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Maternal antioxidant concentrations after uncomplicated pregnancies

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Abstract

Background: To analyse the post-partum concentrations of intra- and extra-cellular blood antioxidants in women with uncomplicated pregnancies.

Methods: Whole blood and plasma thiols, plasma vitamin E and C, serum cholesterol and triglyceride, ferric reducing ability of plasma (FRAP) concentrations were compared between women delivered by caesarean section ($n = 17$) or spontaneous delivery ($n = 10$). A repeated mixed model was used for statistical analysis.

Results: The majority of whole blood thiols increased significantly in both groups the first days post-partum. However, within the caesarean group free cysteine, oxidised cysteine, homocysteine and glutathione and plasma cysteine and homocysteine levels dropped significantly after 24 h, while FRAP levels peaked significantly in this group. Plasma vitamin E levels decreased significantly in both groups within 24 to 48 h after delivery. Independent of the way of delivery whole blood and plasma thiols were significantly increased and vitamin E levels were significantly decreased 3 months post-partum while plasma vitamin C levels and FRAP were unchanged compared to ante-partum levels.

Discussion: Decreased plasma vitamin E levels shortly post-partum are associated with decreased lipid peroxidation. The 24 h post-partum drop of some plasma and whole blood thiols in the caesarean group may be due to prolonged fasting.

Keywords: *Antioxidants, thiols, glutathione, vitamin E, vitamin C, pregnancy*

Introduction

In uncomplicated pregnancies lipid peroxide concentrations increase with advancing gestational age, possibly caused by a pronounced physiological hyperlipidemia and production of lipid peroxides in the placenta.[1] In addition, increased levels of reactive oxygen species (ROS), released by respiratory burst of neutrophiles are found in uncomplicated pregnancies,[2] although also decreased levels compared to the non-pregnant state are reported.[3] In parallel with the rise in lipid peroxidation various antioxidants increase during uncomplicated pregnancy, such as vitamin E, vitamin C, glutathione peroxidase, ceruloplasmin, red blood cell amino thiols, and net antioxidant activity.[4–6] One of the most important intracellular antioxidants is glutathione, which can maintain the redox balance of cells, either acting alone or in combination with glutathione peroxidases and glutathione S-transferases.[4] In healthy people the formation of ROS is effectively counteracted by the antioxidant defence.

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Unless properly counteracted an excess of ROS causes oxidative stress, may result in glutathione depletion, lipid peroxidation, or membrane damage.[7]

Labour is associated with generation of ROS and lipid peroxidation,[8] possibly due to repetitive ischemia and reperfusion.[9] Within 24 to 48 h after normal pregnancy a decrease is reported of lipid peroxides and antioxidant activity.[10] However, Nakai et al. (2000) found a significant increase of the antioxidants superoxide dismutase and catalase in the first 24 h after delivery, which was ascribed to the physical stress and pain of labour.[11]

In general, data on the post-partum course of the different antioxidant levels are scarce. Vitamin E concentrations are decreased at 3 months post-partum, though the time of onset of this decrease is unknown.[6] The post-partum courses of other antioxidants, such as vitamin C and plasma and whole blood glutathione levels as well as other thiols have never been studied before.

The knowledge of the concentration courses of thiols and other antioxidants in uncomplicated pregnancies is necessary for understanding the role of a disturbed oxidant–antioxidant balance in pregnancies complicated by preeclampsia and/or HELLP syndrome. Hypertensive disorders of pregnancy are complications of pregnancy associated with a suboptimal function of the placenta coinciding with increased oxidative stress.[12,13] Subsequently, secondary preventive strategies early in pregnancy in women at high risk may be developed. The antioxidant system probably reacts on the changes occurring during pregnancy, especially during complicated pregnancy. Once the longitudinal patterns of antioxidants, such as those of the thiols and vitamins C and E, in uneventful pregnancies are known, a next step would be determination and interpretation of levels of these antioxidants during complicated pregnancies.

Therefore we studied the concentrations of the antioxidants vitamin E, vitamin C, plasma and whole blood thiols, as well as antioxidant capacity, by measuring the FRAP, in the peripartum period and throughout the puerperium of women with uncomplicated pregnancies.

Material and methods

Subjects

The study was performed between October 2000 and September 2001 at the University Medical Center Nijmegen, the Netherlands. The experimental protocol was approved by the Medical Ethical Review Committee and each subject gave written informed consent.

