Pathophysiology of Hypertension during Preeclampsia:
Linking Placental Ischemia with Endothelial Dysfunction

Jeffrey S Gilbert, Michael J. Ryan, Babbette B. LaMarca, Mona Sedeek, Sydney R. Murphy, and Joey P. Granger
Department of Physiology and Biophysics
And
Center for Excellence in Cardiovascular-Renal Research
University of Mississippi Medical Center, Jackson, MS

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To whom correspondence should be addressed:
Joey P. Granger, Ph.D.
Department of Physiology and Biophysics
University of Mississippi Medical Center
2500 North State Street
Jackson, MS 39216-4505
Phone: 601-984-1821
FAX: 601-984-1817
email: jgranger@physiology.umsmed.edu
Abstract

Studies over the last decade have provided exciting new insights into potential mechanisms underlying the pathogenesis of preeclampsia. The initiating event in preeclampsia is generally regarded to be placental ischemia/hypoxia, which in turn results in the elaboration of a variety of factors from the placenta that generates profound effects on the cardiovascular system. This host of molecules includes factors such as, soluble fms-like tyrosine kinase-1, the angiotensin II type-1 receptor autoantibody, and cytokines such as tumor necrosis factor-α which generate widespread dysfunction of the maternal vascular endothelium. This dysfunction manifests as enhanced formation of factors such as endothelin, reactive oxygen species, and augmented vascular sensitivity to angiotensin II. Alternatively, the preeclampsia syndrome may also be evidenced as decreased formation of vasodilators such as nitric oxide and prostacyclin. Taken together, these alterations cause hypertension by impairing renal pressure-natriuresis and increasing total peripheral resistance. Moreover, the quantitative importance of the various endothelial and humoral factors that mediate vasoconstriction and elevation of arterial pressure during preeclampsia remains to be elucidated. Thus, identifying the connection between placental ischemia/hypoxia and maternal cardiovascular abnormalities in hoped of revealing potential therapeutic regimens remains an important area of investigation and will be the focus of this review.
Introduction

Preeclampsia (PE), a pregnancy specific syndrome characterized by new onset hypertension and proteinuria, is a considerable obstetric problem and a significant source of maternal and neonatal morbidity and mortality (12; 95). While PE and related hypertensive disorders of pregnancy continue to affect approximately 8% of all pregnancies, the incidence of preeclampsia has seen a 40% increase in recent years (81). Moreover, it has recently been recognized that women whom endure preeclampsia are at a greater risk for cardiovascular disease than non-preeclamptic women and the men that fathered those preeclamptic pregnancies (37). Despite thorough characterization of the preeclamptic syndrome and a suite of contributing circulating factors (12; 77; 80; 95), the mechanisms underlying the pathogenesis of this troubling condition remain nebulous.

Interestingly, it has been proposed that not only are increased circulating factors responsible for much of the preeclamptic syndrome, but they may also predispose the maternal cardiovascular system to subsequent endothelial dysfunction as the mother ages (20).

The uncertainties regarding the mechanisms of preeclampsia are at least partially attributable to difficulties faced in the development of suitable animal models for mechanistic research of this disease (72). Consequently, it is held by many that more effective strategies for prevention and treatment of preeclampsia shall be forthcoming with the recent progress in developing animal models that allow careful mechanistic investigation of the underlying pathophysiological mechanisms involved in preeclampsia (32).
Placental Ischemia/Hypoxia and the Etiology of Preeclampsia.

Although the pathophysiology of preeclampsia remains undefined, placental ischemia/hypoxia is widely regarded as a key factor. Inadequate trophoblast invasion leading to incomplete remodeling of the uterine spiral arteries is considered to be a primary cause of placental ischemia. Thus, the poorly perfused and hypoxic placenta is thought to synthesize and release increased amounts of vasoactive factors such as soluble fms-like tyrosine kinase-1 (sFlt-1), cytokines, and possibly the angiotensin II type 1 receptor-auto antibodies (AT1-AA). Figure 1 illustrates a model by which these and other candidate molecules are thought to induce widespread activation/dysfunction of the maternal endothelium in vessels of the kidney and other organs that ultimately results in hypertension.

