Reduced Selenium Concentrations and Glutathione Peroxidase Activity in Preeclamptic Pregnancies

Hiten D. Mistry, Vicky Wilson, Margaret M. Ramsay, Michael E. Symonds, Fiona Broughton Pipkin

Abstract—Preeclampsia is pregnancy-specific, affecting 2% to 7% of women, and is a leading cause of perinatal and maternal morbidity and mortality. Preeclampsia may also predispose the fetus to increased risks of adult cardiovascular disease. Selenium, acting through the selenoprotein glutathione peroxidases, has critical roles in regulating antioxidant status. Recent reports implicate poor maternal selenium status as a nutritional factor predisposing the mother to preeclampsia but the fetus and placenta have not been studied in tandem. Measurement of selenium concentrations, expression, and activity levels of glutathione peroxidase and markers of oxidative stress were performed on maternal and umbilical venous blood samples or the placenta from 27 normal pregnant, 25 preeclamptic, and 22 healthy age-matched nonpregnant women. The results of this study revealed highly significant reductions in serum selenium concentrations and plasma glutathione peroxidase activity in pregnancy per se compared to nonpregnant controls. Moreover, these levels were further decreased in the preeclamptic mothers and babies compared to normal pregnancies. Umbilical venous selenium was particularly low (42.1 ± 11.8 and 29.0 ± 9.9 μg/L; mean ± SD; P < 0.05). Both mother and baby had significantly increased levels of markers for oxidative stress in the preeclamptic group. The placental glutathione peroxidase activity and immunohistochemical staining were also reduced in the preeclampsia placentae. Oxidative stress associated with preeclampsia may be a consequence of reduced antioxidant defense pathways specifically involving glutathione peroxidases, perhaps linked to reduced selenium availability. Reduced glutathione peroxidases could be associated with increased generation of toxic lipid peroxides contributing to the endothelial dysfunction and hypertension of preeclampsia. (Hypertension. 2008;52:881-888.)

Key Words: pregnancy ■ human ■ preeclampsia ■ hypertension ■ oxidative stress ■ selenium ■ glutathione peroxidase ■ placenta

Preeclampsia is estimated to occur in 2% to 7% of all pregnancies and is a leading cause of maternal and perinatal mortality and morbidity in the Western world. The effects of the disease are not restricted to pregnancy, as it also predisposes both the mother and baby to adult cardiovascular disease. Preeclampsia is now commonly regarded as being a state of oxidative stress (see: ). It is thought that excessive production of reactive oxygen species (ROS), secondary to reduced placental perfusion, results in oxidative stress, playing a critical role as a possible mediator of endothelial cell dysfunction, hypertension, and thus clinical manifestations of preeclampsia.

The trace element selenium is an essential component of the antioxidant selenoproteins, including glutathione peroxidases (GPx). These remove the products of attack by hydroperoxides and oxidized lipoproteins, and so limit adverse effects on the endothelium. Various forms of GPx are found in vertebrates: the cellular and cytosolic GPx (GPx1), the cytosolic gastrointestinal GPx (GPx2), the extracellular plasma GPx (GPx3), and the phospholipid hydroperoxide GPx (GPx4). Two studies have shown decreases in maternal serum or toenail selenium concentrations in preeclamptic patients compared to normal pregnant controls, and other studies have reported significant reductions in maternal plasma and placental GPx activities in preeclamptic patients. We are not aware of any studies linking selenium and GPx in the mother, placenta, and baby. We hypothesized that fetal selenium and GPx concentrations would also be reduced in preeclamptic pregnancy, which could contribute to present morbidity and future cardiovascular risk. We have therefore conducted a cross-sectional study to explore these factors in tandem comparing patients and their babies from normal and preeclamptic pregnancies and, when appropriate, nonpregnant controls.