Participants were healthy nulliparous and multiparous women with uncomplicated, normotensive pregnancies. Exclusion criteria were smoking during pregnancy and the puerperium, essential hypertension, diabetes, multiple pregnancy, and a history of premature birth (<37 weeks gestation), fetal growth retardation, preeclampsia and HELLP syndrome (haemolysis, elevated liver enzymes and low platelets). Normotension was defined as a blood pressure below 140/90 mmHg, preconceptionally and throughout pregnancy.

Seventeen healthy women with uncomplicated, normotensive pregnancies, who delivered by elective caesarean section, either in case of breech presentation or in case of repeat caesarean section, and ten healthy, pregnant women with uncomplicated, normotensive pregnancies, who all delivered vaginally and spontaneously, were included before onset of labour. All caesarean sections were performed under spinal anaesthesia. According to protocol, the first 24 h after caesarean section all women in our hospital are only allowed water and tea for oral intake.

Blood samples

Women were studied longitudinally and blood was sampled within one week before delivery and at seven occasions after delivery; e.g. 6 h, 12 h, 24 h, 48 h, 72 h, 6 weeks, and 3 months post-partum. Blood was collected into sterile evacuated blood collection tubes containing ethylenediaminetetra-acetic acid (EDTA) or into dry collection tubes for serum. Blood was sampled after fasting of at least 8 h, except for the samples 6, 12 and 24 h post-partum of the vaginal group. Whole EDTA blood was processed within half-an-hour for the analysis measurement of free and oxidised thiols as prepared for analysis and stored at -80°C until analysis.[14,15] The remaining whole blood was centrifuged at 1500*g* for 15 min and the obtained blood thus obtained for measurement of (total) thiols, ferric reducing ability of plasma (FRAP), vitamin C (ascorbic acid), and vitamin E (α-tocopherol) was stored either at -80°C until analysis (vitamin C, vitamin E and FRAP) or at -30°C until analysis (plasma thiols).

Processed plasma and whole blood samples were analysed for the concentration of cysteine, homocysteine, glutathione, and cysteinylglycine by high performance liquid chromatography (HPLC) as described previously.[14,15]

FRAP levels were measured spectrophotometrically and were expressed as nmol Fe^{2+} equivalent/ml.[16] For the analysis of vitamins C and E a HPLC method was used, as described earlier.[17]

Since vitamin E has the same carrier system in plasma as cholesterol and triglycerides, which increase substantially during pregnancy,[18] vitamin E levels were also calculated as the ratio vitamin E to cholesterol and the ratio vitamin E to triglycerides. Some investigators consider the ratio vitamin E to cholesterol a more relevant marker than vitamin E alone.[19] Haemoglobin, haematocrite, serum cholesterol and triglyceride concentrations were

measured at the routine clinical chemistry laboratory of our hospital using standardised methods.

Statistical methods

Concentration Course During the First 3 days after Delivery. At forehand we expected these laboratory parameters to exert a fast decline, followed by a slower recovery. Therefore we used a repeated mixed model on each parameter, separately, while accounting for the way of delivery (being either vaginal delivery or caesarean section).

At first, we found that for nearly all laboratory parameters the model was statistical significantly improved when a quadratic term in time was included in the linear part of the model (Likelihood-Ratio test). This indicates that the recovery, e.g. increase or decrease, after stress of this parameter was reached already within 3 days post-partum. Furthermore, no significant improvement was reached when the best model found above was extended with either a higher-order-time term, or an exponential term in time or a broken linear term in time. Inspection of the residuals, using a saturated model, did not show deviation from normality of any of the parameters which would have motivated to use a quadratic or logarithmic transformation. After all, the following final mixed model was used for each laboratory parameter, separately. The dependent variable was the laboratory parameter. The independent random variable was "women" and the independent continuous variable was linear time (and quadratic time, respectively) and the independent class variable was the way of delivery (vaginal and caesarean). Also the interaction term between way of delivery and the time variable(s) was (were) included in the model, allowing for differences in the course between the ways of delivery.

The initial model we used is as follows:

$$Y_i(t_{ij}) = \beta_1 C_i + \beta_2 nC_i + (\beta_3 C_i + \beta_4 nC_i)^* t_{ij} + (\beta_5 C_i + \beta_6 nC_i)^* t_{ij}^2 + b_{1i} + b_{2i}^* t_{ij} + b_{3i}^* t_{ij}^2 + \varepsilon_{ij}$$

where i refers to subject and j to the time after partus, with the fixed effects β, the random effects b, C and nC are indicator variables for caesarean and non-caesarean and ε_{ij} is the normal distributed residual with mean zero. Note that this implies that differences between women may vary over time.

