

Symposium on 'Micronutrients through the life cycle'

Could antioxidant supplementation prevent pre-eclampsia?

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Pre-eclampsia is a disorder characterised by pregnancy-induced hypertension and new-onset proteinuria occurring in the second half of pregnancy. Worldwide, approximately 2–3% of all pregnant women develop pre-eclampsia. The condition is a major cause of maternal and fetal morbidity and mortality. Abnormal placentation is an important predisposing factor for pre-eclampsia, while endothelial activation appears to be central to the pathophysiological changes, possibly indicative of a two-stage disorder characterised by reduced placental perfusion and a maternal syndrome. There is increasing evidence that pre-eclampsia is associated with both increased oxidative stress and reduced antioxidant defences, which has led to the hypothesis that oxidative stress may play an important role in the pathogenesis of pre-eclampsia, perhaps acting as the link in a two-stage model of pre-eclampsia. In support of this hypothesis a small, but important, preliminary study has shown a highly significant ($P = 0.02$) reduction in the incidence of pre-eclampsia in women at risk who were taking a supplement of vitamins C and E from mid-pregnancy. Furthermore, these findings support the hypothesis that oxidative stress is at least partly responsible for the endothelial dysfunction of pre-eclampsia. Several larger multicentre trials are currently underway to evaluate the efficacy, safety and cost benefits of antioxidant supplementation during pregnancy for the prevention of pre-eclampsia in both low- and high-risk women, including women with diabetes. The results of these trials are awaited with interest.

Antioxidants: Pre-eclampsia: Oxidative Stress: Pregnancy: Diabetes

Chappell *et al.* (1999) have reported in a small, but important, study a highly-significant reduction in the incidence of pre-eclampsia in women at risk who were taking a vitamin C and E supplement (adjusted odds ratio 0.39 (95% CI 0.17, 0.90), $P = 0.02$). Antioxidant supplementation in these women has also been shown to be associated with changes in indices of oxidative stress and placental function (Chappell *et al.* 2002*b*). Primarily, the results of this trial indicate that antioxidants may be beneficial in the prevention of pre-eclampsia and support the emerging concept that oxidative stress plays a role in the pathophysiology of pre-eclampsia. Several multicentre trials are currently underway to confirm these results in larger groups of both low-risk and high-risk women. The present review gives an overview of pre-eclampsia, discusses the role of oxidative stress in the pathophysiology of this disorder and illustrates why antioxidants may play a role in pre-eclampsia prophylaxis.

Pre-eclampsia

Pre-eclampsia is a disorder of pregnancy characterised by pregnancy-induced hypertension (≥ 140 mmHg systolic and/or ≥ 90 mmHg diastolic blood pressure) and new-onset proteinuria (≥ 300 mg protein/d) occurring in the second half of pregnancy (Brown *et al.* 2001). Pre-eclampsia has several predisposing risk factors including: primiparity; age < 20 years or > 40 years; high BMI; personal and family history of pre-eclampsia; multiple pregnancy; pre-existing medical conditions such as chronic hypertension, renal disease, autoimmune disease, anti-phospholipid syndrome and diabetes mellitus (Duckitt & Harrington, 2005). Complications of pre-eclampsia include haemolysis, elevated liver enzymes and low platelets (termed HELLP syndrome), and eclampsia, where eclampsia is characterised by one or more convulsions superimposed on pre-eclampsia. Worldwide, approximately

Abbreviations: MDA, malondialdehyde; SOD, superoxide dismutase.

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3% of all pregnant women develop pre-eclampsia, of whom 1.9% will develop eclampsia. Although its greatest impact is in the developing world, where >90% of the most serious pre-eclampsia-related maternal and fetal morbidity and mortality occurs (Villar *et al.* 2003), hypertensive disease of pregnancy is the second highest cause of maternal death in the UK (Lewis & Confidential Enquiry into Maternal and Child Health, 2004). Furthermore, as delivery is the only cure, pre-eclampsia is responsible for $\leq 15\%$ of preterm births and consequently increases infant mortality and morbidity (Meis *et al.* 1998).

Pathogenesis of pre-eclampsia

The exact cause of pre-eclampsia, often referred to as a 'disease of theories', remains unknown. However, the placenta plays a major role in the pathophysiology of pre-eclampsia, and it has, therefore, long been referred to as a placental condition (Redman & Sargent, 2003*a*). In normal pregnancy major alterations occur in the spiral arteries to allow increased blood supply to the intervillous space in order to meet the needs of the foeto-placental unit during the later stages of pregnancy. Pre-eclampsia is characterised by failure of spiral artery remodelling (Brosens *et al.* 1972), a phenomenon associated with incomplete endovascular trophoblast invasion in early pregnancy (Pijnenborg *et al.* 1991, 1996) that results in a dramatic reduction in blood flow into the intervillous space.

