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The pathophysiology of smoking during pregnancy: a systems biology approach

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1. ABSTRACT

This article focuses on a systems biology approach to studying the pathophysiology of cigarette smoking during pregnancy. Particular emphasis is given to the damaging role of oxidative stress. Cigarette smoking exerts multiple adverse affects but abundant evidence, mostly in adults, suggests that oxidative stress and free radical damage is a major pathophysiological factor. Smoking during pregnancy is known to contribute to numerous poor birth outcomes, such as low birth weight, preterm birth as well as life-long health and developmental problems. It is clinically important to know the separate contributions that cigarette derived-nicotine and smoking-induced free oxidative stress make to these poor outcomes. Surprisingly, the extent to which smoking dependent oxidative stress contributes to these poor outcomes is not well studied but the application of redox proteomics should be useful. Considerable biochemical evidence suggests that antioxidants, such as tocopherols and ascorbate, could be useful in minimizing oxidative stress induced pathology to the developing fetus in those women who, despite medical advice, continue to smoke. Nevertheless, this suggestion has yet to be tested in well-designed clinical studies.

2. INTRODUCTION

2.1. A systems biology approach to maternal smoking induced oxidative stress

This article will focus on the pathophysiological roles of maternal smoking induced oxidative stress on the placenta and developing fetus. Smoking is perhaps the most potent and clinically relevant form of oxidative stress. Oxidative stress results when an organism's exposure to reactive oxygen species (ROS) or reactive nitric oxide species (RNOS) exceeds the organism's protective antioxidant mechanisms. The damaging consequences of maternal smoking are likely to depend on many factors such as diet, the use and composition of prenatal vitamins as well as the degree and "timing" of smoking. Gracie *et al.* (1) have detailed the practical benefits of an integrated systems biology approach, with "omics" technology, to studying the etiological factors giving rise to preterm birth. The assertions made by Gracie *et al.* (1) are both persuasive and equally applicable to studying the role of maternal smoking induced oxidative stress. A systems biology approach is one in which an organism is viewed as an integrated and interacting network of genes, proteins and biochemical reactions. Instead of analyzing individual components of an

organism, a systems biology approach focuses on many of the components, their internal interactions as well as their interactions with the environment. This article will attempt to identify a targeted set of parameters that would be useful for a systems biology approach to studying the pathophysiology of cigarette smoking during pregnancy.

2.2. The health related consequences of smoking during pregnancy

Despite aggressive smoking cessation programs, many women continue to smoke during pregnancy. Indeed, nearly 12% of pregnant women in the U.S. smoke (2) with rates exceeding 30% in some populations and regions (3, 4). Pregnant smokers are at increased risk for pregnancy complications and put their children at substantially elevated risk for poor birth outcomes and long term health and developmental problems. Ectopic pregnancy, placenta previa and abruption, stillbirth, and premature rupture of membranes have all been associated with smoking (5-8). For a smoke-exposed fetus that survives to delivery, the increased likelihood of adverse birth outcomes is significant. Dozens of studies have detailed the strong association between pregnancy smoking and decreased birth weight, with decrements attributable exclusively to smoking ranging from 190 g (9, 10) to over 250 g (3, 11, 12) and to 350 g or more for heavy smokers (13-15). A recent study demonstrated that each additional cigarette smoked per day in the third trimester led to an 11.6. g decrease in birth weight (16). Consequently, smoking accounts for 20% to 30% of all low birth weight births in the U.S. each year (17). In addition, the risk of preterm birth is nearly doubled for smokers (18, 19), however, as noted and quantified by multiple investigative teams, the impact of smoke exposure on gestational age does not appear to be as strong as the effect on birth weight (10, 13, 20). Thus, it appears that the impact of smoking on birth weight is more a result of an influence on fetal growth rather than simply an abbreviated gestation.

Pregnancy smoking has additional negative immediate and short term impacts on child health and medical needs. Increased rates of low birth weight and preterm delivery among smokers produce a significant economic cost through neonatal intensive care unit (NICU) admission and treatment. Indeed, compared to babies born to non-smokers, babies born to smokers are anywhere from 20% to 90% more likely to be admitted to the NICU (3, 21). Additionally, smoke-exposed newborns have an increased risk for respiratory dysfunction, cranial hemorrhage, and necrotizing enterocolitus (22-24). Finally, an increased likelihood of infant mortality is associated with heavy pregnancy smoking, with exposure accounting for 5% of perinatal deaths (24 in 40), and as many as one third of cases of Sudden Infant Death Syndrome (SIDS) (25).

