Oxidative Stress in the Pathogenesis of Preeclampsia (44447)

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Abstract. **The etiology and pathogenesis of the pregnancy syndrome preeclampsia remain poorly understood. There is substantial evidence to suggest that the diverse manifestations of preeclampsia, including altered vascular reactivity, vasospasm, and discrete pathology in many organ systems, are derived from pathologic changes within the maternal vascular endothelium. With the theme of endothelial cell dysfunction emphasized, this review focuses on the role of oxidative stress (an imbalance favoring oxidant over antioxidant forces) in the pathogenesis of preeclampsia. Data are summarized regarding 1) the role of the placenta in preeclampsia; 2) evidence and mechanisms of oxidative stress in the preeclampsia placenta; 3) markers of oxidative stress in the maternal circulation; and 4) the potential role of maternal dyslipidemia in generation of oxidative stress. A recurrent theme is that free radical reactions, promoted by "cross-talk" between the diseased placenta and maternal dyslipidemia, promote a vicious cycle of events that make cause and effect difficult to distinguish but likely contribute to the progression of preeclampsia.** [P.S.E.B.M. 1999, Vol 222]

Preeclampsia is a human pregnancy-specific disorder that adversely affects the mother (by vascular dysfunction) and the fetus (by intrauterine growth restriction). The incidence of preeclampsia is between 3% and 10% of pregnancies, and there is no evidence that this has changed appreciably during the last century. Preeclampsia is characterized by vasospasm, increased peripheral vascular resistance, and thus reduced organ perfusion (1). The syndrome is polymorphic in that virtually every organ system can be affected. Preeclampsia is diagnosed by the new development of hypertension (usually $\leq$140/90 mm Hg), significant proteinuria, and remission of these signs after delivery (2). Eclampsia is the occurrence of seizures in a preeclamptic patient that cannot be attributed to other causes (2). Even without progression to eclampsia, the syndrome presents substantial risk to mother and baby. Preeclampsia is the leading cause of maternal mortality in developed countries and is associated with a five-fold increase in perinatal mortality. The major cause of fetal compromise is reduced uteroplacental perfusion (1). The only intervention that effectively reverses the syndrome is delivery. A large portion of the perinatal mortality is consequently due to iatrogenic prematurity. Up to 15% of preterm births are a result of preeclampsia (3). The combination of hypertension plus proteinuria markedly increases the risk of perinatal morbidity and mortality over that of hypertension alone (4). Hypertension is a result of the disease and is not usually of pathogenic importance, and it cannot explain the diverse laboratory and clinical features of preeclampsia (5, 6). For research purposes especially, a rigorous classification scheme is advocated in which preeclampsia is distinguished from transient gestational hypertension by the lack of significant proteinuria in the latter disorder (2). *Chesley's Hypertensive Disorders in Pregnancy, Second Edition*, is recommended for further reading on the clinical spectrum and epidemiology of preeclampsia (7).

About 10 years ago, Roberts *et al.* (8) formally proposed that maternal endothelial cell dysfunction is the key event resulting in the diverse clinical manifestations of preeclampsia. Evidence has since accumulated to support a major role of the endothelium in preeclampsia (9, 10). The mechanisms involved in induction of endothelial cell dysfunction are poorly understood. Abnormal placentation is clearly involved in the genesis of both preeclampsia and

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fetal intrauterine growth restriction (IUGR) (11, 12). Preeclampsia (with or without IUGR), however, is distinguished from IUGR (without preeclampsia) by extension of disturbances into the maternal vasculature (6, 12). It has been proposed that product(s) of the fetal-placental unit enter the circulation and then initiate the maternal pathophysiologic changes of preeclampsia (8). However, there is increasing evidence that both feto-placental and maternal factors interact in manifesting endothelial cell dysfunction and its clinical manifestations (9, 13, 14).

One hypothesis receiving increased attention is that placental and maternal free radical reactions promote a cycle of events that compromise the defensive functioning of the vascular endothelium in preeclampsia. Since the time that data relevant to this hypothesis were initially reviewed (15), a significant body of new information has been generated. The present review begins with some free radical terminology and then focuses on preeclampsia.

Free Radicals and Reactive Oxygen Species

A free radical is any molecule capable of independent (usually brief) existence that contains one or more unpaired electrons (16). Most free radicals in biology fit within the broader category of reactive oxygen species (ROS), which include not only oxygen-containing free radicals, such as hydroxyl radical (HO·), superoxide anion radical ($O_2^{\cdot -}$), and nitric oxide (NO·), but also reactive molecules that do not contain unpaired electrons, such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and peroxynitrite anion (ONOO–). The highly reactive primary products of lipid peroxidation, lipid hydroperoxides, are formed when free radicals attack polyunsaturated fatty acids or cholesterol in membranes or lipoproteins. Alternatively, they can be formed by cyclooxygenase or lipoxygenase (17). Lipid hydroperoxides function in normal physiology by regulating enzymes and redox-sensitive genes (18, 19). However, uncontrolled lipid peroxidation can result in cellular dysfunction and damage. Lipid peroxidation has received a great deal of attention in preeclampsia (15, 20). Many endothelial changes of potential relevance to preeclampsia can be induced by lipid peroxidation in experimental systems. Some examples are listed in Table I.

