

PRE-ECLAMPSIA—STILL A DISEASE OF THEORIES

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Abstract: Pre-eclampsia is still one of the leading causes of maternal and fetal morbidity and mortality. Despite active research for many decades, the etiology of this disorder exclusive to human pregnancy is an enigma. Recent evidence suggests there may be several underlying causes or predispositions leading to endothelial dysfunction and causing the signs of hypertension, proteinuria, and edema—findings that allow us to make the diagnosis of the “syndrome” of pre-eclampsia. It is obvious that a single mechanism responsible for the syndrome pre-eclampsia does not exist. Instead, several mechanisms can act together and even multiply each other. The search for the underlying cause of this disorder and for a clinical marker to predict which women will develop pre-eclampsia is ongoing, with its prevention being the ultimate goal.

Key words: Pre-eclampsia, vascular factors, oxidative stress, genetics, angiogenesis

INTRODUCTION

Hypertensive disorders in pregnancy constitute a major risk factor for maternal mortality as well as fetal wastage and morbidity in the United States and in countries worldwide. They are the second leading cause of maternal mortality in the United States, representing almost 15% of pregnancy-related deaths and occurring in 3%-10% of pregnancies^{1,2)}. This is especially true in underdeveloped nations. About 20% of perinatal mortality and morbidity are related to hypertensive disorders in pregnancy³⁾.

Classifications of hypertensive disorders in pregnancy have varied in the past and led to some confusion in both the clinical management and research efforts

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toward the etiology of these disorders⁴⁾. Currently, a classification established by the National Institutes of Health Working Group on High Blood Pressure in Pregnancy²⁾ is used in the United States and recommended by the International Society for the Study of Hypertension in Pregnancy :

- 1) *Chronic hypertension* :
Hypertension present before pregnancy or first diagnosed before 20 weeks' gestation
- 2) *Preeclampsia-eclampsia* :
Hypertension unique to pregnancy (blood pressure >140 mmHg systolic or 90 mmHg diastolic)
Diagnosed after 20 weeks' gestation
Associated by new onset proteinuria (≥ 0.3 g/24 h)
Eclampsia, if seizures occur
- 3) *Pre-eclampsia superimposed upon chronic or preexisting hypertension* :
New onset or acutely worse proteinuria, a sudden increase in blood pressure, thrombocytopenia, or elevated liver enzymes after 20 weeks' gestation in a woman with preexisting hypertension
- 4) *Gestational hypertension* :
Hypertension first diagnosed after 20 weeks' gestation, not accompanied by proteinuria
 - a) *Transient hypertension*
The hypertension resolves by 12 weeks' postpartum
 - b) *Chronic hypertension*
The hypertension does not resolve by 12 weeks' postpartum.

HISTORY

Hippocrates first described the condition when he wrote in one of his aphorisms "*convulsions take place from either repletion or depletion*". Hippocrates had observed the sudden and unexpected appearance of maternal grand-mal seizures, which occur when preeclampsia progresses to eclampsia, the word being derived from the Greek word for *lightning*.

Presumably eclampsia was first confused with epilepsy and not described as a separate entity until 1739. The use of the term has been attributed to Gutsch in 1776, but this has not been well documented. Nonetheless, it was many years more before it was universally accepted as separate from epilepsy or hysteria.

Even so, it was not until about 150 years ago, when protein could be measured in the urine and by the introduction of blood pressure measurements at the turn of the 20th century, that the forerunner to eclampsia became apparent.

The triad of hypertension, proteinuria, and edema was termed preeclampsia. Because of the toxins that were believed to be in the pregnant woman's body, this disorder was also commonly called "toxemia of pregnancy," a term coined at least

150 years ago, but not currently used in today's nomenclature. Pre-eclampsia is now unanimously viewed as a multisystem disorder, as increases in blood pressure are rarely responsible for multi-organ dysfunction⁵⁾.

There is a vast diversity of additional symptoms and complications associated with pre-eclampsia. These can include cerebral edema⁶⁾, neurological manifestations (including headache, confusion, paralysis, coma, visual loss, and seizures)⁷⁾, liver capsule distension⁸⁾, renal failure^{9,10)}, pulmonary edema^{10,11)}, thrombocytopenia¹²⁾, coagulopathy¹³⁾, hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome¹⁴⁾, and nausea¹⁵⁾.

EPIDEMIOLOGY

Pre-eclampsia is a pregnancy-specific syndrome that is the principal cause of maternal and fetal morbidity and mortality^{2,16)}, accounting for almost 15% of pregnancy-associated maternal deaths²⁾.

In the United States, from the years 1979 to 1986, pre-eclampsia was the second leading cause of maternal death¹⁾ and ranks between the second and third leading cause of maternal death in more recent years¹⁶⁾.

Risk and predisposing factors :

Numerous maternal factors can predispose women to the disorder pre-eclampsia ; these may be genetic, behavioral, or environmental. The list of predisposing factors includes, besides the history of a previous pre-eclampsia, hypertension, diabetes, increased insulin resistance, increased testosterone, black race, and increased blood homocysteine concentration (Table 1).

In industrialized countries, pre-eclampsia complicates 3%-5% of pregnancies¹⁷⁾ and is more likely to occur at both extremes of reproductive age, but is greatest in women younger than 20 years of age¹⁾.

Primigravid women are at higher risk for pre-eclampsia¹⁸⁾. The incidence of pre-eclampsia in multiparous women is lower than in primiparous women, but higher if the multiparous woman has a different partner^{19,20)}. This finding supports the hypothesis that risk is reduced with repeated exposures to specific antigens from the same partner. However, the protective effect against pre-eclampsia of a previous pregnancy with the same partner was likely confounded by the time interval between births. Skjaerven *et al.*²¹⁾ showed that the risk of pre-eclampsia in subsequent pregnancies was related to the time that had elapsed since the index pregnancy, not to a change of partners. When the birth interval was greater than 10 years, the risk of the multiparous woman was identical to that of a primiparous woman.

Also, recent information suggests that a short interval of sexual cohabitation before conception is associated with an increased risk of pre-eclampsia²²⁾. Women with longer durations of sexual cohabitation before conception may be exposed to

Table 1. Risk factors for pre-eclampsia

Preconceptional and/or chronic risk factors	
<i>Partner-related risk factors :</i>	
Nulliparity	Limited sperm exposure
Primipaternity	Donor insemination
Teenage pregnancy	Partner who fathered a pre-eclamptic pregnancy in another woman
<i>Maternal-specific risk factors :</i>	
Previous pre-eclampsia	Interval between pregnancies
Family history	Patient requiring oocyte donation
Increasing maternal age	
<i>Presence of specific underlying disorders :</i>	
Chronic hypertension	Low maternal birth weight
Renal disease	APC resistance (factor V Leiden)
Obesity	Protein S deficiency
Insulin resistance	Antiphospholipid antibodies
Gestational diabetes	Hyperhomocysteinaemia
Diabetes mellitus Type-1	
<i>Exogenous factors :</i>	
Smoking (risk decrease)	Structural congenital anomalies
Stress	Hydrops fetalis (hydropic placenta)
Urinary tract infection	Hydatiform moles
Pregnancy-associated risk factors	Chromosomal anomalies (trisomy 13, triploidy)
Multiple pregnancy	

paternal antigens and presumably become more tolerant.

Pre-eclampsia is more likely to occur in women with underlying hypertension or other chronic illnesses such as autoimmune disease, renal disease, and diabetes. The risk of superimposed pre-eclampsia upon already existent hypertension is approximately 25%²³⁾. Women with a strong family history of hypertension are also more susceptible to this syndrome²⁴⁾.

Additionally, women with thrombophilias, both inherited and acquired, may be more likely to develop pre-eclampsia²⁵⁻²⁸⁾. An association with pre-eclampsia has been suggested for women who have antiphospholipid syndrome^{25,29,30)}, factor V Leiden mutation^{25-27,32)} (whereby this mutation especially seems to be associated with hemolysis, elevated liver enzymes, low platelets [HELLP] syndrome³³⁻³⁵⁾ activated protein C resistance^{25,26,36)}, and hyperhomocysteinemia^{26,37)}.

Women who are carriers of certain other inherited metabolic disorders, aside from those that predispose to thrombophilia, also appear to be more likely to develop pre-eclampsia. Specifically, women who are heterozygous carriers for beta-oxidation disorders appear to be at a higher risk for pre-eclampsia as well as other complications of pregnancy³⁸⁾. Mothers with long chain 3-hydroxyacyl-coenzyme A dehydrogenase (LCHAD) deficiency not only have a higher risk of pre-

eclampsia, and acute fatty liver of pregnancy, but also HELLP syndrome, as well as intrahepatic cholestasis and hyperemesis gravidarum^{38,39}.

Other lipid abnormalities may be associated with the development of pre-eclampsia. Wetzka *et al.*⁴⁰ found higher levels of triglycerides and lipoproteins in women with severe pre-eclampsia with or without HELLP syndrome.

Interestingly, these are also risk factors for other endothelial diseases, particularly atherosclerosis, and the late complications of diabetes mellitus⁴¹. Pre-eclampsia, atherosclerosis, and diabetes also share a common dyslipidemia. Increased triglycerides, decreased high density lipoproteins (HDL), and an increased concentration of small dense low density lipoproteins (LDL) are characteristic of these disorders. In addition, although Chesley *et al.*⁴² showed that pre-eclampsia does not cause cardiovascular disease, the work of Fisher *et al.*⁴³ indicates that pre-eclamptic women have a higher risk of cardiovascular disease in later life. This finding supports common risk factors for pre-eclampsia and atherosclerosis, with normal pregnancy being a screening test indicating the absence of these factors.

Pre-eclampsia occurs only when placental tissue is present, and more often with an excess of placental tissue, even without the presence of a fetus. Women with multiple gestations (multiple placentas), i.e., twins and triplets, are more likely to develop pre-eclampsia⁴⁴, than women with a partial or complete molar pregnancy⁴⁵. Interestingly, women with hydropic or extremely edematous fetuses (and hydropic, edematous placentas) may also show signs and symptoms of pre-eclampsia⁴⁶ that have actually been noted to resolve before delivery if the fetal hydrops resolves⁴⁷.