Prenatal exposure to cigarettes has also been shown to have long-term developmental consequences. Physical health effects well into childhood include growth restriction, abnormal neuromotor tone, increases in respiratory infections, asthma, otitis media, and obesity (26-30). Long-term effects on cognitive development, including learning problems and delayed academic achievement, have also been reported (31-33). Finally, prenatal cigarette exposure has been implicated in significantly increased rates of behavioral and mental health problems, including attention deficit hyperactivity disorder, conduct disorders, depressive and anxiety disorders, criminal behavior, and substance use, abuse, and dependence (34-44).

3. MATERNAL SMOKING AND OXIDATIVE STRESS

Cigarette smoke contains large numbers of free radicals (45) as well as other oxidants and toxicants (46, 47). Considerable evidence demonstrates that adult smoking increases oxidative stress biomarkers such as breath pentane (48), plasma protein carbonyls (49), F2 isoprostanes (50) and increased vitamin E consumption through an oxidative stress pathway (51-53). It is likely that smoking induced intrauterine growth retardation and low birth weight (as well as other poor outcomes) are caused by multiple toxic factors in cigarette smoke but it is, nevertheless, important to know the degree to which oxidative stress plays a role.

Even in the absence of additional oxidative stress from maternal smoking, there is compelling evidence that both the developing fetus and the placenta exhibit a state of enhanced oxidative stress (54). Myatt and Cui (54) suggest that this increased oxidative stress is associated with an increased placental mitochondrial production of ROS as well the placental production of nitric oxide (NO). As outlined in Figure 1, nitric oxide rapidly reacts with superoxide radicals to produce peroxynitrite which is highly reactive and damaging to placental functions (54). Peroxynitrite is thought to react *in vivo* with the tyrosine residues of proteins to form nitro-tyrosine. Little is known, however, about the protein targets of placental oxidative stress and the absence of this knowledge is a major gap in our understanding of functional damage at the molecular level.

3.1. Oxidative stress episodes during pregnancy

It is likely that there are specific times during pregnancy when endogenous placental oxidative stress could be pronounced and amplified by an additional oxidative stress from smoking. In early pregnancy both the placenta and fetus are in a hypoxic environment with pO_2 levels less than 20 mm Hg at eight weeks of gestation. There is, however, a marked increase in the pO_2 (to 50 mm Hg) of the intervillous space at the end of the first trimester when the invading trophoblast promotes the opening of the maternal endometrial blood vessels. As detailed by Myatt and Cui (54), this process has all the "earmarks" of an ischemia-reperfusion oxidative stress injury and is accompanied by the increased placental expression of nitro-tyrosine amino acid residues which is consistent with increased peroxynitrite production. The work of Hung *et al.* (55) reinforces the potential role that oxidative stress plays in placental ischemia-reperfusion injuries. These investigators used the term human placenta as a model for ischemia-reperfusion and found a rapid generation of ROS when hypoxic placental tissues were reoxygenated (55).

