View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Universität München: Elektronischen Publikationen

British Journal of Nutrition (2010), **103**, 1648–1656
doi:10.1017/S0007114509993746
© The Authors 2010

Influence of fish oil or folate supplementation on the time course of plasma redox markers during pregnancy

Corinna Franke[1], Hans Demmelmair[1], Tamas Decsi[2], Cristina Campoy[3], Milagros Cruz[3], Juan A. Molina-Font[3], Klaus Mueller[4] and Berthold Koletzko[1]*

[1]*Division of Metabolism and Nutrition, Dr. v. Hauner Children's Hospital, Munich, Germany*
[2]*Department of Pediatrics, University of Pécs, Hungary*
[3]*Department of Pediatrics, University of Granada, Spain*
[4]*Department of Pediatrics, University of Frankfurt am Main, Germany*

(Received 25 November 2008 – Revised 1 December 2009 – Accepted 9 December 2009 – First published online 9 March 2010)

Maternal supplementation with long-chain PUFA, to improve infant neurological development, might cause additional increase of oxidative stress. Pregnant women aged 18–41 years were randomised into one of four supplementation groups. From week 22 on, they received supplements containing either modified fish oil (*n* 69), 5-methyl-tetrahydro-folate (*n* 65), both (*n* 64), or placebo (*n* 72). Plasma Trolox-equivalent antioxidative capacity (TEAC), concentrations of α-tocopherol, retinol, β-carotene, free thiol groups, uric acid and thiobarbituric acid-reactive substances (TBARS) were determined at weeks 20 and 30 and at delivery. The studied antioxidants showed no significant differences between the four supplementation groups. At week 30 plasma TBARS levels were found to be significantly higher in the fish oil group (0·80 (SEM 0·04) μmol/l) than in the folate (0·67 (SEM 0·03) μmol/l; $P=0·024$) and control (0·69 (SEM 0·04) μmol/l; $P=0·01$) groups. Concentrations of retinol and free thiol groups decreased during pregnancy, whereas uric acid increased and β-carotene as well as TEAC showed only minor changes. Fish oil supplementation during the second half of pregnancy appears not to decrease antioxidant status. The increased TBARS levels at week 30 may indicate a period of increased oxidative stress in plasma at this time.

Pregnancy: DHA: Oxidative stress: Thiobarbituric acid-reactive substances

Oxidative stress occurs as a result of an increase in oxidant generation, a decrease in antioxidant protection, or a failure to repair oxidative damage. Damage to cells results from reactive oxygen species-induced alteration of PUFA in membrane lipids, proteins and DNA. During pregnancy, oxidative stress increases, but there is also an increase of antioxidants and anti-oxidative enzymes[1,2]. Imbalances between oxidants and the antioxidative system may be associated with the onset of pre-eclampsia[3,4] and with an increased risk of miscarriage[1]. Furthermore, an inverse correlation between the maternal oxidative stress biomarkers malondialdehyde (for lipid peroxidation) and 8-hydroxydeoxyguanosine (for DNA peroxidation) and neonatal birth weight has been demonstrated[5].

Increased availability of *n*-3 long-chain PUFA, for example, DHA, during the perinatal period has been reported to improve cognitive and visual development of the infant[6–8]. The fetus accumulates up to 50 mg DHA per d in brain and adipose tissue during the last 3 months of gestation[9]. Fish oil supplementation in pregnancy was found to slightly prolong mean duration of gestation time and to markedly lower the risk for early preterm delivery[10,11]. However, long-chain PUFA are susceptible to peroxidation[12] and additional oxidative stress might be caused by a high dietary intake of *n*-3 fatty acids without adequate antioxidative protection[13].

In women of childbearing age adequate folate supply reduces the incidence of neural tube defects in infants and in the general population. Folate supplementation can reduce plasma concentrations of homocysteine[14]. During pregnancy this might improve placental vascularisation and hence maternal–fetal substrate transfer. In line with this hypothesis Böhles *et al.* showed a negative correlation between maternal plasma homocysteine and DHA-percentage in the erythrocyte membrane phospholipids of their newborns[15]. Thus a combined supplementation with folate and *n*-3 long-chain PUFA seems reasonable. As several reactions of homocysteine metabolism (for example, formation of homocysteine) promote the formation of reactive oxygen species, folate supplementation might beneficially influence redox markers[16].

The aim of the present study was to compare oxidative stress and antioxidant levels in pregnant women with and without an *n*-3 long-chain PUFA supplementation, considering a potentially confounding influence of folate supplementation.

Since redox status cannot be adequately assessed from a single analytical parameter, we analysed a set of biomarkers

Abbreviations: TBARS, thiobarbituric acid-reactive substances; TEAC, Trolox-equivalent antioxidant capacity; w20, week 20 ± 1 of gestation; w30, week 30 ± 1 of gestation.

* **Corresponding author:** Professor Berthold Koletzko, fax +49 89 5160 3336, email office.koletzko@med.uni-muenchen.de

Downloaded from https://www.cambridge.org/core. IP address: 138.246.2.185, on 03 Mar 2017 at 14:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0007114509993746

in the plasma of pregnant women participating in a randomised clinical trial on the effect of fish oil supplementation during the second half of pregnancy on pregnancy outcome[17]. Vitamin E is the major lipid-soluble, peroxidation chain-breaking antioxidant[18]. β-Carotene is an effective scavenger of peroxyl radicals[19] and it is a precursor of retinol, which is of essential importance for growth and development of cells and tissues. Uric acid is a powerful scavenger of singlet oxygen and other radicals[20]. Thiol groups, mainly from glutathione, are susceptible to oxidative changes and play an important role in antioxidative reactions[21]. Because there are more antioxidants and interactions in the aqueous phase of plasma, we measured Trolox-equivalent antioxidant capacity (TEAC) in plasma as an integrative parameter. In addition, the plasma concentration of thiobarbituric acid-reactive substances (TBARS) was determined as a marker of lipid peroxidation.

Subjects and methods

Subjects and enrolment

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the local Ethical Committees of the participating centres. From November 2001 to March 2003, pregnant women were recruited in Granada (Spain), Pécs (Hungary) and Munich (Germany). Women attending antenatal care clinics for ultrasound examinations between week 12 and week 20 of gestation were approached by study personnel, informed about the aims and nature of the study and invited to participate. Subject information includes an oral explanation by the physician and a written informed consent which was given to the subject. The criteria for inclusion were: age at study entry of 18–41 years, uncomplicated singleton pregnancy and weight at study entry of 50–95 kg. Women taking folate supplements after week 16 of gestation or fish oil supplements since they became pregnant were excluded from the study. Furthermore, for this analysis all women who smoked during pregnancy were excluded because smoking may enhance oxidative stress. Details of the study protocol and execution have been previously reported[17].