The estimated regression parameters with standard errors of each variable are used to calculate the average level per hour of women in each group with confidence bands. And these levels are further presented in figures.

For the course of whole blood cysteine a third-degree polynomial in a linear mixed model was used. In contrast to other parameters the third degree polynomial fitted better to the data, although like using other parameterisations the fit was still not satisfactorily. Especially the caesarean group deviates, most possible due to a prolonged fastening up to 24 h after delivery, as the time pattern in the vaginal group could be quadratic. However all of these models were consistent concerning the statistical significance of the differences between the groups.

Concentration course 6 weeks and 3 months after delivery. The difference in mean levels of the laboratory parameter at 6 weeks and 3 months post-partum compared to the mean level at the time of delivery was analysed using a repeated mixed model, somewhat different from the one described above. The dependent variable was the laboratory parameter. The independent random variable was "women" and the independent class variables were way of delivery (vaginal and caesarean) and time (three levels: at delivery, 6 weeks after delivery and 3 months after delivery). Also the interaction term between way of delivery and the time variable was included in the model. The appropriate, adjusted Tukey-Kramer contrast test was used to test for specific differences in mean levels.

The estimated mean levels of the laboratory parameters with 95% confidence intervals are presented by each group and by each point of measurement.

A p-value below 0.05 was considered statistically significant.

Results

The patient characteristics are presented in Table I. Of all these, only gestational age at delivery was slightly, but significantly lower in the caesarean group than in

Table I. Clinical and demographic characteristics of the study groups.

	Caesarean group (n=17)	Vaginal group (n=10)
Age (years)	32 (27–38)	33 (26–41)
Primiparity	3 (18%)	7(70%)
Gestational age at delivery (weeks)	38^{+6} (37^{+6}–41^{+2})*	40 (38–42^{+3})
Length of labour (hours)	–	5.0 (3.5–15)
Lactation		
First days post-partum	15 (88%)	7 (70%)
Six weeks post-partum	12 (71%)	5 (70%)
Three months post-partum	9 (53%)	5 (50%)
Diastolic blood pressure (mmHg)	70 (60–80)	63 (55–80)
Smoking before pregnancy	2 (12%)	2 (20%)

Data are presented as medians (min–max) or numbers (%). Differences between groups are tested for statistical significance by using the Mann-Whitney U-test.
$\star p < 0.05$.

the vaginal group. During pregnancy none of the women smoked, but one woman of the caesarean group restarted smoking two months after delivery. No significant differences between baseline levels of the two groups were found in any of the biochemical parameters.

Concentration course during the first 3 days after delivery

The analysis of whole blood levels of free cysteine showed that none of the parameterisations of time adequately fitted to the data. A further inspection showed that this was due to the mean level of free cysteine at 24 h, being remarkable low in women who were delivered by caesarean section. This time pattern is visualised in Figure 1 showing the estimated and observed mean values of whole blood cysteine.

A similar pattern was observed in the mean whole blood levels of oxidised cysteine, homocysteine and glutathione, although to a far lesser extent, since these parameters fitted adequately to the quadratic model.

During the first 72 h following delivery a significant increase ($p < 0.05$) of whole blood levels of free cysteine, oxidised cysteine, and oxidised homocysteine was observed in women who were delivered vaginally, whereas in the caesarean group mean whole blood levels of free cysteine and oxidised cysteine and oxidised homocysteine increased after an initial significant drop of levels (Figure 1).

Furthermore, statistical significant differences ($p < 0.05$) in the time pattern, as well in rise as in decline, between both groups were observed for the plasma thiols (cysteine, homocysteine, cysteinylglycine and glutathione), FRAP, cholesterol and ratio of vitamin E to cholesterol.

During the first 3 days the mean levels of plasma thiols, FRAP and ratio of vitamin E to cholesterol remained unchanged in the group of vaginal delivery. However, among women who were delivered by caesarean section the first 3 days after delivery were characterised by a temporary fall in concentrations of plasma cysteine and homocysteine (Figures 1 and 2), a temporary increase of FRAP and the ratio of vitamin E to cholesterol, whereas plasma cysteinylglycine and glutathione concentrations increased linearly during this time period.

In both groups the mean cholesterol levels reached their lowest point within 3 days after delivery, although in the caesarean group levels are somewhat lower as compared to the vaginal group. All other parameters, except those mentioned above, showed no difference in the time patterns between both delivery groups. As a result, irrespective of way of delivery, vitamin E (Figure 2), ratio of vitamin E to triglycerides, haemoglobin and haematocrite decreased quadratically during the first 72 h after delivery, resulting in a bottom level near 36 h after delivery. Furthermore, a statistically significant linear increase in the mean whole blood levels of free homocysteine and cysteinylglycine was observed, whereas whole blood levels of free glutathione and serum triglyceride concentrations were characterised by a significant linear decrease.

