Studies published in Hypertension and other journals over the last few years have provided exciting new insights into potential mechanisms underlying the pathogenesis of hypertension during preeclampsia. Although numerous factors including genetic, immunologic, behavioral, and environmental influences have been implicated in the pathogenesis of preeclampsia, the main focus of this Hypertension Highlight is to review recent studies that link endothelial dysfunction and hypertension in preeclampsia. The pathophysiologic processes that underlie preeclampsia has been proposed to occur in 2 stages: stage 1, reduced placental perfusion, and stage 2, the maternal clinical syndrome. Placental ischemia/hypoxia is believed to result in the release of a variety of placental factors that have profound effects on blood flow and arterial pressure regulation. These factors include a host of molecules such as the soluble VEGF receptor-1 (sFlt-1), the angiotensin II type-1 receptor autoantibody (AT1-AA), and cytokines such as tumor necrosis factor (TNF)-α which in turn generate widespread dysfunction of the maternal vascular endothelium. This dysfunction manifests as enhanced formation of factors such as endothelin, reactive oxygen species (ROS), thromboxane, 20-HETE, and augmented vascular sensitivity to angiotensin II. In addition, preeclampsia is also associated with decreased formation of vasodilators such as nitric oxide (NO) and prostacyclin. These alterations in vascular function not only lead to hypertension but multi-organ dysfunction, especially in women with early onset preeclampsia. Preterm preeclampsia remains a leading cause of maternal death and perinatal morbidity. Moreover, it has recently been recognized that women who endure preeclampsia are at a greater risk for cardiovascular disease later in life. Therefore, the identification of the connection between placental ischemia/hypoxia and maternal cardiovascular abnormalities is an important area of investigation. In addition, the quantitative importance of the various endothelial and humoral factors that mediate vascular dysfunction and hypertension during preeclampsia remains to be elucidated.

Endothelial Dysfunction and Preeclampsia

The maternal vascular endothelium appears to be an important target of factors that are triggered in preeclampsia. Endothelium-derived relaxing and contracting factors play an important role in the regulation of vascular resistance and blood pressure. When abnormalities in the production or action of these factors occur, the vasculature is predisposed to vasoconstriction, leukocyte adherence, mitochondrial dysfunction, prooxidation, vascular inflammation. Further, markers of endothelial dysfunction may serve as predictors of the syndrome in women that develop preeclampsia because many are often elevated weeks before observation of clinical manifestations.

Potential Mediators of Endothelial Dysfunction

Nitric Oxide

NO production is significantly elevated in normal pregnancy. Experimental studies also suggest that NO production plays an important role in the cardiovascular adaptations of pregnancy. Because NO is an important physiological vasodilator in normal pregnancy, it follows that NO deficiency during preeclampsia has been implicated in the disease process. Although numerous studies indicate chronic NOS inhibition in pregnant rats produces hypertension associated with peripheral and renal vasoconstriction, proteinuria, intrauterine growth restriction, and increased fetal morbidity, it is unclear whether an NO deficiency occurs in women with preeclampsia. Much of the uncertainty in this area of research originates from the difficulty in directly assessing the activity of the NO system in the clinical setting. Recently, Noris et al suggested that L-arginine depletion, caused by arginase II overexpression, may orient NO synthesis toward oxidant species in placenta in preeclampsia. In addition, McCord and colleagues reported that a relative deficiency of arginine in peripheral blood mononuclear cells may favor superoxide and peroxynitrite production and contribute to oxidative and nitrosative stress in preeclampsia. In a study by Conrad and colleagues, under conditions that were carefully monitored to reflect endogenous production and not dietary intake, there was no evidence for a decrease in NO production by measure of plasma or urinary excretion of nitrite and nitrate. In contrast, previous studies have indicated that serum levels of nitrite and nitrate, metabolites...
of NO, are increased relative to severity of the disorder in women that develop preeclampsia.24,26–28 Elevated asymmetrical dimethylarginine (ADMA) concentration before clinical onset of preeclampsia also suggests a role of this NO synthase inhibitor in the pathophysiologic condition of preeclampsia.26