A wide spectrum of ROS function as signal transducers in normal physiology (19); however, their overproduction may result in, or be the result of, numerous human health problems (21, 22). Untangling cause and effect is confounded by the evanescence of ROS and the high potential for experimental artifact. For example, lipid hydroperoxides are generated during exposure of blood and tissues to oxygen *ex vivo* (22). Another problem is the lack of gold stan-

Table I. Dysfunction in Preeclampsia Mimicked by Experimental Lipid Peroxidation

Dysfunction in preeclampsia	Peroxidation in experimental models
1. Evidence of endothelial structural injury: • Glomerular capillary endothelium • Umbilical endothelium	1. Acute exposure to lipid peroxides damages endothelial cells (187)
2. Proteinuria	2. Intrarenal infusion of hydrogen peroxide induces reversible proteinuria in rats (188)
3. Convulsions during eclampsia	3. Eclampsia-like convulsions and intravascular thrombosis in term rats fed a diet deficient in vitamin E and containing lipid peroxides beginning Day 13 of gestation (189)
Endothelial functional/biochemical changes	
4. Vasoconstriction and increased sensitivity to pressor agonists	4. Lipid peroxides or oxidized LDL increase artery sensitivity to agonists (190, 191)
5. Impaired endothelial-dependent relaxation of isolated arteries (31, 32)	5. Oxidized LDL inhibits endothelial-dependent vasodilation (192, 193)
6. Reduced prostacyclin (PGI_2) production by vessels (194)	6. Increased lipid peroxidation via vitamin E deprivation decreases PGI_2 production (195)
7. Increased circulating cellular fibronectin (38)	7. Peroxides induce tissue release of cellular fibronectin (196)
Preeclampsia serum/plasma alters endothelial cell function *in vitro*	
8. Preeclampsia plasma increases endothelial production of nitric oxide (197, 198)	8. Oxidized LDL at low concentration increases nitric oxide production from endothelial cells in culture (199, 200)
9. Preeclampsia plasma induces biphasic release of PGI_2 from endothelial cells in culture (increased at 24 hr; decreased at 72 hr) (201)	9. Oxidized LDL or hyperlipidemic sera increase endothelial PGI_2 production at 24 hr but inhibit during longer incubations (48–72 hr) (202, 203)
Functional changes in red blood cells	
10. Hemolysis and increased red cell osmofragility (204)	10. Lipid peroxidation promotes osmofragility and hemolysis (205)
11. Decreased calcium-ATPase activity (206)	11. Lipid peroxides and other ROS inhibit calcium-ATPase *via* modification of protein thiols (207)

dard methods to evaluate oxidative stress (23, 24). We currently lack incontrovertible evidence that oxidative stress contributes to progression of preeclampsia. Clarity has been hindered by the lack of suitable animal models (25) and unique difficulties in obtaining longitudinal tissue samples in this low-prevalence pregnancy disorder.

Endothelial Cell Dysfunction in Preeclampsia

Several lines of evidence indicate that adverse changes in structure and function of the maternal vascular endothelium account for the altered vascular reactivity, activation of the coagulation cascade, and multisystem damage that occurs in preeclampsia. The endothelial changes are more appropriately described as dysfunction or activation (an altered state of endothelial cell differentiation in response to sublethal injury or cytokine stimulation (26)) rather than damage (10). Pathologic changes in the endothelial cells that line the renal glomerular capillaries (glomerular endotheliosis) are a consistent feature in women with preeclampsia (27, 28). The cells become larger, may contain lipid droplets, and often occlude the capillary lumen (29). These changes are reversible after delivery, signifying that repair processes take place after some influence is removed (30).

Normal human pregnancy is characterized by profound changes in the cardiovascular system, including decreased vascular reactivity and reduced vascular tone. An increase in reactivity and a reduction in relaxation capacity of resistance arteries occurs with preeclampsia. For example, subcutaneous resistance arteries isolated from women with normal pregnancy exhibit enhanced bradykinin-mediated (endothelium-dependent) relaxation relative to nonpregnancy, but this pregnancy change is absent in women with preeclampsia. The attenuation of relaxation may result from decreased production of endothelial vasodilator nitric oxide (31). An endothelium-dependent, nitric oxide-independent component may be involved in other vascular beds (32). The hypertension, increased blood pressure responsiveness to vasoconstrictors (33, 34), and reports of retinal arteriolar vasospasm/ischemia (35, 36) in preeclampsia are also consistent with endothelial cell dysfunction.

A variety of substances indicative of endothelial dysfunction are increased in the blood or urine of women with preeclampsia (9, 10). Many of these substances including serum soluble VCAM-1 (37) and cellular fibronectin (38), are elevated weeks before (as well as during) clinically evident preeclampsia. Multiple circulating factors may provoke the spectrum of endothelial changes, including altered lipoproteins (9, 39, 40). There are also numerous reports that substances in plasma or serum from women with preeclampsia alter the function of endothelial cells in culture in ways relevant to the endothelial pathology of the disease (9, 39, 41).

There is no convincing evidence that changes in classically defined hormones account for the vascular changes of preeclampsia. It has been proposed that deficient nitric oxide (NO·) production or availability may contribute to the pathophysiology of preeclampsia (42). However, there are reports of decreased (43), unchanged (44), and increased (45–47) circulating nitric oxide degradation products (nitrate and nitrite) in preeclampsia. The conflicting data on nitric oxide in normal and preeclamptic pregnancies have been reviewed (48). Present discussion of nitric oxide will be restricted primarily to its interactions with superoxide anion radical ($O_2^{\cdot -}$) to form the profoundly reactive peroxynitrite anion ($ONOO^-$).

Role of the Placenta in Preeclampsia

Evidence points to the placenta as a key source of factors that lead to the maternal endothelial cell dysfunction in preeclampsia (11). This is evident in that the clinical signs and lesions of preeclampsia remit within days after termination of pregnancy. The disease can occur in anembryotic pregnancy (hydatidiform mole), suggesting that the presence of a fetus is not strictly necessary (49). In rare cases of extrauterine (abdominal) pregnancy, in which delivery of the fetus is not followed by delivery of the placenta, the signs of preeclampsia persist postpartum until the placenta is resorbed (50, 51).

