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F_2-Isoprostanes, tocopherols and normal pregnancy

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Abstract
This study investigates oxidative stress and antioxidants in normal human pregnancy and post-partum period. Thirty-seven healthy women with normal pregnancies were included. Both urinary and serum samples were collected throughout the pregnancy and post-partum period. Oxidative stress was estimated by measuring the reliable *in vivo* marker, namely 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$, an F_2-isoprostane) and antioxidant status was evaluated by measuring α- and γ-tocopherol in serum samples. Pregnancy was associated with successively increased levels of 8-iso-$PGF_{2\alpha}$ with advancing gestational age. The median post-partum value corresponded to the values observed in early gestation and a significant decrease was observed from late pregnancy to the post-partum period. Lipid-adjusted α- and γ-tocopherol levels decreased with advancing gestational age. This longitudinal study suggests that mild oxidative stress is involved in normal human pregnancy.

Keywords: *Pregnancy, free radicals, oxidative stress, antioxidants, isoprostanes, human*

Introduction

In recent years many studies have been published on the aetiology of pre-eclampsia in humans, focusing on the role of oxidative stress. The results remain contradictory [1–4]. However, very little is known on the involvement of oxidative stress in normal human pregnancy. Before conclusions can be drawn about the involvement of oxidative stress in pre-eclampsia and other pregnancy-related conditions, its role in normal pregnancy should be established.

Oxidative stress is an imbalance between increased formation of free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), and the antioxidant defence systems in the body. Free radical-mediated lipid peroxidation occurs when poly-unsaturated fatty acids with one or two double bonds, which are more susceptible to oxidation due to their instability, react with oxygen. Isoprostanes, a family of prostaglandin derivatives, are generated *in vivo* by free radical catalysed oxidation of arachidonic acid [5]. One of the major F_2-isoprostanes, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), is increased in several syndromes associated with oxidant injury and estimation of isoprostanes is now widely regarded as a reliable biomarker for *in vivo* measurement of oxidative stress [6–8]. Long-term administration of high doses of vitamin E decreases isoprostane formation in studies on humans and in experimental animal models [9–11].

In pregnant Japanese women, F_2-isoprostanes are significantly increased in late pregnancy compared to non-pregnancy [12]. In the same study, lipid-adjusted α- and γ-tocopherol levels showed a significant decrease in mid- to late-stage pregnancy compared to non-pregnancy. However, this study included only 19 women with uncomplicated pregnancy and all samples were taken in the third trimester. Other studies have also described involvement of oxidative stress in normal pregnancy, but few have followed the women throughout the pregnancy and none have

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studied a consistent *in vivo* biomarker of oxidative stress, such as isoprostanes [13–15]. In addition, we have previously observed a progressive increase of the cyclooxygenase-catalysed arachidonic acid metabolized product $PGF_{2\alpha}$ throughout pregnancy in bovines, which provides insight on the important role of enzymatically-catalysed lipid oxidation in pregnancy [16]. The described findings imply that increased lipid peroxidation and oxidative stress in pregnancy might have some biological role in animal as well as in human pregnancies [12,16].

The primary aim of this study was to evaluate the presence of oxidative stress in normal pregnancy estimated by measuring F_2-isoprostanes in biological samples collected from women throughout pregnancy and the post-partum period. In addition, α- and γ-tocopherol levels were measured throughout the pregnancy.

