Free Radical Biology & Medicine, Vol. 30, No. 7, pp. 715–721, 2001

0891-5849/01/$–see front matter

PII S0891-5849(00)00516-5

Original Contribution

BLOOD GLUTATHIONE REDOX STATUS IN GESTATIONAL HYPERTENSION

ILONA NÉMETH,* HAJNALKA ORVOS,† and DOMOKOS BODA*

*Department of Pediatrics and †Department of Obstetrics and Gynecology, Albert Szent-Györgyi Medical and Pharmaceutical Center, University of Szeged, Szeged, Hungary

(*Received* 28 *August* 2000; *Revised* 1 *December* 2000; *Accepted* 14 *December* 2000)

Abstract—Gestational hypertension during the third trimester reflects an exaggerated maternal inflammatory response to pregnancy. We hypothesized that oxidative stress present even in normal pregnancy becomes uncompensated in hypertensive patients. A glucose-6-phosphate dehydrogenase (G6PD) activity sufficient to meet the increased reductive equivalent need of the cells is indispensable for defense against oxidative stress. The erythrocyte glutathione redox system was studied, where G6PD is the only NADPH source. The glutathione (GSH) redox status was measured both in vivo and after an in vitro oxidative challenge in pregnant women with gestational hypertension ($n = 19$) vs. normotensive pregnant subjects ($n = 18$) and controls ($n = 20$). An erythrocyte GSH depletion with an increase in the oxidized form (GSSG) resulted in an elevated ratio GSSG/GSH (0.305 ± 0.057; mean $\pm$ SD) in hypertensive pregnant women vs. normotensive pregnant or control subjects (0.154 ± 0.025; 0.168 ± 0.073; $p < .001$). In hypertensive pregnant patients, a "GSH stability" decrease after an in vitro oxidative challenge suggested a reduced GSH recycling capacity resulting from an insufficient NADPH supply. The erythrocyte GSSG/GSH ratio may serve as an early and sensitive parameter of the oxidative imbalance and a relevant target for future clinical trials to control the effects of antioxidant treatment in women at increased risk of the pre-eclampsia syndrome.

Keywords—Oxidized/reduced glutathione, Hemoglobin, Glutathione recycling, Glucose-6-phosphate dehydrogenase, Free radicals

INTRODUCTION

Hypertensive disorders are relatively common complications of pregnancy (6–8%) and make a significant contribution to neonatal morbidity and mortality [1]. Gestational hypertension, a new onset of hypertension manifested after 20 gestational weeks, without proteinuria, is currently considered to be the first stage of a severe multisystem endothelial disorder, the pre-eclampsia syndrome, characterized by an increased blood pressure, proteinuria, and edema [1,2]. Pre-eclampsia is more than simply hypertension, a consequence of disturbed placentation with a reduced placental perfusion [3,4]. Hypertension has been explained as a disturbed endothelial control of the vascular tone with an imbalance in the thromboxane/prostacyclin ratio or an impaired effect of the vasodilator nitric oxide (NO), which causes maternal vasodilatation during normal pregnancy [5,6].

In addition to the endothelial dysfunction, a systemic activation of the maternal inflammatory cell responses becomes evident in the pre-eclampsia syndrome [7]. Phagocytes (granulocytes and monocytes) produce and secrete reactive oxygen species as a nonspecific immune defense mechanism [8,9]. An abnormally activated clotting system and an affected complement system have been revealed [10], as has an increased release of the proinflammatory cytokines and fibronectin degradation products into the circulation [11]. Furthermore, toward the end of pregnancy, an inflammatory response to pregnancy is present even in normal cases [12]. Thus, according to a new concept, the clinical symptoms of pre-eclampsia arise when a universal maternal intravascular inflammatory response to pregnancy decompensates because either the stimulus or the maternal response is too strong [7]. Therefore, a generalized

Address correspondence to: Ilona Németh, MD, PhD, Department of Pediatrics, Albert Szent-Györgyi Medical and Pharmaceutical Center, University of Szeged, Szeged, Hungary, H-6701, POB 471; Tel: +36 (62) 545332/1112; Fax: +36 (62) 545329; E-Mail: nemethil@pedia.szote.u-szeged.hu.

endothelial cell dysfunction is part of a more extensive inflammatory process [7].

Heterogenous causes have been implicated in the pre-eclampsia syndrome [13,14]. There are both placental and maternal factors that predispose to an excessive maternal inflammatory response syndrome [7]. Placental hypoxia, as a result of poor placentation, or the maternal constitution (obesity, hyperlipidemia, and insulin resistance) and pre-existing diseases are factors predisposing to an excessive maternal response [7]. Recurrence of the hypertensive disorder in subsequent pregnancies and its more frequent prevalence in certain families [15] suggest a genetic predisposition to the exaggerated maternal response to pregnancy [7].

