Preeclampsia is associated with increased susceptibility of serum lipids to copper-induced peroxidation in vitro

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Background. Several reports suggest preeclampsia to be associated with oxidative stress. In view of potential experimental artifacts in these studies, we tested the effect of preeclampsia on the oxidizibility of maternal serum lipids, using an optimized ex vivo method.

Methods. This prospective study included 28 pregnant women with preeclampsia and 28 women matched for maternal and gestational age with normal pregnancies. Venous blood was drawn from the consenting women. Serum levels of lipid peroxidation products and the kinetics of copper-induced oxidation ex vivo were monitored spectroscopically by continuous recording of absorbance at 245 nm.

Results. The initial optical density (OD) at 245 nm, attributed to preformed products of lipid peroxidation, was higher in the women with preeclampsia than in the controls (1.26 ± 0.02 vs. 1.17 ± 0.02 OD units; p = 0.01). The lag phase preceding oxidation, reflecting resistance of serum lipids to oxidation, was significantly shorter in the preeclampsia group than in the controls (47.4 ± 2.3 vs. 57.6 ± 4.0 min; p = 0.027).

Conclusions. High levels of serum hydroperoxides and increased susceptibility of serum lipids to copper-induced peroxidation ex vivo indicate preeclampsia to be associated with high oxidative stress. The role of this high oxidizibility in the pathogenesis of preeclampsia has yet to be evaluated.

Key words: preeclampsia; lipid peroxidation; oxidative stress

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Preeclampsia is a unique disease of unknown etiology that complicates 5–7% of pregnancies (1). Despite extensive research, there are currently no effective means of either predicting or preventing this disease. Furthermore, the mechanism responsible for this pathology is not fully understood. Several lines of evidence indicate that vascular endothelial dysfunction is important in the pathogenesis of preeclampsia, and that such dysfunction may be caused by uncontrolled lipid peroxidation (2). Oxidative stress, due to an imbalance between prooxidants and antioxidants, has been shown to aggravate a variety of diseases (e.g. atherosclerosis) and contribute to their progression (3). The role of oxidative stress in the pathogenesis of preeclampsia is consistent with the high levels of oxidized lipids in decidual-placental tissues (4–6), as well as in the circulation (7–9) of women with established preeclampsia. Moreover, there is evidence (4,10) that patients with preeclampsia have reduced

Abbreviations:
SEM, standard error of the mean; OD: optical density; Vmax: maximal rate of accumulation of absorbing products; tmax: timepoint at which maximal rate is achieved; ODmax: maximal accumulation of absorbing products; OD initial: absorbing products before oxidation.
antioxidative capacity when compared to normal pregnant women.

Nonetheless, the clinical relevance of all these analyses of oxidation products and antioxidants is limited by probable experimental errors caused by the need for processing of the tissue studied (i.e. fractionation of plasma lipids) and the possibility of continuing peroxidation of blood lipids upon their exposure to oxygen \textit{ex vivo} during processing. Furthermore, it is commonly believed that in relating to ‘oxidative stress’, the most relevant factor that can be studied \textit{ex vivo} is the ‘oxidizibility’ of the plasma lipids, namely their susceptibility to oxidation upon exposure to various inducers of oxidation. This attribute depends in a very complex fashion on the level of prooxidants, including preformed hydroperoxides, and on water-soluble and lipid-soluble antioxidants (such as vitamin C and vitamin E, respectively). In view of the proposed importance of ‘oxidizibility’, much effort has been devoted to the development of \textit{in vitro} assays for its evaluation, mainly in low density lipoprotein (LDL). Such assays avoid the need to measure the concentrations of antioxidants and preformed hydroperoxides. The lengthy procedure involved in LDL fractionation and the possible modification of the lipids during isolation of LDL limit the clinical usefulness of the kinetic data. Our previously developed spectroscopic method, capable of monitoring the kinetics of copper-induced peroxidation of serum lipids \textit{ex vivo} (11,12), does not involve any processing of the serum, aside from freezing and thawing, which we have shown (13) to have no effect on the kinetics of oxidation. Furthermore, in our previous work, we have shown that the kinetics of copper-induced oxidation of serum lipids in unfractionated serum \textit{ex vivo} (11,12), does not involve any processing of the serum, aside from freezing and thawing, which we have been shown (13) to have no effect on the kinetics of oxidation. Furthermore, in our previous work, we have shown that the kinetics of copper-induced oxidation of serum lipids in unfractionated serum, as monitored at 245 nm, correlates with the kinetics of copper-induced oxidation of the LDL and of a mixture of the LDL and high density lipoprotein (HDL) extracted from the same sera (11). These correlations obviated the need to fractionate LDL from the sera, using conditions that might alter the lipid composition and the oxidative state of LDL lipids. We suggest that the major kinetic parameters determined by this optimized \textit{ex vivo} method, namely the lag preceding oxidation and the maximal rate of peroxidation, are likely to be more relevant to the lipid ‘oxidizibility’ \textit{in vivo} than the respective parameters obtained for fractionated LDL.

