Oxidative Stress and Antioxidant Status in Fetal Circulation in Preeclampsia

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ABSTRACT: Preeclampsia is associated with oxidative stress in maternal circulation. The purpose of this study was to explore oxidative stress and antioxidants in the fetal circulation in preeclampsia. Women with preeclampsia ($n = 19$) or uncomplicated pregnancies ($n = 33$) delivered by cesarean section were included. Blood was sampled separately from the umbilical vein and artery. 8-Iso-prostaglandin $F_{2\alpha}$ (8-isoprostane), a stable product of lipid peroxidation, is a reliable marker of oxidative stress. Concentration of total 8-isoprostane in cord plasma was analyzed by gas chromatography–mass spectrometry. Antioxidant status was evaluated measuring ferric reducing ability of plasma and vitamin E. There was no difference between preeclampsia and control groups regarding median plasma concentration of 8-isoprostane in umbilical vein (955 versus 780 pg/mL, $p = 0.41$) or in umbilical artery (233 versus 276 pg/mL, $p = 0.65$). Concentration of 8-isoprostane was much higher in plasma from the umbilical vein than artery, suggesting placenta as the source of 8-isoprostane. Median ferric reducing ability of plasma concentration was higher in preeclampsia than in controls, both in the umbilical vein and artery. Median vitamin E concentration in the umbilical vein was higher in preeclampsia, but no difference was found in the umbilical artery. In conclusion, no evidence of increased oxidative stress, evaluated by 8-isoprostane concentration, was found in fetal circulation in preeclampsia. (*Pediatr Res* 60: 560–564, 2006)

Preeclampsia is a pregnancy specific disorder, characterized by hypertension and proteinuria developing in the second half of the pregnancy. For the fetus, the preeclampsia syndrome could have serious consequences, including increased risk of fetal growth restriction and induced premature delivery due to disease progression on the maternal or fetal side. Maternal endothelial dysfunction is believed to be the common final pathway leading to the syndrome of preeclampsia. A dominating hypothesis is that agents produced by the relatively hypoxic placenta is transferred to the maternal circulation, causing endothelial dysfunction (1). Several candidates have been proposed as possible agents that directly or indirectly could mediate the endothelial dysfunction, including lipid peroxidation products (2,3), activated leukocytes (4), placental debris (5), or sFlt1, a vascular endothelial growth factor (VEGF) antagonist (6). To what extent such placental

agents affect the fetus, or are present in the fetal circulation at increased concentrations in preeclampsia, has not been fully explored. Few studies have addressed this issue, with conflicting results (7–10). If endothelial activating agents are present in the fetal circulation, they could cause immediate effects, such as endothelial activation, or even long-term consequences, such as the programming of adult diseases. The concept of intrauterine fetal programming, that an individual's adult health could be influenced by conditions and growth *in utero*, is convincingly documented in epidemiologic studies, including effects on blood pressure, cardiovascular risk factors and insulin resistance (11).

In studies of long-term effects of intrauterine exposure to preeclampsia, an increased risk of hypertension in the adolescent offspring (12,13) as well as decreased risk of breast cancer have been reported (14), but the mechanisms are unknown (15).

Oxidative stress is defined as an imbalance between free radical damage and antioxidant protection. The F2-class of isoprostanes includes 8-isoprostane, which is a stable product of free radical-catalyzed peroxidation of arachidonic acid in cell membrane phospholipids (16). 8-Isoprostane is considered a highly reliable and stable marker of oxidative stress, and is increased in many diseases associated with oxidative stress, such as diabetes and atherosclerosis (17,18). Increased oxidative stress has been proposed as a factor contributing to the endothelial dysfunction and pathophysiological changes in preeclampsia (3). Evidence for this concept includes studies demonstrating elevated levels of oxidative stress-markers in preeclampsia compared with normal pregnancies, in the placental tissue (19,20), in the maternal circulation (2,21), as well as in exhaled breath (22).