Perhaps the most prominent molecule postulated to play a key role in the pathogenesis of preeclampsia is sFlt-1. Several lines of evidence support the hypothesis that the ischemic placenta contributes to endothelial cell dysfunction in the maternal vasculature by inducing an alteration in the balance of circulating levels of angiogenic/anti-angiogenic factors such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), and sFlt-1. Although recent data suggest circulating sFlt-1 concentrations may presage the clinical onset of preeclamptic symptoms, several studies indicate that placental hypoxia and poor placental perfusion may initiate this imbalance of angiogenic factors. Nevertheless, it remains unclear whether impaired placental perfusion initiates preeclamptic symptoms such as hypertension, endothelial dysfunction and increased sFlt-
1 or if inadequate placental development occurs initially and is followed by a pathological rise in sFlt-1 expression and secretion (41).

Hypertension associated with preeclampsia develops during late pregnancy and remits after delivery or termination of the pregnancy, suggesting the placenta is a central culprit in the disease. The foremost hypothesis regarding the initiating event in preeclampsia is postulated to be reduced placental perfusion that in turn, leads to widespread dysfunction of the maternal vascular endothelium. While numerous other factors including genetic, immunological, behavioral, and environmental influences have been implicated in the pathogenesis of preeclampsia (95; 96), the main focus of this review is to describe the links between placental ischemia/hypoxia and the cardiovascular dysfunction that is widely recognized as a part of the preeclampsia syndrome.

Reduced Uterine Perfusion Pressure as a Model to Study the Pathophysiology of Hypertension during Pregnancy

The physiological mechanisms that mediate the alterations in cardiovascular and renal function that are requisite for a successful pregnancy have been studied in great detail. Nonetheless, experimental data investigating the mechanisms underlying preeclampsia have been limited because of the difficulty in performing mechanistic studies in pregnant women. While several animal models have been developed to study preeclampsia, none to date completely represent the protean characteristics of the human syndrome. Furthermore, information on the mechanisms mediating the long-term increase in vascular resistance and arterial pressure associated with placental ischemia is lacking.
Experimental induction of chronic uteroplacental ischemia appears to be a promising animal model to study potential mechanisms of preeclampsia since reductions in uteroplacental blood flow in a variety of animals leads to a hypertensive state that has many of the manifestations present in preeclamptic women (5-7; 9-11; 18; 22; 31; 33; 48; 49; 61; 94). It is important to note that the reduced uterine perfusion pressure (RUPP) model of placental ischemia induced hypertension in pregnancy most closely relates to severe premature/preterm preeclampsia and not late-onset preeclampsia. The former is characterized by intrauterine growth restriction (IUGR), hypertension and proteinuria, while the latter is not regarded as mediated by placental ischemia and is usually not associated with IUGR. Thus, a considerable strength of the RUPP model is it allows mechanistic investigation of the preterm ischemic placenta and the factors it elaborates.

The relationship between reduced utero-placental perfusion and hypertension during pregnancy has been demonstrated in a variety of animals, mostly those with hemochorial placentation. Previous studies have shown that chronic RUPP via partial ligation or placement of silver clips on the lower abdominal aorta or uterine arteries results in proteinuric hypertension in the baboon (18; 19; 61), rhesus monkeys (22; 124), rabbit (5; 59), and the dog (6; 35). Our laboratory has established a RUPP model in the pregnant rat (7; 9-11; 31; 33; 48; 49; 94). In this model, utero-placental perfusion is reduced by approximately 40% by the placement of silver clips on the aorta and ovarian arteries on day 14 of a 21 day gestation (94). On gestation day 19, the RUPP animals display increased mean arterial pressure (MAP), decreased glomerular filtration rate (GFR), decreased renal pressure natriuresis, decreased renal plasma flow and proteinuria, and endothelial dysfunction (7; 9-11; 26; 31; 33; 48; 49). Recently, we demonstrated that
these pregnant RUPP rats have increased total peripheral resistance, decreased cardiac
d index and decreased uteroplacental blood flow (94) indicating that these animals have
marked cardiovascular dysfunction similar to what is observed in preeclamptic women
(17; 70; 94; 109). We have also recently shown that RUPP hypertension in the rat is
associated with an imbalance of angiogenic factors, in particular increased sFlt-1 and
decreased VEGF and PI GF (30). Thus, these models of preeclampsia have many of the
features common in preeclamptic women and provide researchers with a valuable
substrate from which to investigate the mechanisms of hypertension during pregnancy.