There is considerable evidence of an increased free radical activity both in normal pregnancy and in pregnancy-induced hypertension [16–19]. Oxidative stress was recently postulated as a link in the two-stage model of pre-eclampsia [20].

Red blood cells (RBCs) containing millimolar concentrations of both glutathione (GSH) and hemoglobin (Hb) circulate in the direct vicinity of the activated blood cells and the endothelial surface, with a membrane permeable to oxidants. The intracellular GSH redox system, with a high GSH level and a far lower concentration of the oxidized form (GSSG), is predominantly responsible for the protection of both Hb and the RBC membrane against oxidation and hemolysis. Interrelated enzyme systems function to achieve the efficient recycling of GSSG to GSH and to provide the reducing equivalent, NADPH. The activity of the first and key enzyme of the hexose-monophosphate shunt (HMP), glucose-6-phosphate dehydrogenase (G6PD; E.C.1.1.1.49), is indispensable for the defense against oxidative stress [21].

We hypothesized that the GSH redox system of the RBCs may be an early and highly sensitive indicator of oxidative stress. The RBCs are unique targets with which to assess the sufficiency of G6PD activity because the RBCs acquire NADPH exclusively from the HMP shunt. A method of calibrated in vitro oxidative challenge was evolved by Beutler in order to recognize patients with defective G6PD activity [22].

The aim of the present study was to assess the redox status of GSH, the signs of Hb denaturation, and the GSH recycling capacity of RBCs from patients with pregnancy-induced hypertension as compared with those from normotensive pregnants and nonpregnant controls. The relationships of the ratio GSSG/GSH to the usual indicators of oxidative stress (the levels of plasma free thiols, ceruloplasmin, and lipid peroxidation products) were also established.

MATERIALS AND METHODS

Subjects

Pregnant women with mild hypertension but without superimposed pre-eclampsia were detected at routine obstetric visits after 24 weeks of gestation (35.5 $\pm$ 2.3 weeks; mean $\pm$ SD). In the patients who were included in the study, an increased blood pressure was measured for the first time during their present pregnancy. Five of them had had transient hypertension during a previous pregnancy. Therefore, they had not been treated either occasionally or regularly with antihypertensive drugs before the study. Nineteen subjects (age: 26.3 $\pm$ 1.2 years; parity: 1.5 $\pm$ 0.2) met the criterion of gestational hypertension, defined as an increase of at least 30 mm Hg in the systolic or 15 mm Hg in the diastolic blood pressure as compared with the values obtained before 20 weeks of gestation, or an absolute blood pressure of $>$140/90 mm Hg if the earlier blood pressure was not known [1]. Neither proteinuria nor any impairment in renal function was observed (defined as $>$500 mg per 24-h urine collection, and plasma creatinine values). The body weight before pregnancy was 64.2 $\pm$ 1.7 kg. There was a $>$15 kg rise in body weight during pregnancy in 14 patients, with a body weight of 83.7 $\pm$ 1.5 kg at the time of the study. Thrombocytopenia, an increased packed cell volume, and an abnormal liver enzyme activity were not seen. The patients seemed obese rather than edematous.

Eighteen women with a clinically normal pregnancy, matched for maternal age (25.3 $\pm$ 1.3 years), parity (1.2 $\pm$ 0.1), and gestational age (35.2 $\pm$ 1.3 weeks), but with a significantly lower body weight (69.5 $\pm$ 1.2 kg; $p < .05$) at the time of the study, acted as pregnant controls. The body weight before pregnancy was 60.5 $\pm$ 1.4 kg. The control women (65.5 $\pm$ 1.8 kg, $n = 20$) were normotensive age-matched members of the laboratory staff, including eight cases with an increased body mass index. As no inhomogenity was observed in the biochemical data of the control population related to the body weight was assessed, the data were combined.

This study was previously approved by the Ethical Committee of the University.

Biochemical analysis of the GSH and Hb redox systems

Highly sensitive and specific separate determinations of GSSG and GSH+GSSG concentrations were carried out by a previously published method [23]. Samples for GSSG measurement (25 μl of whole blood collected over EDTA) were hemolyzed within 15 min after venipuncture with cold buffer in the presence of N-ethyl-

maleimide (NEM; final concentration 20 mM in 0.01 M phosphate buffer containing 5 mM EDTA). The reaction of NEM with GSH results in the formation of a stable complex, which prevents its possible oxidation to GSSG. After incubation with NEM for 60 min at 25°C, metaphosphoric acid (final concentration 3%, w/v) was used to precipitate proteins. The supernatant can be stored at −20°C (max. 6 d) until the spectrophotometric determination of GSSG. As NEM is an inhibitor of glutathione reductase, it was separated from the supernatant by gel filtration with Sephadex G-10 immediately before GSSG measurement.

