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# The transient increase of oxidative stress during normal pregnancy is higher and persists after delivery in women with pre-eclampsia

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**Abstract**

*Objectives:* Glutathione, an intracellular tripeptide, functions in the protection of cells against free radicals and toxins of endogenous and exogenous origin. To maintain the intracellular redox status in presence of reactive oxygen species, glutathione (GSH) and other thiols are oxidized. The oxidative status of thiols is reflected by the free-to-oxidized ratio and is a real-time measure for oxidative stress. Previously, we have reported abnormal ratios for the thiols cysteine (Cys), homocysteine (Hcy) and cysteinylglycine (CysGly) in women with pre-eclampsia. The aims of this study were to confirm our previous findings in a different case–control cohort and more importantly to determine whether these differences persist postpartum.
*Study design:* At onset of disease and at 6–8 weeks postpartum we analyzed whole blood of 41 women with pre-eclampsia and of 31 women with normotensive pregnancies for the free-to-oxidized ratio of thiols by the assessment of free and oxidized thiol levels using high performance liquid chromatography. Differences between values were determined using either the paired *t*-test (antepartum versus postpartum) or the *t*-test (pre-eclampsia versus normotensive pregnancy).
*Results:* Antepartum levels of free GSH as well as the free-to-oxidized ratios of Hcy were lower in pre-eclampsia and normotensive pregnancy when compared with corresponding postpartum values ($P < 0.0001$ and $P < 0.01$, respectively). Moreover, the free-to-oxidized ratio for Hcy was significantly lowered in pre-eclamptic compared with normotensive women, during as well as after pregnancy (both $P \leq 0.01$).
*Conclusion:* The data suggest that pregnancy is a state of higher oxidative stress when compared to the postpartum period. In women with pre-eclampsia, oxidative stress is higher and persists in the postpartum period.


*Keywords:* Pre-eclampsia; Oxidative stress; Glutathione; Homocysteine; Cardiovascular disease

## 1. Introduction

In reproduction the glutathione/glutathione dependent enzyme system plays an important role in the defense against oxidative damage by scavenging of free radicals, thereby leading to oxidation of glutathione [1]. To maintain the cellular redox-balance the oxidized form of glutathione can either be converted to reduce glutathione or excreted when excessive amounts are formed [2]. The presence of the free sulfhydryl (–SH) group also confers potent antioxidant properties to other amino thiols including cysteine and homocysteine [3]. However, in presence of free transition metals (auto)oxidation of thiols will lead to the formation of

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superoxide, thus amino thiols may also contribute to oxidative stress. Cysteine is more easily oxidized than homocysteine in this process, but can subsequently be reduced by homocysteine. The dynamic equilibrium between reduced and oxidized amino thiols is termed the redox thiol status and elevated levels of oxidized thiols reflect a pro-oxidant environment [3].

Pre-eclampsia, classically defined by pregnancy-induced hypertension and concurrent proteinuria, is a severe complication of pregnancy with a prevalence of 5–7% and significant maternal as well as fetal mortality and morbidity [4]. Although the pathogenesis is not yet elucidated, disturbed trophoblast invasion and subsequent reduced placental perfusion are considered to contribute to a state of oxidative stress in the placenta [5]. At time of disease pre-eclampsia is characterized by maternal oxidative stress that has been proposed to give rise to the dysfunction of vascular endothelium, a hallmark of the disease [4]. It has been suggested that pregnancy may act as a vascular stress test for the development of cardiovascular diseases (CVD) in later life, since women who have previously experienced pre-eclampsia are more susceptible to CVD than those who have not [6,7]. Although the mechanism remains unknown, both oxidative stress and endothelial activation are common characteristics [8,9]. In addition, elevated plasma homocysteine is widely accepted to be one of the major risk factors of CVD [10] and has recently been associated with a pro-oxidant state [11].

Oxidative stress in women with pre-eclampsia has been investigated by many others. However, methodologies applied in these studies, such as estimating the levels of individual antioxidants or markers for lipid peroxides, all have the major drawback that only one side of the oxidative stress balance is investigated [12]. In contrast, the use of the free-to-oxidized ratio of thiols will provide a 'real-time' reflection of the oxidative stress balance. In an earlier study we have shown that the free-to-oxidized ratios for cysteine and homocysteine can be used as markers for oxidative stress in pre-eclampsia. However, no significant differences were found for glutathione, but there was a large inter-individual variation in free glutathione levels among subjects [13]. The principal aim of this study was to determine whether this oxidative stress persists postpartum in women who experienced pre-eclampsia and to confirm our previous findings in a newly recruited cohort. Therefore, we have assessed the antepartum and postpartum thiol redox status in blood of women with normotensive pregnancies and pregnancies affected by pre-eclampsia.

## 2. Materials and methods

The Institutional Medical Ethical Review Committee approved the study protocol and all subjects investigated provided written informed consent. Pre-eclampsia was defined as pregnancy-induced hypertension (diastolic blood pressure >90 mmHg on two or more occasions each more than 4 h apart) with proteinuria (protein/creatinine ratio ≥0.30 g/10 mmol) according to the standard of the International Society for the Study of Hypertension in Pregnancy [14]. Forty-one patients with pre-eclampsia were recruited at the onset of disease of which five subjects participated in earlier studies by our group [13,15]. The control group consisted of 31 women with uncomplicated pregnancy outcome, who were recruited upon admission to the hospital at term before delivery by caesarean section. Participants were recruited at the University Medical Center Nijmegen between 1999 and 2001. Characteristics of the study populations are described in Table 1.

Whole blood, anti-coagulated with EDTA, was collected by venipuncture immediately after admittance to the hospital, before any treatment had been started, and again at 6–8 weeks after delivery. Within 1 h of sampling, whole blood was handled for the assessment of oxidized and free

Table 1
Pregnancy characteristics of women with pre-eclampsia and normotensive controls

| | Normotensive controls ($n = 31$) | Pre-eclamspia ($n = 41$) |
|---|---|---|
| Maternal age (years) | 33 (27–41) | 29 (21–38)* |
| Primipara | 11 (35) | 32 (78)* |
| Birth weight (g) | 3305 (2335–4675) | 943 (370–3045)† |
| Gestational age (weeks$^{+days}$) | $39^{+1}$ ($37^{+6}$ to $42^{+1}$) | $28^{+3}$ ($22^{+4}$ to $37^{+5}$)† |
| Hemoglobin (mmol/L) | 7.3 (6.3–8.5) | 7.7 (5.0–9.0) |
| Hematocrit | 0.34 (0.28–0.48) | 0.36 (0.28–0.45) |
| Diastolic BP (K5; mmHg) | 68 (55–80) | 110 (90–135)† |
| Protein/creatinine ratio (mg/10 nmol) | | 4.2 (0.3–27.4) |
| LDH (IU/L) | | 586 (92–3870) |
| ALAT (IU/L) | | 34 (8–936) |
| ASAT (IU/L) | | 63 (12–1291) |
| Platelet count ($\times 10^9$/L) | | 121 (20–378) |
| Uric acid (μmol/L) | | 0.4 (0.2–0.56) |
| Creatinine (μmol/L) | | 71 (49–146) |

Data are presented as median (range), except for parity, which is expressed as number (percentage). Abbreviations: BP, blood pressure; LDH, lactic dehydrogenase enzyme activity; ALAT, alanine aminotransferase enzyme activity; ASAT, aspartate aminotransferase enzyme activity. Differences between pre-eclampsia and normotensive controls were analyzed using the Wilcoxon–Mann–Whitney test: *$P < 0.001$; †$P < 0.0001$.