Participants who agreed to participate were randomised without stratification into one of four dietary supplementation groups separately at each centre. To ensure that the different supplementation groups are nearly equally represented in each centre, randomisation was performed in blocks of twenty numbers. For this purpose twenty envelopes containing cards with one of the four numbers according to the supplementation groups were prepared and put into a closed box. By drawing envelopes, supplementation group numbers were assigned to the subject identity number. This procedure was performed identically for each study centre. After allocation to the dietary group, women were provided correspondingly with ninety sachets of 15 g, of which they had to consume one per d. At the second investigation date in week 30 ± 1 (w30) of gestation a further batch of ninety sachets was provided for the rest of pregnancy.

Thus, from week 22 of gestation onwards participants received milk-based supplements containing either modified fish oil providing 500 mg DHA and 150 mg EPA per d (fish oil group), or 400 μg 5-methyl-tetrahydro-folate (MTHF) per d (folate group), both in combination (fish oil + MTHF; fish oil + folate group) or placebo (control group). All supplements provided the estimated additional requirements for minerals and vitamins during the second half of pregnancy (Table 1).

Table 1. Nutrition, mineral and vitamin content of the supplements according to manufacturer's analysis (nutrient supply per sachet of 15 g)

Group...	Fish oil	Folate	Fish oil + folate	Control
DHA (mg)	500	–	500	–
EPA (mg)	150		150	
5-MTHF (μg)	–	400	400	–
Energy				
kJ	297	293	297	293
kcal	71	70	71	70
Protein (g)	2·5	2·9	2·5	2·9
Fat (g)	3·1	2·9	3·1	2·9
Carbohydrates (g)	8·2	8·0	8·2	8·0
Vitamin A (μg)	330	330	330	330
Vitamin D (μg)	1·5	1·5	1·5	1·5
Vitamin E (mg)	3	3	3	3
Thiamin (mg)	0·36	0·36	0·36	0·36
Riboflavin (mg)	1·5	1·5	1·5	1·5
Niacin (mg)	4·5	4·5	4·5	4·5
Vitamin B_6 (mg)	1·9	1·9	1·9	1·9
Vitamin B_{12} (μg)	3·5	3·5	3·5	3·5
Vitamin C (mg)	270	270	270	270
Ca (mg)	300	300	300	300
P (mg)	240	240	240	240
Mg (mg)	93	93	93	93
Zn (mg)	3	3	3	3
I (μg)	66	66	66	66

5-MTHF, 5-methyl-tetrahydro-folate.

Non-fasting maternal venous blood samples for the laboratory analyses were collected at week 20 ± 1 (w20) of gestation, before supplementation started, at w30 and at the time of delivery using EDTA as anticoagulant. The plasma samples were stored at −80°C until assayed. At the same time points a well-trained physician performed standardised interviews with the woman to assess data about socio-economic status, obstetrical history, intercurrent diseases and maternal smoking habits. Additionally, maternal height, weight and blood pressure were measured. At w20 and w30 participating women completed a FFQ to assess the DHA and folate intake with their habitual diet. Details of the nutritional evaluation have previously been reported[22].

Analytical procedures

TBARS, TEAC, free thiol-groups, total protein and uric acid concentrations from all samples of each woman were analysed during one and the same day.

TBARS concentrations were determined by reaction with 2-thiobarbituric acid, based on the method of Knight *et al.*[23]. Ortho-phosphoric acid (500 μl; 0·44 M), 100 μl plasma and 200 μl 2-thiobarbituric acid solution (60 mg per 10 ml water) were pipetted into reaction vials. The mixture was heated in a water-bath for 1 h to 100°C. After cooling, a 100 μl sample was added to a 100 μl methanol–NaOH mixture (0·45 ml 1 M-NaOH per 4·55 ml methanol).

Downloaded from https://www.cambridge.org/core. IP address: 138.246.2.185, on 03 Mar 2017 at 14:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0007114509993746

After centrifugation a 50 μl portion of the supernatant fraction was used for HPLC with fluorescence detection (excitation, 550 nm; emission, 532 nm) for the measurement of TBARS[23]. External calibration with 1,1,3,3-tetraethoxypropane was used to quantify TBARS in the plasma samples. Intra- and inter-assay CV were 2·6 and 8·8 %, respectively.

Plasma α-tocopherol, retinol and β-carotene concentrations were analysed at the Department of Paediatrics, University of Frankfurt am Main (Germany) by an established HPLC method with UV detection after extraction of lipids into hexane[24]. An external standard was applied for quantification. Plasma α-tocopherol is given as concentration and in relation to plasma lipids (α-tocopherol:cholesterol + TAG ratio).

Free thiol-groups were determined using Ellman's reagent (5,5′-dithio-bis 2-nitrobenzoic acid)[25]. Micro-plate wells were filled with 165 μl water, 60 μl phosphate-saline buffer (0·1 M), 15 μl plasma or standard and 60 μl Ellman's reagent (10 mM in 0·15 M-NaCl and 0·1 M-Na_3PO_4). Blanks were measured with each plasma sample, containing distilled water instead of Ellman's reagent, and one blank containing pure water instead of plasma. The reaction was allowed to proceed during incubation at room temperature for at least 15 min on a shaker plate, before absorption was measured at 405 nm (photometer anthos ht III; Labtec Instruments, Wals, Austria). A five-point calibration curve was prepared daily using fresh cysteine solution. Intra- and inter-assay CV averaged 6·6 and 8·9 %, respectively.

The measurement of TEAC is based on the inhibition of the formation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^+$) radical cations by antioxidants[26]. PBS buffer (506 μl; 5 mM; pH 7·4), 36 μl myoglobine (70 μM), 300 μl 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (500 μM) and 8·4 μl plasma were combined. The mixture was incubated for 3 min at 30°C. The reaction was started by the addition of 150 μl H_2O_2 (450 μM), which was prepared fresh every day, and after 3 min absorbance at 734 nm was read. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid diluted in 2·5 mM-PBS) was used for calibration. The intra- and inter-assay CV averaged 1·1 and 4·7 %, respectively.

Cholesterol, TAG, total protein and uric acid were analysed with an automatic Hitachi analysis system (Fa. Boehringer, Mannheim, Germany), using enzymic assays for cholesterol, TAG, uric acid and a colour test for total protein, respectively.

