Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial[1–3]

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ABSTRACT

Background: Pregnant women usually meet their increased energy needs but do not always meet their increased micronutrient requirements. The supply of both folic acid and docosahexaenoic acid (DHA) has been related to positive pregnancy and infant outcomes.

Objective: We aimed to assess whether fish-oil (FO) supplementation with or without folate from gestation week 22 to birth improves maternal and fetal n−3 long-chain polyunsaturated fatty acid (n−3 LC-PUFA) status.

Design: We conducted a multicenter (Germany, Hungary, and Spain), randomized, double-blind, 2 × 2 factorial, placebo-controlled trial. From gestation week 22 until delivery, 311 pregnant women received daily a preparation with FO [0.5 g DHA and 0.15 g eicosapentaenoic acid (EPA)], 400 μg methyltetrahydrofolic acid (MTHF), FO with MTHF, or placebo. Outcome measures included maternal and cord plasma DHA and EPA contents at gestation weeks 20 and 30 and at delivery, indicators of pregnancy outcome, and fetal development.

Results: FO significantly ($P < 0.001$) increased maternal DHA and EPA (% by wt), as shown by 3-factor repeated-measures ANOVA (ie, MTHF, FO, and time) with adjustment for maternal baseline DHA and EPA. In addition, FO significantly ($P < 0.001$) increased cord blood DHA (% by wt; 2-factor ANOVA). MTHF was significantly ($P = 0.046$) associated with increased maternal DHA (% by wt). There was no FO × MTHF interaction for the time course of DHA or EPA ($P = 0.927$ and 0.893). Pregnancy outcomes and fetal development did not differ significantly among the intervention groups.

Conclusions: FO supplementation from gestation week 22 until delivery improves fetal n−3 LC-PUFA status and attenuates depletion of maternal stores. MTHF may further enhance maternal n−3 LC-PUFA proportions. *Am J Clin Nutr* 2007;85:1392–400.

KEY WORDS Pregnancy, long-chain polyunsaturated fatty acids, LC-PUFA, folate, randomized controlled trial, fetal docosahexaenoic acid, DHA, eicosapentaenoic acid, EPA

INTRODUCTION

The last trimester of pregnancy is characterized by markedly higher substrate requirements for rapid fetal growth. Healthy pregnant women in European countries usually meet their increased energy and protein needs, but the relative increase in reference intakes for numerous micronutrients is far higher than that for energy (1, 2).

The average dietary supply of n−3 long-chain polyunsaturated fatty acids (n−3 LC-PUFAs) to pregnant women consuming European diets is rather low (3). Docosahexaenoic acid (DHA) is an indispensable component of all cell membranes and is incorporated in high concentrations in the membrane phospholipids of brain and retina (4). Randomized controlled trials (RCTs) found that DHA availability during the perinatal period may be associated with long-term cognitive and visual development (5, 6). Beneficial effects of DHA on postpartum depression also have been reported (7, 8). Therefore, it was proposed to provide pregnant women with supplemental DHA and other n−3 LC-PUFAs (9, 10).

Another critical nutrient is folate, which is an important cofactor of the homocysteine remethylation to methionine. Poor folate supply leads to higher plasma homocysteine concentrations, which are related to placental abruption, stillbirth, and increased rates of very low birth weight (VLBW) or preterm delivery (11–14). Therefore, supplementation with folate not

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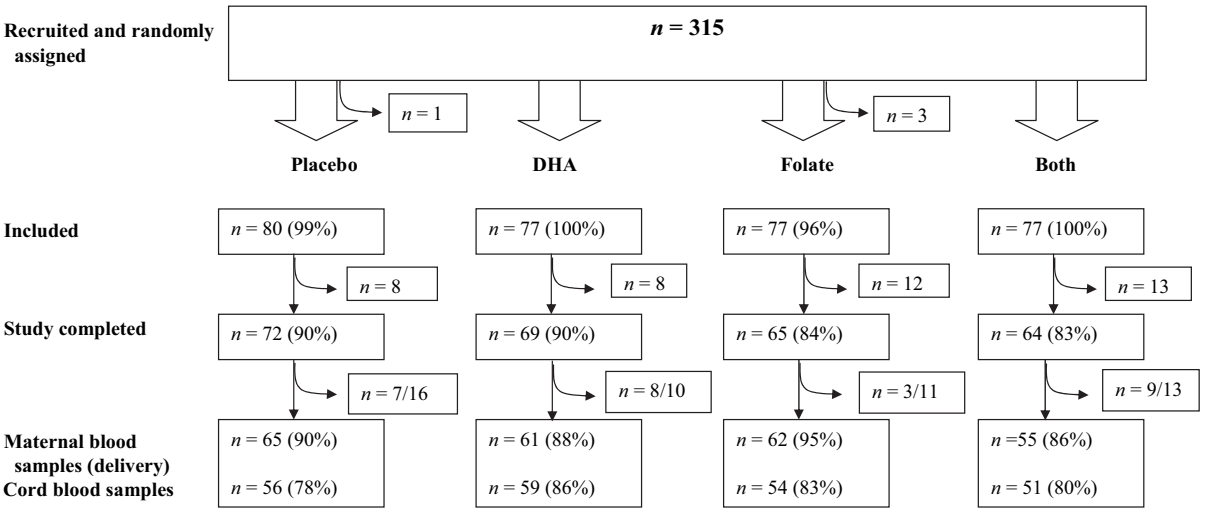