ETIOLOGY/PATHOPHYSIOLOGY

Pre-eclampsia, a life-threatening disease unique to pregnancy has been called a disease of theories. Even today, the etiology of pre-eclampsia is unknown, widely speculated about, and studied. A completely satisfactory, unifying hypothesis has not emerged. It is likely that there may be several etiologies or underlying predispositions with effects that result in the common group of signs and symptoms we can find with the syndrome pre-eclampsia.

Vasospasm or increased vascular reactivity and endothelial cell dysfunction may be the final common pathway of several different pathophysiologic mechanisms⁴⁸. Nonetheless, the inciting organ in the syndrome of pre-eclampsia is the placenta⁴⁹. The major underlying mechanism of pre-eclampsia is inadequate placentation with resultant placental ischemia⁵⁰. According to this thesis, abnormal cytotrophoblast invasion of the spiral arteries of the uterus leads to failure of remodeling of these vascular channels into more spacious, lower resistance vessels. As a result, uteroplacental blood flow is compromised⁵¹.

The characteristic pathologic finding in the pre-eclamptic placenta results from shallow or "inadequate" interstitial invasion by the cytotrophoblastic cells and limited endovascular invasion⁵². Thus, the trophoblast fails to develop into vascu-

lar cells, as they do in normal pregnancy. In consequence, the spiral arteries of the uterus remain small and narrow, with high resistance to flow, resulting in a failure of the uterine blood supply to adequately nourish the placenta^{52,53}. These vessels, in pre-eclamptic patients, are estimated to be only about 40% of the diameter of those in normal pregnancy. This leads to placental hypoxia resulting in the villi of pre-eclamptic patients demonstrating abnormalities associated with growth in an environment of low oxygen tension.

In the past, several pathophysiologic mechanisms have been proposed suggesting that pre-eclampsia has multifactorial origins (Fig. 1):

- 1) An imbalance of vasodilative and vasoconstrictive substances^{54–56} resulting in peripheral vasoconstriction, which causes the reduced organ perfusion^{3,57}
- 2) Oxidative stress caused by an increased production of free radicals or by a deficiency of protective antioxidative substances^{58–60}
- 3) Immunologic defects such as antiphospholipid syndrome^{29,30} or angiotensin-1 receptor antibody^{61,62}
- 4) An excessive maternal inflammatory reaction to pregnancy^{59,63–65}
- 5) Coagulopathies and thrombophilias^{25–28,31,32,36}
- 6) Genetic mutations may play an important role in the pathogenesis of pre-eclampsia^{66,67}
- 7) Alterations in angiogenesis due to hypoxia and/or alterations in levels of angiogenic factors leading to inadequate placentation and immature vessel formation^{49,68–80}

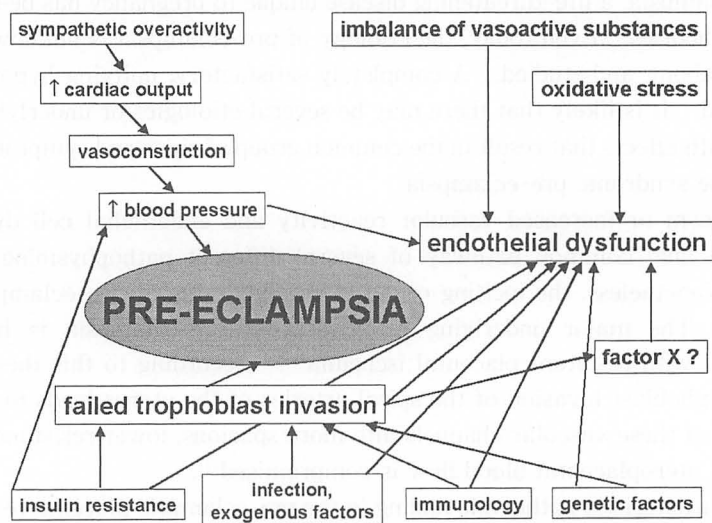


Fig.1. Etiologic and pathophysiologic mechanisms in the development of pre-eclampsia.

VASCULAR FACTORS

During pregnancy, extensive haemostatic changes occur in the utero-placental circulation and, in pregnancies complicated by pre-eclampsia, a restricted physiological adaptation of the utero-placental blood vessels leads to increased vascular resistance and reduced blood flow. Nearly every vasoactive substance (which additionally may interact with each other) has been investigated with respect to its possible involvement in the pathogenesis of pre-eclampsia (Fig. 2). This categorization includes the prostaglandins, the renin-angiotensin-aldosterone axis, nitric oxide (NO), atrial natriuretic peptide (ANP), endothelin, adrenomedullin, and vasopressin (Table 2).

Nitric Oxide :

In the last years, extensive study of the nitric oxide (NO) system has been performed. Both, animal experiments and studies in pregnant women, have strongly suggested an important role for the nitric oxide synthase (NOS) system in pre-eclampsia. NO is a small, molecular weight mediator with diverse functions that include vasodilation, inhibition of platelet aggregation, and vascular remodeling.

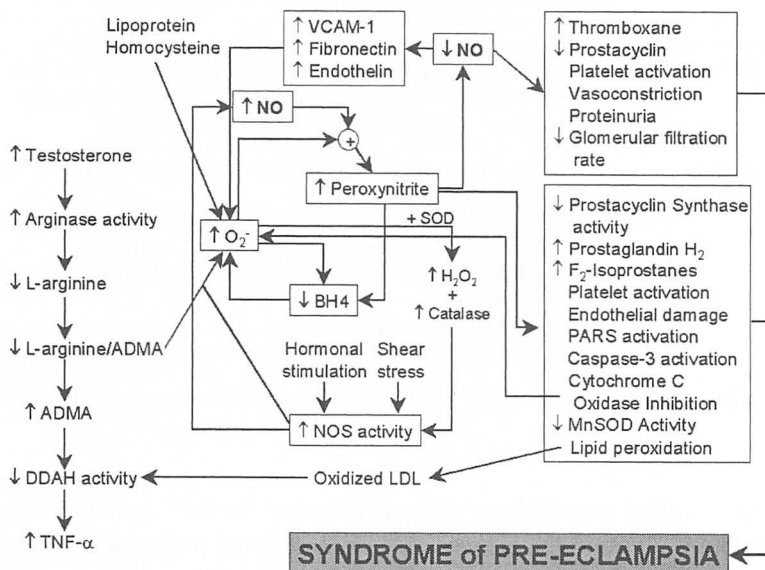


Fig. 2. Interactions involved in the pathophysiology and leading to the clinical syndrome of pre-eclampsia. Abbreviations: ADMA=asymmetrical dimethylarginine; BH4=tetrahydrobiopterin; DDAH=dimethylarginine dimethylaminohydrolase; H₂O₂=hydrogen peroxide; LDL=low-density lipoproteins; (Mn) SOD=(manganese) superoxide dismutase; NO=nitric oxide; NOS=nitric oxide synthase; O₂⁻=superoxide anion; PARS=poly(ADP-ribose) synthetase; TNF-α=tumor necrosis factor alpha; VCAM-1=vascular cell adhesion molecule-1

Table 2. Circulating vasoactive substances in pregnancy and pre-eclampsia

Vasoactive Substance (s)	Alterations in Pre-eclampsia
Nitric oxide (NO)	<p><u>Rat</u> : Chronic NOS inhibition results in renal and peripheral vasoconstriction, proteinuria, increased fetal morbidity, and intrauterine growth restriction.</p> <p><u>Human</u> : Studies report variable results (NO production and NOS inhibition), perhaps related to different vascular beds examined. NO metabolite levels increase through normal and particularly abnormal pregnancy, predominantly in the fetal compartments, suggesting that NO production is an additional instrument in the fetal control of the intrauterine environment</p>
Endothelin (ET)	<p><u>Human</u> : Most studies have shown an increase in ET in plasma and placental tissue of pre-eclamptic women.</p> <p>ET-1 induces oxidative stress and alters secretion of vasoactive substances in human endothelial cells.</p>
Prostaglandins	<p>Postulate : An imbalance $TXA_2 > PGI_2$, is pathogenetic.</p> <p>However, use of prostaglandin inhibitors (low-dose aspirin) only effective in high risk group</p>
Renin-Angiotensin-Aldosterone system (RAAS)	<p><u>Rat</u> : Chronic RAAS blockade throughout pregnancy does not play an important role in the hypertensive response to chronic reductions in uterine perfusion pressure.</p> <p><u>Human</u> : Plasma renin activity and angiotensin II are lower than normal in pre-eclampsia.</p> <p>Role in causation of pre-eclampsia remains unclear.</p>
Atrial natriuretic peptide (ANP)	<p>Controversy results about increased maternal ANP levels.</p> <p>Placental ANP production and levels of pro-ANP mRNA do not differ between pre-eclampsics and normal pregnant females.</p> <p>→ Changes in ANP levels may be a secondary effect.</p>
Adrenomedullin	<p>Levels of adrenomedullin do not differ from those measured in normal pregnant women.</p> <p>Levels in amniotic fluid and umbilical vein plasma have been reported to be several-fold higher in pre-eclampsia.</p> <p>Lower adrenomedullin mRNA expression in placental villi from pre-eclamptic women.</p> <p>Adrenomedullin may be involved in the adaptation of the vascular system to pregnancy and in the regulation of placental vascular tone.</p>

* Abbreviations : NOS=nitric oxide synthase ; TXA_2 =thromboxane ; PGI_2 =prostacyclin

NO, originally called endothelium-derived relaxing factor⁸¹⁾ results from the enzymatic action of NOS, which converts L-arginine, in the presence of oxygen, to L-citrulline and NO (Fig. 3). Molecular oxygen and NADPH are cosubstrates in this reaction. Three NOS enzymes have been sequenced : 1) the constitutive enzyme present in the vascular endothelium (eNOS or NOS-3), 2) neuronal cells (nNOS or NOS-1), and 3) several other cell types⁸²⁾. The other is an inducible enzyme (iNOS or NOS-2) that has been found in macrophages and neutrophils and is activated by bacterial endotoxin or cytokines (e.g., IL-1, interferon- γ)⁸²⁾. Human placental

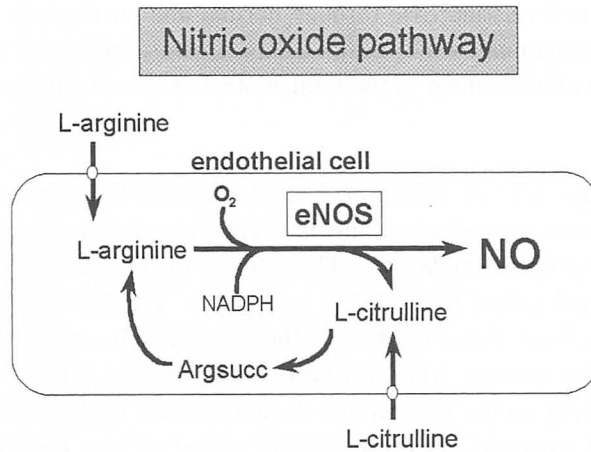


Fig. 3. Intracellular pathway of nitric oxide

syncytiotrophoblast is known to express eNOS but not iNOS. NO isoenzyme is also expressed on villous endothelial cells and NO produced from these cells is thought to be an important mediator for spiral artery transformation⁸³.

