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Is There a Role for Isofurans and Neuroprostanes in Pre-Eclampsia and Normal Pregnancy?

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Abstract

Pre-eclampsia is a complex disorder of pregnancy that adversely affects the mother and baby. Arachidonic acid and docosahexaenoic acid are essential for fetal development and can undergo free radical oxidation to F_2-isoprostanes (F_2-IsoPs) and isofurans (IsoFs); and F_4-neuroprostanes (F_4-NeuroPs), respectively. These metabolites may be relevant to pre-eclampsia and fetal development. We examined IsoFs, F_4-NeuroPs, and F_2-IsoPs in maternal plasma and cord blood plasma of 23 women with pre-eclampsia and 21 normal pregnancies. Women with pre-eclampsia had significantly elevated maternal IsoFs and F_4-NeuroPs, but not F_2-IsoPs. Cord blood F_4-NeuroPs were elevated among neonates of women with pre-eclampsia. In women with pre-eclampsia, birth weight was predicted by gestation at delivery. The latter was also true in normal pregnancy, but birth weight was negatively related to maternal F_2-IsoPs, IsoFs, and F_4-NeuroPs. We have shown that in women with pre-eclampsia, IsoFs and F_4-NeuroPs are elevated, and cord blood F_4-NeuroPs are increased. The inverse relationship between maternal F_2-IsoPs, IsoFs, and F_4-NeuroPs and birth weight may be relevant as predictors of low birth weight in normal pregnancy. Future studies should examine whether these markers in maternal blood at early stages of pregnancy relate to subsequent maternal, fetal, and neonatal complications. *Antioxid. Redox Signal.* 16, 165–169.

Pregnancy, Pre-Eclampsia, and Oxidative Stress

Pre-eclampsia is a complex disorder of unknown etiology. Nulliparity is a major risk factor for pre-eclampsia, but other factors play an important role in the development of pre-eclampsia. For example, in women who subsequently develop pre-eclampsia, total cholesterol and triglycerides are elevated above those of normal pregnancy. F_2-isoprostanes (F_2-IsoPs) are formed from free radical attack on arachidonic acid (AA) and are considered to be good markers of *in vivo* lipid peroxidation (7). We have shown that plasma F_2-IsoP are raised in pre-eclampsia (1). In addition, increased urinary F_2-IsoPs at 16 weeks is associated with a heightened risk of developing pre-eclampsia, thus suggesting that oxidative stress may be important in the pathogenesis of this syndrome (8). Related compounds, such as isofurans (IsoFs) that are also formed from free radical-induced peroxidation of AA but under conditions of high oxygen tension, and F_4-neuroprostanes (F_4-NeuroPs) that are formed from docosahexaenoic acid (DHA, 22:6 ω 3), are likely to be relevant to neonatal

Innovation

Pre-eclampsia is a life-threatening disorder of pregnancy that adversely affects the mother and baby. Oxidative stress may contribute to the pathogenesis of this syndrome. Free radical oxidation of arachidonic acid and docosahexaenoic acid, both essential for fetal development, generates F_2-isoprostanes (F_2-IsoPs) and isofurans (IsoFs); and F_4-neuroprostanes (F_4-NeuroPs), respectively. We examined IsoFs, F_4-NeuroPs, and F_2-IsoPs in maternal plasma and cord blood plasma of 23 women with pre-eclampsia and 21 normal pregnancies. In women with pre-eclampsia, IsoFs and F_4-NeuroPs were elevated and cord blood F_4-NeuroPs increased. These new and important findings may have important clinical implications. Further studies are required to determine how these markers of oxidative stress in maternal and cord blood relate to subsequent maternal, fetal, and neonatal complications.

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outcomes in complicated pregnancies. DHA is essential for the growth and functional development of the fetal brain and is taken up by the brain, particularly during the last trimester, in preference to other fatty acids. F_4-NeuroPs may be important markers of brain-related oxidative stress (7). The high oxygen requirements of the brain suggest that IsoFs are also likely to be important indicators of brain oxidative stress (7). Although one of the F_2-IsoP isomers, 15-F_{2t}-IsoP, affects vascular and platelet function, the role of F_4-NeuroPs and IsoFs in vascular homeostasis has not been fully characterized. The aim of this study was to compare maternal plasma and cord blood plasma IsoFs, F_4-NeuroPs, and F_2-IsoPs in age-matched women with pre-eclampsia and normal pregnancies.