Methods

Power Calculations

We calculated that a sample of 25 normal pregnant and 25 preeclamptic women would give us a 95% power of detecting a 30% difference in serum selenium concentration.
Subjects
The study population consisted of 3 groups of white women: 27 normal, 25 preeclamptic, and 22 healthy age-matched women. The investigations were approved by the Hospital Ethics Committee of the Nottingham University Hospitals; written informed consent was obtained from each participant. Preeclampsia was stringently defined.15 Medical and obstetric histories were obtained for each woman. The birthweight centile for each baby was computed, correcting for gestation age, sex, maternal parity, and body mass index (BMI).16

Sample Collection
Venous blood samples were taken from mothers before delivery; where possible, umbilical venous samples were also taken, immediately after placental delivery. Samples were taken into chilled tubes containing either EDTA (for GPx assays) or heparin (for thiobarbituric acid reactive substances (TBARS) assay) or into plain glass tubes (for selenium measurement). Plasma and serum were stored at −80°C. Full depth placental tissue samples were collected within 10 minutes of delivery from 3 standardized locations (1 cm from the cord, 1 cm from the periphery, and the middle of the two), avoiding placental infarcts. All membranes were removed and tissue washed in 1×PBS to remove maternal blood contamination; one set was snap frozen and stored at −80°C. Another set was formalin fixed and wax-embedded for immunohistochemical analysis.

Tissue Preparation
Placental tissue fragments (100 mg) were thawed and homogenized in 3 volumes of radio-immunoprecipitation (RIPA) buffer (Sigma-Aldrich) or 0.5% sodium deoxycholate using an Ultra Turrax homogenizer. Samples were centrifuged (3000g, 10 minutes, 4°C) and supernatants were stored at −80°C. Protein concentrations were determined using the Lowry method.17

Biochemical Measurements
Plasma concentrations of TBARS were measured by the method of Urchiyama and Milhara.18 Samples were assayed in duplicate; the within- and between-assay coefficients of variation were 4% and 5%, respectively, with a lower detection limit of 0.2 μmol/L.

Selenium concentrations were determined by a Varian SpectrAA graphite furnace atomic absorption spectrophotometer.19 Standard reference samples (Seronorm and serum control [Nycomed Pharma AS] and UTAK trace element control, normal level) were used. The intra- and interassay coefficients of variances were <5% for both with a lower detection limit of 0.3 μg/L.

mRNA Expression Measurements
Total RNA was extracted from placental tissue using Tri-Reagent (Sigma-Aldrich). After RNA extraction, 1 μg of each sample was reverse transcribed in 20 μL reaction buffer (Roche Diagnostics) in a Touchgene Gradient thermocycler (Techne, Barloworld Scientific Ltd). RNA concentration and quality were verified by gel electrophoresis and spectrophotometrically; all samples had an A260/A280 ratio greater than 1.96 and were stored at −80°C.

Standards for GPx 1, 2, 3, 4, and the housekeeping gene 18S ribosomal RNA were made from cDNA obtained from a randomly selected control placental sample using semiquantitative polymerase chain reaction (PCR). The method used oligonucleotide primers to GPx 1, 2, 3, 4, and 18S genes generating specific intron-spanning products (Table 1). The annealing temperature (60°C) and cycle number (40) of all primers were optimized and used in their linear range. The resultant PCR product was extracted after agarose gel electrophoresis (QiAquick gel extraction kit, Qiagen, cat no. 28704), sequenced, and results cross-referenced against the Genbank website to determine specificity of the target gene. Extracted PCR products were resuspended in nuclease-free water and a 10-fold serial dilution performed. Standards were stored at −20°C until use in quantitative PCR.

Quantitative real time PCR (Techne Quantica 14 real-time thermocycler; Techne, Barloworld Scientific Ltd) using SYBR Green Master Mix (with ROX passive reference dye [Qiagen Ltd]) was used to examine the expression of GPx 1, 2, 3, 4, and 18S. Three negative control reactions were carried out with each set of samples analyzed:1 no RNA template but RT and polymerase provided;2 RNA and polymerase provided but no RT; and3 RNA and RT provided but no polymerase.

GPx Activity Assay
GPx activity was determined by a modified method of Paglia and Valentine20 in both plasma and placental extracts. Briefly, 900 μL of assay mix containing 0.1 mol/L potassium phosphate, pH 7.0; 2 mmol/L EDTA; 0.5 μmol/L glutathione reductase; 10 mmol/L glutathione and 0.3 mmol/L reduced NADPH was placed into a WPA Biotech spectrophotometer set at 340 nm. Diluted tissue extracts or plasma (50 μL of 1/10 dilution) were added to the cuvette along with 50 μL of 20 mmol/L tert-butyl hydroperoxide.