The role of a variety of endothelial, autacoid, and hormonal factors that mediate
the cardiovascular and renal dysfunction produced by chronic reductions in
uteroplacental perfusion pressure will be the primary focus of the remaining portion of
this review. In the present review, we will also focus on the effects of reductions in
uteroplacental perfusion pressure on vascular endothelial function since factors that
emanate from the ischemic placenta are thought to be responsible for widespread chronic
long-term alterations in arterial pressure.

Mediators of Endothelial Dysfunction in Response to Placental Ischemia

The maternal vascular endothelium appears to be an important target of factors that are
triggered by placental ischemia/hypoxia in preeclampsia. The endothelium is a single-cell
lining that covers the luminal side of blood vessels. This strategic location permits it to
signal alterations in hemodynamics and humoral factors by synthesizing and releasing
vasoactive substances. Thus, a critical balance exists between endothelium-derived
relaxing and contracting factors which maintains vascular homeostasis. When this
delicate balance is disrupted, the vasculature is predisposed to vasoconstriction, leukocyte 
adherence, mitogenesis, pro-oxidation, and vascular inflammation (100; 101). Further, 
markers of endothelial dysfunction may serve as predictors of the syndrome in women 
that develop preeclampsia since many are often elevated weeks prior to observance of 
clinical manifestations.

Potential Mediators of Endothelial Dysfunction:

Nitric Oxide (NO). Substantial evidence indicates that NO production is elevated in 
normal pregnancy and that these increases appear to play an important role in the renal 
vasodilatation of pregnancy (97). In contrast, the role of NO in preeclampsia remains 
enigmatic(25; 76; 91). Taken together, these studies underscore the need for continued 
investigation into the (patho)physiological role of NO in preeclampsia.

Studies from several laboratories indicate chronic NOS inhibition in pregnant rats 
produces hypertension associated with peripheral and renal vasoconstriction, proteinuria, 
intrauterine growth restriction, and increased fetal morbidity (23; 43). We have 
previously reported similar cardiovascular perturbations exist in the NO inhibition and 
RUPP models, the latter illustrated in figure 2 (94). In addition, we have also previously 
shown that placental ischemia results in impairment of acetyl choline (ACh) mediated 
vase-relaxation and nitrite/nitrate production in aortic strips are both decreased in the 
RUPP rat compared to normal pregnant rats and shown in Figure 3 (26). Chronic RUPP 
in pregnant rats also decreases renal protein expression of neuronal nitric oxide synthase, 
but not urinary nitrite/nitrate excretion relative to control pregnant rats (9). Although no 
difference in urinary nitrite/nitrate excretion was found between RUPP and control
pregnant rats we have found that basal and stimulated release of nitric oxide from isolated vascular strips were significantly lower in RUPP rats (13). Although, whether there is a reduction in NO production in this spontaneous model of pregnancy-induced hypertension remains unclear.

**Oxidative Stress.** During oxidative stress, an imbalance of pro- and anti-oxidant factors results in endothelial dysfunction, either by direct actions on the vasculature or through reductions in the bioavailability of vasoactive mediators (113). Oxidative stress may mediate endothelial cell dysfunction and contribute to the pathophysiology of preeclampsia based on evidence of increased pro-oxidant activity along with decreased anti-oxidant protection. During preeclampsia, oxidative stress may result from interactions between the maternal component which may include preexisting conditions such as obesity, diabetes, and hyperlipidemia, and/or the placental component which may involve secretion of lipid peroxides (79).

Several important anti-oxidants are significantly decreased in women with preeclampsia. Vitamin C, vitamin A, vitamin E, β-carotene, glutathione levels, and iron-binding capacity are lower in the maternal circulation of women with preeclampsia than women with a normal pregnancy. Interestingly, supplementation does not appear to ameliorate the incidence of preeclampsia in multi-center clinical trials (73; 83). Reduced superoxide dismutase (SOD) levels and decreased SOD activity have been reported in neutrophils and placentas of women with preeclampsia (113). The decrease in SOD levels and activity in women with preeclampsia is important as diminished SOD occurs...
within both the maternal and placental components. Consequently, there appears to be a
decreased total anti-oxidant protective capacity in women with preeclampsia.