Presently, several clinical studies are assessing the effect of antioxidants, such as vitamin C and E, in the prevention of preeclampsia (23), calling for further focus on the fetal situation in preeclamptic pregnancies. Premature infants, with their decreased antioxidant defense, are susceptible to oxidant injury and diseases associated with oxidative stress, such as bronchopulmonary dysplasia and retinopathy of prematurity

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Abbreviations: FRAP, ferric reducing ability of plasma; GC-MS, gas chromatography–mass spectrometry; 8-isoprostane, 8-iso-prostaglandin $F_{2\alpha}$; MDA, malondialdehyde; sFlt1, soluble fms-like tyrosine kinase 1

(24–26). It is thus of interest to explore whether pregnancy complications such as preeclampsia add to the imbalance between pro- and antioxidants in the infant.

The aim of this study was to investigate the concentration of 8-isoprostane in plasma from the umbilical vein and artery of newborns from pregnancies complicated by preeclampsia, compared with uncomplicated pregnancies. We also wanted to evaluate the antioxidant system by measuring vitamin E and total antioxidants, measured as FRAP, in the same groups.

METHODS

Subjects. The included patients in this study were preeclamptic patients and women with uncomplicated pregnancies (called controls), all singleton pregnancies. The study is part of an ongoing biobank collection of patient samples from complicated and uncomplicated pregnancies at Ullevål University Hospital. The women included in the study were all delivered by cesarean section, all were fasting, none were in active labor and none had rupture of fetal membranes or signs of infection. Cesarean section was performed in the preeclamptic women ($n = 19$) because vaginal delivery was not considered appropriate due to disease progression and/or unfavorable cervical ripening. Controls ($n = 33$) were healthy, normotensive women undergoing cesarean section due to breech presentation, previous cesarean section or psychological reasons.

Preeclampsia was defined as rise in blood pressure after 20 wk gestation to $>140/90$ on two or more occasions at least 6 h apart in a previously normotensive woman, combined with proteinuria. Proteinuria was defined as protein dip stick $\geq 1+$ on two or more midstream urine samples at least 6 h apart or a 24-h urine excretion of ≥ 0.3 g protein. Severe preeclampsia was defined according to the criteria of American College of Obstetricians and Gynecologists (ACOG) (27), including preeclamptic women with systolic blood pressure ≥ 160 mm Hg. Pregnancy duration was calculated on the basis of routine ultrasonographic screening between gestational wk 17 and 20. The newborn birth weight percentiles were calculated according to Norwegian birth registry data (28). Medical history and obstetrical information was obtained from hospital records and the patients' national pregnancy charts, as well as by interviewing the patients before cesarean delivery. The study protocol was approved by the Regional Committee of Medical Ethics in Norway, and informed written consent was obtained from each patient.

Biologic samples. During the delivery of the infant, the umbilical cord was double clamped both close to the infant and close to the placenta. Immediately after delivery of the placenta, blood from the umbilical arteries (transporting blood from the fetus to the placenta) and blood from the umbilical vein (transporting blood from the placenta to the fetus) was sampled separately into EDTA-containing vials for vitamin E and FRAP analyses, and into cold tubes containing 1 mg/mL citrate and indomethacin 15 μ M for the 8-isoprostane measurements. The blood samples were kept on ice for maximum 30 min before centrifugation for 10 min at 2000 g at 4°C, and EDTA-plasma was stored at -76°C until FRAP and vitamin E analysis. The plasma for the 8-isoprostane analysis was collected and protected from oxidation by addition of butylated hydroxytoluene (BHT) (Sigma Chemical Co., St. Louis, MO), to prevent *ex vivo* oxidation, at a final concentration of 20 μ g/mL before storage at -76°C .

Total 8-isoprostane. Total 8-isoprostane concentration (free plus esterified 8-isoprostane) was measured by use of electron-capture negative-ion GC-MS by a collaborating laboratory in the United Kingdom, as previously described (29), after shipment on dry ice. All samples were measured in duplicates.

