Oxidative Stress: Changes in Pregnancy and with Gestational Diabetes Mellitus

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Pregnancy is susceptible to oxidative stress and antioxidant defenses can be altered in response to elevated levels of oxidative stress. Limited data in gestational diabetes mellitus (GDM) suggest that products of lipid peroxidation may be increased and antioxidant enzyme activities decreased, although the results have been inconsistent. As in type 2 diabetes mellitus (T2DM), glycemic levels in patients with GDM correlate with concentrations of lipid peroxides. The effects of supplementation with antioxidants or antioxidant-rich food in T2DM are controversial. Whether or not increased antioxidant intake can reduce the complications of GDM in both mother and fetus has not been explored.

Introduction

Oxidative stress is an imbalance between the production of free radicals and the synthesis of antioxidant defenses against them. Free radicals are highly reactive molecules that include reactive oxygen species (ROS), reactive nitrogen species, and reactive chlorine species. The most prominent ROS are the superoxide anion, the hydroxyl, and the peroxyl radicals [1••, 2].

Free radical reactions are essential for host defense mechanisms involving neutrophils, macrophages, and other cells of the immune system; however, excessive production of free radicals can lead to tissue injury and cell death and result in antioxidant depletion [3,4]. The mechanisms of ROS-related DNA, protein, and lipid damage have been well described [2,5]. Superoxide and hydrogen peroxide do not react with DNA directly. Rather, hydrogen peroxide crosses mitochondrial and plasma membranes, reaches the DNA where it reacts with bound transition metals to produce in situ hydroxyl, and causes DNA structural damage. This includes fragmentation, apoptosis, base-pair modifications, and strand breaks with a wide range of biologically toxic effects [6]. Thus, organs including the placenta, liver, and β cells in pancreas and heart are mitochondria rich, a condition that favors oxidative stress [7••,8]. Conversely, ROS can cause modification of proteins and damage to enzymes, receptors, and transporter proteins. Metals like Fe^{++} and CU^{+} reacting with unsaturated fatty acids in the presence of superoxide anion can initiate a lipid peroxidation cascade in biological membranes and lipoproteins by the production of highly reactive hydroxyl. Furthermore, the final aldehyde products of lipid peroxidation can also produce DNA and protein damage by acting as free radicals [1••,2]. Antioxidants are substances or enzymes present in tissues with the capacity to balance or neutralize these free radicals [2].

Gestational diabetes mellitus (GDM) is glucose intolerance that is first recognized during pregnancy; it complicates approximately 4% of all pregnancies in the United States [9]. GDM increases the risk of macrosomia and perinatal morbidity and mortality for the fetus while presaging a long-term risk of developing type 2 diabetes mellitus (T2DM) for the mother [10]. The pathophysiology of GDM remains controversial. Pregnant women with normal glucose tolerance have decreased insulin sensitivity, whereas women with GDM have insulin sensitivity that is further reduced [11]. Women with GDM in late gestation have both increased peripheral insulin resistance and impaired insulin secretion [12]. Characteristics of GDM (*eg*, hyperglycemia, insulin resistance, and hyperlipidemia) are components of T2DM and the metabolic syndrome [13]. The multiple metabolic defects of GDM precede the development of T2DM. Thus, GDM can be considered a prediabetic state [10,13].

Oxidative stress is a component of disorders ranging from cardiovascular disease and cancer, to chronic inflammation, autoimmune disease, and hypertension during pregnancy [14–16]. Although a number of biomarkers of oxidative stress and antioxidant status have been identified, including products of lipid peroxidation and intracellular antioxidants and enzymes, only a few have been studied in detail. Change in most biomarkers is inconsistent; often a biomarker will demonstrate no change, or show decreased or even increased levels in the same disease state [15–17]. There is also controversy about which markers of oxidative stress are the most reliable and the most suitable for clinical practice [4,18]. Although oxidants and antioxidants have been studied extensively in T2DM and its complications, only limited data are available for GDM, a disease of similar pathophysiology.