Subjects and methods

Fifty-nine pregnant women from one outpatient antenatal clinic in the city of Uppsala were recruited into the study during the period 2003–2004. The catchment area includes urban and rural areas and the population consists of women with varying levels of education. Those included in the study were healthy women, aged 18 years or older, with a normal single spontaneous pregnancy at inclusion. Exclusion criteria were lack of Swedish language skills and use of medications other than iron or folic acid supplements. At the antenatal clinic the pregnant women were given written and verbal information about the study by a midwife and all participating women gave their consent. Out of these 59 women seven were withdrawn from the study, four due to miscarriage, one due to relocation and two due to unwillingness to continue participation. Of the fifty-two women who completed the study and were included in the reference material, 37 had pregnancies classified as uncomplicated. Fifteen women had minor complications, four took medications, two were smokers (one of whom took medications and delivered post-term), three delivered pre-term, four delivered post-term and two gave birth to infants that were SGA (small for gestational age). A pre-term delivery was defined as gestational age less than 37 completed weeks at delivery and a post-term delivery as a gestational age more than 42 completed weeks at delivery. All women attended routine antenatal care and samples were taken throughout pregnancy and post-partum. The aim was to collect blood samples at gestational weeks 12, 20, 24, 28, 32, 36 and 40 and 8–10 weeks after delivery for analysis of α- and γ-tocopherol. Urinary samples were collected on the same occasions for measurement of 8-iso-$PGF_{2\alpha}$ and creatinine. Samples collected on the day of delivery were excluded. Blood and urinary samples were collected 6–8 times from all participating women.

Blood samples were collected in serum tubes (LH PST™ II, BD Vacutainer Systems, Plymouth, UK). Urinary samples were collected as midstream spot samples. The collected blood and urine samples were stored for a maximum of 7 h in a refrigerator at 8°C and transported the same day to the laboratory. The blood samples were then centrifuged and stored in a freezer at −70°C until analysis. The urinary samples were stored likewise at −70°C until analysis. The study protocol was approved by the local Ethics Board at the Medical Faculty, Uppsala University.

Measurement of 8-iso-$PGF_{2\alpha}$ (oxidative stress marker)

The urinary samples were analysed for 8-iso-$PGF_{2\alpha}$ by a radioimmunoassay (RIA), as described by Basu [17]. The cross-reactivity of the 8-iso-$PGF_{2\alpha}$ antibody with 15-keto-13, 14-dihydro-8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\beta}$, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, 15-keto-13, 14-dihydro-$PGF_{2\alpha}$, TXB_2, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$ and 8-iso-$PGF_{3\alpha}$ was 1.7, 9.8, 1.1, 0.01, 0.01, 0.1, 0.03, 1.8 and 0.6%, respectively. The detection limit of the assay was 23 pmol/l. The intra-assay CV was 14.5% at low and 12.2% at high concentrations. The urine 8-iso-$PGF_{2\alpha}$ concentrations were corrected for urine creatinine values, which were measured using a commercial kit (IL™ Test by Monarch Instrument).

Measurement of α- and γ-tocopherol (antioxidants)

Serum α- and γ-tocopherol levels were assayed by HPLC with fluorescence detection [18]. In brief, 500 µl plasma were extracted with 500 µl ethanol containing 0.005% butylated hydroxytoluene and 2 ml hexane. A volume of 20 µl of the supernatant was injected into an HPLC column (LiChrospher 100 NH2 250 × 4 mm). The fluorescence detector had an excitation wavelength of 295 nm and an emission wavelength of 327 nm. Serum tocopherol levels were measured with and without adjustment for serum lipid concentrations. Serum cholesterol and triglyceride levels were measured by enzymatic methods using Test Cholesterol Trinard methods 181618-80 in a Monark apparatus (Instrument laboratory analysis, MS, USA). An external serum standard was used in all analyses. Intra-assay CV for α-tocopherol is 4.5% and γ-tocopherol is 7.2%.

Statistical calculations

Data were not normally distributed and, hence, non-parametric methods were used. The gestational period was divided into 2-week intervals and the median of 8-iso-$PGF_{2\alpha}$, α- and γ-tocopherol levels was calculated for each interval. When calculating the median each woman had the same weight, hence if a woman had more than one sample taken within a 2-week interval

the mean value of the samples was used. In order to examine the trend of repeated 8-iso-$PGF_{2\alpha}$, α- and γ-tocopherol measurements during pregnancy, individual linear regression models with gestational week as independent variable were estimated according to the equation $y = a + b^{\star}x$, where a = intercept, b = slope, x = gestational age (weeks) and y = 8-iso-$PGF_{2\alpha}$, α-tocopherol (lipid-adjusted and non-lipid-adjusted) or γ-tocopherol (lipid-adjusted and non-lipid-adjusted), respectively.