For measurement of the total concentration of GSH+GSSG, 25 μl of whole blood anticoagulated with EDTA was immediately hemolyzed in 2.5 ml of cold 0.01 M sodium phosphate buffer containing 5 mM EDTA, pH = 7.5, and stored at −20°C until spectrophotometric analysis (max. 6 d).

The same standard GSH assay mixture was used for the analysis of GSSG and GSH+GSSG concentrations. Reagents were dissolved in 0.1 M sodium phosphate/5 mM EDTA buffer, pH = 7.5; the final volume was 1.0 ml. Components were added in the following sequence: 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) (0.6 μM), GSSG (0.4 ml of supernatant after NEM removal) or GSH+GSSG (25 μl of hemolyzate), glutathione reductase (10 μg), and NADPH (0.2 μM). The combined actions of DTNB and NADPH in the presence of glutathione reductase result in a reaction cycle, the rate of which depends on the concentration of GSH+GSSG recorded at 412 nm during the first 6 min.

GSH (μM) and GSSG (nM) concentrations were calculated by using 5–100 nM GSSG standards and expressed with reference to Hb determined simultaneously by the cyanmethemoglobin method.

The "GSH stability test" according to Beutler [22] was used to measure the recycling capacity of the RBCs after an in vitro oxidative stress. Acetylphenylhydrazine (APH) (0.33 mM) was added to the whole blood sample, together with sufficient glucose. Following incubation at 37°C for 60 min with APH, RBCs deficient in recycling, but not the normal ones, suffer a marked fall in GSH level, accompanied by a pronounced Hb oxidation [22]. Concentrations of oxyHb, metHb, and hemichrome were calculated after absorbance measurements in diluted hemolyzates at 560, 577, 630, and 700 nm as described by Winterbourn [24], together with GSH determination before and after APH incubation.

Other biochemical methods

The proportions of carboxyHb (COHb) and metHb and the total concentration of Hb in the whole blood were measured with a Hemoximeter (Radiometer, Copenhagen, Denmark) within 15 min after venipuncture. The plasma content of the lipid peroxidation products, as fluorescent lipids and conjugated dienes, was estimated according to Ward et al. [25]. The concentration of free SH groups in the plasma was measured with DTNB at 412 nm [26]. Plasma ferroxidase activity was measured according to Johnson et al. [27]. Ten microliters of plasma was added to the reaction mixture (170 μl of 1.2 M sodium acetate buffer, pH 6.0; 270 μl of Chelex100-treated water; 250 μl 1% (w/v) of conalbumin solution; and 300 μl of 400 μM $Fe(NH_4)_2(SO_4)_2$ solution. The oxidase activity was measured spectrophotometrically by continuous monitoring of Fe(III)-conalbumin formation through the absorbance change at 460 nm [27].

Statistical analysis

Clinical data on the patients are reported as means ± standard deviations (x ± SD), while results of biochemical analyses are shown in the figures as means ± standard errors (x ± SEM). Statistical analyses included both parametric (variance analysis, Tukey test and Student's *t* test) and nonparametric tests (Wilcoxon rank test). When the extent of variance between pairs of groups differed significantly ($p < .05$ in the *F* test), we used the Welch test (*d* probe) instead of the *t* test to compare the mean values. Correlations between parameters were characterized by calculation of the linear regression and correlation coefficients. The significance level for all tests was taken as $\alpha = .05$.

RESULTS

There was no difference in the whole blood Hb or COHb concentrations as potential markers of an increased RBC catabolism in the pregnant subjects and the age-matched control women. However, the concentration of metHb in the whole blood revealed an increase in the hypertensive pregnant patients (Table 1).

The GSH level in the normotensive pregnant women was significantly elevated as compared with that in the nonpregnant controls (Fig. 1). In contrast, there was a significantly decreased GSH level in the RBCs from the pregnant women with a hypertensive disorder, together with a simultaneously increased GSSG level (Fig. 1). Elevated ratios of oxidized Hb metabolites (either metHb or hemichrome) to total Hb from hemolyzates were seen in both groups of pregnant subjects as compared with the controls (Fig. 2). In the normotensive pregnant patients, an increased level of metHb was contrasted by a somewhat elevated metHb and a highly elevated ratio of hemichrome in the patients with a hypertensive disorder

Table 1. Concentrations of Some Biochemical Parameters in the Plasma and in the Whole Blood of the Study Population (means ± SD)

	Nonpregnant controls $n = 20$	Normotensive pregnants $n = 18$	Hypertensive pregnants $n = 19$
Whole blood values			
Total hemoglobin (mM)	8.2 ± 0.4	7.8 ± 0.6	7.6 ± 1.0
Carboxyhemoglobin (μM)	199.5 ± 25.1	204.7 ± 32.0	229.2 ± 38.5
Methemoglobin (μM)	80.5 ± 7.4	88.6 ± 8.9	128.9 ± 6.9*#
Plasma values			
Conjugated dienes (OD, 233 nm)	0.52 ± 0.26	0.82 ± 0.31	1.70 ± 0.32*#
Fluorescent lipids (OD, 430 nm)	49.4 ± 19.2	68.67 ± 28.2	136.8 ± 57.4*#
Ceruloplasmin ferroxidase (IU/L)	0.59 ± 0.06	1.16 ± 0.07***	1.210 ± 0.16***&

*$p < .05$; ***$p < .001$ vs. nonpregnant controls; &Welch test used; #$p < .05$ vs. normotensive pregnants.

