Experimental and Clinical Studies of Oxidative Stress in Pre-Eclampsia

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Abstract


Impaired placentation and oxidative stress are proposed to play major roles in the pathogenesis of pre-eclampsia (PE). It has recently been pointed out that PE might be more than one disease and may have several different pathogeneses. This thesis describes a new animal model for PE and examines the role of oxidative stress in early respective late onset PE.

The effects of Suramin injections on day 10 and 11 of pregnancy were investigated in normal and diabetic rats of two strains (U and H), with or without additional vitamin E treatment. Suramin caused placental dysfunction in both rat strains: foetal growth restriction, increased resorption rate, reduced placental blood flow, and decreased maternal blood volume in the placenta. In the U strain Suramin also caused maternal hypertension and reduced renal blood flow. Oxidative stress in the Suramin treated rats was indicated by increased levels of isoprostane 8-iso-PGF$_{2\alpha}$ in the placenta. Antioxidative treatment with vitamin E partly protected against the effects of Suramin. Streptozotocin-induced diabetes seemed to cause similar placential effects as Suramin, and in the diabetic rats the additional effects of Suramin were only moderate. In conclusion, Suramin-injected pregnant rats constitute a valid animal model for placental dysfunction (U and H rats) and PE (U rats).

Oxidative stress was estimated in women with early onset (≤ 32 weeks) or late onset (≥ 35 weeks) PE, in normotensive pregnant women of respective gestational length, and in healthy non-pregnant women. The ratio of PAI-1/PAI-2 was measured in serum, and the amount of isoprostane 8-iso-PGF$_{2\alpha}$ was measured in placenta, serum, and urine. The ratio of PAI-1/PAI-2 and placental isoprostane levels were higher in women with early onset PE compared with all other groups. Serum levels of isoprostane were similar between groups. Urinary levels of isoprostane were similar in all pregnant women, but lower in non-pregnant women. These data indicate that pregnancy increases general oxidative stress, and that early onset, but not late onset PE, causes increased oxidative stress also in placental tissue. The pathogeneses of early and late onset PE are, therefore, not likely to be identical.

Keywords: Pre-eclampsia, Oxidative stress, Diabetes, Placenta, Rat, Antioxidants, Suramin, Vitamin E, Vitamin C, PAI-1, PAI-2

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“Sivistyneen ihmisen tärkein velvollisuus on olla hilpeällä mieellä.”
T.T.
List of papers

This thesis is based on the following papers, referred to in the text by their Roman numerals:


IV. Peppi Nash, Anna-Karin Wikström, Solveig Nordén Lindeberg, Ulf J. Eriksson, and Matts Olovsson: Increased placental isoprostanes in early but not in late onset pre-eclampsia, Manuscript
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Abbreviations

AIDS Acquired immune deficiency syndrome
ANOVA Analysis of variance
BMI Body mass index
COX Cyclo-oxygenase
CRP C-reactive protein
DIC Disseminated intravascular coagulation
DNA Deoxyribonucleic acid
ET1 Endothelin 1
HD H-strain rat, diabetic
HDS H-strain rat, diabetic, treated with 100 mg/kg Suramin
HDSE H-strain rat, diabetic, treated with 100 mg/kg Suramin + 5% vitamin E
HELLP Hemolysis, elevated liver enzymes, low platelets
HIV Human immunodeficiency virus
HLA-C Human lymphocyte antigen type C
HN H-strain rat
HNS H-strain rat, treated with 100 mg/kg Suramin
HNSE H-strain rat, treated with 100 mg/kg Suramin + 5% vitamin E
ISSHP International Society for the Study of Hypertension in Pregnancy
KIR Killer immunoglobulin-like receptor
L-NAME N-nitro-L-arginine methyl ester
NK Natural killer cell
NO Nitric oxide
PAI Plasminogen activator inhibitor
ROS Reactive oxygen species
SD Standard deviation
SEM Standard error of mean
sFlt1 Soluble fms-like tyrosine kinase 1
STBM Syncytiotrophoblast microvilli
TBARS Thiobarbituric acid reactive substances
UD U-strain rat, diabetic
UDS U-strain rat, diabetic, treated with 100 mg/kg Suramin
UDSE U-strain rat, diabetic, treated with 100 mg/kg Suramin + 5% vitamin E
UN U-strain rat
UNs U-strain rat, treated with 60 mg/kg Suramin
UNS U-strain rat, treated with 100 mg/kg Suramin
UNSE U-strain rat, treated with 100 mg/kg Suramin + 5% vitamin
Introduction

History

Eclampsia - convulsions specifically related to pregnancy - is first noted in the Hippocratic writings (430 - 330 BC) [1]. Pregnancy related convulsions are also mentioned in writings of other ancient cultures, and eclampsia is later referred to for example by Celsus (100 AD), Mauriceau with collaborators (1668) and Denman (1768). In the mid 1800’s proteinuria was linked to eclampsia, and after that it was thought to be mainly a renal disease. At the same time an association to oedema, blurred vision, and headache were remarked upon. By the end of the 19th century hypertension was also linked to eclampsia. The combination of hypertension, proteinuria and often oedema, which preceded convulsions, came to be known as pre-eclampsia. [40,101,103]

Subsequent studies of pre-eclampsia concentrated on characterizing hypertension and renal dysfunction in patients, until a few decades ago the research focus shifted to the pathophysiology and numerous systemic manifestations of the disease [142].

The definition of pre-eclampsia

Generally, pre-eclampsia is defined as the debut of hypertension and proteinuria after the 20th week of pregnancy. The exact definition, however, has varied in different studies, as demonstrated by Chappell and collaborators [37]. In particular, the threshold values of blood pressure have varied in different study cohorts, and sometimes proteinuria has not been required for the diagnosis. A defined increase in blood pressure, even if the absolute value of blood pressure is considered normal, has also been used as inclusion criteria. It seems reasonable, that for different purposes slightly different definitions of the disease are appropriate. For clinical practice a definition with a low risk for false negative cases is to be preferred. However, for scientific pur-
poses a more strict definition with little risk of false positive cases should be used in order to obtain a homogenous study group.

Standardizing the diagnosis of pre-eclampsia is further complicated by variations in measurements of blood pressure and proteinuria. Blood pressure is a continuous variable and difficult to measure accurately. The choice of measuring device, size of the cuff and the choice between Korotkoff sounds IV and V all affect the result. Measuring proteinuria is problematic as well: the usually accepted reading +1 on a dipstick in a random urine sample can, in fact, overestimate the occurrence of significant proteinuria by as much as 50%. A 24-hour urine collection is by far the most reliable way of detecting true proteinuria, but it can be somewhat impractical. [25] A protein/creatinine ratio of single urine sample has been presented as a valid method of detecting proteinuria, however, the protein / creatinine ratio does not seem to correlate with the amount of protein in a 24-hour collection [180].

The International Society for the Study of Hypertension in Pregnancy (ISSHP) defines pre-eclampsia for research purposes as gestational hypertension after 20 weeks of gestation accompanied by proteinuria. Gestational hypertension is defined as systolic blood pressure >140 mm Hg or diastolic blood pressure (determined by Korotkoff sound V) >90 mmHg in a woman who was normotensive before gestational week 20. Proteinuria is defined as the urinary excretion of $\geq 300$ mg protein / 24 hours. [26]

The clinical disease

Symptoms

Pre-eclampsia is a pregnancy specific multiorgan disorder with elaborate effects, and it is potentially life threatening to both the mother and the foetus. It is a major cause of maternal and foetal morbidity and mortality, especially in the developing countries [100]. Pre-eclampsia has a wide selection of symptoms, which spontaneously resolve after delivery.

In addition to hypertension, the haemodynamic changes of pre-eclampsia include reduced plasma volume, increased vasoconstriction and increased peripheral vascular resistance [174]. Renal perfusion and glomerular filtration rate are reduced, as opposed to a normal pregnancy when they are increased [46]. Glomerular capillary endotheliosis is the microscopic characteristic of pre-eclampsia in the kidney [160].
General oedema may be found in pre-eclampsia, often in the upper body and face. The oedema is more prominent than that seen in normal pregnancy and the weight increase due to increased extravasal fluid can be several kilograms per week. The oedema can affect the brain and cause headache and visual disturbances, which are also caused and/or worsened by the high blood pressure.

Pre-eclampsia can occur as anything from a mild, subjectively asymptomatic blood-pressure elevation to a severe disease with fulminant symptoms and can lead to general convulsions of the mother (eclampsia). The progress can be very fast, and can occur before, during or after labour.

Signs of severe pre-eclampsia include a severely elevated blood pressure (> 110 mmHg diastolic or > 160 mmHg systolic pressure), kidney failure (over 5 g of protein excreted in 24 hour urine collection or oliguria – less than 400-500 ml urine produced in 24 hours) and subjective symptoms mentioned above [68]. Laboratory findings indicating severe pre-eclampsia include, in addition to a high amount of protein in urine, elevated creatinine and urate as signs of kidney failure, elevated haemoglobin and hematocrite as a result of hemoconcentration, low platelet count and elevated liver enzymes. One form of severe pre-eclampsia is the HELLP syndrome (Hae-molysis, Elevated Liver enzymes and Low Platelet count), which can lead to disseminated intravascular coagulation (DIC) and is a life threatening condition. Subjective symptoms of affected liver can be pain in the right upper quadrant of the abdomen or in the epigastric area. The elevation of liver enzymes is probably caused by periportal hemorrhagic necrosis. The thrombocytopenia is likely to result from platelet activation and consumption. [46]

The foetal growth is often restricted in pre-eclampsia [151]. This is a result of placental insufficiency, and impaired blood supply to the placenta and the foetus. Women with pre-eclampsia generally have increased resistance in their uterine arteries, which can be illustrated by Doppler ultrasound measurements. Normally by the second trimester of pregnancy the high resistance of the uterine arteries decreases significantly, but persists when the woman is at a greater risk to develop pre-eclampsia [2,21]. The ratio of plasminogen activator inhibitors 1 and 2 (PAI-1 / PAI-2) has been presented as a measure of placental insufficiency [139], and can even be used to detect women at greater risk of developing pre-eclampsia [121]. The severity of the placental insufficiency and its immediate threat to the wellbeing of the foetus can be estimated by a Doppler ultrasound of the umbilical artery.

Treatment

The treatment of pre-eclampsia is mostly symptomatic, since the only known cure for it is delivery and subsequent removal of the placenta. This always
promotes the health status of the mother, but interruption of pregnancy has also to be weighed against the well being of the foetus, which often would benefit from a longer gestation. Today pre-eclampsia is a major cause of preterm delivery in the developed countries [142].