The regression analysis was based on samples collected during gestational weeks 7–40. The median slope, representing the mean change per gestational week, was calculated and the Wilcoxon Signed Rank test was used to test whether the median slope was significantly different from zero.

To compare the levels of 8-iso-$PGF_{2\alpha}$ and antioxidants at gestational weeks 9–10 and 39–40 to post-partum levels the Mann-Whitney U-test was used. Since only few women had measurements done at both gestational weeks 9–10, 39–40 and post-partum a test for comparing independent samples was used.

Results

Characteristics of the women and their newborn infants are presented in Table I. All deliveries took place at Uppsala University Hospital, Sweden, during the period 2004–2005.

Median levels of urinary 8-iso-$PGF_{2\alpha}$ throughout pregnancy and during the post-partum period are presented in Table II and Figure 1. An increase in 8-iso-$PGF_{2\alpha}$ with advancing gestational age is revealed. Table III presents a trend analysis of urinary 8-iso-$PGF_{2\alpha}$ during pregnancy and shows an increase of 0.0030 units per week ($p < 0.001$). The median post-partum value corresponds to the values observed in early gestational weeks 9–10 and a significant decrease is observed from late pregnancy to the post-partum period ($p = 0.014$; Figure 1).

Table I. Maternal and neonatal characteristics.

Characteristics	*n*	Mean	%	SD
Maternal age (years)	37	32		4
BMI (at booking)	37	24		4
Primipara	20		54.1	
Way of delivery				
Vaginal	32		86.5	
Caesarean section	5		13.5	
Caesarean section				
Elective	3		8.1	
Emergency	2		5.4	
Gestational age at delivery (days)		281		8
Foetal gender				
Female	16		43.2	
Male	21		56.8	
Birth weight (grams)	37	3607		452
Apgar $\leq$7 at 5 min	0			

Median levels of lipid-adjusted and non-lipid-adjusted serum α-tocopherol throughout pregnancy and during the post-partum period are presented in Table II and Figures 2 and 3. Table III presents a trend analysis and shows a decrease of 0.0055 units per week ($p < 0.001$) for lipid-adjusted α-tocopherol and an increase of 0.2836 units per week ($p < 0.001$) for non-lipid-adjusted α-tocopherol with advancing gestational age. The median post-partum value of lipid-adjusted α-tocopherol corresponds to the values observed in gestational weeks 9–10 ($p = 0.244$). No significant difference is observed from late pregnancy to the post-partum period ($p = 0.540$; Figure 2). The median post-partum value of non-lipid-adjusted α-tocopherol is higher compared to gestational weeks 9–10 ($p = 0.045$) and a significant decrease is observed from late pregnancy to the post-partum period ($p < 0.001$; Figure 3).

Median levels of lipid-adjusted and non-lipid-adjusted serum γ-tocopherol throughout pregnancy and during the post-partum period are presented in Table II and Figures 4 and 5. Table III presents a trend analysis of lipid-adjusted γ-tocopherol and shows a decrease of 0.0004 units per week ($p < 0.05$) and for non-lipid-adjusted γ-tocopherol an increase of 0.0105 units per week ($p < 0.001$). The median post-partum value of lipid-adjusted γ-tocopherol is increased compared to values observed in gestational weeks 9–10 ($p = 0.015$). It is also significantly increased compared to values in late pregnancy ($p = 0.010$; Figure 4). The median post-partum value of non-lipid-adjusted γ-tocopherol is increased compared to values in gestational weeks 9–10 ($p = 0.002$) and corresponds to values in late pregnancy ($p = 0.531$; Figure 5).