The genesis of preeclampsia is clearly related to deficient trophoblast invasion and failure of uterine artery remodeling (12, 52). In normal pregnancy the spiral arteries feeding the intervillous space of the placenta increase greatly in diameter and become refractory to vasomotor agents. This involves replacement of endothelium by invading trophoblast (the trophoblast cells assuming an endothelial cell adhesion molecule phenotype) and replacement of the internal elastic lamina and smooth muscle by trophoblast and fibrinoid matrix. This transformation is complete by 20 weeks of gestation (53). Along with venous distension, this accounts for the increased blood supply to the intervillous space necessary to meet the demands of the rapidly growing feto-placental unit during the later stages of gestation (54).

Preeclampsia placentas show abnormal expression of integrin molecules that regulate cell-cell and cell-matrix interactions (12, 52). As a result, trophoblastic invasion is inhibited, and spiral artery remodeling is often limited to the decidual portions such that the myometrial segments do not widen and remain contractile (55). Defective spiral artery remodeling in preeclampsia (and in IUGR) likely results in reduced uteroplacental perfusion and foci of placental hypoxia or ischemia (12, 56, 57). Placental infarcts occur with increased frequency in preeclampsia, consistent with focal ischemia (58). Preeclampsia is more common in primigravid twin pregnancies (30% incidence) (59), suggesting that placental perfusion is an important determinant of pregnancy outcome. Many of the ultrastructural changes of preeclamptic placental tissue resemble alterations in placental tissue when placed in hypoxic organ culture (60). Preeclampsia is more common at high altitude suggesting that chronic hypoxia is a predisposing factor (61).

A continuing mystery, however, is how the poor placental remodeling is connected to the maternal syndrome. Placental hypoxia/ischemia could result in release of products into the maternal circulation which then initiate the maternal pathophysiologic changes of preeclampsia. Although it is unclear whether posthypoxic reperfusion oxidative damage occurs in the preeclampsia placenta, there are numerous changes consistent with accentuated oxidative stress in the preeclampsia placenta (Table II).

Oxidative Stress in the Preeclampsia Placenta

Acute Atherosis. Preeclampsia is associated with a distinct pathologic lesion of the decidual arterioles known as acute atherosis. This arteriopathy occurs in regions of spiral and myometrial arteries in which the physiologic transformational changes are absent. Acute atherosis bears a striking resemblance to atherosclerotic lesions of coronary arteries, both showing fibrinoid necrosis of the vessel wall, disruption of the endothelium, aggregates of platelets, and accumulation of lipid-laden macrophages (foam cells) (55, 62, 63). This is considered to be a true atherosclerosis-like change. It can also occur in pregnancies with intrauterine growth restriction without a maternal syndrome (64). The morphology of these vessels suggests parallels with the atherogenic process of carotid arteries, in which low-density lipoprotein (LDL) lipid peroxidation with foam cell formation has a paramount role. However, it remains to be seen whether oxidized lipids are increased in decidual arterial walls in preeclampsia.

Placental Lipid Peroxidation. Lipid peroxidation products are candidate factors that may mediate disturbance of the maternal vascular endothelium (65). Although not examined in spiral arteries directly, a study of women undergoing cesarean section showed significantly higher concentrations of lipid hydroperoxides, phospholipids, and cholesterol in decidua basilis tissues from women with preeclampsia as compared with tissues from a normal pregnancy (66). Immediate postpartum curettage, which involves removal of decidual tissue, results in more rapid clinical recovery from preeclampsia (67). This is consistent with decidual tissue being a source of factors that enter the maternal circulation and contribute to the maternal syndrome. A follow-up study showed that the content of free isoprostane (8-iso-PGF_{2_α}), but not total (free plus esterified) isoprostane, is nearly two-fold elevated in decidua from women with preeclampsia (68). Isoprostanes, are produced specifically by free-radical–catalyzed peroxidation of arachidonic acid (69). Free 8-iso-$PGF_{2\alpha}$ has activities of relevance to preeclampsia, being a potent vasoconstrictor, platelet activator, and mitogen (69).

In vitro production of lipid hydroperoxides and thromboxane are reportedly increased in both trophoblast cell and villous tissues from women with preeclampsia (70–72). Production of 8-iso-$PGF_{2\alpha}$ is also increased in incubated placental tissue from women with preeclampsia compared with tissue from a normal pregnancy (73). However, there has been no direct demonstration that placental peroxidation products accumulate in the maternal circulation.

Table II. Changes Consistent with Oxidative Stress in Preeclampsia Placenta

Marker or activity	Location	Reference no.
Decreased total superoxide dismutase (SOD) activity	Placental homogenate, and mitochondrial and cytosolic fraction	(93, 94)
Decreased Cu, Zn-SOD activity and mRNA expression	Placental cotyledons, excluding chorionic and basal plates	(95)
Decreased glutathione peroxidase activity	Placental tissue homogenate	(74)
Increased immunohistochemical staining for xanthine oxidase holoenzyme	Invasive cytotrophoblast	(90)
Increased xanthine oxidase holoenzyme and specific oxidase isoform activity	Placental bed curettings (containing cytotrophoblast)	(90)
Increased nitrotyrosine immunostaining	Villous vascular endothelium, surrounding smooth muscle and villous stroma	(81)
Increased lipid hydroperoxide concentrations	Placental tissue homogenate	(74)
	Decidua basalis	(66)
Increased lipid hydroperoxide production/secretion	Trophoblast cells and villous tissue	(71)
Increased production of 8-isoprostane	Placental tissue pieces	(73)
Increased malondialdehyde (lipid peroxidation product)	Placental homogenate and mitochondrial and cytosolic fractions; syncytiotrophoblast plasma membranes	(93, 94, 208)
Increased membrane fluidity (possible indicator of lipid peroxidation)	Syncytiotrophoblast plasma membranes	(161)
Increased membrane susceptibility to peroxidation	Syncytiotrophoblast plasma membranes	(208)
Increased maximum amount of peroxidizable material	Placental homogenate	(93)
Presence of lipid-laden macrophages (foam cells)	Decidual arterioles	(55, 62)
Increased elastase-positive neutrophils (marker of neutrophil activation)	Decidua of the placental bed	(172)