Experimental data reveals that there is an intimate relationship between reactive
oxygen species (ROS), NO activity and blood pressure in rats (85). Thus it is not
surprising that there is increased oxidative stress in the hypertensive pregnant RUPP rat
(87-89). While anti-oxidant treatment with vitamins C and E does not decrease blood
pressure, the SOD mimetic, Tempol does attenuate RUPP hypertension (88; 89).
Similarly, apocynin, an NADP(H) oxidase inhibitor, attenuates but does not normalize
the increased blood pressure observed in RUPP hypertension (89) suggesting there may
be other pathways generating reactive oxygen species in this model. While oxidative
stress is implicated in the pathogenesis of preeclampsia, it remains unclear if it is a
primary or secondary mediator of increased blood pressure and deranged renal function.

Endothelin. Another endothelial-derived factor that may play a role in preeclampsia is
the vasoconstrictor, endothelin-1 (ET-1) (63; 67; 102). Since endothelial damage is a
known stimulus for ET-1 synthesis, increases in the production of endothelin may
participate in the pathophysiology of preeclampsia (12). Further, ET-1 is also reported to
increase oxidative stress in placental villi (27). Thus, endothelin may have additional
effects on the maternal cardiovascular system not only by direct actions on the
vasculature but also indirectly via oxidative stress.

Previously, we have investigated the role of endothelin in mediating RUPP
hypertension in conscious, chronically instrumented pregnant rats (11). Further, we have
shown that RUPP elicits increased renal cortical and medullary expression of
preproendothelin and that chronic administration of the selective ET$_A$ receptor antagonist (ABT-627, 5mg/kg/day for 10 days) markedly attenuates the increased mean arterial pressure in these rats (11). In contrast, ETA receptor blockade had no significant effect on blood pressure in the normal pregnant animal suggesting that ET-1 plays an important role in mediating the hypertension produced by chronic RUPP pregnant rats (11). Furthermore, recent work in our laboratory has shown that sera from pregnant rats exposed to chronic RUPP increases ET-1 production by cultured endothelial cell and that this increase is mediated via the angiotensin II type 1 receptor (82). While the exact mechanism linking enhanced production of ET-1 to placental ischemia in pregnant rats or in preeclamptic women remains unknown, possibilities include production of an agonistic AT1 auto antibody, and/or increased TNF-$\alpha$ as we shall describe later.

Arachidonic acid metabolites. Several lines of evidence suggest that changes in the metabolites of arachidonic acid may play a role in mediating the renal dysfunction and increase in arterial pressure during preeclampsia (114). Significant alterations in the balance of prostacyclin and thromboxane production occur in women with preeclampsia (46; 68; 114). Thus, while there is evidence that AA metabolites play a part in preeclampsia their role is not clearly defined.

Experimental studies in animals have endeavored to determine the role of AA metabolites such as thromboxane in preeclampsia. Such evidence derives from studies indicating short-term increases in systemic arterial pressure produced by acute RUPP in pregnant dogs can be prevented by thromboxane receptor antagonism (119). Further, we have previously reported that urinary excretion of thromboxane B2 was increased in the
RUPP rats compared to normal pregnant rats at day 19 of gestation (56). Additionally, inhibition of cytochrome P450 enzymes with 1-aminobenzotriazole (ABT) attenuated the hypertension, increased renal vascular resistance, 20-HETE formation and CYP4A expression in the renal cortex normally observed in the RUPP rat (55). Nevertheless, experimental data is limited in this area and the quantitative importance of prostaglandins in mediating long-term reduction in renal hemodynamics and increased arterial pressure produced by chronic RUPP in pregnant rats remains unclear.