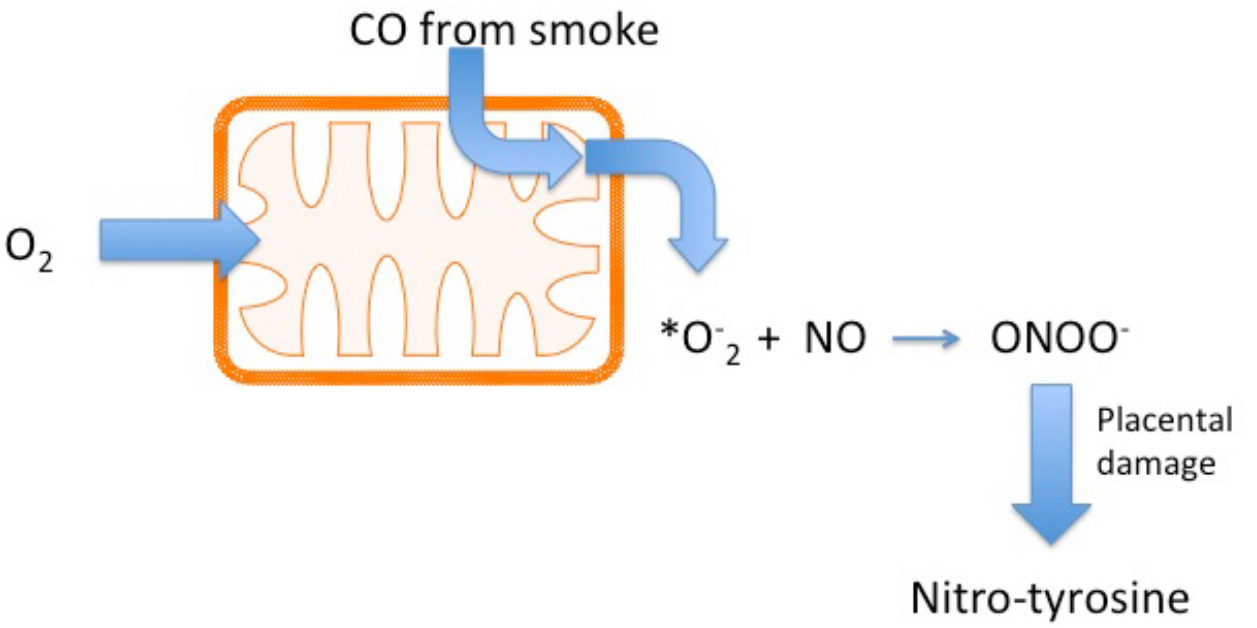

Figure 1. Intrinsic placental oxidative stress is attributed to an increased mitochondrial production of superoxide radical ($*O_2$-), which can react with placental produced nitric oxide (NO) yielding peroxynitrite (ONOO$^-$). ONOO$^-$ is highly reactive and can react with tyrosine residues on placental proteins. Carbon monoxide (CO) from cigarette smoke can enhance the production of $*O_2$- and thereby act as a pro-oxidant.

An important issue is whether there are critical periods during which maternal smoking could be particularly damaging to fetal development (56). Clearly, knowing when placental/fetal oxidative stress is at a maximum could have consequences when considering the intervention interval during which antioxidant supplementation could be clinically useful in preventing ROS or RNS-dependent placental pathologies. Bernstein *et al.* (57) have found that maternal third-trimester cigarette consumption was the strongest predictor of birth weight percentile (partial r = -0.23, P < .001). Recent studies by Iniguez *et al.* (56) shows that pregnant women who continue to smoke beyond 12 weeks of gestation have fetuses (at week 34) with reduced size as measured by fetal weight, femur length, transverse head diameter and abdominal circumference. In contrast pregnant women who stop smoking before 12 weeks of gestation had fetuses at week 34 with only a decreased femur length (56). There appears to be little or no information that correlates fetal growth curves with placental or fetal oxidative stress biomarkers and maternal smoking patterns. To obtain such a meaningful correlation there must be a robust set of validated oxidative biomarkers. We will, therefore, review the most promising of these biomarkers. When available, data relevant to smoking during pregnancy will be summarized.

3.2. Isoprostane 8-epi PGF2α an important biomarker for smoking induced oxidative stress and is reduced by antioxidant supplements

Isoprostanes, a family of eicosanoids produced by the oxidation of biomembrane phospholipids by ROS, are excellent *in vivo* biomarkers of oxidative stress and are increased in smokers (50). The F2-isoprostane, 8-epi-prostaglandin F2α (8-epi PGF2α) is a potent pulmonary and renal vasoconstrictor. Work by Obwegeser *et al.* (58) has shown that 8-epi PGF2α is increased in the umbilical vessels of mothers who smoke during pregnancy compared to values for mothers who do not smoke. These authors suggested that 8-epi PGF2α could be an important biomarker for damaging functional and morphological changes to the umbilical vessels (58).