Glutathione peroxidase, an enzyme that removes hydrogen peroxide and converts lipid hydroperoxides to less reactive alcohols, may be deficient in placental tissue from preeclamptic women. This is seen in conjunction with increased *in vitro* placental production of lipid hydroperoxides and thromboxane A_2 (TXA_2) (20). TXA_2 is a vasoconstrictive and pro-aggregatory prostaglandin normally counterregulated by prostacyclin (PGI_2). Chemical inhibition of placental glutathione peroxidase resulted in increased production of lipid hydroperoxides and an increase in the placental TXA_2 to PGI_2 output ratio (74). Lipid hydroperoxides can inhibit PGI_2 synthase enzyme activity and simultaneously stimulate the cyclooxygenase component of PGH synthase (75) whereas TXA_2 synthase activity is unchanged or even stimulated (20, 76). Since expression of the synthases is not altered in the uteroplacental unit (77), these effects of lipid hydroperoxides could be the source of the decreased placental PGI_2 to TXA_2 production ratio in preeclampsia. The altered prostaglandin ratio might provoke vasospasm with exacerbation of placental ischemia, increased cell damage, and increased lipid peroxidation (amplification of oxidative stress) (15).

Placental Nitrotyrosine, Xanthine Oxidase, and SOD. Peroxynitrite anion ($ONOO^-$) is capable of nitrating proteins and inducing lipid peroxidation (78). Peroxynitrite formation is favored when NO·outcompetes superoxide dismutase (SOD) for $O_2^{\cdot -}$ (78). Nitrotyrosine is the stable reaction product of $ONOO^-$ attack on proteins and thus signals oxidative damage. Nitrotyrosine immunostaining has been detected around foam cells in human atherosclerotic lesions (79) and in lung tissue of newborns with acute lung injury (80). Myatt *et al.* (81) have found greater nitrotyrosine immunostaining in placental villous vascular endothelium, and surrounding vascular smooth muscle and villous stroma in preeclampsia and also in intrauterine growth restriction compared to normal pregnant controls. They also found increased nitrotyrosine residues in the placental vasculature of women with well-controlled diabetic pregnancies (82). Of note, there is an increased incidence of preeclampsia in women with IDDM (59). A variety of cells can be induced to produce both $O_2^{\cdot -}$ and NO· (and consequently $ONOO^-$) by inflammatory stimuli or by postischemic reoxygenation (83, 84). Increased nitrotyrosine immunoreactivity may reflect upregulation of such pathways in preeclampsia (83, 84). However, nitration of tyrosine can occur by other free radical processes. Superoxide-independent pathways have been reported, such as interaction of nitrogen dioxide (NO_2·) with tyrosyl radicals generated by myeloperoxidase during oxidative stress (85).

Changes in xanthine oxidase in the preeclampsia placenta further suggest ischemic or inflammatory injury. The dehydrogenase (type D) form of xanthine oxidase requires NAD and produces uric acid and NADH. During hypoxia/ischemia, this form is increasingly converted to the oxidase (type O form) which requires oxygen and produces uric acid and $O_2^{\cdot -}$ during reoxygenation (86, 87). However, a recent reappraisal of xanthine oxidase in human tissues has suggested that both D and O isoforms can generate reactive oxygen species during posthypoxic reperfusion (88). Immunoreactivity, mRNA, and enzyme activity for the holoenzyme (combined D and O) have been demonstrated in normal human placental trophoblast (although at low levels compared to liver) (89). Remarkably, there is increased immunohistochemical staining for the holoenzyme in invasive, but not villous, trophoblast from preeclamptic pregnancies (90). Placental site curettings (which contain cytotrophoblast) from women with preeclampsia exhibit increased holoenzyme and increased type O activity compared to samples from normal controls (90).

In addition to a role for xanthine oxidase, placental generation ROS in preeclampsia might be facilitated by decreases in superoxide dismutase expression and activity. Total SOD activity in placental homogenates reportedly increases with gestational age (91, 92). Total activity may be decreased in whole placental homogenates (93, 94) and mitochondrial and cytosolic fractions (94) from women with preeclamptic compared to normal gestations. Placental tissue homogenate Cu,Zn-SOD activity and mRNA expression are reportedly decreased in preeclampsia relative to normal pregnancy (95). However, another study found no differences in Cu,Zn- or Mn-SOD immunostaining intensity in placental villous tissue of normal and preeclamptic pregnancies (96). Circulating white and red blood cells from women with preeclampsia have decreased superoxide dismutase activity but not in the concentration of its mRNA, suggesting post-transcriptional reduction (97, 98).

In summary, there appears to be an increase in ROS generation in the placenta of preeclamptic women. There is evidence for increased nitrotyrosine formation in the preeclampsia placenta suggestive of $ONOO^-$ production, perhaps arising from local NO· production coupled with increased xanthine oxidase generation of $O_2^{\cdot -}$ and either regionally decreased or inadequate SOD. Whether this could lead to oxidative stress and/or endothelial dysfunction in the systemic circulation is uncertain. Beneficial/compensatory effects of $ONOO^-$ are also plausible. For example, $ONOO^-$ can lessen leukocyte rolling and adhesion to endothelial cells and inhibit platelet aggregation (99).