Although the placenta is necessary for pre-eclampsia, poor placentation is not the cause of pre-eclampsia, but rather an important predisposing factor (Redman & Sargent, 2000; Sibai *et al.* 2005). Other pregnancies, such as those complicated by intrauterine growth restriction and a subgroup of preterm deliveries, are also associated with abnormal placentation but do not develop pre-eclampsia (Khong *et al.* 1986; Arias *et al.* 1993). This paradox has led to the hypothesis that pre-eclampsia is a two-stage disorder, with reduced placental perfusion representing stage one (Redman 1991), while stage two refers to the multisystemic disorder or maternal syndrome produced in response to reduced placental perfusion (Ness & Roberts, 1996) that is influenced by genetic or environmental maternal constitutional factors (Roberts & Hubel, 1999). Endothelial activation appears to be central to the pathophysiological changes associated with pre-eclampsia (Roberts, 1998; Wareing & Baker, 2003), with circulating markers of endothelial activation increased in pre-eclampsia and in those women destined to develop pre-eclampsia (Taylor *et al.* 1998).

The question remains as to the nature of the link between poor placentation and endothelial activation, for which a number of theories have been put forward (Hubel, 1999; Roberts & Cooper, 2001; Page, 2002; Redman & Sargent, 2003*a*; Levine & Karumanchi, 2005). It has been proposed that an unknown factor excreted from the placenta is central to the pathogenesis of pre-eclampsia, with candidates for this unknown factor including placental debris, apoptotic fragments, lipid peroxidation products or other reactive oxygen species, all of which are able to induce maternal oxidative stress directly or indirectly (Raijmakers *et al.* 2005). It is almost certain, however,

that pre-eclampsia is multi-factorial in origin, with the incidence varying according to genetically-determined maternal constitutional and environmental factors including diabetes, hypertension, increased insulin resistance and raised homocysteine concentrations (Roberts & Cooper, 2001). Many of the predisposing factors for pre-eclampsia listed earlier are also known risk factors for atherosclerosis. Indeed, pre-eclampsia is associated with a highly-atherogenic lipid pattern, with increased plasma triacylglycerol concentrations and decreased HDL-cholesterol concentrations evident before clinical manifestations of the disease (Potter & Nestel, 1979; Lorentzen *et al.* 1995; Hubel *et al.* 1996; Sattar 2003). There is substantial evidence for oxidative stress in atherosclerosis, with oxidative modification of LDL now considered to play a central role in disease pathogenesis (Witztum & Steinberg, 1991); emerging evidence also suggests that lipid changes in pre-eclampsia are associated with increased oxidative stress and endothelial activation (Hubel *et al.* 1996, 1998; Hayman *et al.* 1999; Wetzka *et al.* 1999; Sattar 2003). In addition, normal healthy pregnancy is associated with a systemic inflammatory response, and it is hypothesised that pre-eclampsia represents a continuum of this response, albeit at the extreme end of the spectrum (Redman & Sargent, 2003*b*). Such an inflammatory response can cause or be caused by endothelial dysfunction and oxidative stress. Thus, there is increasing evidence that oxidative stress plays an important role in the pathogenesis of pre-eclampsia, perhaps acting as the link in the two-stage model of pre-eclampsia (Roberts & Hubel, 1999).

Oxidative stress and antioxidant defences

Oxidative stress

Free radical production occurs continuously in all cells as part of normal cellular function. However, excess free radical production originating from endogenous or exogenous sources might play a role in many diseases (Young & Woodside, 2001). Oxidative stress is defined as an imbalance between oxidants and antioxidants in favour of the oxidants, which potentially leads to damage (Sies, 1997). For a disturbance in this balance to occur it follows that there must be either an increase in oxidants or a reduction in antioxidants. Oxidants or reactive oxygen species include free radicals, such as HO \cdot , O $_2^{\cdot-}$ and NO \cdot and also include the reactive molecules H $_2$ O $_2$, the peroxynitrite anion (ONOO $^-$) and HOCl. Free radicals are defined as any molecular species capable of independent existence that contains an unpaired electron (Halliwell & Gutteridge, 1999), and their production occurs continuously in all cells as part of normal cellular function. The most important free radicals in many disease states are oxygen derivatives, particularly O $_2^{\cdot-}$ and HO \cdot . Excess free radical production originating from endogenous or exogenous sources is thought to play a role in many diseases, including atherosclerosis and pre-eclampsia (Hubel, 1999; Young & Woodside, 2001). In particular, lipoprotein particles and membranes characteristically undergo the process of lipid peroxidation giving rise to lipid hydroperoxides. Although lipid hydroperoxides regulate enzymes