Oxidised cysteinylglycine and plasma vitamin C did not show significant differences in time, independent of the way of delivery. The ratios of free to oxidised whole blood cysteine, homocysteine, glutathione, and cysteinylglycine did not change during the first 3 days.

Concentration course 6 weeks and 3 months after delivery

The repeated measurement analysis showed no statistical significant differences in concentration courses of any of the variables between the two delivery groups. This is demonstrated in the examples presented in the Figures 1 and 2, where the observed mean values (95% confidence intervals) in both groups at the three time points (ante-partum, 6 weeks and 3 months post-partum) are compared. Therefore the long-term post-partum course can be described irrespective of the way of delivery.

In Tables II and III the estimated mean values at each of the three time points are shown. In general there were no statistical significant differences between the mean values of the thiols at 6 weeks and 3 months post-partum, whereas both post-partum values are significantly higher compared to the ante-partum value. The mean values of free cysteinylglycine, the ratio of free to oxidised cysteine, vitamin C and FRAP were not significantly different between these three points of measurement, indicating a fairly constant level. The concentration course of plasma glutathione showed a pattern different from any of the other variables, namely the mean level of glutathione increased significantly at 6 weeks compared to ante-partum levels followed by a drop 3 months post-partum to a level that was still significantly higher than that ante-partum. Furthermore, not until 3 months post-partum the mean values of ratio of free to oxidised of both cysteinylglycine and glutathione were significant lower compared to ante-partum levels. This was also observed for vitamin E and the ratio of vitamin E to cholesterol. Three months post-partum only the mean values of haemoglobin and haematocrite were significantly increased compared to 6 weeks.

The mean values of the ratio of vitamin E to triglycerides followed a recovery pattern similar to that of most of the thiols, a full recovery at 3 months after child birth with the increase starting 6 weeks post-partum. This also holds for the mean values of the cholesterol and triglycerides, although decreasing in time.

Whole blood and plasma cysteinylglycine levels are not depicted in Figure 1, though all concentration courses of the cysteinylglycine levels are described above.