Biomarkers of Oxidative Stress and Antioxidant Status

The most reliable direct assessment of free radical synthesis is by in vitro reaction or in cell culture. Direct measurement of ROS in vivo is difficult because ROS are extremely short-lived, have a low concentration, and cannot be directly detected with current techniques [2,18]. Instead, products of oxidative damage to DNA, lipids, and proteins are measured as biomarkers of oxidative stress. Measurement of antioxidants is also used for evaluation and this seems to be logical because oxidative stress may result in the induction or depletion of circulating antioxidant levels [2,7••].

Markers of DNA oxidation and damage

8-hydroxydeoxyguanosine (8-OHdG) is an ROS-induced modification of a purine residue of DNA; it is a sensitive index of oxidative DNA damage and a frequently used biomarker of oxidative stress. Oxidized bases are present in both cells and urine, but urinary excretion of 8-OHdG is considered a marker of total systemic oxidative stress in vivo. Common methods to determine 8-OHdG include the following: high-performance liquid chromatography (HPLC) with electrochemical detector, gas chromatograph-mass spectrometry (GC-MS) with selected ion monitoring, or enzyme-linked immunosorbent assay (ELISA). Sample preparation for HPLC or GC-MS usually requires hydrolysis and derivatization, and carries a risk of auto-oxidizing 8-OHdG that can result in a falsely high background and a low sensitivity [19,20]. Shin *et al.* [19] reported that 8-hydroxyguanine, a free base of 8-OHdG determined by HPLC, was unaffected during work-up and was well correlated with oxidative DNA damage in tissue.

Markers of lipid peroxidation

Lipid peroxides result from the oxidative degradation of polyunsaturated fatty acids and give rise to products that may further increase oxidative damage to cells and tissues. The F2-isoprostanes are a unique series of prostaglandin-like compounds formed in vivo from the free radical-catalyzed peroxidation of arachidonic acid independent of cyclooxygenase [4]. 8-iso-prostaglandin F2α (8-iso-PGF2α) is the most abundant type of these bioactive compounds and is considered to be an accurate marker of endogenous lipid peroxidation [4,21]. Plasma and urine are noninvasive sources for measuring 8-iso-PGF2α, but other tissues, such as placenta, are often used [4,7••]. There are two forms of 8-iso-PGF2α in plasma, free and lipoprotein bound, but only free 8-iso-PGF2α is excreted in urine [17]. Common methods for assay of 8-iso-PGF2α are by ELISA, radioimmunoassay, and GC-MS [4,16,21]. Morris *et al.* [16] pointed out that in vitro auto-oxidation significantly raised levels of isoprostanes and lipid hydroperoxides when plasma samples were stored at -20°C. Adding an inhibitor (β-hydroxytoluene) into plasma samples can prevent in vitro auto-oxidation.

Malondialdehyde (MDA) is another product of the peroxidation chain reaction of unsaturated fatty acids [14]. The conventional assays to measure lipid peroxidation have relied on the thiobarbituric acid (TBA) assay, which involves adding TBA to the sample and colorimetrically measuring the resultant reactive substances (TBARS) [22,23]. The TBA test is calibrated with MDA. Thus, the TBARS reading is sometimes reported as MDA equivalents. This method lacks specificity because many other substances in biologic samples also react with TBA. More recently, MDA concentrations were derived by calculating the third derivative from each sample's absorption spectrum, which mathematically eliminates interference from other biologic compounds [24]. An HPLC method has been modified to separate MDA and TBA in blood samples [25•]. Serum, plasma, blood erythrocytes, and urine can be used to assay MDA.

Markers of protein damage

Damaged proteins can affect the function of receptors, enzymes, and transport proteins, as well as contribute to secondary damage of other biomolecules by inactivating antioxidant defense or repair enzymes [7••]. Protein carbonyl groups result from free radical–induced protein oxidation. The level of protein carbonyl in tissues, cells, and plasma is a relatively stable marker of oxidative damage. Protein carbonyl can be analyzed by a commercially available ELISA kit using blood erythrocytes [7••,26].

Markers of antioxidant status

The first-line defenses against superoxide or hydrogen peroxide–mediated injury are superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase. Selenium-dependent GPX catalyzes the reduction of organic hydroperoxides or hydrogen peroxides by glutathione. Enzymatic methods using erythrocyte or serum samples to determine the activities of SOD, GPX, and catalase have been studied extensively [27–29].