FRAP. FRAP was measured in plasma with a photometer analyzer (Technicon Instruments Corporation, New York, NY), essentially as described (30,31), with the exception that the sample was not diluted with water in the assay (21). All samples were measured in triplicates.

Vitamin E. A 50- μ L internal standard of α -tocopherol was added to 500 μ L of plasma. After addition of 0.5 mL water and 1 mL hexane, the solution was vortexed and centrifuged for 10 min at 4°C. The organic phase was removed and dried under a nitrogen stream at 4°C, and reconstituted in 100 μ L acetonitrile and transferred to WISP auto-injector vials. Aliquots of 40 μ L were injected on to HPLC and detection of tocopherol was carried out using fluorescence (excitation wavelength 295 nm and emission wavelength 430 nm). All samples were measured in duplicates.

Statistics. As all results were not normally distributed, medians are reported, and nonparametric Mann-Whitney *U* test was used for testing differences between groups. Pearson's correlation was used to calculate correlation coefficients. Statistical analyses were performed using Statistical Package for the Social Sciences (version 11.0; SPSS Inc, Chicago, IL). A probability level of ≤ 0.05 was considered statistically significant.

RESULTS

The clinical characteristics of the subjects included in the study are given in Table 1. Due to limited amount of fetal blood samples available, not all 19 included preeclamptic pregnancies described in Table 1 had all analyses performed.

For the preeclampsia group, median pregnancy duration was shorter and newborn weight was lower, but also the median weight percentile for infants born after preeclamptic pregnancies was lower than in the control group. In the control group, all infants were healthy and stayed in the maternity ward with their mothers after delivery. Five infants from preeclamptic pregnancies stayed in the maternity ward and 14 were transferred to the neonatal intensive care unit, as expected from their low gestational age ranging from 199 to 244 d. In the intensive care unit, three of these infants required ventilator, another six needed continuous positive airway pressure, and three infants were given antibiotics due to invasive procedures, but there was no culture proven sepsis. All infants in the preeclampsia group (as well as in the control group) survived the neonatal period.

The individual concentrations of total 8-isoprostane in plasma from umbilical vein and artery for the two study groups are illustrated in Figure 1. The group values for umbilical vein and artery concentrations of 8-isoprostane, FRAP, and vitamin E are summarized in Table 2. We found no statistically significant difference between the preeclampsia and control groups regarding median concentration of total 8-isoprostane in umbilical vein or in umbilical artery. As illustrated in Figure 1, there was a statistically significant

Table 1. Clinical characteristics for the patient groups included

	Preeclampsia ($n = 19$)	Control ($n = 33$)	<i>p</i> Value
Maternal age (y)	31.5 (19–39)	32.5 (22–38)	0.39
BMI before pregnancy (kg/m^2)	22.8 (19.4–36.7)	22.5 (18.3–28.9)	0.41
BMI at delivery (kg/m^2)	28.6 (22.5–39.5)	29.7 (23.6–37.5)	0.44
Gestational age (d)	235 (199–271)	271 (259–283)	$<0.001^*$
BP at delivery, systolic	160 (148–196)	120 (100–140)	$<0.001^*$
BP at delivery, diastolic	105 (90–119)	75 (60–92)	$<0.001^*$
Birth weight (g)	1719 (870–3856)	3472 (2800–4680)	$<0.001^*$
Birth weight percentile	29 (7–74)	51 (10–99)	0.007*

Values shown are medians and range (minimum and maximum values). Differences between the patient groups were tested using Mann-Whitney *U* test. BMI, body mass index (kg/m^2); BP, blood pressure.

* $p < 0.05$.