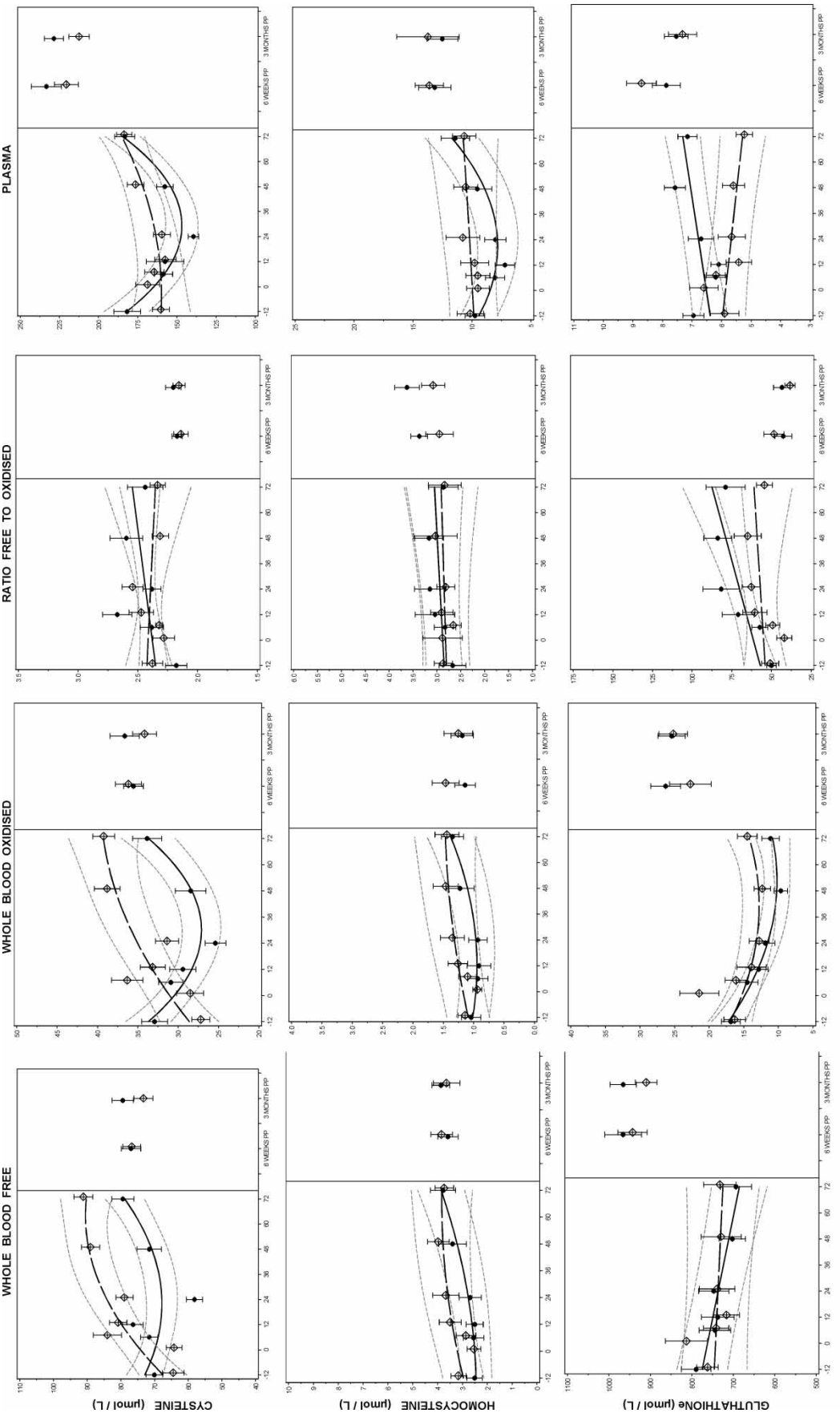

Figure 1. The observed mean levels of whole blood and plasma cysteine, homocysteine and glutathione are depicted from 12 h antepartum upto 3 months post-partum (\scale90% {\tf="Pi3"\char"5E} = vaginal group; • = caesarean group); the vertical bars indicate ± one standard error (SE). For the ease of presentation, the data concerning the vaginal group are shifted to the right. The estimated profiles are presented, a quadratic equation in a linear mixed model (long dashes: vaginal group; solid line: caesarean group). Also the appropriate 95% confidence bands are shown (short dashes). Note: The course of the caesarean group deviates, this is most possible due to a prolonged footing up to 24 h after delivery. PP = postpartum.