Measures of Lipid Oxidation in Pre-Eclampsia and Normal Pregnancy

Compared with normal pregnancy, women with pre-eclampsia did not differ with regard to age, body mass index (BMI), or smoking status (Table 1). Women with pre-eclampsia had significantly higher blood pressure, were more likely to be primigravid (43% *vs.* 10%), and their gestation at delivery was shorter. Proteinuria, determined by urinary dipstick, was >1+ in 58% of the women with pre-eclampsia. Birth weight, length, and head circumference of the babies born to these women were significantly smaller than those of normal pregnancies. The ratio of male to female babies in the two groups was not different. Umbilical cord arterial and venous blood gases did not differ between the groups. Arterial and venous bicarbonate (HCO_3^-) were significantly lower in the pre-eclampsia group, but pH was not different.

Table 1. Characteristics of Women with Normal Pregnancy or Pre-Eclampsia and Neonatal Outcomes

	Normal pregnancy (n=*21*)	*Pre-eclampsia* (n=*23*)
Age (year)	30.4±1.1	28.9±1.2
BMI (kg/m^2)	32.7±1.5	32±1.7[a]
Primigravida (*n*)	2	10[a]
Smokers (*n*)	6	3
Proteinuria ≥1+ dipstick (*n*)	0	14[b]
Systolic BP at delivery (mmHg)	129±3	152±3[a]
Diastolic BP at delivery (mmHg)	80±3	92±1[a]
Gestation at delivery (weeks)	38.8±0.1	33.9±0.9[a]
Neonatal Outcomes		
Gender M/F	9/12	11/13
Birth length (cm)	49.0±0.3	43.0±1.2[a]
Head circumference (cm)	35.2±0.3	30.6±0.8[a]
Umbilical blood gases		
PaO_2 (mmHg)	15.2±1.5	15.7±1.0
PvO_2 (mmHg)	27.5±3.0	23.8±1.6
$PaCO_2$ (mmHg)	56.7±2.1	54.4±1.3
$PvCO_2$ (mmHg)	45.4±1.4	46.5±1.2
Arterial HCO_3^- (mmol/L)	25.9±0.5	22.1±0.7[a]
Venous HCO_3^- (mmol/L)	24.1±0.4	22.1±0.6[a]
Arterial pH	7.28±0.01	7.28±0.01
Venous pH	7.34±0.01	7.32±0.01

Values are mean±SEM.
[a]$p<0.05$.
[b]$p<0.01$.
BMI, body mass index; BP, blood pressure.

Women with pre-eclampsia had increased levels of plasma F_4-NeuroPs and IsoFs compared with normal pregnancy ($p=0.007$ and $p=0.045$, respectively) (Fig. 1A, B). Maternal concentrations of plasma F_2-IsoPs did not differ between the groups (Table 2). Cord blood F_4-NeuroPs (Fig. 1C) were significantly higher in women with pre-eclampsia ($p=0.014$). In contrast, cord blood IsoFs (Fig. 1D) and F_2-IsoPs (Table 2) were not significantly different between the groups. Interestingly, cord blood IsoFs were approximately fivefold higher than levels in maternal plasma.

Univariate regression analyses showed that each of the maternal plasma IsoFs, F_4-NeuroPs, or F_2-IsoPs was not related to levels in cord blood plasma within either the pre-eclampsia or normal pregnancy groups. Cord blood IsoFs, F_4-NeuroPs, and F_2-IsoPs were not significantly correlated with umbilical cord arterial or venous blood gases, birth weight, or head circumference.

Multiple regression analysis was used to explore the relationship between markers of lipid peroxidation and birth weight. Gestation at delivery was a significant predictor of birth weight in both women with normal pregnancies ($p=0.001$) and those with pre-eclampsia ($p=0.0001$) (Table 3). In normal pregnancy, but not pre-eclampsia, inclusion of maternal plasma IsoF, or F_4-NeuroP or F_2-IsoP concentrations significantly improved the model (adjusted $R^2=0.252$ with gestation at delivery alone and $R^2=0.410$ with inclusion of maternal plasma IsoFs, $R^2=0.402$ with F_4-NeuroPs and $R^2=0.496$ with F_2-IsoPs). The model that best explained the variance in birth weight in normal pregnancy included a positive relationship with length of gestation ($\beta=0.596$, $p=0.001$) and a negative relationship with the sum of maternal plasma concentrations of IsoFs, F_4-NeuroPs, and F_2-IsoPs ($\beta=-0.544$, $p=0.002$). This model explained 53.6% of the variance in birth weight in normal pregnancy (Table 3). This relationship was also independent of smoking status, age, BMI, gravida status, and neonatal gender. In pre-eclampsia, gestation at delivery accounted for 81.6% of the variation in birth weight, and inclusion of the lipid oxidation measures did not significantly alter the model.