Markers of Oxidative Stress in the Maternal Circulation

Nitrotyrosine in the Maternal Vasculature. In striking parallel to data in the placenta, immunohistochemical analysis of microvessels from biopsies of subcutaneous fat suggests increased peroxynitrite formation in preeclampsia (100). The percentage of vascular endothelium staining for nitrotyrosine was greater in preeclampsia (73%) than normal pregnancy (3%). Greater staining was also seen just outside the endothelium, possibly due to diffusion of peroxynitrite from the endothelium. In conjunction, the intensity of endothelial cell immunostaining was significantly lower for SOD and higher for nitric oxide synthase (eNOS)

in vessels from women with preeclampsia. These data suggest deleterious oxidative changes in the maternal vasculature.

Lipid Peroxidation Products. There are scores of reports that lipid peroxidation products, primarily measured as thiobarbituric acid-reactive substances (which include malondialdehyde), are increased in plasma/sera of women with preeclampsia (15, 20). There are also reports of increased lipid peroxidation products in platelets (101) from women with preeclampsia. However, most lipid peroxidation assays have sensitivity and specificity problems (23, 24). Morris *et al.* (102) found no evidence that circulating lipid peroxidation products (8-iso-PGF_{2_α}, lipid hydroperoxides, and malondialdehyde) are elevated in preeclampsia once appropriate precautions were taken, including addition of antioxidants, to prevent *in vitro* oxidation. However, these oxidation markers were significantly raised in normal pregnancy (and in preeclampsia) as compared with nonpregnant women, agreeing with several earlier publications (20, 103, 104). Thus, it is possible that pregnancy is a stimilus for lipid peroxidation.

Linoleic acid content in plasma phospholipid and triglyceride fractions decreases from early to late pregnancy in women with preeclampsia relative to normal pregnancy. Progressive lipid peroxidation may explain this change (105). Due to ascorbate and other antioxidants in plasma, susceptible lipids are believed not to undergo significant oxidation in the circulation (106). Circulating peroxidation products may originate from the placenta (20).

Circulating Anti-Oxidized LDL Antibodies. Low-density lipoprotein particles continuously enter and exit the artery wall. In the subendothelial interstitial matrix, the presumed site of LDL oxidation *in vivo*, LDL may be exposed more frequently to cell-derived oxidants and at the same time may be less protected by antioxidants relative to circulating LDL (107). The potential for prolonged contact with LDL is one reason the endothelium is prone to oxidative disturbances. Antibodies directed against oxidized LDL are found in the serum of most people but are increased in disorders associated with oxidative stress (108). Increased autoantibodies to an epitope of oxidized LDL have been described in women with preeclampsia relative to normal pregnancy although a negative report also exits (109, 110). In such studies, it is important to consider the antigenic epitope of oxidized LDL used. Uotila *et al.* (111) found increased titers of serum autoantibodies against copper-oxidized LDL, but not against malondialdehyde-LDL, in preeclampsia. Kurki *et al.* (112) found that antibodies to malondialdehyde-LDL and anticardiolipin were not increased in early in gestation in women who subsequently developed preeclampsia compared with women whose pregnancies remained normal. The pathophysiologic implications of these circulating markers thus remain uncertain.

Ascorbate Oxidative Consumption. Reduced ascorbate (vitamin C) is supremely effective in protecting plasma lipoproteins and other susceptible molecules from peroxidation during exposure to a wide spectrum of water- or lipid-soluble free radicals. The semidehydroascorbate anion radical ($Asc^{\cdot -}$) formed in the process is extremely unreactive, enhancing the antioxidant effectiveness of ascorbate. Plasma ascorbate reserves decrease gradually throughout normal pregnancy (113). A decrease in mean plasma ascorbate concentration in preeclampsia relative to normal pregnancy was noted in 1964 (114) and then in 1994 (115). Table III is from a recent study on concentrations of ascorbate, total thiols (glutathione + protein thiols), and vitamin E (α–tocopherol) in plasma (116). Ascorbate concentrations were 50% lower in preeclampsia relative to normal pregnancy plasma, but total thiols and vitamin E did not differ. These relationships were maintained in the subset of samples obtained at term and without magnesium sulfate administration.

In contrast to ascorbate, plasma vitamin E concentrations increase during normal gestation (102, 103). One likely explanation for the vitamin E increase is the marked gestational increase in circulating lipoproteins. Vitamin E is transported in plasma lipoproteins, thus elevated lipid concentrations generally result in elevated vitamin E (117). Plasma vitamin E concentrations are either unchanged (102, 116) or increased (118) in preeclampsia, even in severe cases. Another study found increased serum vitamin E in severe but not mild preeclampsia relative to normal pregnancy, but with no group differences after normalization to serum cholesterol (119). Increased vitamin E is likely due to the accentuated hyperlipoproteinemia of preeclampsia (40, 120). Lipid-adjustment better reflects the number of α-tocopherol molecules per lipoprotein particle and thus potential impact upon lipoprotein oxidative resistance. Table III

Table III. Plasma Antioxidant Reserves in Women with Preeclampsia and Normal Pregnancy

	Ascorbate nmol/ml	Total thiols nmol/ml	Vitamin E nmol/ml	Vitamin E nmol/μmol lipid[a]
Preeclampsia (n = 12)	11.0 (9.2 to 15.3)	646 (518 to 794)	25.7 (21.8 to 30.6)	2.8 (2.4 to 2.9)
Normal pregnancy (n = 13)	21.2 (16.8 to 26.4)	516 (476 to 598)	21.3 (16.1 to 22.8)	2.4 (2.0 to 3.0)
Significance	$P < 0.002$	NS ($P = 0.05$)	NS ($P = 0.06$)	NS ($P = 0.53$)

Note. Data are medians and interquartile range
NS: not significant
[a] lipid corrected: vitamin E/(cholesterol + triglycerides) in nmol/μmol

indicates no differences in lipid-corrected vitamin E concentrations. These patients reported daily intake of prenatal vitamins containing vitamins E and C during pregnancy, a factor likely to diminish the influence of diet.