FIGURE 1. The numbers of enrolled subjects and blood samples for docosahexaenoic acid analyses. Maternal blood was not obtained in 25 cases. Cord blood could not be drawn in 60 cases because the placental vein had collapsed.

only at the beginning but throughout pregnancy may improve pregnancy outcomes.

Furthermore, animal studies suggest that folate deficiency is associated with decreased DHA (15, 16), which can be reversed by folate administration (17). A significant positive correlation of red blood cell (RBC) folate with plasma DHA was recently shown in men with emotional disorders (18). An interventional trial indicated that a high-dose supply of folate (5000 μg/d) increases dihomo-γ-linolenic acid and arachidonic acid (AA) in patients with continuous ambulatory peritoneal dialysis (CAPD) and hyperhomocysteinemia but not in CAPD patients without hyperhomocysteinemia (19). In an observational study, maternal plasma homocysteine correlated negatively with placental weight and with neonatal RBC DHA concentrations (20). Therefore, we compared the effects of combined supplementation of pregnant women with n−3 LC-PUFAs and folate on maternal and fetal plasma n−3 LC-PUFA and pregnancy outcomes with the separate effects of n−3 LC-PUFA or folate supplementation alone.

To address this question, a multicenter, randomized, double-blind, placebo-controlled European study was designed. Pregnant women were supplemented either with a fish-oil (FO) preparation, methyltetrahydrofolic acid (MTHF), or both or with placebo from gestation week 22 until delivery. Plasma contents of DHA and eicosapentaenoic acid (EPA) were quantified in maternal and cord blood (as a proxy for fetal supply), and pregnancy outcomes were monitored.

SUBJECTS AND METHODS

Study design

The present study was conducted with a 2 × 2 factorial design to assess the effects of increased intake of FO, 5-MTHF, both FO and 5-MTHF, or placebo from gestation week 22 until delivery on pregnancy and birth outcomes. Maternal primary outcome variables were the relative contents of DHA and EPA within plasma phospholipids at gestation week 30 and at delivery and their changes relative to baseline values (gestation week 20). Secondary outcome variables were maternal weight gain and indicators of the course of pregnancy and delivery, including proteinuria, blood pressure, eclampsia, mode of delivery, delivery complications, blood loss, and postnatal depression 8 wk after birth. The primary outcome variable in infants was the relative content of DHA in plasma phospholipids in cord blood. Secondary outcomes were Apgar score, umbilical pH, birth length, and birth weight.

After a careful explanation of the study details, written informed consent was obtained from all participating women. The ethics committee of each participating center approved the study protocol.

Recruitment of subjects

Apparently healthy pregnant women were recruited before gestation week 20 in the Departments of Obstetrics at Ludwig Maximilians University, Munich, Germany; the University of Granada, Granada, Spain; and the University of Pecs, Pecs, Hungary. Inclusion criteria were singleton pregnancy, gestation <20 week at enrollment, and intention to deliver in one of the obstetrical centers. Women with serious chronic illness (eg, diabetes, hepatitis, or chronic enteric disease) or who used FO supplements since the beginning of pregnancy or folate or vitamin B-12 supplements after gestation week 16 were excluded from the study. Recruitment started in November 2001 and continued until March 2003. Pregnant women attending antenatal care clinics for ultrasound examination between gestation weeks 12 and 20 were approached by study personal and invited to participate. The expected delivery date and gestation week had been determined by using Naegeles's rule and were used to determine a woman's eligibility for enrollment in the study. We included women whose body weight was from >50 kg to 92 kg and who were >18–41 y old. After inclusion in the study and before gestation week 22, participating women were randomly assigned to 1 of the 4 intervention groups (**Figure 1**).