Discussion

This is the first longitudinal study in normal human pregnancy that shows successively increased oxidative stress as measured by F_2-isoprostanes in urinary samples collected throughout pregnancy. Isoprostanes have been established as a reliable *in vivo* marker of oxidative stress in both humans and animals [6–8]. We have found a significant increase in F_2-isoprostanes from week 9 throughout pregnancy until week 40 and thereafter a significant decrease to basal levels at post-partum weeks 9–10. In other longitudinal studies, it has been shown that levels of lipid hydroperoxides and/or TBARS (thiobarbituric acid) increase with advancing gestational age [13,14,19].

We have previously shown in a cross-sectional study performed on a Japanese population that F_2-isoprostane levels are increased in plasma and urine in the third trimester of pregnancy compared to non-pregnancy. However, the levels of the F_2-isoprostanes

Table II. Median levels of lipid-adjusted and non-lipid-adjusted 8-iso-$PGF_{2\alpha}$, α- and γ- tocopherol in 2-week intervals throughout pregnancy and during the post-partum period ($n = 37$). Samples collected on the day of delivery are excluded.

				α-tocopherol		γ-tocopherol	
Week	Days	n	8-iso-$PGF_{2\alpha}$ (Nmol/mmol creatinine)	adjusted (mg/mmol)	not adjusted (μg/ml)	adjusted (mg/mmol)	not adjusted (μg/ml)
Pregnancy							
7–8	49–62	4	0.36	2.08	10.1	0.12	0.59
9–10	63–76	10	0.36	1.86	10.9	0.06	0.30
11–12	77–90	19	0.30	2.05	12.2	0.07	0.45
13–14	91–104	1	0.40	2.04	10.4	0.04	0.22
15–16	105–118	3	0.30	2.08	14.3	0.09	0.55
17–18	119–132	1	0.45	2.39	13.8	0.15	0.86
19–20	133–146	23	0.35	1.84	15.5	0.07	0.57
21–22	147–160	12	0.35	1.97	15.7	0.08	0.51
23–24	161–174	8	0.34	1.97	16.7	0.07	0.51
25–26	175–188	28	0.35	1.88	16.1	0.07	0.63
27–28	189–202	15	0.32	1.86	17.0	0.07	0.61
29–30	203–216	19	0.45	1.83	18.9	0.05	0.52
31–32	217–230	26	0.39	1.82	19.0	0.06	0.67
33–34	231–244	12	0.36	1.85	18.3	0.07	0.63
35–36	245–258	28	0.39	1.83	18.7	0.07	0.71
37–38	259–272	10	0.38	1.87	22.0	0.09	0.93
39–40	273–286	20	0.42	1.84	19.5	0.08	0.88
Post-partum							
5–6	35–48	1	0.33	1.57	11.4	0.13	0.94
7–8	49–62	7	0.32	1.85	12.3	0.08	0.57
9–10	63–76	14	0.28	1.77	12.7	0.12	0.89
11–12	77–90	4	0.40	1.97	13.1	0.10	0.69
13–14	91–104	5	0.33	1.66	11.0	0.09	0.68
15–16	105–118	3	0.22	1.74	11.9	0.10	0.72
17–18	119–132	2	0.33	1.79	11.2	0.13	0.83
19–20	133–146	1	0.60	1.77	9.1	0.09	0.47