The activity of the NO system has also been assessed in animal models of placental ischemia and cytokine excess. Placental ischemia in pregnant rats has no effect on urinary nitrite/nitrate excretion relative to control pregnant rats.30,31 However, basal and stimulated release of NO from isolated vascular strips were significantly lower in the pregnant rats with placental ischemia32 Moreover, a recent study by Orshal and Khalil found reduced endothelial NO-mediated vascular relaxation in hypertensive pregnant rats chronically infused with the inflammatory cytokine, interleukin (IL)-6.33

**Oxidative Stress**

In disease states of oxidative stress, an imbalance of prooxidant and antioxidant forces results in endothelial dysfunction, either by direct actions on the vasculature or through vasoactive mediators.34–40 During preeclampsia, oxidative stress may result from interactions between the maternal component which may include preexisting conditions such as obesity, diabetes, and hyperlipidemia, or the placental component which may involve secretion of lipid peroxides.34–40 Oxidative stress may mediate endothelial cell dysfunction and contribute to the pathophysiology of preeclampsia as there is evidence of increased prooxidant activity formation along with decreased antioxidant protection in preeclampsia.

NAD(P)H oxidases are an important source of superoxide in neutrophils, vascular endothelial cells, and cytotrophoblast. Increased expression of NAD(P)H oxidase subunits have been reported in both trophoblast and placental vascular smooth muscle cells in placental tissue of women with preeclampsia.43 Moreover, higher placental NAD(P)H oxidase activity has been reported in women with early-onset preeclampsia as compared with those with late-onset of disease which is consistent with the concept that early-onset preeclampsia is more dependent on placental dysfunction than the later-onset disease.43 Thus, there is considerable evidence to suggest that activation of NAD(P)H oxidase plays an important role in the placental oxidative stress associated with preeclampsia.

Several important antioxidants are significantly decreased in women with preeclampsia. Vitamin C, vitamin A, vitamin E, beta carotene, glutathione levels, and iron-binding capacity are lower in the maternal circulation of women with preeclampsia than women with a normal pregnancy.38 Gandley et al recently suggested that the higher circulating levels of S-nitrosoalbumin in women with preeclampsia reflect a deficiency in ascorbate-mediated release of NO from S-nitrosoalbumin.41 These deficiencies in antioxidants may have important vascular effects in preeclampsia. For example, Ramirez and colleagues recently reported that moderate ascorbate deprivation increases mesenteric artery myogenic responsiveness during pregnancy and that this increase may result from a decrease in NO-mediated modulation of the myogenic contractile response.42

In view of the abnormally low plasma vitamin C concentrations in preeclampsia, investigators suggested that a combination of vitamins C and E may be a promising prophylactic strategy for prevention of preeclampsia.43 However, a recent multi-center clinical trial showed that antioxidant supplementation with vitamins C and E during pregnancy did not reduce the risk of preeclampsia in nulliparous women, the risk of intrauterine growth restriction, or the risk of death.44 Indeed, in one study supplementation with vitamin C and vitamin E increased the rate of babies born with low birthweight.45 Thus the use of high dose vitamin C and vitamin E does not appear to be justified during preeclampsia.45

In addition to increased prooxidant activity formation, there is also evidence for decreased total antioxidant protective capacity in women with preeclampsia. Superoxide dismutase (SOD) levels are decreased and reduced SOD activity has been reported in neutrophils and placentas of women with preeclampsia.38 The decrease in SOD levels and activity in women with preeclampsia is important as diminished SOD occurs within both the maternal and placental components.

**Endothelin**

Another endothelial-derived factor that may play a role in preeclampsia is the vasoconstrictor, endothelin-1 (ET-1). Although some studies have reported no significant changes in circulating levels of ET-1 during moderate forms of preeclampsia, a possible role for ET-1 as a paracrine or autocrine agent in preeclampsia remains worthy of consideration.11,12,23,46–47 Because ET-1 is released toward the vascular smooth muscle in a paracrine fashion, changes in plasma levels of ET may not reflect its local production. Indeed, this is one of the reasons why it has been difficult to ascertain whether preeclampsia is associated with altered ET production. Local synthesis of ET has been assessed in preeclamptic women, and investigators have found preproendothelin mRNA to be elevated in a variety of tissues.11,12 Because of the limitations of clinical studies using selective ET type A receptor antagonists in pregnant women, the importance of locally produced ET in the pathophysiology of preeclampsia remains unclear.