A series of studies in the rat have been performed^{84,85} to examine the postulate that the pregnant animal responds to a chronic reduction in uteroplacental perfusion pressure with a reduction in renal NO synthesis. Evidence has accrued that indicates that NO synthesis is increased in normal pregnancy as are plasma and urine levels of cyclic guanosine 3', 5'-cyclic monophosphate (cGMP), the second messenger of NO⁸⁶. In the rat, it has been possible to show that chronic NOS inhibition results in renal and peripheral vasoconstriction, proteinuria, increased fetal morbidity, and intrauterine growth retardation^{87,88}. Additionally, it has been reported that both eNOS and nNOS are upregulated in pregnant rats⁸⁹. Furthermore, chronic inhibition of NOS reverses systemic vasodilation and glomerular hyperfiltration in the pregnant rat model⁹⁰. Interestingly, chronic reduction in uterine perfusion pressure in the rat model is associated with no differences in whole body NO production and a decrease in the renal protein expression of neuronal NOS in pregnant animals compared with controls⁹¹.

Studies performed in pregnant and pre-eclamptic women have unfortunately produced conflicting results. This may be explained in part by the difficulties in assessing the NOS system in clinical settings, but additionally by the different settings of these studies (different vessels, plasma/serum, amniotic fluid, fetal blood). NO production seems to be reduced using maternal serum^{92,93}, and unchanged or higher in maternal plasma and/or urine⁹⁴⁻⁹⁶. In amniotic fluid, NO levels are higher in pre-eclampsia compared with normal pregnancy⁹⁶. In endothelial cell cultures and placental homogenates, NO production has been reported to be unchanged or higher in pre-eclampsia⁹⁷⁻⁹⁹. Recently a higher NO production has been reported in plasma from umbilical vein and artery⁹⁶.

NO metabolite levels increase through normal and particularly through abnormal pregnancy predominantly in the fetal compartments, suggesting that NO production is an additional instrument in the fetal control of the intrauterine environment.

Endothelins :

The endothelins (ET) are regulatory peptides, distributed in many organ systems and producing potent physiological effects. They are the most powerful vasoconstrictive substances known today¹⁰⁰. Three forms of these 21-amino acid peptides have been described, called ET-1, ET-2, and ET-3. ET-1, the most important of the three peptides, is produced by the endothelium¹⁰¹ and by smooth muscle cells¹⁰².

These peptides interact with two types of receptors: ETA and ETB. ETA receptors are present on the smooth muscle and mediate contraction in response to ET-1. The ETB receptors are present on the endothelium. Both ET-1 and ET-3 are capable of inducing the release of NO and prostacyclin, thereby inducing vascular relaxation. Thus, ETB receptors are able to mediate both vasodilation and vasoconstriction¹⁰³. ET-1 causes increased salt and water excretion, which represents a potentially hypotensive action, but in the vasculature it causes vasoconstriction¹⁰⁴. The relevance of endothelin to the pathogenesis of pre-eclampsia remains unclear. Thus, most studies^{56,104-108} have demonstrated an increase in endothelin in plasma or serum and in placental tissue¹⁰⁹ in pre-eclamptic women. Typically, ET-1 plasma levels are highest during the latter stage of the disease, suggesting that ET may not be involved in the initiation of pre-eclampsia, but rather in the progression of disease into a malignant phase⁷³. Interestingly, immunoreactive endothelin levels have been reported to be higher in the plasma¹¹⁰ and in the umbilical artery and vein blood of pregnant black women than in patients of European origin¹¹¹. Therefore, this could contribute to the higher incidence of hypertension and pre-eclampsia noted in the former population compared with the latter¹⁸. Levels of ET-1 performed in early pregnancy have been reported to have low¹¹² or no predictive value^{108,113} as to the later development of pre-eclampsia. Additionally, in experimental models of pregnancy hypertension, endothelin levels are often not elevated¹⁰⁴. Yet its effect on blood pressure most likely is more accurately described by its local action at the endothelial and vascular level rather than its serum concentration. Therefore, the actions of endothelin in regulating blood pressure are no doubt correlated best with its effects as a paracrine or autocrine factor. Faxen *et al.*¹¹⁴ noted no change in mRNA for ET-1 in myometrium or placenta of patients compared to normotensive pregnant patients. However, the expression of ETA-mRNA was significantly reduced in placenta, whereas that of ETB was unchanged. These data suggested that high circulating levels of ET-1 might have downregulated the ETA receptor. ET-1 levels have been determined by Singh *et al.*¹⁰⁹ to be significantly higher in placental tissues from women with pre-eclampsia than in normotensive pregnancies. Additionally, an ETA receptor antagonist has been reported to lower blood pressure in pregnant rats

in which hypertension was induced by chronic reductions in uterine blood flow¹¹⁵). Napolitano *et al.*¹¹⁶) reported an increased ET-1 expression in cultured human placental trophoblastic cells obtained from pre-eclamptic pregnancies compared with those harvested from the placentas of patients with normal pregnancies. The expression of iNOS was decreased in their studies, whereas that of eNOS was increased. The authors postulated that interactions between the ET and NOS systems could represent an important pathogenetic mechanism in the development of the reduced uteroplacental blood flow associated with pre-eclampsia¹¹⁶). Additionally, we could show, that incubation of human umbilical vein endothelial cells (HUVEC) with serum from pre-eclamptic women results in increased ET-1 production⁹⁷), therefore suggesting, that serum from pre-eclamptic women contains a factor(s) that specifically stimulates ET-1 secretion. Finally, when incubating HUVEC's for 24 hours with ET-1 in different concentrations (0-1,000 pmol/L)¹¹⁷), at lower concentrations (5-50 pmol/L), ET-1 increases the intracellular content of lipid peroxides (LPO), stimulates the secretion of thromboxane A₂ (TXA₂), but inhibits the secretion of prostacyclin (PGI₂). At higher concentrations (100-1,000 pmol/L), ET-1 increases the intracellular content of glutathione, but results in a decrease of LPO and an increase of PGI₂ back to control levels. ET-1 had no effect on NO secretion. Therefore ET-1 is able to induce oxidative stress and alter secretion of vasoactive substances in human endothelial cells. This observation supports the postulate that ET-1 is involved in the progression to a severe phase of the disease.

Prostaglandins :

The prostaglandins and thromboxane A₂ (TXA₂) are a series of biologically active compounds derived from arachidonic acid¹¹⁸). The former are vasodilatory, while the latter is a vasoconstrictor. The prostaglandins are considered to represent important mediators of the minute-to-minute tone of the vasculature acting to offset the vasoconstrictive influence of angiotensin II. The major vasodilator prostaglandin is prostacyclin (prostaglandin I₂ [PGI₂]). TXA₂ is the principal metabolite of arachidonic acid in platelets. This compound is only evanescently present in plasma (its half-life is approximately 30 seconds), so that its effects are largely a function of the microenvironment of its action. TXA₂ is an important contributor to platelet aggregation intravascularly, but also contracts the muscular layer of arteries. Prostacyclin, however, is an inhibitor of platelet aggregation and is also a major vasodilator.

Several lines of evidence suggest that changes in the prostaglandin system may play a role in mediating the renal dysfunction and increase in arterial pressure during pre-eclampsia¹¹⁹). Significant alterations in PGI₂ and TXA₂ production occur in women with pre-eclampsia¹²⁰). Plasma and urine levels of TXA₂ are elevated in women with pre-eclampsia, whereas synthesis of prostaglandins, such as PGI₂, is reduced¹²⁰⁻¹²²).

Additional evidence for a potential role of TXA₂ in pre-eclampsia derives from

a study demonstrating that short-term increases in systemic arterial pressure produced by acute reductions in uterine perfusion in pregnant dogs can be prevented by thromboxane receptor antagonism¹²³⁾. In addition, Wang and coworkers¹²²⁾ reported that there is an abnormal increase of serum lipid peroxides in pre-eclamptic women. They postulated that these substances, which cause oxidative stress and thereby cellular damage, act by inhibiting prostaglandin synthase.

Further evidence of a potential role for TXA_2 is supported by studies in humans indicating that low-dose aspirin may attenuate the development of pre-eclampsia, but unfortunately, a meta-analysis revealed disappointing results¹²⁴⁾. This can be in part explained by the different settings of these studies. Recent evidence suggest that in women at high risk for the disease¹²⁵⁾, low dose aspirin (100 mg/day) has a significant benefit, when started early in pregnancy (before week 16).

Renin-Angiotensin-Aldosterone System :

The renin-angiotensin-aldosterone system (RAAS) plays an important role in the long-term regulation of renal function and arterial pressure during a variety of physiological and pathophysiological conditions. It is a major contributor not only to the sodium and volume status of the intact organism, but also to the maintenance of the blood pressure¹²⁶⁾. Because of its importance in not only the day-to-day but also the minute-to-minute regulation of blood pressure, the RAAS in the pathogenesis of pre-eclampsia has been extensively investigated.

