27 developed pre-eclampsia: 6 early-onset and 21 late-onset forms of the disease. By the second measurement, differences in PlGF concentrations, and by the third measurement, differences

in sFlt-1 concentrations were discovered between those who developed early-onset pre-eclampsia and others. With a cut-off point of 40 the ratio of sFlt-1/PlGF identified all women who developed early-onset pre-eclampsia, with no false positives, 4 to 6 weeks before the clinical diagnosis.

To study the predisposing factors to superimposed pre-eclampsia in women with CHT (Study V) we assessed markers of placental and endothelial function, and maternal cardiac function, and renal tubular injury at all three timepoints in women with CHT (n=90) and healthy controls (n=90). Superimposed pre-eclampsia was diagnosed in women with CHT with new proteinuria over 0.3g per day after 20<sup>+0</sup> weeks of gestation. In women who subsequently developed superimposed pre-eclampsia, plasma syndecan-1 concentrations were lower at 26<sup>+0</sup>-27<sup>+6</sup> weeks (p=0.03) and plasma PlGF concentrations were lower (p=0.002) across gestation, compared to women with CHT without superimposed pre-eclampsia. Urine albumin creatine ratio (ACR) was elevated across gestation in women with CHT who subsequently developed superimposed pre-eclampsia compared with women with CHT only (p=0.007) or healthy controls (p=0.002). At the first timepoint receiver operator curve (ROC) value for ACR for prediction of pre-eclampsia was 0.87 (CI 0.73-1.0).

We investigated the concentrations of total and individual free fatty acids (FFA) in pre-eclamptic and normotensive pregnant women and the relationship between FFA concentrations and insulin sensitivity (Study I). Total FFA concentrations at baseline were 67% higher in pre-eclamptic than normotensive pregnancies (P = 0.0002). This difference was no longer significant after an oral glucose load. At the baseline, the differences between pre-eclamptic and normotensive pregnancies were largest in the concentrations of the oleic (75%), linoleic (129%) and arachidonic (315%) acids.

In the aspirin trial (Study II), we randomised 152 women with risk factors for pre-eclampsia and abnormal uterine artery flow upon Doppler velocimetry to low dose aspirin (100 mg per day) or placebo. We could not demonstrate any differences in the primary or secondary outcomes. Because the number of women identified as high risk by ultrasound was lower than expected, we concluded a meta-analysis of placebo-controlled aspirin trials with women whose uterine artery measurement indicated a high risk. According to the meta-analysis (3 trials, 346 women) low dose aspirin started at or before 16 weeks of gestation reduced the risk of pre-eclampsia (Risk ratio (RR) 0.6, 95% confidence interval (CI) 0.37-0.83), and severe pre-eclampsia (RR 0.3, 95%CI 0.11-0.69).

In conclusion, the risk of pre-eclampsia increased exponentially with respect to the number of risk factors. The sFlt-1/PlGF ratio identified women who develop early-onset pre-eclampsia weeks before the onset of clinical findings. The association between ACR in early pregnancy and superimposed pre-eclampsia is an evidence that pre-existing endothelial dysfunction in women with CHT may contribute to development of pre-eclampsia. It may be used in the prediction of pre-eclampsia in women with CHT already at the 12 weeks of gestation. Reduced syndecan-1 and PlGF antedate the development of pre-eclampsia in women with CHT, which implicate endothelial glycocalyx disturbance and reduced angiogenic capacity in the pathophysiology of superimposed pre-eclampsia. Women with established pre-eclampsia had higher FFA concentrations, which may influence several characteristics of pre-eclampsia, for example increased insulin resistance, endothelial cell dysfunction and altered production of vasoactive substances. According to the meta-analysis, low dose aspirin reduced the risk of pre-eclampsia and severe pre-eclampsia in women with abnormal uterine artery flow. Consequently, low dose aspirin at a dose of 100 mg per day should be recommended to initiate before the 16<sup>th</sup> week of gestation in high-risk women and continued until 35<sup>+0</sup> weeks of gestation.

## LIST OF ORIGINAL PUBLICATIONS

- I. Villa PM, Laivuori H, Kajantie E, Kaaja R. Free fatty acid profiles in pre-eclampsia. *Prostaglandins, Leukotriens and Essential Fatty Acids* 2009; 81:17-21.
- II. Villa PM, Kajantie E, Räikkönen K, Pesonen A-K, Hämäläinen E, Taipale P, Laivuori H. Aspirin in the prevention of pre-eclampsia in high-risk women: a randomised placebo-controlled PREDO Trial and a meta-analysis of randomised trials. *BJOG: An International Journal of Obstetrics and Gynaecology* 2013; 120:64-74.
- III. Villa PM, Hämäläinen E, Mäki A, Räikkönen K, Pesonen A-K, Taipale P, Kajantie E, Laivuori H. Vasoactive agents for the prediction of early- and late-onset pre-eclampsia in a high-risk cohort. *BMC Pregnancy and Childbirth* 2013; 13:110.
- IV. Villa PM, Marttinen P, Gillberg L, Lokki AI, Majander K, Taipale P, Pesonen A-K, Räikkönen K, Hämäläinen E, Kajantie E, Laivuori H. Cluster analysis to estimate the risk of pre-eclampsia in the high-risk Prediction and Prevention of Pre-eclampsia and Intrauterine Growth Restriction (PREDO) study. *PLoS One* 2017; 12(3).
- V. \*Bramham K, \*Villa PM, Laivuori H, Hämäläinen E, Kajantie E, Räikkönen K, Pesonen A-K, Seed P, Dalton RN, Turner C, Wong M, Von Dadlezen P, Roberts J, Poston L, Chappell L. Predisposition to superimposed pre-eclampsia in women with chronic hypertension: a role for endothelial and placental factors. Submitted manuscript. \*Equal contribution to the study and manuscript.

## ABBREVIATIONS

AIM	$\alpha_1$ -microglobulin
ACOG	American College of Obstetricians and Gynecologists
ACR	Albumin:creatinine ratio
ADMA	Asymmetric dimethylarginine
AFABP	Adipocyte fatty acid-binding protein
AHA	American Heart Association
ANOVA	Analysis of variance
Aspirin	Acetylsalicylic acid
AT <sub>1</sub>	Angiotensin-1
BAPS	The Bayesian Analysis of Population Structure
BMI	Body mass index
BNP	B-type natriuretic peptide
CHT	Chronic hypertension
CI	Confidence interval
CKD	Chronic kidney disease
CO	Carbon monoxide
COX-1 and COX-2	Cyclo-oxygenases
EFA	Essential fatty acids
eNOS	Endothelial nitric oxide synthase
FDR	False discovery rates
FFA	Free fatty acid
FGR	Fetal growth restriction
Flt-1	Fms-like tyrosine kinase
FMF	Fetal Medical Foundation
FPR	False positive rate
HbF	Fetal haemoglobin
hCG $\beta$	Beta subunit of human chorionic gonadotropin
HELLP syndrome	Haemolysis, elevated liver enzymes, low platelet count
HLA	Human leukocyte antigen
HO-1	Heme oxygenase-1
IL-8	Interleukin-8
IQR	Interquartile range
IU	International unit
IUGR	Intrauterine growth restriction
I <sup>2</sup>	Degree of inconsistency across studies in a meta-analysis
LCPUFA	Long-chain polyunsaturated fatty acids

LMWH	Low molecular weight heparin
MAP	Mean arterial pressure
MoM	Multiples of median
MSMS	Tandem mass spectrometric method
NAG	N-acetyl- $\beta$ -D-glucosaminidase
NGAL	Neutrophil gelatinase-associated lipocalin
NICE	The National Institute for Health and Care Excellence
NMR	Nuclear magnetic resonance method
NO	Nitric oxide
OR	Odds ratio
PAPP-A	Pregnancy-associated protein A
p-FABP <sub>pm</sub>	Placental membrane fatty acid binding protein
PGH <sub>2</sub>	Prostanoids type 2
PI	Pulsatility index
PIGF	Placental growth factor
PP-13	Placental protein 13
PREDO	Prediction and prevention of pre-eclampsia Study Cohort
RBP	Retinol binding protein
RI	Resistance index
ROC	Receiver operating curve
RR	Risk ratio
SD	Standard deviation
SDMA	Plasma symmetric dimethylarginine
sEng	Soluble endoglin
s-Flt1	Soluble Fms-like tyrosine kinase
SGA	Small for gestational age
TGF- $\beta$	Beta subunit of transforming growth factor
USPSTF	U.S. Preventive Services Task Force
VEGF	Vascular endothelial growth factor
VLBW	Very low birth weight
VLDL	Very low density lipoprotein
WHO	World Health Organization
20 <sup>+5</sup> weeks of gestation	20 weeks and 5 days of gestation

## INTRODUCTION

Pre-eclampsia affects 2-8% of all pregnancies worldwide (1). In Finland, 2.5% of babies are born from pre-eclamptic pregnancies, similar rates have been reported in other Nordic countries (2,3). Pre-eclampsia is a pregnancy-specific condition currently defined as blood pressure above 140 mmHg systolic and/or 90 mmHg diastolic and proteinuria over 0.3mg per day after 20<sup>+</sup> weeks of gestation (4) and may include a wide range of other symptoms and findings. Pre-eclampsia and its consequences are important causes of both maternal and newborn morbidity and mortality. The risks are not only restricted in the period of pregnancy, but long-term maternal risks after pre-eclampsia include chronic hypertension, stroke, and ischemic heart disease (5,6). Children and adults born from pre-eclamptic pregnancies have elevated risks of long-term cardiovascular (7,8) and mental health disorders and cognitive impairments (9,10).

Pre-eclampsia is a multi-systemic syndrome. The aetiology of pre-eclampsia is still largely unknown, but we know more regarding the pathogenesis. The defects in placentation, which later lead to clinical pre-eclampsia, already emerge in the early weeks of gestation. In pre-eclampsia, the remodelling of placental spiral arteries is incomplete. The cytotrophoblast invasion of decidua is shallow, limited in the superficial decidua and the myometrial segments of the spiral arteries remain narrow and high-resistance (11-13). This first stage in the pathogenesis of pre-eclampsia, poor placentation, is followed by the second stage involving endothelial damage. The symptoms of pre-eclampsia reflect this endothelial dysfunction, which is induced by antiangiogenic factors, systemic inflammation, immunologic factors, and hypoxia, which all contribute to the development of this heterogenous condition (13). The result is vasoconstriction, end-organ ischaemia and increased vascular permeability (14).

An imbalance of the angiogenic factors plays a crucial role in the pathogenesis of pre-eclampsia (15). Vascular endothelial growth factor (VEGF) is necessary for glomerular capillary repair and in maintaining the health of the endothelium (13). Placental growth factor (PlGF) is a potent angiogenic growth factor and has a structural homology to VEGF-A. VEGF and PlGF have a common receptor, VEGF receptor-1, also called fms-like tyrosine kinase (Flt-1) on endothelial cells. Its soluble variant, sFlt-1, binds both PlGF and VEGF, and inhibits their binding to endothelial cell surface receptors. In pre-eclamptic women,

expression of sFlt-1 is upregulated (15) and an increase in serum sFlt-1 seems to precede clinical syndrome (16-18).

Metabolic characteristics of pre-eclampsia include reduced glucose utilisation, hyperinsulinemia and hyperlipidemia (19,20) and many other known cardiovascular risk factors (21-24). High plasma lipids may contribute to the development of pre-eclampsia by increasing vascular dysfunction in uteroplacental circulation by advancing lipid changes in the walls of already poorly transformed spiral arteries (25).

Superimposed pre-eclampsia is diagnosed in a pregnant woman with chronic hypertension (CHT) when she develops new-onset proteinuria over 0.3g per day after 20<sup>+0</sup> weeks of gestation. Approximately 26% of women with CHT develop superimposed pre-eclampsia (26). Endothelial dysfunction and abnormalities in cardiac and renal function are proposed to contribute to the development of superimposed pre-eclampsia in women with CHT.

A great effort regarding finding tools to predict pre-eclampsia before the clinical onset of the disease has been made. Demographic factors, biochemical markers, or biophysical findings may offer clinical tools in the prediction. An ideal biochemical marker plays a central role in pathogenesis, is present early on or before the clinical manifestations, is easy and inexpensive to test for, shows a high sensitivity and specificity for the disease, correlates with the severity of the disease, and is non-detectable or present at low levels in normal pregnancies (27).

There is strong evidence that low dose aspirin would prevent pre-eclampsia. Aspirin's preventive function is thought to be due to its favourable effect on prostaglandin production, particularly the prostacyclin-thromboxane ratio, which is in imbalance in pre-eclamptic women before the clinical disease. Other possible routes of the action of low dose aspirin are recently introduced, as well.

# REVIEW OF LITERATURE

## Background

Pre-eclampsia is a pregnancy-specific disorder, affecting 2-8% of all pregnancies (1). It is one of the greatest challenges of obstetrics causing nearly one-fifth of all maternal deaths (28,29) and increasing the risk of adverse outcomes. Pre-eclampsia is a placental disease and symptoms arise from endothelial dysfunction. It is characterised by increased blood pressure and proteinuria, and a broad spectrum of other symptoms. These include headache, visual disturbances, oedema, upper abdominal pain, nausea, and may lead to serious complications, and even death of the mother or fetus. Placental dysfunction may affect fetal growth and wellbeing. At one end of the spectrum is pre-eclampsia with mild symptoms in a term pregnancy, and at the other end is rapidly progressing early-onset pre-eclampsia with severe, even life threatening, symptoms. The only treatment of pre-eclampsia is delivery, which may lead to a very premature birth.

Symptoms typical in pre-eclampsia were mentioned as early as in ancient Greek sources originating from the time before Hippocrates (30). The Coan Prognosis depicted: “In pregnancy, the drowsy headaches with heaviness is bad; such cases are perhaps liable to some sort of fits at the same time” (31). For thousands of years, the disease was known as a convulsive disease of pregnancy and different theories explaining the reasons changed over times (30). In a symposium on eclampsia, held in Giessen in 1901, it was agreed upon that the disease was caused by a toxin. This was followed by several hypotheses on the source of the toxin. The theory was later refuted. However, today we still use the word toxæmia when referring to pre-eclampsia.

## Definitions

Definitions of pre-eclampsia have varied throughout the times. Improved understanding of the pathogenesis of pre-eclampsia has influenced the definitions. Pre-eclampsia is a pregnancy specific condition diagnosed when blood pressure is elevated above 140 mmHg systolic and/or 90 mmHg diastolic and proteinuria over 0.3 mg per day after 20<sup>+0</sup> weeks of gestation (4). Severe pre-eclampsia is diagnosed when blood pressure exceeds 160 mmHg systolic and/or 110 mmHg diastolic or proteinuria is over 5 g per day. Superimposed pre-eclampsia is diagnosed when a pregnant woman with CHT has new-onset proteinuria over 0.3 mg per day after 20<sup>+0</sup> weeks of gestation. When blood pressure is elevated after 20<sup>+0</sup> weeks of gestation without proteinuria, the condition is defined as gestational hypertension. Pre-eclampsia is

commonly divided into early- and late-onset disease and preterm and term subtypes based on the time of delivery or time of the diagnosis (Table 1).

**Table 1. Definitions of pre-eclampsia**

<b>Pre-eclampsia</b>	Systolic blood pressure $\geq 140$ mmHg or diastolic blood pressure $\geq 90$ mmHg after 20 <sup>+0</sup> weeks of gestation and proteinuria $\geq 0,3$ g/day
<b>Early-onset</b>	Delivery (or diagnosis) before 34 <sup>+0</sup> weeks of gestation
<b>Late-onset</b>	Delivery at or after 34 <sup>+0</sup> weeks of gestation
<b>Preterm</b>	Delivery (or diagnosis) before 37 <sup>+0</sup> weeks of gestation
<b>Term</b>	Delivery at or after 37 <sup>+0</sup> weeks of gestation
<b>Severe</b>	Systolic blood pressure $\geq 160$ mmHg or diastolic blood pressure $\geq 110$ mmHg or proteinuria $\geq 5,0$ g/day
<b>Eclampsia</b>	New-onset grand mal seizures in a woman with pre-eclampsia
<b>Gestational hypertension</b>	Systolic blood pressure $\geq 140$ mmHg or diastolic blood pressure $\geq 90$ mmHg after the 20 <sup>th</sup> week of gestation without proteinuria
<b>Chronic hypertension</b>	Systolic blood pressure $\geq 140$ mmHg or diastolic blood pressure $\geq 90$ mmHg before 20 <sup>+0</sup> weeks of gestation without proteinuria
<b>Superimposed pre-eclampsia</b>	New proteinuria over 0.3 g/day after 20 <sup>+0</sup> weeks of gestation in a pregnant woman with chronic hypertension

Recently, pre-eclampsia definitions have been updated. The American College of Obstetricians and Gynecologists (ACOG) proposed, in the Task Force on Hypertension in gestation (32) recommendations that, in the absence of proteinuria, pre-eclampsia could be diagnosed when newly diagnosed hypertension occurs in association with thrombocytopenia, impaired liver function, new development of renal insufficiency, pulmonary oedema, or new-onset cerebral or visual disturbances. The Australasian Society for the Study of Hypertension and the Society of Obstetricians and Gynaecologists of Canada have adopted even broader definitions and include fetal features, such as fetal growth restriction, in the definitions. ACOG suggested that massive proteinuria (greater than 5g) should be removed from the definition of severe pre-eclampsia, and recommends that any one of the following findings indicate severe form of the disease: blood pressure according to the classical definition of

severe pre-eclampsia, thrombocytopenia, impaired liver function, progressive renal insufficiency, pulmonary oedema, new-onset cerebral or visual disturbances. The classic definition could also be updated by taking into account the current knowledge of the pathology of pre-eclampsia. Using vasoactive markers, PIGF and s-Flt1, pre-eclampsia can be divided into angiogenic and non-angiogenic subgroups (33). Early-onset and severe pre-eclampsia seem to predominantly form the angiogenic subtype while the non-angiogenic subtype has milder and later course of the disease (34). Pre-eclampsia can also develop in the postpartum period, including pre-eclampsia with severe systemic organ involvement and seizures (32,35). One-third of all eclamptic seizures occur in the post-partum period (35). These cases are called late postpartum pre-eclampsia and late postpartum eclampsia and the pathophysiology of these phenomena is unknown. The definitions based on pathophysiology are discussed more closely later in the paragraph on pathophysiology.

### **Public health burden**

Pre-eclampsia affects 2-8% of all pregnancies (1). In Finland, according to the combined data from the National Medical Birth Registry and Care Register of Health Care, at the National Institute for Health and Welfare of Finland in year 2013, 2.5% of babies were born from pre-eclamptic pregnancies, of which 24% were severe, and 8% early-onset subtypes of pre-eclampsia. Similar rates have been reported in other Nordic countries. In the Danish hospital discharge register 2.7% of the women who gave birth in Northern Sealand from 1998-2000 had pre-eclampsia (2) and in the Norwegian Medical Birth Registry data of 1.7 million births from years 1967 to 1992, the pre-eclampsia rate was 2.2% (3). While no validation studies have been performed for pre-eclampsia in the Finnish healthcare registers, studies of the Norwegian (36) and Danish (2) registries have been validated using medical records and in both studies the prevalence of pre-eclampsia in the study populations were relatively similar whether obtained from registry or whether based on medical records.

Pre-eclampsia and its consequences are important causes of both maternal and newborn morbidity and mortality. Every year an estimated 62,000 to 77,000 women worldwide die due to pre-eclampsia and its complications. That is almost 18% of all maternal deaths (28,29) most of these in developing countries. For comparison, during years 1972-2005, there were 117 direct maternal deaths (caused by a disease or its management during gestation) in Finland. The maternal mortality ratio was 5.6 per 100,000 (deaths per 100,000 live births) (37). During that time, there were 15 maternal deaths per about 2 million live births due to pre-eclampsia or its complications, 12.4% of all maternal deaths. The significance of organised maternity care is emphasised by the fact that between years 1926-1937 there were

93 maternal deaths per 100,000 births in the Women’s hospital in Helsinki and 264 in Vyborg hospital (38). Although maternal death is rare in developed countries, the burden of pre-eclampsia and its consequences on health care systems is enormous. Women with pre-eclampsia have a 3- to 25-fold increased risk of severe pregnancy complications (39). In developing countries, pre-eclampsia and its complications cause 25% of all stillbirths and neonatal deaths (40). In a Norwegian study of 21,000 pre-eclamptic pregnancies, the risk of fetal death was remarkably high for early-onset pre-eclampsia (41). At 26 weeks of gestation, the relative risk of fetal death was 86-fold higher, compared to pregnancies without pre-eclampsia. This risk declined as gestation advanced, however, though still more than 7-fold higher at 34 weeks of gestation.