Statistical analysis

Data were analysed with SPSS for Windows 12.0 (SPSS Inc., Chicago, IL, USA). Normal distribution was examined using the Kolmogorow–Smirnov test (with Lilliefors correction). One-way ANOVA with *post hoc* Bonferroni correction was used to evaluate differences between supplementation groups for normally distributed data. In the case of non-normal distribution the Mann–Whitney *U* test was applied. Statistical significance was assumed at $P<0·05$. Differences over time were evaluated using a general linear model. Correlations between parameters were estimated by computing Pearson's correlation coefficient in the case of normally distributed values and the Spearman ρ correlation coefficient in the case of other distributions, respectively.

Results

Study participants

From the 311 women enrolled into the study, forty-one women were excluded from the analyses because they did not complete the study. Reasons for dropping out were non-compliance (*n* 2), relocation (*n* 1), aversion to or bad taste of the supplement (*n* 9), and the loss of contact (*n* 2). For the remaining cases, a special reason for drop out could not be identified. From 270 study participants who completed the study[17], samples were available for sixty-five women recruited in Munich, 113 in Granada and fifty-four in Pècs, respectively. Allocation of these women to the different intervention groups was: fish oil group (*n* 69), folate group (*n* 65), control group (*n* 72) and fish oil + folate group (*n* 64). Age and BMI were not significantly different between the four supplementation groups at study entry (Table 2). The four supplementation groups differed at none of the time points in BMI and weight gain during pregnancy. The whole study population showed an average weight gain of 5·8 (SEM 3·8) kg from w20 to w30 and 4·2 (SEM 3·5) kg from w30 until the end of pregnancy. Weight development was not different between the groups.

Effects of fish oil and folate supplementation on maternal plasma

Plasma cholesterol levels were significantly different over time of pregnancy in the whole study population (w20, 5·72

Table 2. Characteristics of the participants in the four supplementation groups*
(Median values and interquartile ranges (IQR))

Group…	Fish oil		Folate		Control		Fish oil + folate		Total study population		
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	*P*
Age at study entry (years)	31·2	27·4–34·0	31·9	26·1–35·2	31·0	28·4–34·8	31·9	28·0–35·2	31·3	27·6–34·8	0·62
BMI (kg/m^2)											
w20	25·2	22·8–28·2	24·3	22·2–27·3	24·2	23·0–26·6	24·5	23·1–27·2	24·6	22·8–27·4	0·42
w30	27·7	25·0–31·6	26·2	24·2–28·3	26·4	25·0–29·1	26·2	24·6–29·1	26·5	24·7–29·5	0·37
Delivery	29·1	26·4–33·0	27·5	25·3–30·3	28·4	26·6–31·1	28·2	26·4–31·2	28·4	26·4–31·3	0·27

w20, Week 20 ± 1 of gestation; w30, week 30 ± 1 of gestation.
* Group-specific statistical differences were assessed by ANOVA (with Bonferroni correction) and the Kruskal–Wallis test.

Downloaded from https://www.cambridge.org/core. IP address: 138.246.2.185, on 03 Mar 2017 at 14:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0007114509993746

Table 3. Plasma levels of total protein, TAG and cholsterol at the studied time points according to supplementation group‡ (Median values and interquartile ranges (IQR))

Group…§	Fish oil			Folate			Control			Fish oil + folate		
	Subjects (*n*)	Median	IQR	Subjects (*n*)	Median	IQR	Subjects (*n*)	Median	IQR	Subjects (*n*)	Median	IQR
Total protein (mg/l)												
w20	59	65·5	62·5–69·1	53	65·9	63·8–69·4	65	66·7	63·8–69·5	52	66·5	63·2–69·1
w30	59	65·5	63·0–69·2	52	66·1	61·9–68·2	63	66·4	62·0–70·0	52	65·2*	61·7–67·0
Delivery	57	61·3†	55·2–65·6	51	60·3†	57·7–65·0	59	60·7†	54·7–65·7	49	61·2†	58·1–65·3
Cholesterol (mmol/l)												
w20	59	5·52	5·02–6·11	53	5·75	5·22–6·42	65	5·46	4·93–6·40	52	5·72	5·13–6·46
w30	59	6·66*	5·98–7·49	52	6·70*	5·81–7·17	63	6·37*	5·67–7·23	52	6·85*	6·02–7·63
Delivery	57	6·01†	5·48–6·95	51	6·24	5·57–7·30	59	5·93†	4·84–6·86	49	6·68	5·88–7·36
TAG (mmol/l)												
w20	59	1·53	1·30–1·87	53	1·64	1·36–2·04	65	1·63	1·43–2·10	52	1·78	1·45–1·98
w30	59	2·09*	1·66–2·59	52	2·43*	1·98–2·83	63	2·30*	1·93–2·87	52	2·25*	1·82–2·49
Delivery	57	2·44	1·81–2·78	51	2·37	1·95–2·68	59	2·42	1·80–2·88	49	2·43†	2·03–2·91

w20, Week 20 ± 1 of gestation; w30, week 30 ± 1 of gestation.
*Median value was significantly different from that at w20 ($P<0·05$).
†Median value was significantly different from that at w30 ($P<0·05$).
‡Statistical differences were calculated with ANOVA and the Mann–Whitney *U* test between the groups. Differences between time points were determined with Student's *t* test and the Wilcoxon test, respectively.
§Differences between the supplementation groups were not found.

(SEM 0·06); w30, 6·64 (SEM 0·08); delivery, 6·24 (SEM 0·09) mmol/l; $P<0·001$). In all supplementation groups the highest plasma mean cholesterol value was found at w30 and the lowest at baseline, before supplementation started (Table 3). A significant difference ($P=0·003$) at w30 was found between women supplemented with fish oil (fish oil and combined groups: 6·78 (SEM 0·10) mmol/l) and non-supplemented women (folate and control groups: 6·18 (SEM 0·17) mmol/l). There were no other significant differences between the supplementation groups. Plasma TAG values increased significantly with advancing pregnancy (w20, 1·72 (SEM 0·04); w30, 2·26 (SEM 0·05); delivery, 2·30 (SEM 0·06) mmol/l; $P<0·01$) without significant group differences at the different time points ($P=0·30$; Table 3).

Supplementation did not affect plasma total protein levels (general linear model: $P=0·723$). But all supplementation groups showed time-dependent changes. With exception of the combined group ($P=0·04$), total plasma protein levels were similar at w20 and w30 and decreased towards delivery in all groups ($P<0·001$; Table 3). Between all time points we found significant correlations for plasma total protein (w20–w30, *r* 0·54; w30–delivery, *r* 0·34; $P<0·001$).