During normal pregnancy, plasma renin concentration, renin activity, angiotensin II (AT II) levels, and aldosterone are all elevated ; however, the vascular responsiveness to AT II appears to be reduced^{9,127)}.

The importance of the RAAS in the regulation of renal function and arterial pressure during pre-eclampsia has not yet been fully elucidated. Plasma renin activity and AT II levels are usually lower than normal throughout pregnancy in patients with pre-eclampsia. Only in the third trimester does the aldosterone level increase in hypertensive pregnancy¹²⁷⁾.

Although circulating levels of AT II may be normal during pre-eclampsia, it is possible that reducing uteroplacental perfusion pressure could increase the renal sensitivity to AT II through reductions in nitric oxide (NO) or prostacyclin synthesis or by enhanced formation of thromboxane.

This suggestion is confirmed by studies indicating enhanced vascular responsiveness to AT II in vessels from animals or humans with pre-eclampsia¹²⁰⁾. Furthermore, the pre-glomerular vessels of the renal circulation become extremely sensitive to the vasoconstrictor actions of AT II when the renal synthesis of NO or prostacyclin is reduced or when thromboxane synthesis is elevated¹²⁸⁾. Increased vascular AT II responsiveness during pre-eclampsia, however, does not prove AT II as an important endogenous mediator of the vasoconstriction or hypertension in experimental models of pre-eclampsia, because increased responsiveness may only reflect low endogenous AT II formation. Neither does the chronic blockade of the RAAS

system seem to play an important role in the hypertensive response to chronic reductions in uterine perfusion pressure in the rat¹²⁹⁾. In addition, the refractoriness to AT II is lost as early as the midtrimester in women who later develop pregnancy-induced hypertension¹³⁰⁾.

Thus, while the RAAS seems to be responsible in a major way for regulating the cardiovascular adaptation that occurs in pregnancy, its role in the causation of pre-eclampsia remains unclear.

Atrial Natriuretic Peptide :

Atrial natriuretic peptide (ANP) has been originally discovered by de Bold *et al.*¹³¹⁾ as a natriuretic factor produced by the myocytes of the atrium. It is secreted from the atria following the splitting of the storage form, a 126-amino acid prohormone (pro-ANP), into an N-terminal moiety of 98 amino acids (N-ANP) and the biologically active hormone, in equimolar amounts¹³²⁾. ANP is diuretic, natriuretic, vasorelaxant and has antiproliferative properties¹³³⁾. It causes intravascular volume contraction, both because of its ability to induce natriuresis and diuresis, as well as because it causes a shift of fluid from the capillary bed to the interstitium¹³⁴⁾, resulting in a reduction in both blood pressure and preload¹³⁵⁾.

In normotensive pregnancy ANP levels trend downward as gestation proceeds from the eighth through the 32nd week, then increase significantly in the 36th week¹³⁶⁾. In hypertensive pregnant women, some investigators reported that ANP levels increase markedly in late pregnancy¹³⁶⁻¹³⁸⁾, but this increase is not a universal finding^{139,140)} and is counterintuitive given the plasma volume contraction seen in pre-eclamptic patients as term approaches¹⁴¹⁾. These increments in plasma ANP may be secondary to some other factors, such as the release of increased amounts of angiotensin II (AT II), endothelin, or catecholamines, either into the circulation or locally in the affected tissues^{142,143)}. The enhanced ANP secretion could represent a defense against additional vasoconstriction and sodium retention in pre-eclamptic patients. ANP is also produced by a small population of human placental trophoblast-like cells¹⁴⁴⁾, suggesting that ANP may be secreted locally or into the fetoplacental circulation and that its effects occur as a result of paracrine or autocrine actions^{144,145)}. However, pro-ANP mRNA levels performed on placental tissue of pre-eclamptic patients do not differ from those of normal pregnant woman¹⁴¹⁾. Therefore, production of ANP by the placenta is not altered at this pre-translational level in pre-eclampsia.

Adrenomedullin :

Adrenomedullin, a member of the calcitonin gene-related peptide family, is a 52-amino acid peptide, originally discovered in human pheochromocytoma tissue¹⁴⁶⁾, that produces blood pressure reduction along with natriuresis and diuresis¹⁴⁷⁾. The latter effect is the result of an ability to increase glomerular filtration rate (GFR) as well as to inhibit distal tubular sodium reabsorption¹⁴⁷⁾. Its hypotensive action is

potent and long lasting¹⁴⁶⁾.

Plasma levels of adrenomedullin progressively increase as pregnancy proceeds¹³⁸⁾. Whereas first trimester adrenomedullin levels did not differ from those of non-pregnant women in either the follicular or luteal phases of the menstrual cycle in studies performed by Minegishi *et al.*¹³⁸⁾, third trimester plasma concentrations were significantly higher than first and second trimester levels. In contrast, Di Iorio *et al.*¹⁴⁸⁾ could not find any significant difference of plasma adrenomedullin levels throughout gestation. Amniotic fluid adrenomedullin concentrations decreased after the first trimester (8–12 weeks of gestation) and were lowest at 13–20 weeks of gestation and then increased at 21–28 weeks of gestation. A further increase was found in samples collected after 37 weeks of gestation¹⁴⁸⁾. In the umbilical vein, adrenomedullin concentration was higher than in the umbilical artery, suggesting that adrenomedullin in the fetal circulation derives from the placenta¹⁴⁸⁾. These findings lead to the conclusion that adrenomedullin may have an important role in human reproduction, from implantation to delivery¹⁴⁸⁾.

In pre-eclamptic patients, third trimester adrenomedullin levels did not differ significantly from those measured in pre-eclamptic patients at 28 to 40 weeks of pregnancy¹³⁸⁾. Additionally, Di Iorio and his co-workers¹⁴⁹⁾ have reported that plasma levels of adrenomedullin from normotensive pregnant patients did not differ from those obtained from pre-eclamptic patients and Hata *et al.*¹⁵⁰⁾ reported that mean levels of adrenomedullin in pre-eclamptic patients were lower than those obtained from normal pregnant women in the third trimester. Interestingly, levels in amniotic fluid and umbilical vein plasma were several-fold higher in pre-eclamptic patients than in normal pregnant women¹⁴⁹⁾. These data are supported by the findings that the umbilical veins in the intrauterine growth restriction (IUGR) group had significantly higher levels of growth restricted mean fetal adrenomedullin than control patients, whereas there was no difference in maternal plasma adrenomedullin levels of the two groups¹⁵¹⁾.

Additionally, adrenomedullin has been identified in human fetoplacental tissues^{152,153)}, in which its presence, determined by immunohistochemical methods, seems to be greater in fetal membranes than in the placenta. The peptide was found to be localized to the amnion and to trophoblast cells¹⁵²⁾. Kanenishi *et al.*¹⁵³⁾ report decreased immunohistochemical adrenomedullin expression in the placentas obtained from pre-eclamptic pregnancies, and most recently Knerr *et al.*¹⁵⁴⁾ reported a significantly lower adrenomedullin mRNA expression in placental villi from pre-eclamptic compared with normotensive women.

Furthermore, Jerat *et al.*¹⁵⁵⁾ reported no significant differences in the response of stem villous arteries taken from normal pregnant patients compared with those from pre-eclamptic patients with respect to their response to adrenomedullin.

Based upon the available data to date, it appears that adrenomedullin may be involved in the adaptation of the vascular system to pregnancy and in the regulation of placental vascular tone. However, controversy exists on the status of circulating

and placental adrenomedullin in pre-eclampsia and of the relative contribution of adrenomedullin to impaired fetoplacental circulation and fetal growth.

NON-VASOACTIVE PEPTIDES

β -human chorionic gonadotropin :

Human chorionic gonadotropin (hCG) is a glycoprotein composed of two non-covalently linked subunits, α and β , and is secreted from the blastocyst and early placental syncytiotrophoblast. Maternal serum level peaks at 8-10 weeks of gestation and then declines to reach a plateau at 18-20 weeks. The free β -hCG circulating in maternal serum corresponds to only about 0.3%-4% of total hCG¹⁵⁶⁾.

In the case of β -hCG, there are several reports of an association with the incidence of pre-eclampsia^{157,158)}. There is general agreement that the placenta remains the main source of hCG in patients with pre-eclampsia, but whether the cause of the high circulating levels of the hormone is placental overproduction is still debated. Some advocate that hCG secretion may be increased as a consequence of abnormal placental invasion or placental immaturity¹⁵⁹⁾. It may also be linked to the trophoblast response to hypoxia with the development of a hypersecretory state¹⁶⁰⁾. Compared with normal pregnancies, the placentas of patients with unexplained elevated maternal hCG levels in the second trimester tend to be larger and to have an increased density of hCG-positive trophoblasts along with an increased intensity of hCG immunostaining within the placental villi¹⁶¹⁾. However, in contrast to that, a small sample study found equivalent expression of β -hCG mRNA in normal and pre-eclamptic placental tissues¹⁶²⁾.

On average, maternal hCG levels are already increased in the second trimester in pregnancies that subsequently develop pre-eclampsia^{157,163-165)}. Because the measurement of hCG levels during the second trimester for Down's syndrome screening has already been incorporated into clinical practice at many antenatal clinics worldwide, thousands of records of midtrimester hCG levels for women attending screening programs and their respective outcomes have permitted the investigation of whether the finding of elevated hCG concentrations in maternal serum is predictive of pre-eclampsia. There are accumulating data from studies that evaluated whether a single elevated hCG value (usually above 2.0 MoM) between 14 and 24 weeks of gestation is predictive of pre-eclampsia^{158,163,166-172)}. The results of these studies are convergent in suggesting that women with elevated hCG levels in the second trimester are at increased risk for pre-eclampsia, but there is divergence regarding the accuracy of this test and, by consequence, its predictive value. Many reasons contribute to the disagreement between the studies. The sensitivity and specificity of the test may change according to the method of assay, the clinical and epidemiological background of the subjects, the gestational age at which samples were collected, and the cutoff chosen to distinguish high from normal hCG levels. Most recent publications have suggested that hCG may be more

predictive for early than late onset pre-eclampsia^{171,172}).