Oxidative stress is considered to be a key etiological factor contributing to preeclampsia, which is the development of hypertension and elevated urine protein levels after the late 2nd or 3rd trimester of pregnancy (59, 60). Chappell *et al.* (61) studied the effects of ascorbate (vitamin C) and vitamin E supplementation on a cohort of women at high risk for developing preeclampsia. Vitamin E is the primary lipid soluble antioxidant in plasma and vitamin C is a key water-soluble plasma antioxidant. These researchers found that: (1) plasma 8-epi PGF2α were initially elevated in high risk women, compared to low risk women and: (2) antioxidant supplementation (1000 mg/day for ascorbate and 400 IU/day for vitamin E) reduced plasma 8-epi PGF2α in the high risk group to the levels observed in the low risk women (61). Moreover, some favorable changes in indices of placental function were observed in the high-risk women taking the antioxidant supplement (61). As detailed below, vitamin E is not a single compound and synthetic vitamin E is also different from natural vitamin E. The form of vitamin E used by Chappell *et al.* (61) was, unfortunately, not specified.

3.3. Vitamin E, maternal smoking and premature placental calcification

Normally, antioxidants such as vitamin E help reduce oxidative stress related damage, but newborns have plasma vitamin E levels significantly lower than those of their mothers (62). Similarly, serum from cord blood has significantly lower tocopherol levels than maternal serum (63-66). For premature or low birth weight infants there is strong evidence for a true vitamin E deficiency state with a recommendation of early treatment (67).

In a small study, Mathews (68) found that cord blood from infants born to smokers had significantly ($p < 0.02$) higher alpha-tocopheryl quinone (alpha-TQ) to alpha-tocopherol ratios (7.21 ± 0.70) compared to the nonsmokers (5.34 ± 0.36). Alpha-TQ is an oxidation product of alpha-tocopherol and a biomarker for oxidative stress (69). Moreover, a negative correlation was observed between maternal weight gain and the alpha-TQ to alpha-tocopherol ratio ($r = -0.410$, $p < 0.05$). These data suggest that oxidative stress induced by smoking during pregnancy is transmitted to the developing fetus (68). As expected, Mathews (68) found that infants born to smokers had a significantly ($p < 0.01$) lower birthweight (2963 ± 135 g, N=15) than infants born to nonsmokers (3570 ± 147 g, N=23). The data from this study needs to be repeated on a much larger cohort.

In a large-scale study of over 1,500 pregnant women, Klesges *et al.* (70) found that smoking during pregnancy was associated with increased placental calcification. In addition, significant reductions in villus calcification were observed with higher dietary intake of alpha-tocopherol. Placental calcification is the deposition of calcium on the placenta and this normally occurs near the end of pregnancy (38 to 42 weeks gestation). Premature calcification occurring at (or before) 36 weeks of gestation is, however, associated with a number of pathologies such as preeclampsia as well as increased neonatal morbidity and mortality. Ultrasonographic examinations have provided strong evidence that maternal cigarette smoking is associated with an increase risk of premature calcification (71, 72) and intrauterine growth retardation (72). Klesges *et al.* (70) have suggested that dietary alpha-tocopherol could help reduce intrauterine growth retardation by reducing villus calcification in mothers that smoke during pregnancy. This hypothesis has not yet been tested in a clinical trial.

Placental calcification can be ultrasonically evaluated by the grading system described by Grannum *et al.* (73) as well as quantitative image analysis method described by Ryan *et al.* (74) which provides a percentage of the placenta that is calcified. The Grannum classification scheme, although widely used, is based on a rather subjective observation of the placenta. Depending upon the presence, location and intensity of calcium depositions, a placenta is given a score of 0 to 3 in order of increasing calcification (73). Calcification regions within the placenta appear as bright regions in the ultrasound image. Ryan *et al.* (74) in the UK, have attempted to overcome some of the subjective aspects of the Grannum grading scale by utilizing a software based approach to quantify 2D ultrasound images of the placental area as input.