Plasma TBARS concentrations at w20 showed no significant differences between the supplementation groups. At w30 we found a significant difference between the DHA group and the folate group ($P=0·047$; Fig. 1). In all four groups, plasma TBARS increased significantly from w30 until the end of pregnancy. Subjects with *n*-3 long-chain PUFA in their supplement, with or without folate, had higher plasma TBARS concentrations at w30 ($P=0·042$) and at delivery than non-*n*-3 long-chain PUFA-supplemented groups ($P=0·030$; Fig. 2). Plasma TBARS level showed no differences from w20 to w30 in groups with the fish oil supplement, but decreased significantly without the fish oil supplement ($P=0·001$; Fig. 2). We found no significant effect of the study supplement on the plasma TBARS levels (general linear model: $P=0·305$). Plasma TBARS correlations between the different time points in the whole study group (w20–w30, *r* 0·35, $P<0·001$; w30–delivery, *r* 0·21, $P=0·03$) and in women with fish oil supplementation (fish oil and combined groups) were statistically significant (w20–w30, *r* 0·22, $P=0·021$; w30–delivery, *r* 0·28, $P=0·006$). However, there was only a significant correlation in the subjects not supplemented with fish oil (folate and control groups) between

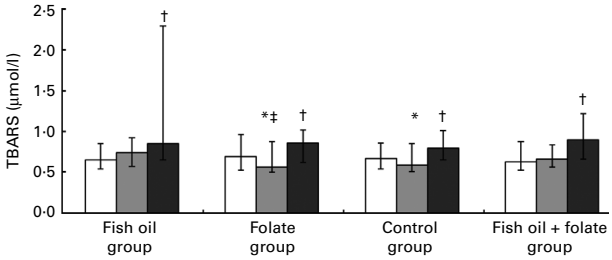

Fig. 1. Maternal thiobarbituric acid-reactive substances (TBARS) plasma levels in the different supplementation groups over time: week 20 ± 1 of gestation (w20; □); week 30 ± 1 of gestation (w30; ▨); delivery (■). Values are medians, with interquartile ranges represented by vertical bars. *Median value was significantly different from that at w20 ($P<0·05$). †Median value was significantly different from that at w30 ($P<0·05$). ‡Median value was significantly different from that of the fish oil group at w30 ($P<0·05$).

Downloaded from https://www.cambridge.org/core. IP address: 138.246.2.185, on 03 Mar 2017 at 14:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0007114509993746

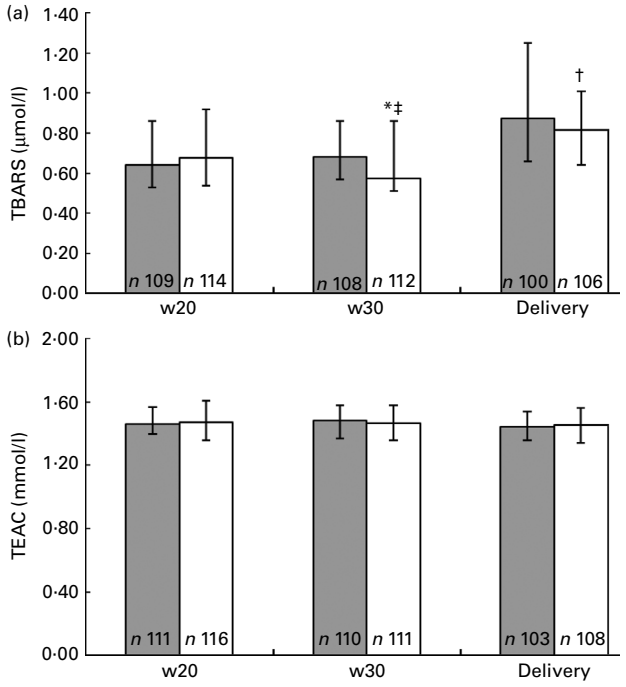

Fig. 2. Plasma thiobarbituric acid-reactive substances (TBARS) (a) and Trolox-equivalent antioxidant capacity (TEAC) (b) values in women with (■) and without (□) fish oil in their supplementation over time: week 20 ± 1 of gestation (w20); week 30 ± 1 of gestation (w30); delivery. Values are medians, with interquartile ranges represented by vertical bars. *Median value was significantly different from that of the women receiving the fish oil supplementation at the same time point (P=0·042). †Median value was significantly different from that of the women receiving the fish oil supplementation at the same time point (P=0·030). ‡Median value was significantly different from that of the women not receiving the fish oil supplementation at w20 (P=0·001).

w20 and w30 (w20–w30, r 0·37, $P<0·001$; w30–delivery, r 0·05; NS). Only at w30 were there significant negative correlations between TBARS and the plasma α-tocopherol concentration (r −0·21; P=0·002) and the α-tocopherol:lipids ratio (r −0·24; $P<0·001$).

The concentration of plasma α-tocopherol was related to total lipids (cholesterol + TAG) and rose between w20 and w30 significantly in all four supplementation groups ($P<0·001$). From w30 until the end of pregnancy the plasma α-tocopherol concentrations showed no differences in all groups (Table 4). Supplementation had no effect on the plasma α-tocopherol:lipid ratio; during the whole intervention time no differences were found between the supplementation groups. Significant intra-individual correlations of the α-tocopherol:lipid ratio in the study population were found between w20 and w30 (r 0·27; $P<0·001$) as well as w30 and delivery (r 0·27; $P<0·001$). Correlations between these time points were also significant for α-tocopherol concentrations (w20–w30, r 0·45, $P<0·001$; w30–delivery, r 0·40, $P<0·001$).

Plasma TEAC was not affected by supplementation at any time point (Table 4). There were no significant changes over time and no differences between fish oil-supplemented and non-fish oil-supplemented women (Fig. 2). Significant intra-individual correlations of TEAC at all time points were found in the total population (w20–w30, r 0·82, $P<0·001$; w30–delivery, r 0·70, $P<0·001$). Plasma TEAC levels correlated significantly with β-carotene levels at w20 (r 0·18; P=0·006) and w30 (r 0·14; P=0·034). Other correlations were obtained between TEAC and retinol (w20, r 0·32, $P<0·001$; w30, r 0·28, $P<0·001$; delivery, r 0·21, P=0·002), TEAC and α-tocopherol (w30, r 0·29, $P<0·001$; delivery, r 0·45; $P<0·001$) as well as between TEAC and total protein (w20, r 0·17, P=0·009; delivery, r 0·36; $P<0·001$). No correlations were found between TEAC and uric acid and the free thiol groups, respectively.