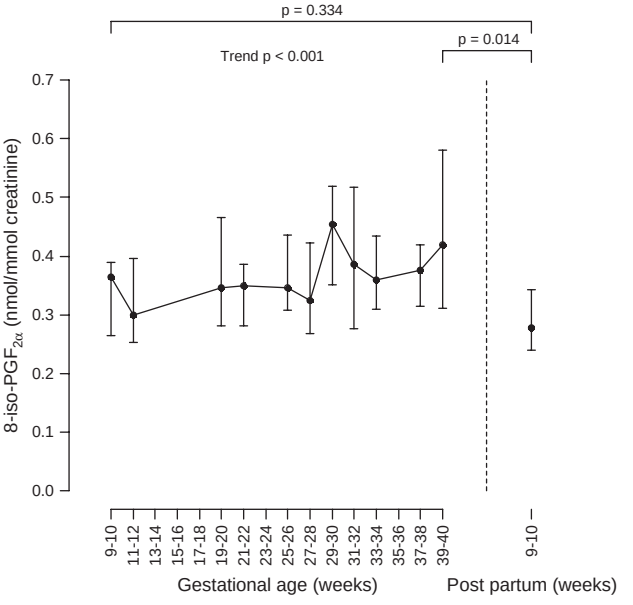

Figure 1. Urinary levels of 8-iso-$PGF_{2\alpha}$ in uncomplicated pregnancies and 9–10 weeks post-partum ($n = 37$). Only values from intervals including samples from at least 10 women are given. Data are presented as median and the 25th percentile to the 75th percentile. The p-value for trend was calculated as described in Table III.

were not investigated in the first and second trimester or during the post-partum period [12]. McKinney et al. [20], who collected urinary samples from gestational weeks 11–33 could also find increased levels of F_2-isoprostanes in normotensive pregnancy compared to non-pregnancy. This study did not measure F_2-isoprostanes in the post-partum period. Another cross-sectional study has shown that lipid hydroperoxides and malondialdehyde (MDA) were significantly increased in normal pregnancy compared to non-pregnancy, but the F_2-isoprostane levels did not change significantly [2]. Likewise, Chappell et al. [21] did not find any significant increase of F_2-isoprostanes in low risk pregnancy. Two other

Table III. Median change per week of 8-iso-$PGF_{2\alpha}$, lipid-adjusted and non-lipid-adjusted α- and γ-tocopherol during gestational weeks 7–40 ($n = 37$).

	Median change per week[a]	p-value[b]
8-iso-$PGF_{2\alpha}$	0.0030	<0.001
α-tocopherol (adjusted)	−0.0055	<0.001
α-tocopherol (not adjusted)	0.2836	<0.001
γ-tocopherol (adjusted)	−0.0004	0.046
γ-tocopherol (not adjusted)	0.0105	<0.001

[a] Median of slope from 37 individual regression lines.
[b] Wilcoxon Signed Rank test.

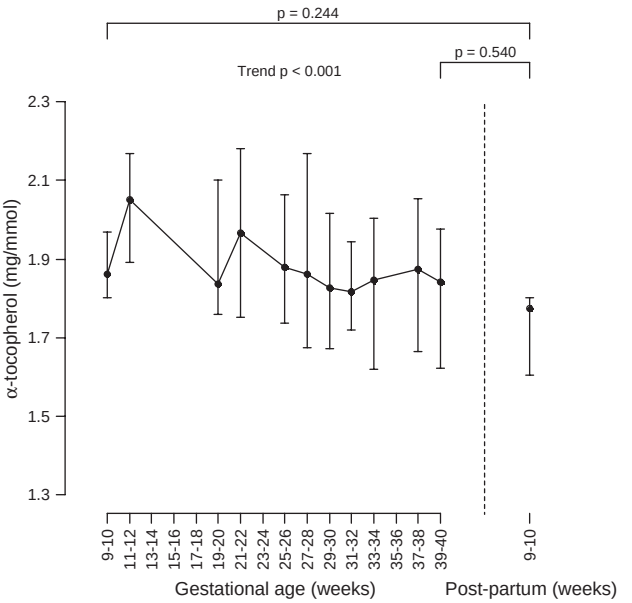

Figure 2. Lipid-adjusted serum α-tocopherol levels in uncomplicated pregnancies and 9–10 weeks post-partum ($n=37$). Only values from intervals including samples from at least 10 women are given. Data are presented as median and the 25th percentile to the 75th percentile. The p-value for trend was calculated as described in Table III.