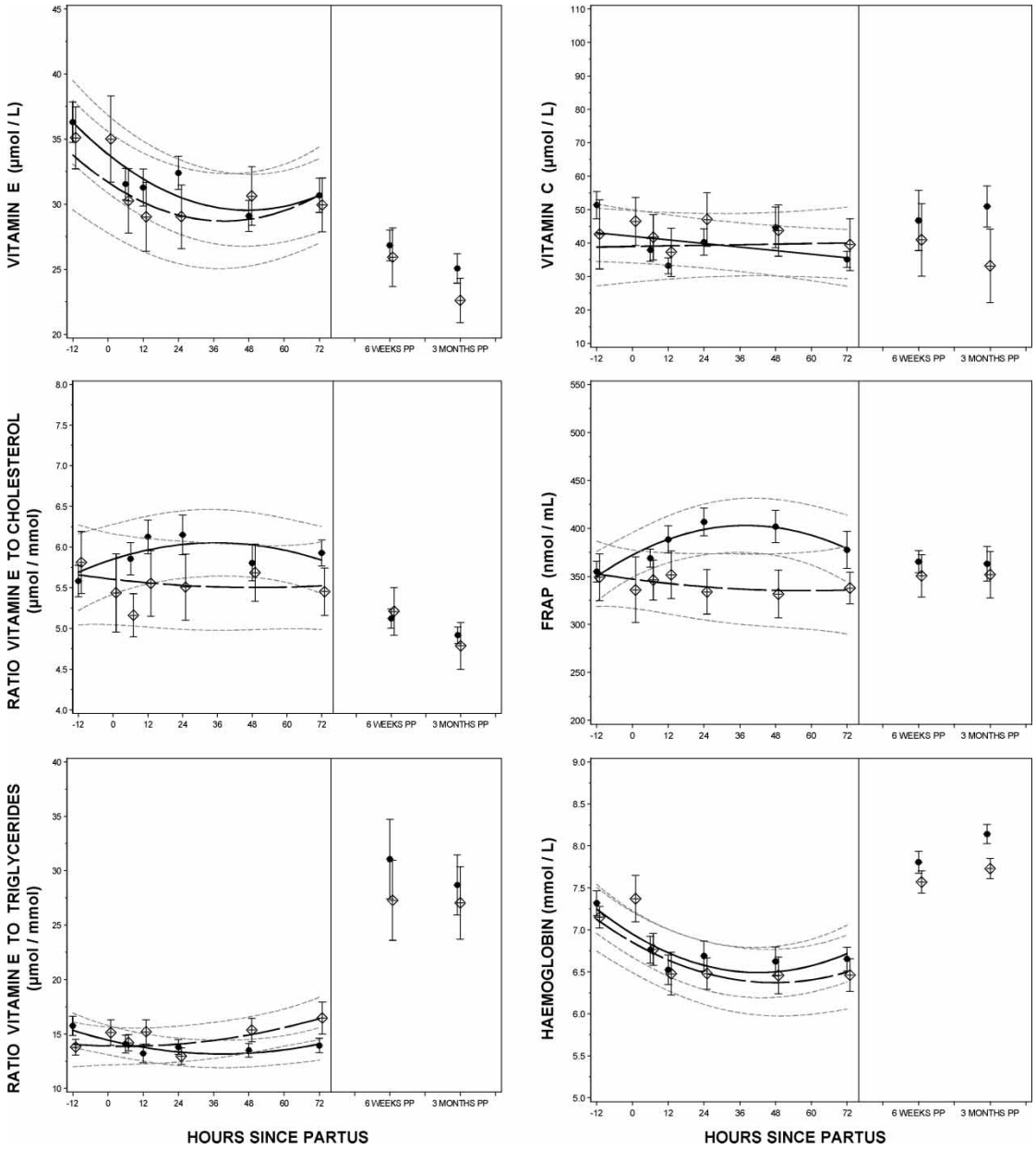

Figure 2. The observed mean levels of vitamin E, vitamin C, ratio vitamin E to cholesterol, ratio vitamin E to triglycerides, FRAP and haemoglobin are depicted from 12 h antepartum upto 3 months post-partum (\scale90% {\tf="Pi3"\char"5E} = vaginal group; • = caesarean group); the vertical bars indicate ± one standard error (SE). For the ease of presentation, the data concerning the vaginal group are shifted to the right. The estimated profiles are presented, a quadratic equation in a linear mixed model (long dashes: vaginal group; solid line: caesarean group). Also the appropriate 95% confidence bands are shown (short dashes). FRAP = Ferric reducing ability of plasma. PP = postpartum.

Discussion

Despite the current interest for the oxidant–antioxidant system during normal and compromised pregnancies, there are only a few studies on antioxidant behaviour in the puerperium. We demonstrated that vitamin E levels started to decrease right after delivery and reached it is lowest level near 36 h postpartum. This corresponds well with the decrease of lipid peroxidation within 24 h post-partum as reported by others.[6] Oostenbrug et al. (1998) sampled maternal blood directly after delivery and found no decrease of α-tocopherol levels, though two other forms of tocopherol, e.g. δ and β + γ tocopherol, which are as well very potent antioxidants, were decreased shortly after delivery as compared to levels found at 32 weeks of gestation.[20] During pregnancy the lipophilic vitamin E is one of the most important antioxidants, protecting against lipid peroxidation.[21] When the burden of free radical damage and lipid peroxidation disappears after delivery, vitamin E levels will gradually decrease to non-pregnant levels, as found in our study where the decline of concentrations continued until 3 months post-partum. During pregnancy there is a major increase of cholesterol and

Table II. The estimated mean levels (95% CI) of whole blood and plasma thiols ante-partum, 6 weeks and 3 months post-partum.

