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 F2-isoprostanes (F2-IsoPs) and isofurans (IsoFs); and F4-neuroprostanes (F4-NeuroPs), respectively. These metabolites may be relevant to pre-eclampsia and fetal development. We examined IsoFs, F4-NeuroPs, and F2-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 F4-NeuroPs, but not F2-IsoPs. Cord blood F4-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 F2-IsoPs, IsoFs, and F4-NeuroPs. We have shown that in women with pre-eclampsia, IsoFs and F4-NeuroPs are elevated, and cord blood F4-NeuroPs are increased. The inverse relationship between maternal F2-IsoPs, IsoFs, and F4-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.

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. F2-isoprostanes (F2-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 F2-IsoPs are raised in pre-eclampsia (1). In addition, increased urinary F2-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 F4-neuroprostanes (F4-NeuroPs) that are formed from docosahexaenoic acid (DHA, 22:6 ω3), are likely to be relevant to neonatal oxidative stress in maternal and cord blood plasma of 23 women with pre-eclampsia and 21 normal pregnancies. In women with pre-eclampsia, IsoFs and F4-NeuroPs were elevated and cord blood F4-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.

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 F2-isoprostanes (F2-IsoPs) and isofurans (IsoFs); and F4-neuroprostanes (F4-NeuroPs), respectively. We examined IsoFs, F4-NeuroPs, and F2-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 F4-NeuroPs were elevated and cord blood F4-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. F₂-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 F₂-IsoP isomers, 15-F₂t-IsoP, affects vascular and platelet function, the role of F₂-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, F₂-NeuroPs, and F₂-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 (HCO₃⁻) were significantly lower in the pre-eclampsia group, but pH was not different.

Women with pre-eclampsia had increased levels of plasma F₂-NeuroPs and IsoFs compared with normal pregnancy (p = 0.007 and p = 0.045, respectively) (Fig. 1A, B). Maternal concentrations of plasma F₂-IsoPs did not differ between the groups (Table 2). Cord blood F₂-NeuroPs (Fig. 1C) were significantly higher in women with pre-eclampsia (p = 0.014). In contrast, cord blood IsoFs (Fig. 1D) and F₂-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, F₂-NeuroPs, or F₂-IsoPs was not related to levels in cord blood plasma within either the pre-eclampsia or normal pregnancy groups. Cord blood IsoFs, F₂-NeuroPs, and F₂-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 F₂-NeuroOr F₂-IsoP concentrations significantly improved the model (adjusted R² = 0.252 with gestation at delivery alone and R² = 0.410 with inclusion of maternal plasma IsoFs, R² = 0.402 with F₂-NeuroPs and R² = 0.496 with F₂-IsoPs). The model that best explained the variance in birth weight in normal pregnancy included a positive relationship with length of gestation (β = 0.596, p = 0.001) and a negative relationship with the sum of maternal plasma concentrations of IsoFs, F₂-NeuroPs, and F₂-IsoPs (β = -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, F₂-NeuroPs, or F₂-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 F₂-NeuroPs formed from free radical oxidation of AA and DHA, respectively. In addition, cord blood F₂-NeuroPs, but not IsoFs, from women with pre-eclampsia are significantly elevated compared with normal pregnancy. Maternal and cord blood F₂-IsoP were not different between the groups.

We have previously shown that plasma F₂-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 F₂-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 F₂-IsoPs were increased in women who had been specifically

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

<table>
<thead>
<tr>
<th></th>
<th>Normal pregnancy (n = 21)</th>
<th>Pre-eclampsia (n = 23)</th>
</tr>
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<tbody>
<tr>
<td>Age (year)</td>
<td>30.4±1.1</td>
<td>28.9±1.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.7±1.5</td>
<td>32±1.7</td>
</tr>
<tr>
<td>Primigravida (n)</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Proteinuria ≥1+ dipstick (n)</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Systolic BP at delivery (mmHg)</td>
<td>129±3</td>
<td>152±3</td>
</tr>
<tr>
<td>Diastolic BP at delivery (mmHg)</td>
<td>80±3</td>
<td>92±1</td>
</tr>
<tr>
<td>Gestation at delivery (weeks)</td>
<td>38.8±0.1</td>
<td>33.9±0.9</td>
</tr>
</tbody>
</table>

**Neonatal Outcomes**

- Gender M/F            | 9/12                     | 11/13                   |
- Birth length (cm)     | 49.0±0.3                 | 43.0±1.2                 |
- Head circumference (cm) | 35.2±0.3   | 30.6±0.8                |

**Umbilical blood gases**

- PaO₂ (mmHg)           | 15.2±1.5                 | 15.7±1.0                 |
- PaCO₂ (mmHg)          | 27.5±3.0                 | 23.8±1.6                 |
- PaO₂ (mmHg)           | 56.7±2.1                 | 54.4±1.3                 |
- PaCO₂ (mmHg)          | 45.4±1.4                 | 46.5±1.2                 |
- Arterial HCO₃⁻ (mmol/L) | 25.9±0.5       | 22.1±0.7             |
- Venous HCO₃⁻ (mmol/L)  | 24.1±0.4                 | 22.1±0.6                |
- Arterial pH            | 7.28±0.01               | 7.28±0.01               |
- Venous pH             | 7.34±0.01               | 7.32±0.01               |

Values are mean±SEM.

* p<0.05.
* p<0.01.
BMI, body mass index; BP, blood pressure.
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 F2-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 F4-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 F4-NeuroPs were not significantly correlated with cord blood F4-NeuroPs in either pre-eclampsia or normal pregnancy, thus suggesting that the origin of cord F4-NeuroPs may be independent of maternal plasma.

In our study, groups were not different for cord blood IsoFs and F2-IsoPs concentrations. Varma et al. (9) also reported no differences in umbilical vein or artery F2-IsoPs in women with pre-eclampsia and age-matched controls. To our knowledge,

Table 2. Maternal Plasma and Cord Blood Plasma F2-Isoprostanes in Normal Pregnancy or Pre-Eclampsia

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>β</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Normal pregnancy</td>
<td></td>
<td></td>
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<tr>
<td>Gestation at delivery (weeks)</td>
<td>0.596</td>
<td>0.001</td>
</tr>
<tr>
<td>Maternal plasma (F2-IsoPs + IsoFs + F4-NeuroPs)</td>
<td>-0.544</td>
<td>0.002</td>
</tr>
<tr>
<td>Adjusted $R^2 = 0.536; F_{2,18} = 12.54, p &lt; 0.0001$</td>
<td></td>
<td></td>
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<tr>
<td>Pre-eclampsia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestation at delivery (weeks)</td>
<td>0.908</td>
<td>0.0001</td>
</tr>
<tr>
<td>Adjusted $R^2 = 0.816; F_{1,23} = 103.3, p &lt; 0.0001$</td>
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</table>

Maternal plasma (F2-IsoPs + IsoFs + F4-NeuroPs) was not a significant predictor in this model.

F4-NeuroPs, F4-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 extraterine 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, F4-NeuroPs, and F2-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, F4-NeuroPs, and F2-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 F4-NeuroPs is more sensitive than measurement of F2-IsoPs in predicting complications during pregnancy. (A fully referenced discussion may be viewed as Supplementary Data, available online at www.liebertonline.com/ars).
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References


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Abbreviations Used

AA = arachidonic acid
BMI = body mass index
BP = blood pressure
DHA = docosahexaenoic acid
F2-IsoPs = F2-isoprostanes
F4-NeuroPs = F4-neuroprostanes
IsoFs = isofurans