(Fig. 2). However, the sum of the two oxidized Hb metabolites (metHb + hemichrome) was significantly higher in the hypertensive than in the normotensive pregnant patients ($p < .01$).

After an in vitro oxidative stress, a significant fall in the residual GSH ratio was observed in the hypertensive pregnant patients as compared either with the controls or with the normotensive pregnant patients (Fig. 3). However, the proportions of oxidized derivatives of Hb (metHb + hemichrome) to total Hb after APH were higher both in the hypertensive pregnant patients and in the normotensive ones (Fig. 3), suggesting an increased oxidative susceptibility of Hb from pregnant women in late pregnancy.

The depletion of erythrocyte GSH and the simultaneous increase in GSSG (Fig. 1) resulted in a highly elevated redox ratio GSSG/GSH in the pregnant subjects with hypertension as compared either with the controls or with the normotensive pregnant subjects (Fig. 4). The concentration of free plasma SH groups displayed a marked decrease in the hypertensive patients, but also in the normotensive pregnant women as compared with the controls (Fig. 4), as a sign of a pro-oxidant state even in normal late pregnancy.

The erythrocyte GSSG/GSH correlated significantly with the usual parameters of an oxidative imbalance in late pregnancy. The plasma level of free thiols ($r = -0.618$, $n = 37$, $p < .01$), and the concentration of fluorescent lipids in the plasma correlated significantly with the redox ratio ($r = 0.596$, $n = 37$, $p < .01$).

Highly significant increases in the ferroxidase activity of the plasma were seen in both the normotensive and the hypertensive pregnant patients (Table 1). The activity of ferroxidase correlated negatively with the level of conjugated dienes in the normotensive pregnant subjects ($r = -0.707$, $n = 18$, $p < .01$) but exhibited a weak positive correlation in the women with hypertensive disorders ($r = 0.456$, $n = 19$, $p < .05$).

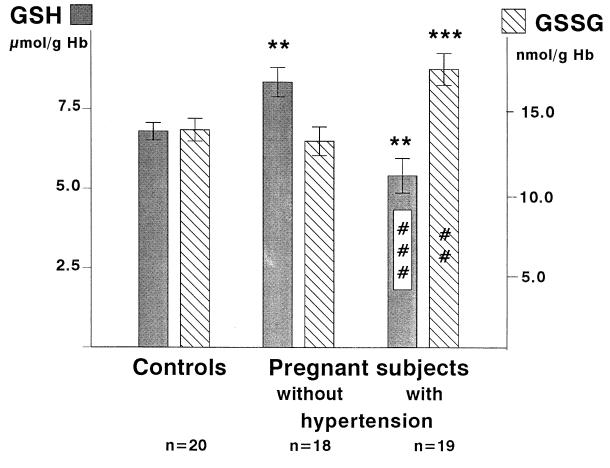

Fig. 1. Concentrations of reduced and oxidized glutathione (GSH and GSSG) in controls and pregnant subjects without/with hypertension (means ± SEM). **$p < .01$; ***$p < .001$ vs. controls; ###$p < .01$; ###$p < .001$ vs. normotensive pregnant subjects.

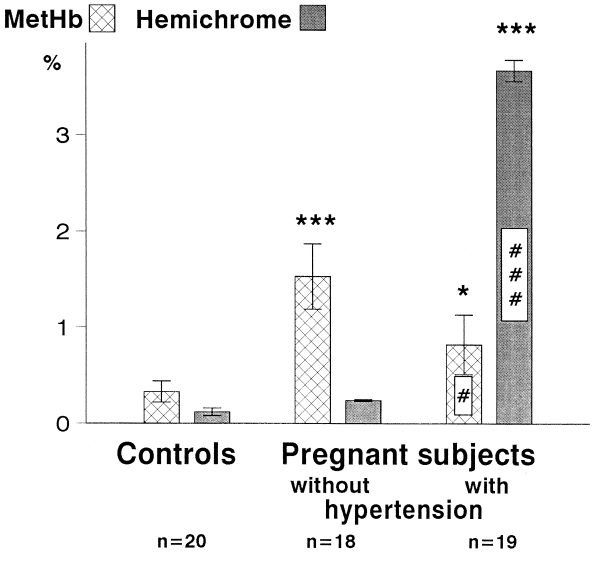

Fig. 2. Ratio of hemoglobin oxidation products (methemoglobin or hemichrome) to total hemoglobin in hemolyzates of controls and pregnant subjects without/with hypertension. *$p < .05$; ***$p < .001$ vs. controls; #$p < .05$; ###$p < .001$ vs. normotensive pregnant subjects.