### Complications

Maternal complications of pre-eclampsia include eclampsia, stroke, placental abruption, HELLP syndrome (hemolysis, elevated liver enzymes, low platelets), disseminated intravascular coagulation, liver haemorrhage or rupture, pulmonary oedema, adult respiratory syndrome, acute renal failure, and death (1,42) (Table 2).

**Table 2. Maternal and fetal complications of pre-eclampsia**

<b>Central nervous system</b>	Eclampsia (seizures), cerebral haemorrhage (stroke), cerebral oedema, cortical blindness, retinal blindness
<b>Renal system</b>	Renal cortical necrosis, renal tubular necrosis
<b>Respiratory system</b>	Pulmonary oedema, laryngeal oedema
<b>Liver</b>	Jaundice, HELLP syndrome*, hepatic rupture
<b>Coagulation system</b>	Disseminated intravascular coagulation
<b>Placenta</b>	Placental dysfunction, placental infarction, placental abruption
<b>Fetus</b>	Death, intrauterine growth restriction, preterm birth and their complications
* Haemolysis, elevated liver enzymes, and lowered platelets	

In general, pre-eclampsia increases the rates of cardiovascular, respiratory, central nervous system, renal, hepatic and other maternal morbidity, and women with early-onset pre-eclampsia have significantly higher rates of maternal morbidity than women with late-onset pre-eclampsia (43). Pre-eclampsia reduces the woman’s health-related quality of life and increases the risk of

postpartum depression (44-46). Perinatal consequences include stillbirth, preterm delivery, FGR, neonatal complications, and later sequelae (47). In Finland, 8% of all pre-eclamptic pregnancies were early-onset, meaning deliveries before 34<sup>+0</sup> weeks and almost a quarter were preterm, before 37<sup>+0</sup> weeks of gestation. Preterm birth in pre-eclampsia is usually due to iatrogenic labour, induced labour or caesarean section, because, at present, ending the pregnancy is the only treatment for pre-eclampsia. Preterm birth prolongs newborn hospitalisation and the earlier the delivery the more pervasive the problems. Complications associated with premature birth include respiratory distress, hypoglycaemia, jaundice, feeding difficulties, and more severe in very premature newborns; kernicterus, seizures, periventricular leucomalasia (48). Preterm delivery, as well as, pre-eclampsia itself, elevates the risk of bronchopulmonary dysplasia (49) and cerebral palsy (46,50).

### **Long term consequences of pre-eclampsia**

The long-term maternal risks after pre-eclampsia are chronic hypertension, stroke, and ischemic heart disease (5,6). Pre-eclampsia in any previous pregnancy is an independent risk-factor for coronary artery disease (51). Women who have had early-onset pre-eclampsia have an almost eight times higher risk of dying from ischemic heart disease than women without hypertensive pregnancy complications (52). Women with recurrent pre-eclampsia may have underlying pathological diseases, which increase their risk for hypertensive and cardiovascular disease (6). Genetic factors that increase the risk of cardiovascular disease may also be linked to pre-eclampsia (52). It has not been studied whether prevention of pre-eclampsia affects the risk of later cardiovascular complications. However, it is important for women to become aware of these risks, since, with lifestyle interventions, it is possible to influence them.

Children and adults born from pre-eclamptic pregnancies have increased risks of long-term cardiovascular and other diseases (7,8). For example, those born from hypertensive pregnancies between years 1934-1944 in Helsinki, had twice the risk of stroke (53) and four times the risk of depression than adults not born from hypertensive pregnancies (10). They had, as well, a lower cognitive ability and greater cognitive decline up to old age (9,54). Children born from pregnancies with gestational hypertension are at an increased risk of type 2 diabetes mellitus in adult life (55) and the association between low birth weight and cardiometabolic disease is well established (56-58). This association is mostly attributable to poor fetal growth, although preterm birth is also independently associated with adult cardiometabolic risk factors. Pre-eclampsia is likely to contribute in part to these associations. For example, a recent individual-participant meta-analysis on blood pressure in adults born preterm at very low birth-weight (VLBW) showed that this association is strongest among VLBW offspring exposed to maternal pre-eclampsia, but it is also present in VLBW offspring whose mothers did not have pre-eclampsia (59). Part of the risks

may reflect common genetic risk factors of pre-eclampsia and cardiovascular diseases, but part is thought to reflect fetal programming during pregnancy. The long-term risk attributable to fetal programming could in theory be reduced through prevention or improved management of pre-eclampsia, but this has not been studied.

## Aetiology and pathogenesis of pre-eclampsia

### Aetiology

Pre-eclampsia is a multi-systemic syndrome. Despite active research and knowledge of the risk factors, the cause of pre-eclampsia is still largely unknown. Different causal models may be speculated (60). In the mono-causality model one known exposure is both necessary and sufficient to cause disease. An example is a single gene with high penetrance. Multiple-causality refers to a model described in terms of sufficient and necessary causes (60,61). In that model, a cause of a disease is an event, condition or characteristics that precedes the disease and is necessary for the disease to occur. A sufficient cause refers to a complete causal mechanism. A disease can be caused by more than one causal mechanism, and recognised causes of the disease are neither necessary nor sufficient to produce the disease. The multiple-causality model can be applied to pre-eclampsia. However, the threshold liability model is suggested by Trogstad and colleagues (60) as the best fitting model on pre-eclampsia based on the current knowledge (Figure 1).

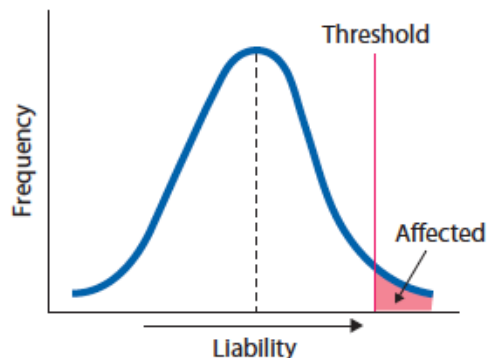


Figure 1. The liability model explaining the risk of pre-eclampsia. Genetic, pregnancy specific and environmental factors all add to the woman's basic liability to pre-eclampsia. Modified from Trogstad et al (60).

The model assumes that genetic action is additive, and that the phenotype reflects the summed effect of a number of genetic and environmental factors, each with small or moderate influence. In that model, maternal susceptibility genes set a liability to pre-eclampsia, which are influenced by fetal genes and environmental factors. Thus, the risk of pre-eclampsia may differ between pregnancies, depending on maternal compatibility with the fetus, change of maternal weight or other environmental or maternal factors that may be subject to change between pregnancies.

### **Pathogenesis**

Regarding the pathogenetic background of pre-eclampsia, we know slightly more than the syndrome's aetiology. The symptoms of pre-eclampsia reflect the maternal endothelial dysfunction, which is a consequence of a complicated cascade of events. The placenta is essential in the development of pre-eclampsia, and pre-eclampsia may develop without a fetus, for example in hydatiniform mole pregnancies (62). The defects in placentation, which later lead to clinical pre-eclampsia, already emerge in the early weeks of gestation. Between the 8<sup>th</sup> to 18<sup>th</sup> weeks of gestation, the cytotrophoblasts of fetal origin invade decidua and enter the lumina of the uterine spiral arteries. These invaded spiral arteries go through remodelling and lose their smooth muscle and become widely dilated, turning into large-caliber capacitance vessels capable of providing adequate placental perfusion (63-65). This remodelling is necessary for normal placental function. In pre-eclampsia, remodelling is incomplete. The cytotrophoblast invasion of the decidua is shallow, limited to the superficial decidua and the myometrial segments of the spiral arteries remain narrow and high-resistance (11-13). Poor placentation is the first stage in the two-stage model of the pathogenesis of pre-eclampsia. The contributors in early placental vascular development and trophoblast invasion may be hypoxia, perturbation of the renin-aldosterone-angiotensin II axis, excessive oxidative stress, inflammation, immune maladaptation, and genetic susceptibility. This first stage takes place in the first half of pregnancy without clinical findings of pre-eclampsia (66) and is followed by the second stage with endothelial damage. The endothelial dysfunction is induced by antiangiogenic factors, systemic inflammation, immunologic factors, and hypoxia, which all contribute to the development of this heterogenous condition (13). It is unknown whether these are interrelated, have synergistic effects or act independently (13). The result is vasoconstriction, end-organ ischaemia and increased vascular permeability (14).

The imbalance of angiogenic factors plays a crucial role in the pathogenesis of pre-eclampsia (15) VEGF is necessary for glomerular capillary repair and in maintaining the health of the endothelium (13) particularly in the kidney, liver, and brain (15) PlGF is a potent angiogenic

growth factor with structural homology to VEGF-A and is thought to amplify VEGF signalling (67,68) (Figure 2).

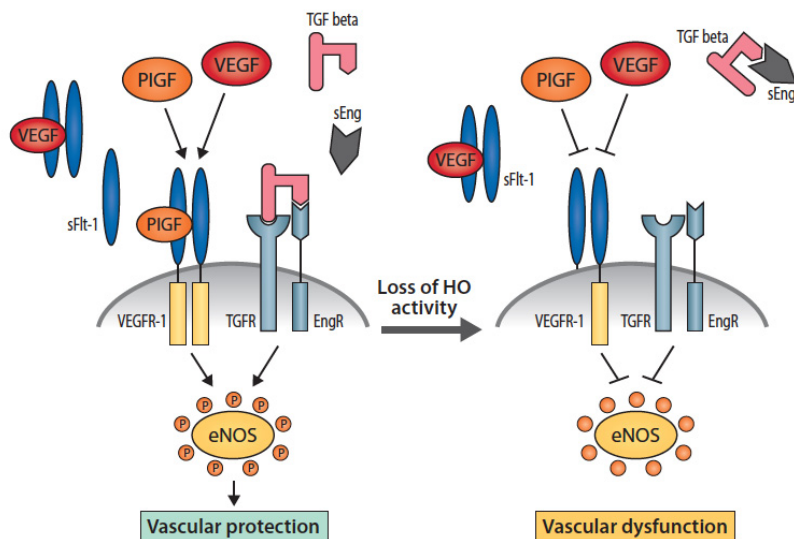


Figure 2. Vascular dysfunction in pre-eclampsia. The endothelial monolayer requires vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and transforming growth factor- $\beta$  (TGF- $\beta$ ) to function normally via activation of nitric oxide (NO). In pre-eclampsia, the VEGF protective signal is comprised due to an excess of soluble Flt-1 (sFlt-1), which is compounded by decrease in the expression of PlGF and a rise in circulating soluble endoglin (sEng), which binds and neutralizes TGF- $\beta$ . eNOS=endothelial nitric oxide synthase, HO=heme oxygenase, VEGFR=vascular endothelial growth factor receptor. Modified from Ahmed A (68).

PlGF is expressed early in pregnancy by the placenta. In men and non-pregnant individuals, low levels are produced by the heart, lung, thyroid, skeletal muscle, and adipose tissue (69). VEGF and PlGF have a common receptor, VEGF receptor-1, also called Flt-1 in endothelial cells. Its soluble variant, sFlt-1, binds to both PlGF and VEGF and inhibits their binding to endothelial cell surface receptors. In pre-eclamptic women, expression of sFlt-1 is upregulated in the placenta (15) and an increase in serum sFlt-1 seems to precede the clinical syndrome (16-18). Maynard and colleagues (15) first presented that sFlt-1 given to pregnant rats induced hypertension, proteinuria and glomerular endotheliosis (typical histological findings in pre-eclamptic kidneys). Excessive anti-angiogenic activity causes pre-eclampsia-like syndrome in non-pregnant experimental animals and non-pregnant humans being treated with VEGF antagonists for cancer (70). An experimental study of extracorporeal apheresis of sFlt-1 in early-onset pre-eclampsia has been conducted, and it

lowered sFlt-1 levels, reduced proteinuria and stabilised blood pressure without any adverse effects (71).

Another anti-angiogenic, placenta-derived factor, soluble endoglin (sEng), is upregulated in pre-eclampsia (68,72,73). Both sFlt-1 and sEng are released from syncytiotrophoblasts of the placenta. sEng binds and neutralises transforming growth factor (TGF- $\beta$ ), a proangiogenic and an anti-inflammatory factor (74) sEng acts synergistically with sFlt-1. In pre-eclamptic women, they contribute to the endothelial dysfunction and hypertension, proteinuria, glomerular endotheliosis, HELLP syndrome, and restriction of fetal growth (73). The molecular mechanisms that regulate their release are not thoroughly identified, except that cytokines and angiotensin-1 (AT<sub>1</sub>) receptor autoantibodies increase (75) and heme oxygenase-1 (HO-1) inhibits their release (76).

Recently, the HO-enzyme has garnered interest in pre-eclampsia research and may play an important role in the syndrome's pathogenesis. HO has antioxidant and anti-inflammatory effects, and tissue protective actions, which lead to the inhibition of atherogenic processes in the cardiovascular system. The HO enzyme exists in two forms, HO-1 and HO-2 (77). The products of HO are bilirubin and carbon monoxide (CO). Bilirubin has anti-complement and anti-oxidant effects. CO has anti-platelet (78) and vasodilatory properties, and it reduces perfusion pressure in the placenta. HO-1 is upregulated in states of hypoxia and ischaemia. HO-1 promotes angiogenesis (79) and increased expression of the HO-1 coding gene Hmox-1 inhibit the production of sFlt-1 and sEng (68). The expression (80) and activity of Hmox-1 are suppressed in the pre-eclamptic placenta.

Endothelin-1 is a 21 amino acid peptide secreted by vascular endothelial cells and placental syncytiotrophoblasts, and is the most potent vasoconstrictor known (81,82). It is elevated in pregnant women with pre-eclampsia, and some studies indicate that the level of circulating endothelin-1 correlates with symptom severity and plasma sFlt-1 levels (83,84). In pregnant rats, hypertension induced by placental ischaemia, chronic infusion of sFlt-1, tumour necrosis factor- $\alpha$ , or angiotensin II type 1 receptor can be completely attenuated by antagonism of the endothelin-1 receptor type-A found in vascular smooth muscle (83,84). Therefore, it is suggested that endothelin-1 could be the common pathway linking factors produced by placental ischemia to elevations of blood pressure and may be a future target for pharmacological interventions (81,82).

## **Defining pre-eclampsia on the basis of pathogenesis**

Since knowledge of the pathogenesis of pre-eclampsia has increased, it may become necessary and practical to update the definitions of the disease. Pre-eclampsia has been classified into early-onset and late-onset subtypes on the basis of the timing of the clinical findings. Division into placental and maternal pre-eclampsia has also been used. Both classifications express the same phenomenon of two extremities of this syndrome with differing in the onset of the pathophysiological changes and clinical findings, the severity of the disease, the presence or absence of placental dysfunction and FGR and differences in the predisposing clinical risk factors. These subgroups (early/late, or placental/maternal), are also different in genetic risk and inheritance (85).

Egbor and colleagues compared placental morphology in pregnancies complicated by early- and late-onset pre-eclampsia with controls (86). Placentas of early-onset pre-eclampsia differed significantly from those of late-onset pre-eclampsia by weight, volume of intervillous space, terminal villous volume and surface areas of the terminal villi (86). Placentas from late-onset pre-eclampsia were morphologically similar to placentas from gestational-age-matched controls, suggesting two subtypes of pre-eclampsia and supporting the hypothesis of predominantly maternal and placental disease.

Staff and colleagues have proposed redefining pre-eclampsia on the basis of placental contribution to the syndrome (33). They reasoned that current definitions of high blood pressure and proteinuria are clinical signs, which are tertiary, maternal features. Placental markers, like PIGF, reflecting placental pathology, and FGR may be more sensitive and specific diagnostically. In women with FGR, similar changes in the vasoactive markers as in placental pre-eclampsia are seen, reflecting poor placentation. It is unknown why poor placentation does not always stimulate hypertension and proteinuria, but only FGR. It is suggested that this reflects interaction of fetal and maternal predisposing factors (33).

## **Prediction of pre-eclampsia**

A great effort on finding tools to predict pre-eclampsia before the clinical onset of the disease has been committed. Demographic factors, biochemical markers, or biophysical findings may offer clinical tools in the prediction. An ideal biochemical marker plays a central role in a disease's pathogenesis, applies early or before the clinical manifestations, is easy and inexpensive to test for, shows a high sensitivity and specificity to the disease in question, correlates with the severity of the disease, and is not-detected or detected in only low levels in normal pregnancies (27). The pathogenesis of the pre-eclamptic placenta initiates in the first trimester and the process begins to emerge early. From a preventive point of view, to be able to influence this process it is important

to identify the at risk women in early gestation. However, it is unlikely that any single biomarker would perform well in the prediction of this multi-systemic syndrome involving variety of risk factors and heterogeneous aetiological and pathophysiological factors among women who go on to develop pre-eclampsia.

### Clinical risk factors

Clinical risk factors may be used in the prediction of pre-eclampsia. Duckitt and Harrington conducted a systemic review of 52 controlled studies to determine the risk of pre-eclampsia associated with factors that may be present at antenatal booking (Table 3) (85). They reported that the risk factors causing the highest risk for pre-eclampsia are a history of pre-eclampsia and the presence of antiphospholipid antibodies.

<b>Risk factors</b>	<b>Increased risk</b>
<b>Nulliparity</b>	3 fold <sup>a,b</sup>
<b>Pre-eclampsia</b>	7-8 fold <sup>a,b</sup>
<b>Multifetal pregnancy</b>	3 fold <sup>b</sup>
<b>Prior placental abruption</b>	2 fold <sup>b</sup>
<b>Prior stillbirth</b>	2 fold <sup>b</sup>
<b>Chronic hypertension</b>	5-10 fold <sup>a,b</sup>
<b>Obesity</b>	3 fold <sup>a,b</sup>
<b>Diabetes – insulin dependent</b>	4 fold <sup>a,b</sup>
<b>Maternal age over 40</b>	1.5 -2 fold <sup>a,b</sup>
<b>Antiphospholipid antibodies*</b>	3-10 fold <sup>a,b</sup>
<b>Systemic lupus erythematosus</b>	3 <sup>b</sup>
<b>Renal disease</b>	2-3 fold <sup>a,b</sup>
<b>Family history of pre-eclampsia</b>	3 fold <sup>a</sup>
<b>Use of assisted reproductive technology</b>	2 fold <sup>b</sup>
* anticardiolipin or lupus anticoagulant antibodies	
<sup>a</sup> Duckitt and Harrington 2005	
<sup>b</sup> Bartsch et al 2016	

Pre-eclampsia in the first pregnancy increased the risk of pre-eclampsia seven-fold in the second pregnancy (85). Recurrent pre-eclampsia and the presence of antiphospholipid antibodies (anticardiolipin or lupus anticoagulant or both) increased the risk of pre-eclampsia almost ten-fold. Pre-existing diabetes and a pre-pregnancy body mass index (BMI) of 35 kg/m<sup>2</sup> or over almost quadrupled the risk. Nulliparity, a family history of pre-eclampsia, and a

twin pregnancy almost tripled the risk. Neither the chorionicity nor the zygosity alters the risk in a twin pregnancy, however (87,88). Maternal age 40 years or over, BMI 30 kg/m<sup>2</sup> or over at booking, and a systolic blood pressure of 130mmHg or higher at booking doubled the risk. The meta-analysis by Bartsch and colleagues showed parallel findings (89). Duckitt and Harrington also reported an increased risk of pre-eclampsia with pre-existing renal disease, chronic autoimmune disease, and a period of ten years or more between pregnancies, but they were not able to specify the magnitude of the increase.

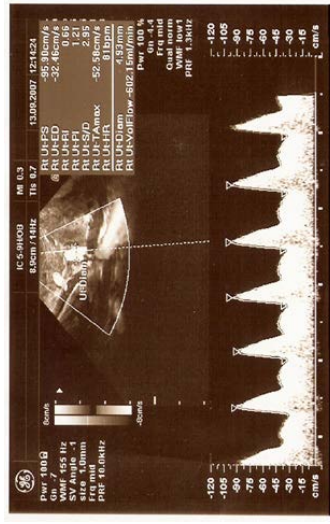
### **Uterine artery Doppler ultrasound measurements**

Uterine artery blood flow can be measured by Doppler ultrasound examination. In the non-pregnant uterus and in very early pregnancy, the uterine arteries have high flow resistance, with high systolic and low diastolic blood flow. A physiological early diastolic notch may be present. Resistance to blood flow gradually drops during gestation as a greater trophoblastic invasion takes place. An abnormally high resistance can persist in women who go on to develop pre-eclampsia and intrauterine growth restriction (IUGR). The early defects of placentation and the resulting abnormalities in uteroplacental blood flow characteristic of pre-eclampsia are reflected in the uterine arteries by an increased resistance to blood flow (90). This results in an abnormal waveform pattern that can be evaluated by Doppler ultrasound measurements (91,92) analysing the resistance or pulsatility indices (RIs or PIs) or persistence of a unilateral or bilateral diastolic notch (Figure 3).