and redox-sensitive genes in normal physiology (Smith *et al.* 1991; Sen & Packer, 1996), uncontrolled lipid peroxidation can result in cellular dysfunction and damage, and as such oxidative stress is associated with damage to a wide range of molecular species, including lipids, proteins and nucleic acids (Djordjevic, 2004).

Antioxidant defences

A complex web of antioxidant defence systems play a key role in protecting against oxidative damage (Young & Woodside, 2001), and it is thought that these processes are disordered in many conditions, implicating oxidative stress as a cause of tissue damage. Antioxidant defence systems include the chain-breaking antioxidants, such as vitamin C and vitamin E, and the antioxidant enzymes, such as catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase (SOD).

Chain-breaking antioxidants are small molecules that can receive an electron from a radical or donate an electron to a radical with the formation of stable by-products that in turn will not readily accept an electron from or donate an electron to another molecule, preventing the further propagation of the chain reaction (Halliwell, 1995). Chain-breaking antioxidants include lipid-phase and aqueous-phase chain-breaking antioxidants.

Lipid-phase chain-breaking antioxidants, the most important of which is probably vitamin E (Esterbauer *et al.* 1991), scavenge radicals in membranes and lipoprotein particles and are central to the prevention of lipid peroxidation. In lipoproteins and cell membranes vitamin E traps peroxy radicals, breaking the chain reaction of lipid peroxidation by minimising the formation of secondary radicals (Burton & Ingold, 1986). Vitamin E exists in eight forms, α -, β -, γ - and δ -tocopherols and α -, β -, γ - and δ -tocotrienols, each of which are lipid soluble and have antioxidant properties, and of these forms α -tocopherol is the most abundant in man and the most potent antioxidant.

Aqueous-phase chain-breaking antioxidants directly scavenge radicals present in the aqueous compartment. Vitamin C or ascorbate is the most important aqueous-phase chain-breaking antioxidant (Levine *et al.* 1999), but is also an essential cofactor for several enzymes catalysing hydroxylation reactions, such as those in the synthesis of collagen. In its role as an antioxidant ascorbate scavenges HO^\bullet , $\text{O}_2^{\bullet-}$, aqueous peroxy radicals, H_2O_2 , HOCl and singlet oxygen and undergoes a two electron reduction, initially to the relatively stable semidehydroascorbyl radical and subsequently to dehydroascorbate, which is relatively unstable and hydrolyses readily to diketogulonic acid, which is subsequently broken down to oxalic acid.

It is now well established that there is synergy between vitamins C and E. *In vitro*, ascorbate has been shown to reduce the α -tocopherol radical, a relatively stable radical that is formed during the chain-breaking action of α -tocopherol, and as such plays a role in the regeneration of tocopherol (Stoyanovsky *et al.* 1995; May *et al.* 1998). This interaction between vitamin C and vitamin E has been confirmed *in vivo* by Hamilton *et al.* (2000), who

have reported that supplementation of healthy adults with ascorbic acid increases ascorbic acid and lipid-standardised α -tocopherol levels in plasma, and that supplementation with α -tocopherol is associated with increased plasma ascorbic acid concentration, as well as improved vitamin E status.

Oxidative stress and pre-eclampsia

Early research in experimental models has demonstrated that acute exposure to lipid peroxides can damage endothelial cells (Cutler & Schneider, 1974). Indeed, much of the dysfunction evident in pre-eclampsia can be mimicked by lipid peroxidation in experimental models, as outlined by Hubel (1999).

Normal healthy pregnancy is associated with a transient increase in reactive oxygen species production, an increase that is counterbalanced by an increase in antioxidant capacity (Raijmakers *et al.* 2005). It is proposed that in normal pregnancy the embryo develops in a low O_2 environment until completion of embryogenesis to protect differentiation cells from oxidative stress. Thereafter, the maternal intervillous circulation is established following a burst of oxidative stress (Burton & Jauniaux, 2004). While this physiological event plays a role in stimulating normal placental differentiation, it may also serve as a factor in the pathogenesis of pre-eclampsia (Jauniaux *et al.* 2000), when an imbalance in oxidative stress and antioxidant capacity leads to impaired trophoblast invasion, impaired spiral artery remodelling and an ischaemia-reperfusion-type phenomenon leading to chronic oxidative stress in the placental unit (Burton & Jauniaux, 2004; Raijmakers *et al.* 2005).