Figure 1. 8-Isoprostane concentrations (pg/mL) in umbilical vein and artery from preeclampsia ($n = 19$) and control ($n = 33$) pregnancies. Horizontal bars represent median values.

higher median concentration of 8-isoprostane in the umbilical vein compared with the umbilical artery for both pregnancy groups (preeclampsia group: umbilical vein 955 pg/mL *versus* umbilical artery 233 pg/mL, $p = 0.002$; control group: umbilical vein 780 pg/mL *versus* umbilical artery 276 pg/mL, $p < 0.001$). We found no correlation between gestational age and 8-isoprostane concentration in the umbilical vein or artery for the whole group or for the preeclampsia group alone (data not shown). There was no correlation between FRAP and 8-isoprostane or between vitamin E and 8-isoprostane concentrations in the umbilical artery or vein (data not shown).

Median FRAP concentration was higher in the preeclampsia group than in the control group, both in the umbilical vein and artery. Median vitamin E concentration was higher in the preeclampsia group in the umbilical vein plasma compared with the control group, whereas no difference between the two study groups was found in the umbilical artery (Table 2).

There was no statistically significant difference between the subgroups of severe preeclampsia ($n = 13$) and moderate preeclampsia ($n = 6$) regarding fetal median 8-isoprostane, FRAP, or vitamin E concentrations, neither for the umbilical vein, nor for the umbilical artery (data not shown).

We found no statistically significant correlation between the concentrations of 8-isoprostane in umbilical vein and umbilical artery (Pearson -0.12 , $p = 0.50$). In contrast, the levels of FRAP in umbilical vein and artery correlated positively for the whole group (Pearson 0.83 , $p < 0.001$) and for the preeclampsia group separately (Pearson 0.97 , $p < 0.001$), and the same was found for vitamin E concentrations (for the whole group: Pearson 0.84 , $p < 0.001$; for the preeclampsia group: Pearson 0.86 , $p = 0.003$).

DISCUSSION

In a previous publication by our group, we have reported the concentrations of the oxidative stress marker 8-isoprostane in the systemic circulation of the mothers of the newborns included in the present study (21). We reported that the preeclampsia group had a 62% higher median maternal plasma concentration of 8-isoprostane compared with the control group (354 and 218 pg/mL, $p = 0.02$). In the present study, we did not find that the increased maternal oxidative stress in preeclampsia was reflected in the fetal circulation; median 8-isoprostane concentration was not increased compared with control pregnancies, neither in umbilical vein nor artery. The much higher concentration of 8-isoprostane in umbilical vein plasma compared with umbilical artery plasma (Fig. 1), suggests that the placenta, and not the fetus, is the main source of fetal circulating 8-isoprostane. No correlation between previously published maternal (21) and fetal (neither umbilical vein nor artery) 8-isoprostane plasma concentration was found (neither for the whole patient group nor for the preeclampsia and control subgroups).

Few other studies have explored oxidative stress markers in the fetal circulation in preeclampsia. Kato *et al.* (8) reported a 26% higher mean concentration of malondialdehyde (MDA), another marker of oxidative stress, in preeclamptic pregnancies compared with controls, samples taken from umbilical vein at cordocentesis unaffected by labor. In contrast, Bowen *et al.* (7) found no difference between preeclampsia and control concentrations of MDA in umbilical vein. Recently pentosidine, an advanced glycation end product, which can also be used as a marker of oxidative stress, was explored in fetal circulation in preeclampsia (10). Mean pentosidine concentration was found to be 45% higher in blood from umbilical vein at delivery from infants born after preeclamptic pregnancies compared with controls, supporting a concept of elevated oxidative stress in the fetal circulation in preeclampsia.

To our knowledge, ours is the first study exploring oxidative stress in plasma separately from the umbilical vein and artery in preeclampsia. Other studies have used samples from the umbilical vein, which possibly does not give the most representative estimate of the fetal situation. Also, 8-isoprostane is considered a superior marker of oxidative stress. Other oxidized lipid products, such as MDA, represent more unstable products of lipid peroxidation, less specific and more likely to undergo auto-oxidation *ex vivo* (32).