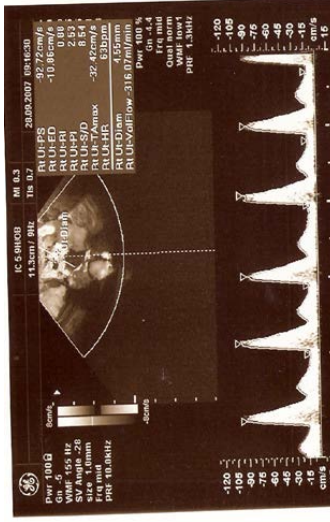
The utility of these measurements in the prediction of pre-eclampsia and small for gestational age (SGA) have been studied extensively. In a systematic review and meta-analysis by Cnossen and colleagues (93) the use of uterine artery Doppler ultrasonography in pre-eclampsia prediction was evaluated from 74 studies on pre-eclampsia (total of 79,547 women) and 61 studies of SGA (total of 41,131 women). That meta-analysis revealed that abnormal uterine artery waveforms are a better predictor of pre-eclampsia than of SGA. The PI, alone or combined with notching, is the most predictive Doppler index. According to that meta-analysis, and other research, uterine artery Doppler ultrasonography provides a more accurate prediction when performed in the second trimester (after the 16<sup>th</sup> week of gestation) than in the first trimester. An elevated PI with notching was the best predictor of pre-eclampsia in the second trimester in both high- and low-risk women (positive likelihood ratio of 21.0 among high-risk patients, and 7.5 among low-risk patients in the second trimester, and negative likelihood ratio 0.82 and 0.59, respectively) in Cnossen and colleagues meta-analysis.

Figure 3. Waveform patterns of uterine artery Doppler measurements.

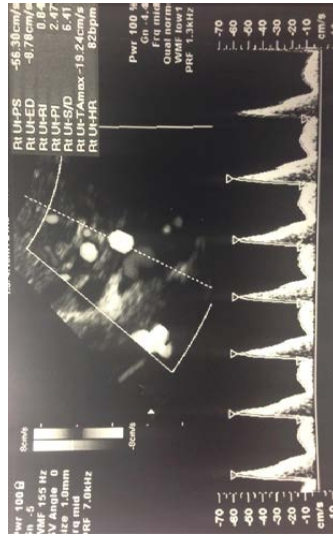
a. First degree notch with normal pulsatility index.



b. First degree notch with high pulsatility index.



c. Second degree notch with high pulsatility index.



d. Second degree notch with high pulsatility index and low diastolic flow.



The best predictor of severe pre-eclampsia in low-risk women was PI (positive and negative likelihood ratio 15.6 and 0.23, respectively) and bilateral notching (positive and negative likelihood ratio 13.4 and 0.37, respectively). Doppler assessment showed no diagnostic characteristics in the prediction of severe pre-eclampsia in high-risk women or pre-eclampsia in the first trimester altogether. Doppler ultrasound measurement of uterine arteries, however, has a significant role in the risk-analysis algorithms, which combine different indices in the first trimester to predict the risk for pre-eclampsia.

### **Biochemical markers**

#### *Placental angiogenic and anti-angiogenic factors*

In normal pregnancy, in the early first trimester, vasoactive markers produced by the placenta are elevated, with sFlt-1 concentrations over ten-fold and PlGF over two-fold higher than in the non-pregnant state. In men and non-pregnant women, low levels of PlGF are produced by heart, lung, thyroid, skeletal muscle, and adipose tissue (69). In pregnancies ending in early miscarriage, both sFlt-1 and PlGF concentrations, measured at gestational weeks six to ten, are significantly lower than in pregnancies that result in live birth (94). The concentration of PlGF continues to increase in the maternal blood until the end of the second trimester and decreases during the last two months of normotensive pregnancies, whereas sFlt-1 stays constant until the last two months, when it starts to increase (16,70,95) (Figure 4). In normal pregnancy, PlGF concentrations decrease with maternal weight, and in cigarette smokers (96).

Maynard and colleagues (15) first reported that elevated concentrations of sFlt-1 circulates in the plasma of pre-eclamptic women, which leads to reduced PlGF and VEGF concentrations and in endothelial dysfunction. Levine and colleagues (16) first showed that changes in PlGF and sFlt-1 concentrations occur earlier and are more pronounced in women who will develop pre-eclampsia compared to normotensive pregnancies. In pregnancies with early-onset pre-eclampsia and pre-eclamptic pregnancies associated with an IUGR, these changes are further exaggerated (16,96). Therefore, these biomarkers have been of high research interest. Neither of these, however, performs in the first trimester as a sole marker in the prediction of pre-eclampsia. PlGF, though,

has an important role in risk estimation algorithms, which seem to work well in the prediction of early-onset pre-eclampsia (Table 4).

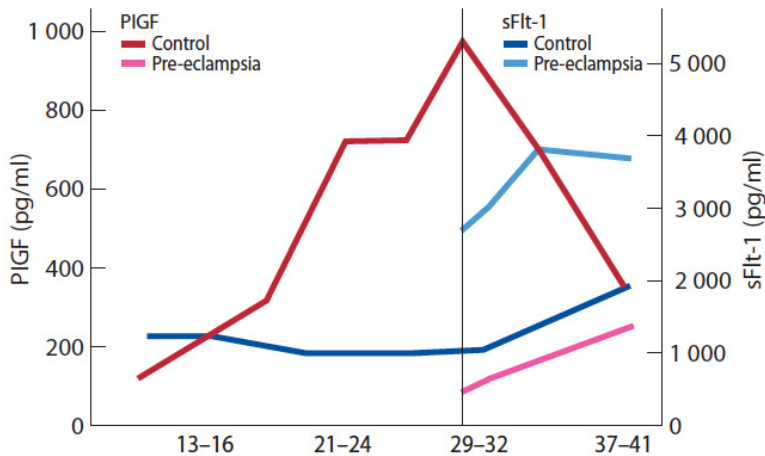


Figure 4. PIGF and sFlt-1 in normal pregnancy and in pregnancy with established preeclampsia. In normal pregnancy, PIGF concentrations rise until 29-32 weeks of gestation and fall then towards term. sFlt-1 increases towards the end of gestation. At term both markers approach the concentrations of established pre-eclampsia. Modified from Redman C and Staff C (70).

In the second and third trimester, these vasoactive markers provide predictive information. They may be used as a tool when planning a monitoring schema for those women who are at increased risk of pre-eclampsia or show the first clinical signs of the disease. Stepan and colleagues suggested in a review that the sFlt-1/PIGF ratio could be used in the follow-up of women at high risk for pre-eclampsia at an asymptomatic stage and in clinical decisions with women having signs and symptoms of pre-eclampsia (97).

#### *Pregnancy-associated protein A*

Pregnancy-associated protein A (PAPP-A) is mainly produced by the placenta and its concentration increases until the end of pregnancy (27). PAPP-A reflects placental function. It is involved in growth and repair processes. Its concentration has an interesting dual meaning (98) in

healthy conditions, like normal pregnancy, PAPP-A is increased and contributes to anti-inflammatory, anabolic and reparative processes, raised concentrations indicate a healthy state. In active disease states, for example in acute coronary symptoms, raised levels relate to ongoing morbidity and poor outcomes, and may indicate a compensatory increase in expression. Interestingly, one of PAPP-A's main functions is to allow free insulin-like growth factor to interact with its cell membrane protein, and consequently activates the synthesis of NO, with its multiple protective actions on the cardiovascular system.

In pregnancy, PAPP-A measurement is used in the screening for fetal chromosomal abnormalities at gestational weeks 11<sup>+0</sup>- 13<sup>+6</sup> together with human chorionic gonadotropin- $\beta$  (hCG $\beta$ ) and nuchal translucency thickness (99). In pregnancies affected by trisomy 21, PAPP-A levels are lower than in pregnancies with normal fetal chromosomes (100). Lower levels of PAPP-A in the first trimester in pregnancies with fetuses with normal chromosomes are associated with increased risk for pre-eclampsia, IUGR, SGA and preterm delivery (101-103). With extremely low PAPP-A concentrations (under 0.3 multiples of median (MoM)), the risks of aneuploidy, spontaneous abortion, preterm delivery, and SGA newborn increase with decreasing concentrations (104). In the first trimester the screening performance of PAPP-A is only 10-20% as a single predictive marker for pre-eclampsia (105,106). However, it performs better in risk estimate algorithms with other predictive variables.

### *Placental protein 13*

Placental protein-13 (PP-13) is produced by placental trophoblast cells (107). In normal pregnancies, serum PP-13 levels slowly rise with gestational age (27). In women who subsequently develop pre-eclampsia, PP-13 levels are lower in the first trimester (108,109) and with early-onset or severe pre-eclampsia, these findings are more pronounced (108,110). Different studies have produced differing information on the performance of PP-13 as a predictive marker (111,112). Detection rates are not high enough to enable using PP-13 as a single biochemical marker in the prediction of pre-eclampsia. For example, when combining PP-13 with Doppler ultrasound PI measurement, the prediction rate in early pregnancy was 71% at a false positive rate of 10% in a study by Khalil and colleagues (112). Another study reported a prediction rate of 90% in the prediction of early-onset pre-eclampsia at 11<sup>+0</sup>-13<sup>+6</sup> weeks of gestation in combination with Doppler ultrasound PI with a false positive rate of 10% (110).

### *Free fetal haemoglobin and $\alpha_1$ -microglobulin*

Free, extracellular fetal haemoglobin (HbF) may be involved in the pathogenesis of pre-eclampsia, and the heme- and radical scavenger  $\alpha_1$ -microglobulin (A1M) in the physiological defence against HbF (27,113). Increased Hb production has been demonstrated in pre-eclamptic placentas (114,115). It is hypothesised that Hb genes may be overexpressed in a subpopulation of cells in the pre-eclamptic placenta, and Hb may be released into the placenta blood vessel lumen (114). Free Hb, and its metabolites heme and iron are potent toxins and cause endothelial damage and inflammation (116,117). Placental cells produce haematopoiesis stimulating agents in response to reduced perfusion and possibly local hypoxia (114). Several Hb and heme detoxification systems have been described (117). Free heme and radical neutralising A1M is produced by liver and placenta (118). Its expression is up-regulated following exposure to Hb, heme, and reactive oxygen species (115). In women who subsequently develop pre-eclampsia, both HbF and A1M are significantly elevated in the first trimester (115). There are promising results of studies evaluating HbF and A1M in the prediction of pre-eclampsia in the first trimester (115,119). Combining these two biomarkers with maternal clinical risk factors may serve as a prediction model for pre-eclampsia (120).

### **Genetic risk factors**

Pre-eclampsia tends to run in families. The estimated heritability is 54 %, according to a Swedish twin register study (121). Both maternal and paternal factors have been shown to contribute to the risk (122). A woman born from a pre-eclamptic pregnancy has over twice the risk of pre-eclampsia compared to a woman without such a history. A man born from a pre-eclamptic pregnancy has a 1.5-times increased risk of fathering a pre-eclamptic pregnancy than a man without such a history, and moreover, if a woman's sibling is born from a pre-eclamptic pregnancy, her risk of pre-eclampsia is doubled (122) (Figure 5). There are also clear differences in the prevalence of pre-eclampsia between ethnical groups (123) for example between white American and African-American women.

Since fetus expresses both maternal and paternal genes, mechanisms have evolved to protect the fetus from immune rejections by the mother. It is speculated that an inappropriate maternal immune response might influence trophoblast invasion and lead to pre-eclampsia (124).

Trophoblasts express an unusual repertoire of human leukocyte antigens (HLA). Interactions of maternal, paternal and fetal genotypes of HLA may play an important role in the pathogenesis of pre-eclampsia (124,125).

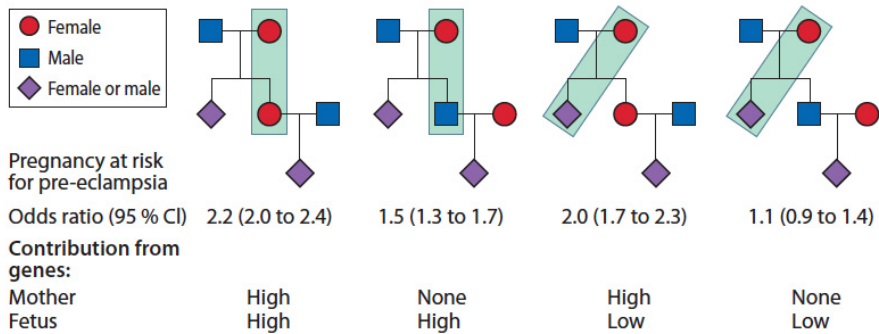


Figure 5. Risk of parenting a pregnancy with pre-eclampsia. Modified from Skjaerven R et al (122).

The majority of candidate gene studies have been underpowered and results inconsistent. Family linkage studies, however, have identified susceptibility loci. For example, in Finnish pre-eclampsia families susceptibility loci have been found in chromosomes 2 and 9 (126,127). However, individual genes have not been pinned down. New genome-wide strategies provide opportunities to identify common and rare sequence variants associated with pre-eclampsia (128,129). The InterPregGen consortium (130) has reported the discovery of the first genome-wide significant susceptibility locus in the offsprings of pre-eclamptic pregnancies. The locus is near the *fms*-related tyrosine kinase gene (*FLT1*) encoding Flt-1, which has been implicated in pre-eclampsia. Moreover, Lokki and colleagues (131) recently reported findings of maternal *FLT1* sequence variants associated with lower pre-eclampsia susceptibility. Women carrying these variants may also have a lower incidence of heart failure later in life.

At present, there are no genetic tests available for the prediction of pre-eclampsia. However, family history of pre-eclampsia has been included in the risk prediction models. Recent promising findings may, in the future, assist in the prediction of pre-eclampsia and offer new opportunities in pre-eclampsia prevention.

## **Risk prediction models**

Since pre-eclampsia is a multi-system syndrome with a great diversity of aetiological factors, it is unlikely, that a single biomarker could be found to predict the disease. Consequently, different prediction algorithms have been generated. In these, maternal history, mean arterial pressure (MAP), PI of uterine arteries measured with Doppler ultrasound, and biochemical markers in different combinations are utilised (Table 4). With all combinations of predictive variables, prediction of early-onset pre-eclampsia performs better than prediction of late-onset pre-eclampsia. In Onwudine's work (132) uterine artery PI together with maternal history and MAP find all women who will develop early-onset pre-eclampsia when 10% false positives is accepted, and 96 % with only uterine artery PI measurements. From studies by others (93,96) however, sole uterine artery PI does not perform as well. An efficient combination of variables for early prediction of early-onset pre-eclampsia is maternal factors, (maternal history and characteristics) included with PIGF concentrations, uterine artery PI, and MAP. PAPP-A (96,133,134) does not improve the performance of this combination. The best performance in the prediction study of late-onset pre-eclampsia by Poon and colleagues (135) is a combination of maternal factors, uterine artery PI, MAP and a selection of biochemical markers which partially differ from the ones used in the prediction of early-onset disease: PIGF, activin-A, and P-selexin (135). MAP (132) or cardiac output (136,137) which are already elevated early in the pregnancy in women who will later develop pre-eclampsia, do not serve as solitary variables in the prediction of pre-eclampsia.

In a recent prospective study (138) a screening based on clinical risk factors suggested in The National Institute for Health and Care Excellence (NICE) and ACOG recommendations (4,32) was compared to a risk algorithm developed by the Fetal Medicine Foundation (FMF), with a multivariate logistic model combining maternal factors, biophysical (MAP, uterine artery PI) and biochemical (PIGF) measurements by O'Gorman and colleagues (134). The FMF method detected 100% of the very early-onset (under 32<sup>+0</sup> weeks of gestation) and 75% of preterm (under 37<sup>+0</sup>) pre-eclampsia, with a false positive rate (FPR) of 10.0%. The NICE guidelines detected 41% and 35% respectively, with a FPR of 10.2% and ACOG guidelines 94% and 90% , respectively, with a FPR of 64.2%.

## **Possible predictive factors of superimposed pre-eclampsia in women with chronic hypertension**

Superimposed pre-eclampsia is diagnosed in a pregnant woman with CHT when new-onset proteinuria over 0.3 g per day is detected after 20<sup>th</sup> weeks of gestation. Approximately 26% of women with CHT develop superimposed pre-eclampsia (26). It is associated with adverse outcomes for both the mother and fetus (139). Women with CHT who develop superimposed pre-eclampsia have significantly higher rates of perinatal morbidity, they significantly more often deliver SGA infants and before 32 weeks of gestation compared to women with CHT without pre-eclampsia (140). Endothelial dysfunction and abnormalities in cardiac and renal function are proposed to contribute to the development of superimposed pre-eclampsia.

In hypertensive men and non-pregnant women, the relationship between microalbuminuria and reduced NO bioavailability in association with impaired endothelial flow-mediated dilatation is well described. NO plays an important role in maintaining vascular homeostasis. It inhibits smooth muscle contraction and growth, platelet aggregation and leukocyte adhesion to the endothelium (141). It is a highly reactive nitrogen radical, a gas with a short half-life. Blood vessels produce NO by endothelial nitric oxide synthase (eNOS) from L-arginine. eNOS is activated by blood flow induced shear stress across the vascular endothelial cell layer and it leads to continuous NO release (142). ADMA is an endogenous NOS inhibitor, it competes with L-arginine in the NO synthesis pathway. In normal pregnancy, ADMA concentrations fall until 24<sup>th</sup> weeks of gestation, consistent with gestational changes in blood pressure, and rise to pre-pregnancy levels at term (143). Some studies report that the albumin:creatinine ratio (ACR) and ADMA are elevated prior to onset of pre-eclampsia compared to normal pregnancies (144,145). However, conflicting results exist (146,147) and studies focusing on pregnant women with CHT are few.

**Table 4. Algorithms for early prediction of pre-eclampsia.**

Study	Predictive factors	Subgroups	Detection rate (%,(95%CI)) for fixed false positive rate (FPR)	
			FPR 5%	FPR 10%
<b>O’Gorman N et al. 2016</b>	Maternal factors	Early-onset pre-eclampsia < 32	42 (30-55)	53 (40-65)
		Preterm pre-eclampsia < 37	36 (30-41)	49 (43-55)
		Term pre-eclampsia ≥ 37	28 (24-31)	38 (34-41)
	Maternal factors plus UtaPI, MAP, PAPP-A, PlGF	Early-onset pre-eclampsia < 32	82 (70-90)	89 (79-96)
		Preterm pre-eclampsia < 37	64 (58-70)	75 (70-80)
		Term pre-eclampsia ≥ 37	33 (30-37)	48 (44-52)
<b>Park F et al. 2013</b>	Maternal factors, MAP, UtaPI, PAPP-A	Early pre-eclampsia < 34	42 (15-72)	92 (62-99)
<b>Poon L et al. 2010</b>	Maternal factors	Early-onset pre-eclampsia < 34	40 (5-69)	47 (13-79)
		Late-onset pre-eclampsia ≥ 34	37 (27-48)	48 (24-68)
	Maternal factors plus UtaPI, MAP, biochemical (early: PlGF; late: PlGF, activin-A, P-selexin)	Early-onset pre-eclampsia < 34	89 (70-97)	92 (75-99)
		Late-onset pre-eclampsia ≥ 34	45 (36-57)	66 (55-75)
<b>Onwudine N et al. 2008</b>	Maternal factors	Early-onset pre-eclampsia < 34	30 (10-55)	35 (24-46)
		Late-onset pre-eclampsia ≥ 34		
	Uta-PI	Early-onset pre-eclampsia < 34	96 (78-99)	41 (30-52)
		Late-onset pre-eclampsia ≥ 34		
	MAP	Early-onset pre-eclampsia < 34	65 (43-84)	40 (29-52)
		Late-onset pre-eclampsia ≥ 34		
	<u>Combined screening</u>	Early-onset pre-eclampsia < 34	100 (85-100)	56 (41-64)
		Late-onset pre-eclampsia ≥ 34		

**Akolekar R et al. 2008**

Maternal factors	Early-onset pre-eclampsia <34 Late-onset pre-eclampsia ≥34	39 (18-63) 30 (21-40)	49 (20-70) 44 (34-54)
PIGF	Early-onset pre-eclampsia <34 Late-onset pre-eclampsia ≥34	28 (13-47) 19 (12-30)	52 (33-71) 33 (24-43)
PAPP-A	Early-onset pre-eclampsia <34 Late-onset pre-eclampsia ≥34	24 (10-44) 8 (4-16)	41 (24-61) 18 (11-28)
Uta-PI	Early-onset pre-eclampsia <34 Late-onset pre-eclampsia ≥34	38 (21-58) 16 (10-25)	66 (46-82) 28 (19-38)
<u>Maternal factors plus:</u> PIGF	Early-onset pre-eclampsia <34 Late-onset pre-eclampsia ≥34	55 (36-74) 29 (20-39)	62 (42-79) 52 (33-71)
PAPP-A	Early-onset pre-eclampsia <34 Late-onset pre-eclampsia ≥34	52 (33-71) 30 (20-39)	69 (49-85) 47 (37-57)
Uta PI	Early-onset pre-eclampsia <34 Late-onset pre-eclampsia ≥34	69 (49-85) 30 (21-40)	76 (57-90) 51 (41-61)
PAPP-A, UtaPI	Early-onset pre-eclampsia <34 Late-onset pre-eclampsia ≥34	69 (49-85)	72 (53-87)
PIGF, UtaPI	Early-onset pre-eclampsia <34 Late-onset pre-eclampsia ≥34	76 (57-90) 30 (21-40)	90 (73-98) 49 (39-59)
PIGF, PAPP-A, UtaPI	Early-onset pre-eclampsia <34 Late-onset pre-eclampsia ≥34	76 (57-90)	86 (68-96)

UtaPI = uterine artery pulsatility index, MAP = mean arterial pressure, PAPP-A = pregnancy-associated protein A, PIGF = placental growth factor

The glycocalyx is a negatively-charged mesh of membranous glycoproteins, proteoglycans, glycoaminoglycans and associated plasma proteins on the endothelial cell surface (148). Its principal glycosaminoglycans (heparin sulphate and hyaluronic acid) and core proteins (syndecans and glypicans) are degraded in vascular diseases, leading to breakdown of the vascular permeability barrier, enhanced access of leukocytes to the arterial intima that propagate inflammation, and alteration of endothelial mechanotransduction mechanisms that protect against disease (149). Vascular endothelial glycocalyx is a crucial regulator of vascular function, and its damage has been implicated in reperfusion oxidative injury, inflammation, atherosclerosis and diabetes (149-151). Disruption of the glycocalyx in the non-pregnant state is also associated with proteinuric disease (152) salt overload (148) and high concentrations of atrial natriuretic peptide (153). These all are features of CHT and potentially relevant to the development of superimposed pre-eclampsia. Syndecan-1 (a transmembrane heparin sulphate peptidoglycan) is a major constituent of the endothelial glycocalyx. Syndecan-1 and sialic acid are shed in response to sheddases (e.g. heparanase, matrix metalloproteinase) and neuraminidase enzymatic activity respectively (149).