Rather than measuring the concentration of a specific antioxidant, total antioxidant capacity (TAC), which reflects the oxygen radical absorbance capacity or capacity of plasma sample to inhibit an oxidant reaction, has been widely used [30,31]. TAC does not always include all of the major antioxidants. The assay method described by Toescu *et al.* [31] suggested that substances contributing to TAC included uric acid, vitamins E, A, and C, and thiols. Uric acid may be one of the major determinators of TAC depending on the method. Thus, in that study, uric acid–corrected TAC gave an indication of the potential change in other contributors to the overall antioxidant capacity.

Vitamin E is a well-known antioxidant. Its protective actions are mainly exerted on long-chain polyunsaturated fatty acids in cellular and subcellular membranes. Vitamin C, a water-soluble antioxidant vitamin, plays a pivotal role in several biological processes, in addition to readily scavenging reactive oxygen and nitrogen species [32,33]. HPLC is the most reliable method for quantifying vitamin E or C in plasma or in erythrocytes.

It is believed that elevated levels of biomarkers of DNA, protein, and lipid oxidation represent oxidative stress, whereas altered concentrations of antioxidants suggest that the defense system is working, with increased concentrations suggesting upregulation in response to oxidative stress and lower levels suggesting depletion from utilization. Multiple measures should be used to evaluate oxidative stress and antioxidant status rather than a single biomarker because of the limitations of the various assays, as described earlier.

Oxidative Stress and Antioxidant Status in Normal Pregnancy

Normal pregnancy is characterized by a high energy demand for many bodily functions and an increased oxygen requirement by different organs, including the fetoplacental unit [1••]. Physiologic increases in insulin resistance during late gestation result in an increase in circulating lipids (*eg,* triglycerides, free fatty acids, total cholesterol and low-density lipoprotein [LDL] levels) [11,12,30,31,34]. Increased plasma LDL is associated with increased lipid hydroperoxides in normal pregnancy [30,31]. Thus, as found in T2DM, increased susceptibility of LDL particles to peroxidation and an imbalance between oxidized LDL and protective antioxidants (*eg,* vitamin E) may occur [35].

Moreover, human placenta is rich in mitochondria, hemomonochorial, and highly vascularized with high metabolic rate. Thus, with increased leakage of electrons from the mitochondrial respiratory chain there is increased generation of ROS in normal pregnancy. This physiologic response, in the presence of transition metals, potentially contributes to damage of DNA, lipids, and proteins by ROS [1••,2,16,23]. Therefore, pregnancy is a condition where there is increased susceptibility to oxidative stress.

Many studies have compared biomarkers of oxidative stress in normal pregnant women and nonpregnant control subjects [16,17,22,23]. Results of such studies suggest that erythrocyte or plasma MDA and lipid peroxides are generally higher during the second trimester [23] as well as late in gestation [16,22,23]; higher levels of salivary 8-isoprostane concentrations (but not plasma) have been noted during the second trimester [17]. Conversely, the activity levels of antioxidant enzymes (*eg,* erythrocyte or plasma SOD and GPX) and their substrates (*eg,* selenium, glutathione) are usually decreased [22,23,27].

During late gestation, erythrocyte or plasma SOD and GPX activity either increases, declines, or shows no change [27–29,36•]. In our prospective epidemiologic study (n = 408 normotensive nondiabetic gravidas), we found erythrocyte GPX activity increased by 15% between entry (week 16) and the third trimester [36•]. In contrast, Zachara *et al.* [27] and Mihailovic *et al.* [23] found that erythrocyte and plasma GPX activity gradually declined with gestation. Numerous studies have compared markers of lipid peroxidation in women with complicated and uncomplicated pregnancies, usually at a single point following diagnosis [15,16,37]. Longitudinal observations during pregnancy are limited and results are conflicting [27–29,34]. For example, Patrick *et al.* [34] found that serum MDA concentration did not change across pregnancy when samples were taken at five time points during gestation, whereas Carone *et al.* [28] and Loverro *et al.* [29] reported erythrocyte TBARS levels increased in the third trimester compared with levels taken earlier in gestation ($P < 0.001$ and $P < 0.05$).