3.4. Placental and fetal hypoxia, carbon monoxide and oxidative stress

Bush *et al.* (75) performed a detailed quantitative study of the effects of maternal smoking on placental morphology. Their goal was to determine the extent to which smoking-induced morphological changes resemble those caused by other conditions with known preplacental hypoxia. There is compelling evidence that maternal smoking induces fetal hypoxia since fetal hematocrits are elevated (75). Moreover, carbon monoxide, which is present in cigarette smoke at levels from 10 to 50 ppm, binds to hemoglobin (Hb) forming carboxyhemoglobin (HbCO) resulting in a leftward shift of the oxyhemoglobin dissociation curve resulting in decreased oxygen delivery to target tissues. These facts suggest that carbon monoxide induced placental hypoxia should induce compensatory adoptive changes in placental morphology (75). Nevertheless, the changes in placental morphology observed with maternal smoking do not resemble the adoptive changes observed in pregnancies with known preplacental hypoxia (75). The smoking associated changes in placental morphology include a thickening of the trophoblast component of the villous membrane and a decrease in the volume of fetal capillaries: these changes compromise oxygen delivery to the fetus rather than being adaptive (75). These findings led Bush *et al.* (75) to speculate the existence of a "non-hypoxic" effect of maternal smoking on the placenta. Oxidative stress may well be this "non-hypoxic" effect.

Although the evidence linking carbon monoxide in cigarette smoke to fetal hypoxia is strong, it is interesting that carbon monoxide may also act as a pro-oxidant (see Figure 1). Using an animal model, Piantadosi *et al.* (76) found that carbon monoxide exposure (50 ppm) induces mitochondrial ROS and RNOS as well as elevated protein nitro-tyrosine levels when compared to hypoxia alone. These authors attributed these effects to carbon monoxide meditated deregulation of the calcium-dependent mitochondrial pore transition.

Richter *et al.* (77) recently explored a very relevant animal model looking at the effects of chronic hypoxia (13% O_2) on placental oxidative stress in pregnant rats. Placental levels of 4-hydroxynonenal (4-HNE), a well characterized biomarker for lipid peroxidation, was elevated in rats exposed to hypoxia compared to levels in rats subject to normoxic pregnancy. Ascorbate (vitamin C) in the rats' drinking water was able to prevent the hypoxia induced increase in placental 4-HNE and also increased birth weight. The authors suggest that "antioxidant treatment may provide useful intervention to improve placental function and protect fetal growth in pregnancy complicated by fetal hypoxia" (77).

4. WHAT IS THE BEST FORM OF VITAMIN E FOR WOMEN WHO SMOKE DURING PREGNANCY?

The data presented above strongly suggest that

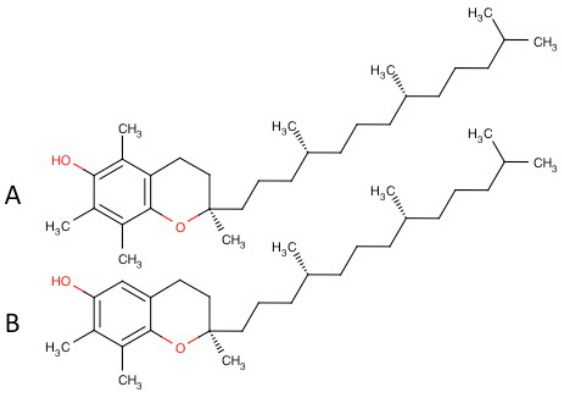

Figure 2. Panel A (top) shows the structure of RRR-alpha-tocopherol, which is the primary plasma lipid-soluble antioxidant. Panel B (bottom) shows the structure of RRR-gamma-tocopherol, which is the primary dietary form of vitamin E. RRR-gamma-tocopherol has one less methyl group on the chroman head group (left most part of the structure).

vitamin E supplementation could be useful for women who continue to smoke during pregnancy. Work presented by Smedts *et al.* (78) suggests, however, that a high maternal vitamin E intake is associated with an increased risk of congenital heart defects. The work by Smedts (78) has some major flaws since it did not include vitamin E intake from supplements and did not distinguish the different chemical and stereochemical forms of vitamin E. It would be important, therefore, to establish oxidative stress biomarkers that correlate with reduced intrauterine growth retardation, low birth weight, umbilical artery resistance (see below) and placental calcification in pregnant smokers which can then be used in future studies to determine the optimal level and chemical form(s) of vitamin E for use in prenatal nutrition, particularly for pregnant smokers.