Cardiac dysfunction, defined by left ventricular systolic and diastolic dysfunction, and left ventricular mass index and wall thickness, has been described in women prior to the onset of pre-eclampsia (154) however, the contribution of pre-existing cardiac disease in women with CHT to superimposed pre-eclampsia is poorly understood. CHT is also associated with abnormalities in renal function and undiagnosed chronic kidney disease (155), but the involvement of subclinical glomerular and renal tubular injury to superimposed pre-eclampsia development is unknown.

### **Free fatty acids and metabolic adaptations in pregnancy**

In the non-pregnant state, higher concentrations of free fatty acids (FFA) are associated with lipid accumulation in multiple tissues, including liver, skeletal muscle, heart, and pancreatic  $\beta$ -cells (156). This intracellular lipid accumulation in non-adipose tissues is associated with steatohepatitis, skeletal muscle insulin resistance, and  $\beta$ -cell dysfunction (157). In pregnancy, the plasma FFA composition and concentration are dynamic and depend on many factors, including the state of the pregnancy, obesity, insulin resistance, and other metabolic and dietary conditions (158). In the first and second trimesters of pregnancy, the mother's metabolism is in an anabolic state. Through enhanced lipogenesis and hyperphagia, fat

deposit accumulation increases (159). As the pregnancy enters the third trimester, metabolism switches into a catabolic state, responding to rapid fetal growth. Physiological hyperlipidemia involves a rise in blood triglycerides and cholesterol (160,161). Maternal triglycerides do not cross the placenta, but, by the hydrolysing action of lipases, they are released to the fetus as FFAs (159). Lipolytic activity of the adipose tissue becomes enhanced late in the second trimester, increasing plasma levels of FFA and glycerol (162). There is a positive linear relationship between plasma FFAs and maternal age, nulliparity and prepregnant BMI (163). It is thought that maternal hypertriglyceridemia benefits the fetus and newborn in several ways (159). Under fasting conditions, triglycerides can rapidly be used for energy by ketone body synthesis in the liver, and they also provide a source of essential fatty acids to the fetus from the maternal diet. Essential fatty acids (EFA) and long-chain polyunsaturated fatty acids (LCPUFA) are important in cell growth and development (164) and in mediating intracellular gene expression (165). These are important in fetal development during the third trimester and in the neonatal period. The fetus depends on placental transport completely for the supply of linoleic and  $\alpha$ -linolenic acid EFAs. More than 90% of fetal fat deposition occurs in the last 10 weeks of gestation (166). LCPUFAs are higher in fetal than in maternal circulation and the composition is different (164,167). Fetal production of LCPUFAs, arachidonic acid and docosahexaenoic acid, is very low, but there is active uptake by the placenta (166). The fetal brain and retina are very rich in LCPUFAs, especially arachidonic acid and docosahexaenoic acid (168-170) which the placental membrane fatty acid binding protein (p-FABP<sub>pm</sub>) preferentially binds to compared with linoleic and oleic acid (171). Modification of pregnant mothers' dietary fat intake does not affect the cord and neonatal lipid levels (172).

Metabolic characteristics of pre-eclampsia include reduced glucose utilisation, hyperinsulinemia and hyperlipidemia (20) and many other known cardiovascular risk factors (21-24). These metabolic changes are seen both in normal pregnancies and in pre-eclampsia, but are exaggerated in pre-eclampsia, and are likely to have evolved to meet the metabolic demands of the growing fetus (159). Circulating FFAs are key regulators of glucose metabolism and have been shown to increase in pre-eclamptic women long before clinical onset of the disease (173-177). High plasma lipids may contribute to the development of pre-eclampsia by increasing vascular dysfunction in the uteroplacental circulation by advancing lipid changes in the walls of already poorly transformed spiral arteries. The atherogenic haemodynamic flow patterns in these pre-eclamptic spiral arteries may play a role in aggregating lipid deposition in the vessel wall (25). High concentrations of FFAs may influence several characteristics of pre-eclampsia, such as increased insulin resistance, disturbed endothelial cell function and altered production of vasoactive substances.

## **Prevention of pre-eclampsia**

Since placental changes in pre-eclampsia begin in the first trimester, women at highest risk, those who would benefit most from prevention of the disease, should be identified in early gestation. Due to the multifactorial nature of pre-eclampsia, it might be that in the future different subgroups of pre-eclampsia will be recommended individual preventive medication.

### **Acetylsalicylic acid**

#### *Background*

Acetylsalicylic acid, or aspirin, is believed to have been used as early as 1534 BC, since the use of 'salix', aspirin-related compounds derived from willow tree bark, were documented on papyrus scrolls used by Egyptian physicians (178). In 1758, Reverend Edward Stone studied willow tree bark for the relief of headaches, myalgia and fever. The Nobel Prize in 1971 was awarded to Samuelson and Bergström for demonstrating the mechanism of action and clinical aspects of aspirin. After that, research on its antiplatelet effects was undertaken. In 1979, it was observed that pregnant women who took aspirin did not develop pre-eclampsia as often as other pregnant women (179). Results of a small randomised trial were published in the year 1985. This trial reported that 150 mg aspirin, started at 12<sup>th</sup> weeks of gestation, prevented pre-eclampsia, IUGR and perinatal death (180).

The preventive effect of aspirin is thought to be due to its favourable effect on prostaglandin production, particularly the prostacyclin:thromboxane ratio, which is in imbalance in pre-eclamptic women before the clinical disease. Vasodilatory prostacyclin is produced in endothelial cells, and vasoconstrictive and aggregatory thromboxane in platelets. In normal pregnancy there is a predominance of prostacyclin (181). Arachinoid acid is a precursor of these prostaglandins. By the action of cyclo-oxygenases (COX-1 and COX-2), arachinoid acid is converted to prostanoids (PGH<sub>2</sub>), which are further converted to prostaglandins. Low dose aspirin irreversibly inhibits COX-1 synthase. Since COX-1 synthase is the main cyclo-oxygenase in platelets, the production of thromboxane is efficiently inhibited. Inhibition of COX-2 synthase in the blood vessel wall requires considerably higher doses of aspirin and the production of prostacyclin is intact with the low dosage (182). Vainio and colleagues (183) studied this dose-dependent inhibition and the favourable effect on prostacyclin:thromboxane ratio was within the 0.5-2.0 mg/kg dose range.

Other possible routes of action of low dose aspirin have emerged. One of these is through the HO-1 pathway. Aspirin stimulates the expression and enzymatic activity of HO-1 in endothelial cells, presumably via NO-dependent and COX-independent pathways (184,185).

The increased HO-1 activity leads to the ensuing formation of bilirubin and CO, and may contribute to and explain the antioxidant and antiatherogenic activities of aspirin (186). Aspirin also reduces ADMA concentration (184,185) consequently increasing endothelial NO synthase activity. Furthermore, aspirin dose-dependently inhibits sFlt-1 production in cytotrophoblasts via COX-1 inhibition (187).

Low dose aspirin has side effects as often as placebo (188) and it is safe in pregnancy due to the knowledge of its use on tens of thousands of pregnant women. Aspirin is contraindicated in patients with an allergy to aspirin or a history of peptic ulcer. One to two out of every one thousand users of aspirin develop intestinal haemorrhage (189). There are no long-term follow-up studies of the children after mothers' consumption of low dose aspirin during pregnancy.

#### *Studies on pre-eclampsia prevention by low dose aspirin*

Large meta-analyses suggest that low dose aspirin and other antiplatelet agents would benefit women with an increased risk of pre-eclampsia. The Paris collaboration (190) meta-analysis of 32,217 mothers included randomised studies regardless of the inclusion criteria. The effect was moderate but consistent. The relative risk (RR) of pre-eclampsia was 0.9 in women receiving anti-platelet agents compared to control women. The authors recommended low dose aspirin in high-risk women. The Cochrane review (191) updated in 2007, accepted studies that included women with an increased risk of pre-eclampsia and demonstrated a 17% reduction in the incidence of the disease. Another meta-analysis (192) included 27 studies (11,348 women) in which the initiation of the low dose aspirin treatment could be identified. In those women who started low dose aspirin at 16 weeks of gestation or earlier had significantly reduced risk of pre-eclampsia compared to control women (RR 0.47, 95%CI 0.34-0.65). In women who started aspirin after 16 weeks of gestation there was no effect on the risk (RR 0.81, 95%CI 0.63-1.03). According to meta-analyses, low dose aspirin prevents early-onset and severe pre-eclampsia, but does not have an effect on late-onset and non-severe pre-eclampsia (193-196). The evidence of low dose aspirin in the prevention of pre-eclampsia has been determined to be so strong that several organisations and expert groups have released recommendations on its initiation in high-risk women (32,197-200) (Table 5).

#### *Aspirin resistance*

In cardiovascular literature, the concept of aspirin resistance is well identified. Some patients seem to develop severe cardiovascular-related events despite the antithrombotic therapy. It is

suggested that these patients are aspirin ‘resistant’, their platelets are not affected or the response to aspirin therapy is different compared to those who benefit from it. Some of these patients require a larger dose of aspirin to develop the expected antiplatelet effect. The reason behind this phenomenon is unclear. A too low aspirin dose, compliance issues, differences in abilities to absorb aspirin, or an underlying genetic disposition are suggested explanations (201-203). In pregnancy, enhanced platelet turnover may be an underlying cause (178). According to a meta-analysis (204) of aspirin resistance and cardiovascular risk, the patients who are aspirin resistant have about a four-fold greater risk of long term cardiovascular, cerebrovascular, or vascular morbidity while on aspirin compared to patients with a normal aspirin response. In that meta-analysis, 28% of the 2980 patients were classified as aspirin resistant by platelet function assays. Since low dose aspirin completely inhibits COX-1 pathway, those assays that target aspirin COX pathways are appropriate in demonstrating aspirin resistance (178).

## **Statins**

Statins, used to treat high blood cholesterol are currently being studied in the prevention of pre-eclampsia (205). They have many positive effects, but have previously been contraindicated during pregnancy (206). Since more experience has accumulated, it seems that statins can be safe during pregnancy (207). Statins have a positive effect on cytokine and free radical oxide production and immunomodulation, and they have anti-inflammatory effects. They increase the production of Flt-1 in endothelial cells by stimulating factor 1-alpha. Statins induce HO-1 transcription and expression. HO-1 has anti-inflammatory and cell-protective actions, and the HO-1 pathway inhibits vasoconstrictive sFlt-1 and sEng (208).

## **Low molecular weight heparin**

Women with placenta-mediated complications in earlier pregnancies have an increased risk of both recurrent placenta-mediated complications (209) and venous thromboembolism in subsequent pregnancies (210). Vice versa, women with previous venous thromboembolic complications have an increased risk of placenta-mediated pregnancy complications (211). These are all associated with placental vascular thrombosis. Antithrombotic low molecular weight heparin (LMWH) has been studied in the prevention of pre-eclampsia combined with and without low dose aspirin. The Cochrane review of LMWH in improving maternal and infant health outcomes did not show an effect in the prevention of pre-eclampsia (212) and in smaller trials, including women with thrombophilia, results have been similar (213).

**TABLE 5. Recommendations for low dose aspirin in the prevention of pre-eclampsia**

<b>Recommendation</b>	<b>Dose (mg per day)</b>	<b>Recommended starting point</b>	<b>Indications</b>
<b>American Congress of Obstetricians and Gynecologists (ACOG)</b>	60-80	During the late first trimester	Medical history of early-onset pre-eclampsia with preterm delivery (under 34 weeks of gestation) or pre-eclampsia in more than one prior pregnancy (In a statement from July 2016, ACOG supported the broader list of risk factors by USPSTF)
<b>World Health Organization (WHO)</b>	75	12 <sup>th</sup> to 20 <sup>th</sup> weeks of gestation	High risk women (i.e., history of pre-eclampsia, diabetes, chronic hypertension, renal or autoimmune disease, multiple pregnancy)
<b>The UK National Institute for Health and Care Excellence (NICE)</b>	75	12 <sup>th</sup> week of gestation	High risk women (i.e. those with a history of hypertension in a previous pregnancy, chronic kidney disease, type 1 or 2 diabetes, or chronic hypertension) and if more than one of the moderate risk factors exists (first pregnancy, age $\geq$ 40 years, pre-pregnancy interval over 10 years, BMI $\geq$ 35 kg/m <sup>2</sup> , family history, multifetal pregnancy)
<b>American Heart Association (AHA)</b>	Low dose aspirin	12 <sup>th</sup> week of gestation	Chronic hypertension, previous pregnancy-related hypertension
<b>U.S. Preventive Services Task Force (USPSTF)</b>	81	After 12 weeks of gestation	Previous pre-eclampsia, diabetes, chronic hypertension, renal disease, autoimmune disease and previous pre-eclampsia with adverse outcome, multiple pregnancy, and if more than one of the moderate risk factors exists (history of an SGA infant, first pregnancy, age $\geq$ 35 years, pregnancy interval over 10 years, BMI $\geq$ 35 kg/m <sup>2</sup> , family history, multifetal pregnancy)

BMI = body mass index, SGA = small for gestational age

Combined treatment of LMWH with low dose aspirin, started in the first trimester, seems to prevent early-onset hypertensive diseases and SGA in women with inherited thrombophilia (214). According to a recent meta-analysis, LMWH combined with aspirin compared to sole low dose aspirin seems to reduce the risk of early-onset pre-eclampsia and SGA considerably more efficiently in women with a history of pre-eclampsia (215). Because of small number of women in these studies larger clinical trials are required to confirm these findings and for recommendations in clinical work.

### **Antioxidants and other dietary supplementation**

Low dietary calcium and low serum calcium concentrations are associated with pre-eclampsia. Large trials on the benefit of calcium supplementation, 1.5-2 g per day, in prevention of pre-eclampsia have not supported this treatment in healthy nulliparous women (216) nor in women with a calcium deficient diet (217). However, the findings are controversial. In the World Health Organisation (WHO) randomised trial of over 8,000 women with a calcium deficient diet (217) treatment significantly reduced the risk of serious complications from pre-eclampsia, which included maternal and neonatal morbidity and death and preterm delivery; the latter among young women. Daily calcium supplementation of 1.5-2.0 grams is thereby recommended by the WHO in the second half of pregnancy in those women whose dietary intake is low. In Cochrane review from 2014 (218) with meta-analysis of 13 trials and over 15,000 women, calcium supplementation ( $\geq 1\text{g/day}$ ) was associated with a significant risk reduction of pre-eclampsia, particularly among women with a low calcium diet. However, the authors advise to interpret these results cautiously since they believe the result is overestimated due to a small-study effect or publication bias. In Finland, calcium supplementation, 0.5-1.0 g per day, is recommended to pregnant women, depending on their diet (219).

Oxidative stress, an imbalance between pro-oxidant and antioxidant forces, plays a part in the pathogenesis of pre-eclampsia. Various studies have attempted to find out if antioxidants have an effect on pre-eclampsia prevention. Vitamins C, E or D do not reduce the risk of pre-eclampsia. In a 2008 Cochrane Review of antioxidants in the prevention of pre-eclampsia (220) a meta-analysis of 9 trials and 5,500 women, vitamins C and E did not have a preventive effect. A meta-analysis by Rossi and Mullin (221) did not show any effect of vitamin C and E in preventing pre-eclampsia in subgroups of high-risk or low-risk women. In the International Trial of Antioxidants in the Prevention of Pre-eclampsia (INTAPP) (222) 2647 women were randomised to 1 g vitamin C and 400 IU vitamin E daily. The trial was

discontinued due to a concern of adverse pregnancy outcomes such as fetal loss or perinatal death and preterm prelabor rupture of membranes. No effect was seen in the prevention of pre-eclampsia, as was found in another placebo-controlled trial (223) with similar doses of vitamins C and E, however, in that study significantly more low birthweight babies were born in the antioxidant group.

Studies of vitamin D are contradictory. A recent prospective cohort (224) with 1710 pregnant women suggest that vitamin D concentrations at 15<sup>th</sup> weeks of gestation are not associated with development of pre-eclampsia.

### **Physical exercise and obesity management**

Regular physical exercise has various health benefits and it protects from cardiovascular diseases through a wide variety of mechanisms. By having an impact on many risk factors common to pre-eclampsia, physical exercise may also be important in the prevention of pre-eclampsia. Regular exercise reduces obesity-related markers and the risk of type 2 diabetes by improving insulin sensitivity, endothelial dysfunction, lipid profile, oxidative stress and inflammatory markers (225-228). Exercise reduces visceral fat, which increases insulin sensitivity. A meta-analysis including 15 studies with 180,000 participants investigating the effect of pre-pregnancy and early-pregnancy physical activity on the risk of pre-eclampsia suggested a reduced risk with increasing levels of physical activity (229). Women in the higher levels of physical activity had a 20 to 33% reduction in the risk of developing pre-eclampsia. The protective effect of physical exercise on the incidence of pre-eclampsia is based on its effect on the improvement of the functional capacity of placenta and endothelial function (230) glycemic control and insulin resistance (231) antioxidant defences, pregnancy-induced inflammation suppression, blood pressure reduction, and levels of total cholesterol, triglycerides (232,233) and leptin (234).

## **AIMS OF THE STUDY**

1. To investigate the concentrations of total and individual free fatty acids in pre-eclamptic and normotensive pregnant women and to study the relationship between free fatty acid concentrations and insulin sensitivity (Study I).
2. To explore predictive factors concerning the onset and severity of pre-eclampsia by using cluster analysis in a prospectively-collected cohort of women with known clinical risk factors for pre-eclampsia (Study IV).
3. To study the concentrations of sFlt-1 and PlGF and their ratio (sFlt-1/PlGF) in predicting pre-eclampsia in prospectively-collected serial serum samples from a cohort of pregnant women with clinical risk factors for pre-eclampsia, and controls with special reference to early- and late-onset disease (Study III).
4. To study the contribution of maternal (endothelium, cardiac, renal) and placental factors to the development of superimposed pre-eclampsia in women with chronic hypertension (Study V).
5. To study the effect of low dose aspirin (100mg) started at 12<sup>+0</sup> to 13<sup>+6</sup> weeks of gestation on the prevention of pre-eclampsia and intrauterine growth restriction in high-risk women identified by abnormal uterine artery flow (Study II).

## **MATERIALS AND METHODS**

### **Patients and study design for the free fatty acid profiles in the pre-eclampsia study (Study I)**

The study participants, 21 nulliparous, previously healthy pre-eclamptic women and 11 normotensive, non-proteinuric women, were recruited in the maternity clinics, the prenatal clinic and the antenatal ward of Helsinki University Central Hospital between January 1996 and February 1998. Patients with severe pre-eclampsia were not included. At examination, the range of gestational ages was 32.3-39.3 (median 36.4) in the pre-eclamptic women and 29.0-38.3 (median 34.4) in the control group. There were no differences in the pre-pregnancy BMI and weight gain.

