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Effect of docosahexaenoic acid and eicosapentaenoic acid supplementation on oxidative stress levels during pregnancy

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Abstract

Docosahexaenoic acid (DHA) is an indispensable component of cell membranes with high requirements during pregnancy. DHA supplementation is thought to enhance oxidative stress because of increased likelihood of lipid peroxidation. We estimated the oxidative stress levels in two groups of pregnant women who received daily supply of required vitamins with ($n = 23$) or without ($n = 23$) 500 mg of DHA and 150 mg of eicosapentaenoic acid (EPA) from 20 weeks of gestation to the time of delivery. Urinary excretions of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage and of malondialdehyde (MDA), a marker of lipid peroxidation, were measured at 20, 30 weeks and at the time of delivery. Urinary MDA excretion remained unchanged throughout the study period in both groups. Urinary 8-OHdG excretion at delivery was significantly higher than at 20 and 30 weeks ($p < 0.05$), but there were no group differences at the three time points. There were no differences between the two groups in plasma α-tocopherol levels. We conclude that under the conditions studied, a daily supplementation of 500 mg DHA and 150 mg EPA with vitamins to pregnant women did not enhance lipid peroxidation or oxidative DNA damage.

Keywords: *Long-chain polyunsaturated fatty acids, docosahexaenoic acid, malondialdehyde, 8-hydroxy-2′-deoxyguanosine, oxidative stress, pregnant woman*

Introduction

Evidence accumulates that the quality of nutrient supply to pregnant women affects maternal health and well-being, pregnancy outcome, the rate of complications and fetal growth [1]. Long-chain polyunsaturated fatty acids (LCPUFA) are required in relatively large amounts during pregnancy. Docosahexaenoic acid (DHA; C22:6$n-3$), a major $n-3$ LCPUFA in fish oil, is an indispensable component of all cell membranes that is incorporated in relatively high concentrations into the brain and other membrane rich tissues of the fetus [2]. Depletion of dietary DHA is associated with adverse neurological outcomes in animals [3], suggesting that variations in maternal LCPUFA stores have the potential to affect fetal development [4]. A series of controlled studies has demonstrated that DHA availability during early development is associated with long-term cognitive and visual development [5–7]. Furthermore, $n-3$ LCPUFA have a wide range of biological effects, including beneficial effect on lipoprotein metabolism, platelet function, endothelial function, vascular reactivity, cytokine production and coagulation

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[8,9]. However, there are concerns that an added supply of $n-3$ LCPUFA might enhance oxidative stress with potential untoward effects during pregnancy. Oxidative stress can be defined as the imbalance between free radical damage and antioxidant protection [10]. Levels of peroxidation markers, such as lipid peroxides and malondialdehyde (MDA), are higher in pregnant than in non-pregnant women [11], and the placenta has been identified as an important source of lipid peroxides [12].

LCPUFA including DHA are susceptible to peroxidation because of their high degree of unsaturation [13,14], and they might enhance peroxidative damage also in proteins and DNA. 8-Hydroxy-2′-deoxyguanosine (8-OHdG) is produced by oxidation of the nucleoside deoxyguanosine and is subsequently excreted directly into urine. Urinary 8-OHdG excretion has been identified as a sensitive marker for oxidative DNA damage [15]. Excessive oxidative stress was suggested to be causally involved in the development of pre-eclampsia and fetal growth retardation [16–18], but there is only limited information on the potential effects of DHA supplementation on oxidative stress in pregnant women. Therefore, we measured urinary 8-OHdG and MDA excretion as markers of oxidative damage in pregnant women with or without DHA supplementation. Additionally, plasma α-tocopherol, the major lipid soluble antioxidant vitamin and the fatty acid composition of plasma phospholipids were quantified.

Materials and methods

Study population and intervention

The study was performed as a double-blind randomized controlled trial. Pregnant women were enrolled prior to the 20th week of gestation at the University Hospital of Granada, Spain, between 15 November 2001 and 15 July 2002. Healthy women with an uncomplicated singleton pregnancy between 18 and 40 years of age at study entry, who did not use fish oil supplements since beginning of pregnancy, were eligible for this study. Women who smoked were excluded.

Pregnant women were randomized double blind at 20 ± 1 weeks of gestation to receive one of the dietary supplements. The supplements *Blemil plus* (Laboratories Ordesa, Barcelona, Spain) are milk based and contain vitamins in amounts meeting the estimated additional requirements during the second half of pregnancy [19] and were provided as sachets of 15 g for mixing with water. The DHA group received the supplement with 500 mg DHA and 150 mg eicosapentaenoic acid (EPA; C20:5$n-3$) from modified fish oil (Pronova Biocare, Lysaker, Norway), whereas, the other group was given a placebo with negligible contents of DHA or EPA (control group) (Table I).