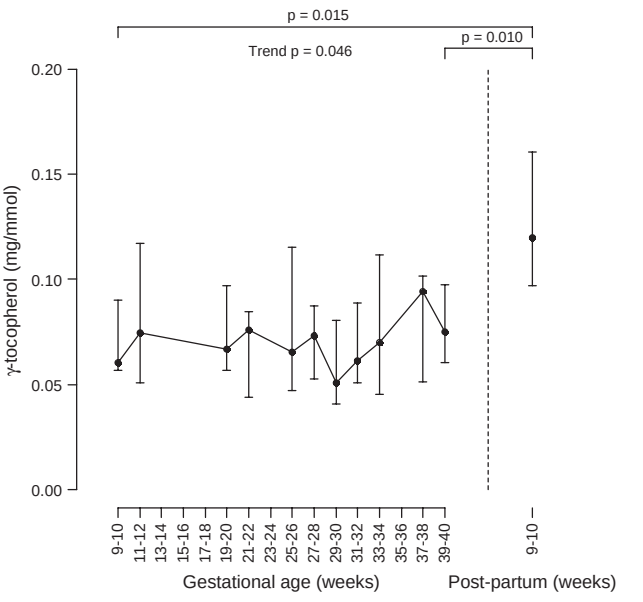

Figure 4. Lipid-adjusted serum γ-tocopherol levels in uncomplicated pregnancies and 9–10 weeks post-partum ($n=37$). Only values from intervals including samples from at least 10 women are given. Data are presented as median and the 25th percentile to the 75th percentile. The p-value for trend was calculated as described in Table III.

cross-sectional studies have reported increased levels of lipid peroxides and/or TBARS during pregnancy [22,23].

To our knowledge the current finding that the high levels of F_2-isoprostanes observed in late pregnancy decrease to basal levels in the post-partum period has not been reported previously. However, other studies have shown similar changes concerning levels of lipid peroxides and/or TBARS [24,25]. Together, these and the current results indicate that mild oxidative stress is associated with normal pregnancy and that the levels of various biomarkers of oxidative stress including F_2-isoprostanes decrease during the post-partum period.

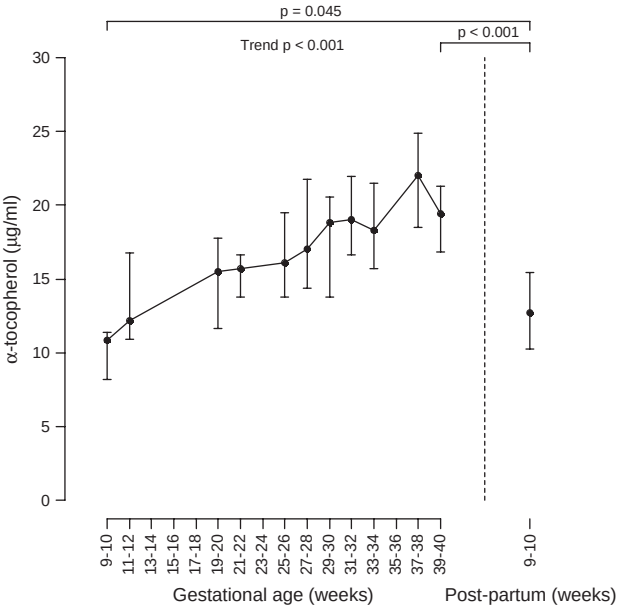

Figure 3. Non-lipid-adjusted serum α-tocopherol levels in uncomplicated pregnancies and 9–10 weeks post-partum ($n=37$). Only values from intervals including samples from at least 10 women are given. Data are presented as median and the 25th percentile to the 75th percentile. The p-value for trend was calculated as described in Table III.