Do IsoFs, F_4-NeuroPs, or F_2-IsoPs Have a Role in Pre-Eclampsia and Pregnancy?

This study has shown for the first time that women with pre-eclampsia have significantly elevated levels of maternal plasma IsoFs and F_4-NeuroPs formed from free radical oxidation of AA and DHA, respectively. In addition, cord blood F_4-NeuroPs, but not IsoFs, from women with pre-eclampsia are significantly elevated compared with normal pregnancy. Maternal and cord blood F_2-IsoP were not different between the groups.

We have previously shown that plasma F_2-IsoPs were significantly elevated in women with proteinuric pre-eclampsia, matched for age and gestation with normal pregnancies (1, 2). These data were confirmed in several subsequent (4, 5), but not all, studies (6). The finding in our current study that plasma F_2-IsoPs were not significantly increased in women with pre-eclampsia relative to normal pregnancy likely relates to differences in the severity of the condition. In previous reports, we had shown that plasma F_2-IsoPs were increased in women who had been specifically

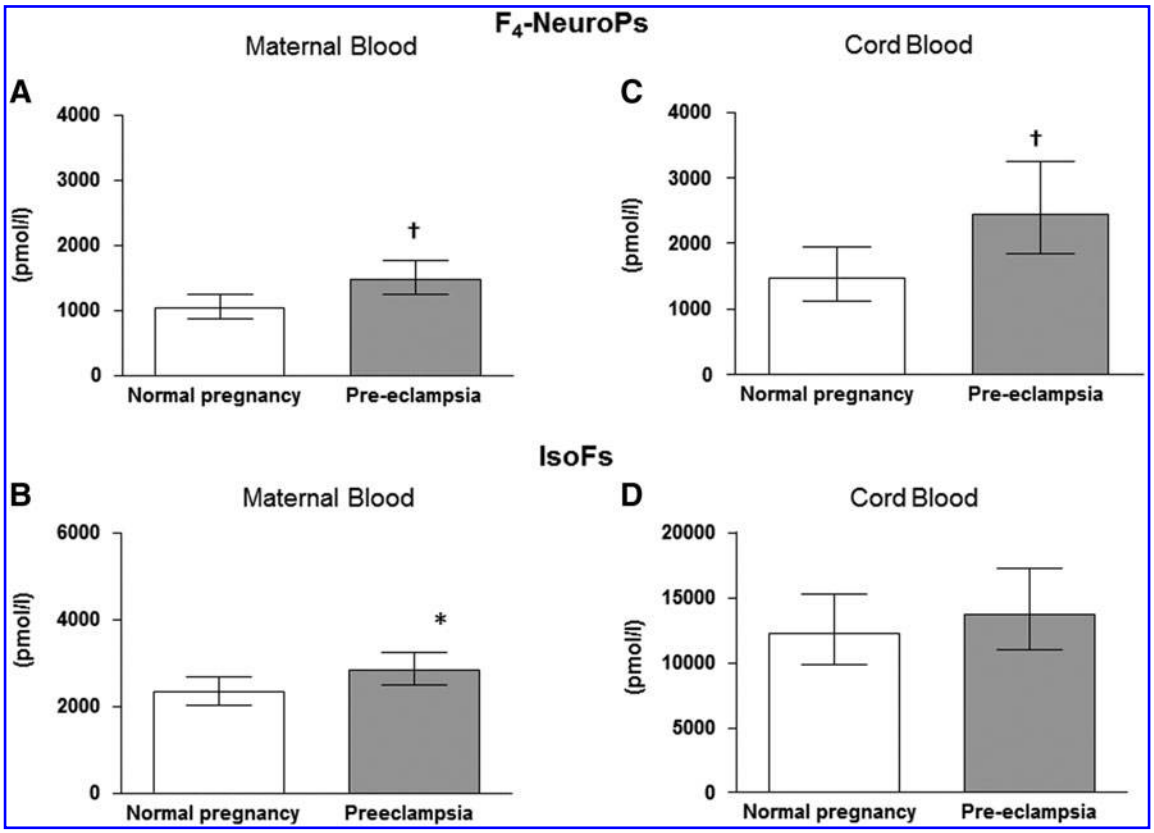

FIG. 1. Maternal blood F_4-NeuroPs (A) and IsoFs (B), and cord blood F_4-NeuroPs (C) and IsoFs (D) in women with pre-eclampsia or normal pregnancy. Values are geometric mean and 95%CI. $^*p \le 0.05$, $^\dagger p \le 0.001$. F_4-NeuroPs, F_4-neuroprostanes; IsoFs, isofurans.