### **Measurements**

FFAs were measured in conjunction with a 75 mg oral glucose tolerance test, with serum samples collected at baseline and at 60 and 120 min. No women had gestational diabetes as defined with concurrent Finnish criteria (fasting:  $\leq 4.5$  mmol/L; at 1 h:  $\leq 9.1$  mmol/L; at 2 h  $\leq 7.9$  mmol/L).

As previously reported (19), the intravenous glucose tolerance test, using the minimal model technique, was used to measure whole-body insulin sensitivity. A dose of glucose (0.3g/kg glucose) was injected intravenously after overnight fast, followed by a bolus of insulin at 20 min (0.03IU/kg insulin). Blood samples were collected at baseline with 5-min interval, and 4, 6, 8, 10, 22, 29, 37, 67, 90, 180 min after administration of the glucose bolus. Insulin sensitivity was evaluated from using disappearance curves of glucose and insulin with the Minimal Model computer program and expressed in  $\text{min}^{-1}$  per  $\mu\text{U/mL}$ .

### **FFA determinations**

Lipids were first extracted from the serum samples using the Folch and Lees method. The extract was dried and reconstituted in chloroform (E.Merck, Darmstadt, Germany). Then, lipid extract was applied to separate small amino acid columns employing a Gilson automatic sample pretreatment device (Middleton, Wisconsin, USA). The FFA fraction was eluted with 2% acetic acid in diethyl ether and ether fractions were then evaporated in vacuo and FFAs

methylated with HCl-methanol reagent. Isolated FFAs were then determined employing a gas chromatograph equipped with a FID detector.

### **Statistical analysis**

Data were analyzed by using the SPSS 14.0 software program (SPSS Inc). FFA concentrations were log transformed to attain normality. An independent-samples t-test was used to calculate differences between subjects, and adjusted for gestational age and pre-pregnancy BMI by linear regression. A paired-sample t-test was used to calculate differences within subjects. All P-values are two-sided.

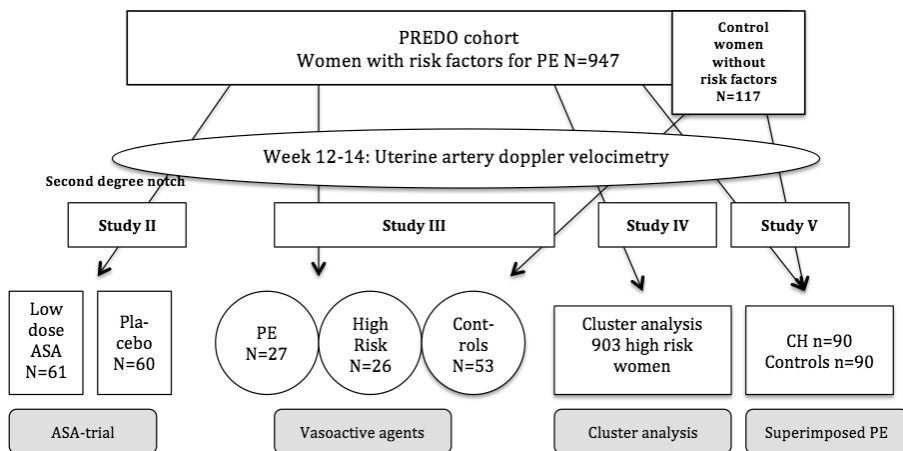
## **PREDO - Prediction and prevention of pre-eclampsia Study Cohort (Studies II-V)**

The multidisciplinary PREDO Project ‘Prediction and Prevention of Pre-eclampsia’ has three study arms: obstetric, genetic and psychological. The entire PREDO Project is described in a Cohort Profile publication (235). This thesis is based on the obstetric arm, for which we recruited 947 women with risk factors for pre-eclampsia and 117 pregnant women without known risk factors as a comparison group at 12<sup>+0</sup> to 13<sup>+6</sup> weeks of gestation between September 2005 and December 2009. We refer to these groups as high-risk and a control group, respectively. The psychological arm includes an additional subsample of 3698 women who were enrolled regardless of their risk status (236-238). The development and health of the children is being followed up (239-241), however that data is not part of this thesis. The recruitment took place when these women attended the first ultrasound screening in one of the ten hospital maternity clinics participating in the PREDO Project; Women’s Hospital, Kätilöopisto Maternity Hospital and Jorvi Hospital in Helsinki University Central Hospital, Kanta-Häme Central Hospital, Päijät-Häme Central Hospital, Tampere University Hospital, Kuopio University Hospital, Northern Karelia Central Hospital, Hyvinkää Hospital and Iisalmi Hospital. Table 6 illustrates the number of participants recruited from each hospital. We also enrolled the spouse of each study participant (biological father of the child) for the genetic arm. The flow-chart of Studies II-V of this thesis is shown in Figure 6.

**Table 6.**  
**Number of participants recruited from each hospital**

Hospitals in PREDO Project	Number of participants recruited
Helsinki University Central Hospital:	
<b>Women's Hospital</b>	402
<b>Kätilöopisto Maternity Hospital</b>	292
<b>Jorvi Hospital</b>	112
Other hospitals:	
<b>Hyvinkää Hospital</b>	52
<b>Kanta-Häme Central Hospital</b>	28
<b>Päijät-Häme Central Hospital</b>	12
<b>Tampere University Hospital</b>	16
<b>Kuopio University Hospital</b>	80
<b>Northern Karelia Central Hospital</b>	50
<b>Iisalmi Hospital</b>	38

**Figure 6.** Flow chart of Studies II-V. These studies are part of the Prediction and Prevention of Pre-eclampsia (PREDO) Project.



PE = pre-eclampsia, ASA = aspirin, CH = chronic hypertension

## Inclusion and exclusion criteria and definitions

The inclusion and exclusion criteria of the at-risk group of women in the PREDO cohort in Table 7. Women with one or more risk factors for pre-eclampsia were invited to participate unless any of the exclusion criteria were present.

**Table 7. Inclusion and exclusion criteria in women recruited in the at-risk group of the PREDO Project**

<b>Inclusion criteria</b>
Age under 20 or age over 40
Obesity (BMI over 30 kg/m <sup>2</sup> )
Chronic hypertension
Sjögren's syndrome, lupus erythematosus
<u>A history of one of the following conditions:</u>
Gestational diabetes
Pre-eclampsia
Small for gestational age (birthweight < -2 SD)
Fetus mortus (fetal death after 22 weeks of gestation or > 500 g weight in a previous pregnancy)
<b>Exclusion criteria</b>
Allergy to aspirin, tobacco smoking (during pregnancy), multiple pregnancy
A history of one of the following: asthma, peptic ulcer, placental ablation, inflammatory bowel disease (Crohn's disease, colitis ulcerosa), rheumatoid arthritis, haemophilia or thrombophilia (previous venous or pulmonary thrombosis or coagulation abnormality)
BMI = body mass index, SD = standard deviation

## Samples collected

Blood and urine samples were collected at 12<sup>+0</sup> to 13<sup>+6</sup>, 18<sup>+0</sup> to 20<sup>+0</sup> and 26<sup>+0</sup> to 28<sup>+0</sup> weeks of gestation and stored at -80°C.

## Ultrasound measurements

We measured uterine artery blood flow by colour Doppler ultrasound transvaginally from all participants at 12<sup>+0</sup> to 13<sup>+6</sup> weeks of gestation. We identified the uterine artery at the level of the internal cervical os as it approaches the uterus laterally. Women who had a bilateral pre-diastolic second-degree notch were allocated to the medication group. Women who did not fulfil the criteria for the medication group were allocated into follow-up groups. Those women who did not have any of the inclusion criteria and who did not have a bilateral second degree pre-diastolic notch in the uterine artery blood flow examination were recruited into the control group.

## **Outcomes**

Outcome diagnoses of the PREDO cohort were: pre-eclampsia (systolic blood pressure  $\geq 140$  mmHg or diastolic  $\geq 90$  mmHg and proteinuria  $\geq 0,3$  g/day or dipstick equivalent in two consecutive measurements), severe pre-eclampsia (systolic blood pressure  $\geq 160$  mmHg or diastolic blood pressure  $\geq 110$  mmHg or proteinuria  $\geq 5,0$  g/day), early-onset pre-eclampsia (diagnosis  $< 34^{+0}$  gestation), CHT (blood pressure  $\geq 140/90$  mmHg or medication for hypertension before 20 weeks of gestation), gestational hypertension (systolic blood pressure  $\geq 140$  mmHg or diastolic blood pressure  $\geq 90$  mmHg after 20<sup>th</sup> weeks of gestation without proteinuria), gestational diabetes (diet treated/ insulin treated), HELLP -syndrome, eclampsia, SGA ( $< -2$  standard deviation (SD), birthweight SD score as a continuous variable calculated according to Finnish standards (242)), normal pregnancy, and fetus mortus (fetal death after 22weeks of gestation or  $>500$ g weight) in a previous pregnancy. Each individual outcome diagnosis was determined by a jury, which consist of two physicians and a study nurse, who met face-to-face and reviewed the hospital and maternity clinic records of each participant.

## **Patients and methods for the cluster analysis (Study IV)**

### **Participants and outcomes**

Those 903 women who had risk factors for pre-eclampsia were included in the cluster analysis. Women (n=69) who were randomised to receive low-dose aspirin were excluded from this substudy.

Primary outcomes for the study were pre-eclampsia (blood pressure  $\geq 140/90$  mmHg at two consecutive measurements and proteinuria  $\geq 0.3$  g/24hours) (4), gestational hypertension (new onset hypertension after 20 weeks of gestation, without proteinuria), and birth weight SD score as a continuous variable calculated according to Finnish standards (242).

Secondary outcomes were early-onset pre-eclampsia (delivery before 34<sup>+0</sup> weeks of gestation), late-onset pre-eclampsia (delivery at or after 34<sup>+0</sup> weeks of gestation), preterm pre-eclampsia (delivery before 37<sup>+0</sup> weeks of gestation), term pre-eclampsia (delivery at or after 37<sup>+0</sup> weeks of gestation), intermediate pre-eclampsia (delivery between 34<sup>+0</sup>-36<sup>+6</sup> weeks of gestation), severe pre-eclampsia (blood pressure  $\geq 160$  mmHg systolic and/or  $\geq 110$  mmHg diastolic and/or proteinuria  $\geq 5$  g/24hours), SGA (birthweight  $< -2$ SD), gestational diabetes (diet or insulin treated), CHT ( $\geq 140/90$  mmHg or medication for hypertension before 20 weeks of gestation), HELLP syndrome and fetal demise (fetal death after 22<sup>nd</sup> gestational week or over 500g weight).

## Methods

We applied a Bayesian clustering algorithm based on mixtures of binary variables, see, e.g. (243) using an implementation available in the Bayesian Analysis of Population Structure (BAPS) software (244) to classify the study participants on the basis of their risk factors. The algorithm detected 25 clusters, corresponding to different risk factor combinations. For each cluster detected, we computed the risk ratio of each disease outcome, relative to the risk in the general population. The significance and CIs of the risks in the different clusters were computed using the exact binomial test (function *binom.test* in the R software). The false discovery rates (FDR) were computed using the function *p.adjust* in R.

The risk for pre-eclampsia and its subtypes in the general Finnish population for these outcomes were estimated according to data from the National Institute for Health and Welfare registers (Medical Birth Register and Care Register for Health Care) from the year 2013; pre-eclampsia 2.5% (of those 24% severe, 8% early-onset, 15% intermediate, 77% term, 23% preterm) with frequencies obtained by request from the register authorities, and gestational hypertension 4.4%, SGA 2.3%, and gestational diabetes 9%.

## Vasoactive agents for the prediction of early- and late-onset pre-eclampsia (Study III)

### Subcohort

In this substudy we included 26 women with risk factors for pre-eclampsia who eventually developed the disease. The first control group was formed from 26 women with risk factors for pre-eclampsia who did not develop the disease. They were chosen by computerised randomization. The second control group was formed with 53 women without clinical risk factors and without pre-eclampsic pregnancy. One woman without risk factors was included in the pre-eclampsia group, since she developed the disease.

### Sample collection and assays

Serum samples from all three trimesters were included in the analysis. Serum sFlt-1 and PIGF concentrations were determined with an automated ElecSys 2010 immunoanalyzer (Roche Diagnostics, Germany).

## **Statistical analysis**

Data were analysed with the SPSS 19.0 program. For comparisons between groups Student t-tests and analysis of variances (ANOVA) were used as appropriate. For multiple comparisons Bonferroni adjustment was used. The nonparametric Kruskal-Wallis test was used since the sizes of the groups differed and for correction: post hoc test pairwise comparisons with adjusted significance and to compare concentrations (PIGF and sFlt-1) and the sFlt-1/PIGF ratio between the four groups.

The change in the concentration (PIGF and sFlt-1) or ratio (sFlt-1/PIGF) between measurements was calculated for each individual and for comparison of sequential changes between the first and second, and the second and third measurement. For comparison of these changes between the four study groups the non-parametrical Kruskal-Wallis test was used. To determine the predictive value of PIGF and the sFlt-1/PIGF ratio at the second and third measurement point, receiver -operating characteristic (ROC) analysis was performed.

## **Patients and methods for the analysis of predisposing factors of superimposed pre-eclampsia in chronic hypertension (Study V)**

All women of the PREDO project with CHT were included in this substudy and those healthy controls with the greatest sample availability. Samples from the entire cohort (522 samples from 90 women with CHT and 90 healthy controls at 3 timepoints) were analysed for plasma markers of placental function (PIGF), maternal cardiac function (B-type natriuretic peptide; BNP) and renal tubular injury (neutrophil gelatinase-associated lipocalin; NGAL).

### **Nested case-control study**

Additional markers were measured in a nested case-control study using samples which were available at all visits from 12 women who developed superimposed pre-eclampsia, 24 women with CHT who did not develop superimposed pre-eclampsia matched for BMI and age and 24 randomly selected healthy controls. There were no demographic differences between control women who were included or excluded from the case-control study.

## Validation cohort

Concentrations of plasma biomarkers that were significantly different between women with and without superimposed pre-eclampsia were validated in a second cohort of women with CHT and/or chronic kidney disease (CKD) with and without superimposed pre-eclampsia (at time of diagnosis), women with pre-eclampsia without pre-existing disease and healthy controls (245).

## Biochemical analysis

Analysed biomarkers and methods used in Table 8.

**Table 8. Measured biomarkers and methods used**

Functional entity	Sub entity	Biomarker measured	Method
<b>Endothelial function</b>	Glycocalyx	Syndecan-1	ELISA
		Plasma-free sialic acid	Chromatographic stable isotope dilution electrospray (MSMS) method
	Functional markers	Plasma asymmetric dimethylarginine (ADMA)	MSMS method
<b>Cardiac function</b>		Urine albumin	Laser nephelometric immunoassay
		Plasma B-type natriuretic peptide (BNP)	Triage CardioRenal Test (Alere)
<b>Renal function</b>		Plasma symmetric dimethylarginine (SDMA)	MSMS method
		Plasma cystatin C	Particle-enhanced laser nephelometric immunoassay
		Plasma neutrophil gelatinase-associated lipocalin (NGAL)	Triage CardioRenal Test
<b>Placental function</b>		Urine and plasma N-acetyl- $\beta$ -D glucosaminidase (NAG) activity	LC electrospray MSMS
		Urine retinol binding protein (RBP)	Particle-enhanced laser nephelometric immunoassay
		Placental growth factor (PlGF)	Triage PlGF Test

ELISA = the enzyme-linked immunosorbent assay, MSMS = tandem mass spectrometric method, LC = liquid chromatography

## Statistical analysis

Demographic data are presented as medians (interquartile range, IQR) or frequencies (percentages). A Q-Q plot was used to explore distribution normality, and logarithmic transformations were used where appropriate. T-tests or the Mann-Whitney test were used to compare parametric and non-parametric differences between groups, respectively, and

Fisher’s exact test for contingency tables. To avoid multiple testing of the same hypothesis, data were corrected for inclusion of women who had provided multiple samples, using interval regression analysis with random-effect modeling for individual clustering. Relationships between biomarkers, maternal age, and BMI was examined with Spearman’s correlation.

ROC areas were used for comparison of potential predictive performance of biomarkers for development of superimposed pre-eclampsia.

### Sample size calculation

A sample size of 12 cases and 24 controls was calculated as adequate to detect a one SD difference in means of the concentration of any biomarker between cases and controls to give 81% power at the 5% significance level.

## Aspirin in the prevention of pre-eclampsia – aspirin trial and meta-analysis (Study II)

### Randomisation and blinding

This was a randomised placebo-controlled double-blinded trial. Randomisation was performed by the Tampere University Hospital Pharmacy. Aspirin and placebo tablets were prepared by a pharmaceutical company, as a paid service (Orion, Espoo Finland). Women with one or more risk factors (inclusion criteria Table 7) and bilateral second degree uterine artery notch were randomized to 100 mg per day aspirin or placebo from 12<sup>th</sup> to 35<sup>th</sup> weeks of gestation.

Primary and secondary outcomes of the aspirin trial are presented in Table 9.

**Table 9. Outcomes in the aspirin trial**

<b>Primary outcomes</b>
Pre-eclampsia (blood pressure $\geq$ 140 mmHg systolic or $\geq$ 90 mmHg diastolic and proteinuria $\geq$ 0.3 g/24 hours or dipstick equivalent in two consecutive measurements)
Gestational hypertension
<b>Secondary outcomes</b>
Early-onset pre-eclampsia (diagnosed before 34 <sup>+0</sup> weeks of gestation)
Preterm pre-eclampsia (diagnosed before 37 <sup>+0</sup> weeks of gestation)
Severe pre-eclampsia (blood pressure $\geq$ 160 mmHg systolic or $\geq$ 110 mmHg diastolic and proteinuria $\geq$ 5.0 g/24 hours)
Small for gestational age fetus (birthweight $<$ -2 SD)
Severe diagnosis (early-onset pre-eclampsia and/or severe pre-eclampsia and/or SGA fetus)
SD = standard deviation

## **Meta-analysis**

All prospective, randomised, controlled trials, that met the following criteria were included in the meta-analysis: 1) included women that had abnormal uterine artery Doppler flow velocimetry measurements and 2) aspirin started at or before the 16<sup>th</sup> week of gestation with doses between 50 and 150 mg/day. The control group had to be allocated to either on placebo or no treatment. Through the literature search, we identified 1414 eligible studies. Only two studies (246,247) fulfilled the abovementioned inclusion criteria in addition to our study. The results were available for a total of 346 women.

The outcome measures for the meta-analysis were pre-eclampsia, severe pre-eclampsia, preterm and term pre-eclampsia.

## **Statistical methods**

Continuous variables were tested for normality. Highest proteinuria values were log transformed to attain normality. Independent sample t-tests were used for continuous variables between study groups and  $\chi^2$  tests for categorical variables. Two-tailed P-values less than 0.05 were considered statistically significant. To compare the risk of each outcome between the aspirin and placebo groups RRs were calculated.

We expected an incidence of 25% for pre-eclampsia among the study participants based on a previous study (246). We calculated that with a power of 0.80 and an alpha of 0.05 we would be able to confirm or exclude a reduction in incidence to 10% in groups of 80 participants each. For groups of 60 and 61 participants, which was the number included in the analysis, the corresponding power is 0.62.

Studies included in the meta-analysis were combined and analysed using Comprehensive Meta-analysis V 2.0 software (Biostat Inc., Englewood, NJ, USA). Individual RRs were calculated for each study, and pooled for global analysis with 95% confidence intervals (CI). In case of heterogeneity global RR were calculated according to Der Simonian and Laird random effect models. Heterogeneity between studies was analysed with Higgins'  $I^2$  and considered to be high if over 50%. Since heterogeneity for both term and preterm pre-eclampsia was 75%, a random effects models was used for all outcomes. For pre-eclampsia, heterogeneity was 14%, and for severe pre-eclampsia, it was 0%. Because of the small number of studies, a funnel plot analysis to assess publication bias was not conducted.

## RESULTS

### Free fatty acid profiles in pre-eclampsia (Study I)

#### Total FFA concentrations

Total FFA concentrations at baseline were 67% higher in pre-eclamptic (569.4  $\mu\text{mol/l}$ ) than in normotensive pregnancies (341.7  $\mu\text{mol/l}$ ) ( $P = 0.0002$ ) (Table 10). This difference was no longer significant after an oral glucose load, which at 2 h had reduced FFA concentrations by 40% in pre-eclamptic women, but only 24% in control women ( $P$  for interaction = 0.084).