Although the physiologic role of oxidative stress in normal pregnancy is still poorly understood, the findings of biomarkers in the maternal circulation suggest that pregnancy is susceptible to oxidative stress and that the placenta is one of its sources [2,7••]. In normal pregnancy, it seems plausible that the antioxidant defense system may be able to compensate through induction and increased activity of antioxidant enzymes as well as nonenzymatic free radical protection and scavenging (*eg,* by protein thiols, and vitamins E and C) [16,28–30]. Several factors potentially contribute to these inconsistent results. First, it is possible that there is an insufficient increase in antioxidant defenses to offset the increase in oxidative stress and lipid peroxidation—this may explain the reduced circulating levels of antioxidants in some studies [23,27]. Second, maternal circulating lipid levels and plasma volume can influence the values of biomarkers as well [30,34,38]. For example, increased maternal lipids potentially induce lipid peroxidation; expanded plasma volume may lower the concentration of antioxidant enzymes and other measures but poor plasma volume expansion would have the opposite effect. Third, most previous studies were done with small samples of women (n = 10 to 40) with blood samples collected at differing stages of gestation. Thus, longitudinal studies with larger sample size are needed to provide more reliable information on the onset of and changes in oxidative stress during pregnancy.

Oxidative Stress and Antioxidant Status in GDM

Increased markers of oxidative stress have been found in patients with a variety of conditions that complicate pregnancy [15,17]. The link between T2DM, a condition which has clinical characteristics similar to GDM [8,10,39], and complications of T2DM with oxidative stress has been well described [4,19]. Evidence suggests increases in the biomarkers of ROS damage and in abnormalities of the antioxidant defenses with T2DM [3,4]. Hyperglycemia is a widely known cause of increased oxidative damage [39,40]. Mononuclear cell and serum 8-OHdG and 8-hydroxyguanine levels are significantly increased in T2DM [3,19], and urinary 8-iso-PGF2α concentration is higher [4]. In addition, hyperinsulinemia and insulin resistance, not necessarily accompanied by overt hyperglycemia, are associated with increased oxidative stress [41,42]. For

example, Facchini *et al.* [42] found that insulin resistance in healthy subjects was positively correlated with levels of lipid peroxides and negatively correlated with concentrations of α-tocopherol and the carotenoids. Recently, our prospective study of 408 normal pregnant women showed that erythrocyte GPX activity at approximately weeks 16 and 30 was positively associated with markers of insulin resistance (increased fasting insulin concentration and the homoeostasis model assessment for insulin resistance), suggesting a link between insulin resistance and antioxidant defenses in nondiabetic pregnancy [36•].

However, although correlations are present, it is unclear whether the relationship between diabetes and oxidative stress is causal [8,39,41]. It is known that hyperglycemia increases free radical production through several mechanisms: nonenzymatic glycation, glucose auto-oxidation, and intracellular activation of polyol pathways [8,39,40]. Enhanced oxidative stress and sharply reduced antioxidant defenses may impair insulin action and secretion. Thus, it is possible that oxidative stress could play a role in the onset and the progression of diabetes and its complications [8,41,42].

In contrast, the relation of GDM to oxidative stress is a neglected research area (Table 1). The importance of the oxidant/antioxidant balance is far from clear. Several in vitro studies have used placental tissue to measure oxidative stress after a GDM diagnosis [5,7••,21,43]. Coughlan *et al.* [7••] found that release of 8-isoprostane by placental tissue was twofold greater in women with GDM (n = 12) compared with pregnant control subjects; SOD activity and protein carbonyl content were also elevated. The same author also reported that when placental tissue was exposed to superoxide-generating substances (*eg*, xanthine and xanthine oxidase), the capacity of GDM placental tissue to respond to oxidative stress was reduced [5]. Similarly, Lappas, *et al.* [21] showed that in incubated placenta, adipose, and skeletal muscle tissues, release of 8-isoprostane was greater in women with GDM (n = 8) compared with pregnant control subjects (n = 6) [21].