Further experiments tested whether blood-borne factors from women with preeclampsia accelerate the oxidation of ascorbate. Freshly obtained, EDTA-anticoagulated whole blood from women with normal and preeclamptic pregnancies (sampled prior to labor and $MgSO_4$) were incubated, and plasma aliquots were harvested at successive time intervals. The time-dependent changes in endogenous ascorbate and total thiol concentration were then measured by electron paramagnetic resonance (EPR) spectroscopy. The median time required for half-consumption of ascorbate in preeclampsia blood was significantly less (median 95 min vs. 360 min) (116). No time-dependent decrease in thiols was evident.

During its antioxidant action, ascorbate undergoes two consecutive one electron oxidations to dehydroascorbic acid with intermediate formation of the ascorbate radical. Ascorbate radical is detectable by electron paramagnetic resonance (EPR) spectroscopy whereas ascorbate and dehydroascorbate are EPR-silent. The initial signal amplitude of ascorbate radical is directly proportional to the overall rate of ascorbate oxidation, whereas the signal duration is inversely proportional. Ascorbate radical thus serves as a gauge of ongoing oxidative stress in plasma (121). EPR spectroscopy was used to measure temporal changes in ascorbate radical signal amplitude in plasma after initial equalization of ascorbate concentrations by addition of exogenous ascorbate. Figure 1 illustrates that the ascorbate radical signal amplitude was initially greater in preeclampsia plasma and then, in contrast to normal pregnancy plasma, decreased progressively during the recording interval. An ascorbate-oxidizing activity is thus increased in blood from women with preeclampsia, with at least a portion present in the plasma (independent of blood cells). Iron chelators had no effect on the ascorbate radical signal suggesting that free iron is not the catalyst for ascorbate oxidation (116). However, the copper (Cu^{2+}) chelator cuprizone extends the ascorbate radical lifetime in a majority of preeclampsia (but not normal pregnancy) plasma samples, eliminating differences between groups. This may reflect decreased ability of Cu-binding proteins to sequester Cu in redox-inactive form (122).

Dyslipidemia and Oxidative Stress in Preeclampsia

Lipid alterations may promote oxidative stress in preeclampsia (65, 123, 124). In particular, the insulin resistance syndrome ("syndrome X"; a cluster of abnormalities including dyslipidemia, obesity, and resistance to insulin-stimulated glucose uptake) may have an important role in the pathogenesis of preeclampsia (125), as it does in nonpregnancy cardiovascular disease (126).

Lipid Changes in Normal and Preeclamptic Pregnancies. During the first half of normal pregnancy, increased maternal adipose fat accumulation sets the stage for the subsequent physiologic hyperlipidemia of later gestation (127). Plasma concentrations of very low density lipoprotein (VLDL) and LDL increase progressively with gestational age as reflected by increases in serum triglycerides and cholesterol (128, 129). Reversal of pregnancy lipid changes is essentially complete by 6 weeks postpartum (129). Gestational increases in estrogen are thought to promote hepatic production of VLDL triglyceride (130). The release of free fatty acids from adipocytes into the circula-

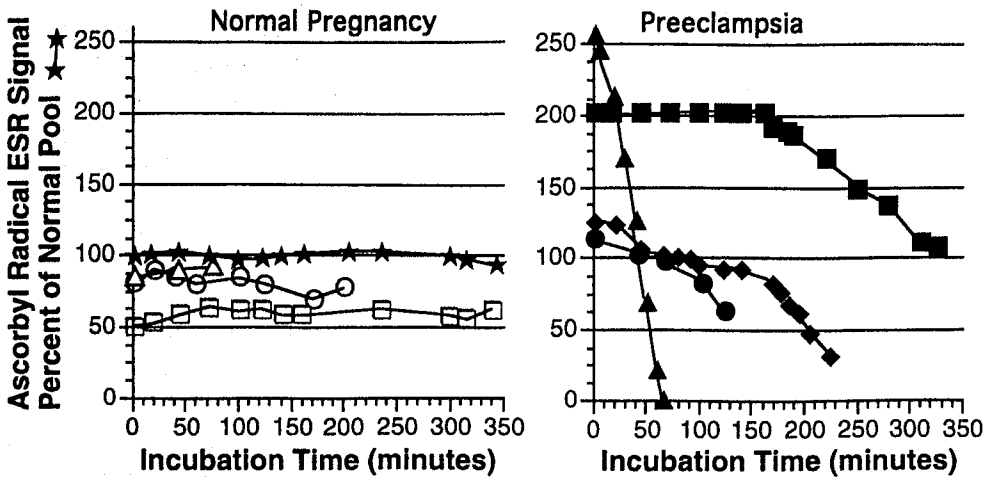

Figure 1. Temporal changes in ascorbate radical signal amplitude in preeclampsia and normal pregnancy plasma after normalization of ascorbate concentrations by addition of exogenous ascorbate. Ascorbate concentrations in plasma were normalized (1 m*M*) and then ascorbate radical spectra continuously recorded. No exogenous oxidation catalysts were added. Signal amplitude is proportional to the steady-state concentration of ascorbate radical. Data are expressed as a percentage of the initial signal intensity measured in a plasma pool composed of plasma from six women with normal pregnancies. (★★) pregnancy pool (n = 6); (○○ △△ □□) individual normal pregnancy plasma samples. (◆◆) preeclampsia pool (n = 6); (▲▲ ■■ ●●) individual preeclampsia samples. The initial ascorbate radical signal amplitude is higher in preeclampsia plasma and then, in contrast to normal plasma, decreases progressively indicating increased ascorbate oxidation.

tion increases due to the insulin resistance of late gestation (130). Activities of adipose tissue lipoprotein lipase and hepatic lipase are substantially decreased during normal pregnancy (due to insulin resistance and estrogen, respectively). The net result is impaired removal of triglyceride-rich lipoproteins from the circulation (130). Circulating triglycerides cannot cross the placental barrier (127, 131). However, lipoprotein lipase in the human placenta (not suppressed during pregnancy) may ensure release of free fatty acids for transfer to the fetus (132).