			Post-partum	
		Ante-partum	6 weeks	3 months
Whole blood	Free cysteine (μmol/l)	67.26 (63.10–71.43)	76.88 (72.41–81.33)[a]	76.36 (71.58–81.14)[a]
	Oxidised cysteine (μmol/l)	30.05 (27.72–32.39)	35.85 (33.76–37.93)[a]	35.39 (32.68–38.10)[a]
	Free to oxidized cysteine	2.28 (2.14–2.42)[a]	2.15 (2.08–2.23)[a]	2.18 (2.08–2.27)[a]
Plasma	Total cysteine (μmol/l)	171.0 (158.7–183.3)	227.0 (212.6–241.2)[a]	220.5 (210.8–230.1)[a]
Whole blood	Free homocysteine (μmol/l)	2.83 (2.35–3.33)	3.71 (3.07–4.36)[a]	3.76 (3.12–4.39)[a]
	Oxidised homocysteine (μmol/l)	1.09 (0.86–1.33)[a]	1.30 (1.01–1.59)[a]	1.22 (0.91–1.53)[a]
	Free to oxidized homocysteine	2.77 (2.36–3.18)	3.16 (2.83–3.49)[a]	3.35 (2.96–3.75)[a]
Plasma	Total homocysteine (μmol/l)	9.94 (8.53–11.36)	13.38 (11.31–15.46)[a]	13.12 (10.41–15.84)[a]
Whole blood	Free cysteinyl–glycine (μmol/l)	8.15 (7.61–8.69)[a]	10.18 (8.70–11.66)[a]	8.85 (8.14–9.56)[a]
	Oxidised cysteinyl-glycine (μmol/l)	1.85 (1.61–2.10)	2.52 (2.23–2.81)[a]	2.31 (2.08–2.54)[a]
	Free to oxidized cysteinyl-glycine	4.63 (4.22–5.04)[a]	4.17 (3.70–4.64)[a,b]	3.96 (3.56–4.35)[b]
Plasma	Total cysteinyl-glycine (μmol/l)	31.04 (28.92–33.17)	37.81 (35.17–40.45)[a]	38.66 (36.22–41.10)[a]
Whole blood	Free glutathione (μmol/l)	775.8 (723.4–828.2)	954.3 (888.1–1020.4)[a]	937.8 (890.6–984.9)[a]
	Oxidised glutathione (μmol/l)	16.59 (14.40–18.78)	24.50 (20.75–28.25)[a]	25.31 (22.20–28.42)[a]
	Free to oxidized glutathione	50.36 (44.49–56.24)[a]	45.44 (36.72–54.15)[a,b]	40.82 (33.33–48.30)[b]
Plasma	Total glutathione (μmol/l)	6.43 (5.82–7.04)	8.29 (7.54–9.04)	7.43 (6.78–8.08)

a,*b*: same letter, horizontally, indicate no statistical significant difference between the points of measurement.
Note: Only the overall mean levels are presented at each point of measurement, because the difference between both groups were never statistical significant using the repeated mixed model.

triglycerides levels, whereas these levels gradually decrease after delivery to non-pregnant levels.[22] Triglycerides declined faster than vitamin E concentrations, resulting in an increased ratio of vitamin E to triglycerides after an initial decrease during the first 3 days. The ratio of vitamin E to cholesterol acted in the opposite way, e.g. a decrease after an initial increase. The courses of these ratios illustrate that vitamin E itself is diminished after delivery and cannot only be explained by decreased concentrations of triglyceride and cholesterol. Since only cholesterol levels are directly related to vitamin E levels and not triglyceride levels, the vitamin E to cholesterol ratio is probably the most important ratio.[18]

Vitamin C exhibits multiple antioxidant properties and is one of the most important, extracellular antioxidants in humans.[23] It contributes in the defence against lipid peroxidation due to its ability to regenerate vitamin E by reducing α-tocopherol radicals to α-tocopherol.[24] In our study the ante-partum levels of vitamin C were not higher as compared to non-pregnant levels in healthy individuals (normal range 50–200 μM).[23] Furthermore, we found no change of vitamin C levels in the puerperium. Various studies on vitamin C levels during normal pregnancy reported a fairly constant level of vitamin C throughout pregnancy despite a seasonal variation of vitamin C in some regions, and a rise in concentrations up to 6 months post-partum as summarised by Dostolova.[25] Nevertheless, Woods et al. reported that vitamin C concentrations were significantly lower in women during delivery as compared to women who underwent an elective caesarean section.[9] They postulated that this was due to generation of ROS, caused by repetitive ischemia and reperfusion of uterine tissue after each contraction.

Remarkably we did not find an effect of labour, neither for plasma vitamin C and E, nor for plasma

Table III. The estimated mean levels (95% CI) of haematological parameters, vitamins, cholesterol and triglycerides ante-partum, 6 weeks and 3 months post-partum.