**Table 10. Free fatty acids in women with pre-eclampsia and normotensive pregnant women at baseline and at 2 hours after oral glucose intolerance test.**

	Pre-eclampsia geometric mean ( $\mu\text{mol/l}$ )	Normotensive geometric mean ( $\mu\text{mol/l}$ )	Mean difference % (95% confidence interval)	P-value	P-value, adjusted by BMI and gestational diabetes
<b>Total free fatty acids</b>					
<i>Baseline</i>	569.4	341.7	67 (30; 114)	0.0002	0.003
<i>2h</i>	342.7	259.3	32 (-4; 82)	0.08	0.04
<b>Myristic acid (14:0)</b>					
<i>Baseline</i>	19.8	22.3	-12 (-32; 6)	0.36	0.73
<i>2h</i>	12.1	20.4	-41 (-59; 13)	0.009	0.04
<b>Palmitic acid (16:0)</b>					
<i>Baseline</i>	173.0	112.7	48 (4; 110)	0.03	0.06
<i>2h</i>	116.8	92.6	-14 (-37; 16)	0.31	0.53
<b>Palmitoleic acid (16:1 n-7)</b>					
<i>Baseline</i>	24.0	16.3	48 (4; 110)	0.03	0.06
<i>2h</i>	9.0	10.5	-14 (-37; 16)	0.31	0.53
<b>Stearic acid (18:0)</b>					
<i>Baseline</i>	65.1	47.0	39 (5; 83)	0.02	0.51
<i>2h</i>	47.3	45.9	3 (-29; 50)	0.88	0.82
<b>Oleic acid (18:1 n-9)</b>					
<i>Baseline</i>	171.1	97.8	75 (33; 131)	0.0002	0.003
<i>2h</i>	81.4	67.1	21 (-11; 66)	0.22	0.62
<b>Linoleic acid (18:2 n-6)</b>					
<i>Baseline</i>	70.4	30.8	129 (61; 225)	<0.0001	0.0005
<i>2h</i>	41.7	16.9	146 (55; 291)	0.0004	0.003
<b>Arachidonic acid (20:4 n-6)</b>					
<i>Baseline</i>	12.4	3.0	315 (130; 664)	<0.0001	0.001
<i>2h</i>	7.9	2.7	193 (59; 440)	0.001	0.02

BMI = body mass index

### **Individual FFA concentrations**

Table 10 shows the concentrations of total and individual FFAs at baseline and at 2 h. At baseline, the concentrations of individual FFAs were all higher in women with pre-eclampsia, with the exception of myristic acid. The differences between pre-eclamptic and normotensive pregnancies were largest in the concentrations of the oleic (75%), linoleic (129%) and arachidonic (315%) acids. After the oral glucose tolerance test, the concentrations of linoleic and arachidonic acids remained significantly higher in women with pre-eclampsia, whereas myristic acid concentrations were lower. After adjusting for BMI before pregnancy and gestational age the differences of FFA concentrations between pre-eclamptic and control subjects remained similar.

There was a group of FFAs, which were not identified. The concentration of undetermined FFAs was 5.9% in the pre-eclamptic group and 3.4% in the control group.

### **FFA concentrations and insulin sensitivity**

Insulin sensitivity in pre-eclamptic women was 37% lower ( $P=0.009$ ) than in control women as previously reported (20).

### **Cluster analysis in the prediction of pre-eclampsia (Study IV)**

Of the 903 women with risk factors for pre-eclampsia, 86 (9.5%) developed the disease. Of those with pre-eclampsia, 10 (11.6%) had early-onset disease and 36 (41.9%) severe disease. 465 women (51.5%) did not meet any of the primary or secondary outcome criteria, whereas 438 (48.5%) had one or more of these pregnancy complications.

In the control group of 110 women without risk factors, 2 developed pre-eclampsia (1 of them severe and the other non-severe late-onset pre-eclampsia). Of women in the healthy control group, 87% did not meet any of our primary or secondary outcome criteria.

Women with pre-eclampsia in a previous pregnancy, CHT, a SGA newborn, or type 1 diabetes mellitus were at high risk of early-onset, severe, preterm and intermediate pre-eclampsia. Pre-eclampsia in a previous pregnancy and obesity (BMI over 30 kg/m<sup>2</sup>) were the most important single risk factors for term pre-eclampsia. Obese women had an increased risk of any (Odds ratio (OR) 2.1, 95% CI 1.1-3.6), term (OR 2.3, 95% CI 1.1-4.2) and severe (OR 5.2, 95% CI 2.1-10.5) pre-eclampsia. No association with preterm or early-onset pre-eclampsia was observed. Obesity combined with other risk factors, CHT, and type 1 diabetes mellitus further increased the risk of pre-eclampsia. Type 1 diabetes mellitus without other risk factors increased the risk of preterm, but not term pre-eclampsia. Age over 40 or under 20

in a healthy woman without other risk factors did not increase the risk of pre-eclampsia in our cohort. Systemic lupus erythematosus was an inclusion criterion for 4 women and Sjögren's syndrome for 13 women. None developed pre-eclampsia. Women with pre-eclampsia significantly more often had labour induced or caesarean sections, and their newborns had lower Apgar scores.

The risk of pre-eclampsia increased exponentially (linearly on the logarithmic scale) as the number of risk factors increased.

### Vasoactive agents in the prediction of pre-eclampsia (Study III)

We studied PlGF and sFlt-1 (also known as VEGF-1) and the sFlt-1/PlGF ratio in 3 timepoints (12-14, 18-20 and 26-28 weeks of gestation) in 53 women with high pre-eclampsia risk and 53 healthy controls. From these women, 27 developed pre-eclampsia, 6 early-onset and 21 late-onset forms of the disease.

#### PlGF

There were no significant differences in PlGF concentrations between early-onset, late-onset and control groups in the first trimester. By 18<sup>th</sup> to 20<sup>th</sup> weeks of gestation, significant differences in PlGF concentrations occurred between those who developed early-onset pre-eclampsia and those who developed late-onset pre-eclampsia and controls without risk factors for pre-eclampsia (Table 11).

**Table 11. Concentrations of placental growth factor (PlGF) in the three measurements during pregnancy**

Weeks + days of gestation	Early-onset pre-eclampsia (n=6)	Late-onset pre-eclampsia (n=21)	Controls with risk factors (n=26)	Controls without risk factors(n=53)
12 <sup>+0</sup> to 14 <sup>+0</sup>	29.4 (21.0-41.1)	35.3 (30.0-41.3)	37.9 (31.6-45.5)	40.9 (35.3-47.3)
18 <sup>+0</sup> to 20 <sup>+0</sup>	71.9 (52.8-98.1)*	136.7 (112.7-165.8)	132.4 (113.8-154.0)	137.3 (120.9-155.9)
26 <sup>+0</sup> to 28 <sup>+0</sup>	44.6 (25.6-77.8)*	274.2 (222.4-338.1)	271.5 (224.9-327.6)	383.8 (332.3-443.3)

Concentrations (geometric mean, 95% confidence interval) of PlGF in all four study groups.

\*P < 0.05 compared to late-onset pre-eclampsia, controls with risk factors and without risk factors

### sFlt-1

There were no significant differences in sFlt-1 concentrations between early-onset, late-onset and control groups in the first and second trimester. sFlt-1 was higher at the third measurement (26-28 weeks of gestation) in those women who developed early-onset pre-eclampsia (Table 12). sFlt-1 concentrations were higher at all three timepoints in women who developed severe, late-onset pre-eclampsia than in women who developed non-severe, late-onset pre-eclampsia.

**Table 12. Concentrations of soluble vascular endothelial growth factor receptor (sFlt-1) in the three measurements during pregnancy**

Weeks + days of gestation	Early-onset pre-eclampsia (n=6)	Late-onset pre-eclampsia (n=21)	Controls with risk factors (n=26)	Controls without risk factors (n=53)
12 <sup>+0</sup> to 14 <sup>+0</sup>	1006.7 (715.0-1417.8)	924.5 (760.3-1124.3)	930.7 (803.2-1078.4)	1020.0 (875.0-1189.0)
18 <sup>+0</sup> to 20 <sup>+0</sup>	1147.1 (592.2-2221.3)	918.1 (772.9-1090.7)	922.1 (748.4-1143.9)	969.8 (831.8-1128.3)
26 <sup>+0</sup> to 28 <sup>+0</sup>	4847.3* (1318.6-17819.2)	1054.4 (887.2-1252.9)	1034.9 (876.2-1222.4)	1141.3 (975.9-1334.8)

Concentrations (geometric mean, 95% confidence interval) of sFlt-1 all four study groups.

\*P < 0.05 compared to late-onset pre-eclampsia, controls with risk factors and without risk factors

### sFlt-1/PlGF ratio

The ratio of sFlt-1/PlGF was higher at 26-28 weeks of gestation in the group of women who later developed early-onset pre-eclampsia (Table 13). The sFlt-1/PlGF ratio separated women who developed early-onset pre-eclampsia from other pre-eclamptics and controls explicitly. According to our study, all women who developed early onset pre-eclampsia could be identified 4.0 to 6.3 weeks before clinical diagnosis of the disease.

**Table 13. Soluble vascular endothelial factor receptor-1 (sFlt-1) and placental growth factor (PlGF) ratio (sFlt-1/PlGF ratio) in the three measurements during pregnancy**

Weeks + days of gestation	Early-onset pre-eclampsia (n=6)	Late-onset pre-eclampsia (n=21)	Controls with risk factors (n=26)	Controls without risk factors (n=53)
12 <sup>+0</sup> to 14 <sup>+0</sup>	34.4 (22.2-53.3)	26.6 (21.3-33.2)	24.8 (19.7-30.5)	25.9 (22.3-28.0)
18 <sup>+0</sup> to 20 <sup>+0</sup>	15.9 (6.6-38.8)	6.7 (5.2-8.7)	6.9 (5.3-8.9)	7.1 (6.1-8.2)
26 <sup>+0</sup> to 28 <sup>+0</sup>	108.8* (49.5-292.1)	4.0 (3.0-5.4)	3.8 (3.0-4.9)	3.0 (2.5-3.6)

Concentrations (geometric mean, 95% confidence interval) of the sFlt-1/PlGF ratio placental growth factor in all four study groups.

\*P < 0.05 compared to late-onset pre-eclampsia, controls with risk factors and without risk factors.

## **The role of maternal and placental factors in the risk of superimposed pre-eclampsia in chronic hypertension (Study V)**

### **Biomarker data in women with CHT with and without superimposed pre-eclampsia**

Plasma syndecan-1 concentrations were lower at 26<sup>+0</sup>-27<sup>+6</sup> weeks ( $p = 0.03$ ) in women who subsequently developed superimposed pre-eclampsia compared to women with CHT without superimposed pre-eclampsia. This was not seen at earlier gestational timepoints, nor was there any difference over the assessed gestational age range between women with CHT and healthy controls.

Plasma PIGF concentrations (across gestation) were lower in women with CHT who developed superimposed pre-eclampsia compared to those who did not ( $p = 0.002$ ) and was also discriminatory for superimposed pre-eclampsia at 26<sup>+0</sup>-27<sup>+6</sup> weeks ( $p < 0.0001$ ).

The ACR was elevated (across gestation) in women with CHT who subsequently developed superimposed pre-eclampsia compared with women with CHT ( $p = 0.007$ ) or healthy controls ( $p = 0.002$ ), and discriminated between women with CHT who did and did not develop superimposed pre-eclampsia at 12<sup>+0</sup>-13<sup>+6</sup> and 26<sup>+0</sup>-27<sup>+6</sup> weeks ( $p = 0.009$  and  $p = 0.006$  respectively).

There were no differences in any other marker between women with CHT who did and did not develop superimposed pre-eclampsia.

### **Comparisons between women with CHT without superimposed pre-eclampsia and healthy controls**

Plasma NGAL and serum cystatin C concentrations were significantly higher across gestation in women with CHT without superimposed pre-eclampsia than healthy controls ( $p < 0.0001$  and  $p = 0.008$  respectively).

Creatinine clearance was higher across gestation in women with CHT without superimposed pre-eclampsia than healthy controls ( $p = 0.03$ ).

There were no differences in PIGF concentrations between healthy controls and women with CHT without superimposed pre-eclampsia, nor any other markers of cardiac, renal or endothelial function.

## Gestational profile

PIGF concentrations increased with gestation in healthy controls and women with CHT with and without superimposed pre-eclampsia ( $p < 0.0001$ ), except between the second and third timepoints in women who subsequently developed superimposed pre-eclampsia ( $p = 0.70$ ). Similarly, the syndecan-1 concentration also increased with gestation in healthy controls and women with CHT without superimposed pre-eclampsia ( $p < 0.01$ ).

Plasma sialic acid and N-acetyl- $\beta$ -D glucosaminidase (NAG) concentrations also increased with gestation ( $p < 0.0001$ ), but there were no differences between healthy controls and those with CHT with and without superimposed pre-eclampsia.

There were no changes with gestation in the ACR in healthy controls or women with CHT who developed superimposed pre-eclampsia.

BNP concentrations fell as gestation advanced in women with CHT without superimposed pre-eclampsia ( $p = 0.011$ ) and healthy controls ( $p = 0.005$ ) but did not decrease with gestation in women with subsequent superimposed pre-eclampsia.

There were no changes with gestation in creatinine clearance in women with CHT without superimposed pre-eclampsia or healthy controls, but women who developed superimposed pre-eclampsia had a significant increase in creatinine clearance at 18<sup>+0</sup>-19<sup>+6</sup> compared to 12<sup>+0</sup>-13<sup>+6</sup> weeks ( $p = 0.007$ ) and then lower values at 26<sup>+0</sup>-27<sup>+6</sup> weeks ( $p < 0.0001$ ).

Women with CHT with and without superimposed pre-eclampsia demonstrated a decrease in plasma symmetric dimethylarginine (SDMA) clearance at 26<sup>+0</sup>-27<sup>+6</sup> compared to 18<sup>+0</sup>-19<sup>+6</sup> weeks' ( $p = 0.045$  and  $p = 0.001$  respectively), which was not evidenced in healthy controls.

Cystatin C concentrations increased with gestation in all groups ( $P < 0.0001$ ) with no differences between groups.

There were significant increases in the urine NAG:creatinine ratio with gestation in healthy control women ( $p < 0.0001$ ) and women with CHT without superimposed pre-eclampsia ( $p < 0.0001$ ); the change with gestation was less marked in women with CHT who developed superimposed pre-eclampsia (26<sup>+0</sup>-27<sup>+6</sup> vs 12<sup>+0</sup>-13<sup>+6</sup> weeks,  $p = 0.034$ ), but did not discriminate between groups. Similarly, the urinary retinol binding protein (RBP):creatinine ratio increased with gestation in all groups ( $p < 0.001$ ), but no differences between subgroups were seen.

There were no significant differences between groups or with gestation in plasma ADMA concentrations or the SDMA:ADMA ratio.

### **Prediction of superimposed pre-eclampsia**

The highest observed ROC areas for the prediction of superimposed pre-eclampsia were for ACR at 12<sup>+0</sup>-13<sup>+6</sup> weeks 0.87 (95% CI 0.73-1.0) and PIGF at 26<sup>+0</sup>-27<sup>+6</sup> weeks 0.78 (95% CI 0.55-1.00).

### **Validation cohort**

Samples were taken at 37.1 weeks of gestation (interquartile range (IQR): 32.2, 38.3) for women with CHT with superimposed pre-eclampsia, at 34.0 weeks (28.3, 37.3) for women without superimposed pre-eclampsia, at 36.2 weeks (34.2, 38.1) for women with pre-eclampsia without pre-existing disease, and at 37.9 weeks (31.2, 39.2) for healthy controls.

PIGF concentrations were lower in women with superimposed pre-eclampsia than those with CHT and/or CKD ( $p = 0.003$ ), and in women with pre-eclampsia without pre-existing disease compared with healthy controls ( $p < 0.001$ ).

Syndecan-1 was lower with superimposed pre-eclampsia than those with CHT and/or CKD ( $p = 0.001$ ) and after adjustment for gestation remained significantly lower ( $p = 0.005$ ).

Syndecan-1 was also lower in healthy controls than women with CHT and/or CKD without superimposed pre-eclampsia ( $p = 0.045$ ) and after adjustment for gestation ( $p = 0.045$ ). There were no differences in syndecan-1 concentrations ( $p = 0.615$ ) between women with pre-eclampsia compared with healthy controls even after adjustment for gestation.

## Aspirin in the prevention of pre-eclampsia in high-risk women (Study II)

Of the recruited 947 women with risk factors for pre-eclampsia in the PREDO cohort, 152 (16.1%) were allocated into the aspirin trial since they had a bilateral second-degree diastolic notch in the uterine artery flow examination. Of the 152 randomised women initially recruited into the aspirin trial, 121 were included in the final analysis. The baseline characteristics of the 61 women randomly allocated into the aspirin group and 60 women in the placebo group are presented in Table 14. Their pregnancy characteristics are presented in Table 15.

**Table 14. Baseline characteristics of the women in the aspirin trial**

Characteristics	Aspirin group (n=61)	Placebo group (n=60)
Age years (SD)	30.8 (5.3)	31.0 (5.1)
BMI before pregnancy kg/m <sup>2</sup> (SD)	27.9 (6.6)	29.7 (7.8)
Primiparous, n (%)	19 (26.2%)	9 (15.0%)
<b>Educational attainment</b>		
Elementary or less	3 (7.8%)	1 (2.4%)
High school or vocational school	7 (17.5%)	15 (35.7%)
Intermediate	13 (32.5%)	13 (31.0%)
University	17 (42.5%)	13 (31.0%)

SD, standard deviation. BMI, body mass index. Continuous data presented as mean (SD).

**Table 15. Pregnancy characteristics of the women in the aspirin trial**

<b>Characteristics</b>	<b>Aspirin group (n=61)</b>	<b>Placebo group (n=60)</b>	<b>P-value</b>
<b>Antihypertensive medication, n (%)</b>			
<b>Before 20 weeks of gestation</b>	4 (6.6%)	3 (5.0%)	0.8
<b>After 20 weeks of gestation</b>	7 (11.5%)	9 (15.0%)	
<b>Weigh gain during pregnancy, kg (SD)</b>	11.7 (4.7)	12.1 (4.9)	0.6
<b>Gestational diabetes, n (%)</b>			
<b>Diet</b>	10 (16.4%)	9 (15.0%)	0.6
<b>Insulin</b>	1 (1.6%)	3 (5.0%)	
<b>Oral glucose tolerance test performed</b>	6 (9.8%)	5 (8.3%)	
<b>Highest systolic blood pressure, mmHg (SD)</b>	142.5 (19.6)	146.2 (21.9)	0.3
<b>Highest diastolic blood pressure, mmHg (SD)</b>	92.1 (11.8)	95.1 (12.5)	0.2
<b>Highest proteinuria, g/day*</b>	3.3	1.3	0.1
<b>Mode of delivery, n (%)</b>			
<b>Vaginal</b>	47 (77.0%)	43 (71.7%)	0.8
<b>Elective caesarean section</b>	3 (4.9%)	3 (5.0%)	
<b>Caesarean section during labour</b>	11 (18.0%)	14 (23.3%)	
<b>Apgar score at 5 min</b>	9.0 (0.8)	8.9 (0.8)	0.7
<b>Umbilical artery pH below 7.15, ** n (%)</b>	7 (12.5%)	4 (7.4%)	0.6
<b>Newborn birthweight, g (SD)</b>	3413 (630)	3321 (871)	0.5
<b>Placental weight, g (SD)</b>	602 (131)	585 (150)	0.5
<b>Continuous data presented as mean (standard deviation, SD).</b>			
* Geometric mean			
** No umbilical artery pH was below 7.00			

Of those 31 women who discontinued the study, 4 had a miscarriage (3 in the aspirin group and 1 in the placebo group). Eleven women were lost to follow-up or discontinued for various nonmedical reasons (7 of these were in the aspirin group and 4 in the placebo group). Five women decided to discontinue the aspirin trial because of a medical condition (3 of these women were in the placebo group and 2 in the aspirin group). Eleven participants were additionally excluded from the analysis because of noncompliance with the study protocol. The pregnancy outcome for all of these 31 discontinued women is known. We conducted an intention-to-treat analysis, in which we included all randomised women, except the ones who miscarried. The results of the intention-to-treat analysis did not differ from the analysis made without the excluded women.

### Primary outcomes in the aspirin trial

Primary outcomes are shown in Table 16. Altogether 19 (15.7%) women were diagnosed with pre-eclampsia, 8 in the aspirin group and 11 in the placebo group (RR 0.7, 95% CI 0.3-1.7). Sixteen women were diagnosed with gestational hypertension, 10 in the aspirin and 6 in the placebo group (RR 1.6, 95% CI 0.6-4.2). The birthweight SD score in the aspirin group was -0.1 (SD = 1.1) and in the placebo group -0.3 (1.3) (P = 0.3).