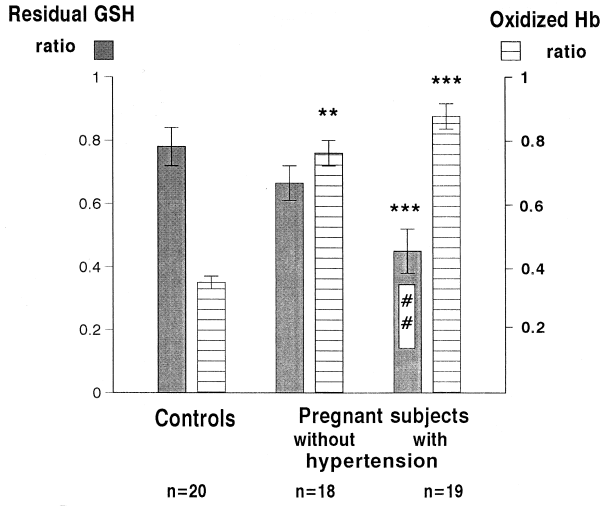

Fig. 3. Ratio of GSH remaining after in vitro oxidative stress with acetylphenylhydrazine and ratio of hemoglobin oxidation products (methemoglobin+hemichrome) to total hemoglobin in hemolyzates of controls and pregnant subjects without/with hypertension. $^{**}p < .01$; $^{***}p < .001$ vs. controls; $^{\#\#}p < .01$; $^{\#\#\#}p < .001$ vs normotensive pregnant subjects.

DISCUSSION

The clinical status of our patients met the criteria of mild gestational hypertension without superimposed preeclampsia [1,20]. The results obtained in this study on the concentrations of thiols in the RBCs and plasma, the antioxidant activity of ceruloplasmin, and the lipid peroxidation parameters confirmed the previous conclusions that a pro-oxidant status develops in late pregnancy, which is further enhanced in pregnancy-induced hypertension [16–19,28]. In addition, significant correlations were found between the usual parameters of an oxidative imbalance and the erythrocyte GSSG/GSH ratio, our proposed index.

As a new result, an increased oxidative susceptibility of Hb to either auto-oxidation or an in vitro oxidative stress was demonstrated in late pregnancy, even without a hypertensive disorder. Hb readily undergoes one-electron oxidation and reduction, and it can act as a source or sink of free radicals [24]. The oxidation of oxyHb yields superoxide and metHb, a reversible oxidation product. If the globin structure is destabilized, metHb can convert to hemichrome, an irreversible oxidation metabolite, which has a potential destabilizing effect on the RBC membrane [29]. No direct signs of either Hb denaturation or RBC membrane destruction were evident in the whole blood, although the level of free thiols in the plasma was reduced, as an early sign of a compromised antioxidant status in the normotensive pregnant subjects.

On the other hand, an increased GSH concentration with an appropriate (i.e., relatively increased) recycling capacity was seen as an adaptation to maintain the adequate redox milieu of the RBCs. Enhanced GSH levels and resistance to oxidation were demonstrated in different cells after an oxidant insult mediated by an increased G6PD expression [30,31]. An efficient level of GSH is crucial for the maintenance of an adequate metabolism, antioxidant protection of Hb, and the membrane integrity of the RBCs. After exhaustion of the antioxidant systems, the RBCs are the sources of free Hb/heme, which can inhibit the vasodilator effect of NO [32]. Enhanced RBC destruction is a frequent complication of mild preeclampsia, even in the absence of overt intravascular hemolysis [33].

In the hypertensive pregnant patients, we observed several signs of an uncompensated oxidative stress status for both the Hb and the GSH redox systems. This was manifested by a minimally increased metHb concentration in the whole blood, an elevated level of hemichrome in the hemolyzate, an increased ratio GSSG/GSH, and a pronounced GSH fall after in vitro APH loading, with a simultaneous increase in oxidized Hb derivatives.

The elevated ratio GSSG/GSH in the RBCs from the hypertensive pregnant subjects was equally due to a depleted GSH content and a highly elevated GSSG concentration. As a new result, a reduced GSH recycling capacity of the RBCs was demonstrated in the hypertensive pregnant patients, which could be a harmful factor, predisposing them to oxidative hemolysis. We suggest that a misadaptation to the normal pregnancy-induced oxidative stress in hypertensive patients may be a consequence of either a redox-sensitive inactivation of G6PD, mediated by oxidants from activated neutrophils [34], or an insufficient induction of the enzyme G6PD [31].