The placenta and oxidative stress

There is substantial evidence of oxidative stress in the pre-eclamptic placenta (for review, see Hubel, 1999). In brief, many studies have shown increased placental levels of reactive oxygen species such as $\text{O}_2^{\bullet-}$ (Sikkema *et al.* 2001; Wang & Walsh, 2001) and, in general, lower placental antioxidant capacity (Poranen *et al.* 1996; Wang & Walsh, 1996; Zusterzeel *et al.* 1999; Sahlin *et al.* 2000). Furthermore, higher placental levels of lipid peroxidation (Gratacos *et al.* 1998; Madazli *et al.* 2002), oxidative protein damage (Zusterzeel *et al.* 2001) and isoprostanes (Staff *et al.* 1999; Walsh *et al.* 2000), as well as evidence of peroxynitrite formation (Myatt *et al.* 1996), provide further evidence of placental oxidative stress in pre-eclampsia (Raijmakers *et al.* 2005). In a study of pre-eclamptic placental tissue homogenates Vanderlelie *et al.* (2005) have demonstrated increased levels of lipid peroxidation and increased protein carbonyl concentrations, together with reduced levels and activities of antioxidant enzymes, including SOD and glutathione peroxidase, suggesting that the placenta is likely to be central to oxidative stress in pre-eclampsia, given the decreased enzymic antioxidant capacity and increased oxidation in placental tissue.

The maternal circulation and oxidative stress

There is also substantial evidence of oxidative stress in the maternal circulation, with studies reporting reduced levels of antioxidants, reduced antioxidant enzymes and increases in products of oxidation. Oxidative stress in the maternal circulation may be a result of placental oxidative stress, either directly or indirectly. The atherogenic lipid profile of women with pre-eclampsia may also predispose to oxidative stress (Raijmakers *et al.* 2004).

Over 40 years have passed since the first report of a decrease in plasma concentrations of maternal ascorbate in pre-eclampsia (Clemetson & Andersen, 1964; Hubel, 1999). After a further 30 years, Mikhail *et al.* (1994) reported that plasma levels of reduced ascorbic acid are markedly decreased in patients with mild and severe pre-eclampsia. The association between decreased ascorbate and pre-eclampsia has been confirmed by several other studies (Hubel *et al.* 1997; Sagol *et al.* 1999; Panburana *et al.* 2000; Chappell *et al.* 2002a; Llurba *et al.* 2004).

During normal pregnancy vitamin E concentrations rise, a phenomenon probably related to increased lipoproteins during pregnancy since vitamin E is transported in circulating lipoproteins (Wang *et al.* 1991; Traber, 1994; Morris *et al.* 1998; Hubel, 1999). Studies have reported increased (Zhang *et al.* 2001; Llurba *et al.* 2004), unchanged (Hubel *et al.* 1997; Morris *et al.* 1998; Williams *et al.* 2003) and decreased (Mikhail *et al.* 1994; Sagol *et al.* 1999; Panburana *et al.* 2000) levels of α -tocopherol in pre-eclampsia, with the decreased levels associated only with severe pre-eclampsia. Concentrations of triacylglycerol-rich lipoproteins are increased in pre-eclampsia compared with healthy pregnant controls (Sattar *et al.* 1997; Cekmen *et al.* 2003), which, together with the association of vitamin E with lipoproteins and the importance of reporting corrected measures of α -tocopherol, may explain the inconsistency in the literature in relation to vitamin E levels and pre-eclampsia. Studies in which vitamin E levels are corrected for lipoproteins have shown both increased levels (Llurba *et al.* 2004) and no difference (Hubel *et al.* 1997) in pre-eclampsia.