Table I. The nutrient content, fatty acid and vitamin composition of the two daily supplements.

Nutrient content (per sachet)	Control	DHA
Energy (kcal)	70	71
Protein (g)	2.9	2.9
Carbohydrate (g)	8.0	8.2
Fat (g)	2.9	2.9
DHA (C22: 6$n-3$) (mg)	0.0	500
EPA (C20: 5$n-3$) (mg)	0.0	150
α-tocopherol (mg)	3.0	3.0
Vitamin A (μg)	240	240
Vitamin D (μg)	0.50	0.50
Vitamin C (mg)	30	30

The supplements were taken daily from week 20 until delivery. Their habitual diet of the subjects was not restricted, but they were asked to follow the recommendations for pregnant women.

After enrollment in the study, obstetrical history, urine and blood samples were obtained basally, before supplementation start (time point 20 week), at week 30 ± 1 of gestation (time point 30 week) and at the time of delivery (time point delivery). Blood samples were drawn into vacutainers containing EDTA and centrifuged immediately. Plasma and urine samples were stored at -80°C until assay.

Analytical methods

Urinary MDA was determined by high performance liquid chromatography (HPLC) of the adduct obtained with thiobarbituric acid (TBA) [14]. Urinary MDA concentrations were expressed as nmol MDA-TBA adduct/mg creatinine. The concentration of 8-OHdG was determined using an enzyme linked immunosorbent assay kit (8-OHdG check, Japan Institute for the Control of Aging, Shizuoka, Japan). The specificity of the assay has been established [20], and the determination range is 0.64–2000 ng/ml. All urine samples were analyzed in duplicate. The coefficient of variation of this assay was 4.5%. Urinary 8-OHdG concentrations are expressed as nanogram 8-OHdG/mg creatinine. Plasma α-tocopherol was determined by HPLC and plasma phospholipid fatty acids were quantified by gas liquid chromatography. Fatty acids were calculated as weight percent of all detected plasma phospholipids fatty acids with 14–24 C atoms.

Statistical analyses

Results are given as mean ± standard error of mean (SEM). Differences between groups were analyzed with Student's-*t* test. Difference from baseline was tested by two-way repeated measures analysis of variance and *post hoc* Tukey–Kramer test if indicated.

Table II. General characteristics of the pregnant women and offspring (mean ± SEM).

	Control group (n=23)	DHA group (n=23)
Pregnant women		
Age at entry (years)	30.42 ± 0.94	29.97 ± 1.10
Previous number of pregnancies	1.65 ± 0.20	1.78 ± 0.23
Gestational age (week)		
Entry	19.73 ± 0.16	19.80 ± 0.17
Delivery	39.89 ± 0.30	39.42 ± 0.36
Body weight (kg)		
20 week	66.31 ± 1.66	67.05 ± 1.74
30 week	72.34 ± 1.86	72.81 ± 1.88
Delivery	76.55 ± 1.98	77.54 ± 2.05
Body mass index at 20 week (kg/m^2)	25.32 ± 0.65	25.62 ± 0.80
Weight of placenta (g)	533.91 ± 20.33	527.73 ± 24.36
Offspring		
Sex (M/F)	7/16	9/14
Birth weight (kg)	3.39 ± 0.74	3.26 ± 0.96
Body length (cm)	50.84 ± 0.48	50.47 ± 0.45
Head circumference (cm)	34.94 ± 0.43	34.28 ± 0.41

Correlations were evaluated according to Pearson correlation. Statistical significance was considered at $p < 0.05$. All statistical analyses were performed with StatView 5.0, (Abacus Concepts, Inc., Berkeley, CA).

Results

Samples of 46 pregnant women were analyzed. There were no significant differences in clinical characteristics of pregnant women and offspring between the control group and the DHA group (Table II).

At study entry there were no differences between the two groups in plasma α-tocopherol level and plasma phospholipid DHA and EPA levels. After supplementation, the levels of DHA were significantly higher in the DHA supplemented group than in the control group. EPA levels at 30 week were significantly higher in the supplemented group than in the control group but not at delivery (Table III).