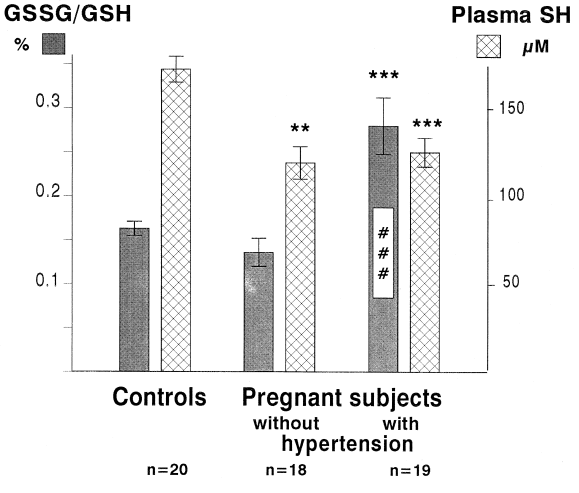

Fig. 4. Erythrocyte glutathione redox ratio (GSSG/GSH) and plasma free thiol concentration in controls and pregnant subjects without/with hypertension. $^{***}p < .001$ vs. controls; $^{\#\#\#}p < .001$ vs. normotensive pregnant subjects.

A further possibility for an inadequate adaptation to the oxidative stress of pregnancy might be a delayed induction of the 32 kD oxidative stress protein, heme oxygenase-1 isoform (HOX-1). This enzyme catalyzes the degradation of endothelial toxic heme to the antioxidant biliverdin and the vasodilator carbon monoxide (CO). CO has been implicated in the control of vascular tone, akin to NO [35]. HOX-1-derived CO contributed to the suppression of an acute hypertensive response in vivo under experimental conditions [36]. In a recent study on human placentas, an induction of HOX-1 was protective against cytotoxic damage and promoted the vessel relaxation [37]. Furthermore, HOX-1 protein was significantly reduced in placentas from pregnancies complicated with pre-eclampsia, as compared with gestationally matched normal pregnancies [37].

A significant decrease in blood GSH level has previously been reported in severe forms of pre-eclampsia complicated with the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) [38]. The RBC glutathione balance has also been measured by means of NMR in severe states of pre-eclampsia, without the separate estimation of GSSG [39]. The rate of GSH oxidation tended to be related to the severity of the illness and the susceptibility to hemolysis [39]. Our results in pregnant subjects with mild gestational hypertension provide further evidence on the altered GSH redox status in an earlier stage of the illness, when the multisystem disorders are not present, but the normal vascular adaptations to pregnancy are compromised.

Therefore, we suggest that the erythrocyte ratio GSSG/GSH may serve as a measurable index of the oxidative stress status during pregnancy. The ratio may be a relevant target for future clinical trials to control the effects of antioxidant treatment in women at increased risk of the pre-eclampsia syndrome. In a recent study, Chapell et al. reported the beneficial effect of preventive vitamin C and E supplementation on the frequency or recurrence of the pre-eclampsia syndrome [40]. As ascorbate and GSH provide a cycle for the regeneration of vitamin E, the main membrane antioxidant in the RBCs, the ratio GSSG/GSH would presumably be a useful biochemical indicator to give further evidence of the effect. Similar studies on patients with severe pre-eclampsia syndrome are warranted to prove the usefulness of the ratio GSSG/GSH as a sensitive parameter of prognostic value.

Acknowledgements — We are indebted to Professor János Herczeg and Assistant Professor Sándor Sávay from the Department of Obstetrics and Gynecology at Albert Szent-Györgyi Medical and Pharmaceutical Center for making available the serum and urine samples from pregnant women under their careful obstetric control. This study was supported financially by the Hungarian Research Fund (OTKA F20262; T22562) and the Hungarian Ministry of Welfare (ETT 625/13; 631/08).

REFERENCES

[1] American College of Obstetrics and Gynecology. Hypertension in pregnancy. *ACOG Technical Bulletin* no. 219; Washington, DC, ACOG; 1996.

[2] Roberts, J. M.; Taylor, R. N.; Musci, T. J.; Rodgers, C. M.; Hubel, C. A.; McLaughlin, M. K. Preeclampsia: an endothelial cell disorder. *Am. J. Obstet. Gynecol.* **161:**1200–1204; 1989.

[3] Roberts, J. M.; Redman, C. W. G. Preeclampsia: more than pregnancy-induced hypertension. *Lancet* **341:**1447–1451; 1993.

[4] Redman, C. W. Current topic: preeclampsia and placenta. *Placenta* **12:**301–308; 1991.

[5] Wang, Y.; Walsch, S. W.; Guo, J.; Zhang, J. The imbalance between thromboxan and prostacyclin in preeclampsia is associated with an imbalance between lipid peroxides and vitamin E in maternal blood. *Am. J. Obstet. Gynecol.* **165:**1695–1700; 1991.