Most studies of antioxidants in newborns report that concentrations of antioxidants are low, and even lower in premature infants (24,26,33). The fetal concentration of lipid soluble

Table 2. 8-Isoprostane, FRAP, and vitamin E concentrations in umbilical vein and artery

	Preeclampsia	Control	<i>p</i> Value
8-Isoprostane (pg/mL) umbilical vein plasma	955 (636–1050) ($n = 16$)	780 (576–1000) ($n = 33$)	0.41
8-Isoprostane (pg/mL) umbilical artery plasma	233 (221–346) ($n = 15$)	276 (238–320) ($n = 21$)	0.66
FRAP (μ mol/L) umbilical vein plasma	1101 (913–1191) ($n = 14$)	899 (807–966) ($n = 31$)	0.001*
FRAP (μ mol/L) umbilical artery plasma	1137 (885–1187) ($n = 10$)	881 (832–981) ($n = 14$)	0.001*
Vitamin E (μ mol/L) umbilical vein plasma	5.7 (3.6–7.1) ($n = 14$)	3.6 (3.3–4.5) ($n = 31$)	0.001*
Vitamin E (μ mol/L) umbilical artery plasma	4.7 (3.3–8.8) ($n = 10$)	3.7 (2.6–4.9) ($n = 13$)	0.19

Results are shown as medians (and 95% confidence interval of medians). Differences between the patient groups were tested using Mann-Whitney *U* test.

* $p < 0.05$.

antioxidants is probably limited by placental transport proteins, and these antioxidants are found in lower concentrations in fetal than maternal circulation (33,34). Vitamin E scavenges lipid soluble peroxide radicals and is the most important lipid soluble, chain-breaking antioxidant. It is consumed during peroxidation, and increased lipid peroxidation in preeclampsia could result in decreased plasma levels of vitamin E, described by some (35,36), but not all authors (37) in the maternal circulation in preeclampsia. In our previously published study of the mothers of the newborns reported in the present study, we did not find any difference in maternal vitamin E concentrations between preeclampsia and controls (21). In preeclampsia, the fetal antioxidant status is largely unexplored. Lower maternal levels of carotenoids was found in preeclamptic women, whereas no differences were found between preeclampsia and controls in their infants' umbilical vein cord blood (38); cord values were about one tenth of maternal values. We found one publication on umbilical vein vitamin E levels in preeclampsia, reporting no difference between preeclampsia and controls (7). In contrast, we found in the present study significantly higher concentrations of vitamin E in umbilical vein plasma in preeclampsia compared with controls (Table 2). In the umbilical artery, which might more correctly reflect the situation in the fetus, transporting blood from the fetus to the placenta, we found no statistically significant difference in vitamin E concentrations between the groups. We found that the median concentrations in the umbilical artery were about 10% of our previously reported median maternal values [38.5 μ M for both preeclampsia and control groups (21)]. Vitamin E concentration could be depending on the total lipid concentration (34), which might be higher in cord plasma in preeclampsia. Due to limited cord blood sample volumes, we have not measured total infant lipid concentrations in this study.

No agreed "golden standard" exists for analysis of antioxidant capacity. The FRAP assay, which measures the total concentration of redox-active compounds (*i.e.* antioxidants), is easy, rapid to perform and require little plasma volume. The modified FRAP assay (31) measures water-soluble antioxidants (including uric acid, vitamin C and bilirubin) as well as most fat-soluble antioxidants. FRAP is regarded a reliable measurement of over-all ability to resist oxidative damage (30). In our present study of fetal antioxidant capacity, median FRAP concentration was actually higher in the infants of the preeclampsia group compared with controls, despite lower median gestational age than the control group. FRAP levels in both umbilical artery and vein correlated positively with previously analyzed (21) maternal FRAP levels (Pearson correlation 0.9, $p < 0.001$). Uric acid concentration is elevated in maternal circulation in preeclampsia, which could influence maternal FRAP concentrations. We did not measure fetal uric acid concentrations in our study and are not aware of studies exploring this. It is therefore a limitation of this study that we are unaware of a potential effect on fetal FRAP by a possibly elevated uric acid concentration in infants of mothers with preeclampsia.