Urinary MDA excretions in both groups at 30 week (control: 1.31 ± 0.21, DHA: 1.27 ± 0.10 nmol/mg Cr) tended to be higher than those at 20 week (control: 1.03 ± 0.08, DHA: 1.12 ± 0.12 nmol/mg Cr) and at delivery (control: 1.01 ± 0.16, DHA: 1.03 ± 0.12 nmol/mg Cr) but were not significantly different, nor were there group differences (Figure 1). Urinary 8-OHdG excretion in both groups at delivery (control: 12.38 ± 0.72, DHA: 13.19 ± 1.03 ng/mg Cr) were significantly higher than at 20 week (control: 9.29 ± 0.69, DHA: 9.81 ± 0.79 ng/mg Cr) and at 30 week (control: 8.97 ± 0.69, DHA: 7.73 ± 0.63 ng/mg Cr) but there were no significant differences between the groups at any of three time points (Figure 1).

At the time of study entry there was a significant correlation between urinary 8-OHdG and MDA excretion ($r = 0.34$, $p = 0.02$) (Figure 2) but not at 30 week and at delivery. The measured urinary biomarker levels did not correlate with DHA and EPA levels as well as plasma α-tocopherol level.

Table III. Plasma α-tocopherol level, plasma phospholipid DHA and EPA composition (% by wt) at 20, 30 weeks of gestation and at delivery (mean ± SEM).

	Control group (n=23)	DHA group (n=23)
α-tocopherol (μmol/l)		
20 week	17.58 ± 0.64	18.44 ± 0.78
30 week	25.36 ± 1.26	25.12 ± 1.15
Delivery	23.24 ± 1.53	25.11 ± 1.22
DHA (C22: 6n − 3) (% by wt)		
20 week	6.14 ± 0.31	6.26 ± 0.31
30 week	5.72 ± 0.19	7.76 ± 0.42*
Delivery	6.02 ± 0.39	7.65 ± 0.35*
EPA (C20: 5n − 3) (% by wt)		
20 week	0.44 ± 0.09	0.39 ± 0.09
30 week	0.37 ± 0.04	0.58 ± 0.05*
Delivery	0.33 ± 0.05	0.39 ± 0.03

* $p < 0.01$ compared with control group. Student's-t test.

Discussion

Pregnancy is a physiological state accompanied by a high metabolic demand and elevated requirements for tissue oxygen [21] and causes an increase of ROS production [22]. Moreover, the placenta is a major source of oxidative stress because of its enrichment with PUFA [23]. Falkay et al. suggested that the increase in the lipid peroxide levels was due to the increased prostaglandin synthesis in the placenta [24]. Lipid peroxidation is enhanced in the second trimester and tapers off later in gestation and decrease after delivery [25]. Monitoring of the oxidative stress in pregnant women is important to enable an understanding of the relationship between oxidative stress and pregnancy

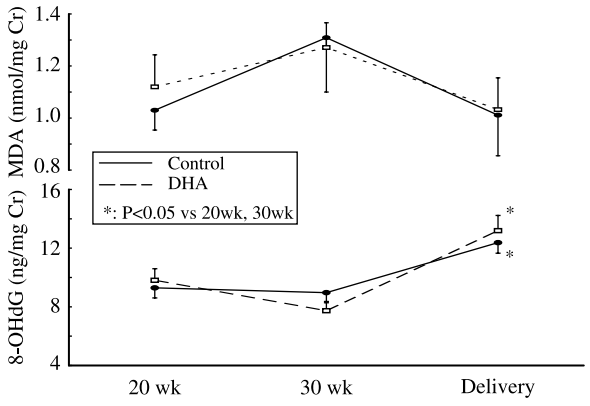

Figure 1. Effect of the DHA and EPA supplementation on urinary MDA and 8-OHdG excretion (mean ± SEM). * $p < 0.05$, two-way repeated measures analysis of variance with the *post hoc* analysis by the Tukey–Kramer test.