[6] Sladek, S. M.; Magness, R. R.; Conrad, K. P. Nitric oxide and pregnancy. *Am. J. Physiol.* **272:**R441–R463; 1997.

[7] Redman, C. W.; Sacks, G. P.; Sargent, I. L. Preeclampsia: an excessive maternal inflammatory response to pregnancy. *Am. J. Obstet. Gynecol.* **180:**499–506; 1999.

[8] Greer, I. A.; Haddad, N. G.; Dawe, J.; Johnstone, F. D.; Calder, A. A. Neutrophil activation in pregnancy-induced hypertension. *Br. J. Obstet. Gynecol.* **96:**978–982; 1989.

[9] Himmelfarb, J.; Hakim, R. M.; Holbrook, D. G.; Leeber, D. A.; Ault, K. A. Detection of granulocyte reactive oxygen species formation in whole blood using flow cytometry. *Cytometry* **13:** 83–89; 1992.

[10] Perry, K. G.; Martin, J. N. Abnormal hemostasis and coagulopathy in preeclampsia and eclampsia. *Clin. Obstet. Gynecol.* **35:** 338–350; 1992.

[11] Vince, G. S.; Starkey, P. M.; Austgulen, R.; Kwiatkowski, D.; Redman, C. W. Interleukin-6, tumor necrosis factor and soluble tumor necrosis factor receptors in women with preeclampsia. *Br. J. Obstet. Gynecol.* **102:**20–25; 1995.

[12] Sacks, G. P.; Studena, K.; Sargent, I. L.; Redman, C. W. Normal pregnancy and preeclampsia both produce inflammatory changes in peripherial blood leukocytes akin to those of sepsis. *Am. J. Obstet. Gynecol.* **179:**80–86; 1998.

[13] Ness, R. B.; Roberts, J. M. Heterogenous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. *Am. J. Obstet. Gynecol.* **175:**1365–1370; 1996.

[14] Solomon, C. G.; Carroll, J. S.; Okamura, K.; Graves, S. W.; Seely, E. W. Higher cholesterol and insulin levels are associated with increased risk for pregnancy-induced hypertension. *Am. J. Hypertens.* **12:**276–282; 1999.

[15] Ros, H. S.; Lichtenstein, P.; Lipworth, L.; Cnattingius, S. Genetic effects on the liability of developing preeclampsia and gestational hypertension. *Am. J. Med. Gen.* **91:**256–260; 2000.

[16] Hubel, C. A.; Roberts, J. M.; Taylor, R. N.; Musci, T. J.; Rodgers, G. M.; McLaughlin, M. K. Lipid peroxidation in pregnancy: new perspectives on preeclampsia. *Am. J. Obstet. Gynecol.* **161:**1025–1034; 1989.

[17] Wisdom, S. J.; Wilson, R.; McKillop, J. H.; Walker, J. J. Antioxidant systems in normal pregnancy and in pregnancy-induced hypertension. *Am. J. Obstet. Gynecol.* **165:**1701–1704; 1991.

[18] Hubel, C. A.; Kozlov, A. V.; Kagan, V. E.; Evans, R. W.; Davidge, S. T.; McLaughlin, M. K.; Roberts, J. M. Decreased transferrin and increased transferrin saturation in sera of women with preeclampsia: implications for oxidative stress. *Am. J. Obstet. Gynecol.* **175:**692–700; 1996.

[19] Morris, J. M.; Gopaul, N. K.; Endersen, M. J. R.; Knight, M.; Linton, E. A.; Dhir, S.; Anggard, E. E.; Redman, C. W. G. Circulating markers of oxidative stress are raised in normal pregnancy and preeclampsia. *Br. J. Obstet. Gynecol.* **105:**1195–1199; 1998.

[20] Roberts, J. M.; Hubel, C. A. Is oxidative stress the link in the two-stage model of preeclampsia? *Lancet* **354:**788–789; 1999.

[21] Pandolfi, P. P; Sonati, F.; Rivi, R.; Mason, P.; Grosveld, F.; Luzzatto, L. Targeted disruption of the housekeeping gene encoding glucose-6-phosphate dehydrogenase (G6PD): G6PD is dis-