The decrease in A340 was determined over a 3-minute period, and rate calculations were performed. GPx activity was standardized against protein concentrations and expressed as mmol/min/mg protein or mmol/min/mL for plasma samples; the inter- and intraassay variations were <5%.

Immunohistochemistry
Immunohistochemical analysis was performed on 5-μm serial sections of paraffin-embedded placental tissue using the Dako Envision visualization system (Dako). All GPx antibodies were purchased from Autogen Bioclear. The GPx1 antibody (polyclonal) was used at 1:1000 dilution, GPx3 (monoclonal) at 1:100, and GPx4 (polyclonal) at 1:250. Positive control tissues were thymus, prostate, and tongue, respectively. Antibodies to cytokeratin (CK-7) and CD-68 (both monoclonal; Santa Cruz Biotechnology) were used at 1:1 and 1:50 to confirm positive GPx staining in cytrophoblast and Hofbauer cells, respectively. A negative control was performed for each test section by omitting incubation in the primary antibody. Quantification was performed at ×400 magnification (Leica DM RB microscope) using a previously described method that allows counting of positively stained cells/areas and requires no corrections based on other estimated quantities21; Improvision Openlab 4.0.2 software was used for quantification. All analysis was performed blinded as to group by the same assessor using a reference slide to check for consistency.

Statistical Analysis
All tests were performed using SPSS for Windows version 14.0. Summary data are presented as means±SDs or median (interquartile range) as appropriate. Between group comparisons were made using 1-way ANOVA (with post hoc Gabriels test if significant)/Kruskal-Wallis tests or 2-tailed Student t test/Mann–Whitney U-tests depending on the distribution. Correlations between the parameters were tested with either Pearson or Spearman Ranks correlation tests. Discriminant function analysis (DFA)22 using the method of Wilks was used to determine which of the markers studied allowed best discrimination between normal pregnancy and preeclampsia. The null hypothesis was rejected where P<0.05.

Table 1. Primers, Sequences, Product Sizes, and BLAST Sequence Numbers for the Different GPx Measured in This Study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequences (F=Forward; R=Reverse)</th>
<th>BLAST Sequence No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx1</td>
<td>F: CACCAAGTTTGGCATCA R: CCGTTCACCTGCGACTTC</td>
<td>NM_000581</td>
</tr>
<tr>
<td>GPx2</td>
<td>F: TGTTGCCCTGCTCTGAGG R: GTTCTCGTGATGTCAAA</td>
<td>NM_002083</td>
</tr>
<tr>
<td>GPx3</td>
<td>F: GATGGAGGACTACATCC R: ACCAGTGTGCAAGGTC</td>
<td>NM_002084</td>
</tr>
<tr>
<td>GPx4</td>
<td>F: GGCATAAGCTGCAATTGCG R: GCAGTCTCTTCTCTATCA</td>
<td>NM_002085</td>
</tr>
</tbody>
</table>

Master Mix (with ROX passive reference dye [Qiagen Ltd]) was used to examine the expression of GPx 1, 2, 3, 4, and 18S. Three negative control reactions were carried out with each set of samples analyzed:1 no RNA template but RT and polymerase provided;2 RNA and polymerase provided but no RT; and3 RNA and RT provided but no polymerase.
The normal pregnancy group gave birth without any pregnancy complications, to infants weighing significantly different between pregnancy groups (Table 2). No pregnant woman was taking medication other than paracetamol or prescribed antihypertensive agents. BMI was not taking any medication, including oral contraceptives. No pregnant woman was taking medication other than paracetamol or prescribed antihypertensive agents. BMI was not significantly different between pregnancy groups (Table 2). The normal pregnancy group gave birth without any pregnancy complications, to infants weighing >2500 g, delivered 37 weeks or later. Overall, the preeclamptic women all had moderate to severe disease, without HELLP, and had lower gestational ages at delivery than the control group ($P<0.0001$); (Table 2). All Caesarean sections in the control group, and 5 in the PE group, were performed under epidural anesthesia as emergencies during labor; 6 PE women had elective Caesarean sections for worsening maternal or fetal condition. All neonates from both pregnancy groups survived.