The aim of the present study was to evaluate the effect of preeclampsia on the resistance of maternal serum lipids to oxidative stress \textit{in vitro}.

\textbf{Materials and methods}

\textit{Subjects and study design}

Preeclampsia was diagnosed on the basis of the following criteria: (i) diastolic blood pressure of at least 90 mmHg on two occasions 6 h apart, and (ii) proteinuria of 300 mg protein in 24 h or two episodes of 100 mg/dL protein reading on a urinary dipstick test. Gestational age was determined by the best obstetric estimate, which combined last menstrual period dating with the earliest available ultrasonographic examination.

The study group consisted of 28 pregnant women with preeclampsia and the control group consisted of 28 normal pregnant women, matched for age and gestational age (Table I). The parity of the two groups was also similar. As expected, the mean arterial systolic and diastolic blood pressure of the women with preeclampsia were significantly higher (Table I). Of the 28 women diagnosed with preeclampsia only five were diagnosed to have severe preeclampsia (diastolic blood pressure >110 mmHg). Other criteria for severe preeclampsia (i.e. systolic blood pressure >160 mmHg, proteinuria >5 g/24h, pulmonary edema, impaired liver function, right upper quadrant pain, oliguria <30 mL/h, thrombocytopenia <100 000/μL or cerebral/visual impairment) were not met by any of the women in the study group. The remaining 23 women were diagnosed with mild preeclampsia.

\begin{table}[h]
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\begin{tabular}{lcc}
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& Preeclampsia (n=28) & Control (n=28) & *p-value \\
\hline
Age (years) & 29.2 ± 1.1 & 29.5 ± 0.8 & 0.220 \\
Gestational age (weeks) & 35.3 ± 1.0 & 36.1 ± 1.1 & 0.370 \\
Primapara (%) & 64 & 57 & \\
Multipara (%) & 36 & 43 & 0.790 \\
Systolic blood pressure (mmHg) & 151.7 ± 2.9 & 108.0 ± 2.2 & <0.001 \\
Diastolic blood pressure (mmHg) & 102.0 ± 1.4 & 69.4 ± 1.5 & <0.001 \\
\hline
\end{tabular}
\caption{Maternal characteristics (mean ± SEM)}
\end{table}

*Nonpaired two-tailed \textit{t}-test.  \\
†Fisher’s exact test.
Venous blood was drawn from each woman and serum was prepared, frozen immediately and stored at −70°C. All women were not in labor during blood sampling.

The study was approved by the medical center’s institutional review board and informed consent was obtained from each woman.

Copper-induced oxidation of serum samples (11)
Copper-induced oxidation was monitored at 37°C by continuous recording of absorbance at 245 nm, using a Kontron (Uvikon 933) double-beam spectrophotometer equipped with a 12-position automated sample changer. Measurements were carried out in quartz cuvettes (optical pathway 1 cm) after addition of CuCl₂ (final concentration 100 μM) to a solution containing 720 μM sodium citrate in phosphate-buffered saline (NaCl 146 mM, NaH₂PO₄ 3.3 mM, Na₂HPO₄ 3.3 mM, pH = 7.4) and 30 μL serum in a final volume of 1.5 mL (12). The time-dependent increase of absorbance at 245 nm is due to the formation of conjugated dienic hydroperoxides and 7-ketocholesterol during lipid peroxidation (11,14). The kinetic profiles were analyzed in terms of the following attributes (Fig. 1): the ‘lag’ preceding oxidation, which reflects the resistance of serum lipids to oxidative stress (12); the maximal rate of accumulation of absorbing products (Vₘₐₓ), determined from the first derivative of the time course of absorption (11); and the maximal accumulation of absorbing products (ODₘₐₓ), which is dependent primarily on the total concentration of oxidizable lipids. This method has been validated by us previously (11). We have demonstrated strong correlation between the lag preceding oxidation in unfractionated serum (as described above) and the widely accepted method of isolated LDL oxidation (15).

Statistical methods
All continuous variable distributions were analyzed and proved normal by the one-sample Kolmogorov–Smirnov test. Differences between these parameters in the study groups were evaluated by the nonpaired two-tailed student t-test. Differences between proportions were analyzed by the Fisher exact test. p-values <0.05 were considered statistically significant. Calculations were performed using the SPSS software package (SPSS Inc, Chicago, IL).

Results
As shown in Table I, there were no differences between the two groups in maternal age, gestational age and parity.

Representative kinetic profiles of copper-induced oxidation of unfractionated sera of one woman with normal pregnancy and of one woman with preeclampsia are shown, respectively, in Fig. 1.