Mean plasma triglyceride and free fatty acid concentrations undergo near doubling in women with preeclampsia relative to normal pregnancy (105, 133). Roughly one-third of women with preeclampsia develop plasma triglyceride values above 400 mg/dl (133), greater than the 90th percentile of randomly selected women at 36 weeks gestation (128). The dyslipidemia begins months before, and thus is not a consequence of, clinically evident preeclampsia (105, 134, 135). This is suggestive of a pathogenic role for dyslipidemia. Women with chronic hypertension during pregnancy do not usually display dyslipidemia (135). Fasting serum triglycerides correlate with serum malondialdehyde, a lipid peroxidation product, in women with preeclampsia (133). The hypertriglyceridemia of preeclampsia is also accompanied by increased prevalence of smaller, denser LDL particles (40, 136) and decreased HDL cholesterol (136, 137). The mechanisms underlying the dyslipidemia of preeclampsia are poorly understood. Heightened gestational insulin resistance (124, 125) may accentuate the suppression of lipoprotein lipase activity and increase the mobilization of free fatty acids from visceral adipocytes.

Hypertriglyceridemia and Small Dense LDL. There is strong support for a causal relationship between cholesterol and coronary artery disease. Nevertheless, up to half of patients with coronary artery disease may have cholesterol levels in the normal range, suggesting involvement of other factors (138). Hypertriglyceridemia is emerging as a major risk factor (138–140). Elevated triglycerides may compromise vascular function in several ways. For example, triglyceride-rich lipoproteins have prothrombotic activity (140).

Hypertriglyceridemia also shifts the spectrum of LDL subclasses toward proportional increases in smaller, denser, more atherogenic LDL particles (139). Prospective studies have shown that small LDL size is a risk factor for coronary heart disease (138, 141). A direct pathophysiologic role is suggested by several findings. Relative to the large buoyant variety, small dense LDL particles more readily infiltrate into arterial tissue (the presumed site of LDL oxidation), and exhibit enhanced adhesiveness to artery intimal proteoglycans (142). In addition, smaller denser LDL particles are intrinsically more susceptible to oxidation (143). Compared with more buoyant particles, small dense LDLs show greater capacity to provoke changes in vascular cells in culture consistent with vasoconstrictive effects *in vivo* (144, 145).

The normal pregnancy rise in plasma total triglyceride is associated with a progressive shift from predominantly large and buoyant LDL to intermediate and small dense LDL, with reversal by 6 weeks postpartum (146). The size of the predominant LDL subclass is significantly decreased in preeclampsia relative to normal pregnancy, correlating inversely with plasma triglycerides (40, 136). LDL size differences between normal pregnancy and preeclampsia are less striking than those between normal pregnancy and nonpregnancy (40, 146). The physiologic significance of small-sized LDL in pregnancy remains unknown.

Genetic Susceptibility. Susceptibility to preeclampsia is highly heritable. Population studies have shown a strong familial susceptibility to preeclampsia (147–149). At present there is no consensus as to the best genetic model to explain this increased risk. The dominant hypothesis is that preeclampsia involves multiple susceptibility genes and environmental influences, with endothelial dysfunction as a common end point (13, 150, 151).

In addition to possible hormonal suppression of lipoprotein lipase, Caucasian women with preeclampsia have a substantially increased prevalence of functional mutations in the lipoprotein lipase gene relative to normal pregnancy and population controls (152). Heterozygous lipoprotein lipase deficiency is thought to play an important role in the pathogenesis of coronary artery disease (153). By decreasing lipoprotein lipase activity, these mutations promote the dyslipidemic triad of increased triglyceride, decreased HDL cholesterol, and predominance of small dense LDL (154). Heterozygous lipoprotein lipase deficiency alone may be insufficient in general to cause overt dyslipidemia. However, the dyslipidemic phenotype is promoted by interaction of these mutations with factors such as pregnancy, obesity, or diabetes, which challenge the lipolytic system by increasing hepatic secretion of VLDL (153). Since triglyceride-rich lipoproteins are prothrombotic (140), functional mutations in the lipoprotein lipase gene may fit with the cluster of thrombophilic mutations (155) associated with preeclampsia. However, there are presently no reports on postheparin lipoprotein lipase enzyme activity or mass in women before, during, or after preeclampsia. Since women with a history of preeclampsia-eclampsia are at increased risk for cardiovascular disease in later life (156) and manifest a more adverse lipoprotein profile in later life (157), it will also be of interest to examine the association of lipase mutations with remote prognosis.

Maternal and Placental Interactions

In trophoblasts and macrophages of the normal placenta, scavenger receptor activity (uptake of oxidized LDL) greatly exceeds "native" LDL receptor activity (158). LDL might be prone to oxidation during its relatively slow traversal through the intervillous space in direct contact with trophoblast cells (158). The aldehydic lipid peroxidation product, 4-hydroxynonenal, has been found in trophoblast cells of the normal human placenta, and it might be derived

from lipoprotein oxidation (159). The progressively smaller denser LDL formed during pregnancy should be increasingly susceptible to oxidation, and oxidation might occur during transit through the placenta.