Limited evidence suggests that in GDM oxidative stress is accompanied by modified antioxidant capacity [25•,31,37,44]. Kamath *et al.* [37] collected maternal erythrocytes immediately after delivery and found that proteolytic activity, an indicator of protein oxidation, was increased in women with GDM (n = 20). Erythrocyte MDA concentration was higher in GDM, but this was not statistically significant. Peuchant *et al.* [25•] reported that at 26 to 32 weeks of gestation, maternal plasma and erythrocyte MDA was increased and GPX decreased in GDM (n = 16) as well as in type 1 diabetes mellitus. However, no differences in erythrocyte and plasma GPX, catalase activity, or TBARS levels were found during the third trimester (n = 3) [15]. The only study to longitudinally assess biomarkers of oxidative stress by trimester was reported by Toescu *et al.* [31]. They found that serum lipid and lipid hydroperoxide levels were elevated at each trimester in women with T2DM, type 1 diabetes mellitus, and GDM compared with pregnant control subjects, whereas uric acid–corrected TAC concentration was reduced significantly. Epidemiologic data also support a relation between reduced serum vitamin C and increased risk of developing GDM [32].

The maternal glycemic level is correlated with biomarkers of oxidative stress in GDM, including the following: erythrocyte MDA with hemoglobin A_{1c} levels ($r^2 = 0.352$, $P < 0.001$) [25•] and 8-isoprostane in placental tissue with maternal glucose concentration (hour 2 of the oral glucose tolerance test, $r^2 = 0.26$, $P < 0.03$) [7••].

These limited data suggest that oxidative stress may be involved in progression to or pathogenesis of GDM [21]. The reduced antioxidant defenses reported in the few extant studies of women with GDM may reflect a protective response to existing oxidative stress [25•]. It is unclear if the level of glycemic control in GDM is associated with the severity of oxidative stress as it is in T2DM [4]. Longitudinal studies over the course of gestation are needed in order to determine whether oxidative stress triggers the onset and the progression of GDM, and if improved glycemic control will reduce the severity of oxidative stress.

Can Antioxidant Supplementation Improve GDM and Its Outcomes?

Gestational diabetes mellitus represents nearly 90% of all pregnancy complicated by diabetes; in the United States there are approximately 135,000 cases annually [9]. Thus, it is important to find effective means of predicting and preventing GDM. There is some evidence in both T2DM and GDM to suggest an imbalance between pro-oxidant and antioxidant status. Theoretically, long-term antioxidant supplementation should reverse this imbalance, and could be an attractive therapeutic strategy for treating and preventing diabetes and its complications [45,46].

Higher dietary antioxidant intake is associated with a reduced risk of T2DM [45], and supplementation with vitamins E and C has been shown to improve insulin action [46,47]. However, randomized trials of antioxidant supplements, or of antioxidant-rich diets, have yielded generally disappointing results [48,49]. For example, a 12-year study of beta-carotene supplementation (50 mg of beta-carotene on alternate days) found no difference in T2DM between treatment and placebo groups [49].

Data on the effects of antioxidant supplementation on biomarkers of oxidative stress also are conflicting [4,48]. In T2DM, one study showed that urinary 8-isoprostane excretion was reduced by 37% (n = 10) after vitamin E administration (600 mg/d) for 14 days [4]. Patients treated for 8 weeks with RRR-α-tocopheryl acetate supplements at pharmacologic dose showed decreased LDL oxidizability as measured by lipid peroxide formation [50]. However, recently, a study allocating healthy subjects to a 25-day intervention that increased antioxidant intake by diet (higher fruit and vegetable intake, n = 16), by use of nutri-

Table 1. Summary of reported data on changes in oxidative stress and antioxidant biomarkers in women with GDM compared with non-GDM pregnancy*