**Table 16. Study outcomes in aspirin and placebo groups, and relative risk (RR) with 95% confidence intervals (CI) for each outcome**

	Aspirin n=61	Placebo n=60	RR	95%CI
Primary outcomes				
<b>Pre-eclampsia</b>	8 (13.1%)	11 (18.3%)	0.7	0.3-1.7
<b>Gestational hypertension</b>	10 (16.4%)	6 (10.0%)	1.6	0.6-4.2
Secondary outcomes				
<b>Early-onset pre-eclampsia</b>	1 (1.6%)	4 (6.7%)	0.2	0.03-2.1
<b>Preterm pre-eclampsia</b>	3 (4.9%)	5 (8.3%)	0.6	0.2-2.4
<b>Severe pre-eclampsia</b>	3 (4.9%)	8 (13.3%)	0.4	0.1-1.3
<b>Small for gestational age</b>	2 (3.3%)	6 (10.0%)	0.3	0.1-1.6
<b>Severe diagnosis</b>	4 (6.6%)	10 (16.7%)	0.4	0.1-1.2
Early-onset pre-eclampsia = diagnosed before 34 <sup>+0</sup> Preterm pre-eclampsia = diagnosed before 37 <sup>+0</sup> Severe pre-eclampsia = blood pressure ≥ 160 systolic and/or ≥110 diastolic and/or proteinuria ≥ 5g/24hour Small for gestational age = birthweight < -2 SD Severe diagnosis = early-onset pre-eclampsia and/or severe pre-eclampsia and/or small for gestational age				

### Secondary outcomes in the aspirin trial

Secondary outcomes are shown in Table 16. There was one woman with early-onset pre-eclampsia in the aspirin group and four in the placebo group (RR 0.2, 95% CI 0.03-2.1). Severe pre-eclampsia was diagnosed in three women in the aspirin group and eight women in the placebo group (RR 0.4, 95% CI 0.1-1.3). In the aspirin group two newborns were diagnosed as SGA compared with six in the placebo group (RR 0.3, 95% CI 0.1-1.6). These diagnoses were, in part, seen in the same participants. Four women in the aspirin group and

ten in the placebo group had one or more of these severe diagnoses (early-onset pre-eclampsia and/or severe pre-eclampsia and/or SGA) (RR 0.4, 95% CI 0.1-1.2). Three women in the aspirin group and five in the placebo group (RR 0.6, 95% CI 0.2-2.4) developed preterm pre-eclampsia (diagnosed before 37+0 weeks of gestation). Mean gestational age in the aspirin group was 39.1 weeks (SD = 0.8) and in the placebo group 38.9 (SD = 3.0) (P = 0.6). None of these differences were statistically significant. One woman in the placebo group had HELPP syndrome with early-onset pre-eclampsia. None had eclampsia.

Among the 795 women with clinical risk factors who were included in the follow-up groups, but whose uterine artery Doppler ultrasound waveforms did not fulfil the criteria of the aspirin trial, 66 (8.3%) women developed pre-eclampsia, 24 (3.0%) of these were diagnosed with severe pre-eclampsia and 16 (2.0%) with early-onset pre-eclampsia. Eighty-nine women were diagnosed with gestational hypertension (11.1%). Twenty-four (3.0%) newborns were born SGA and ten (1.3%) women both gave birth to an SGA newborn and were diagnosed with pre-eclampsia. In the control group of 110 women without known risk factors for pre-eclampsia, two (1.8%) developed pre-eclampsia.

### **Adverse effects**

One participant reported sudden deafness in one ear at 24 weeks of gestation. The medication was discontinued and her randomisation code was opened: this participant had received placebo. No other adverse effects were reported.

### **Results of the meta-analysis**

The meta-analysis included two additional studies (246,248). In the meta-analysis aspirin started at or before 16 weeks of gestation in women with abnormal uterine artery measurements, as an indication of increased risk, aspirin significantly reduced the risk of pre-eclampsia (RR 0.6, 95% CI 0.37-0.83), and severe pre-eclampsia (RR 0.3, 95% CI 0.11-0.69). Aspirin did not reduce the risk of preterm (RR 0.2, 95% CI 0.02-1.26) or term pre-eclampsia (RR 1.0, 95% CI 0.25-4.26).

## DISCUSSION

### Main findings

In our cohort, 10.0% of the women with and 1.8% of the women without known risk factors developed pre-eclampsia. Of all pre-eclamptic women, 22.3% had early-onset pre-eclampsia. The most important risk factors for severe and preterm pre-eclampsia in our cohort were pre-eclampsia or an SGA newborn in a previous pregnancy, CHT, and type 1 diabetes mellitus. Pre-eclampsia in a previous pregnancy was the most important risk factor for term pre-eclampsia. Obesity increased the risk for term pre-eclampsia and severe pre-eclampsia. Term and preterm pre-eclampsia had different risk profiles. The more risk factors a pregnant woman had, the stronger her risk of developing pre-eclampsia was. The risk increased exponentially as the number of risk factors increased.

We found that pre-eclamptic women had higher circulating FFA concentrations, which, despite insulin resistance, were suppressed by oral glucose loading. Arachidonic, linoleic, oleic and palmitic acids were higher in pre-eclamptic women compared to controls. This may have an important role in the endothelial cell dysfunction seen in pre-eclampsia, and may also have a role in disturbance of the prostaglandin metabolism, which is associated with pre-eclampsia.

In the group of women with CHT, 15% developed superimposed pre-eclampsia. As an implication of endothelial dysfunction and reduced placental angiogenic capacity, these women had reduced syndecan-1 and PIGF concentrations and an elevated overnight urine:creatinine ratio compared to hypertensive women who did not develop pre-eclampsia and controls without CHT. No differences between groups appeared in renal tubular or cardiac function. Findings of endothelial dysfunction and reduced placental angiogenic capacity are likely predisposing factors for superimposed pre-eclampsia in pregnant women with hypertension.

In our cohort, we showed that PIGF can be used in the prediction of early-onset pre-eclampsia from the 18<sup>th</sup> to 20<sup>th</sup> weeks of gestation and the sFlt-1/PIGF ratio from the 26<sup>th</sup> to 28<sup>th</sup> weeks of gestation. From 26 weeks of gestation, the sFlt-1/PIGF ratio was able to identify all women who later had early-onset pre-eclampsia, without false positives. We also found that serum

sFlt-1 is already significantly elevated in the first trimester in women who developed late-onset, severe pre-eclampsia compared to those who developed late-onset, non-severe pre-eclampsia.

Our placebo-controlled randomized aspirin trial was limited by a small sample size. Of the women who had risk factors for pre-eclampsia, 16.1% were estimated to be high-risk by second degree diastolic notches in the uterine artery velocity waveforms and were randomised in the aspirin trial. In the placebo group, 18.3% developed pre-eclampsia in comparison to 13.1% in the medication group. Although both early-onset and severe pre-eclampsia and SGA infants were more common in the placebo group than in the low-dose aspirin group, the differences between the two study groups were not significant. For the difference to be significant, the risk ratio in the early-onset group needed to be 0.12, instead of 0.2. We conducted a meta-analysis of similar studies, and according to that, low-dose aspirin started at or before the 16<sup>th</sup> gestational week prevents pre-eclampsia and severe pre-eclampsia, with number needed to treat (NNT) 6 and 12, respectively.

### **Strengths and limitations of the study**

The strengths of the study include the well-characterized study cohorts. In the study of FFA profiles (Study I) the case and control groups were relatively homogenous, women with gestational diabetes were excluded, and insulin sensitivity was assessed by an intravenous glucose tolerance test. The sample size of the study was limited due to the labor-intensive design. Moreover, we did not measure FFA in cord blood, which would have added on the provided information on FFA metabolism from the fetal side of the placenta.

The PREDO study cohort used in studies II-V consists of prospectively recruited women with risk factors for pre-eclampsia and controls without known risk factors. However, the number of women who developed pre-eclampsia was rather small, even though we studied women with increased risk of pre-eclampsia. The aspirin trial (Study II) was limited because of the relatively small sample size. The main reason for that was the use of second-degree uterine artery notch as a criterion in selecting women in the medication group. Our objective was to distinguish women with the highest risk, however, the number of women who fulfilled this inclusion criterion was unexpectedly small, only 16%. Furthermore, the number of women who developed pre-eclampsia was also unexpectedly small. The rationale for conducting the meta-analysis was to incorporate the findings of our study with the scientific literature despite

the small sample size. The high homogeneity for pre-eclampsia and severe pre-eclampsia suggested valid findings.

We initiated the study of vasoactive markers (Study III) at a point when only some of the total participants had final outcomes verified by the jury, which limited the available sample size. Since we did not collect samples after the 30<sup>th</sup> week of gestation we were unable to study the changes of the biomarkers in women who developed late-onset pre-eclampsia close to the established disease.

For the cluster analysis (Study IV), we had limited information on the onset, severity and recurrence of pre-eclampsia in the previous pregnancies. Therefore, we were unable to incorporate this information of possible previous pre-eclampsia in estimating the risk.

In the study of predisposing factors to superimposed pre-eclampsia (Study V), *in vivo* assessment of function using cardiac indices, endothelial function and formal measurement of glomerular filtration would have confirmed the relationship between pre-existing disease and the development of superimposed pre-eclampsia.

## **Interpretation of the study**

In the PREDO cohort 10.0% of the women with risk factors for pre-eclampsia developed the disease. Of those, 11.6% had early-onset pre-eclampsia and 41.9% had severe disease. Half of the women at risk met one or more of the primary or secondary outcome criteria. In contrast, from 110 control women without known risk factors for pre-eclampsia, 2 developed pre-eclampsia (1 had severe and 1 non-severe late-onset pre-eclampsia). Of the control women, 87% did not meet any of the primary or secondary outcome criteria.

In our study, women who developed early-onset and/or severe pre-eclampsia had different risk profiles compared to term pre-eclampsia. CHT was a stronger predictor of early-onset and severe pre-eclampsia. The results were consistent with earlier studies (249). A pre-existing endothelial dysfunction that predisposes to placental vascular insufficiency may contribute to the development of superimposed pre-eclampsia in CHT. Maternal diabetes-related vascular and metabolic dysfunction predisposes women with type I diabetes mellitus to pre-eclampsia (250). In our cohort, women with type I diabetes mellitus had an increased risk of severe, early-onset and preterm pre-eclampsia. Klemetti and colleagues (250) reported an increased risk for pre-eclampsia in women with diabetes classified as White's class B to F. Classes B to F include diabetes which existed before pregnancy to disease with diabetic nephropathy. A history of giving birth to an SGA newborn increased a risk of early-onset, preterm and severe pre-eclampsia. Conversely, preterm pre-eclampsia in a previous

pregnancy has been shown to be associated with having a SGA newborn in a subsequent pregnancy without pre-eclampsia (251) which reflects the common placenta-derived pathogenesis of these outcomes (252). The outcome is modified by the absence or presence of maternal metabolic factors. Previous pre-eclampsia was a strong risk factor for all types of pre-eclampsia. According to earlier studies, women with early-onset pre-eclampsia have even higher risk of recurrent pre-eclampsia than women with late-onset disease (85,253). Moreover, women with a previous preterm pre-eclampsia have an increased risk of adverse pregnancy outcomes in the second pregnancy even in the absence of pre-eclampsia (254). A previous normal pregnancy is associated with a reduced risk of pre-eclampsia in subsequent pregnancy (255). The strongest risk factor for term pre-eclampsia was previous pre-eclampsia, and accompanied with high BMI, the risk increased. Altogether, the risk for pre-eclampsia increased exponentially as the number of risk factors increased.

### **Vasoactive agents in the prediction of pre-eclampsia in the first trimester**

Our study of the vasoactive agents was relatively small, however, there are only a few comparable studies with prospectively-collected serial samples. We studied PIGF and sFlt-1 at three timepoints (12-14, 18-20 and 26-28 weeks of gestation). We did not show differences between early-onset, late-onset and control groups in the first trimester. In larger studies, it has been shown that women who develop preterm or term pre-eclampsia already have significantly lower concentrations of PIGF in the first trimester (96,256-259). Others have demonstrated low first trimester PIGF concentrations only in women who developed early-onset or preterm preeclampsia (260,261). Recently, in a larger substudy of the PREDO project (Murtoniemi et al., unpublished), women with subsequent early-onset pre-eclampsia already had significantly lower PIGF concentration than women who developed late-onset pre-eclampsia or controls in the first trimester samples. In a recent study by Vieira and colleagues (262) low plasma PIGF concentrations in early pregnancy was associated with subsequent preterm and term pre-eclampsia in obese women, but only with preterm pre-eclampsia in non-obese women. However, PIGF concentrations are not sensitive enough to be used alone in the prediction of pre-eclampsia in the first trimester. Combined with clinical risk factors and physical measurements, PIGF performs well in the early prediction of pre-eclampsia. Different calculation models have been introduced and some are in commercial use. These tests promise significant specificity and sensitivity. These calculation models are more sensitive in predicting early-onset and severe pre-eclampsia. The pathology of those subgroups is more of placental origin. The group of women with late-onset pre-eclampsia is

more heterogenous, they more often have the maternal type or the non-angiogenic type of pre-eclampsia without early placental changes and markedly reduced PIGF concentrations.

### **Vasoactive agents in the prediction of pre-eclampsia in the second and third trimester**

By the 18<sup>th</sup> to 20<sup>th</sup> weeks of gestation there were differences in PIGF concentrations between those who developed early-onset pre-eclampsia and those who developed late-onset pre-eclampsia and controls. In all three groups PIGF concentration rose between the first and second sampling, however, in the early-onset group PIGF concentration rose less steeply, and before the third measurement, the concentration almost halved. In the late-onset group, PIGF concentrations rose in the same manner as in the control groups. As with PIGF, in the analysis of sFlt-1, similar groups were seen, with significant differences only between the early-onset pre-eclampsia group and others. There were only slightly rising concentrations of sFlt-1 in the longitudinal samples of the other groups, but in the early-onset pre-eclampsia group, the sFlt-1 concentration rose steeply between the second and third sampling, indicating impending clinical disease. This is in line with most other studies (17,258,263). One study found a sequential change of sFlt-1 between the first and second trimester to be strongly predictive of pre-eclampsia (264).

We were able to identify, four to six weeks prior to the clinical diagnosis of pre-eclampsia, all women who developed early-onset disease. A cut-off value of 40 for the sFlt-1/PIGF ratio distinctly identified women who developed early-onset from those who developed late-onset disease. This same finding has been observed by others (97). The sFlt-1/PIGF ratio could be used in the follow-up of women at high risk of pre-eclampsia in an asymptomatic stage (97) and in clinical decision making in women with signs and symptoms of pre-eclampsia (17,265-268). Stepan and colleagues (97) suggested that in asymptomatic high-risk women with an sFlt-1/PIGF ratio under 38, pre-eclampsia could be ruled out for at least a week. Women with a ratio between 38-85 are at high risk of developing pre-eclampsia within 4 weeks in an early-onset setting and should be followed up in 1 to 2 weeks time. In late-onset pre-eclampsia, the intermediate result of the ratio (38-110) is suggestive of impending placental dysfunction and induction of delivery should be considered. Additionally, the sFlt-1/PIGF ratio may add valuable information regarding the clinical course and progression of the established pre-eclampsia. In women with suspected or diagnosed pre-eclampsia, measurement of the sFlt-1/PIGF ratio can assist in clinical decisions. Again, a ratio under 38 rules out pre-eclampsia for at least 1 week. In women with a ratio over 85 in an early-onset situation and over 110 in a late-onset situation, a diagnosis of pre-eclampsia or other placenta-related disorder is highly probable. In that case, repeated measurements 2-4 days apart would distinguish if the disease will progress rapidly, with a steeply rising sFlt-1/PIGF ratio, or if it will be rather stable with

ratio not elevating steeply, whereupon a very frequent follow-up is not needed. In the stable situation, the authors recommend sFlt-1/PlGF ratio re-measurement in two weeks. A severely elevated sFlt-1/PlGF ratio, over 655 in early-onset and over 201 in late-onset pre-eclampsia, are associated with the need to deliver within 48 hours.

The PreOS study (269) experimented with the influence of the sFlt-1/PlGF ratio on clinical decision-making in more than 200 women with suspected pre-eclampsia. They reported that, in clinical situations, it influenced the decision making towards appropriate hospitalisation. In the PreOS study, among women who had suspected pre-eclampsia, only one in five actually developed pre-eclampsia. They also reported an increase in pre-eclampsia-related maternal and fetal outcomes with increasing sFlt-1/PlGF ratio. Rana and colleagues (270) prospectively studied 616 women who were evaluated with suspected pre-eclampsia. They concluded that of the 167 women presenting with pre-eclamptic symptoms before 34 weeks of gestation, the sFlt-1/PlGF ratio significantly improved the prediction of adverse outcomes occurring within 2 weeks. From women who had a Flt-1/PlGF ratio over 85, 86.5% delivered within 2 weeks, compared with 15.5% of women with ratio under 85. Furthermore, in women with an atypical pre-eclampsia presentation, with relatively normal blood pressure or no proteinuria, this test performed well. The sFlt-1/PlGF ratio was inversely correlated with the remaining duration of pregnancy.

It seems that the sFlt-1/PlGF ratio has the potential to assist in planning the actions in high-risk women and in women with established pre-eclampsia. However, the different laboratory assays may give differing concentrations of sFlt-1 and PlGF, which impacts the cut-off values. The threshold values of singleton pregnancies do not work in twin pregnancies (271). As a clinical implement in the screening of threatening pre-eclampsia, the sFlt-1/PlGF ratio is promising and in need of clinical evaluation.

### **Superimposed pre-eclampsia and the role of endothelial and placental factors**

We studied endothelial, cardiac, renal and placental biomarkers in longitudinal samples of women with CHT to assess their relative contribution to the development of superimposed pre-eclampsia. Few previous studies have explored the influence of pre-existing maternal disease on developing superimposed pre-eclampsia (272). In healthy controls and in women with CHT without superimposed pre-eclampsia, plasma syndecan-1 and sialic acid concentrations increased with gestation. Previously, syndecan-1 protein expression has been demonstrated in the placenta, localised to the syncytiotrophoblast interface (273,274). Elevated plasma concentrations of syndecan-1 in normal pregnancy could reflect placental release. However, increased endothelial permeability, evident even in a healthy pregnancy, is

suggestive of systemic glycocalyx changes. We found that women who developed superimposed pre-eclampsia were characterised by a failure to sustain a normal gestational increase in the plasma syndecan-1 concentration, a marker of endothelial glycocalyx function, in parallel with the reduction of PIGF. Furthermore, women with CHT or CKD had lower plasma syndecan-1 concentrations after diagnosis of superimposed pre-eclampsia compared to those without. This may reflect a decline in placental or systemic synthesis of glycocalyx constituents and an inability to maintain the protective glycocalyx barrier in those with pre-existing endothelial injury. Reduced plasma syndecan-1 concentrations may also occur as a precursor to the onset and contribute to the clinical manifestations of pre-eclampsia. The glycocalyx barrier may become exhausted and thereby contribute to the endothelial manifestations. The estimation of glycocalyx function in women with CHT has not been previously done. In pregnancy, and particularly in pre-eclamptic compared to healthy women, modulators of the glycocalyx, including shear stress, tumour necrosis factor- $\alpha$ , reactive oxygen species and matrix metalloproteinases increase (275). We found an increase of NAG in all groups in our study (Study V), a surrogate biomarker of lysosomal enzyme release, including neuraminidase. These lysosomal enzymes are capable of endothelial glycocalyx disruption. Syndecan-1 is a natural competitive inhibitor of neuraminidase, hence, a reduction in syndecan-1 concentration in those with superimposed pre-eclampsia could lead to further glycocalyx destruction. There is also evidence for reduced expression of syndecan-1 by trophoblasts in women with pre-eclampsia, which reflects reduced placental synthesis (273,276).

Women who developed superimposed pre-eclampsia had lower plasma levels of PIGF compared to other groups. This is consistent with earlier findings (277) but not all (278). PIGF-2 (a splice variant of PIGF) has a potent heparan binding domain (279) which may contribute to glycocalyx maintenance and could also contribute to a reduction in syndecan-1 concentration in those who develop pre-eclampsia.

Microalbuminuria is strongly associated with several factors (e.g., obesity) that are prevalent in women with CHT, and endothelial dysfunction is a proposed common mediator. The overnight urinary ACR was predictive in hypertensive women who developed pre-eclampsia compared to those who did not. This offers an inexpensive way, already in the early pregnancy, to evaluate the risk of pre-eclampsia when planning how to monitor the pregnancy of a women with CHT. This association between ACR in early pregnancy and later developing superimposed pre-eclampsia supports the implication that pre-existing systemic endothelial injury plays a role in the development of pre-eclampsia. However, other markers of endothelial, cardiac or renal function did not differ significantly between women with CHT

who did or who did not develop superimposed pre-eclampsia. Women with CHT without superimposed pre-eclampsia had higher glomerular filtration rates estimated by creatinine clearance than healthy controls. This may reflect impaired autoregulation. In early hypertension, which occurs prior to development of CKD, hyperfiltration is an identified feature, which may be the consequence of fewer functional nephrons (280).