Epidemiology

Pre-eclampsia affects 3-5% of all pregnancies [140]. It is predominant among primiparous women [31], but also multiparous women whose pregnancy is fathered by a new partner have higher risk for pre-eclampsia [97]. Robillard and collaborators showed that a long history of sexual cohabitation with the father decreases the risk for developing the disease [144]. Thus, the risk for pre-eclampsia might be primipaternity rather than primiparity – a theory that later studies have both questioned [177] and confirmed [50]. Based partly on these observations, immunological factors are thought to contribute to the pathogenesis of pre-eclampsia.

There is a genetic factor in the aetiology of pre-eclampsia. Women whose mothers or sisters have had pre-eclampsia have higher risk to develop the disease themselves [11,41]. Likewise, if the father was born in a pregnancy complicated by pre-eclampsia, the risk is increased [59]. Thus it can be concluded that both the maternal and foetal genome seem to be involved in the aetiology of the syndrome.

Multiple pregnancies increase the risk for pre-eclampsia. The prevalence in twin pregnancies has been reported to be 14% [33,155]. In the case of a rapidly growing hydatid mole pre-eclampsia can occur in as many as 70% of the cases, often with an earlier onset than 20 weeks of gestation [41]. It is speculated, that in these cases the reason for increased prevalence of pre-eclampsia would be the increased amount of placental tissue.

Pre-eclampsia in a previous pregnancy, primary hypertension and diabetes mellitus all increase the risk for pre-eclampsia. In their study Caritis and collaborators noted the prevalence of pre-eclampsia in these conditions to be 18%, 25%, and 20%, respectively [33]. Obesity, which is becoming more common among pregnant women, increases the risk for pre-eclampsia, as well as its risk factors hypertension and gestational hypertension [90].

It is also interesting, that women whose pregnancy has been complicated by pre-eclampsia have an increased risk of cardiovascular disease later in life [179].
Diabetes mellitus

Diabetes is an endocrine disease with rapidly increasing numbers of affected people in the world. Approximately 5-10% of all cases are type 1 diabetes, in which an autoimmune destruction of pancreatic beta cells leads to absence of insulin production and a lifelong dependency on insulin treatment. 90-95% of cases of diabetes are type 2, which is a metabolic disease often connected to obesity and hypertension. It is characterised by insulin resistance, and the patients are not necessarily dependent on insulin therapy. Gestational diabetes is a condition of increased blood sugar values and insulin resistance during pregnancy, which disappears after delivery or termination of pregnancy. Women who have had gestational diabetes have a markedly increased risk to develop type 1 or type 2 diabetes [95].

Women with diabetes mellitus are at increased risk to be affected by pre-eclampsia. Type 1 diabetes seems to increase this risk by 3-4 times [45,71,75], while type 2 and gestational diabetes slightly less [53,146]. Oxidative stress in diabetes [12,154,178] may contribute to this increased risk of pre-eclampsia.

Pathophysiology of pre-eclampsia

The aetiology of pre-eclampsia remains unclear in spite of extensive studies. Placental and foetal ischemia [78], disturbances of the coagulation and immune systems [134], and alterations in cytokine-mediated trophoblast and endothelial maturation [32,85] have been suggested as factors contributing to the development of the syndrome. In addition, a genetic component in the aetiology of pre-eclampsia has been inferred from clinical studies [11,168,186]. In recent years, oxidative stress in the maternal and placental tissue has been considered to have an important role for the development of the syndrome [6,76,104,131,166,170,173].

The placenta

The presence of a placenta is essential for the development of pre-eclampsia, which can occur even in case of a hydatide mole or an abdominal pregnancy [9,61,110]. It spontaneously resolves after the delivery and removal of the placenta – in the case of an abdominal pregnancy, where the placenta was left intact after the delivery of the foetus, symptoms of pre-eclampsia persisted until the removal of the placenta 99 days later [123].
In normal pregnancy the spiral arteries are invaded by trophoblasts, and as a result lose their muscular wall and ability to contract. In pre-eclampsia, however, the trophoblast invasion is absent or limited in depth, and the spiral arteries retain their contractility.

A widely recognized characteristic of pre-eclampsia is so called shallow placentation [190]. In a normal pregnancy the trophoblast cells of the foetal placenta migrate deep into the muscular layer of the uterus. They infiltrate the spiral arteries and convert them into widened vessels, unable to contract. This provides an expanded and non-restricted blood flow to the placenta. In placentas from pre-eclamptic pregnancies the migration of trophoblasts is abnormal and only reaches shallow depths of the decidua, and the trophoblasts may even fail to invade some of the spiral arteries altogether [24,88,124] (Figure 1). Thus the spiral arteries preserve their vasoactivity, and this may result in impaired blood flow to the placenta and cause relative hypoxia associated with oxidative stress and increased apoptosis [114]. However, this type of failed placentation is not specific to pre-eclamptic pregnancies, but can occur also in pregnancies with intrauterine growth restriction or premature birth when the mother remains healthy [10,88], or in the case of miscarriage [89].

Immunology

In normal pregnancy the maternal immune system carefully balances between maintaining adequate protection towards harmful pathogens and allowing the foetus – an allograft from the maternal perspective – to develop successfully. One aspect of the immunological adaptations in pregnancy is
the shifting of T-lymphocyte profile from pro-inflammatory towards suppressor phenotype [51,148]. In pre-eclampsia, however, the inflammatory type may be dominant, thus the systemic inflammatory process becomes excessive and causes the clinical symptoms [135].

Syncytiotrophoblast microvilli (STBM) are known to damage endothelial cells in vitro [159]. Knight with collaborators demonstrated that during pregnancy STBM are shed into maternal circulation, and they detected increased amounts of STBM in pre-eclampsia [92]. The STBM in maternal circulation are probable triggers of the inflammatory reaction in pregnancy, and the abundance of STBM in pre-eclampsia is a likely contributing factor to the disease [135].

Maternal immune system, especially the natural killer cells (NK) in the uterus, may also affect the trophoblast invasion. The killer immunoglobulin-like receptors (KIR) of the NK cells recognize polymorphic human lymphocyte antigens of the trophoblasts (HLA-C), and are either inhibited or stimulated. There are several phenotypes of both KIR and HLA-C, the types of which affect the response in the NK cells. Certain combinations seem unfavourable to trophoblast invasion, and are likely to be more common in pre-eclampsia. [109,136]

Oxidative stress

Oxidative stress can be defined as an imbalance between antioxidants and pro-oxidants in favour of the latter [69]. Most common pro-oxidants are reactive oxygen species (ROS), which cause damage to proteins, lipids and DNA. Although the damaging effects of ROS can be desirable, as when activated phagocytes produce ROS to kill bacteria, they are more often strictly controlled by antioxidant defence systems. There are many antioxidants both produced in the body and derived from the diet, and usually the antioxidant defences and the pro-oxidant forces are in well controlled balance. [70]

ROS can attack polyunsaturated fatty acids and trigger lipid peroxidation. This can cause a chain reaction of free radical formation and further lipid peroxidation, which may enhance membrane permeability and contribute to endothelial injury [43]. A major isoprostane 8-iso-PGF$_{2\alpha}$ is produced through non-enzymatic free radical -catalyzed peroxidation of arachidonic acid, and is considered a reliable indicator of lipid peroxidation and oxidative stress [16,143].

There is abundant evidence of increased oxidative stress and lipid peroxidation as well as decreased antioxidant capacities in pre-eclampsia. The level of isoprostanes is reported to be raised in the placenta [170] and in maternal
plasma [106]. Placental superoxide, a potent pro-oxidant, has also been shown increased in pre-eclampsia [156]. Malondialdehyde, which is a metabolite of lipid hydroperoxide and thus a marker of oxidative stress and lipid peroxidation, has been shown to be increased in pre-eclampsia [152,169,171]. Free iron can accelerate free radical reactions, and in pre-eclampsia serum iron concentrations and percent saturation of iron binding capacity are increased while total iron-binding capacity is lower than in normal pregnancy [77]. In pre-eclampsia antioxidant capacity has been shown to be decreased in placenta [173] and in serum [149,152]. However, some studies question the role of oxidative stress and lipid peroxidation in pre-eclampsia [52,137].

Antioxidant supplementation is an interesting approach to prevent pre-eclampsia. Although preliminary studies of the efficacy of vitamins C and E in this respect were promising [39], recent reports from two randomized controlled trials showed no prophylactic or protective effect on the incidence of pre-eclampsia by a combination of these two antioxidants [128,147].

Two-stage model of pre-eclampsia

A well supported theory sees pre-eclampsia as a two-stage disease [141]. The first, preclinical, stage takes place on the 6th – 18th week of pregnancy, and consists of insufficient trophoblast invasion of the spiral arteries. This results in preserved vasoactivity of the spiral arteries and may lead to fluctuations in oxygen tension in the placenta. This would generate an ischemia – reperfusion – type insult, which causes oxidative stress and stimulates apoptosis of syncytiotrophoblasts [78,79]. Increased apoptosis [8] is suggested to lead to abundant placental debris in maternal circulation, which in turn would trigger an abnormal systemic inflammatory response in the mother [51,135]. The result would be the second, clinical, stage of pre-eclampsia with generalized endothelial dysfunction, which would lead to vasoconstriction, activation of the coagulation system and extravasation of fluids [133,174].

Oxidative stress seems a likely mediator between the two stages [80,141]. However, the maternal response is likely to be influenced by several other factors, such as genetic, behavioural, and environmental. Identification of the factors connecting the two stages might enable development of therapies for pre-eclampsia.

The maternal syndrome of pre-eclampsia might be more than one disease [115,119,175]. There are major differences between late onset pre-eclampsia without distinct foetal morbidity and early onset pre-eclampsia, which is associated with low birth weight and preterm delivery. Recently the two-stage model has been discussed as the origin of severe early onset disease,
but is thought to be less relevant for near term disease [130]. Poor placentation is more strongly associated with early onset than with late onset disease [3,54]. The excess of syncytiotrophoblast debris in maternal circulation is also greater in early than late onset pre-eclampsia [67]. Late onset pre-eclampsia is associated with increased insulin resistance, while early onset pre-eclampsia is not [47]. Since pre-eclampsia can be the result of heterogeneous causes, it seems important to separate early onset and late onset pre-eclampsia when investigating pathophysiology of the disease.