The term "Vitamin E" does not refer to a single unique organic compound but rather to all tocopherols and tocotrienols and their chemical derivatives (79). Naturally occurring vitamin E is primarily four tocopherols (alpha-, beta- delta- and gamma-) and four tocotrienols (alpha-, beta- delta- and gamma-) with specific chirality. Naturally occurring tocopherols and tocotrienols all have the R-configuration. Human plasma and tissues primarily contain only RRR-alpha- and RRR-gamma-tocopherols (see Figure 2). A typical USA diet contains about 2 to 4 times more RRR-gamma-tocopherol than RRR-alpha-tocopherol. Despite the fact that the primary form of dietary vitamin E in the USA diet is RRR-gamma-tocopherol, most clinical research has been performed with synthetic vitamin E, which is all-racemic-alpha-tocopherol (or all-racemic-alpha-tocopheryl acetate). The rationale for using alpha-tocopherol in clinical experiments lies in the fact that this isoform is present in plasma from fasting individuals at levels 5-10 fold higher than gamma-tocopherol. Most prenatal vitamin supplements contain only synthetic all-racemic-alpha-tocopheryl acetate. Synthetic all-racemic-alpha-tocopherol is an equimolar mixture of eight stereoisomers with only 1/8 being the natural RRR-alpha-tocopherol. Infants can discriminate between natural RRR-alpha-tocopherol and synthetic all-racemic-alpha-tocopherol with the natural form having twice the biological activity (79).

It is increasingly recognized, however, that gamma-tocopherol has unique biochemical properties with important health related significance (80-83). McCarty *et al.* (82) have suggested that gamma-tocopherol might promote endothelial nitric oxide (NO) synthase (eNOS) function by protecting tetrahydrobiopterin (an NO synthase substrate) from peroxynitrite oxidation. Diminished eNOS functioning is thought to be an important factor contributing to low birth weight in pregnant smokers (58). Gamma-tocopherol might be particularly important in reducing oxidative stress for pregnant smokers since cigarette smoke is particularly high in RNOS. Gamma-tocopherol, but not alpha-tocopherol quenches RNOS to form 5-nitro-gamma-tocopherol. Gamma-tocopherol in human plasma exposed to cigarette smoke *in vitro* is converted into 5-nitro-gamma-tocopherol (84). Similarly, smokers have twice the plasma 5-nitro-gamma-tocopherollevels of nonsmokers (84).

4.1. Vitamin E intake from prenatal vitamin supplements and diet

Prenatal vitamin supplements contain a wide range of vitamin E levels: from zero to 100 IU with 15 IU of RRR-alpha-tocopherol being the RDA for pregnant women. About half of the commercial supplements use all-racemic-alpha-tocopheryl ester and half use an RRR-alpha-tocopheryl ester (acetate or succinate). In most OB&GYN clinics in the U.S., pregnant mothers are provided with prenatal vitamins or a prescription for such supplements, but the particular product varies from one physician and pharmacy to the next. Very few prenatal vitamin supplements contain gamma-tocopherol and there is almost no clinical information available on the efficacy of such supplements on fetal outcomes in women who smoke during pregnancy. However, should an appropriate formulation be available, several studies have revealed that from 74% to over 90% of women do adhere to recommendations to take prenatal vitamins daily (85-87). Reported obstacles to compliance include various adverse GI events and pill size (88, 89).

Supplementation with alpha-tocopherol alone lowers plasma levels of gamma-tocopherol but increases plasma alpha-tocopherol levels (90). This observation could be used to monitor compliance with taking prenatal supplements containing alpha-tocopherol by monitoring the levels of alpha-tocopherol and the ratio of alpha- to gamma-tocopherol in the maternal plasma samples. A high level of alpha-tocopherol and a high ratio of alpha- to gamma-tocopherol are a good marker for taking a supplement with alpha-tocopherol. It is not known if this phenomenon also occurs in plasma from cord blood. It should also be noted that the lowering of plasma gamma-tocopherol by taking a supplement with only alpha-tocopherol might not be ideal for human health. Using an animal model, Traber *et al.* (91) have demonstrated that a high dietary intake of alpha-tocopherol, but not gamma-tocopherol, can stimulate the cytochrome P-450 (CYP3a) metabolism of gamma-tocopherol thereby decreasing its

level in plasma and tissues.