Alexander et al recently examined the role of ET-1 in mediating the hypertension in a placentical ischemic rat model of preeclampsia.48 Using an RNase protection assay, they found that renal expression of preproendothelin was significantly elevated in both the medulla and the cortex of pregnant rats with chronic reductions in uterine perfusion pressure (RUPP) compared with control pregnant rats. Moreover, they reported that chronic administration of the selective ET\textsubscript{A} receptor antagonist, ABT627 markedly attenuated the increase in mean arterial pressure in pregnant rats with RUPP. In contrast, ET\textsubscript{A} receptor blockade had no significant effect on blood pressure in the normal pregnant animal. These findings suggest that ET-1 plays a major role in mediating the hypertension produced by chronic reductions in uterine perfusion in pregnant rats.

Sera from pregnant rats exposed to chronic RUPP increases ET-1 production by cultured endothelial cells.49 The exact mechanism linking enhanced renal production of ET-1 to placental ischemia in pregnant rats or in preeclamptic
women is unknown. One potential mechanism for enhanced ET-1 production is via transcriptional regulation of the ET-1 gene by TNF-α. TNF-α is elevated in preeclamptic women and has been implicated in the disease processes. LaMarca and colleagues recently reported that chronic infusion of TNF-α in pregnant rats significantly increases blood pressure. The increase in arterial pressure produced by a 2- to 3-fold elevation in plasma levels of TNF-α in pregnant rats is associated with significant increases in local production of ET-1 in the kidney, placenta, and vasculature. Moreover, the increase in mean arterial pressure in response to TNF-α is completely abolished in pregnant rats treated with an ETα receptor antagonist. Collectively, these findings suggest that endothelin, via ETα receptor activation, plays an important role in mediating TNF-α–induced hypertension in pregnant rats.

**Arachidonic Acid Metabolites**

Although significant alterations in the balance of prostacyclin and thromboxane production occur in women with preeclampsia, the importance of arachidonic acid metabolites in the pathophysiology of this disease has yet to be fully elucidated. Experimental studies in animals have attempted to determine the role of AA metabolites in response to placental ischemia. Increases in systemic arterial pressure produced by acute placental ischemia in pregnant dogs can be prevented by thromboxane receptor antagonism. Although urinary excretion of thromboxane B2 is increased in response to placental ischemia in pregnant rats, acute administration of a thromboxane receptor antagonists failed to alter blood pressure. In contrast, inhibition of cytochrome P450 enzymes with 1-aminobenzotriazole (ABT) attenuated the hypertensive and increased renal vascular resistance, 20-HETE formation, and CYP4A expression in the renal cortex normally observed in the placental ischemic pregnant rat.

**Renin-Angiotensin System**

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. In contrast, during preeclampsia there appears to be a marked increase in the sensitivity to Ang II. Although the mechanisms underlying these observations remain unclear, there is growing evidence to suggest that dysregulation of the tissue-based and circulating renin-angiotensin system (RAS) may be involved in the pathophysiology of preeclampsia.

Recent studies in preeclamptic women demonstrate increased circulating concentrations of an agonistic autoantibody to the angiotensin type 1 receptor (AT1-AA). In addition to being elevated during preeclampsia, the AT1-AA has also been reported to be increased in postpartum women. Hubel and colleagues demonstrated that the AT1-AA does not regress completely after delivery and that the increase in AT1-AA correlated with insulin resistance and sFlt-1. The importance of AT1-AA after preeclampsia, especially in the context of increased cardiovascular risk, remains to be determined.

Interestingly, the AT1-AA appear to be responsible for a variety of effects in several different tissues ranging from increased intracellular Ca2+ mobilization to monocyte activation and stimulation of IL-6 production from mesangial cells. Another effect that has recently been attributed to the AT1 receptor is stimulation of sFlt-1 expression from trophoblast cells. Although 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.

**Cytokines**

Several groups have also suggested a potential role for inflammatory cytokines that in the etiology of preeclampsia. Freeman and colleagues recently examined changes in inflammatory markers prospectively during pregnancy and the current inflammatory status of women who had a pregnancy complicated by preeclampsia 20 years previously against matched controls and found that preeclampsia was associated with short- and long-term changes in inflammatory status. Whereas Armanini and Calo suggested that aldosterone could play an important role in the genesis of this increased susceptibility of inflammatory process in preeclampsia, other factors such as obesity, diabetes, and placental ischemia could also be involved.