Animal models of pre-eclampsia

Studies using animal models of pre-eclampsia would be of great value in trying to reveal the aetiology and pathogenesis of the disease and finding preventive or curative treatments for it. Pre-eclampsia seems to occur spontaneously only in primates [13], but conditions mimicking the human disease have been induced to various animals in numerous experimental studies [127].

One widely used method of inducing pre-eclampsia like condition in animals is reducing the blood flow to the uterus. Placing a contracting clip around the aorta appears to lead to hypertension and occasionally proteinuria in pregnant animals of several species [127]. Surgically reducing the blood flow in lower aorta of pregnant baboons by 55-60% resulted in hypertension and glomerular lesions, and proteinuria was present in three of nine treated animals [34]. In rhesus monkeys a similar aortic constriction resulted in hypertension, proteinuria and glomerular endotheliosis in four of seven treated monkeys [44]. However, the depth of interstitial trophoblast invasion was increased [189], as opposed to impaired trophoblast invasion and shallow placentation in human pre-eclampsia. This indicates that although the end result of aortic constriction might resemble the second stage of pre-eclampsia, the first stage is not satisfactorily created. In the rat, the condition acquired by aortic constriction seems to resemble only mild pre-eclampsia with increased blood pressure and more frequent proteinuria but without foetal morbidity [73]. When also the uterine arcades of ovarian arteries were constricted, pregnant rats showed symptoms of hypertension, proteinuria, and foetal growth restriction [7], but changes resembling HELLP-syndrome in maternal status were absent [83].

The synthesis of nitric oxide (NO), a potent vasodilator, and responsiveness to it are up-regulated during normal pregnancy, and have been considered a potential factor involved in the pathogenesis of pre-eclampsia [28]. Inhibiting the synthesis of NO by continuous administration of N-nitro-L-arginine methyl ester (L-NAME) to pregnant rats has been reported to yield increased
blood pressure, proteinuria, intrauterine growth restriction and foetal death [18,185]. However, when the blood pressure of unstressed and unrestrained pregnant rats receiving continuous L-NAME infusion was measured daily with radiotelemetry, the blood pressure of the treated rats did not differ from the controls during the last five days of pregnancy [27]. Also, as opposed to human pre-eclampsia, the L-NAME treated pregnant rat does not present with oxidative stress in the placenta [172], and has decreased rather than increased plasma level of endothelin-1 [188].

HELLP syndrome in human pre-eclampsia resembles generalized Shwartzman reaction, which is characterized by thrombocytopenia and DIC, and can be caused in pregnant rats by endotoxin [113]. Low dose Endotoxin infusion to pregnant rats on pregnancy day 14 yielded increased blood pressure and enhanced proteinuria, but the impact on foetal outcome was minimal [60]. In a different study, however, endotoxin failed to cause hypertension in pregnant rats [150].

Renal disease and maternal diabetes are known risk factors of pre-eclampsia. These conditions have been reproduced in rats by adriamycin (nephropathy) and streptozotocin (diabetes). When adriamycin treated rats become pregnant, they show clinical signs resembling superimposed pre-eclampsia, although placental and renal histology remain normal [126]. When rats on pregnancy day 6 were injected with 30 mg/kg streptozotocin, they developed diabetes, hypertension, increased proteinuria, and foetal growth restriction [81]. Although very interesting, these two models are not optimal for studies of pre-eclampsia, since both are complicated by other interfering medical conditions.

Beauséjour and collaborators added 1.8% NaCl to drinking water of pregnant rats during the last week of gestation, and demonstrated hypertension and proteinuria together with foetal growth restriction in these rats, as well as increased oxidative stress and apoptosis in the placenta [19]. This model is likely to simulate only the second stage of pre-eclampsia, since the interference is timed only after the placenta is already formed.

The soluble fms-like tyrosine kinase 1 (sFlt1) acts as an antagonist of vascular endothelial growth factor (VEGF) and placental growth factor (PIGF), and has been shown to be increased in pre-eclampsia [74,105,161]. SFlt1 is proposed as a mediating factor secreted by the placenta and causing endothelial dysfunction. When sFlt1 was infused to pregnant rats, these developed hypertension, proteinuria, and glomerular endotheliosis, supporting the suggestion that sFlt1 might be the factor secreted by the placenta and causing endothelial damage in pre-eclampsia [105]. This is a promising animal model, and although excess placental sFlt1 may contribute to the pathogenesis of pre-eclampsia, other factors are likely to be involved in the process.
Although numerous attempts to create animal models of pre-eclampsia have been made and many of them have contributed to our knowledge about the pathogenesis of the syndrome, these experimental models may not be optimal in testing preventive treatments for the two-stage disease pre-eclampsia is today considered to be. Most of the previous animal models of pre-eclampsia seem to produce the second stage of the disease, and it is possible that even though the acquired clinical condition resembles that of human pre-eclampsia, the pathophysiologic changes leading to this condition are not identical to the human syndrome. A valid model originating in impaired placentation – i.e. the first stage of pre-eclampsia – and resulting in symptoms identical to the second stage of pre-eclampsia has not previously been described, but would be valuable for treatment trials.

The rat placenta

The rat gestation is 21-22 days long. In the beginning, the yolk sac is responsible for the nutrition of the foetus, but its importance decreases when the chorioallantoic placenta is formed around days 9-11 of pregnancy.

![Diagram of rat and human placentas](image)

**Figure 2** Haemochorial placentas of rats (left) and humans (right). In the haemotrichorial rat placenta foetal blood (FC = foetal capillary) is separated from the maternal by two layers of syncytiotrophoblasts (ST) and one layer of cytotrophoblasts (CT). In the haemomonochorial human placenta there is but one syncytiotrophoblast layer, the cytotrophoblast layer being discontinuous.
The rat placenta is in many ways similar to the human placenta. Both are discoid in shape, and both are haemochorial [163,181]: The labyrinthine rat placenta is haemotrichorial and the villous human placenta haemomono-chorial, referring to the number of trophoblast layers separating the maternal and the foetal circulations [55] (Figure 2). During pregnancy, the uterine spiral arteries of both species are subject to trophoblast invasion, although in the human endovascular invasion follows interstitial invasion, while in the rat the endovascular invasion happens first [125]. The resulting vascular changes are remarkably similar in rats and humans [30]. All in all, the human placenta has more in common with the rat placenta than with villous placentas of other species [164], thus the rat seems to offer a suitable model for studies of both normal and disturbed human placentation.

Suramin

Suramin (Figure 3) is a polysulfonated naphtylurea, which was originally developed in 1916 as a trypanocidal agent in a research program on the anti-parasite activity of aminonaphtalene-sulfonic azo dyes. It was first used against trypanosomiasis, but because of its poor penetration into the central nervous system it was not very effective in disseminated disease. Later it was used also in the treatment of onchocerciasis. In 1980’s Suramin was discovered to inhibit the reverse transcriptase of retroviruses, including HIV. It was then used in clinical trials to treat AIDS, but did not seem to improve the immunologic status of the patients. It did, however, cause regression of disseminated Kaposi’s sarcoma and a stage IV small cleaved cell lymphoma. Suramin was discovered to be toxic to many human tumour cell lines, and was then used in clinical trials as an anti-neoplastic drug against different types of cancer. [49,94]
Suramin is a potent inhibitor of angiogenesis and antagonizes several growth factors, such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), several of which are known to participate in placentation. [23,64,191]. Suramin may, therefore, affect placentation by inhibiting angiogenesis in the uterus [192].

Suramin has been suggested to act as a teratogen in rats when administered in high doses [98]; however, when the drug was administered in a comparative study to both rats and mice it was only teratogenic in the latter species [107]. The early studies also reported that the compound preferentially accumulates in yolk sac lysosomes and does not appear to reach the embryo. It was later shown that a dose of 250 mg/kg given on gestational day 8 or 9 disturbs yolk sac function in rats [62]. It has also been reported, that Suramin is remarkably non-toxic to endothelial cells in culture even in high concentration [22].

In addition to its anti-angiogenetic effects, Suramin blocks the binding of lipoproteins to cell membrane receptors [96] and diminishes phospholipid uptake by inhibiting the enzyme aminophospholipid translocase [167].

It is reasonable to assume, that Suramin would inhibit the formation of placenta when administered to pregnant rats. To administer Suramin on gestational days 10 and 11 seems beneficial, since this is the time of maturation of the chorioallantoic placenta, and a time when the role of the yolk sac dramatically diminishes. Compromising the placentation in pregnant rat might be a representative animal model of the two stages of pre-eclampsia.
Aims

The aim of this thesis was to answer the following questions:

Papers I-III

Is it possible to induce pre-eclampsia in pregnant rats with Suramin?

Is there a difference in Suramin susceptibility between different rat strains?

Does maternal diabetes worsen the outcome in Suramin treated pregnant rats?

Does antioxidant treatment have a normalizing effect in Suramin treated pregnant rats?

Paper IV

Is pregnancy associated with oxidative stress?

Does pre-eclampsia increase oxidative stress?

Is the degree of oxidative stress different in early and late onset pre-eclampsia?

Is early onset pre-eclampsia a more severe disease compared to late-onset pre-eclampsia?
Materials and Methods

Papers I - III

Animals
Female Sprague-Dawley rats from two closely related strains denoted U and H were randomly selected from the stock. There are minor genetic differences between these two strains, and their main difference in foetal outcome is an increased rate of resorptions and malformations in the U compared to the H strain when the female rats have been made diabetic prior to pregnancy [56].

Both non-diabetic (UN and HN) and diabetic (UD and HD) rats were used in the study. Diabetes was induced by intravenous injections of 40 mg/kg Streptozotocin, and rats whose blood glucose level was over 20 mmol/l seven days after the injection, were considered diabetic and included in the study.

Subgroups, denoted with E, of both the non-diabetic and diabetic rats received vitamin E enriched food immediately before and throughout pregnancy. The enriched food was prepared by grinding the standard food pellets and adding powdered DL-α tocopherol hydrogen succinate to a final concentration of 5%.

The blood pressure of all the female rats was measured on several occasions before and throughout pregnancy with a tail-cuff blood pressure monitoring system. This estimates the systolic blood pressure by occluding the blood-flow in the tail artery. When the occlusion is slowly released, the pressure at which the pulse first appears is measured and equals to the systolic blood pressure.
Pregnancy

The female rats were mated overnight with male Sprague-Dawley rats of respective strain. Day 0 of pregnancy was defined as the day when spermatozoa were detected in a vaginal smear. Some of the rats in the study did not become pregnant in spite of a positive vaginal smear, and these rats were included as special non-pregnant controls.