More recently, Wong *et al.* (92) have conducted a clinical metabolomic investigation of high dose (400 mg/day) RRR-alpha-tocopheryl acetate with ten adult males. These investigators found that phospholipid metabolism was altered with an increased blood level of lysophosphatidylcholines which is known to have proinflammatory activity (92).

In the past, measuring vitamin E intake using a food frequency questionnaire (FFQ) was problematic since there was insufficient information about the content of different vitamin E isoforms. The FFQ developed by the National Cancer Institute now utilizes a nutrient database that has specific information about the different levels and chemical forms of tocopherol. A comprehensive evaluation of vitamin E intake from prenatal supplements, other multivitamin supplements or specific vitamin E supplements would be very useful.

5. REDOX PROTEOMICS, PROTEIN CARBONYLS AND SMOKING DURING PREGNANCY

It is well established that oxidative stress can give rise to oxidatively modified forms of proteins such as protein carbonyls (49, 93). Protein carbonyls are an excellent biomarker for oxidative stress since they are relatively stable and can be measured with high sensitivity (10 femtomoles). Marangon *et al.* (49) found higher levels of protein carbonyls in the plasma of smokers compared to matched controls. Despite its health related significance, the effects of smoking during pregnancy on protein carbonyl levels, in either maternal or cord blood samples, have not been reported. Similarly, there is no information on the potential of a high tocopherol/ascorbate intake to diminish protein carbonyl levels in smoking mothers or their newborns.

In addition to the potential value of measuring total plasma carbonyl levels (a measure of systemic oxidative stress), it might be more important to know the specific protein targets of carbonylation as well as the specific sites of protein oxidation. Such information would be critical for understanding how oxidative stress could potentially alter the functions of specific proteins and potentially provide specific oxidative stress biomarkers for specific pathophysiological states such as smoking induced intrauterine growth retardation. The newly emerging area of redox proteomics is ideal for this task (94-96). A key question is whether or not the presence of oxidatively modified proteins plays a central etiological role in smoking induced fetal damage or is a secondary epiphenomena.

Colombo *et al.* have recently reviewed the use of comparative and redox proteomics in the studying the pathophysiology of smoke exposure (95) but very little information is available concerning mothers who smoke during pregnancy. The use of affinity chromatography for isolating and identifying carbonylated proteins by tandem mass spectrometry looks like a very promising technique (93). This method is useful for both identifying oxidized proteins and for determining the specific sites of oxidation on the proteins (93). In this procedure, proteins in plasma are first treated with biotin hydrazide which selectively reacts with protein carbonyls. The biotinylated (oxidized) proteins are subsequently isolated by use of avidin affinity chromatography and further fractionated by a C8 reverse phase column. The fractions are then trypsinized and the peptide identified by a combination of electrospray reverse phase LC-MS/MS and databases searches. This is very powerful technology but it has not yet been applied for characterizing the specific proteins altered by smoking during pregnancy (either cord blood or maternal blood).

6. IS NICOTINE REPLACEMENT THERAPY (NRT) EFFECTIVE?

It is has long been suggested that many of the adverse effects of smoking during pregnancy can be attributed to restricted blood flow in the vascular beds of the placenta (97). Restricted blood flow caused by smoking could be due to chronic nicotine exposure, increased levels of vasoactive substances (e.g., 8-epi PGF2α) or structural alterations in the placenta (e.g. accelerated placental calcification) (97). Among these factors it is generally thought that nicotine is the primary "culprit" causing low birth weight since nicotine is a vasoconstrictor that could impair blood flow to the placenta and limit the supply of nutrients and oxygen to the developing fetus.

If nicotine were the primary culprit causing low birth weight then the widespread use of nicotine gum or the nicotine patch (i.e., nicotine replacement therapy or NRT) in pregnant women could be problematic. Similarly, the use of E-cigarettes during pregnancy could likewise be problematic (98) but very little scientific literature exists on this issue. Wisborg *et al.* (99) found that nicotine patches had no influence on smoking cessation during pregnancy but actually increased birth weight in comparison with pregnant women provided with placebo. A more recent randomized trial found the nicotine patches did not increase smoking abstinence and had no significant effects on the rates of preterm birth, low birth weight or congenital abnormalities (100). Although, these studies did not demonstrate any short-term ill effects of NRT, they cannot be considered conclusive since compliance was low and the studies did not address potential long-term consequences. As reviewed by Bruin *et al.* (101), there is minimal clinical data on the long-term effects of NRT.