Maternal retinol levels decreased in the whole study population with increasing duration of pregnancy ($P<0·001$; w20, 2·74 (SEM 0·08) μmol/l; w30, 2·18 (SEM 0·07) μmol/l; delivery, 1·99 (SEM 0·07) μmol/l). Significant changes between w20 and w30 were found in all four groups (Table 4) and a significant change between w30 and delivery was found in the fish oil group (P=0·008). Splitting women into subjects with (fish oil and combined groups) and without fish oil supplementation (folate + control groups) resulted in significant differences between w20 and w30 in both groups (with fish oil: P=0·002, w20, 2·70 (SEM 0·11) μmol/l; w30, 2·2 (SEM 0·10) μmol/l; without fish oil: $P<0·001$, w20, 2·72 (SEM 0·11) μmol/l; w30, 1·99 (SEM 0·09) μmol/l) and between w30 and delivery only in fish oil-supplemented women (P=0·002; delivery, 1·86 (SEM 0·12) μmol/l). Between supplementation groups we found no significant differences at any of the time points. Intra-individual correlations of the plasma retinol levels were significant between all time points (w20–w30, r 0·40, $P<0·001$; w30–delivery, r 0·23; $P<0·001$).

The plasma β-carotene levels showed different developments in women with and without fish oil supplementation. Both the folate and control groups showed a significant decrease between w20 and w30 (P=0·014; P=0·002), as well as all subjects without fish oil supplementation taken together (control and folate groups: w20, 0·52 (SEM 0·04) μmol/l; w30, 0·41 (SEM 0·04) μmol/l; $P<0·001$). In all groups, the lowest plasma β-carotene concentration was found at the end of pregnancy. There were no significant differences between the four supplementation groups at any of the time points. Comparing women with fish oil (fish oil and combined groups) and those without fish oil supplementation (control and folate groups), we found a significant difference at w30 (P=0·03) and delivery (P=0·04). In both cases, women with fish oil supplementation had higher β-carotene concentrations (with fish oil: w30, 0·51 (SEM 0·04) μmol/l; delivery, 0·42 (SEM 0·03) μmol/l; without fish oil: w30, 0·42 (SEM 0·04) μmol/l; delivery, 0·38 (SEM 0·04) μmol/l). Correlations between the time points were significant (w20–w30, r 0·60; w30–delivery, r 0·75; $P<0·001$) in the whole study population.

Plasma free thiol groups decreased until the end of pregnancy, but no differences were found between the four supplementation groups, as well as between fish oil-supplemented (fish oil and combined groups) and non-fish oil-supplemented (control and folate groups) subjects. A significant decrease between w30 and delivery was found in all four groups (Table 4). Correlations were significant between all time points (w20–w30, r 0·49; w30–delivery, r 0·45; each $P<0·001$). At all time points we found significant correlations between the concentrations of total protein and the free thiol groups (w20, r 0·22, P=0·001; w30, r 0·34;

Downloaded from https://www.cambridge.org/core. IP address: 138.246.2.185, on 03 Mar 2017 at 14:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0007114509993746

Downloaded from https://www.cambridge.org/core. IP address: 138.246.2.185, on 03 Mar 2017 at 14:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0007114509993746

Table 4. Plasma levels of redox parameters at the studied time points according to supplementation group‡
(Median values and interquartile ranges (IQR))

Group...	Fish oil			Folate			Control			Fish oil + folate		
	Subjects (*n*)	Median	IQR	Subjects (*n*)	Median	IQR	Subjects (*n*)	Median	IQR	Subjects (*n*)	Median	IQR
Thiol groups (μmol/l)												
w20	58	200	173–243	51	213	181–256	62	217	170–271	52	216	171–272
w30	58	196	157–256	51	204	140–252	61	203	172–269	52	205	145–294
Delivery	57	156†	117–209	51	161†	132–194	58	183†	126–238	49	178†	127–236
TEAC (mmol/l)												
w20	59	1·46	1·40–1·56	53	1·49	1·37–1·64	63	1·45	1·36–1·55	52	1·48	1·39–1·62
w30	58	1·49	1·41–1·57	50	1·47	1·36–1·59	61	1·46	1·37–1·57	52	1·47	1·36–1·59
Delivery	57	1·43	1·37–1·55	52	1·48	1·35–1·58	56	1·43	1·34–1·54	47	1·45	1·36–1·53
Uric acid (μmol/l)												
w20	59	178	155–208	53	190	158–217	65	186	158–214	52	173	137–201
w30	59	200*	178–232	52	194	161–225	63	203*	172–232	52	188*	155–220
Delivery	57	274†	229–312	51	259†	214–315	59	266†	226–291	49	257†	214–294
Tocopherol:TAG + cholesterol ratio												
w20	59	5·44	4·82–6·06	53	5·27	4·54–5·79	64	5·26	4·47–5·98	51	5·14	4·68–5·85
w30	57	5·99*	5·12–7·10	50	5·43*	4·50–6·98	61	6·11*	4·98–7·30	50	6·24*	5·07–7·66
Delivery	55	5·71	4·78–7·03	51	5·42	4·41–6·24	57	5·53	4·57–7·30	48	5·48	4·72–7·12
β-Carotene (μmol/l)												
w20	59	0·39	0·29–0·54	54	0·42	0·26–0·65	65	0·41	0·25–0·63	51	0·47	0·26–0·70
w30	59	0·41	0·24–0·61	51	0·30*	0·17–0·59	65	0·30*	0·19–0·47	50	0·41	0·21–0·70
Delivery	55	0·34	0·20–0·69	53	0·31	0·19–0·54	58	0·28	0·18–0·45	48	0·41	0·24–0·63
Retinol (μmol/l)												
w20	59	2·38	1·84–3·56	54	2·51	1·71–3·60	65	2·57	1·84–3·51	51	2·30	1·79–3·62
w30	58	2·04*	1·66–2·87	51	1·69*	1·20–2·51	65	1·91*	1·35–2·91	50	2·19*	1·46–2·89
Delivery	55	1·52†	1·05–2·46	53	1·74	1·13–2·58	58	1·75	1·41–2·65	48	1·81	1·39–2·79

w20, Week 20 ± 1 of gestation; w30, week 30 ± 1 of gestation; TEAC, Trolox-equivalent antioxidant capacity.
*Median value was significantly different from that at w20 ($P<0·05$).
†Median value was significantly different from that at w30 ($P<0·05$).
‡Statistical differences were calculated with ANOVA and the Mann–Whitney *U* test between the groups. Differences between time points were determined with a general linear model (Bonferroni correction) and the Wilcoxon test, respectively.

delivery, r 0·44, $P<0·001$). The ratio of thiol groups per total protein did not differ between the supplementation groups.