On day 10 and 11 of pregnancy the rats were given intraperitoneal injections of either saline or Suramin. The control rats (UN, UD, HN and HD) received 0.3 ml of saline. The Suramin was dissolved in saline (100 mg/ml) and each rat received doses of 60 mg/kg (UNs) or 100 mg/kg (UNs, UNSE, UDS, UDSE, HNS, HNSE, HDS, HDSE).

On gestational day 19 a 24h-urine sample was collected with the help of a metabolic cage, and on gestational day 20 the rats were weighed and sedated with ether and intraperitoneal injection of thiobutabarbital sodium.

The systolic blood pressure of the rats was measured with the tail cuff both before and after anaesthesia, and the mean arterial pressure was measured in the anaesthetized rats via a polyethylene catheter inserted into the right carotid artery and connected to a pressure transducer. Blood flow in a randomly selected placenta and the left kidney was measured by laser-Doppler flowmetry.

Blood was drawn from the aorta and centrifuged for 10 min in test tubes with heparin, and the serum was stored at -70 ºC until analyzed. The pregnant rat was killed by cervical dislocation, and the left kidney was weighed and preserved in 4% formalin. The outcome of pregnancy was evaluated and randomly selected placentas were preserved in formalin or washed with saline and frozen.

Biochemical analyses

**Protein** concentration in rat urine was determined by the method of Lowry and collaborators using bovine serum albumin as standard. [102]

The concentration of metabolites of nitric oxide, nitrites and nitrates, in rat serum was measured using a Bioxytech® NO-540™-kit (Bio-Stat Research, Stockport, UK), following the manufacturer's instructions.

**Endothelin-1** amount in rat serum was measured using a human Endothelin-1 Enzyme Immunometric Assay Kit (Catalog No. 90020, Assay Design, Inc. Ann Arbor, MI, USA), following the manufacturer's instructions.
The concentrations of isoprostane 8-iso-PGF$_{2\alpha}$ were measured in placental tissue using a commercial kit (Cayman Chemical Company, Ann Arbor, MI, USA) following manufacturer’s instructions. Small bits of placental tissue from the frozen placentas were homogenized with 500 µl of water, and the amount of isoprostanes was determined as a ratio to protein in the sample, which was determined by the method of Lowry and collaborators [102].

**Morphology of placentas and kidneys**

The placentas and the kidneys of the rats were dehydrated in graded ethanol series and embedded in paraffin. Entire placenta and kidney were sectioned into 7-µm-thick sections, mounted on glass slides, and stained with hematoxylin-eosin.

The overall morphology of the rat placentas was evaluated by visual impression under a light microscope. The thickness of the labyrinthine walls was categorized according to its visual appearance as thin / moderate / thick, and the type dominating on the slide was noted and assigned a numerical value (1, 2, 3, for thin, moderate, thick, respectively). The size of maternal blood lacunae was similarly judged as normal / large / very large, and indexed (1, 2, 3 for normal, large, very large, respectively). We also measured the distance from 7 - 10 randomly picked, easily identifiable foetal blood vessels of each placenta to the nearest maternal blood lacuna.

Four sections of each placenta were also examined by planimetric analysis in a MOP-Videoplan image analysis system (Kontron Bildanalyse, Munich, Germany) fitted with a Leitz microscope and a colour monitor. The total area of both maternal and foetal blood volume was measured on visual fields running diagonally through the section.

Six or seven kidneys were randomly selected from each experimental group and evaluated in a light microscope. One section of each kidney was randomly selected, and the glomeruli were counted. The glomeruli were categorized according to their degree of morphological damage: normal, moderately damaged, or greatly damaged. 100 normal glomeruli were randomly selected from each kidney and the number of cells in each glomerulus was counted, and the average cell count per glomerulus was calculated.

**Statistics**

Means ± SEM were calculated and comparisons between groups were made using ANOVA and Fisher’s protected least significant difference test (PLSD) as a post hoc test. Chi-2 test was used to compare the placental morphology and glomerular damage. A p-value of less than 0.05 was considered
significant and a numerical difference between two means with a p-value in the interval $0.05 < p < 0.1$ was referred to as a “tendency”.

Ethics

Approval for the experiments was obtained from the animal ethics committee at Uppsala University. “Principles of laboratory animal care” (NIH publication no. 85-23, revised 1985) was followed.

Paper IV

Women

Six groups of women were recruited among patients treated at the Uppsala University hospital during the time period 2001-2005:

1) Non-pregnant - healthy non-pregnant women. They were recruited at the reproduction centre among women who visited the centre for infertility based on male or tubal factors. They later became pregnant and delivered successfully.

2a) Early control - healthy pregnant women who were recruited during a routine visit to an antenatal clinic in gestational weeks 24-32. Only those, whose pregnancy continued normally and resulted in a full-term delivery of a healthy child with normal weight, were included.

2b) Early control - healthy pregnant women who delivered in gestational week 24-32 due to cervical insufficiency. These women were admitted to the hospital because of imminent premature delivery. Women with clinical and laboratory signs of infection were excluded.

3) Early onset pre-eclampsia - pregnant women diagnosed with pre-eclampsia before gestational week 32 and delivered prematurely.

4) Late control - healthy pregnant women delivering in gestational week 36-42. Women in this group could be planned to deliver with a caesarean-section (e.g. for breech presentation or previous caesarean-sections), or vaginally. In the latter case they contacted the hospital at term before the active phase of labour, and they were enrolled if the delivery was estimated to occur within a few days. A planned vaginal delivery could be converted to caesarean-section according to the practice at the clinic.

5) Late onset pre-eclampsia - pregnant women diagnosed with pre-eclampsia in gestational week 35 or later.

Pre-eclampsia was defined according to the guidelines of ISSHP [26]. Only women with singleton pregnancies were included, and women with essential
hypertension, diabetes mellitus or other chronic disease were excluded from the study.

Available data were collected from the patient files. Small / large for gestational age was defined as birth weight 2 SDs below / above the mean birth weight for gestational age according to the charts used at the hospital (pre-term charts by Niklasson A, Karlberg P, 1999 and term charts by Albertsson Wikland K, Karlberg J, 1999).

Samples

Serum and urine samples were collected from each subject upon entering the study. None of the patients was in labour at the time of sampling.

From patients in groups 1-4 (except 2a) placental samples were collected immediately after delivery. Placental pieces were rinsed in saline, dried between sheets of paper tissue and snap-frozen in liquid nitrogen.

PAI-1 / PAI-2 ratio

PAI-1 and PAI-2 were measured with commercial ELISA kits no.s 822 and 823 (American Diagnostica Inc. Stamford, CT, USA) in accordance with the manufacturer’s instructions. The samples were diluted 10-40 times and analyzed in antibody coated wells of ELISA microplates.

Concentration of isoprostane 8-iso-PGF$_{2\alpha}$

The concentrations of isoprostane 8-iso-PGF$_{2\alpha}$ were measured in placental tissue, serum, and urine using a commercial kit (Cayman Chemical Company, Ann Arbor, MI, USA) following manufacturer’s instructions. The amount of placental isoprostanes was determined as a ratio to protein in the sample, which was determined by the method of Lowry and collaborators [102].

Concentration of vitamin E and C

The concentrations of vitamin E were measured as described by Simán and Eriksson [157]. 500 µl of serum was analyzed, and the values were compared to values of standard samples prepared from α-tocopherol. Vitamin C concentrations were determined using the method of Jagota and Dani [84]. The analyses were done on 200 µl of serum, and standard samples were prepared in distilled water from ascorbic acid.
Statistics
Means ± SEM were calculated and ANOVA was used for overall comparisons and Fisher’s protected least significant difference test (PLSD) was used for pair-wise comparisons between means of the study groups. Chi-square and Fisher’s exact tests were used for comparisons between proportions. A p-value of less than 0.05 was considered significant.

Ethics
The protocol for the study was approved by the local ethics committee of the medical faculty of Uppsala University, and informed consent was obtained from each patient included in the study.
Results & Discussions

Papers I - III

Blood pressure
The mean blood pressure measured with the tail cuff method showed marked intra- and inter-individual variation in all groups. Both the U and H rats had initially similar mean blood pressure, and there were no differences between groups in the blood pressure measured in non-anaesthetized rats during pregnancy. When the rats were anaesthetized on gestational day 20, the blood pressure of the U rats seemed to follow a pattern, both when measured with the tail cuff (systolic pressure) and the indwelling catheter (mid-arterial pressure). The pattern suggested that the blood pressure was increased by high dose Suramin and normalized by vitamin E treatment. This pattern was seen in both non-diabetic and diabetic U rats. In the anesthetized H rats, however, no differences in the blood pressure were seen after Suramin or vitamin E treatment, except for a tendency towards elevated blood pressure after Suramin treatment in the diabetic H rats. (Table 1)

The lack of differences in blood pressure in non-anaesthetized rats is likely to be due to difficulties in standardizing various stress factors at the time of the measurements. There were large variations in the same rat on consecutive measurements, and the tail cuff method of estimating systolic pressure has been previously documented to be difficult and somewhat unreliable [29]. Some authors, however, consider the this method to be of value [122], and indeed, when the rats in the present study were anaesthetized, an increased blood pressure in the UNS and UDS groups was revealed. This finding is further strengthened by a recent study, in which indwelling blood pressure measuring chips were surgically inserted in female U rats. After recovery, they were mated and given intraperitoneal injections of Suramin (100 mg/kg) or saline on days 10 and 11 of pregnancy. The blood pressure was monitored continuously, and the Suramin treated rats showed symptoms of increased blood pressure in late pregnancy (Carlström et al, in preparation).
These data indicate that Suramin treatment increases the blood pressure of the U rats, and vitamin E treatment inhibits this increase. The blood pressure of pregnant H rats, however, is not affected by Suramin.

Analysis in serum
The serum nitrite concentration (Table 1) was not affected by Suramin or vitamin E treatment in any of the study groups, however, the diabetic rats of both rat strains had lower concentrations of serum nitrites than the non-diabetic rats. Also, the serum nitrite concentration of the non-diabetic H rats was lower than that of the non-diabetic U rats.