Renin-Angiotensin System (RAS). The RAS plays an important role in the long-term regulation of renal function and arterial pressure during a variety of physiological and pathophysiological conditions, and pregnancy and preeclampsia are no exception. During normal pregnancy, plasma renin concentration, renin activity, and angiotensin II (Ang II) levels are all elevated, yet vascular responsiveness to ANG II appears to be reduced (12; 92). In contrast, during preeclampsia there appears to be a marked increase in the sensitivity to Ang II (92), although the mechanisms underlying these observations remains unclear.

Recent studies in preeclamptic women have revealed several exciting findings regarding the RAS. Abdalla and colleagues have shown that the Ang II type 1 (AT1) receptor forms heterodimers with the bradykinin B2 receptor and results in enhanced Ang II sensitivity (1; 2). Further, these authors have shown the AT1-B2 heterodimers are present in greater abundance in preeclamptic women; suggesting that this heterodimerization may play a part in the long observed increased Ang II sensitivity in preeclampsia (1; 2). Another intriguing observation regarding the involvement of the
RAS in the pathophysiology of preeclampsia is the demonstration of increased circulating concentrations of an agonistic autoantibody to the Ang II type 1 receptor (AT1-AA) in preeclamptic women (111; 112). Interestingly, the AT1-AA appear to be responsible for a variety of effects in several different tissues ranging from increased intracellular Ca\(^{++}\) mobilization to monocyte activation and stimulation of IL-6 production from mesangial cells (16; 99; 115; 120). Another effect that has recently been attributed to the AT1 receptor is stimulation of sFlt-1 expression from trophoblast cells but not endothelial cells via calcineurin signaling (123). While these findings potentially implicate AT1 as a central mediator of several pathways in preeclampsia, both the specific mechanisms that lead to excess production and the mechanisms whereby AT1-AA increases blood pressure during pregnancy remain unclear. Consequently, this has become an area of intense interest.

Increased vascular responsiveness to Ang II during preeclampsia does not prove it is an important endogenous mediator of vasoconstriction or hypertension in experimental models of preeclampsia as it could merely reflect low endogenous Ang II formation. As such, the importance of increased Ang II to the control of renal function and blood pressure during preeclampsia remains to be determined. Early experiments in our laboratory indicated that chronic oral administration of converting enzyme inhibitor (Enalapril, 250 mg/L for 6 days) decreased MAP to a similar extent in pregnant rats with reduced uterine perfusion pressure (RUPP) and normal pregnant rats, suggesting that the RAS does not play a major role in mediating the hypertension produced by chronic reductions in uterine perfusion pressure in pregnant rats (7). Nevertheless, we have recently found that AT1 receptor antagonism attenuated the blood pressure response to
placental ischemia and that RUPP rats have increased circulating levels of the AT1-AA (54). As described above, we have also shown that serum from pregnant rats exposed to reductions in uterine perfusion enhances endothelin production by endothelial cells via by AT1 receptor activation (82). While the initial findings by our laboratory and others are exciting there remains much to be investigated with respect to the manner in which the AT1-AA contributes to the pathophysiology of preeclampsia.

Cytokines. Several groups have suggested that the etiology of preeclampsia may involve a hypoxia-induced up-regulation of placental inflammatory cytokines (24; 78; 116). While IL-6 and TNF-α are reportedly elevated in preeclamptic women, the importance of these cytokines in mediating the cardiovascular and renal dysfunction in response to placental ischemia during pregnancy remains unclear. We have previously shown that serum levels of TNF alpha and IL-6 are elevated in RUPP rats and that chronic infusion of TNF-α (Figure 5) or IL-6 into pregnant rats at concentrations similar to what is observed in preeclamptic women, increases arterial pressure and decreases renal plasma flow and glomerular filtration rate (8; 29; 49). Further, we have found that low dose infusion of TNF-α results in decreased renal nNOS expression (8) while also increasing ET-1 mRNA in the kidney, placenta, and vasculature (49). Likewise, we have also reported that ET-1 is complicit in the mediation of this form of PIH as the increased MAP in response to TNF-α is completely abolished in pregnant rats treated with an ET\textsubscript{A} receptor antagonist (49). Collectively, these findings suggest that TNF-α induced hypertension in pregnant rats is mediated in part by endothelin, via ET\textsubscript{A} receptor
activation. These studies also suggest that selective ET$_A$ receptor antagonists for the
treatment of hypertension in women with preeclampsia should receive further attention.