A number of studies have assessed other antioxidants in pre-eclampsia, with variable findings. Decreased levels of β -carotene (Mikhail *et al.* 1994; Palan *et al.* 2001), lycopene (Palan *et al.* 2001) and retinol (Zhang *et al.* 2001) have been reported in women with pre-eclampsia, while another study has reported increased retinol levels (Williams *et al.* 2003). Williams *et al.* (2003), who sampled women in the early postpartum period, have also noted decreases in pre-eclampsia risk with increasing concentrations of α -carotene, β -carotene, β -cryptoxanthin, lutein and zeaxanthin, although such a relationship was not observed by Zhang *et al.* (2001). Differences in study design, differences in population characteristics (such as maternal age, race or ethnicity), overall dietary intake habits, use of prenatal multivitamins and other nutritional supplements, and limited statistical power are likely to have contributed to the variability in results across studies.

Studies investigating the changes in enzymic antioxidants during pre-eclampsia have yielded varying results. Decreased levels of erythrocyte SOD activity (Kumar &

Das, 2002; Atamer *et al.* 2005; Ilhan *et al.* 2002), plasma SOD activity (Mutlu-Turkoglu *et al.* 1998; Aydin *et al.* 2004; Yildirim *et al.* 2004) and vascular SOD (Roggensack *et al.* 1999) have been reported, while other studies have reported an increased (Llurba *et al.* 2004) or unchanged (Diedrich *et al.* 2001) erythrocyte SOD activity in patients with pre-eclampsia. Similar levels of erythrocyte catalase activity have been reported in women with pre-eclampsia as compared with women with a normal healthy pregnancy (Loverro *et al.* 1996; Kumar & Das, 2002), although one study has shown increased activity (Atamer *et al.* 2005). Several studies have reported increased levels of erythrocyte glutathione peroxidase (Uotila *et al.* 1993; Diedrich *et al.* 2001; Kumar & Das, 2002; Orhan *et al.* 2003; Llurba *et al.* 2004), while other studies have reported no differences in plasma glutathione peroxidase levels between pregnant women with pre-eclampsia and pregnant women who are normotensive (Diedrich *et al.* 2001; Funai *et al.* 2002). As glutathione peroxidase is a protective enzyme it has been suggested that glutathione peroxidase expression is induced to prevent excessive lipid peroxidation resulting from low SOD and catalase activities (Raijmakers *et al.* 2005).

While these studies have clearly demonstrated varying results, one study by Loverro *et al.* (1996) has assessed the pro-oxidant:antioxidant status and has demonstrated an increased pro-oxidant:antioxidant status in pregnancy complicated by pre-eclampsia when compared with normal pregnant women. Furthermore, a recent study by Scholl *et al.* (2005) has reported that high total antioxidant capacity in early pregnancy is associated with a 3-fold reduction in risk of pre-eclampsia, supporting the hypothesis that low antioxidant status precedes the recognition of pre-eclampsia. From the evidence to date there appears to be an overall shift towards oxidative stress in pre-eclampsia in relation to antioxidants and enzymic antioxidants.

Many studies have also investigated markers of oxidative stress, such as oxidation products of lipoproteins and proteins, in pregnancies complicated by pre-eclampsia. Malondialdehyde (MDA) is a major metabolite of lipid peroxide breakdown and is measured by the assay of thiobarbituric acid-reacting substances. There are numerous reports in the literature of increased levels of MDA or thiobarbituric acid-reacting substances in pre-eclampsia (Uotila *et al.* 1993; Loverro *et al.* 1996; Mutlu-Turkoglu *et al.* 1998; Ilhan *et al.* 2002; Aydin *et al.* 2004; Atamer *et al.* 2005). However, a small study by Morris *et al.* (1998), which controlled for *in vitro* auto-oxidation, and a recent study by Llurba *et al.* (2004), which measured erythrocyte MDA, have both reported no significant difference in MDA between patients with pre-eclampsia and controls who are normotensive. Furthermore, as MDA is also a by product of cyclooxygenase activity in platelets (Hamberg *et al.* 1975) it may be possible that such increases are associated with increases in platelet activity observed in hypertensive disorders of pregnancy (Nadar & Lip, 2004).

Other markers of lipid peroxidation have also been investigated in pre-eclampsia. Isoprostanes are isomers of enzymically-formed prostaglandins (Morrow *et al.* 1990)