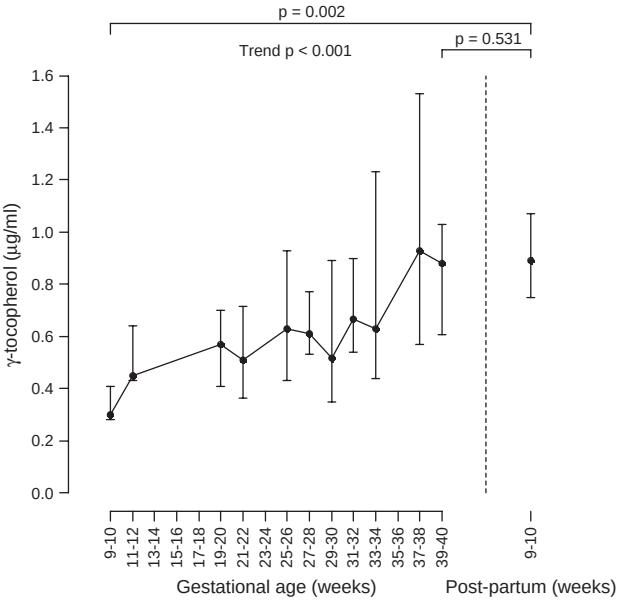

Figure 5. Non-Lipid-adjusted serum γ-tocopherol levels in uncomplicated pregnancies and 9–10 weeks post-partum ($n=37$).fs Only values from intervals including samples from at least 10 women are given. Data are presented as median and the 25th percentile to the 75th percentile. The p-value for trend was calculated as described in Table III.

The present study showed significantly increased non-lipid-adjusted levels of α- and γ-tocopherol from week 9 throughout pregnancy until week 40. Our findings concerning α-tocopherol are in agreement with the results from Roes et al. [15]. A longitudinal study has also shown that the antioxidative status, as measured by total antioxidant capacity (TAC), decreased significantly in the first trimester and then increased throughout pregnancy to reach normal non-pregnant values post-partum [19]. In a cross-sectional study, non-lipid-adjusted levels of vitamin E were found to be significantly higher in late normal pregnancy compared to non-pregnancy [2]. These might be due to the higher levels of circulating serum lipids (cholesterol and triglycerides) found during pregnancy. Our results concerning lipid-adjusted tocopherols reveal, in contrast to Chappell et al. [21], a significant decrease from week 9 throughout pregnancy until week 40. Moreover, in a previous cross-sectional study performed on a Japanese population, lipid-adjusted α- and γ-tocopherol levels were significantly lower in late pregnancy compared to non-pregnancy [12]. These observed reductions of lipid-adjusted antioxidants might indicate a consumption of tocopherols in response to increased oxidative stress *in vivo* during pregnancy.

Lipid peroxidation through the free radical pathway is generally described as unfavourable to mammalian health and often related to pathological consequences. However, it is also known that regulated free radical reactions in the body are beneficial, specifically for cell signalling, cell generation and degeneration, cellular homeostasis and defence against microorganisms, etc. [26–28]. In this longitudinal study, we have found increased levels of F_2-isoprostanes and decreased levels of lipid-adjusted tocopherols throughout normal human pregnancy. It suggests that free radical-mediated lipid peroxidation in mild form might have a role in human pregnancy.

This study has its limitations, since only 37 of the 59 recruited women finally had uneventful pregnancies. The planned periods for blood and urinary sampling were not always adhered to, which is the reason that 2-week periods were used when accounting for the results. The effect is that the number of measurement periods during pregnancy increases with fewer samples per period.

In conclusion, we have found that oxidative stress as measured by 8-iso-$PGF_{2\alpha}$ increases throughout normal human pregnancy. The antioxidant levels as measured by lipid-adjusted α- and γ-tocopherol decrease. The results indicate that mild oxidative stress might be involved in normal pregnancy. Moreover, it is of importance to know the observed increase of oxidative stress in normal pregnancy when pregnancy complications, e.g. pre-eclampsia and gestational diabetes, are to be studied.

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