Animal experiments suggest, however, that nicotine by itself can contribute to impaired fertility, type 2 diabetes, obesity, high blood pressure, neurobiological problems, and respiratory diseases in offspring (101). Moreover, recent data by Xiao *et al.* (102) shows that pregnant rats exposed to nicotine throughout gestation have offspring with evidence of vascular oxidative stress during adulthood. This oxidative stress was attributed to nicotine dependent fetal programming (102). Although the clinical relevance of this finding is not certain, this research suggests that nicotine itself can result in an eventual oxidative stress in offspring. Collectively, these animal data suggest that NRT is not very effective at promoting smoking

cessation and is potentially associated with long-terms problems.

It could be argued that smoking during pregnancy is more harmful than NRT alone since it involves both nicotine and the additional oxidative stress associated with the ROS and RNOS in smoke itself. Clearly it would also be useful to know if oxidative stress makers decrease in pregnant women who were smokers but elect to use NRT (or E-cigarettes) during pregnancy. This issue needs to be studied since prenatal cigarette exposure is clearly of considerable health related significance and a major factor in ongoing and future health care costs.

7. SMOKING INDUCED ALTERATIONS IN UMBILICAL BLOOD FLOW

It is has been suggested that many of the adverse effects of smoking during pregnancy can be attributed to restricted blood flow in the vascular beds of the placenta (97). Restricted blood flow caused by smoking could be due to chronic nicotine exposure, increased levels of vasoactive substances (e.g., 8-epi PGF2α) or structural alterations in the placenta (e.g. accelerated placental calcification) (97). Doppler ultrasonography has proven to be a valuable noninvasive technique for evaluating umbilical artery blood flow. Albuquerque *et al.* (97) have found that both the systolic/diastolic (S/D) ratio and the resistance index (defined as the difference between the maximal systolic and diastolic flow velocities, divided by the systolic flow velocity) to be significantly higher in smokers compared to non-smokers (97). The half-life of plasma nicotine is about two hours and it would be expected that any vasoconstrictive effect due to nicotine alone would diminish after two hours after smoking. Albuquerque *et al.* (97) found, however, no correlation between the resistance index and the time after smoking a cigarette with observations being made from about 10 min to 5 hours after smoking. These data suggest that the vascular resistance induced by smoking is chronic in nature. Oxidative stress-induced placental damage is a good candidate for chronic induced vascular damage.

8. SUMMARY

In summary, a compelling argument can be made for the involvement of oxidative stress in many of the poor birth outcomes associated with smoking during pregnancy. Smoking induced oxidative stress could alter signal transduction pathways, damage macromolecules, produce vasoactive compounds (e.g., isoprostanes), alter both placental morphology (e.g., placental calcification) and blood flow, and contribute to intrauterine growth retardation and low birth weight. Oxidative stress in pregnant smokers could potentially be diminished by supplementation with antioxidants (such as vitamin E), possibly reducing the adverse immediate and long-term consequences of prenatal cigarette exposure. The optimal level and chemical form of vitamin E intake during pregnancy remains an unresolved question. This review article has identified a number of promising oxidative stress biomarkers. The application of redox proteomics looks particularly promising since it could provide a degree of molecular detail that is lacking in most systemic biomarkers of oxidative stress.

9. ACKNOWLEDGEMENTS

All authors contributed equally to this paper.

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Abbreviations: 4-HNE : 4-hydroxynonenal; 8-epi PGF2alpha : 8-epi-prostaglandin F2alpha; alpha-TQ : alpha-tocopheryl quinone; eNOS : endothelial nitric oxide synthase; FFQ : food frequency questionnaire; NRT : nicotine replacement therapy; RNOS : reactive nitric oxide species; ROS : reactive oxygen species

Key Words: Smoking, Oxidative Stress, Biomarkers, Vitamin E, Gamma-Tocopherol, Ascorbate, Pregnancy, Smoking, Nicotine, Low Birth Weight, System Biology, Placenta, Calcification, Carbon Monoxide, Hypoxia, Prenatal Vitamins, Redox, Proteomics, Carbonyls, Review

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