that are formed *in situ* in cell membranes following free radical attack on the arachidonic acid backbone (Meagher & Fitzgerald, 2000), and thus are markers of oxidative stress. Several studies have measured isoprostanes in both plasma and urine with varying results. Higher plasma $F_{2\alpha}$ isoprostane concentrations have been reported in pregnant women with pre-eclampsia when compared with pregnant women who are normotensive (Barden *et al.* 1996, 2001; McKinney *et al.* 2000; Chappell *et al.* 2002a), while others have shown no change (Morris *et al.* 1998; Ishihara *et al.* 2004). Urinary $F_{2\alpha}$ isoprostane concentrations have also been measured, with studies reporting no change (Ishihara *et al.* 2004) or a reduction (Barden *et al.* 1996; McKinney *et al.* 2000) in pregnant women with pre-eclampsia as compared with controls who are normotensive. Interestingly, the studies reporting a reduction in urinary concentrations have also reported increased plasma concentrations of $F_{2\alpha}$ isoprostanes (Barden *et al.* 1996; McKinney *et al.* 2000), perhaps reflecting impaired renal clearance in pre-eclampsia (Barden *et al.* 1996). While these studies measured isoprostanes in patients with pre-eclampsia, several studies have measured isoprostanes before the onset of pre-eclampsia. Regan *et al.* (2001) in a nested case-control study have reported no difference in urinary isoprostanes before or at diagnosis of pre-eclampsia. Chappell *et al.* (2002b) have reported higher plasma isoprostanes in high-risk women when compared with low-risk women, with levels in high-risk women falling to those of low-risk women following antioxidant supplementation. Finally, a recent study by Scholl *et al.* (2005) has reported increased urinary isoprostane in early pregnancy in women who eventually develop pre-eclampsia, such that higher isoprostane excretion is associated with a 5-fold increase in the risk of pre-eclampsia.

The peroxidation of unsaturated fatty acids is accompanied by the formation of conjugated dienes and thus these compounds are markers of lipid peroxidation. Elevated levels of conjugated dienes have been reported in women with pre-eclampsia (Garzetti *et al.* 1993; Uotila *et al.* 1993).

As well as causing lipid peroxidation reactive oxygen species may also induce damage to proteins. Increases in protein carbonyls (oxidation products of proteins) have been reported in several studies of pre-eclampsia (Zusterzeel *et al.* 2000, 2002; Serdar *et al.* 2003). In contrast, a recent study by Llorba *et al.* (2004) has shown a marked decrease in plasma protein carbonyls in women with pre-eclampsia when compared with controls who are normotensive, and have reported no difference between groups in the analysis of products of advanced protein oxidation.

NO reacts with O_2^- to form the powerful oxidant ONOO⁻, which modifies tyrosine in proteins to create nitrotyrosine, and thus nitrotyrosine acts as a marker for peroxynitrite (Beckman & Koppenol, 1996). Roggensack *et al.* (1999) have demonstrated increased nitrotyrosine immunostaining in the maternal vasculature of women with pre-eclampsia, suggesting increased peroxynitrite formation; 73% of the vessels in women with pre-eclampsia compared with 3% of the vessels in women with a normal pregnancy. The authors conclude that this

increased nitrotyrosine immunostaining together with observations of decreased SOD and increased NO synthase may be indicative of oxidative stress leading to endothelial cell dysfunction in women with pre-eclampsia.

While evidence supporting the contribution of oxidative stress to endothelial dysfunction in pre-eclampsia remains inconsistent, the lack of comparative methods and the use of small and heterogeneous study groups are likely to explain the lack of definitive evidence. A recent study by Llorba *et al.* (2004) has assessed oxidative stress using a variety of measures and techniques and has concluded that mild oxidative stress is evident in blood from women with pre-eclampsia, oxidative processes seem to be counteracted by the physiological activation of antioxidant enzymes and the high plasma vitamin E levels may prevent further oxidative damage. While it could not be concluded that oxidant stress might pathogenically contribute to pre-eclampsia, Llorba *et al.* (2004) agree that other sources of oxidative stress such as the placenta, which were not assessed in their study, may underlie the existence of oxidative stress and the genesis of endothelial dysfunction.

Pre-eclampsia in pregnancies complicated with diabetes

As previously outlined, pre-eclampsia has several predisposing risk factors, including: primiparity; age <20 years or >40 years; high BMI; multiple pregnancy; chronic conditions such as diabetes mellitus. A wide range of risk factors is perhaps suggestive of a heterogeneous disorder and hence the aetiology may differ according to the predisposing risk factor or factors.