		Post-partum	
	Ante-partum	6 weeks	3 months
Haemoglobin (mmol/l)	7.23 (7.02–7.45)	7.69 (7.48–7.89)	7.93 (7.75–8.12)
Hematocrite (l/l)	0.34 (0.33–0.35)	0.37 (0.36–0.38)	0.39 (0.38–0.40)
Cholesterol (mmol/l)	6.38 (5.87–6.89)	5.18 (4.69–5.67)[a]	4.94 (4.56–5.31)[a]
Triglycerides (mmol/l)	2.56 (2.29–2.83)	1.14 (0.85–1.42)[a]	1.02 (0.79–1.25)[a]
Vitamin E (μmol/l)	35.88 (33.09–38.69)	26.66 (24.24–29.08)	23.83 (21.79–25.87)
Ratio vitamin E/cholesterol	5.64 (5.26–6.03)	5.18 (4.90–5.46)	4.85 (4.59–5.11)
Ratio vitamin E/triglycerides	14.62 (13.32–15.93)	29.291 (23.77–34.82)[a]	27.86 (23.29–32.42)[a]
Vitamin C (μmol/l)	45.5 (36.6–54.5)[a]	44.9 (30.8–59.1)[a]	43.1 (31.6–54.6)[a]
FRAP (nmol Fe^{2+} eq/ml)	352.09 (328.01–376.18)[a]	357.98 (334.68–381.27)[a]	357.47 (326.71–388.22)[a]

a,*b*: same letters, horizontally, indicate no statistical significant difference between the points of measurement. FRAP = ferric reducing ability of plasma.

and whole blood thiols. Since in our study the first blood sample was obtained 6 h after delivery, a possible effect of labour might have been disappeared already. Moreover, the ratios of free to oxidised thiols, as a measure of oxidative stress,[26] were not changed indicating that there was no significant increase of oxidative stress.

A second intriguing finding was the drop of the whole blood levels of free and oxidised cysteine, oxidised homocysteine and plasma cysteine and homocysteine 24 h after caesarean section, which most likely might be explained by the prolonged fasting of 24 h after caesarean section. Impairment of the nutritional status, by fasting may result in lower levels of sulphur containing amino acids, such as cysteine.[27] Since cysteine is the rate limiting amino acid for synthesis of glutathione, fasting might result in a significant decrease of hepatic glutathione concentration.[28] Inadequate intracellular concentrations of glutathione are associated with a compromised antioxidant defence system and an insufficient capacity to combat free radicals.[29] In animal studies general anaesthesia or hypoxia are associated with increased formation of lipid peroxides and simultaneous depletion of plasma vitamin E and glutathione levels.[30] Since all women in the caesarean group received spinal anaesthesia, this probably did not affect our study results.

Increased FRAP concentrations 24 h after delivery in the caesarean group might be a reaction on the decreased levels of free cysteine and glutathione.

The third important finding of this study are the changes noted in the late puerperium; i.e. 3 months after delivery plasma levels of cysteine, homocysteine, cysteinylglycine, and glutathione are increased, which most likely is due to the diminished plasma volume as compared to the pregnant values.[31] This is in line with a previous report from our group, demonstrating lower plasma thiol levels during uncomplicated pregnancies compared to levels in non-pregnant women.[15] Plasma glutathione showed a peak level at 6 weeks post-partum, which was not due to outliners as can be seen from the confidence intervals, being as wide at all three points of measurement knowing ante-partum, 6 weeks post-partum and 3 months post-partum, respectively. Lowered ratios of free to oxidised levels of glutathione and other thiols indicate a shift in redox balance toward more oxidised levels, and are interpreted as a direct marker of oxidative stress.[26] Reduced glutathione is considered as a very potential scavenger of free radicals and peroxides.[32] Three months post-partum a lowered ratio of free to oxidised glutathione and cysteinylglycine levels was demonstrated which might indicate the presence of oxidative stress. The source of oxidative stress is unclear, and is not caused by re-start of smoking since this involves only one woman in the caesarean group. Multivitamin supplementation was only taken by four women (15%) in the caesarean group and probably also did not affect our results. Since the majority of the study population ($n = 22$) were breast feeding we cannot draw any conclusion on the effect of lactation on parameters of oxidative stress.

The large interindividual range of thiol and vitamins C and E concentrations between the participants and the small numbers in both groups caused relatively wide confidence intervals. Therefore, extrapolation of the data has to be done with caution. However, due to the longitudinal character of the study, we expect the course of the concentrations not to change substantially when larger numbers would be included.

Haemoglobin and haematocrite concentrations dropped significantly shortly after delivery, most probably due to post-partum haemorrhage and by shift of extra vascular body water into the circulation.[33] From previous studies these concentrations are known to normalise to non-pregnant levels 4 to 6 months after delivery,[33] as was confirmed in our study.

In conclusion, plasma vitamin E levels are lowered between 24 and 48 h after delivery. Antioxidant capacity does not seem to decrease within 24 h post-partum. However, fasting after caesarean section may negatively influence concentrations of some important plasma and whole blood thiols.

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