But nevertheless, only when hCG was incorporated into a multifactorial model (including body mass index, parity, and age) did the sensitivity of the test prove effective with a specificity of 71%¹⁷³).

Inhibin A and activin A :

Inhibins are glycoproteins that were first isolated from ovarian follicular fluid and named after their ability to inhibit the pituitary secretion of follicle stimulating hormone (FSH). Inhibins A and B are heterodimers composed by an α subunit and a β A or β B subunit, respectively, linked by a disulfide bridge¹⁷⁴). Inhibin-related proteins comprise activins, which are homodimers composed by the same β subunits of the inhibin molecule, and follistatin, a binding protein with affinity for inhibins and activins via the β subunit. Activins are peptides that act as growth and differentiation factors in many cells and tissues. Inhibins and activins are members of the transforming growth factor (TGF)- β superfamily, a group of structurally similar but functionally diverse growth factors¹⁷⁵).

Inhibin α and β A subunits are widely localized in the cytotrophoblast and syncytiotrophoblast¹⁷⁶), and the intensity of the hybridization signal for inhibin α and β A subunit mRNA increases throughout pregnancy, peaking in extracts prepared from term placentas¹⁷⁶). Although the decidua¹⁷⁷), membranes¹⁷⁸), and fetus all produce inhibin, the placenta is the major source¹⁷⁹). In consonance with placental expression, maternal serum inhibin A and activin A concentrations increase progressively during gestation, especially in the last trimester¹⁸⁰).

Maternal serum activin A and inhibin A levels are substantially increased in the presence of hypertensive disorders¹⁸¹⁻¹⁸⁵). Although this might happen only because of hemoconcentration or decreased urinary clearance, activin A levels begin to rise significantly before the onset of hematological or renal manifestations of clinical disease^{181,186,187}). The most probable mechanism for the high activin A and inhibin A concentrations in patients with pre-eclampsia is increased placental production^{184,188}). This increased placental production is more likely to represent a placental response than a primary overproduction, but the mechanism which increases the activin A level in pre-eclampsia is yet unknown.

The above mentioned observations that inhibin A and activin A levels increase before onset of the disease^{181,186,187}) led to the theory that they might be diagnostic and prognostic markers of pre-eclampsia¹⁸¹). Silver and coworkers¹⁸³) found that inhibin A and activin A levels were higher in women with pre-eclampsia and observed that before 34 weeks of gestation there was a more pronounced difference in the average levels of both analytes between normal and complicated pregnancies. Studies have indicated that inhibin A is elevated several weeks before the onset of clinical signs of pre-eclampsia^{184,187,189-191}). Women with an inhibin A concentration exceeding 2.0 MoM between 15 and 19 weeks of gestation were more likely to develop pre-eclampsia, to deliver a small-for-gestational-age infant, or to have a

stillbirth or neonatal death¹⁹⁰). Lambert-Messerlian *et al.*¹⁶⁴) observed that inhibin A levels tended to be higher when the onset of pre-eclampsia occurred within a shorter interval after collection of the second trimester screening sample and suggested that second trimester inhibin A would be more effective in predicting early onset rather than late onset disease. Inhibin A has been reported to be particularly sensitive in predicting the occurrence of pre-eclampsia before 34 weeks, when the impact of the disease on maternal-fetal outcome is worse¹⁸⁷). Compared with inhibin A, activin A seems to be a more sensitive marker at 21–25 weeks¹⁸⁷).

Altogether, the studies evaluating second trimester inhibin A and activin A measurements to predict pre-eclampsia suggest that these markers have limited sensitivity and low positive predictive value when applied to low risk populations, but it may add significant information when used in combination with other screening modalities such as Doppler ultrasound^{192,193}).

Leptin :

Leptin was initially introduced as an adipocyte-derived hormone that regulates energy metabolism via its hypothalamic receptor¹⁹⁴). Subsequent studies revealed various physiologic functions of leptin. It plays an essential role especially in reproduction by regulating gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus¹⁹⁵).

Leptin is also produced by placental trophoblast and is secreted both in maternal and fetal circulation¹⁹⁶). During pregnancy, leptin levels show marked changes, suggesting the placenta as a putative source of production of leptin in addition to adipose tissue. Maternal plasma leptin levels rise sharply during the first trimester^{197,198}) and decline back to normal values after delivery¹⁹⁹). Serum leptin levels increase in the second and third trimester, and may contribute to the inhibition of increased food intake, body weight, and body fat¹⁹⁸).

Elevated maternal leptin levels have been described in women with pre-eclampsia in the third trimester^{200–203}) but not at delivery²⁰³). The rise in total leptin represents an increase of free leptin levels, as the bound fraction is paradoxically decreased²⁰¹). The most probable mechanism of leptin increase in pre-eclampsia is increased placental production²⁰⁴) (Fig. 4), with placental hypoxia and inflammatory mediators being important stimulators²⁰²). This explains why pre-eclampsia subverts the physiological relationship between adiposity and leptin levels in pregnant women²⁰⁵).

A longitudinal study showed increased leptin levels beginning at 20 weeks of gestation in women who subsequently developed pre-eclampsia, suggesting that leptin may be an early marker of the disease²⁰⁰).

Neurokinin B :

Recently, elevated levels of neurokinin B (NKB) have been reported to cause pre-eclampsia²⁰⁶). NKB belongs to the tachykinin family. These neuropeptides

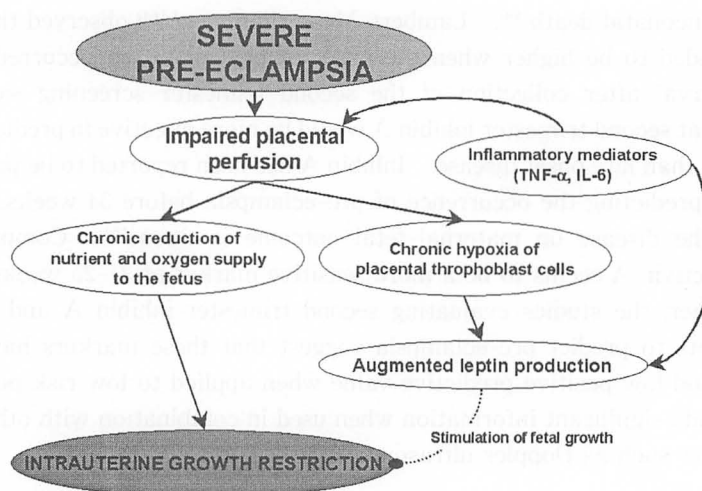


Fig. 4. Relationship between placental leptin production and intrauterine growth restriction in severe pre-eclampsia.

have been implicated in a variety of biological functions from smooth muscle contraction²⁰⁷, vascular reactivity^{208,209}, pain transmission²¹⁰, neurogenic inflammation²¹¹, and the activation of the immune system²¹². They are considered normally to be restricted to nervous tissue and to exert their effects peripherally only by release from nerve endings in the target tissue, activating neurokinin (NK) receptors, of which there are three. NKB binds preferentially to the NK₃-receptor, but in higher doses is also able to activate other neurokinin receptors.

In animal studies, NKB has been found to cause contraction of the hepatic portal vein²⁰⁸, venoconstriction of the mesenteric bed²⁰⁹, and increases in heart rate²¹³, which are all potentially hypertensive effects. A model of its physiological role in normal pregnancy and its potential involvement in pre-eclampsia²¹⁴ has been proposed: NKB is thought to play a physiological role in establishing the early trophoblast. It may be an important regulator of placental perfusion by serving to dilate the uterine spiral arteries. By causing vascular changes, NKB might not only increase maternal blood pressure but also shunts blood from certain organs, such as the liver and the mesenteric beds, to the uterus and placenta in order to maintain a sufficient blood supply to these organs. In pre-eclampsia, it is supposed that if the defective trophoblastic invasion does not rectify itself after the first trimester, then the placenta may start to secrete NKB into the maternal circulation in ever-increasing amounts. The incomplete invasion of the trophoblasts could lead to a situation where the uterine spiral arteries are unresponsive and cannot be dilated. Therefore an adequate blood supply to the placenta fails to occur. Raised levels of NKB may then lead to maternal hypertension and damage the kidneys and liver. At very elevated concentrations, peripheral NK1-receptors (on platelets, neutrophils or monocytes and in the brain) may be activated and in this way, NKB could also cause

the symptoms and complications usually found in pre-eclamptic patients.

In contrast to the results reported by Page *et al.*²⁰⁶⁾, we found lower levels of NKB in women with pre-eclampsia compared with normotensive pregnant women²¹⁵⁾. Our findings are supported by the results published by Li and co-workers²¹⁶⁾, who found an increased expression of placental neutral endopeptidase 3.4.24.11 (NEP) in pre-eclampsia, suggesting that this enzyme may be involved in the pathophysiological changes of pre-eclampsia. As NKB is a substrate for endogenous tachykinin inhibitors, especially NEP²⁰⁷⁾, decreased NKB levels in pre-eclampsia could be due to changes in its inhibitor. Therefore, the role of NKB in pre-eclampsia, if any, remains to be determined.

OXIDATIVE STRESS AND LIPID METABOLISM

Oxidative stress and hyperlipidemia are common themes among many chronic disorders such as atherosclerosis, diabetes, neurodegenerative disorders, and cancer. Pre-eclampsia has also been reported to be associated with oxidative stress⁶⁰⁾ and hyperlipidemia²¹⁷⁾, as are risk factors for pre-eclampsia such as obesity, hypertension, and diabetes¹⁸⁾.