### Results

#### Subjects

Table 2 describes the demographic, obstetric, and pregnancy data of the 74 participants. Both pregnancy groups conceived spontaneously and carried singleton pregnancies. Parous controls had all delivered healthy babies without any pregnancy complications. None of the nonpregnant controls were taking any medication, including oral contraceptives, No pregnant woman was taking medication other than paracetamol or prescribed antihypertensive agents. BMI was not significantly different between pregnancy groups (Table 2). The normal pregnancy group gave birth without any pregnancy complications, to infants weighing >2500 g, delivered 37 weeks or later. Overall, the preeclamptic women all had moderate to severe disease, without HELLP, and had lower gestational ages at delivery than the control group ($P<0.0001$); (Table 2). All Caesarean sections in the control group, and 5 in the PE group, were performed under epidural anesthesia as emergencies during labor; 6 PE women had elective Caesarean sections for worsening maternal or fetal condition. All neonates from both pregnancy groups survived.

#### Biochemical and Molecular Measurements

The values of plasma TBARS, serum selenium concentrations, and plasma GPx activities are given in Table 3. Maternal plasma TBARS levels were significantly higher in preeclampsia compared to both normal pregnancy and non-pregnant controls ($P<0.0001$); no significant differences were observed between nonpregnant and normal pregnancy samples. Umbilical venous TBARS concentrations were also higher in preeclamptic than normal pregnancy, the difference approaching statistical significance ($P=0.058$).

There was a highly-significant trend for decreasing plasma selenium concentrations from nonpregnant to normal pregnant and preeclamptic women (Table 3; Kendall’s $\tau$, $P<0.001$). Selenium concentrations were significantly reduced in umbilical venous samples in preeclampsia by

### Table 2. Demographic and Pregnancy Data of Subject Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nonpregnant</th>
<th>Normal Pregnant</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td>30±10.2</td>
<td>29±6.8</td>
<td>32±5.8</td>
</tr>
<tr>
<td>Primipara, n (%)</td>
<td>...</td>
<td>16 (59.3)</td>
<td>17 (68)</td>
</tr>
<tr>
<td>Booking body mass index, kg/m²</td>
<td>22.7±4.8*</td>
<td>26.6±5.8</td>
<td>27.3±5.6</td>
</tr>
<tr>
<td>Max. systolic blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>labor, mm Hg</td>
<td>...</td>
<td>116±4.3</td>
<td>159±8.8†</td>
</tr>
<tr>
<td>Max. diastolic blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>outside labor, mm Hg</td>
<td>...</td>
<td>76±2.8</td>
<td>98±4.9†</td>
</tr>
<tr>
<td>Proteinuria, g/L</td>
<td>...</td>
<td>...</td>
<td>1.0 (0.3, 11.5)</td>
</tr>
<tr>
<td>Gestation age at delivery, wks</td>
<td>...</td>
<td>40±1.1</td>
<td>36.4±3.8†</td>
</tr>
<tr>
<td>Caesarean section, n (%)</td>
<td>...</td>
<td>4 (15)</td>
<td>11 (44)†</td>
</tr>
<tr>
<td>Birthweight, kg</td>
<td>...</td>
<td>3.55 [3.25, 3.86]</td>
<td>2.92 [1.92, 3.51]†</td>
</tr>
<tr>
<td>Birthweight centile</td>
<td>...</td>
<td>45 [23, 67]</td>
<td>35 [2, 87]</td>
</tr>
<tr>
<td>Placental/birthweight ratio</td>
<td>1.9±0.3</td>
<td>2.3±1.1</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean±SD or median [IQR] as appropriate, except for proteinuria: (median (min, max) and parity and Caesarean sections (No. (percentage)). *$P<0.05$ between nonpregnant and both pregnancy groups; †$P<0.05$ between normal and preeclamptic pregnancies.