Diabetes mellitus and, more specifically, type 1 diabetes are associated with increased oxidative stress and antioxidant depletion (Dominguez *et al.* 1998; Martin-Gallan *et al.* 2003), which are at least partly related to the prevailing level of glycaemia (Giugliano *et al.* 1996). More specifically, glycated Hb levels have been shown to correlate with MDA levels in mothers with diabetes (Kamath *et al.* 1998; Peuchant *et al.* 2004). Furthermore, studies in pregnancy have shown greater oxidative stress in pregnancies complicated by diabetes when compared with pregnancies that are normal. Peuchant *et al.* (2004) have reported higher levels of plasma and erythrocyte-free MDA levels and lower levels of plasma vitamin E, erythrocyte vitamin A and glutathione peroxidase activity in women with diabetes when compared with controls. In addition, Toescu *et al.* (2004) have reported that corrected total antioxidant capacity is lower and lipid hydroperoxides are higher throughout pregnancies complicated by diabetes compared with pregnancies that are normal. In a recent study of patients with pregestational diabetes conducted by Wender-Ozegowska *et al.* (2004) MDA concentrations were found to be higher in patients with elevated glycaemia and patients with an unfavourable outcome. On the other hand, subjects with a favourable neonatal outcome were found to have a higher activity of antioxidant enzymes than those with an unfavourable outcome, throughout the whole course of pregnancy. The authors have concluded that oxidative stress is one of

Table 1. Ongoing randomised placebo-controlled trials of antioxidants to prevent pre-eclampsia*

Study	Countries	n	Inclusion criteria	Gestation	Intervention	Primary outcome	Progress	Principal investigators
DAPIT	UK	756	Type 1 diabetes and pregnant, single	8–22 weeks	1000 mg vitamin C and 400 mg vitamin E daily	Incidence of pre-eclampsia	In progress	D. McCance
VIP	UK and Holland	2400	Abnormal uterine artery doppler at 18–22 weeks; previous history of pre-eclampsia; HELLP, eclampsia; chronic hypertension; anti-phospholipid syndrome; systemic lupus erythematosus; diabetes; chronic renal disease; multiple pregnancy; BMI > 30 kg/m ² in first pregnancy	14–22 weeks	1000 mg vitamin C and 400 mg vitamin E daily	Incidence of pre-eclampsia	Finished recruiting May 2005	L. Poston; A. Shennan
WHO	India, Peru, Vietnam, South Africa	1700	Similar to VIP	14–22 weeks	1000 mg vitamin C and 400 mg vitamin E daily	Incidence of pre-eclampsia	In progress	J. Villar
CAPPS	USA	10 000	Nulliparous; single pregnancy	9–16 weeks	1000 mg vitamin C and 400 mg vitamin E daily	Gestational hypertension and one other pre-eclampsia-associated outcome	In progress	J. M. Roberts
INTAPP	Canada	12 500 (10 000 low risk; 2500 high risk)	Low risk: nulliparous, single pregnancy; High risk: diabetes; chronic hypertension; multiple pregnancy; history of pre-eclampsia	12–18 weeks	1000 mg vitamin C and 400 mg vitamin E daily	Gestational hypertension with or without proteinuria and its adverse conditions	In progress	W. Fraser
Antioxidant therapy to prevent pre-eclampsia	Brazil	734	Chronic hypertension or history of pre-eclampsia (excludes diabetics)	12–20 weeks	1000 mg vitamin C and 400 mg vitamin E daily	Incidence of pre-eclampsia	In progress	J. A. Spinnato II
ACTS	Australia	1870	Nulliparous; single pregnancy	14–22 weeks	1000 mg vitamin C and 400 mg vitamin E daily	Small-for-gestational age; incidence of pre-eclampsia; serious adverse outcome, infant	Finished recruiting January 2005	C. Crowther, G. Dekker, R. Haslam, J. Robinson

DAPIT, Diabetes and Pre-eclampsia Intervention Trial; VIP, Vitamins In Pre-eclampsia Study; CAPPS, Combined Antioxidant and Preeclampsia Prevention Study; INTAPP, International Trial of Antioxidants for the Prevention of Preeclampsia; ACTS, Australian Collaborative Trial of Supplements with vitamin C and vitamin E for the prevention of pre-eclampsia; HELLP, haemolysis, elevated liver enzymes and low platelet count. *Details are correct to the best of the authors' knowledge at the time of publication.

several important factors contributing to the unfavourable outcome of a pregnancy complicated by diabetes. Furthermore, placental homogenates of pregnant women with diabetes have increased levels of MDA and glutathione, while SOD activity is markedly diminished (Kinalski *et al.* 1999). Orhan *et al.* (2003) have reported increased maternal erythrocyte glutathione S-transferase activity and glutathione peroxidase activity and increased thiobarbituric acid-reacting substances in women with diabetes, while women with hypertension and pre-eclampsia show increases only in erythrocyte glutathione peroxidase activity and thiobarbituric acid-reacting substances. Taken together, such evidence of oxidative stress in pregnancies complicated by diabetes may explain why rates of pre-eclampsia are two to four times higher in women who have diabetes and increase with the complexity of diabetes (Garner *et al.* 1990; Hanson & Persson, 1998). These lines of evidence support the hypothesis that oxidative stress plays a key role in the pathophysiology of pre-eclampsia in mothers with diabetes.