Oxidative stress may be defined as an imbalance between oxidant and antioxidant forces in favour of oxidation. Oxidative stress is considered to be an important final pathway in causing endothelial damage and is evident in the maternal circulation of pre-eclamptic women. The increased oxidative stress in pre-eclampsia may on one side be due to elevated free radical generation²¹⁸⁾. Circulating levels of lipid peroxides (LPO), as estimated by malondialdehyde (MDA) or conjugated dienes, are abnormally elevated and graded in proportion to the severity of the disorder²¹⁹⁾. Maternal plasma levels of isoprostanes, which are considered accurate markers of oxidative stress and lipid peroxidation, are increased²²⁰⁾. Serum levels of iron are also elevated²²¹⁾, which leads to oxidant stress because transition metals such as the ferrous ion (Fe^{2+}) initiate peroxidation of lipids according to the Fenton reaction. On the other hand, a decrease in levels of antioxidants and/or impaired regeneration of reduced forms of antioxidants^{222–224)} may lead to oxidative stress, although not all investigators find decreased levels of antioxidants^{223,225,226)}.

In pre-eclampsia, maternal predispositions could also interact with the poorly perfused intervillous space to generate reactive oxygen species. It seems likely that pre-eclamptic women miss a protective factor X or mechanism, which normally protects the pregnant woman²²⁷⁾, making the endothelium more sensitive to possible noxes. Several suggestions have been advanced to explain the transfer of oxidative stress from the intervillous space to the systemic circulation. Activated neutrophils and monocytes are present in pre-eclampsia. These cells could be activated by oxidative stress in the intervillous space and then generate free radicals on contact with endothelium²²⁰⁾. Again, the consequences of this interaction are defined by maternal factors (decreased antioxidants, sensitized endothelium, lipoproteins espe-

cially sensitive to oxidation). Transfer of oxidative stress could also be secondary to the formation of stable products of lipid peroxidation (e.g., malondialdehyde) or by oxidized fragments of syncytiotrophoblast entering the systemic circulation. Finally, the hypoxic placenta might produce cytokines with the potential to generate oxidative stress. Placentas of women with pre-eclampsia are characterized by oxidative stress, evidenced by abnormally elevated tissue levels and production rates of LPO, as well as an imbalance in the arachidonic acid metabolites, thromboxane, and prostacyclin^{125,126,228}. Evidence indicates that the imbalance of increased thromboxane and decreased prostacyclin is caused by placental oxidant stress because oxidized lipids stimulate thromboxane synthesis while inhibiting prostacyclin synthesis. Thromboxane is a potent vasoconstrictor and prostacyclin is a potent vasodilator, so this imbalance restricts uteroplacental blood flow. Further evidence of placental oxidative stress is the finding that placental isoprostane levels are significantly increased in pre-eclampsia²²⁹. The cause of these placental abnormalities is not known yet.

Administration of antioxidants to women in early pregnancy decreases oxidative stress, endothelial activation, and the frequency of pre-eclampsia, which lends support to the potential role of oxidative stress in pre-eclampsia²³⁰.

IMMUNE MARADAPTATION

In 1976, Drs. James R. Scott and Alan A. Beer²³¹ wrote in a review article titled "Immunologic aspects of pre-eclampsia", the following terse but incisive introduction to their review article: "The normal pregnant state represents the only natural and successful transplantation of living tissue from one person to another, but one has to look no farther than pregnancies complicated by Rh isoimmunization to realize that immunologic homeostasis between the mother and fetus is not always perfect." Dekker and Sibai²³² have championed the idea that "genuine" pre-eclampsia is a disease of first pregnancies. They point out the fact that the incidence of pre-eclampsia is low in women who have had a previously normal pregnancy. Interestingly, even a prior abortion may provide protection against this disease²³³. However, if a woman changes sexual partners, the immunity conferred by multiparity is lost^{19,20,234,235}. Repeated exposure to sperm from the same individual may also be a preventive factor in the development of pre-eclampsia^{20,22}, and artificial insemination increases the risk of this disorder^{20,236,237}. Although not well understood, the hypothesis propounded to explain these protective effects of sperm exposure is that T cells within the genital tract may recognize antigens without the need for binding to class I human leukocyte antigen (HLA) on antigen-presenting cells, allowing trophoblasts lacking classical HLA to be recognized²³⁸. In addition, a transient state of T lymphocyte hyporesponsiveness to paternal class I HLA has been reported, which may impact this immune reaction²³⁹. A lower level of messenger RNA for HLA-G has been noted in trophoblasts from pre-eclamptic

patients than from normal pregnant patients²⁴⁰⁾, but this could be the result of fewer trophoblast cells in pre-eclamptic patients²⁴¹⁾.

Implantation and placentation present an immune challenge because of the semi-allogenic nature of the conceptus. Decidualization of the endometrium itself has features in common with an inflammatory response⁶⁴⁾. During decidualization, infiltration by uterine natural killer cells occurs, and these interact with the non-polymorphic HLA class I antigens expressed by invading extravillous trophoblast. Candidates for mediators of the immune maladaptation in pre-eclampsia include cytokines (especially tissue necrosis factor [TNF]- α and interleukin [IL]-2 and IL-6)²³³⁾. Additionally, enzymes released by activated neutrophils, such as elastase and oxygen-free radicals, including LPO, have been thought to be implicated²³³⁾.

Redman *et al.*⁶⁴⁾ hypothesized an interesting theory: They suggest that the endothelial dysfunction is a part of a more generalized intravascular inflammatory reaction involving intravascular leukocytes, as well as the clotting and complement systems, and proposed that such an inflammatory response is already well developed in normal pregnancy and that pre-eclampsia arises when a universal maternal intravascular inflammatory response to pregnancy decompensates in particular cases, which may occur because either the stimulus or the maternal response is too strong.

GENETICS OF PRE-ECLAMPSIA

The familial nature of preeclampsia-eclampsia has been appreciated since at least the 1800s. In the last decades numerous papers report on the familial nature of pre-eclampsia^{67,242,243)}.

The inheritance patterns of pre-eclampsia have been described as Mendelian (autosomal recessive, autosomal dominant with incomplete penetrance), polygenic/multifactorial, or mitochondrial. Additionally, a unique type of inheritance has been postulated that involves an interaction between the genetic components of the mother and those of the father, manifesting through the fetal-placental unit, and possibly through imprinting. But until now, the exact inheritance pattern is still unknown^{67,244)}, no single gene has been identified that explains a clear Mendelian inheritance, and genomic scans of women with pre-eclampsia have yielded varying results. No doubt pre-eclampsia is a syndrome with underlying genetic heterogeneity. It is most likely that genes or mutations in certain genes will predispose women to develop pre-eclampsia, and that these loci will vary in different populations. The most plausible genetic model to date postulates that maternal genes dictate a woman's susceptibility for the expression of the pre-eclamptic phenotype, whereas expression of the phenotype in a woman with a given genetic susceptibility might depend on the genetic load from the trophoblast and possibly on environmental factors^{245,246)}. This susceptibility is dictated by genes and their interaction with environment, but it will be transferred into biochemical and molecular changes to be

found in the syndrome of pre-eclampsia and perhaps in later life.

Fetal (paternal) contribution :

Theoretically, both mother and fetus (and therefore the father) may contribute to the risk. Pre-eclampsia may reflect problems in the close biological interaction between the two subjects²⁴⁵⁾. Current knowledge on the epidemiology of pre-eclampsia, like the particularly high risk in first pregnancies, points primarily to an effect of maternal factors¹⁹⁾.

Although it is likely that the fetus may also contribute to the pathophysiology of the syndrome pre-eclampsia, few studies have focused on the contribution of fetal and/or paternal genes^{247,248)}. Lie *et al.*²⁴⁷⁾ suggested that paternal genes (as expressed in the fetus) contribute also strongly to the mother's risk of pre-eclampsia. Mothers who were pregnant by a partner who fathered a pre-eclamptic pregnancy in another woman had nearly twice the risk in their own pregnancy²⁴⁷⁾. Esplin and co-workers²⁴⁸⁾ reported that both men and women who were the product of a pregnancy complicated by pre-eclampsia were significantly more likely than control men and women to have a child who was the product of a pregnancy complicated by pre-eclampsia.

Candidate Genes :

The search for genes that increase maternal susceptibility to pre-eclampsia is ongoing. Besides thrombophilic mutations a number of genes have been supposed to be involved in the pathogenesis of pre-eclampsia. Potential genes or chromosome locations are chosen for study based upon their pathophysiologic plausibility or their linkage to pre-eclampsia by genome-wide scanning⁶⁷⁾.

Thrombophilia :

A characteristic feature of pre-eclampsia is the maternal hypercoagulable state and intravascular coagulation, which is evidenced by increased platelet consumption and reduced platelet lifespan. Several pro-coagulant factors like tissue plasminogen activator (tPA)^{249,250)}, plasminogen activator inhibitor (PAI)²⁴⁹⁻²⁵²⁾, von-Willebrand factor²⁵³⁾ and anticardiolipin antibodies^{29,30)} are changed in pre-eclampsia. Several endothelial cell-associated anticoagulant proteins have also shown to be associated in pre-eclampsia. A decrease in antithrombin III (AT III) occurs in pre-eclampsia²⁵⁾ and AT III deficiency is more frequent in pre-eclamptic women (odds ratio of 7.2)²⁸⁾. Protein C deficiency has been reported to be associated with pre-eclampsia compared with control women (odds ratio 21.5)²⁸⁾. Additionally, protein S deficiency is more frequent in pre-eclampsia (12.3% versus 0.6% in normal pregnancy, odds ratio 12.7)²⁸⁾.

Thrombophilic mutations such as factor V Leiden, G20210A prothrombin mutation, and methylenetetrafolate reductase mutation have been reported to be underlying factors for the development of pre-eclampsia, although this association

has not shown up that clearly on a molecular level so far. In a Dutch study on women with severe early onset pre-eclampsia, more than 50% of women had at least one underlying thrombophilic disorder²⁵⁾. Kupfermanc *et al.*²⁷⁾ reported a combined prevalence (67%) of inherited and acquired thrombophilic disorders in women with severe pre-eclampsia. Women with thrombophilia delivered at an earlier gestational age, and their neonates' birth weights were lower compared with those of women without thrombophilia²⁷⁾. Van Pampus *et al.*²⁶⁾ also showed a high prevalence of hemostatic abnormalities of about 40% in women with a history of severe pre-eclampsia. Recently, Alfirevic *et al.*²⁸⁾ summarized in their review on the association between maternal thrombophilia and adverse pregnancy outcome that "women with adverse pregnancy outcomes are more likely to have a positive thrombophilia screen."