Although inflammatory cytokines such as IL-6 and TNF-α have been reported by some laboratories to elevate in preeclamptic women, it has been uncertain whether moderate and long-term increases in cytokines during pregnancy could result in elevations in blood pressure. However, recent studies indicate that chronic infusion of TNF-α or IL-6 into pregnant rats at concentrations similar to what is observed in pre-
Angiogenic Factors
Considerable clinical evidence has accumulated that preeclampsia is strongly linked to an imbalance between proangiogenic such as vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) and antiangiogenic factors such as soluble fms-like tyrosine kinase (sFlt-1) in the maternal circulation. Both plasma and amniotic fluid concentrations of sFlt-1 are increased in preeclamptic patients, as well as placental sFlt-1 mRNA. 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 (eg, hypertension, proteinuria). 

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 PIGF concentrations similar to that observed in the preeclamptic patients. Subsequently, similar observations using adenovirus transfection have been reported in the mouse.

Recently, Li and coworkers showed that VEGF infusion attenuates the increased blood pressure and renal damage observed in pregnant rats overexpressing sFlt-1. Thus, this study suggests that sFlt-1 and alterations in angiogenic factors may contribute to the clinical symptoms observed in preeclampsia; however, these observations did not shed any light on the mechanisms whereby sFlt-1 overexpression occurs in preeclampsia. To this end, we have recently demonstrated that uteroplacental ischemia increased plasma and placental sFlt-1 and this is associated with decreased VEGF and PIGF in the late gestation pregnant rat. Similarly, Makris and colleagues have reported uteroplacental ischemia increases sFlt-1 in the baboon as well.

An additional antiangiogenic factor, soluble endoglin (sEng), has also been revealed as a factor in the pathogenesis of preeclampsia. Endoglin is a component of the transforming growth factor (TGF)-β receptor complex and is a hypoxia inducible protein associated with cellular proliferation and NO signaling. sEng, on the other hand, has been shown to be antiangiogenic as it is thought to impair TGF-β1 binding to cell surface receptors. Recent work investigating sEng has furthered progress with respect to the role of antiangiogenic factors in preeclampsia. 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 compliments clinical observations that sEng is an important factor in the pathogenesis of preeclampsia. Moreover, recent clinical evidence also suggests that sEng may also presage the onset of preeclampsia.

Metabolic and Dietary Factors
There are other comorbid conditions such as obesity, diabetes, hyperlipidemia, and hyperhomocysteinemia 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, insulin resistance, and glucose intolerance and the occurrence of preeclampsia. In fact, several authors have suggested insulin resistance may presage the manifestation of preeclampsia, whereas Thadhani et al have proposed that insulin resistance during pregnancy may interact with other conditions such as impaired angiogenesis to generate a preeclamptic phenotype.

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. This significantly increased plasma triglycerides in women with preeclampsia correlates with an increased plasma of concentrations low-density lipoproteins. 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 were no definitive data indicating whether or not metabolic derangements were sequelae or potential contributors to placental ischemia, Gilbert et al recently tested this question in a placental ischemic model. Data obtained from the model suggest that metabolic derangements similar to the metabolic syndrome X are not a direct consequence of reduced uterine perfusion. Rather, it appears that factors associated with metabolic abnormalities may contribute to cardiovascular dysfunction in preeclampsia rather than resulting from poor placental perfusion.

Several clinical studies have also shown that women with higher plasma homocysteine (hyperhomocysteinemia) levels early in pregnancy have a higher incidence of preeclampsia and intrauterine growth restriction (IUGR). Powers and colleagues recently suggested that the vasculature during pregnancy may manifest increased sensitivity to homocysteine. They found that endothelial-dependent vasodilation in pregnant mice is more sensitive to the effect of increased homocysteine than arteries from nonpregnant mice and that this effect of homocysteine appears to result from a loss in NO-mediated relaxation attributable to oxidative inactivation of the NO synthase cofactor, tetrahydrobiopterin.

Homocysteine concentrations are affected by nutritional deficiencies, particularly decreased folic acid and B12, leading to increased homocysteine. Patrick et al recently reported that homocysteine and folic acid are inversely related in Black women with preeclampsia. The importance of folate intake is also highlighted by a recent study by Torrens and colleagues where they reported that folate supplementation during pregnancy improves offspring cardiovascular dysfunction induced by protein restriction in laboratory animals.

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References


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