### Table 3. Maternal and Umbilical Venous TBARS, Selenium Concentrations, and GPx Activities

<table>
<thead>
<tr>
<th>Biochemical Measurement</th>
<th>Nonpregnant</th>
<th>Normal Pregnant</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal TBARS, µmol/L</td>
<td>0.40 [0.2, 0.7]</td>
<td>0.45 [0.2, 0.8]</td>
<td>1.2 [0.6, 1.6]$§</td>
</tr>
<tr>
<td>Umbilical venous TBARS,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>µmol/L (median [IQR])</td>
<td>...</td>
<td>0.6 [0.2, 0.8]</td>
<td>0.8 [0.5, 1.0]</td>
</tr>
<tr>
<td>Maternal selenium, µg/L</td>
<td>69.8±11.7*</td>
<td>58.4±14.9</td>
<td>39.7±13.8§</td>
</tr>
<tr>
<td>Umbilical venous selenium,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>µg/L (median ±SD)</td>
<td>42.1±11.8</td>
<td>29.0±9.9†</td>
<td></td>
</tr>
<tr>
<td>Maternal plasma GPx activity,</td>
<td>0.82 [0.7, 0.9]*</td>
<td>0.53 [0.4, 0.6]</td>
<td>0.32 [0.1, 0.4]$§</td>
</tr>
<tr>
<td>per mL (median [IQR])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Umbilical venous GPx activity,</td>
<td>...</td>
<td>0.51 [0.4, 0.6]</td>
<td>0.39 [0.2, 0.5]†</td>
</tr>
</tbody>
</table>

Data are shown as mean±SD or median [IQR] as appropriate.

*$P<0.005$ between nonpregnant and both pregnancy groups; †$P<0.02$, ‡$P<0.005$, §$P<0.0001$ between normal and preeclamptic pregnancies.
comparison with samples from babies of normotensive mothers ($P<0.0001$). There was also a highly significant trend for decreasing plasma GPx activity from nonpregnant, to normal pregnant and preeclamptic women (Table 3; Kendall’s $\tau$, $P<0.001$), mirrored in significantly lower umbilical venous plasma GPx activities in preeclampsia than in normal pregnancy samples ($P<0.005$). To ensure that the observed differences in both selenium concentration and GPx activities were not related to gestation age at delivery, we also compared these data with controls only for the 15 preeclamptic women and their babies who delivered at ≥37 weeks’ gestation. All comparisons remained statistically significantly different at between $P<0.001$ and $P<0.0001$. There was a highly-significant overall correlation between maternal serum selenium concentrations and placental GPx activity (Figure 1a and 1b; $r=0.49$; $P<0.005$ and $r=0.38$; $P=0.005$, respectively), but no significant association within individual groups, or between umbilical venous serum selenium and GPx activity. Mode of delivery did not significantly affect either maternal or fetal selenium concentration or GPx activity ($P>0.2$ for all comparisons).

No significant differences were observed in placental mRNA expression levels of GPx1, 3, and 4 ($P>0.1$; Table 4). As expected, no biologically significant placental GPx2 mRNA expression was observed. Significantly lower activity of placental GPx activity was seen in preeclampsia compared to normal pregnancy placenta ($P<0.0001$; Table 4). No significant differences in placental GPx activities were seen between sampling sites (Freidman test $P>0.1$), thus mean values are represented. A significant negative correlation was observed between placental GPx activity and maternal TBARS concentrations in preeclampsia only (Figure 2).

Because of the large spread of the corrected birthweight centiles in the preeclampsia group (Table 3), we examined maternal GPx activity in mothers who gave birth to infants above and below the 50th centiles. Univariate analysis of variance indicated that both pregnancy group and centile above/below 50th centile significantly react with maternal GPx activities ($P<0.001$, $P=0.021$, respectively; Figure 3).

Immunohistochemistry

Immunohistochemistry of GPx 1, 3, and 4 indicated positive expression in cytotrophoblast and Hofbauer cells (Figure 4). Quantification revealed that the expression was significantly reduced in preeclampsia for all 3 GPx antibodies (GPx1 and GPx3: $P<0.05$; GPx4: $P<0.001$; Figure 4). Location of positively stained areas was confirmed by staining with CK-7 and CD-68 antibodies (Figure 5). No significant differences in expression were observed from the different placental sampling sites for any of the antibodies tested.