Some studies failed to find any association of thrombophilic mutations with pre-eclampsia²⁵⁴⁻²⁵⁶⁾, but this might be explained by the genetic heterogeneity in the different populations studied and/or the different diagnostic criteria used^{28,257)}. To summarize, screening for thrombophilic mutations seems to be justified in high risk women, but not as a general screening of all pregnant women²⁸⁾.

Factor V Leiden Mutation :

Factor V Leiden deficiency has been associated with an increased risk of pre-eclampsia^{25-27,31,32)}, whereby this mutation especially seems to be associated with HELLP syndrome³³⁻³⁵⁾. The Leiden mutation of factor V (G1691A) was first described in a subgroup of individuals who had activated protein C deficiency²⁵⁸⁾. The presence of the G1691A mutation is a relatively common missense mutation in which substitution of arginine in place of glutamine changes the factor V protein into one that resists inactivation by activated protein C. The prolonged procoagulant activity of factor V in the heterozygote state increases the carrier to thromboembolic complications. The factor V Leiden frequency is found in about 5% of Caucasians, whereas this mutation is only rarely, if at all, found in people of Asian or African ancestry²⁵⁹⁾.

G20210A prothrombin mutation :

Poort and co-workers²⁶⁰⁾ described in 1996 the G20210A prothrombin mutation. This guanine-to-adenine mutation at nucleotide 20210 in the 3'-untranslated region of the prothrombin gene is also associated with an increased risk of venous thromboembolism. In that case, factor Xa/Va-complex cannot convert prothrombin to thrombin²⁶⁰⁾.

The prevalence of this mutation is reported in about 2% of Caucasians^{260,261)}. Again, this mutation is rarely found in people with Asian or African ancestry²⁶¹⁾.

Few studies focussed on the association of the G20210A prothrombin mutation with pre-eclampsia. Although the role of this mutation remains unclear, women who are found to be heterozygous carriers of that mutation are reported to have a

2-fold higher risk (odds ratio 2.4) for pre-eclampsia²⁸⁾.

Methylenetetrafolate Reductase :

Mutations in the methylenetetrafolate reductase (MTHFR) gene in a non-pregnant population have been associated with a modest elevation in plasma homocysteine concentration. The latter has been implicated in vascular injury and an increased risk for cardiovascular disease²⁶²⁾. Hyperhomocysteinemia has been reported to be involved in the pathogenesis of pre-eclampsia^{25,26,263)}. In women with pre-eclampsia, hyperhomocysteinemia can be found in 14.8% compared with 4.5% in control women (odds ratio 2.2)²⁸⁾. Because vascular resistance to uterine blood flow and resultant ischemia is thought to be causative in pre-eclampsia, investigators have targeted the MTHFR gene as a susceptibility gene for pre-eclampsia. Especially the association of a common missense mutation, a C to T substitution at nucleotide 677 (T677), in the MTHFR gene with pre-eclampsia has been investigated^{28,624)}. Heterozygous carrier frequency is estimated to be 42.2% in pre-eclamptic women compared with 38.6% in the controls (odds ratio 1.2)²⁸⁾. The homozygous carrier frequency is 13.4% in pre-eclampsia and 10.3% in normal pregnant women (odds ratio 1.7)²⁸⁾.

Candidate genes involved in hemodynamic changes of pregnancy :

Beside thrombophilic mutations a variety of candidate genes have been targeted as susceptibility genes for pre-eclampsia⁶⁷⁾.

Angiotensinogen :

Angiotensinogen (AGT) is a precursor to angiotensin II, a highly vasoactive compound that is important in the regulation of blood pressure and intravascular volume. Ward and colleagues²⁶⁵⁾ were the first to describe an association between pre-eclampsia and a molecular variant in the maternal AGT gene (T235). The substitution of threonine for methionine in the variant appears to be linked to an A(-6) promoter mutation that causes increased AGT expression, which in turn leads to higher levels of angiotensin II. This association was confirmed by Arngrimsson *et al.*²⁶⁶⁾, while others could not confirm this association²⁶⁷⁾. Recently, it has been reported that the AGT T235 allele predisposes women toward abnormal physiologic change, potentially beginning the cascade of events leading to pre-eclampsia²⁶⁸⁾. Finally it has been postulated that an angiotensin II type 1 receptor gene expression in the fetus may contribute to the etiology of pre-eclampsia. It was unclear whether susceptibility is conferred by the fetal genotype acting alone, or by allele sharing by mother and fetus²⁶⁹⁾.

Endothelial Nitric Oxide Synthase (eNOS) :

Another interesting candidate gene is the nitric oxide synthase (NOS) gene. By microsatellite amplification, Arngrimsson *et al.*²⁷⁰⁾ found evidence for a linkage of

pre-eclampsia with a microsatellite (D7S505) within intron 13 of chromosome 7q36 encoding the endothelial nitric oxide synthase (eNOS) gene in a population of Scottish and Icelandic origin. The linkage results reported by Guo *et al.*²⁷¹⁾, supported the possibility that a susceptibility locus for pre-eclampsia resides in the 7q36 region, however, they failed to support the notion that the eNOS gene itself is responsible for susceptibility to pre-eclampsia. Yoshimura *et al.*²⁷²⁾ identified a G to T conversion at nucleotide position 894 within exon 7 of the eNOS gene resulting in replacement of glutamic acid with aspartic acid at codon 298 (Glu298Asp). Subsequently, they investigated the association of this mutation with pre-eclampsia²⁷³⁾ and found a higher frequency of this mutation in women with severe pre-eclampsia, which was recently confirmed by others^{274,275)}. Savvidou *et al.*²⁷⁵⁾ reported an association of the eNOS Glu298Asp polymorphism is related to differences in endothelium-dependent dilation at 12 weeks' gestation. The presence of this polymorphism was found to be correlated with lower flow-mediated dilation of the brachial artery, a vascular abnormality purported to predict pre-eclampsia. They suggested a role of this mutation in the normal vascular adaptation to pregnancy and pre-eclampsia. Tempfer and coworkers²⁷⁶⁾ reported that a polymorphism in the eNOS gene, segregating with lower NO metabolites, is associated with a six times greater risk of developing pre-eclampsia.

The association of the eNOS gene with pre-eclampsia, however, could not be confirmed by other studies^{271,277)}. Even Arngimsson *et al.*²⁷⁸⁾ couldn't confirm their observation when publishing a genome-wide scan for pre-eclampsia using the same eNOS markers.

Candidate genes involved in oxidative stress :

Endothelial dysfunction is characteristic for the syndrome pre-eclampsia and oxidative stress has been implicated to cause endothelial damage. Marked dyslipidemia may contribute to the endothelial cell dysfunction in pre-eclampsia. A sequence variation in the lipoprotein (LPL) gene has been reported to be associated with pre-eclampsia²⁷⁹⁾. Carriers of N291S or combined D9N/–93T → G mutations in the LPL gene which both predispose to dyslipidemia and cardiovascular disease, are at substantially increased risk of pre-eclampsia²⁷⁹⁾. This finding could not be confirmed by Kim *et al.*²⁸⁰⁾, although in a small sub-group of patients, the N291S mutation was associated with an increased risk for nulliparous hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome.

Oxidative stress may be caused by toxic compounds such as lipid peroxides (LPO) and oxygen-free radicals. Therefore, enzymes that scavenge and detoxify substances were felt to possibly be involved. Epoxide hydrolase is a liver microsomal enzyme, involved in metabolism of endogenous and exogenous toxins. Genetic polymorphisms in the gene (EPHX) coding for this enzyme have been associated with the degree of its activity. Specifically, two polymorphisms, 113Tyr3His in exon 3, and 139His 3 Arg in exon 4 have been associated with

decreased activity. Zusterzeel *et al.*²⁸¹⁾ found that those women with pre-eclampsia were nearly twice as likely as the healthy women (29% vs. 16%) to have the high activity variant of microsomal epoxide hydrolase. Furthermore, these polymorphisms did not seem to increase the risk for concurrent development of the syndrome of HELLP²⁸¹⁾.

Recently, association of pre-eclampsia of the P1b-1b genotype of the glutathione-S-transferase (GST) gene, which encodes a detoxifying biotransformation enzyme, was studied in a Dutch study population²⁸²⁾. This genotype, possibly resulting in a lower glutathione-S-transferase detoxification capacity of oxidative stress-related factors, was more frequent in pre-eclamptic patients than in controls²⁸²⁾.

Candidate genes involved in immunogenetics :

The HLA-G genotype is another interesting candidate gene, which may be associated with pre-eclampsia. Whereas previous studies couldn't find any association between HLA-G polymorphisms and pre-eclampsia^{283,284)}, recently, O'Brien *et al.*²⁸⁵⁾ reported that the distribution of *HLA-G* polymorphisms was different between normal and pre-eclamptic cytotrophoblast samples.

Results on genes encoding pro-inflammatory cytokines and genes participating in the regulation of the immune response, like tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) are also controversial: Whereas Chen *et al.*²⁸⁶⁾ found an association between the TNF-T1 allele and pre-eclampsia, others did not find this association²⁸⁷⁻²⁸⁹⁾.

Additionally, recent study on polymorphisms in the IL-1 and interleukin-1 receptor antagonist (IL1ra) gene and pre-eclampsia or HELLP syndrome did not show association with either gene^{290,291)}.

In summary, it is quite obvious that pre-eclampsia has a complex inheritance pattern, one similar to chronic illnesses such as diabetes, hypertension, and asthma. The genetics of chronic illness involve multiple disease susceptibility loci as well as environmental gene interactions. The potential of the paternal (fetal) component further complicates the elucidation of the inheritance mode of pre-eclampsia. Furthermore, imprinting or differences in methylation patterns may be involved. The finding of etiologic or susceptibility genes in pre-eclampsia will not only allow early identification of susceptible women, but may one day allow detection of unknown protein products that will direct future investigators to the elusive cause or causes of this disorder.