Nitric oxide is a potent vasodilator, which is generated by NO synthase (NOS) enzymes, and causes vasodilatation via cyclic guanidine monophosphate (cGMP). Nitrites and nitrates are metabolites of NO, and are considered to reflect the activity of the NO system. Although NO is a tempting candidate in the pathogenesis of pre-eclampsia, results from studies measuring levels of its metabolites in serum or urine of pre-eclamptic women have been contradictory [28]. Reports of increased NO synthesis [20] and increased levels of NO metabolites [116,183] in pre-eclampsia may indicate, that the NO pathway is a compensatory mechanism which can be activated in varying degree in response to the hypertension present in pre-eclampsia.

The lack of differences between treatment groups in nitrite concentrations in the current model indicates that the increased blood pressure after Suramin treatment is not mediated by the NO pathway.

The serum endothelin-1 concentrations (ET-1; Table 1) were increased by the high dose Suramin treatment in the non-diabetic U rats, but only without additional vitamin E treatment. In the other study groups Suramin and vitamin E treatment did not affect the ET-1 concentrations. Diabetes, however, increased the ET-1 concentration in both rat strains.

ET-1 is a potent vasoconstrictor produced by vascular endothelial cells, and its concentration is demonstrated to be increased in serum of pre-eclamptic women [63,116,183]. These findings are in concert with the increased ET-1 levels and increased blood pressure in the UNS rats.

Pregnancy outcome
The resorbed foetuses in a rat pregnancy can be seen equivalent to a human miscarriage. The number of live foetuses was slightly decreased in the non-diabetic U rats treated with the high dose of Suramin, but not if they were given vitamin E enriched food. In the other study groups Suramin or vitamin E did not seem to affect the number of live fetuses. The percentage of
### Table 1

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Blood pressure tail cuff</th>
<th>Blood pressure arterial</th>
<th>S-nitrite (µM)</th>
<th>S-ET1 (µM)</th>
<th>Nr of foetuses</th>
<th>Resorptions %</th>
<th>Foetal weight (g)</th>
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<tbody>
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<td>UN</td>
<td>12</td>
<td>136 ± 8</td>
<td>108 ± 4</td>
<td>15.4 ± 1.3</td>
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<td>11.8 ± 0.5</td>
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<td>4.1 ± 0.1</td>
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<tr>
<td>UNs</td>
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<td>144 ± 9</td>
<td>117 ± 14</td>
<td>12.3 ± 1.0</td>
<td>6.6 ± 0.7</td>
<td>11.4 ± 0.8</td>
<td>5.3</td>
<td>3.8 ± 0.1 *†</td>
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<td>160 ± 7 *</td>
<td>125 ± 5 *</td>
<td>14.0 ± 1.2</td>
<td>10.5 ± 1.8 *</td>
<td>9.4 ± 1.1 *</td>
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<td>85 ± 9 †</td>
<td>19.3 ± 4.2</td>
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<td>13.8 *</td>
<td>2.7 ± 0.3 *</td>
</tr>
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<td>UD</td>
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<td>120 ± 14</td>
<td>60 ± 9</td>
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<td>91 ± 5 *</td>
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<td>109 ± 3</td>
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<tr>
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<td>2.5</td>
<td>3.2 ± 0.2 *</td>
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<tr>
<td>HNSE</td>
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<td>158 ± 6</td>
<td>113 ± 6</td>
<td>14.4 ± 1.9</td>
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<td>10.3 ± 1.4</td>
<td>1.8</td>
<td>3.0 ± 0.2 *</td>
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<tr>
<td>HD</td>
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<td>98 ± 7</td>
<td>7.7 ± 2.4</td>
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<tr>
<td>HDS</td>
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<td>5.6</td>
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<td>HDSE</td>
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<td>8.9</td>
<td>2.2 ± 0.3 *</td>
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</tbody>
</table>

* p < 0.05 when compared to the respective control group (UN, UD, HN or HD)
† p < 0.05 when compared to UNS or UDS, as appropriate

### Table 2

<table>
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<tr>
<th></th>
<th>n</th>
<th>Placental blood flow (TPU)</th>
<th>Isoprostanes in placenta (pg/mg)</th>
<th>n</th>
<th>Placental blood flow (TPU)</th>
<th>Isoprostanes in placenta (pg/mg)</th>
</tr>
</thead>
<tbody>
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<td>15.6 ± 0.9</td>
<td>94 ± 14</td>
<td>7</td>
<td>34 ± 1</td>
<td>128 ± 32</td>
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<tr>
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<td>203 ± 29 †</td>
<td>HN</td>
<td>7</td>
<td>30 ± 1 *</td>
</tr>
<tr>
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<td>11.3 ± 0.8 *</td>
<td>259 ± 26 *</td>
<td>HNS</td>
<td>7</td>
<td>30 ± 1 *</td>
</tr>
<tr>
<td>UNSE</td>
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<td>14.7 ± 0.7 †</td>
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<td>HNSE</td>
<td>7</td>
<td>30 ± 1 *</td>
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<tr>
<td>UD</td>
<td>6</td>
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<td>259 ± 21</td>
<td>HD</td>
<td>6</td>
<td>29 ± 1</td>
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<tr>
<td>UDS</td>
<td>6</td>
<td>11.9 ± 0.8 *</td>
<td>234 ± 91</td>
<td>HDS</td>
<td>4</td>
<td>28 ± 3</td>
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<tr>
<td>UDSE</td>
<td>6</td>
<td>14.6 ± 1.8</td>
<td>152 ± 33 *</td>
<td>HDSE</td>
<td>5</td>
<td>29 ± 1</td>
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</tbody>
</table>

TPU = tissue perfusion units
* p < 0.05 when compared to the respective control group (UN, UD, HN or HD)
† p < 0.05 when compared to UNS
resorptions of the total number of implantations was increased in the non-diabetic U rats after high dose Suramin treatment, with or without additional vitamin E. The diabetic U rats had a considerably higher resorption rate as the non-diabetic rats, and an additional Suramin treatment failed to have a significant effect. The resorption rate of the H rats was not affected by Suramin or vitamin E treatment, but there was a tendency towards higher resorption rate in the diabetic H rats. (Table 1)

It is possible, that the increased resorption rate is a result of an embryotoxic reaction to Suramin. However, Suramin accumulates in the yolk sac lysosomes, and does not seem to reach the embryo [98]. Its teratogenic effects are mediated by disturbing the yolk sac function and are demonstrated on higher doses than used in the current study, given a few gestational days earlier [62]. Also, the most common malformation caused by Suramin in the experiment by Freeman and collaborators [62] was hydrocephalus (19% of foetuses), which was not detected in this study. Although it is not possible to exclude direct embryotoxic reaction to Suramin in the present study, it is not likely to be the sole explanation to current findings. Instead, the changes in the placental circulation described below are likely moderators of the Suramin induced increase in resorption rate in the U rats, which correlates well with the increased risk of miscarriage and stillbirth in pre-eclampsia. H rats, however, present a resistance to Suramin induced resorptions.

The mean foetal weight of the non-diabetic U and H rats was reduced by the high dose Suramin treatment, regardless of vitamin E treatment, and similar effects were seen in the diabetic H rats. However, in the diabetic U rats the intrauterine growth restriction after Suramin treatment seemed to be absent. The foetal weight of the diabetic U rats was similar to that of UNS rats, whereas the foetal weight of the diabetic H rats was intermediate to that of the HN and HNS rats. (Table 1) It is likely that diabetes had already restricted the foetal growth to the maximum, thus masking the expected effect of Suramin. It seems that the diabetic H rats have a better capacity for foetal growth than the diabetic U rats, and thus the additional Suramin treatment causes a further restriction of the foetal growth.

Placental perfusion

In the non-diabetic U and H rats the high dose Suramin treatment decreased placental perfusion, but the additional vitamin E treatment prevented this decrease only in the U rats. The diabetic U rats had similar placental perfusion than the non-diabetic U rats, showing a decrease after Suramin treatment which was inhibited by vitamin E. In the diabetic H rats, however, the placental blood flow was lower than in the non-diabetic H rats and was not affected by Suramin or vitamin E treatment. The placental blood flow was higher in all the H rats compared to the U rats. (Table 2)
Although a decreased placental blood flow in pregnant diabetic U rats has previously been demonstrated [58], it was not seen in the present study. In the previous experiment placental blood flow was estimated with microspheres, a method which provides information on the net influx of maternal blood to the placenta. The laser Doppler estimations in the present study give a collective account of the blood flows (maternal and foetal) in the placenta and showed the major decrease in blood flow to be caused by Suramin, whereas maternal diabetes was found not to affect placental blood flow in U rats. It may be, that diabetes per se decreases the net influx of maternal blood into the placenta (at least in the U rats), and that Suramin administration primarily decreases intraplacental circulation of maternal and foetal blood. This is further indicated by the notion, that placental blood flow was closely related to the (later described) blood volume in the placenta.

Worth to note is the difference in the placental blood flow between the non-treated U and H rats: the non-treated U rats have a placental blood flow of approximately 45 % of that of the H rats. The H rats seem to be more resistant to the compromising effects of Suramin, presenting with less resorptions and only a moderate foetal growth restriction (i.e. more modest placental dysfunction). Thus, it seems conceivable that the higher functional reserve capacity in the placentas of the H rats at least partly protected the mother and foetus from the effects of Suramin.

Placental isoprostanes

In the non-diabetic U rats the concentration of the isoprostane 8-iso-PGF$_{2\alpha}$ in placental tissue was increased by Suramin treatment. This increase was similar regardless of the dose of Suramin, but was decreased by additional vitamin E treatment. In the non-diabetic H rats the placental isoprostane concentration was increased by Suramin, but not affected by vitamin E treatment. In the diabetic U and H rats the concentration of placental isoprostanes was higher than in the UN and HN rats, but was not affected by Suramin treatment. The concentration of placental isoprostanes was decreased by vitamin E treatment in the diabetic U rats, but not in the diabetic H rats. (Table 2)

The increased concentration of the isoprostane 8-iso-PGF$_{2\alpha}$ in the placenta indicates enhanced lipid peroxidation and increased oxidative stress. The reliability of isoprostanes as measures of oxidative status in tissues and bodily fluids has been questioned, since it has been shown that isolated rat kidney glomeruli are able to produce the isoprostane 8-iso-PGF$_{2\alpha}$ via a cyclooxygenase (COX) dependent pathway [91]. However, this pathway has not been demonstrated in placental tissue, and strong co-variations between isoprostanes and other measures of oxidative stress (TBARS, carbonylated proteins) have been noted in rat foetal and maternal tissues [35]. Further-
more, embryonic tissue exposed to diabetic environment shows evidence in favour of decreased, rather than increased, COX activity [176]. Taken together, it seems reasonable to assume that the isoprostane measurements in the present study reflect, at least to a major extent, a state of oxidative stress in the Suramin-treated and diabetic rats.