Discriminant Function Analysis

Stepwise DFA, applied to the whole sample, using maternal serum selenium concentrations and plasma GPx activity, produced a significant Wilks’ lambda test ($P<0.0001$; Table 5). DFA using these measurements correctly predicted...
82% of the subjects, with correct classification of 84.6% of normal pregnancies and 78.3% of preeclamptic patients (Table 5).

Discussion

The significantly raised concentration of TBARS, a surrogate measure of lipid peroxidation, in the maternal circulation in preeclampsia confirms previous reports. However, the previous studies have focused on maternal parameters, and only a single study appears to have been carried out in the fetus. We showed that umbilical venous concentrations of TBARS were also significantly increased after preeclamptic pregnancy, indicative of increased oxidative stress. Defective placentation in preeclampsia could prevent the active placental transport of these lipid peroxides away from the fetus, thus increasing the levels of TBARS in the umbilical venous samples. Prenatal exposure to raised lipid peroxidation might be one of the factors predisposing the baby of the preeclamptic mother to later cardiovascular disease. Although the numbers of Caesarean sections were significantly higher in the preeclamptic group, Mehmetoglu et al found no significant differences in TBARS concentrations between vaginal deliveries and Caesarean sections in normotensive pregnancy.

Worryingly, the serum selenium concentrations in the nonpregnant women were lower than the recommended levels, as observed in other studies (eg, Reference 27), suggesting that the selenium concentrations may not be high enough for optimum GPx activities even in the nonpregnant population of the United Kingdom. Selenium concentrations are further reduced in normal pregnancies (Table 3), illustrating a possible increased requirement for selenium during pregnancy. This could also be attributable to inadequate absorption from the gastrointestinal tract, or inadequate renal reabsorption given the increased glomerular filtration rate of pregnancy. Our study also showed a further significant reduction in maternal selenium concentrations in the preeclamptic samples compared to both the normal pregnant and nonpregnant controls; this is in line with other studies which measured serum selenium in samples with widely varying gestational ages, and in toenail selenium concentrations respectively. These reduced selenium concentrations might adversely affect the functional activities of the selenoproteins, compromising protection against oxidative stress. Also, a recent report linked increased selenium intake over 2 years with significantly decreased excretion of the major thromboxane metabolite. An early imbalance between thromboxane and prostacyclin synthesis has been implicated in the pathogenesis of preeclampsia over the last 20 years (eg, References 29, 30).

The fetus acquires its selenium via placental transport; in the present study, selenium concentrations were significantly lower in the neonates of both groups, as compared to matched maternal serum. Selenate and sulfate share a saturable transport mechanism across the human placenta, via the sodium-independent NaS2 transporter, but we are not aware of any studies of PE placentae.

The reduced selenium in the maternal and fetal circulations in preeclampsia are associated with lower maternal and umbilical venous plasma GPx activities observed in the preeclamptic samples, supporting the hypothesis that insufficient antioxidant defense may be a contributing factor to the pathophysiological mechanisms associated with oxidative stress and preeclampsia. The reduced plasma activities in the babies born to preeclamptic pregnancies in this study parallel the observation of reduced erythrocyte GPx. Our results thus indicate a potentially-important reduction in a component of the global antioxidant defense levels in both the mother and baby.
Small size at birth has been postulated to increase the risks of cardiovascular disease in later life. Maternal plasma GPx activity clearly differed by pregnancy outcome, in terms both of the presence or absence of preeclampsia and whether the birthweight centile was above or below 50% (Figure 3). The higher plasma GPx in smaller babies was unexpected, but might reflect an adaptive response in these intrauterine growth restricted babies to try and protect them from oxidative stress. To our knowledge, this is a novel observation.