pensable for pentose synthesis but essential for defense against oxidative stress. *EMBO J.* **1458:**5209–5215; 1995.
[22] Beutler, E. The glutathione instability of drug-sensitive red cells. A new method for the in vitro detection of drug sensitivity. *J. Lab. Clin. Med.* **49:**84–95; 1957.
[23] Németh, I.; Boda, D. Blood glutathione redox ratio as a parameter of oxidative stress in premature infants with IRDS. *Free Radic. Biol. Med.* **16:**347–353; 1994.
[24] Winterbourn, C. C. Oxidative reactions of hemoglobin. *Methods Enzymol.* **186:**265–273; 1990.
[25] Ward, P. A.; Till, G. O.; Haterill, J. R.; Annesley, T. M.; Kunkel, R. G. Systemic complement activation, lung injury, and products of lipid operoxidation. *J. Clin. Invest.* **76:**517–527; 1985.
[26] Koster, J. P.; Biemond, P.; Swaak, A. J. Intracellular and extracellular sulphydryl levels in rheumatoid arthritis. *Ann. Rheum. Dis.* **45:**44–46; 1986.
[27] Johnson, D. A.; Osaki, S.; Frieden, E. Micromethod for the determination of ferroxidase (Ceruloplasmin) in human serums. *Clin. Chem.* **13:**142–150; 1967.
[28] Chen, G.; Wilson, R.; Cumming, G.; Walker, J. J.; Smith, W. E.; McKillop, J. H. Intracellular and extracellular antioxidant buffering levels in erythrocytes from pregnancy-induced hypertension. *J. Hum. Hypertens.* **8:**37–42; 1994.
[29] Jarolim, P.; Lahav, M.; Liu, S. C.; Palek, J. Effect of hemoglobin oxidation products on the stability of red blood cell membrane cytoskeletons and the associations of skeletal proteins: correlation with a release of hemin. *Blood* **76:**2125–2131; 1990.
[30] Salvemini, F.; Franzé, A.; Iervolino, A.; Filosa, S.; Salzano, S.; Ursini, M. V. Enhanced glutathione levels and oxidoresistance mediated by increased glucose-6-phosphate dehydrogenase expression. *J. Biol. Chem.* **29:**2750–2757; 1999.
[31] Ursini, M. V.; Parrella, A.; Rosa, G.; Salzano, S.; Martini, G. Enhanced expression of glucose-6-phosphate dehydrogenase in human cells sustaining oxidative stress. *Biochem. J.* **323:**801–806; 1997.
[32] Sarrell, P. M.; Lindsay, D. C.; Poole-Wilson, P. A.; Collins, P. Hypothesis: inhibition of endothelium-derived relaxing factor by haemoglobin in the pathogenesis of preeclampsia. *Lancet* **33658:** 1030–1032; 1990.
[33] Entman, S. S.; Kamban, J. R.; Bradley, C. A.; Cousar, J. B. Increased levels of carboxyhemoglobin and serum iron as an indicator of increased red cell turnover in preeclampsia. *Am. J. Obstet. Gynecol.* **156:**1169–1173; 1987.
[34] Ullrich, O.; Reinheckel, T.; Sitte, N.; Grune, T. Degradation of hypochlorite-damaged glucose-6-phosphate dehydrogenase by the 20S proteosome. *Free Radic. Biol. Med.* **2758:**487–492; 1999.
[35] Maines, M. D. The heme oxygenase system: a regulator of second messenger gases. *Annu. Rev. Pharmacol. Toxicol.* **35:**517–554; 1997.
[36] Motterlini, R.; Gonzales, A.; Foresti, R.; Clark, J. E.; Green, C. J.; Winslow, R. M. Heme oxygenase-1-derived carbon monoxide contributes to the suppresion of acute hypertensive responses in vivo. *Circ. Res.* **83:**568–577; 1998.
[37] Ahmed, A.; Rahman, M.; Zhang, X.; Acevedo, C. H.; Nijjar, S.; Rushton, I.; Bussolati, B.; St. John, J. Induction of placental heme oxygenase-1 is protective against TNF alpha–induced cytotoxicity and promotes vessel relaxation. *Mol. Med.* **6:**391–409; 2000.
[38] Knapen, M.; Mulder, T. P.; Van Rooij, I. A.; Peters, W. H.; Steegers, E. A. Low whole blood glutathione levels in pregnancies complicated by preeclampsia or the hemolysis, elevated liver enzymes, low platelet syndrome. *Obstet. Gynecol.* **92:**1012–1015; 1998.
[39] Spickett, C. M.; Reglinski, J.; Smith, W. E.; Wilson, R.; Walker, J. J.; McKillop, J. Erythrocyte glutathione balance and membrane stability during eclampsia. *Free Radic. Biol. Med.* **24:**1049–1055; 1998.
[40] Chapell, L. C.; Briley, A. L.; Shennan, A. H.; Poston, L. Effect of antioxidants on the occurrence of preeclampsia in women at increased risk: a randomised trial. *Lancet* **354:**810–816; 1999.

ABBREVATIONS

APH—acetylphenylhydrazine
CO—carbon monoxide
G6PD—glucose-6-phosphate dehydrogenase
GSH—reduced glutathione
GSSG—oxidized glutathione
GSSG/GSH—glutathione redox ratio
Hb—hemoglobin
HMP—hexose-monophosphate shunt
NO—nitric oxide
RBCs—red blood cells