PRACENTAL ANGIOGENESIS

Research on the subject of pre-eclampsia has revolved around placental growth and angiogenesis, as both are central to the etiology of the disease. The formation of new vessels is crucial for many physiologic pathways in pregnancy and can be

divided into two major stages termed vasculogenesis and angiogenesis (Fig. 5)²⁹².

Vasculogenesis:

This earliest stage of vascular development, beginning at day 21 postconception, includes the differentiation, expansion, and coalescence of vascular endothelial cell precursors into the initial vascular network. The early vascular plexus forms from mesoderm by differentiation of angioblasts, which subsequently generate primitive blood vessels. Factors of the fibroblast growth factor (FGF) family are crucial to form angioblasts and hematopoietic cells. Vascular endothelial growth factor (VEGF)-receptors and sufficient levels of their ligands seem to be needed to maintain angioblast differentiation and therefore for the vasculogenic process. Induction of VEGF-receptor 2 (KDR=kinase insert domain containing receptor) may thus initiate angioblast differentiation, whereas the quantity and activity of VEGF ligands determine angioblast survival.

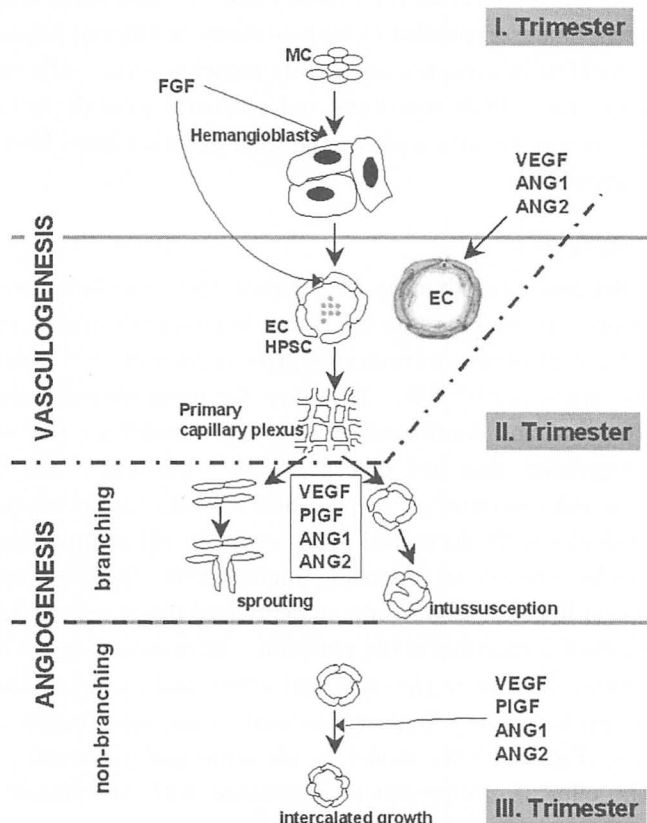


Fig. 5. Vasculo- and angiogenesis in pregnancy. Abbreviations: MC=mesodermal cell; FGF=fibroblast growth factor; EC=endothelial cell; HPSC=hematopoietic stem cell; VEGF=vascular endothelial growth factor; ANG1/2=angiopoietin-1/-2; PlGF=placenta growth factor.

Angiogenesis :

The primary plexus is then remodeled by a process referred to as angiogenesis. Further blood vessels are generated by branching and differential growth of the vessels, forming a more mature system with larger and smaller vessels. This process is termed *pruning*, as the resulting pattern resembles a tree. Beside the vascular endothelial growth factor (VEGF) family, angiopoietins as well as their receptors act during branching (true sprouting of capillaries from pre-existing vessels or intussusceptive growth) and non-branching (intercalated growth) angiogenesis. Branching angiogenesis occurs both in the yolk sac and the embryo and predominantly in the first and second trimester in situations of relative hypoxia. Non-branching angiogenesis occurs predominantly in the third trimester in situations of relative normoxia. The formed vasculature is further differentiated by recruitment of pericytes and smooth muscle cells and remodeled into a more major tree-like hierarchy containing vessels of different sizes.

From fibroblast growth factor (FGF) and VEGF as endothelial cell mitogens, the study of angiogenesis has expanded to include many additional agonists, receptors, and inhibitors working in complex and subtle mechanisms. The key factors are the VEGF family, the VEGF receptors and placental growth factor (PlGF). In addition, several newer factors such as the angiopoietins have been shown to be important in angiogenesis⁷²⁾.

Regulation of angiogenesis :

Metabolic demands are thought to regulate the vascularisation of placenta, tissues, and tumors. It is a common belief that hypoxia is a crucial etiologic factor for late spontaneous abortion, intrauterine growth restriction (IUGR), pre-eclampsia, and abruptio placentae^{68,73,74,293)}. However, the term *placental hypoxia* might be too simple. Kingdom and Kaufmann⁶⁸⁾ proposed a model for the origins of fetal hypoxia and suggested that *pre-placental*, *utero-placental*, and *post-placental* hypoxia should be differentiated: *Pre-placental* hypoxia (as occurs in high altitude, anemia, or smoking) with decreased PO₂ levels in all compartments (mother-placenta-fetus) which results in branching angiogenesis. In late onset *pre-eclampsia*, *utero-placental* hypoxia also results in decreased placental and fetal PO₂ levels and branching vessel formation in the placenta. In contrast, in IUGR with absent or reversed enddiastolic flow in the umbilical artery and in early onset *pre-eclampsia*, higher PO₂ levels can be found in placental tissue, resulting in non-branching vessel formation (Fig. 6). This model is plausible and substantiated in clinical practice. In the clinical circumstances associated with *pre-placental* and *utero-placental* hypoxia, the result is a reduction of villous oxygen content. Adaptation in the form of increased angiogenesis and trophoblast proliferation takes place. This produces greater amounts of highly vascularized terminal villi (branching angiogenesis), which lowers capillary-mediated impedance to blood flow. Opposite

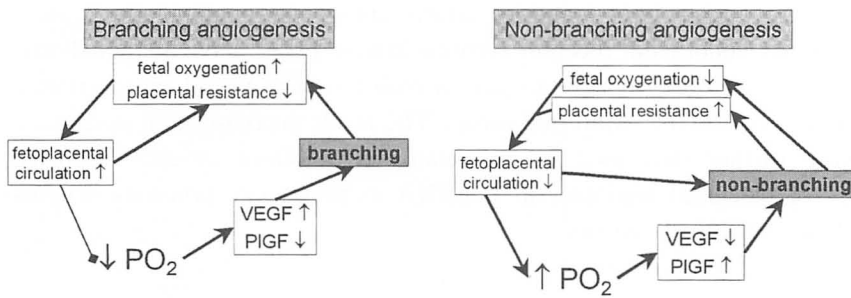


Fig. 6. Placental oxygen content, angiogenic growth factors, and angiogenesis.

to that is the situation of severe preterm IUGR and early onset pre-eclampsia. Here, normal terminal villi have not formed²⁹⁴. The fetoplacental and uteroplacental circulation is compromised. The result is a rising intraplacental oxygen content, which, in turn, results in a suppressed angiogenic drive to form terminal villi. Abnormal, non-branching angiogenesis results, conferring an increase, rather than the normal decrease in vascular impedance²⁹⁴. Oxygen extraction from these malformed villi is reduced²⁹⁵. This situation is what Kingdom and Kaufmann refer to as placental *hyperoxia*⁶⁸.

Angiogenic growth factors :

Of the variety of growth factors, the vascular endothelial growth factor (VEGF) family has been most extensively studied. Placental VEGF expression declines as pregnancy advances^{296–298} and is upregulated under hypoxic conditions and in pre-eclampsia^{75,297,298}. VEGF mediates its action via two tyrosine kinase receptors: fms-like tyrosine kinase (flt-1) and kinase insert domain-containing receptor (KDR). When binding to flt-1, VEGF stimulates endothelial nitric oxide synthase (eNOS) and leads to inhibition of cytotrophoblast proliferation. Placental growth factor (PlGF) shares 53% homology with VEGF, is produced exclusively in the placenta, and acts only via flt-1²⁹⁹. In contrast to VEGF, placental PlGF expression is stimulated by oxygen and PlGF levels increase with advanced gestational age^{297,298}.

In pre-eclampsia maternal VEGF serum or plasma levels have been reported to be increased^{71,300–301} and this increase correlates with the severity of the disease³⁰¹. Other sources reported decreased VEGF levels in pre-eclampsia^{69,70,302}, which might be explained by the interference with binding proteins, different gestational age, or the fact that VEGF is bound to a soluble VEGF-receptor, which has been reported to be elevated in pre-eclampsia^{76–78}. VEGF has shown to increase endothelial production of vasoactive substances and has vasodilative effects on resistance vessels^{303–305}.

Maternal PlGF is decreased in pre-eclampsia^{69,70,78–80}. Recent studies indicate evidence that PlGF may be useful for the prediction of pre-eclampsia^{306–308}.

Recently, angiopoietin-1 and its natural antagonist, angiopoietin-2, as well as their vascular endothelial receptor tyrosine kinase TIE-2, have attracted attention. The angiopoietins are thought to play a major role in vessel maturation, acting downstream to VEGF. Maternal serum TIE levels decrease with gestational age and were further decreased in pre-eclampsia³⁰⁹⁾. Geva *et al.*⁷²⁾ reported an increase of placental angiopoietin-1 mRNA as pregnancy proceeds, whereas angiopoietin-2 mRNA decreased.

CONCLUSION AND OUTLOOK

Despite tremendous work, the enigma pre-eclampsia still exists and it is still a "disease of theories." It is obvious that a single mechanism responsible for the syndrome pre-eclampsia does not exist. It is more likely that some or all of the described mechanisms can act together and even multiply each other. Additionally, in different populations, different mechanisms may be more important due to the genetic heterogeneity. Several underlying risk factors and possible pathophysiologic mechanisms have been elucidated and, although small, possibilities of earlier detection and also prophylaxis have been developed.

The complicated etiology of pre-eclampsia calls for studies that take into account the following:

- Early onset and late onset of the disease, which both give rise to different villous morphologies and are two separate and distinct patient groups³¹⁰⁾
- The contribution from both the mother and the fetus (and therefore the father)

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