Placental morphology

Morphological changes were observed in the trophospongium and the labyrinth layers of the placentas of both the Suramin-treated and diabetic rats. Maternal diabetes and Suramin treatment seemed to compromise the placentation in a similar manner: the morphological changes caused by Suramin were similar to those caused by diabetes.

The trophospongium

The trophospongium of the placentas of diabetic rats is known to contain cystic spaces [5,66,129]. These cysts seem to derive from groups of degenerating glycogen cells [120]. Glycogen, which is normally not detected in the term rat placenta, is known to accumulate in the placentas of diabetic rats, and can be a sign of placental dysmaturity [4,65]. In the present study numerous large cysts were noted in all the diabetic rat placentas, without a noticeable effect of additional Suramin or vitamin E treatment. In the placentas of the non-diabetic control rats only a few small cysts were seen, but in the Suramin treated rats, with or without additional vitamin E treatment, these cysts were somewhat larger and more abundant. This may indicate that Suramin treatment inhibits the maturation of the placenta.

The labyrinth

The thickness of the labyrinthine walls (Table 3) and the average distance between foetal blood vessels and maternal blood lacunae (Table 3) were increased in the non-diabetic rats after Suramin treatment, but not when vitamin E treatment was also administered. Both these parameters were or tended to be increased also by maternal diabetes. In the diabetic U rats Suramin treatment unexpectedly decreased the thickness of the labyrinthine walls and the distance between maternal and foetal circulations, and additional vitamin E treatment further exaggerated the decrease. In the diabetic H rats, however, the treatments had no effect on these parameters.

These seemingly conflicting results might indicate that the primary effect of Suramin is to increase the barrier between the maternal and foetal vascular spaces, and that the introduction of diabetes complicates the condition and unmasks a genetic difference in the ability of physiological adaptation to increased demands between the two rat strains. It is possible, that the placenta of the diabetic U rat is compromised to such an extent, that an additional stress (i.e. Suramin treatment) results in necrosis of the placental tis-
sue, which causes the thinner walls of the diabetic U rats. The diabetic H rats, on the other hand, can still handle the additional Suramin treatment without this ultimate damage.

The size of maternal lacunae tended to be increased in the Suramin treated non-dibetic rats, and was increased when vitamin E was also administered. The maternal lacunae were enlarged in the diabetic rats, and Suramin and vitamin E treatments further increased their size. (Table 3)

In three of the four study groups (non-diabetic U and H rats and diabetic H rats) the blood volume in the placenta was decreased by Suramin treatment, but remained normal when vitamin E was administered. In the diabetic U rats a tendency to similar changes in the blood volume were noted. The same effect was seen both in the total blood volume in placenta and the ratio between foetal and maternal blood volume (Table 3). Diabetes per se had no effect on the placental blood volume.

The observed growth retardation of the foetus after Suramin treatment might be explained by the increased diffusion distance in the Suramin-treated non-diabetic rats. Although the Suramin induced thickening of labyrinthine walls and increase in the distance between foetal blood vessels and maternal blood lacunae were largely corrected by vitamin E treatment, the foetal growth was not enhanced. This discrepancy may be partly explained by the morphological finding, that in the Suramin and vitamin E-treatment group the single maternal blood volume lacunae were larger than those in the control group. This may indicate that the actual contact surface between the two compartments remains diminished although the foetal / maternal blood volume ratio and the thickness of the placental barrier are corrected.

Suramin injections were given at a time of the formation of the chorioallantoic placenta, and this caused decreased maternal blood volume. Since Suramin is a known inhibitor of angiogenesis [23,64], it is likely to have restricted the development of the uterine vasculature. This effect of Suramin could be blocked by administration of vitamin E, and increased levels of isoprostanes were noted in the placentas of Suramin treated rats. It is, therefore, likely, that oxygen radical formation is a component of the Suramin effect.

**Renal weight**

The Suramin treated non-diabetic U and H rats had heavier kidneys than the control rats (UN, HN), regardless of dose of Suramin or vitamin E treatment. Interestingly, Suramin did not seem to have an effect on renal weight in non-
Table 3

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Thickness of walls in labyrinth</th>
<th>Diffusion distance</th>
<th>Size of maternal lacunae</th>
<th>Blood volume in placenta foetal / maternal ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>UN</td>
<td>11</td>
<td>2.00</td>
<td>10.4 ± 0.4</td>
<td>1.00</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>UNS</td>
<td>12</td>
<td>2.62 *</td>
<td>14.4 ± 0.6 *</td>
<td>1.23</td>
<td>7.6 ± 0.8 *</td>
</tr>
<tr>
<td>UNSE</td>
<td>6</td>
<td>1.50 †</td>
<td>10.1 ± 0.9 †</td>
<td>2.17 *</td>
<td>3.9 ± 0.8 †</td>
</tr>
<tr>
<td>UD</td>
<td>5</td>
<td>2.63</td>
<td>11.9 ± 0.8</td>
<td>1.25</td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td>UDS</td>
<td>5</td>
<td>1.70 *</td>
<td>10.2 ± 0.5</td>
<td>1.90 *</td>
<td>5.5 ± 0.6</td>
</tr>
<tr>
<td>UDSE</td>
<td>5</td>
<td>1.13 *</td>
<td>8.5 ± 0.5 *</td>
<td>2.13 *</td>
<td>4.3 ± 1.1</td>
</tr>
<tr>
<td>HN</td>
<td>6</td>
<td>1.83</td>
<td>9.0 ± 0.1</td>
<td>1.33</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>HNS</td>
<td>6</td>
<td>2.83 *</td>
<td>11.4 ± 0.7 *</td>
<td>1.67</td>
<td>9.6 ± 1.3 *</td>
</tr>
<tr>
<td>HNSE</td>
<td>6</td>
<td>1.83</td>
<td>9.5 ± 0.5 †</td>
<td>2.17 *</td>
<td>3.5 ± 0.7 †</td>
</tr>
<tr>
<td>HD</td>
<td>5</td>
<td>2.30</td>
<td>10.9 ± 0.8</td>
<td>1.50</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>HDS</td>
<td>4</td>
<td>2.50</td>
<td>10.3 ± 0.6</td>
<td>1.88 *</td>
<td>5.9 ± 1.1 *</td>
</tr>
<tr>
<td>HDSE</td>
<td>5</td>
<td>1.90</td>
<td>11 ± 0.7</td>
<td>2.30 *</td>
<td>2.3 ± 0.6 †</td>
</tr>
</tbody>
</table>

* p < 0.05 when compared to the respective control group (UN, UD, HN or HD)
† p < 0.05 when compared to UNS, HNS or HDS, as appropriate

Table 4

<table>
<thead>
<tr>
<th></th>
<th>Renal weight (g)</th>
<th>Renal perfusion (TPU)</th>
<th>Damaged glomeruli (%)</th>
<th>Renal weight (g)</th>
<th>Renal perfusion (TPU)</th>
<th>Damaged glomeruli (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UN</td>
<td>0.83 ± 0.01</td>
<td>46 ± 2</td>
<td>4.6</td>
<td>HN</td>
<td>0.85 ± 0.06</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>(n=11)</td>
<td></td>
<td>(n=6)</td>
<td></td>
<td></td>
<td>(n=6)</td>
<td></td>
</tr>
<tr>
<td>UNS</td>
<td>1.04 ± 0.03 *</td>
<td>32 ± 2 *</td>
<td>9.0 *</td>
<td>HNS</td>
<td>1.12 ± 0.05 *</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>(n=12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(n=6)</td>
<td></td>
</tr>
<tr>
<td>UNS</td>
<td>1.12 ± 0.08 *</td>
<td>33 ± 3 *</td>
<td>15.5 *</td>
<td>HNSE</td>
<td>1.17 ± 0.07 *</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(n=6)</td>
<td></td>
</tr>
<tr>
<td>UD</td>
<td>1.42 ± 0.05</td>
<td>45 ± 3</td>
<td>16.3</td>
<td>HD</td>
<td>1.21 ± 0.03</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>(n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(n=5)</td>
<td></td>
</tr>
<tr>
<td>UDS</td>
<td>1.60 ± 0.09</td>
<td>29 ± 2 *</td>
<td>17.7</td>
<td>HDS</td>
<td>1.38 ± 0.09</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>(n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(n=4)</td>
<td></td>
</tr>
<tr>
<td>UDSE</td>
<td>1.55 ± 0.05</td>
<td>39 ± 4 *</td>
<td>9.6 *</td>
<td>HDSE</td>
<td>1.53 ± 0.13</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>(n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(n=5)</td>
<td></td>
</tr>
</tbody>
</table>

TPU = tissue perfusion units
* p < 0.05 when compared to the respective control group (UN, UD, HN or HD)
† p < 0.05 when compared to UNS
pregnant female rats. The diabetic rats of both strains had considerably heavier kidneys than the non-diabetic controls, without effect of different treatments. (Table 4)

The renal hypertrophy presented after Suramin treatment correlated with the dose of the drug. Similarly, in the case of diabetes the weight of the rat kidney correlates with the blood sugar level [153].

Proteinuria and renal perfusion

Proteinuria, a diagnostic criterion for pre-eclampsia, was present in all the rats in the study, regardless of pregnancy or treatment. In fact, the protein excretion tended to be increased by pregnancy rather than by Suramin administration. As a consequence, proteinuria is not a valuable parameter in evaluating Suramin treated rats as a model for pre-eclampsia. Instead, investigating blood flow in the kidney seems a better method of evaluating this model. Decreased renal blood flow in human pre-eclampsia [42,182] has been suggested to reflect changed autoregulation of blood flow [93] or, alternatively, to be a consequence of renal vasospasm and / or partial stenosis of the main renal and segmental arteries [108].