The kinetic parameters of copper-induced oxidation in the two groups are listed in Table II. As is evident from these data, the lag preceding oxidation was significantly shorter in the women with preeclampsia than in the normal pregnant women (47.4 ± 2.3 vs. 57.6 ± 4.0 min, p = 0.027), whereas the maximal rate of accumulation of oxidation products was significantly slower in the women with preeclampsia (5 × 10⁻³ ± 3 × 10⁻⁴ vs. 6 × 10⁻³ ± 3 × 10⁻⁴ OD units/min, p = 0.020). The maximal accumulation of absorbing products was similar in the two groups (0.695 ± 0.03 vs. 0.667 ± 0.02 OD units, p = 0.44). In addition, the initial OD at 245 nm before onset of oxidation was significantly higher in sera of women with preeclampsia when compared to normal pregnant women (1.26 ± 0.02 vs. 1.17 ± 0.02 OD units;
tested whether the lower level of uric acid in women with preeclampsia, we considered. In view of the expected elevated levels of uric acid, preformed hydroperoxides should be considered as a major factor have yet to be discovered. Nonetheless, it is widely accepted that uric acid is a major prooxidant such as polyunsaturated fatty acids and preformed hydroperoxides should be considered. In view of the expected elevated levels of uric acid in women with preeclampsia, we tested whether the lower $V_{\text{max}}$ may be attributed to the potent circulating antioxidant. Uric acid had no effect on the kinetic parameters of copper-induced oxidation even at supraphysiological levels (1190 μM/L) (data not shown), ruling out the possibility that uric acid is a major factor in determining oxidizibility. The identity and the mode of action of this major factor have yet to be discovered.

An important limitation of our study relates to its relevance to the subendothelial space where lipid peroxidation occurs (16). We speculate that the lipid radicals formed in this space leak into the blood and their effects are detected by our assay.

Regardless of the actual mechanism responsible for the high susceptibility of serum lipids to oxidation in preeclampsia, it may be concluded that preeclamptic patients are subject to high oxidative stress. Hence, antioxidants may possibly improve the prognosis of women with preeclampsia. To date, there are conflicting reports (10,17–22) regarding the levels of antioxidants in the sera of women with preeclampsia and their activity. Only one controlled randomized study

| Table II. Kinetic parameters of copper-induced oxidation (mean ± SEM) |
|-----------------------------|-----------------------------|-----------------------------|
|                             | Preeclampsia ($n = 28$)     | Normal pregnancy ($n = 28$) |
| Lag preceding oxidation (min) | 47.4 ± 2.3                  | 57.6 ± 4.0                  |
| $V_{\text{max}}$ (OD 245 nm/min) | $5 \times 10^{-3} \pm 3 \times 10^{-4}$ | $6 \times 10^{-3} \pm 3 \times 10^{-4}$ |
| OD$_{\text{max}}$ (OD 245 nm) | 0.695 ± 0.030               | 0.667 ± 0.017               |
| OD$_{\text{initial}}$ (245 nm) | 1.259 ± 0.022               | 1.169 ± 0.015               |

*Nonpaired two-tailed $t$-test.

$p = 0.013$). Notably, we observed a significant negative correlation between the initial OD, as measured at 245 nm, and the lag preceding oxidation in the group of normal pregnant women ($r = -0.43$, $p = 0.02$), but not in the group of women with preeclampsia ($r = 0.12$, $p = 0.52$).

Discussion

The major finding of the present study is that in women with preeclampsia, the lag preceding oxidation is significantly shorter when compared to normal pregnant women, indicating that preeclampsia is associated with enhanced susceptibility of serum lipids to copper-induced oxidation $\text{ex vivo}$. This finding accords with the hypothesis that oxidative stress has a role in preeclampsia.

Nonetheless, the maximal rate of accumulation of oxidation products, $V_{\text{max}}$, observed during the phase of accelerated peroxidation, was significantly slower in the sera of women with preeclampsia. This observation appears to be inconsistent with the enhanced propensiy of the serum lipids for oxidation, indicated by the shorter lag times. To explain this disparity, putative changes in plasma content of natural antioxidants, prooxidants such as polyunsaturated fatty acids and preformed hydroperoxides should be considered. In view of the expected elevated levels of uric acid in women with preeclampsia, we tested whether the lower $V_{\text{max}}$ may be attributed to this potent circulating antioxidant. Uric acid had no effect on the kinetic parameters of copper-induced oxidation even at supraphysiological levels (1190 μM/L) (data not shown), ruling out the possibility that uric acid is a major factor of the oxidation/antioxidation balance in preeclampsia. Other possibilities should be investigated to explain this observed discrepancy between the major oxidation parameters. Nonetheless, it is widely accepted that uric acid is a major parameter indicating oxidative stress.

Another finding of importance is that the values for the maximal accumulation of absorb-
(23) has shown that supplementation with vitamins C and E is beneficial in the prevention of preeclampsia and only in women at increased risk of the disease. Further elucidation of the possible benefit of antioxidant supplementation for prevention and treatment of preeclampsia requires more research.

References


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