Antioxidant trials in pre-eclampsia: past and present

To date three trials have investigated the potential use of antioxidants in the prevention or treatment of pre-eclampsia. A non-randomised trial by Stratta *et al.* (1994) has found no benefit of 100–300 mg vitamin E/d in fourteen women with pre-eclampsia. Similarly, in a preliminary trial by Gulmezoglu *et al.* (1997) no difference was found among fifty-six women randomised to 800 mg vitamin E, 1000 mg vitamin C and 200 mg allopurinol compared with placebo. Both these studies, however, have concluded that an earlier commencement of therapy before the onset of pre-eclampsia might have been preferable. By contrast, the results of a randomised placebo-controlled clinical trial of antioxidants in women at high risk of pre-eclampsia (Chappell *et al.* 1999) are of considerable importance. Among 283 women randomisation to vitamin C (1000 mg/d) plus vitamin E (400 mg/d) at 16–22 weeks gestation was found to reduce the rate of pre-eclampsia from 17% to 8% (adjusted odds ratio 0.39 (95% CI 0.17, 0.90)). Vitamin supplementation was also reported to be associated with a 21% decrease in plasminogen-activator inhibitor-1:plasminogen-activator inhibitor-2 during gestation (95% CI 4, 35; $P = 0.015$). In this study the high-risk women in the placebo arm who developed pre-eclampsia were found to have lower plasma vitamin C concentrations ($P < 0.002$) compared with normal pregnant controls and these concentrations returned to normal on supplementation (Chappell *et al.* 2002a). Plasma concentrations of the $F_{2\alpha}$ isoprostane were found to be raised in the high-risk placebo group but fell to concentrations comparable with those for the controls after supplementation with vitamins C and E (Chappell *et al.* 2002b).

In light of these findings the hypothesis that antioxidant supplementation may reduce pre-eclampsia in low- and high-risk women, including pregnancies in women with diabetes, is realistic. Currently, there are several large multicentre trials in progress to determine the efficacy of antioxidant therapy in the prevention of pre-eclampsia

in both high- and low-risk women, as outlined in Table 1. The result of these trials, which together will recruit approximately 30 000 patients, are awaited with interest and will determine whether antioxidants are effective in preventing pre-eclampsia in all populations, or whether such therapy will be population specific.

Concluding remarks

The debate on the exact role of oxidative stress in the pathophysiology of pre-eclampsia continues (Regan *et al.* 2001; Hubel *et al.* 2002; Poston & Mallet, 2002). Increasing evidence suggests that a disruption in the oxidative stress–antioxidant balance in pregnancy is likely to contribute to, and the placenta is likely to be central to, oxidative stress in pre-eclampsia (Vanderlelie *et al.* 2005). The preliminary study by Chappell *et al.* (1999), showing a highly-significant ($P = 0.02$) reduction in the incidence of pre-eclampsia in women at risk who take a vitamin C and vitamin E supplement from mid pregnancy, has provided the strongest evidence to date that oxidative stress is implicated in the pathogenesis of pre-eclampsia and that supplementation with antioxidants during pregnancy may prevent or postpone its occurrence.

Pre-eclampsia is likely to be a heterogeneous disease (Sibai, 1998; Dekker & Sibai, 2001; Vatten & Skjaerven, 2004), and thus it is possible that the pathogenesis of pre-eclampsia may differ in women with different risk factors. The pathogenesis in women with pre-existing vascular disease, such as diabetes mellitus, may not be the same as that for nulliparous women. Likewise, the pathogenesis of early-onset pre-eclampsia (before 34 weeks gestation) may differ from that of pre-eclampsia developing at term (Sibai *et al.* 2005). Taking these factors into consideration it is possible that antioxidants may not prevent pre-eclampsia in all patients. This issue highlights the importance of the trials currently in progress to assess the efficacy, safety and cost effectiveness of antioxidants for pregnant women at low and high risk of pre-eclampsia, in which several risk factors are being investigated, including diabetes (Holmes *et al.* 2004, Hathcock *et al.* 2005).

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