Another mechanism by which cytokines may contribute to hypertension during pregnancy is through modulation of sympathetic nerve activity. Previous studies have shown that women with preeclampsia have increased sympathetic tone (34; 86). Although few studies have examined the effect of specific inflammatory cytokines on blood pressure and the regulation of sympathetic activity, one recent experiment has demonstrated that an acute forebrain infusion of TNF-$\alpha$ in rats increased arterial pressure, heart rate and renal sympathetic nerve activity; effects mediated by prostaglandins in the paraventricular nucleus (122). Similarly, another study showed that intracisternal or intravenous infusion of IL-1 beta increases blood pressure in a prostaglandin dependent manner in rats (121). Whether chronic elevations in inflammatory cytokines contributes to the increased sympathetic activity during preeclampsia remains to be determined.

**Angiogenic factors.** While VEGF is primarily recognized for its potent angiogenic and mitogenic effects on endothelial cells it has also been recognized as an important contributor to cell homeostasis; in particular with respect to the balance of oxidative stress (3; 4). VEGF exerts its actions mainly by 2 receptors, VEGFR-1 and VEGFR-2, also known as Flt-1 and the kinase domain region (Flk/KDR), respectively. A soluble and endogenously secreted form of Flt-1 is produced mainly in the placenta by alternative splicing and contains the extracellular ligand-binding domain but not the transmembrane and cytoplasmic portions (21; 44; 45). sFlt-1 disrupts VEGF signaling either by binding VEGF and PlGF or by forming heterodimers with the KDR receptor (45). While sFlt-1 is
not a vasoconstrictor, it does significantly inhibit the dilatory actions of both VEGF and PlGF \textit{in vitro} and chronic elevations in circulating concentrations results in increased blood pressure (60; 66). An additional anti-angiogenic factor, soluble endoglin (sEng), has also been revealed as a factor in the pathogenesis of preeclampsia (64; 108). Endoglin is a component of the TGF-\(\beta\) receptor complex and is a hypoxia inducible protein associated with cellular proliferation and NO signaling (38; 52). sEng, on the other hand, has been shown to be anti-angiogenic as it is thought to impair TGF-\(\beta\) binding to cell surface receptors (38; 108).

Considerable clinical evidence has accumulated that preeclampsia is strongly linked to an imbalance between pro-angiogenic (VEGF, PlGF) and anti-angiogenic (sFlt-1) factors in the maternal circulation (47; 50; 51; 66; 74; 93; 103; 104; 117). Both plasma and amniotic fluid concentrations of sFlt-1 are increased in preeclamptic patients, as well as placental sFlt-1 mRNA (41; 47; 60; 65; 66; 107; 110). Recently, studies have reported that increased sFlt-1 may have a predictive value in diagnosing preeclampsia as concentrations seem to increase before manifestation of overt symptoms (e.g. hypertension, proteinuria) (47; 51). Similarly, recent clinical evidence also suggests that sEng may also presage the onset of preeclampsia (50).

In an elegantly designed study reported several years ago, Maynard et al. reported that exogenous administration of sFlt-1 into pregnant rats via adenovirus mediated gene transfer resulted in increased arterial pressure and proteinuria, and decreased plasma free VEGF and PlGF concentrations similar to that observed in the preeclamptic patients (66). The authors also showed that sFlt-1 impaired VEGF and PlGF induced vasorelaxation (66). Subsequently, similar observations using adenovirus transfection have been
reported in the mouse (60). Recently, we have developed a model of increased circulating sFlt-1 in pregnant rats using recombinant sFlt-1 delivered via osmotic mini-pump placed intraperitoneal and found that the dams are hypertensive, have smaller placentae and fetuses, are proteinuric, and show evidence of impaired vascular function on day 18 of gestation (J.P.G. unpublished observations). While these studies have established the importance of sFlt-1 as an important preeclamptic factor and further studies are needed to elucidate mechanisms governing the expression and actions of this protein.