recruited on the basis of having proteinuria >2+ on dipstick testing (mean proteinuria >2 g/day). Proteinuria was less pronounced in the women with pre-eclampsia in the current study, with 58% having a dipstick reading >1+. Another reason for the divergent results between studies may be the different timings of measurements. Our previous studies measured plasma F_2-IsoPs in pre-eclampsia and normal pregnancy at the same gestational age of ~30 weeks. The collection of cord blood samples in the current study necessitated all samples being taken at delivery, which was ~34 and 39 weeks in pre-eclampsia and normal pregnancy, respectively.

We have shown for the first time that cord blood F_4-NeuroPs from the oxidation of DHA were significantly elevated in pre-eclampsia relative to normal pregnancy. DHA is an essential fatty acid for fetal development. Studies have shown that apart from using DHA and AA from maternal fat deposits, the fetus is able to synthesize these fatty acids. Our study is not able to determine whether oxidation of DHA occurred within the fetus or the placenta. We found that maternal F_4-NeuroPs were not significantly correlated with cord blood F_4-NeuroPs in either pre-eclampsia or normal pregnancy, thus suggesting that the origin of cord F_4-NeuroPs may be independent of maternal plasma.

In our study, groups were not different for cord blood IsoFs and F_2-IsoPs concentrations. Varma *et al.* (9) also reported no differences in umbilical vein or artery F_2-IsoPs in women with pre-eclampsia and age-matched controls. To our knowledge,

Table 2. Maternal Plasma and Cord Blood Plasma F_2-isoprostanes in Normal Pregnancy or Pre-Eclampsia

	Normal pregnancy (n = 21)	*Pre-eclampsia* (n = 23)
Maternal plasma F_2-IsoPs (pmol/L)	2061 (1844, 2300)	2276 (2052, 2522)
Cord blood plasma F_2-IsoPs (pmol/L)	2071 (1799, 2411)	2482 (2126, 2899)

Values are geometric mean and 95% confidence intervals.
F_2-IsoPs, F_2-isoprostanes.

Table 3. Regression Models Examining Predictors of Birth Weight in Normal Pregnancy or Pre-Eclampsia

Predictor variable	*β*	p-*value*
Normal pregnancy		
Gestation at delivery (weeks)	0.596	0.001
Maternal plasma (F_2-IsoPs + IsoFs + F_4-NeuroPs)	−0.544	0.002
Adjusted $R^2 = 0.536$; $F_{2,18} = 12.54$, $p < 0.0001$		
Pre-eclampsia		
Gestation at delivery (weeks)	0.908	0.0001
Adjusted $R^2 = 0.816$; $F_{1,23} = 103.3$, $p < 0.0001$		

Maternal plasma (F_2-IsoPs + IsoFs + F_4-NeuroPs) was not a significant predictor in this model.
F_4-NeuroPs, F_4-neuroprostanes; IsoFs, isofurans.

ours is the first study to measure cord blood IsoFs. We showed that although IsoFs were not significantly different between the groups, their concentration in cord blood was approximately fivefold higher than that in maternal plasma. The very high levels of cord blood IsoFs most likely reflect the oxidative challenge presented at birth, when there is a transition from a relatively low intrauterine oxygen environment to a significantly higher extrauterine oxygen environment. This may have masked the ability to show any differences between the groups. An oxidative challenge is likely to be exacerbated by the low efficiency of natural anti-oxidant systems in the newborn.

We examined predictors of birth weight in babies born to women with pre-eclampsia and normal pregnancy by using multiple regression analysis. Gestation at delivery was not surprisingly a major positive predictor of birth weight in both groups. In normal pregnant women, maternal plasma IsoFs, F_4-NeuroPs, and F_2-IsoPs were negatively related to birth weight, and each independently improved the model. Birth weight in normal pregnancy was best predicted by a model that included the sum of maternal IsoFs, F_4-NeuroPs, and F_2-IsoPs, and gestation at delivery. This model accounted for 53.6% of the variation in birth weight. In contrast, in pre-eclampsia, gestation at delivery accounted for 81.6% of the variation in birth weight, and inclusion of the lipid oxidation measures did not significantly contribute. Our data are in accordance with a previous study which showed that increased markers of lipid peroxidation and DNA damage were related to lower birth weight in full-term deliveries (9).