In the U rats (non-diabetic and diabetic) Suramin decreased the blood flow in the kidney, respective to dose, without an additional effect of vitamin E treatment. Interestingly, there was no difference in renal blood flow between the non-pregnant UN and UNS rats and the pregnant UN rats, which indicates a pregnancy-specific effect of Suramin on the kidney. Diabetes did not affect the renal blood flow in either of the rat strains, and all the H rats regardless of treatment had similar renal blood flows. (Table 4)

Renal morphology

Glomerular endotheliosis has been described in pre-eclampsia [160], and was considered pathognomonic for the disease until similar morphological changes were found even in renal biopsies from healthy pregnant women [165]. Investigation with a light microscope did not reveal any specific glomerular damage in the Suramin-treated or diabetic rats. The damage found in these rats was similar to that in the controls. The glomeruli denoted "normal" displayed normal morphology and filled Bowman's space. The greatly damaged glomeruli were shrunken and filled their Bowman's space up to a third, or less. The morphology of the moderately damaged glomeruli was intermediate to the morphology of the normal and severely damaged glomeruli. An increased number of damaged glomeruli after Suramin treatment was observed in the non-diabetic U and H rats (Table 4). In the diabetic U and H rats the number of damaged glomeruli was increased, but no additional effect of Suramin treatment was seen. The effects of additional vita-
min E treatment were somewhat contradictory: it increased the amount of damaged glomeruli in non-diabetic U rats and diabetic H rats, had no effect in non-diabetic H rats, and decreased the number of damaged glomeruli in diabetic U rats. The absolute number of glomeruli did not differ between groups.

In the Suramin treated non-diabetic U and H rats, with or without vitamin E treatment, several of the tubular lumens were slightly enlarged, and the walls contained small vacuoles, which sometimes were abundant. In the UN and HN rats these vacuoles were rarely observed. In contrast, in the kidneys of the diabetic rats we found wide and enlarged tubules with a few vacuoles in the tubular walls. In the Suramin-treated diabetic U and H rats, with or without vitamin E treatment, the tubules were also enlarged, and the walls of these contained considerably more small vacuoles. The renal hypertrophy after Suramin treatment or in case of maternal diabetes seemed to be mostly due to tubular enlargement, as it has previously been described in diabetic rat pregnancies [117,193].

Summary

The main finding of this study was that Suramin injections (two doses of 100 mg/kg given intraperitoneally on gestational days 10 and 11) to pregnant rats cause placental dysfunction: growth restriction of the foetus, increased resorption rate, reduced placental blood flow, and decreased maternal blood volume in the placenta. In the U strain Suramin also causes maternal hypertension and reduced renal blood flow, thus inducing a condition similar to human pre-eclampsia. In the H strain, however, these maternal symptoms are absent. The morphological changes after Suramin-treatment in the placentas and kidneys are similar in the pregnant non-diabetic U and H rats. Therefore, the differences in response to the treatment between the two rat strains must be in their reaction to these changes. This resembles the situation in humans, where shallow placentation is seen in pregnancies not only complicated by pre-eclampsia, but also in cases of intrauterine growth restriction or miscarriage [89].

U strain was clearly more susceptible to Suramin than the H strain. It is likely that the U strain possesses a genetic predisposition to Suramin exposure. Since the basal placental blood flow in the U rats was about half of that in the H rats, it is tempting to speculate that this is a main reason for the differences seen between the strains. The U strain has a generally lower placental blood flow rate compared with the H strain, and thereby presumably less, and in case of maternal diabetes effectively no, functional reserve to compensate for a Suramin-induced disturbance. In this context it is interesting to remember, that previous experiments have found the U strain to be more sensitive to the teratogenic effects of maternal diabetes than other strains.
The nature of this teratogenic predisposition is currently being investigated in a gene linkage study (Nordquist et al. in preparation) but in the light of the current work it seems possible that the lower placental reserve in U rats might contribute to the susceptibility for malformations.

Diabetes in the mother caused accumulation of placental isoprostanes, as in previous studies [57,158,176]. We know that vitamin E administration is able to alleviate the hampered foetal growth, as well as diminish the increased resorption and malformation rates of diabetic rats [36,157,158]. In the present study, however, vitamin E treatment failed to affect foetal weight and resorption rates of Suramin-treated normal and diabetic rats. One may ask, whether a combination therapy with both vitamin E and C had been more effective. This is, of course, not impossible. However, since the combination therapy proved no more effective than vitamin E alone in preventing malformations in the offspring of diabetic U rats [36,157], it is likely that the results of the current study would have been similar even if combination therapy had been used. The current results suggest that the Suramin effects on the foetus may, after all, in part be an acute toxic effect not involving oxygen radicals. Diabetes, however, causes diminished growth by instituting a state of chronic oxidative stress in the mother and offspring, a condition that can be affected by antioxidant treatment. On the other hand, vitamin E administration normalized the increased blood pressure and diminished placental blood flow caused by Suramin injections both in normal and diabetic U rats. Clearly some aspects of Suramin exposure seem to be mediated by oxygen radical generation.

The morphological changes caused by Suramin were similar to those caused by diabetes, and it might be that both maternal diabetes and Suramin treatment compromise the placentation in a similar manner. This is not entirely surprising, since, after all, streptozotocin-induced diabetic rat has been proposed as an animal model for pre-eclampsia [81]. The effects of Suramin on the diabetic rats were rather modest; presumably the combined effect of Streptozotocin and Suramin is less than the effect of both compounds separately since they – at least partly – may have shared mechanisms of function. The increased levels of placental isoprostanes and partial curative effect of vitamin E treatment in diabetic and / or Suramin treated rats suggest that oxidative stress might be one of these common mechanisms.

In conclusion, Suramin-injected pregnant rats seem a valid animal model for placental dysfunction (U and H rats) and pre-eclampsia (U rats). The damage to the embryo and the pregnant rat is associated with (placental) oxidative stress, but is only partly diminished by vitamin E treatment. If antioxidant treatment is to be used to block placental-associated damage to embryo and mother, the dosage and means of administration need to be further investigated. In addition, the similarity of placental damage caused by maternal
diabetes and Suramin treatment suggests that one important effect of untreated maternal diabetes in the rat may be impaired placentation, leading to oxidative stress, morphological damage, and compromised placental function.

Paper IV

Background data

Background data of the women are presented in Table 5. The women of all groups were of similar age, and the parity was similar in all pregnant groups. Smoking habits were similar between the non-pregnant women and the pregnant women during the first trimester. The patients with early onset pre-eclampsia had a higher BMI at early pregnancy than the early controls, and all women with pre-eclampsia had higher BMI than the non-pregnant women. While the blood pressure in early pregnancy was normal in all groups, it was higher in the pre-eclampsia groups than in the respective controls.

At delivery, the blood pressure of patients with pre-eclampsia was, naturally, higher than that of the controls. The women with early onset pre-eclampsia had higher systolic blood pressure than the women with late onset pre-eclampsia, even though they were more often treated with antihypertensive medication. They also were more likely to receive more than one drug. This might be an indication of a more aggressive disease in the early onset case, although it might just reflect the clinical practice of prolonging the preterm pregnancy to the maximum, while the treatment of choice in near-term pre-eclampsia is delivery.

The gestational age was similar between the early pre-eclampsia group and the early controls, but in the late pre-eclampsia group the gestational age was on average 12 days shorter than in the late control group. In the early pre-eclampsia group 56% of the infants were small for gestational age, but in the late pre-eclampsia group only 5%. In the control groups no infants were small for gestational age. This overrepresentation of small for gestational age infants in the early pre-eclampsia group is well in concordance with previous studies [118,132,175,184], and illustrates how early onset pre-eclampsia is associated with more compromised foetal well-being. Increased CRP in only the early onset pre-eclampsia group suggests as well, that early onset pre-eclampsia is a more severe condition.
Table 5

<table>
<thead>
<tr>
<th></th>
<th>Non-pregnant</th>
<th>early control</th>
<th>early pre-eclampsia</th>
<th>late control</th>
<th>late pre-eclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>31⁺</td>
<td>18</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>Age (mean ± SEM)</td>
<td>30 ± 0.9</td>
<td>31 ± 0.9</td>
<td>31 ± 1.0</td>
<td>33 ± 1.3</td>
<td>30 ± 1.3</td>
</tr>
<tr>
<td>Primipara (%)</td>
<td>45</td>
<td>67</td>
<td>35</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>7</td>
<td>19</td>
<td>22</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>BMI (mean ± SEM)</td>
<td>23 ± 0.7</td>
<td>23 ± 0.6</td>
<td>27 ± 1.3₇</td>
<td>25 ± 0.9</td>
<td>27 ± 1.3₉</td>
</tr>
<tr>
<td>BP* (mean ± SEM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 1st trimester, systolic</td>
<td>113 ± 2.1</td>
<td>123 ± 3.4f</td>
<td>113 ± 2.4</td>
<td>121 ± 3.0f</td>
<td></td>
</tr>
<tr>
<td>- 1st trimester, diastolic</td>
<td>69 ± 1.2</td>
<td>77 ± 1.9f</td>
<td>71 ± 1.8</td>
<td>76 ± 1.9f</td>
<td></td>
</tr>
<tr>
<td>- at delivery, systolic</td>
<td>120 ± 3.3</td>
<td>150 ± 4.2fg</td>
<td>114 ± 6.9</td>
<td>131 ± 7.0f</td>
<td></td>
</tr>
<tr>
<td>- at delivery, diastolic</td>
<td>77 ± 2.3</td>
<td>95 ± 2.9f</td>
<td>76 ± 2.5</td>
<td>91 ± 1.6f</td>
<td></td>
</tr>
<tr>
<td>Any BP medication (%)</td>
<td>83g</td>
<td>71g</td>
<td></td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Gestational age (days, mean ± SEM)</td>
<td>28.2 ± 0.4g</td>
<td>28.6 ± 0.7fg</td>
<td>39.7 ± 0.4f</td>
<td>37.9 ± 0.4f</td>
<td></td>
</tr>
<tr>
<td>SGA / AGA / LGA (n)</td>
<td>0 / 30 / 1</td>
<td>10 / 8 / 0fg</td>
<td>0 / 15 / 2</td>
<td>1 / 18 / 1</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>5 ± 1</td>
<td>21 ± 7fg</td>
<td>4 ± 1</td>
<td>7 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

a includes 22 women with term delivery (group 2a) and 9 with preterm delivery (group 2b)
b BMI = body mass index in early pregnancy
c Blood pressure, mmHg
d at sampling for women with term delivery in early control group, at delivery for all others.
e SGA / AGA / LGA = small / appropriate / large for gestational age, respectively.
f p < 0.05 when compared to respective controls (early or late)
g p < 0.05 when early control compared to late control, or early pre-eclampsia to late pre-eclampsia
h p < 0.05 when compared to non-pregnant