Recently, Li and coworkers showed that VEGF infusion attenuates the increased blood pressure and renal damage observed in pregnant rats over-expressing sFlt-1 (53). Thus, from this study it can be gleaned that sFlt-1 plays a role in the hypertension and renal dysfunction in preeclampsia; however, these observations did not shed any light on the matter of pathologic sFlt-1 over-expression. To this end, we have recently demonstrated that uteroplacental ischemia increased plasma and placental sFlt-1 and this is associated with decreased VEGF and PlGF in the late gestation pregnant rat (30), Figure 6). Similarly, Makris and colleagues have reported uteroplacental ischemia increases sFlt-1 in the baboon as well (61).

Recent work investigating sEng has furthered progress with respect to the role of anti-angiogenic factors in preeclampsia (108). Venkatesha et al. have shown that sEng inhibits in vitro endothelial cell tube formation to a similar extent as sFlt-1. Further, the authors reported in vivo data in the pregnant rat indicating that adenovirus mediated increase of sFlt-1 and sEng in concert exacerbated the effects of either factor alone and resulted in fetal growth restriction, severe hypertension and nephritic range proteinuria.
Thus, there is compelling experimental evidence that complements clinical observations that sEng is an important factor in the pathogenesis of preeclampsia.

Metabolic factors. There are other comorbid conditions that have been proposed as potential contributors to endothelial dysfunction in preeclampsia. Recent studies have indicated a relationship between elements of the metabolic syndrome such as elevated serum triglycerides and free fatty acids (40; 57), insulin resistance (14; 36; 62; 90; 118), and glucose intolerance (39; 98) and the occurrence of preeclampsia. In fact, several authors have suggested insulin resistance may presage the manifestation of preeclampsia (98; 118) while Thadhani et al. have proposed that insulin resistance during pregnancy may collude with other conditions such as impaired angiogenesis to generate a preeclamptic phenotype (105).

Fatty acids may contribute to endothelial dysfunction by serving as substrates to generate lipid peroxides that are significantly increased in plasma from women with preeclampsia (69). Therefore, the generation of free radicals, lipid peroxides, and reactive oxygen species may be an important mechanism contributing to endothelial dysfunction in preeclampsia (101). Although plasma levels of lipids are increased during normal pregnancy, plasma concentrations of both triglyceride-rich lipoproteins and nonesterified fatty acids are significantly increased in women that develop preeclampsia relative to normal pregnant women (57; 58). This significantly increased plasma triglycerides in women with preeclampsia correlates with an increased plasma of concentrations low-density lipoproteins (84). The nature of this correlative data has provided difficulty in
determining a causal effect for abnormal lipid metabolism in the pathogenesis of preeclampsia.

Because there was no definitive data indicating whether or not metabolic derangements were sequelae or potential contributors to placental ischemia, we recently tested this question in our RUPP model. Data obtained from the RUPP model suggest that metabolic derangements similar to the metabolic syndrome X are not a direct consequence of reduced uterine perfusion pressure (31). Rather, it appears that factors associated with metabolic abnormalities may contribute to cardiovascular dysfunction in preeclampsia rather than resulting from poor placental perfusion (31). Further studies are underway to determine what influence obesity may exert during experimental placental ischemia.

Summary. Despite being one of the leading causes of maternal death and a major contributor of maternal and perinatal morbidity, the mechanisms responsible for the pathogenesis of preeclampsia remain enigmatic. The initiating event in preeclampsia has been postulated to be reduced uteroplacental perfusion as a result of abnormal cytotrophoblast invasion of spiral arterioles. Placental ischemia is thought to lead to widespread activation/dysfunction of the maternal vascular endothelium that results in enhanced formation of endothelin, thromboxane, and superoxide, increased vascular sensitivity to angiotensin II, and decreased formation of vasodilators such as nitric oxide and prostacyclin. These endothelial abnormalities, in turn, cause hypertension by impairing renal-pressure natriuresis and increasing total peripheral resistance (summarized in figure 1). The quantitative importance of the various endothelial and
humoral factors in mediating vasoconstriction and increased arterial pressure during preeclampsia remains unclear.