Our findings support the hypothesis that pre-existing endothelial dysfunction, assessed by overnight ACR, contributes to the development of superimposed pre-eclampsia in women with CHT. Reduced shedding of syndecan-1 and decreased PIGF concentrations antedate disease development, as well, implicate endothelial glycocalyx disturbance and reduced angiogenic capacity in the pathophysiology of pre-eclampsia.

### **Free fatty acids**

We found that total concentrations of FFAs were significantly higher in those women who were diagnosed with pre-eclampsia than in healthy controls. Of the individual fatty acids, arachidonic, linoleic, oleic and palmitic acids were significantly higher in pre-eclamptic patients. These high concentrations of FFAs may influence several characteristics of pre-eclampsia, for example increased insulin resistance, endothelial cell dysfunction and altered production of vasoactive substances.

Most earlier studies have only evaluated total FFA concentrations in pregnant women, however, individual FFAs have divergent effects. FFA concentrations were already altered 10-20 weeks before the onset of pre-eclampsia in Lorentzen's and colleagues study (173,177). They showed elevated levels of palmitic, oleic and linoleic acids in women who later developed pre-eclampsia, as well, as in pre-eclamptic women (177). They also showed that there were no differences between early- and late-onset pre-eclampsia groups in FFA concentrations. Insulin resistance (20) and increased adrenergic activity (281), characteristics of pre-eclampsia, are both associated with increased lipolytic activity in adipocytes, which possibly underlies the increased circulating levels of FFAs in pre-eclampsia. We did not find a relationship between total FFA concentrations and insulin sensitivity, as was expected. In pre-eclamptic women, higher insulin sensitivity was associated with higher total FFA concentrations. However, this relationship was no longer significant after adjustment for BMI and gestational age. The interplay between insulin sensitivity and FFAs in pre-eclamptic women may be regulated by other factors, for example placental hormones. A comparable phenomenon is seen with adipokines, adiponectin and adipocyte fatty acid-binding protein (AFABP), which are all elevated in pre-eclamptic women (282,283). In the non-pregnant

state, low adiponectin and high AFABP concentrations are associated with insulin resistance (284) however, in pregnancy they show no relationship with insulin sensitivity (282,283).

#### *FFA and endothelial function*

In the non-pregnant state, altered lipid metabolism is a known cause of endothelial dysfunction, potential vascular remodelling and atherosclerosis (285). Altogether, an increase in plasma glucose, insulin and lipids is associated with endothelial dysfunction (285). An increased molar FFA to serum albumin ratio leads to enhanced endothelial cell uptake of FFAs and enhanced flux of FFAs into the liver. FFAs are esterified into triglycerides in both the endothelial cells and in the liver (286). Consequently, the liver produces increased amounts of triglyceride-rich very low density lipoprotein (VLDL) particles into the plasma, further enhancing the accumulation of triglycerides in the endothelial cells (287). *In vitro* exposition of endothelial cells to hyperlipidemic pre-eclamptic sera increases the cellular triglyceride content and is accompanied by altered endothelial function (288). The accumulation of triglycerides in endothelial cells is a common feature of pre-eclampsia (19) and may harm endothelial cell function. The mechanisms behind the abnormal elevation of triglycerides and FFAs in pre-eclampsia are unknown. One theory proposes that the increased insulin resistance, common in pre-eclampsia, results in elevated FFAs and triglycerides by increasing fatty acid mobilisation from visceral fat, promoting overproduction of VLDLs by the liver, and suppressing postheparin lipoprotein lipase (160,162,289).

#### *FFAs, prostaglandins, and inflammation*

Arachidonic acid, which we found to be increased in pre-eclamptic women, is a precursor of vasoactive prostaglandins (prostacyclin and thromboxane). Prostacyclin is produced mainly in endothelial cells and is a vasodilator and inhibits platelet aggregation (290). Thromboxane is produced mainly in platelets and has vasoconstrictive and platelet aggregatory effects (291). Pre-eclampsia is characterised by disturbances in the prostacyclin-thromboxane ratio. Previous studies show that increased arachidonic and linoleic acid in pre-eclamptic women, as seen in our study, could lead to a disturbed prostacyclin-thromboxane ratio. High linoleic acid concentrations impede the ability of the endothelium to produce prostacyclin and inhibit platelet aggregation (292). Endothelial cells exposed *in vitro* to linoleic acid show a concentration-dependent reduction in the release of prostacyclin (292). In a study by Lorentzen and colleagues reduced prostacyclin release was demonstrated when endothelial

cells were treated with sera from pre-eclamptic women (288). The preventative effect of low dose aspirin on pre-eclampsia is through its action on prostaglandins. It irreversibly inhibits the production of thromboxane by the platelets, but does not affect the production of prostacyclin.

An alternative pathway of arachidonic acid metabolism produces lipoxygenase products, leukotrienes (293). Leukotrienes could partly be responsible for the inhibition of prostacyclin production, hypertension and the increased endothelial permeability seen in pre-eclampsia (294,295). Leik and Walsh (296) showed that when placental smooth muscle cells are exposed to linoleic acid in conditions of oxidative stress, the production of interleukin-8 (IL-8), a potent inflammatory mediator, is stimulated. Furthermore, the production of IL-8 is increased in the placental vascular smooth muscle cells of pre-eclamptic women when exposed to linoleic acid (297).

Our samples for FFA analysis were collected at the time of the diagnosis, thus, assumptions on the performance of the FFAs in the early prediction of pre-eclampsia can not be made based on this study. However, it has been shown by others that elevated serum triglycerides and FFAs are already increased before 20 weeks of gestation in women who go on to develop pre-eclampsia (173,176). These elevated FFA concentrations return to non-pregnant levels two to three days after labour in both normal and pre-eclamptic pregnancies (174). FFA profiling may be a potential predictive method. With a metabolomics method such as nuclear magnetic resonance (NMR) and mass spectrometry, it is possible to measure the distribution of lipids in large sets of samples. In future, this may assist in the identification of different lipid risk profiles of pre-eclampsia in the early weeks of gestation.

### **Low dose aspirin in the prevention of pre-eclampsia**

Low dose aspirin started in the 12<sup>+0</sup> to 13<sup>+6</sup> weeks of gestation did not prevent pre-eclampsia in our high-risk cohort. In the placebo group, we had more women diagnosed with early-onset and severe pre-eclampsia, and more SGA newborns, than in the low dose aspirin group, however, the differences between groups were not statistically significant. We performed a meta-analysis of similar aspirin trials. Only two additional placebo-controlled trials with women whose uterine artery measurements indicated a high risk had been conducted earlier. In these studies, low dose aspirin had been started at gestational weeks 12<sup>+0</sup> to 16<sup>+0</sup>. According to the meta-analysis, women in the low dose aspirin group had a risk ratio of 0.6 (95% CI 0.4-0.8) for pre-eclampsia and 0.3 (95% CI 0.1-0.7) for severe pre-eclampsia.

A wide range of studies on aspirin in the prevention of pre-eclampsia has been published. There are considerable differences between these trials and discrepancies in conclusions. Studies differ on the weeks of gestation the medication is initiated, the dose, the risk status, and on the subgroup analyses. In the earlier trials particularly, low-dose aspirin was started in any week of gestation, even when the first symptoms of pre-eclampsia already emerged. At present, according to meta-analyses, there is evidence that medication should be started before 16<sup>+0</sup> weeks of gestation (192,195,298) however, there are contradictory studies (190,299,300). There are two active phases of trophoblast invasion during placentation, the first of which ends at the 10<sup>th</sup> week of gestation. The second active phase starts between the 14<sup>th</sup> and 15<sup>th</sup> weeks of gestation and ends around the 18<sup>th</sup> week of gestation (65,301,302) however, active endovascular trophoblasts are seen as late as 22<sup>th</sup> weeks. Aspirin is thought to have a positive influence on the development of the placenta when medication is initiated well before the end of the second active phase. The current recommendations suggest the initiation between 12<sup>+0</sup> to 16<sup>+0</sup> weeks of gestation (Table 5). It may be speculated whether the optimal time of initiation is even earlier. Because all medication in the first trimester of gestation, the period of organogenesis, is generally avoided, at this point, this would seem a challenging proposal. However, low-dose aspirin is already used in the first weeks of gestation, and for example, pre-conceptionally as part of in vitro fertilisation treatments (303-305). No problems have been reported. One small study of very early-onset aspirin treatment, at 8 to 10 weeks of gestation, has been published (306). That included 82 women with aspirin treatment and 82 controls. The risk of hypertensive disorders was reduced to 0.07 (95%CI 0.01-0.5). Meher and colleagues speculated in their recent meta-analysis (299) that aspirin should be initiated even after 16 weeks of gestation among high risk women for whom it has not yet been initiated.

The dose of aspirin is important. A wide range, between 50-150 mg per day, is used. Vainio and colleagues (183) studied the favourable dose of aspirin that inhibits the production of vasoconstrictive thromboxane in platelets, but does not affect the production of vasodilatory prostacyclin in the vessel wall. They gave 0.5, 1.0 and 2.0 mg/kg/day of aspirin to hypertensive and control women and measured major stable metabolites in their urine. They concluded that with a daily dose of 0.5-2 mg/kg aspirin, the prostacyclin:thromboxane ratio improved. This means that 100 mg of aspirin is appropriate for most pregnant women. In speculating on the dose, we should take into account the phenomenon of aspirin resistance (307). In patients with cardiovascular disease, the prevalence of aspirin resistance is estimated to be significant; according to a meta-analysis, 28% of patients had aspirin resistance (204). The existence of aspirin resistance should be considered when initiating aspirin treatment to high-risk women. Platelet function assays targeted to aspirin-COX pathways may be used to

demonstrate platelet function, and compliance to treatment may be determined by measuring salicylate levels, however, these have not been assessed in pregnant women (178).

Aspirin trials and meta-analyses can be divided into two types based on the risk status of women included. The treatment may be initiated in women who are estimated to belong in a high-risk group or to a general group of women with an unknown risk status. In large trials or meta-analyses on general populations of pregnant women, the effect of aspirin does not seem very high (190,300). When limited to an at-risk group of women, the effect is higher (192,194,195,298,308) as seen also in our meta-analysis (Table 17). However, by conventional methods of evaluating the risk status, medical history and physiological measurements, the nulliparous women are largely left out of these preventive procedures. By determining the risk status with a model of medical history, uterine artery measurements and predictive markers in all pregnant women at first ultrasound screening, alongside the trisomy screen, low dose aspirin could be offered to women at high risk.

As our understanding of the pathology of pre-eclampsia has improved, subgroup analyses in aspirin trials and meta-analyses has become more common. Pre-eclampsia is not one disease, but a syndrome with different aetiologies and pathological processes. Pre-eclampsia is divided by its onset or time of iatrogenic delivery, severity, existence of vasoactive agents, and by estimation of placental or maternal disease. The clinical disease is due to a common pathway, endothelial dysfunction. Due to the differences, there might not be one preventative medication. It seems that aspirin's preventative action works most efficiently in the placental/early-onset/severe/angiogenic subgroup. The explanation for this may be regarding aspirin's actions on the placenta via prostacyclin:thromboxane ratio, stimulation of the expression and enzymatic activity of HO-1 in endothelial cells (184,185) reduction of ADMA concentration (184,185) and dose-dependent inhibition of sFlt-1 production in cytotrophoblasts via COX-1 inhibition (187).

Meta-analysis is a powerful tool in summarising data and increasing the sample size. However, there are problems which should be taken into account when planning and interpreting results of a meta-analysis (309). There may be bias through the identification and selection of studies, quality of the included studies, large degree of heterogeneity of included studies, small study effect and publication bias. Due to these problems with meta-analysis interpretation, a desire for a large-scale aspirin trial has emerged (310). The ASPRE trial has been established to answer the questions left after meta-analyses, and interesting results have recently been announced from the ASPRE project (311).

**Table 17. Meta-analyses of low-dose aspirin trials.**

	<b>Number of participants</b>	<b>Risk status at the initiation of aspirin</b>	<b>Initiation of medication</b>	<b>Subgroups</b>	<b>Risk ratio (95% CI)</b>
<b>Duley L et al. 2004</b> Cochrane database Updated 2007	59 trials 37,560 participants	No limitations	1st to 3rd trimester	Pre-eclampsia Aspirin dose > 75mg/day Aspirin dose ≤ 75 mg/day	0.83 (0.77-0.89) 0.66 (0.51-0.80) 0.88 (0.81-0.95)
<b>Askie LM et al. 2007</b> PARIS Collaborative Group	31 trials 32,217 participants	No limitations	1st to 3rd trimester	Pre-eclampsia	0.90 (0.83-0.98)
<b>Bujold E et al. 2010</b>	9 trials 764 participants	High-risk women	Before 16 <sup>+0</sup> weeks of gestation	Pre-eclampsia Severe pre-eclampsia	0.47 (0.34-0.65) 0.09 (0.02-0.37)
	18 trials 10,584 participants		After 16 <sup>+0</sup> weeks of gestation	Pre-eclampsia Severe pre-eclampsia	0.81 (0.63-1.03) 0.26 (0.05-1.26)
<b>Rossi AC et al. 2011</b>	7 trials 10,729 participants	High-risk women	1st to 3rd trimester	Pre-eclampsia	0.72 (0.51-1.00)
	3 trials 9901 participants	Low-risk women		Pre-eclampsia	0.82 (0.65-1.04)
<b>Roberge S et al. 2012</b>	4 trials 392 participants	High-risk women	Before 16 <sup>+0</sup> weeks of gestation	Severe pre-eclampsia Non-severe pre-eclampsia	0.22 (0.08-0.57) 0.81 (0.33-1.98)
<b>Roberge S et al. 2012</b>	5 trials 556 participants	High-risk women	Before 16 <sup>+0</sup> weeks of gestation	Pre-eclampsia before 37 <sup>+0</sup> Pre-eclampsia after 37 <sup>+0</sup>	0.11 (0.04-0.33) 0.98 (0.42-2.33)
<b>Villa PM et al. 2013</b>	3 trials 346 participants	High-risk women	Before 16 <sup>+0</sup> weeks of gestation	Pre-eclampsia Severe pre-eclampsia	0.55 (0.37-0.82) 0.27 (0.11-0.69)
<b>Xu T et al. 2015</b>	29 trials, 21,403 6 trials, 3867	High-risk women	1st to 3rd trimester	Pre-eclampsia Severe pre-eclampsia	0.71 (0.57-0.87) 0.37 (0.23-0.61)

<b>Roberge S et al. 2016</b>	21 trials 5130 participants	High-risk women	Before 16 <sup>+</sup> weeks of gestation	Pre-eclampsia	0.57 (0.43-0.75)
				Severe pre-eclampsia	0.47 (0.26-0.83)
				Fetal growth restriction In above groups the effect was dose-dependent	0.56 (0.66-0.70)
	27 trials 15,779 participants	High-risk women	After 16 <sup>+</sup> weeks of gestation	Pre-eclampsia	0.81 (0.66-0.99)
				Severe pre-eclampsia	0.85 (0.64-1.14)
				Fetal growth restriction There was no dose-dependency	0.95 (0.86-1.05)
<b>Meher S et al. 2017</b>	17 trials 9241 participants	High risk women	Before 16 <sup>+</sup> weeks of gestation	Pre-eclampsia	0.9 (0.79-1.03)
	22 trials 21,429	High risk women	After 16 <sup>+</sup> weeks of gestation	Pre-eclampsia	0.9 (0.83-0.98)

ASPREE is a prospective randomised controlled trial with a plan to recruit a large number of participants (over 30,000) who are screened for pre-eclampsia risk. From the screening, those 10% of women who were considered in the high-risk group, were randomized to initiate aspirin with 150 mg dose per day or placebo, between the 12<sup>th</sup> to 16<sup>th</sup> weeks of gestation. From the preliminary results, with approximately 800 women in each study group, the risk of early-onset pre-eclampsia (before 34 weeks) (unpublished) and preterm pre-eclampsia (before 37 weeks) (311) were significantly lower in the aspirin group. The reduction of pre-eclampsia was reported 80% and 62%, respectively.

#### *Cost-benefit of low dose aspirin treatment*

The cost-benefit of aspirin treatment has been analysed by Werner and colleagues (312). They created a decision model to evaluate four approaches to aspirin prophylaxis in the United States in approximately 4 million pregnant women: no prophylaxis, universal prophylaxis, ACOG recommendations, and U.S. Preventive Services Task Force (USPSTF) recommendations (Table 5). They included the costs associated with aspirin, pre-eclampsia, preterm birth, and potential aspirin-associated adverse effects and concluded that the USPSTF approach was the most cost-beneficial and the second was universal prophylaxis. These would reduce morbidity, save lives, and lower health care costs much more effectively than the ACOG approach, with markedly tighter limits of aspirin administration. Therefore, this outcome supports the recommendation for quite unrestricted use of low dose aspirin when using clinical risk factors as the only tool in risk -evaluation.

## **FUTURE DIRECTIONS**

Further studies in low dose aspirin are needed:

- The most effective aspirin dose is still undetermined; for example, it is unknown whether it should be adjusted based on body size. The effect of aspirin resistance should also be taken into account.
- There is little information about the long-term influence of aspirin in the child's future health. Longitudinal follow-up of previous and future studies are needed to evaluate that.

Further studies on the prediction of pre-eclampsia:

- First trimester prediction models of pre-eclampsia combined with low dose aspirin treatment should be tested in clinical work and the impact on morbidity of the women and newborns should be studied.
- Studies should examine how the follow-up of at-risk women (determined by sFlt-1/PlGF ratio measurements in the second trimester) could influence and aid in clinical decision making and in the follow-up of women with established pre-eclampsia.
- New techniques may be introduced in pre-eclampsia prediction from metabolomics and proteomics.
- In the future, risk profiling that could individually suggest a specific prophylaxis, for example aspirin or a statin, on the basis of the woman's profile may be possible.

## CONCLUSIONS

In PREDO cohort early-onset and late-onset pre-eclampsia had different risk profiles. Women who have had pre-eclampsia in a previous pregnancy, or SGA newborn in a previous pregnancy, or who had CHT or type 1 diabetes mellitus were at the highest risk of early-onset pre-eclampsia, severe pre-eclampsia and preterm pre-eclampsia. Pre-eclampsia in a previous pregnancy and a BMI over 30 kg/m<sup>2</sup> were the most important risk factors for term pre-eclampsia. As the number of risk factors increased, the risk of pre-eclampsia increased exponentially.

Low serum PIGF predicted early-onset pre-eclampsia from 18 weeks of gestation. There were no differences in PIGF in the first trimester samples between the study groups. However, in larger studies, in which PIGF is combined with clinical risk factors and physical measurements, PIGF performs well in the prediction of pre-eclampsia already in early pregnancy.

The sFlt-1/PIGF ratio identified women who went on to develop early-onset pre-eclampsia weeks before the onset of clinical findings. This may help to plan individual follow-up in high-risk women. The sFlt-1/PIGF ratio measurements can also help in the clinical decision making among women with signs and symptoms of pre-eclampsia.

Higher overnight urinary ACR was predictive of superimposed pre-eclampsia in women with CHT already in the first trimester. This association between ACR in early pregnancy and superimposed pre-eclampsia suggests that pre-existing endothelial dysfunction in women with CHT contribute to the development of pre-eclampsia.

Reduced shedding of syndecan-1 and reduce PIGF concentrations antedated disease development in women with CHT, implicating the role of endothelial glycocalyx disturbance and reduced angiogenic capacity in the pathophysiology of superimposed pre-eclampsia.

Circulating FFA concentrations were higher in pre-eclamptic than in control women. Despite insulin resistance, FFAs were suppressed by oral glucose loading. Among individual FFAs, arachidonic- and linoleic acid were at the highest concentrations. These may influence several

characteristics of pre-eclampsia, for example increased insulin resistance, endothelial cell dysfunction and altered production of vasoactive substances.

We did not show any effect of low dose aspirin on prevention of pre-eclampsia in the PREDO study cohort. However, the number of women with pre-eclampsia and, consequently, study power were unexpectedly low. In a meta-analysis of studies, that included women with abnormal uterine artery measurements and treatment started before 16<sup>+0</sup> weeks of gestation, low dose aspirin reduced the risk of pre-eclampsia and severe pre-eclampsia. Moreover, recent studies by others support the initiation of low dose aspirin before 16<sup>+0</sup> weeks of gestation in high-risk women. Low dose aspirin reduces the risk of early-onset and severe pre-eclampsia and, based on current knowledge should be recommended to initiate in high-risk women before 16<sup>+0</sup> weeks of gestation.

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A handwritten signature in black ink, appearing to be 'Pia Villa', with a long horizontal flourish extending to the right.

Pia Villa

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