Table 6

<table>
<thead>
<tr>
<th></th>
<th>Non-pregnant</th>
<th>Early control</th>
<th>Early pre-eclampsia</th>
<th>Late control</th>
<th>Late pre-eclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-1 / PAI-2</td>
<td>0.64 ± 0.06</td>
<td>2.10 ± 0.37*</td>
<td>0.36 ± 0.06</td>
<td>0.49 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>8-iso-PGF₂α in placenta (pg/mg)</td>
<td>50 ± 7</td>
<td>81 ± 13 *</td>
<td>44 ± 8</td>
<td>53 ± 9</td>
<td></td>
</tr>
<tr>
<td>8-iso-PGF₂α in serum (pg/ml)</td>
<td>178 ± 20</td>
<td>199 ± 17</td>
<td>171 ± 26</td>
<td>161 ± 23</td>
<td>174 ± 29</td>
</tr>
<tr>
<td>8-iso-PGF₂α in urine (pg/ml)</td>
<td>282 ± 64*</td>
<td>559 ± 71</td>
<td>675 ± 107</td>
<td>527 ± 177</td>
<td>693 ± 165</td>
</tr>
<tr>
<td>Vitamin E in serum (µM)</td>
<td>19.5 ± 2.9*</td>
<td>23.7 ± 2.4</td>
<td>28.1 ± 2.8</td>
<td>32.1 ± 2.6</td>
<td>33.4 ± 2.5</td>
</tr>
<tr>
<td>Vit E / cholesterol (µM /mM)</td>
<td>4.6 ± 0.7</td>
<td>4.1 ± 0.4</td>
<td>4.7 ± 0.2</td>
<td>5.0 ± 0.4</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>Vitamin C in serum (µM)</td>
<td>35.2 ± 1.6</td>
<td>33.9 ± 2.3</td>
<td>32.7 ± 2.1</td>
<td>33.1 ± 2.9</td>
<td>34.3 ± 5.5</td>
</tr>
</tbody>
</table>

Mean ± SEM
* p < 0.05 compared to all other groups
PAI-1 / PAI-2

The ratio of PAI-1 / PAI-2 was similar in the late PE group and the late controls, but higher in the early PE group when compared to all other groups (Table 6). No PAI-2 was detected in the serum of the non-pregnant women. An increased PAI-1 / PAI-2 ratio in pre-eclampsia has been reported in previous studies [48,151] and seems to predate the clinical disease by several weeks [38,121]. In these studies however, early and late onset pre-eclampsia were not separated. These novel results indicate, that placental dysfunction is mostly related to early onset pre-eclampsia.

Isoprostane concentrations

The level of 8-iso-PGF$_{2\alpha}$ in the placental tissue was higher in the early pre-eclampsia group than in the other groups (Table 6). The late pre-eclampsia group and the early and late controls had similar levels of placental 8-iso-PGF$_{2\alpha}$. Two earlier studies of the association between pre-eclampsia and 8-iso-PGF$_{2\alpha}$ levels in placental tissue showed increased levels in pre-eclampsia [162,170]. In these studies the patients were, however, not subgrouped into early and late onset pre-eclampsia. The current study shows, that only early onset pre-eclampsia is associated with increased placental oxidative stress.

In serum, however, the levels of 8-iso-PGF$_{2\alpha}$ did not differ between groups (Table 6). This result might be misleading, since blood is biologically active and there is a continuous turnover of isoprostanes, which might give either false high or false low levels of 8-iso-PGF$_{2\alpha}$ when analysed [17]. In this study the samples were not stored equally long before being processed and deep frozen, even if the majority of samples were frozen within a few hours of collecting. This means that in vitro formation of 8-iso-PGF$_{2\alpha}$ could have occurred to a different degree in the different serum samples. Some previous studies do support that the blood levels of 8-iso-PGF$_{2\alpha}$ are similar in healthy pregnant women and women with pre-eclampsia [38,82,111], whereas other studies show higher levels in women with pre-eclampsia [14,15,106,121]. In the light of the present study, this discrepancy might be at least partly explained by differences in gestational age of the pre-eclampsia patients in the different studies. Also, Rogers and collaborators showed that serum levels of 8-iso-PGF$_{2\alpha}$ in women who developed pre-eclampsia were increased at gestational weeks 24-32, but not at 34-37 weeks [145].

Non-pregnant women had significantly lower levels of 8-iso-PGF$_{2\alpha}$ in urine compared with pregnant women, and there were no significant differences in urine 8-iso-PGF$_{2\alpha}$ between women with PE and their controls, even if there was a tendency towards higher levels in women with PE (Table 6). An adjustment for urinary creatinine levels did not change these results. There are previous studies with similar results [82,106,137], but even those which
show increased urinary isoprostane levels in pre-eclampsia [14,15]. The finding of an elevated level of 8-iso-PGF$_{2\alpha}$ in urine in pregnant compared with non-pregnant women is consistent with reports from previous studies [82].

The observed tendency to higher urinary isoprostane levels in pre-eclampsia might be caused by differences in BMI, since urinary isoprostane levels are known to correlate with BMI [86]. However, since the BMI of the women with late pre-eclampsia and the late controls was similar, this is not likely. Even though the smoking habits between study groups did not differ significantly, smoking is a possible disturbing factor: The number of cigarettes smoked is known to correlate with isoprostane levels [112,138], and unregistered changes in smoking habits during pregnancy may have interfered with the results.

It is probable that the 8-iso-PGF$_{2\alpha}$ levels in urine reflect the general body level of oxidative stress more reliably than its levels in serum, since isoprostane formation and break down do not seem to occur in urine [17]. These findings thus support that pregnancy increases general oxidative stress, but there seems to be no difference between healthy pregnant women and women with pre-eclampsia.

Early onset pre-eclampsia causes increased oxidative stress also in placental tissue, while late onset disease does not. It is, therefore, likely, that there are differences in the pathogeneses of early and late onset pre-eclampsia, as has been previously proposed [115,119,175].

**Vitamins E and C in serum**

Serum levels of vitamin E were slightly higher in all pregnant groups compared to non-pregnant women, however, when the vitamin E concentration was adjusted to serum cholesterol no differences were seen between groups (Table 6). This is in accordance with the findings of Morris and collaborators who discovered higher serum levels of vitamin E in pregnant compared to non-pregnant women, without differences between pregnant women with or without pre-eclampsia [111]. Others have also reported similar vitamin E concentration in the serum of pre-eclamptic compared to healthy pregnant women [38,72]. However, there are also previous studies reporting both decreased [87,152] and increased [169] serum levels of vitamin E in pre-eclampsia.

Serum levels of vitamin C did not differ between the groups (Table 6). Several previous studies have shown decreased vitamin C levels in women with pre-eclampsia compared to healthy pregnant women [38,87,99]. Zhang and collaborators connected such findings to lower dietary intake of vitamin C
The lack of differences in the vitamin C levels in the current study might be explained by similar dietary intake of vitamin C among the women. After all, all pregnant women included in the study had participated in the standard antenatal follow-up, as part of which they had received information and recommendations of healthy diet.

General discussion

The multifactorial origins of pre-eclampsia are reflected in the many experimental models for the disease. The numerous ways of inducing a pre-eclampsia like condition to laboratory animals may all be demonstrations of different causes or underlying factors of the human syndrome. The complexity of pre-eclampsia and the mystery of its underlying cause make finding curative or preventive treatments very difficult.

It seems likely that pre-eclampsia is, in fact, a group of conditions with different aetiological backgrounds but with similar clinical manifestations. The early onset form of pre-eclampsia is more likely to originate in incomplete trophoblast invasion and impaired placentation and is frequently associated with intrauterine growth restriction of the foetus. The onset of the disease often occurs already during the second trimester, and therefore an early pre-eclampsia is a great challenge to the obstetrician. Being able to prevent, or at least postpone, the onset of the disease, or to withhold its progress, would greatly improve the wellbeing of the newborn and its mother.

Oxidative stress has been a very promising candidate as an intervention site in the pathogenesis of pre-eclampsia, and preliminary studies of the efficacy of vitamins C and E in reducing the risk for the disease were positive [39]. However, two recent large randomized controlled trials failed to show a decrease in the occurrence of pre-eclampsia in women receiving vitamin E and C supplementation [128,147]. In these studies the average gestational age at inclusion was 18.6 and 17.1 weeks, and placentation was thus already almost completed, suggesting that the vitamin treatment could have prevented only the second stage of pre-eclampsia. One could speculate that the excess antioxidants might prove helpful in the first stage of pre-eclampsia, if administered at an earlier time during pregnancy, when the trophoblasts are invading the spiral arteries and the placental characteristics of pre-eclampsia are being formed.

The modest benefits of vitamin E to the Suramin treated U rats suggest, however, that even if vitamin supplementation to prevent human pre-eclampsia was introduced already in early pregnancy, the results might be similarly disappointing. It is possible, that although dietary supplementation
of vitamins E and C increases the amount of these antioxidants in serum, it has little effect in placental tissue, which the present study points out as a major location of oxidative stress. Perhaps antioxidant treatment per se is a valid method in preventing pre-eclampsia, but the problem to be solved is how to gain access for the compounds to the placental tissue. The Suramin treated U rat may be useful in future attempts to find a therapeutic route to block the onset and progress of pre-eclampsia.
Conclusions

Papers I-III

Suramin treatment of pregnant rats causes placental dysfunction, which in rats of the U strain resembles human pre-eclampsia. Suramin treated pregnant rats of the U strain may serve as an experimental model of pre-eclampsia.

Maternal diabetes marginally worsens the outcome in Suramin treated pregnant rats.

Vitamin E treatment of pregnant rats diminishes the effects of Suramin on mother but not on offspring.

Streptozotocin-induced diabetes in the rat may result in impaired placentaion, thereby leading to oxidative stress, morphological damage, and impaired placental function.

Paper IV

Pregnancy increases general oxidative stress.

Early onset but not late onset pre-eclampsia causes increased oxidative stress in placental tissue.

Early onset pre-eclampsia is a more severe condition than late onset pre-eclampsia.

The pathogenesis of early and late onset pre-eclampsia is likely to be different.
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References


100. Lopez-Jaramillo P, Garcia RG, Lopez M (2005) Preventing pregnancy-induced hypertension: are there regional differences for this global problem? J Hypertens. 23:1121-1129
103. MacGillivray I (1983) Pre-Eclampsia The Hypertensive Disease of Pregnancy
140. Roberts JM, Cooper DW (2001) Pathogenesis and genetics of pre-eclampsia. Lancet 357:53-56
141. Roberts JM, Hubel CA (1999) Is oxidative stress the link in the two-stage model of pre-eclampsia? Lancet. 354:788-789


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