Study	N (GDM or controls)	Sources of sample§	Gestation, *wk*	Major biomarkers	Findings (women with GDM vs non-GDM)
Tissue samples					
Kinalski *et al.* [43]	19/13	Placenta	Immediately after delivery	MDA, SOD, GPX	MDA increased ($P < 0.0001$), SOD decreased ($P < 0.0001$)
Coughlan *et al.* [7••]	12/11	Placenta	15 min after delivery	8-isoprostane, SOD, GPX, protein carbonyl	Placental release of 8-isoprostane ~ twofold greater in GDM. SOD and protein carbonyl increased ($P < 0.004$ and $P < 0.04$), GPX NS
Lappas *et al.* [21]	12/10	Placenta, adipose, skeletal muscle	10 min after delivery	8-isoprostane	Basal 8-isoprostane release (8 GDM/ 6 controls) increased in all tissues, LPS stimulated increase in adipose and skeletal tissues ($P < 0.05$ for both)
Coughlan *et al.* [5]	5/5	Placenta	15 min after delivery	8-isoprostane	Basal 8-isoprostane release was twofold greater in GDM, response to oxidant challenge was reduced¶
Maternal blood samples					
Chaudhari *et al.* [44]	20/20	Erythrocyte, hemolysate	Trimester 3	MDA, SOD	MDA increased ($P < 0.001$), SOD decreased ($P < 0.01$)
Peuchant *et al.* [25•]	16/27	Plasma and erythrocytes	Trimester 3	MDA, SOD, GPX, vitamins A, E	Free MDA increased (both samples $P < 0.05$), erythrocyte GPX decreased ($P < 0.01$), other biomarkers NS
Toescu *et al.* [31]	12/17	Plasma	Trimesters 2 and 3	TAC, LHP	TAC (uric acid corrected) decreased ($P < 0.001$), total TAC and LHP NS
Orhan *et al.* [15]	3/16	Plasma and erythrocytes	Trimester 3	GSH, GPX, CAT, TBARS	All measures NS
Kamath *et al.* [37]	20/15	Erythrocytes	Immediately after delivery	MDA, proteolytic activity	Proteolytic activity increased ($P < 0.001$), MDA NS
Sobki *et al.* [33]	46/40‡	Plasma	Labor	MDA, vitamin E (α and γ)	Maternal vitamin E (γ) increased in GDM under control diet ($P < 0.05$), GDM receiving insulin treatment NS, MDA NS
Zhang *et al.* [32]†	33/722	Plasma	13	Ascorbic acid	Lower ascorbic acid increased risk of GDM ~ threefold

*Other comparison groups in the studies, including T1DM and T2DM, are not listed in the table.
†A prospective study in 755 pregnant women, in which 33 developed GDM.
‡27 patients with GDM under a control diet and 19 receiving insulin treatment.
§Other sample sources (eg, cord blood) are not listed in the table.
¶Experimental oxidant challenge (see text).
CAT—catalase; GDM—gestational diabetes mellitus; GSH—reduced glutathione; GPX—glutathione peroxidase; LHP—lipid hydroperoxide; LPS—lipopolysaccharide; MDA—malondialdehyde; NS—nonsignificant; SOD—superoxide dismutase; TAC—total antioxidant capacity; TBARS—thiobarbituric acid reactive substances; T1DM—type 1 diabetes mellitus; T2DM—type 2 diabetes mellitus.

tional supplements ($n = 12$), or by placebo ($n = 15$) showed increased erythrocyte GPX activity in the dietary group. Protein carbonyl formation was increased in both dietary and nutritional supplement groups, whereas other markers of oxidative stress and antioxidant status (including urinary 8-isoprostane excretion, plasma MDA, erythrocyte antioxidant enzyme activity, and total antioxidant capacity) were largely unaffected [48].

Whether antioxidant supplementation or eating an antioxidant-rich diet can improve oxidative stress in GDM is not known. Vitamins E and C have anti-inflammatory properties and chronic inflammation is involved in the etiology of T2DM and GDM. Based on the positive effects of preliminary data in T2DM, it is possible that antioxidant supplementation with vitamins E or C may enhance antioxidant capacity as well as reduce inflammation [2,46,47].

Conclusions

Evidence based on circulating biomarkers indicates that there is an increase in oxidative stress during normal pregnancy. Changes in antioxidant enzyme activity are inconsistent, may be influenced by gestational age of sampling, by assay methods, or by small sample size. Limited data on GDM suggest increased levels of oxidative stress, which are generally accompanied by an insufficient antioxidant defense response.

Maternal glycemic levels are correlated with biomarkers of oxidative stress in patients with GDM. Whether antioxidant supplementation can improve oxidative stress in GDM is not known. Although the results are preliminary and sometimes controversial, antioxidant supplementation could improve insulin action and reduce levels of lipid peroxidation in T2DM. Thus, longitudinal studies or randomized clinical trials over the course of gestation are needed to determine 1) whether oxidative stress triggers the onset and the progression of GDM, and 2) whether antioxidant supplementation can improve the imbalance between oxidative stress and antioxidant status, as well as glycemic control in patients with GDM.

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