In the whole study population, plasma uric acid level showed a slight increase between w20 and w30 (510 (SEM 14) to 551 (SEM 8) μmol/l; $P<0·001$) and a stronger increase towards the end of pregnancy (739 (SEM 13) μmol/l; $P<0·001$). There was no significant effect of the supplementation on maternal uric acid level during intervention time (general linear model: $P=0·60$). Except the folate group ($P=0·07$), all groups showed a significant increase between w20 and w30 ($P\leq0·001$). Between w30 and delivery, all four groups showed a significant increase ($P<0·001$). Significant correlations with $P<0·001$ were found between w20 and w30 (r 0·65) as well as w30 and delivery (r 0·60) in the total study population.

Dietary intake of DHA

The analysis of the FFQ showed no significantly different DHA intake with habitual diet between the supplementation groups at w20 and w30. DHA intake in the study population was 30·1 mg per 1000 kJ (126 mg per 1000 kcal) at w20 and 29·4 mg per 1000 kJ (123 mg per 1000 kcal) at w30 on average[22]. We found no significant difference regarding the dietary intakes of folate between the four supplementation groups at w20 nor at w30. The study population showed a folate intake of about 27·7 μg/1000 kJ (116 μg/1000 kcal) at both time points with no significant difference between the groups and change with time.

Discussion

The present study shows a significant increase of plasma TBARS during the second half of pregnancy, independently from supplement allocation. An increase of lipid peroxidation was previously reported with progression of pregnancy, and thus with increasing age of the placenta[27]. While measures of peroxidation are generally higher in pregnant women than in non-pregnant controls, results on the evolution of plasma markers of peroxidation during pregnancy are quite variable[28]. The significant increase of TBARS in our subjects is in line with the reported increase of lipid hydroperoxides between first and third trimester[29], while a significant increase of plasma malondialdehyde concentration with the course of pregnancy was not observed by Patrick *et al.* in American women[30]. A reason for these divergent results might be that the TBARS test is non-specific for malondialdehyde[31]. The mid-pregnancy malondialdehyde concentrations were clearly lower in the women studied by Patrick *et al.* [30] (0·38 μmol/l in white and 0·50 μmol/l in black women) than the TBARS concentrations in the European women in the present study (0·74 μmol/l). Thus antioxidative capacity might have been exhausted earlier in our women. An association between lipid peroxidation products and lipid-soluble antioxidants is reflected in the negative correlations between TBARS and α-tocopherol in the whole study population at w30 (r −0·21; $P=0·002$). Fish oil supplementation induced both significantly higher plasma phospholipid DHA and EPA percentages[17] at w30 and at delivery, while plasma TBARS concentrations were only at w30 significantly increased in the fish oil-supplemented group. This might indicate that the antioxidant system was brought to its limits around w30, but adapted later on or at delivery other factors are the determinants of redox status.

Plasma concentrations of α-tocopherol and the α-tocopherol: lipid ratio increased after w20, which reflects the additional intake of α-tocopherol with the study supplements. While a negative correlation between percentage of total PUFA and vitamin E has been reported in pregnant Italian women[32], we did not find lower α-tocopherol concentrations in the fish oil-supplemented women. The reason seems to be the different amounts of vitamin E intake. None of the studied Italian women took nutritional supplements containing lipid-soluble vitamins[32]. The additional vitamin E intake with the Nutraceuticals for a Healthier Life (NUHEAL) study supplement might have provided α-tocopherol in an amount much higher than the minimal requirement for radical scavenging.

Plasma protection against free radical injury is offered by a wide range of antioxidants with synergistic action. Measurement of all individual antioxidants is not possible; thus we applied the integrative TEAC assay to estimate plasma antioxidative capacity. We did not find significantly lower plasma TEAC in fish oil-supplemented compared with non-supplemented women. In both groups TEAC tended to decrease towards the end of the intervention period. Comparing measured TEAC concentration with antioxidants measured in plasma, we found significant correlations to retinol, β-carotene and total protein levels, but no correlation to uric acid. TEAC was not affected by the progression of pregnancy and the significant changes in uric acid concentration over time were not reflected. Plasma albumin and uric acid are the major determinants of TEAC, but correlations to specific antioxidant concentrations are difficult to establish and depend on the applied radical-generating substrate and reaction time[33–35].

The decrease of plasma retinol concentration during pregnancy is in agreement with the results of Bruinse *et al.* [36] and Cikot *et al.* [37]. Possible reasons for a decrease could be the increasing plasma volume during pregnancy, a decrease in retinol-binding proteins or increased tissue retention. The decreased plasma retinol concentration during advanced pregnancy might also reflect enhanced fetal utilisation[32,38].

Significant differences in plasma β-carotene concentrations were found between fish oil-supplemented and non-supplemented subjects. In contrast to a short-term *n*-3 long-chain PUFA supplementation study[39], we found higher values of plasma β-carotene at w30 and at the time of delivery in fish oil-supplemented women. All groups showed the lowest concentrations at the time of delivery, which might be an indication for a higher level of oxidative stress towards the end of gestation as well as the increased maternal blood volume. Several other factors known to influence β-carotene concentration, such as age, sex, smoking status and residence location, could be excluded as they were not different between the groups ($P>0·05$)[40]. Thus, the reason for the higher β-carotene concentrations in the fish oil-supplemented women remains unclear.

Uric acid plasma concentration increased significantly throughout pregnancy, irrespective of type of supplementation. A similar increase has been reported in other studies and has been interpreted as reflecting a xanthine–xanthine oxidase pathway stimulation[41,42]. Uric acid may act as an

Downloaded from https://www.cambridge.org/core. IP address: 138.246.2.185, on 03 Mar 2017 at 14:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0007114509993746

antioxidant, but there is also evidence that it has pro-oxidative effects[43–45]. Thus, increased levels of uric acid indicate oxidative stress, but it is unclear whether it induces oxidative stress or reflects antioxidant activity. In pregnancy elevated uric acid levels have been associated with increased rates of complications such as pre-eclampsia[42]. In non-smoking pregnant women, uric acid levels of 197 (SEM 15) μmol/l (33·1 (SEM 2·5) mg/l) in weeks 16–24 and 194 (SEM 9) μmol/l (32·6 (SEM 1·5) mg/l) in weeks 24–34 have been reported[46], which is similar to our findings. In contrast, results in smoking pregnant women (202 μmol/l (33·9 mg/l); 214 μmol/l (35·9 mg/l))[46] tended to be higher than the plasma uric acid in our *n*-3-supplemented women (w20, 179 μmol/l (30 mg/l); w30, 196 μmol/l (33 mg/l)). Thus, it appears that the increase with time found in the present study is not related to the *n*-3 supplementation, but rather with falling renal clearance towards the end of pregnancy[46].