In this study of fetal oxidative stress, we limited the eligible study participants to those delivered by cesarean section without labor, to avoid the unpredictable oxidative stress resulting

from various length and intensity of labor (39) and thereby a possible effect on the fetal (and maternal) concentrations of 8-isoprostane or antioxidants.

Before this study, the SD for 8-isoprostane concentrations in umbilical artery and vein were not known. Assuming we wanted to detect at least one standardized difference between the groups, if present, we would need a total number of 30 patients to detect such a difference (with a standard significance level of 5% and a power of 80%) (40). Retrospectively, with mean 8-isoprostane in the umbilical artery (which may better reflect the fetal status than the umbilical vein) of 280 pg/mL, and a SD of 101 pg/mL for the control group, we could have detected a difference between the included preeclampsia and control group of 95 pg/mL (with a power of 80% and a significance level of 5%), if present.

A limitation of our study is that preeclamptic patients and controls were not matched for gestational length, and the median gestational age in the preeclampsia group was lower than in the control group (238 versus 271 d, Table 1). Obtaining umbilical cord blood from a group matching the premature preeclamptic pregnancies is difficult; cesarean section is not done in premature healthy pregnancies and cordocentesis would not be done for research purpose alone. Differences in gestational length between the study groups could potentially affect the conclusions. We did not find any correlation between gestational age and level of total 8-isoprostane in umbilical vein or artery in our whole study group, or in the preeclampsia group when analyzed separately. This is in contrast to a study by Comporti *et al.* (41), showing an inverse correlation between gestational age and concentrations of umbilical vein plasma F2-isoprostanes (measured as free isoprostanes only, not including phospholipid-bound isoprostanes), with higher levels in premature deliveries. The reason for premature delivery in their study was not stated.

In the paper originally describing the GC-MS method used in our study for measuring 8-isoprostane (29), the level of 8-isoprostane in adults was half of what was found in our group of healthy control infants. The median newborn level of 276 pg/mL in the umbilical artery would seem to be from a situation of "increased oxidative stress," if compared directly with adult values. We speculate that this higher level is "physiologic" for the newborn period, and could be a consequence of growth, development and increased metabolism in the fetus.

Intervention with antioxidants (vitamin E and C) has been tried in a clinical study in women at high risk for developing preeclampsia, with effect on reducing the prevalence of preeclampsia. No improved outcome was found in the infants (35), but the study was not powered to test fetal outcome. The authors also demonstrated a reduced longitudinal elevation of the oxidative stress marker 8-isoprostane in the group receiving antioxidants (42). Other interventions, such as reducing blood pressure in preeclamptic women, do not improve outcome for the infants (43). Despite epidemiologic evidence that antioxidants could be beneficial in diseases such as atherosclerosis, similar positive effects in clinical intervention studies have not been demonstrated (44). Antioxidants could also have potential side effects. Vitamin E given to very low birth weight babies, at risk for getting oxygen related diseases,

prevented retinopathy of prematurity, but was associated with an increased risk of sepsis (45). Multivitamins given to healthy infants was recently found to be associated with increased risk of asthma and food allergy (46).

In a recent study of breast-fed, full-term healthy infants, the concentration of F2-isoprostanes was found to be higher in infants than in adults (47). This was interpreted as augmented oxidative stress in infants, and antioxidant therapy was suggested. It has been said that "all studies of premature infants show that they are suffering from oxidative stress, but studies of antioxidants have showed no, minimal or controversial benefit" (26). In the light of this lack of demonstrated benefits and the possible harmful side effects of antioxidants, one has to evaluate carefully the short- and long-term effects on the infants of antioxidant intervention studies to prevent preeclampsia.

In conclusion, we found no difference in median fetal concentration of the oxidative stress marker 8-isoprostane between newborns of preeclamptic and control pregnancies, neither in umbilical cord vein nor artery. A previously demonstrated increased oxidative stress in the maternal circulation in preeclampsia is thus not reflected in the fetal circulation.

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