While recent studies support a role for angiogenic factors, the AT1-AA, cytokines and other factors as potential mediators of endothelial dysfunction, finding the link between placental ischemia and maternal endothelial and vascular abnormalities remains an important area of investigation. Microarray analysis of genes within the ischemic/hypoxic placenta of women with preeclampsia and in animal models of preeclampsia should provide new insights into novel factors that may provide additional links between placental ischemia/hypoxia and hypertension. More effective strategies for the prevention of preeclampsia should be forthcoming once the underlying pathophysiological mechanisms that are involved in preeclampsia are completely understood.
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Figure Legends

Figure 1. Pathways by which reduced uterine perfusion pressure and placental ischemia may lead to endothelial and cardiovascular dysfunction during pregnancy. Placental ischemia results in increased synthesis of soluble fms-like tyrosine kinase-1 (sFlt-1), TNF-α and IL-6, angiotensin II type 1 receptor auto antibodies (AT1-AA) and thromboxane (TX). Elevations in these factors are proposed to result in endothelial dysfunction by decreases in bioavailable nitric oxide, and increased reactive oxygen species (ROS) and endothelin-1 (ET-1), which in turn results in altered renal function, increased total peripheral resistance (TPR) and ultimately hypertension.

Figure 2. Reduced uterine perfusion pressure elicits increased mean arterial pressure, decreased cardiac index (cardiac output/body weight) and increased total peripheral resistance in late gestation rats. All data are expressed as mean ± SEM. Graph adapted from Sholook et al. (94).

Figure 3. Acetycholine (ACh)-induced relaxation of Phe (3x10⁻⁷ mol/L) contraction (A) and the basal and ACh-induced nitrite/nitrate production (B) in endothelium-intact aortic strips of normal pregnant and RUPP rats. Adapted from reference (26).

Figure 4. Effect of Losartan, an AT1 receptor antagonist, on endothelin medium concentration after exposure of HUVECs to serum from RUPP rats or normal pregnant
rats. *P<0.05 vs. control pregnant rats. All data are expressed as mean ± SEM. Adapted from reference (82).

Figure 5. Changes in mean arterial pressure in response to TNF-α infusion and treatment with an endothelin type A receptor (ET_A) receptor antagonist in normal pregnant (NP) rats. (*P<0.05 vs. NP rats). All data are expressed as mean ± SEM. Adapted from references (49).

Figure 6. Plasma concentrations of soluble fms-like tyrosine kinase-1 (sFlt-1) (panel A) were increased in reduced uterine perfusion pressure (RUPP, n = 18) pregnant rats compared to normal pregnant (NP, n = 18) controls at day 19 of pregnancy. Plasma concentrations of vascular endothelial growth factor (VEGF, panel B) and placental growth factor (PlGF, panel C) were decreased in reduced uterine perfusion pressure (RUPP, n = 18) pregnant rats compared to normal pregnant (NP, n = 18) controls at day 19 of pregnancy. All data are expressed as mean ± SEM. Adapted from reference (30).
Figure 1.

- Placental ischemia
  - Decreased uterine placental blood flow
  - sFlt-1
  - Cytokines (TNF-α, IL-6)
  - AT1-AA, TX
  - PIGF, VEGF
  - Endothelial activation/dysfunction
    - NO availability
    - ROS, ET-1
    - Renal pressure natriuresis
    - Total peripheral resistance
  - Hypertension

Copyright Information
Figure 2.

A

MAP (mm Hg)

B

CI (ml/min/kg)

C

TPR (mmHg/ml/min)
Figure 3.

A. Percentage Relaxation

B. Nitrite/Nitrate Production (pmol/mg tissue weight)

Copyright Information
Figure 4.

A

\text{ET-1}
\text{(pg/mg protein)}

\text{Control}

B

\text{ET-1}
\text{(pg/mg protein)}

\text{Losartan Pretreated}

\text{Normal Pregnant} \quad \text{RUPP}
Figure 5.

![Graph showing mean arterial pressure (mmHg) for NP, NP+TNF, NP+ET_A, and NP+TNF/ET_A conditions. The graph indicates a significant increase in mean arterial pressure for NP+TNF compared to NP, NP+ET_A, and NP+TNF/ET_A conditions, marked with an asterisk (*)]
Figure 6.

A

Plasma sFlt-1 (pg/ml)

B

Plasma free VEGF (pg/ml)

C

Plasma PlGF (pg/ml)

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