Plasma thiol groups showed a decrease in all subjects during the intervention time. The present results confirm the results of previews studies[47,48]. The decrease in thiol groups and in total protein levels during pregnancy may reflect the increasing maternal plasma volume and physiological functions of the thiol groups in fetal metabolism, but increases of oxidative stress with pregnancy duration has also been proposed as an explanation[47,49,50]. Our data show a similar decrease in all supplementation groups, which was on average 20 % from baseline to delivery. Thus, if an *n*-3 long-chain PUFA supplementation induced oxidative stress, it was too small to influence plasma thiol concentration.

We conclude that the recommended supply of *n*-3 long-chain PUFA in pregnancy[51,52], here providing a daily supply of 500 mg DHA and 150 g EPA, did not affect water-soluble and lipid-soluble antioxidants. Higher TBARS concentrations in the fish oil group were not associated with a depletion of plasma antioxidants, and the group difference was clearly smaller than the increase with pregnancy duration. Independent of the inclusion of folate the applied dosage of *n*-3 long-chain PUFA supplementation seems to be without adverse effects on antioxidative defence in pregnant women.

Acknowledgements

The authors thank all participating women for their collaboration and all colleagues in the study centres for their support. The present study was financially supported by the Commission of the European Communities within the 5th Framework Programme NUHEAL, CLK1-CT-1999-00888, within the 6th Framework Programmes EARNEST, Food-CT-2005-007036, and EURRECA, FP6-036196-2, and within the 7th Framework Programme NUTRIMENTHE, FP7-212652. This paper does not necessarily reflect the views of the Commission and in no way anticipates the future policy in this area. B. K. is the recipient of a Freedom to Discover Award of the Bristol Myers Squibb Foundation, New York, NY, USA.

All authors have made substantive contributions to the study, and endorse the data and conclusions. The authors contributed as follows: B. K., C. C. and T. D. designed and supervised the study. C. F., K. M., H. D., M. C. and J. A. M.-F. participated in data collection and/or laboratory analyses. C. F. did the statistical analysis and wrote the first draft of the manuscript. B. K., H. D., C. C. and T. D. revised the manuscript and contributed to the final version of the manuscript.

All authors certify that there is no actual or potential conflict of interest in relation to this article.

References

1. Jenkins C, Wilson R, Roberts J, *et al.* (2000) Antioxidants: their role in pregnancy and miscarriage. *Antioxid Redox Signal* **2**, 623–628.
2. Sugino N, Takiguchi S, Kashida S, *et al.* (2000) Superoxide dismutase expression in the human corpus luteum during the menstrual cycle and in early pregnancy. *Mol Hum Reprod* **6**, 19–25.
3. Mutlu-Turkoglu U, Ademoglu E, Ibrahimoglu L, *et al.* (1998) Imbalance between lipid peroxidation and antioxidant status in preeclampsia. *Gynecol Obstet Invest* **46**, 37–40.
4. Perkins AV (2006) Endogenous anti-oxidants in pregnancy and preeclampsia. *Aust NZ J Obstet Gynaecol* **46**, 77–83.
5. Kim YJ, Hong YC, Lee KH, *et al.* (2005) Oxidative stress in pregnant women and birth weight reduction. *Reprod Toxicol* **19**, 487–492.
6. Koletzko B, Agostoni C, Carlson SE, *et al.* (2001) Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal development. *Acta Paediatr* **90**, 460–464.
7. Willatts P & Forsyth JS (2000) The role of long-chain polyunsaturated fatty acids in infant cognitive development. *Prostaglandins Leukot Essent Fatty Acids* **63**, 95–100.
8. Decsi T & Koletzko B (2005) *n*-3 Fatty acids and pregnancy outcomes. *Curr Opin Clin Nutr Metab Care* **8**, 161–166.
9. Lakin V, Haggarty P, Abramovich DR, *et al.* (1998) Dietary intake and tissue concentration of fatty acids in omnivore, vegetarian and diabetic pregnancy. *Prostaglandins Leukot Essent Fatty Acids* **59**, 209–220.
10. Makrides M, Duley L & Olsen SF (2006) Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction *The Cochrane Database of Systematic Reviews 2006*, issue 3, CD003402. http://www.mrw.interscience.wiley.com/cochrane/clsysrev/articles/CD003402/frame.html
11. Olsen SF, Osterdal ML, Salvig JD, *et al.* (2007) Duration of pregnancy in relation to fish oil supplementation and habitual fish intake: a randomised clinical trial with fish oil. *Eur J Clin Nutr* **61**, 976–985.
12. Gitto E, Reiter RJ, Karbownik M, *et al.* (2002) Causes of oxidative stress in the pre- and perinatal period. *Biol Neonate* **81**, 146–157.
13. Herrera E (2002) Lipid metabolism in pregnancy and its consequences in the fetus and newborn. *Endocrine* **19**, 43–55.
14. Homocysteine Lowering Trialists' Collaboration (2005) Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. *Am J Clin Nutr* **82**, 806–812.
15. Böhles H, Arndt S, Ohlenschlager U, *et al.* (1999) Maternal plasma homocysteine, placenta status and docosahexaenoic acid concentration in erythrocyte phospholipids of the newborn. *Eur J Pediatr* **158**, 243–246.
16. Hayden MR & Tyagi SC (2004) Homocysteine and reactive oxygen species in metabolic syndrome, type 2 diabetes mellitus, and atherosclerosis: the pleiotropic effects of folate supplementation. *Nutr J* **3**, 4.
17. Krauss-Etschmann S, Shadid R, Campoy C, *et al.* (2007) Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of