Concluding Remarks and Future Directions

Our study has shown that increased maternal oxidation of both AA and DHA are important determinants of birth weight in normal pregnancy. It is possible that the relationship between these markers of lipid peroxidative stress and fetal growth are present earlier in pregnancy but confounded by other manifestations of pre-eclampsia. Future studies should examine these markers in maternal blood during early stages of pregnancy and relate these to subsequent maternal, fetal, and neonatal complications. Such studies may also elucidate whether measurement of IsoFs and F_4-NeuroPs is more sensitive than measurement of F_2-IsoPs in predicting complications during pregnancy. (A fully referenced discussion may be viewed as Supplementary Data, available online at www.liebertonline.com/ars).

Notes

Patients

Women undergoing caesarian section were recruited at King Edward Memorial Hospital for Women in Perth, Western Australia. All patients gave informed written consent to participate in the study that was approved by the human ethics committee of Women and Newborn Health Service of Western Australia. Twenty three patients with pre-eclampsia, defined using the criteria of International Society for the Study of Hypertension in Pregnancy, were recruited. Normal pregnant women ($n = 21$) undergoing elective caesarian section were matched for age and studied in parallel with the cases. Women with preexisting medical conditions or gestational diabetes were excluded from the study. Analysis was confined to those women who had singleton pregnancies, and all women received spinal anesthesia with bupivacaine and fentanyl.

Blood sampling

Maternal venous blood from the antecubital vein and umbilical cord venous blood obtained after delivery was collected into EDTA, reduced glutathione and butylated hydroxytoluene. Samples were centrifuged at 4°C, and plasma was stored at −80°C until analysis.

Measurement of plasma IsoFs, F_4-NeuroPs, and F_2-IsoPs

IsoFs, F_4-NeuroPs, and F_2-IsoPs were measured by gas chromatography-mass spectrometry using electron capture negative chemical ionization and a modification of our previously reported method (3). Samples were hydrolyzed with 1M potassium hydroxide in methanol, acidified, and applied to pre-washed Certify II cartridges (Varian, Lake Forrest, CA). After washing with methanol/water (1:1) and hexane/ethyl acetate (75:25) IsoFs, F_4-NeuroPs and F_2-IsoPs were eluted with ethyl acetate/methanol (90:10) and dried under vacuum. Samples were derivatized with pentafluorobenzylbromide and N,N-diisopropylethylamine (Sigma Chemicals, St. Louis, MO), dried under nitrogen, and treated with *N,O-bis*-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (Pierce Chemicals, Rockford, IL). IsoFs, F_4-NeuroPs, and F_2-IsoPs were quantitated by using d_4-15-F_{2t}-IsoP (5 ng) as an internal standard (Cayman Chemicals, Ann Arbor, MI) and monitoring ions at *m/z* 569, 573, 585, and 593, for F_2-IsoP, d_4-15-F_{2t}-IsoP, IsoFs, and F_4-NeuroPs, respectively. Standards for IsoF and 4(RS)-F_{4t}-NeuroP were synthesized in our laboratory as previously described.

Statistical analysis

Values are expressed as mean ± SEM or, where data were not normally distributed, geometric mean and 95% confidence intervals. Data were analyzed by using SPSS version 17. Between-group differences in continuous variables were assessed by using a General Linear Model with univariate analysis. Plasma IsoFs, F_4-NeuroPs, and F_2-IsoPs were transformed by using natural logarithms before analysis. Between-group differences in categorical data were analyzed by using a Chi-square test. Regression models were constructed within each group to examine predictors of birth weight. Predictors were selected on the basis that they were significantly correlated with birth weight but not with each other. Birth weight was entered as the dependent variable, and predictor variables (gestation at delivery, and either individual maternal plasma F_2-IsoPs, IsoFs, or F_4-NeuroPs, or Σ F_2-IsoPs, IsoFs, and F_4-NeuroPs) were entered stepwise.

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Abbreviations Used

AA = arachidonic acid
BMI = body mass index
BP = blood pressure
DHA = docosahexaenoic acid
F_2-IsoPs = F_2-isoprostanes
F_4-NeuroPs = F_4-neuroprostanes
IsoFs = isofurans