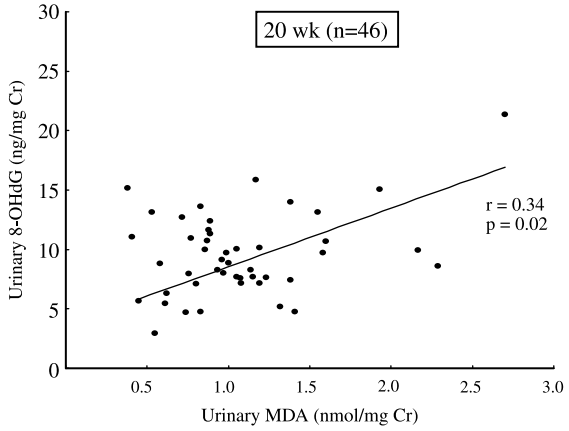

Figure 2. Relationship between urinary 8-OHdG and urinary MDA excretion at 20 weeks of gestation ($r = 0.34$, $p = 0.02$)

outcome [26]. Placental oxidative stress was suggested to play a role in the pathogenesis of pre-eclampsia [12,16] and fetal growth retardation [16–18]. On the other hand, the placenta is a source of antioxidative enzymes to control placental lipid peroxidation during healthy pregnancy. Placental production of lipid peroxides decreases as normal gestation advances, most likely because of an increase in the activity of superoxide dismutase and catalase [27]. Placental antioxidant defense is considered sufficient to control lipid peroxidation in healthy pregnancy [23].

Our data showed that there was a discrepancy between the levels of oxidative DNA damage and lipid peroxidation at delivery. Urinary 8-OHdG excretions at delivery were significantly higher than those at 20 and 30 weeks of gestation whereas urinary MDA excretions did not differ significantly between the time points. A previous study suggested that oxidative stress may influence the placenta or trophoblastic cells differently in respect to DNA damage and to lipid peroxidation [16]. Oxidative DNA damage appears to occur mainly in rapidly growing cells, such as those of the trophoblast of the cell columns, while lipid peroxidation occurs mainly in the superficial cell layers, such as those of the syncytiotrophoblast [16]. Further investigations will be needed to understand the mechanism of the discrepancy, but one possible explanation is that the significant increase of 8-OHdG excretion at the time of delivery may be associated with apoptosis in placenta. Kim et al. [28] reported that bcl-2, an anti-apoptotic protein, expression significantly decreases in placenta after 32 weeks of gestation. Bcl-2 gene expression can protect DNA from oxidative stress [29,30].

In our study, daily supplementation of 500 mg DHA and 150 mg EPA to pregnant women did not significantly influence oxidative DNA damage and lipid peroxidation during the second half of pregnancy. Several human nutritional studies have indicated that supplementation with $n-3$ LCPUFA enhanced the marker of lipid peroxidation at dietary intakes of $n-3$ LCPUFA higher than those supplied in our study [31–33]. However, the influence of $n-3$ LCPUFA supplementation on the oxidative stress is still controversial. Recent studies showed that $n-3$ LCPUFA supplementation reduces oxidative stress level *in vivo* [34,35]. $n-3$ LCPUFA may have both pro- and antioxidant properties depending on experimental conditions, dosage and the antioxidant content of the supplement or background diet. We supplied $n-3$ LCPUFA to pregnant women with vitamins in amounts meeting the estimated additional requirements [19] including Vitamin E (α-tocopherol, 3 mg/day). Vitamin E is known as most important lipid-soluble antioxidant and plays an essential role in protecting cell membrane LCPUFA against oxidation [36]. Vitamin E also has a decreasing effect on 8-OHdG [37] and lipid peroxide production [38] in placenta. We speculate that added supply of Vitamin E may prevent the increase of oxidative stress levels after $n-3$ LCPUFA supplementation in pregnant women.

The measurement of MDA is widely applied as peroxidation marker, but MDA might be absorbed from the diet [39] and it is an unspecific product of peroxidation [40]. Analysis of urinary isoprostans would have provided more specific information on LCPUFA peroxidation [40]. Nevertheless, MDA excretion seems to be a reliable indicator for LCPUFA peroxidation, as there were no other differences in dietary intake according to the dietary protocols (data not shown). Measurement of urinary 8-OHdG has become a well accepted marker of oxidative DNA damage in the human body [15] and it has been measured by several methods such as gas chromatography with mass spectrometric detection [41] and HPLC with electrochemical detection (ECD) [20]. Recently, an ELISA based on monoclonal IgG (N45.1 clone) was developed for estimation of 8-OHdG in urine [15], which made urinary 8-OHdG measurement much easier. Yoshida et al. [42] found a good correlation ($r = 0.88$) between urinary 8-OHdG levels obtained with using ELISA (same kit as in our study) and HPLC-ECD. Urinary 8-OHdG excretion is not affected by the dietary intake of 8-OHdG [43].

In conclusion, under the conditions studied, the daily supplementation of 500 mg DHA and 150 mg EPA with required vitamins to pregnant women from 20 weeks of gestation to the time of delivery did not enhance lipid peroxidation or oxidative DNA damage.

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