Downloaded from https://www.cambridge.org/core. IP address: 138.246.2.185, on 03 Mar 2017 at 14:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0007114509993746

docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. *Am J Clin Nutr* **85**, 1392–1400.
18. Brigelius-Flohe R, Kelly FJ, Salonen JT, *et al.* (2002) The European perspective on vitamin E: current knowledge and future research. *Am J Clin Nutr* **76**, 703–716.
19. Tsuchihashi H, Kigoshi M, Iwatsuki M, *et al.* (1995) Action of β-carotene as an antioxidant against lipid peroxidation. *Arch Biochem Biophys* **323**, 137–147.
20. Ames BN, Cathcart R, Schwiers E, *et al.* (1981) Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc Natl Acad Sci U S A* **78**, 6858–6862.
21. Iciek M, Chwatko G, Lorenc-Koci E, *et al.* (2004) Plasma levels of total, free and protein bound thiols as well as sulfane sulfur in different age groups of rats. *Acta Biochim Pol* **51**, 815–824.
22. Franke C, Verwied-Jorky S, Campoy C, *et al.* (2008) Dietary intake of natural sources of docosahexaenoic acid and folate in pregnant women of three European cohorts. *Ann Nutr Metab* **53**, 167–174.
23. Knight J, Smith S, Kinder V, *et al.* (1988) Urinary lipoperoxides quantified by liquid chromatography, and determination of reference values for adults. *Clin Chem* **34**, 1107–1110.
24. Hess D, Keller HE, Oberlin B, *et al.* (1991) Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. *Int J Vit Nutr Res* **61**, 232–238.
25. Quinlan G, Evans T & Guttridge J (2005) Oxidative damage to plasma proteins in adult respiratory distress syndrome. *Free Rad Res* **20**, 289–298.
26. Motchnik PA, Frei B & Ames BN (1994) Measurement of antioxidants in human blood plasma. *Methods Enzymol* **234**, 269–279.
27. Uotila J, Tuimala R, Aarnio T, *et al.* (1991) Lipid peroxidation products, selenium-dependent glutathione peroxidase and vitamin E in normal pregnancy. *Eur J Obstet Gynecol Reprod Biol* **42**, 95–100.
28. Little RE & Gladen BC (1999) Levels of lipid peroxides in uncomplicated pregnancy: a review of the literature. *Reprod Toxicol* **13**, 347–352.
29. Toescu V, Nuttall SL, Martin U, *et al.* (2002) Oxidative stress and normal pregnancy. *Clin Endocrinol (Oxf)* **57**, 609–613.
30. Patrick TE, Hubel CA & Roberts JM (2004) Evidence of increased oxidative stress, unexplained by lipid changes, is present in nulliparous black women from early gestation. *Hypertens Pregnancy* **23**, 91–100.
31. Janero DR (1990) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic Biol Med* **9**, 515–540.
32. Herrera E, Ortega H, Alvino G, *et al.* (2004) Relationship between plasma fatty acid profile and antioxidant vitamins during normal pregnancy. *Eur J Clin Nutr* **58**, 1231–1238.
33. Cao G & Prior RL (1998) Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clin Chem* **44**, 1309–1315.
34. Van den Berg R, Haenen GRMM, van den Berg H, *et al.* (1999) Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem* **66**, 511–517.
35. Schofield D & Braganza JM (1996) Shortcomings of an automated assay for total antioxidant status in biological fluids. *Clin Chem* **42**, 1712–1714.
36. Bruinse HW & van den Berg H (1995) Changes of some vitamin levels during and after normal pregnancy. *Eur J Obstet Gynecol Reprod Biol* **61**, 31–37.
37. Cikot RJ, Steegers-Theunissen RP, Thomas CM, *et al.* (2001) Longitudinal vitamin and homocysteine levels in normal pregnancy. *Br J Nutr* **85**, 49–58.
38. Clagett-Dame M & DeLuca HF (2002) The role of vitamin A in mammalian reproduction and embryonic development. *Annu Rev Nutr* **22**, 347–381.
39. Foulon T, Richard MJ, Payen N, *et al.* (1999) Effects of fish oil fatty acids on plasma lipids and lipoproteins and oxidant–antioxidant imbalance in healthy subjects. *Scand J Clin Lab Invest* **59**, 239–248.
40. Faure H, Preziosi P, Roussel AM, *et al.* (2006) Factors influencing blood concentration of retinol, α-tocopherol, vitamin C, and β-carotene in the French participants of the SU.VI.MAX trial. *Eur J Clin Nutr* **60**, 706–717.
41. Chappell LC, Seed PT, Kelly FJ, *et al.* (2002) Vitamin C and E supplementation in women at risk of preeclampsia is associated with changes in indices of oxidative stress and placental function. *Am J Obstet Gynecol* **187**, 777–784.
42. Powers RW, Bodnar LM, Ness RB, *et al.* (2006) Uric acid concentrations in early pregnancy among preeclamptic women with gestational hyperuricemia at delivery. *Am J Obstet Gynecol* **194**, 160.
43. Becker BF (1993) Towards the physiological function of uric acid. *Free Radic Biol Med* **14**, 615–631.
44. Glantzounis GK, Tsimoyiannis EC, Kappas AM, *et al.* (2005) Uric acid and oxidative stress. *Curr Pharm Des* **11**, 4145–4151.
45. Sanchez-Lozada LG, Nakagawa T, Kang DH, *et al.* (2006) Hormonal and cytokine effects of uric acid. *Curr Opin Nephrol Hypertens* **15**, 30–33.
46. Lain KY, Markovic N, Ness RB, *et al.* (2005) Effect of smoking on uric acid and other metabolic markers throughout normal pregnancy. *J Clin Endocrinol Metab* **90**, 5743–5746.
47. Wisdom SJ, Wilson R, Mckillop JH, *et al.* (1991) Antioxidant systems in normal pregnancy and in pregnancy hypertension. *Am J Obstet Gynecol* **165**, 1701–1704.
48. Raijmakers MT, Roes EM, Steegers EA, *et al.* (2001) Umbilical cord and maternal plasma thiol concentrations in normal pregnancy. *Clin Chem* **47**, 749–751.
49. Myatt L & Cui X (2004) Oxidative stress in the placenta. *Histochem Cell Biol* **122**, 369–382.
50. Walsh SW (1998) Maternal–placental interactions of oxidative stress and antioxidants in preeclampsia. *Semin Reprod Endocrinol* **16**, 93–104.
51. Koletzko B, Cetin I & Brenna JT (2007) Dietary fat intakes for pregnant and lactating women. *Br J Nutr* **98**, 873–877.
52. Simopoulos AP, Leaf A & Salem N Jr (2000) Workshop statement on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids* **63**, 109–121.

Downloaded from https://www.cambridge.org/core. IP address: 138.246.2.185, on 03 Mar 2017 at 14:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0007114509993746