This is the first study to show the presence of 3 of the main forms of GPx in the human placenta. Wang & Walsh, who did not differentiate the different GPxs, used Northern analysis and reported a significant decrease in expression in pre-eclampsia, but our real-time PCR analysis of individual mRNA expression showed no significant differences in any of the GPxs. However, both the immunohistochemical staining of all 3 GPx antibodies (Figure 4), and placental enzyme activities were significantly lower in the preeclamptic compared to normal placentae. This suggests a possible posttranslational modification/mutation reducing GPx antioxidant activity such as has been observed outside pregnancy. GPx in the cytotrophoblast cells is ideally located to be transported into both the maternal and fetal circulations; these cells are exposed to relatively high oxygen concentrations, and thus the GPxs may also protect the cells from oxidative stress.

Because of the role of Hofbauer cells in vasculogenesis and angiogenesis, the GPxs may play essential roles in antioxidant protection throughout placental development and may indeed be the first line of antioxidant defense, as these Hofbauer cells are found from week 4 of pregnancy until term. The negative association seen between mean placental GPx activities and maternal TBARS concentrations (Figure 2) in preeclampsia provides a further indication that the reduced placental antioxidant defense in the placenta could have functional effects.

The GPxs are selenoproteins, but direct associations between the selenium concentrations and GPx activities have

Table 5. Constant and Discriminant Function Coefficients for the Maternal Predictors

<table>
<thead>
<tr>
<th>Maternal Predictive Variable</th>
<th>Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-4.06</td>
</tr>
<tr>
<td>Serum Se concentrations, μg/L</td>
<td>0.05</td>
</tr>
<tr>
<td>Plasma GPx activity, nmole/min/L</td>
<td>3.47</td>
</tr>
</tbody>
</table>
not previously been investigated with respect to preeclampsia. The significant positive relationships seen in pregnancy per se (Figure 1a and 1b) suggest that at lower maternal selenium concentrations, there is a reduction in GPx; however, as the selenium concentrations increases, the placental GPx activity begins to rise rapidly. This is in line with the reports that the maternal Se concentrations must be clearly deficient to have a detrimental effect on GPx activities,37,38 and this appears to be the case in some of the women suffering from preeclampsia in this study.

Discriminant functional analysis using only maternal biochemical measurements (serum selenium concentrations and plasma GPx activities) classified 82% of the subjects into the correct pregnancy outcomes. Caution must be exercised; this study was performed at the time of delivery, when the disease was established. Longitudinal studies are needed to determine whether similar differences antedate the onset of clinically-detectable disease, that is, whether they are cause or effect. If they do occur early, they would provide a novel addition to the analytes currently suggested as predictive.

**Perspectives**

These data are from this cross-sectional group of women with well characterized moderate-to-severe preeclampsia. The study illustrates how trace quantities of selenium can have very significant physiological effects. There is currently considerable interest in the role of dietary selenium supplementation as prophylaxis for various forms of cancer, such as prostate cancer,39 another condition of aberrant vascular development. If selenium deficiency is confirmed in women suffering from preeclampsia, and continues to be linked with GPx inadequacy, consideration could be given to a randomized controlled trial of selenium supplementation in pregnancy. Furthermore, as assay methods become more readily available, measurements of serum selenium and GPx activity might contribute to the early identification of women at high risk of developing the disease. Further longitudinal studies are required to elucidate a “cause or effect” relationship for these factors.

**Acknowledgments**

We thank all the women who participated in the study and the midwives and doctors whose support made this study possible; John Corrie and Darren Hepworth (University of Nottingham) for help with the selenium measurements; Chrissi Dunster (King’s College, London) for running the TBARS assays; Dr Paula Williams (University of Nottingham) for help and advice regarding immunohistochemical quantification.

**Sources of Funding**

H. Mistry was supported by a studentship from Biotechnology and Biological Sciences Research Council (BBS/SS/P/2003/10412A) and the Nottingham Hospital Special Trustees charity (reference number: RAP 0003; Fund No: N7050).

**Disclosures**

None.

**References**


Reduced Selenium Concentrations and Glutathione Peroxidase Activity in Preeclamptic Pregnancies

Hiten D. Mistry, Vicky Wilson, Margaret M. Ramsay, Michael E. Symonds and Fiona Broughton Pipkin

Hypertension. 2008;52:881-888; originally published online October 13, 2008;
doi: 10.1161/HYPERTENSIONAHA.108.116103

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/52/5/881

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/