If stable peroxidation metabolites are produced during placental oxidative stress and enter the maternal circulation, these could contribute to widespread endothelial dysfunction. Malondialdehyde and 4-hydroxynonenal, for example, are second toxic messengers of lipid peroxidation; exposure of cells in culture to pathophysiologic concentrations of these agents has toxic effects (160). Syncytiotrophoblast membranes from preeclamptics have decreased fluidity, suggesting lipid peroxidation that may predispose to increased syncytiotrophoblast membrane shedding (deportation) into the maternal circulation (161). Syncytiotrophoblast membrane products are increased in the maternal circulation in preeclampsia and may have a profound adverse effect on the vascular endothelium (11, 162, 163). Murai *et al.* (164) have presented data consistent with the idea that a factor associated with syncytiotrophoblast microvillous membranes deported into the maternal circulation, perhaps human placental lactogen, increases maternal fat cell lipolysis with resultant rises in circulating free fatty acid concentrations and, subsequently, endothelial dysfunction in preeclampsia.

Tumor Necrosis Factor (TNFα) Hypoxia promotes excess production of placental tumor necrosis factor (TNFα) (165). Release of this cytokine into the maternal circulation by the hypoxic placenta might promote endothelial dysfunction in preeclampsia (165). Amplification of injurious effects of placental TNFα by increased maternal free fatty acids is then possible. Free fatty acids are highly inflammatory. Unsaturated fatty acids and TNFα cooperatively amplify endothelial oxidative stress and dysfunction *in vitro* (166). Further placental vasospasm and hypoxic production of TNFα could result from TNFα stimulation of mitochondrial and neutrophil ROS production locally (167). In humans and animal models, increased TNFα production by adipose tissue occurs with obesity, insulin resistance, and hypertriglyceridemia (168, 169). This cytokine decreases lipoprotein lipase activity, increases adipose tissue lipolysis, and may be a mediator of insulin resistance (168, 169). Hypothetically, increased production of TNFα from the placenta and/or maternal adipose tissue could contribute to insulin resistance, dyslipidemia, and oxidative stress in preeclampsia.

Inflammatory Responses in Preeclampsia. As reviewed, placental lipid peroxidation products, TNFα, and syncytiotrophoblast membrane fragments are candidate blood-borne agents with potential to cause endothelial cell dysfunction. Redman *et al.* (170) have proposed that endothelial cell dysfunction is part of a more widespread intravascular inflammatory response causing the clinical syndrome preeclampsia. This would involve leukocytes and the clotting and complement systems. Using flow cytometry of whole blood to preclude artifactual leukocyte activation due to their isolation, they have shown that normal pregnancy itself stimulates a robust leukocyte inflammatory response (171). Intracellular reactive oxygen species were significantly increased in monocytes, granulocytes, and lymphocytes in normal pregnancy as compared with nonpregnancy with a further increase evident in preeclampsia. Surface markers were consistent with marked activation of leukocytes in the peripheral circulation during normal pregnancy and further increases in some but not all surface markers in preeclampsia. Differences between normal pregnancy and nonpregnancy were generally more striking than those between normal pregnancy and preeclampsia (171). Thus, inappropriate maternal response to the proinflammatory stimulus of pregnancy might promote preeclampsia. Other pronounced changes in normal pregnancy as compared with nonpregnancy fit with this concept, including increases in circulating triglycerides, free fatty acids, small dense LDL, and lipid peroxidation products. Placental hypoxia, resulting from poor placental perfusion, may predispose to preeclampsia by amplifying the release of inflammatory stimuli into the maternal circulation (170).

Activation of maternal neutrophils during their transit through the placenta could provide a pathway for transfer of oxidative disturbances into the maternal circulation in preeclampsia. Elastase-positive neutrophils (a marker of neutrophil activation) are found in increased numbers in the decidua of the placental bed in women with preeclampsia compared with normal pregnancies. This is seen at the same placental site as the acute atherosis mentioned previously (172). Neutrophil elastase concentrations are increased in the peripheral circulation of women with preeclampsia (173) as well as intrauterine growth restriction (174). A significant correlation exists between plasma neutrophil elastase and von Willebrand factor, a marker of endothelial dysfunction (175). Postischemic reoxygenated cells release factors that induce neutrophils to discharge oxidants ($O_2^{\cdot -}$, H2O2, HOCl, chlorine gas) (87, 176, 177). Such stimulatory factors include components of the complement cascade, adhesion molecules, TNFα, and also certain oxidized and nonoxidized fatty acids (87, 178–183). Nonphagocytic vascular cells, including smooth muscle and endothelial, possess a potent superoxide- and hydrogen peroxide-producing NADH/NADPH oxidase that is related to the neutrophil NADPH oxidase (184). Angiotensin II causes long-term activation of this oxidase *via* the AT_1 receptor (185). Women with preeclampsia have drastically elevated circulating autoantibodies against the AT_1 receptor (186). These autoantibodies bind to the AT_1 receptor and have agonist activity (186). Whether these autoantibodies contribute to vascular cell oxidase activation is currently not known.

Concluding Remarks

Oxidative stress may be the point at which multiple factors converge resulting in endothelial cell dysfunction and the consequent clinical manifestations of preeclampsia.

Abnormal placentation with reduced uteroplacental perfusion might lead primarily to intrauterine growth restriction. Maternal dyslipidemia and/or a primary or secondary decrease of antioxidants might make preeclampsia increasingly likely. Differences in the prevalence of placental versus maternal oxidative stressors in different subsets of women could contribute to the heterogeneity of preeclampsia. Interaction of maternal components, particularly neutrophils and oxidation-susceptible lipids, with placental cells and placental-derived factors may engender feed-forward cycles of oxidative stress and endothelial cell dysfunction. Lesser individual tolerance to a given oxidant/inflammatory burden during pregnancy may be important in development of the disorder. It is hoped that this review will stimulate further investigation.

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