Blockwise randomization was performed by using stratification by center before the study started. Thus, in each center, 20 envelopes containing cards with 1 of 4 numbers (1, 2, 3, or 4) according to the 4 intervention groups were mixed and put into a closed box. By drawing envelopes, intervention group numbers

TABLE 1
Composition of supplements according to manufacturers' analyses[1]

Nutrient supply	Group			
	Placebo	Fish oil	5-MTHF	FO and 5-MTHF
	per 15-g sachet			
DHA (mg)	0	500	0	500
EPA (mg)	—	150	—	150
5-MTHF (μg)	0	0	400	400
Energy (kcal)	70	71	70	71
Protein (g)	2.9	2.5	2.9	2.5
Fat (g)	2.9	3.1	2.9	3.1
Carbohydrate (g)	8.0	8.2	8.0	8.2

[1] 5-MTHF, 5-methyltetrahydrofolate; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. The content of vitamins and minerals was identical for all supplementation groups—minerals: 300 mg Ca, 240 mg P, 93 mg Mg, 3 mg Zn, 66 μg I; vitamins: 330 μg vitamin A, 1.5 μg vitamin D, 3 mg vitamin E, 0.36 mg thiamine, 1.5 mg riboflavin, 4.5 mg vitamin B-3, 1.9 mg vitamin B-6, 3.5 μg vitamin B-12, 270 mg vitamin C.

TABLE 2
Schedule of data collection instruments and assessment of biological material

Data and biological material	Time of collection; age of child
Recruitment information sheet	Antenatal clinic
Obstetrical questionnaire	Gestation weeks 20 and 30; at delivery
Socioeconomic questionnaire	Gestation week 20
Food-frequency questionnaire	Gestation weeks 20 and 30
Pediatric symptoms and illness questionnaire	Birth; 8 and 24 wk old
Colostrum; breast milk sample	Birth; 8 wk old
Visually evoked potentials	8 wk old (Germany and Spain)
Bayley Mental Development Test	6 mo old (Spain)
Skin-prick test	6 mo old (Spain)
Blood collection	Gestation weeks 20 and 30; at delivery
Spot urine sample	Gestation weeks 20 and 30; at delivery
Maternal depression measured by Edinburgh Postnatal Depression Scale	At delivery

were consecutively assigned to subject identity numbers. The procedure was identical for each study center.

Dietary intervention

After allocation to the respective intervention groups, participating women were provided with 90 sachets, each containing 15 g of a milk-based supplement (Blemil Plus Matter; Ordesa Laboratorios, Barcelona, Spain). Each sachet was to be consumed as one daily dose, providing 500 mg DHA and 150 mg EPA (Pronova Biocare, Lysaker, Norway), 400 μg 5-MTHF (BASF, Ludwigshafen, Germany), both, or placebo together with vitamins and minerals in amounts meeting the recommended intakes during the second half of pregnancy for European women (21; **Table 1**). Detailed instructions were given in German, Spanish, or Hungarian on the label of each sachet. Thus, sufficient supplement until the second investigation date at gestation week 30 was provided. A second batch of 90 sachets was then provided for use until delivery. The different supplements were not distinguishable with respect to the appearance of the sachets or to their contents. Subjects were instructed to return leftover sachets to the study center. Compliance was assessed in standardized questionnaires (*see* Data collection) at gestation week 30 and at delivery by asking each subject how many days of dosing she had missed (eg, <6, >5, or none).

Data collection

At study entry, in gestation week 30, and at delivery, a trained physician performed standardized, personal, face-to-face interviews that included information about parity, gravidity, gravidity risks, parental allergic disease (ie, hay fever, atopic eczema, allergic asthma, or other manifestations of type 1 allergies), and maternal smoking habits (average number of cigarettes during the previous week) (**Table 2**). In addition, maternal weight, height, and blood pressure were assessed and a routine laboratory work-up was conducted.

Proteinuria, blood pressure, and eclampsia were documented in gestation weeks 22 and 30 and at delivery, and delivery complications and blood loss at parturition were recorded as safety parameters. All pregnant women underwent safety monitoring, regardless of the availability of blood samples.

Sociodemographic data and information on dietary habits were obtained at study entry with the use of standardized questionnaires. At delivery, information about pregnancy complications, mode of delivery, and delivery complications (eg, proteinuria, blood pressure, occurrence of eclampsia, or estimated blood loss) was obtained in a standardized report form by the study personnel. Data on the infants were collected at birth and at the ages of 2 and 6 mo. Birth data included anthropometric measures such as birth weight, birth length, and head circumference; the Apgar score; and a postnatal clinical examination. Data at the age of 2 mo were collected with the use of a standardized questionnaire and included information on postnatal disease (physician's diagnosis), visits to a pediatrician, and maternal depression (according to the Edinburgh Postnatal Depression Scale). In addition, visually evoked potentials and a Bayley mental development test were performed at the age of 2 and 6 mo, respectively (in Spain only; data not shown).

Collection of biological material

For the assessment of plasma and RBC phospholipid fatty acids, antioxidant status, folate, and vitamin B-12 status, 10 mL maternal venous blood was collected into EDTA at study entry and again at gestation week 30. At delivery, 12 mL maternal blood was collected into EDTA, as was 12 mL venous placental cord blood. Placenta samples (central parenchyma) were collected from each woman and stored at −80 °C until analysis. Breastfeeding mothers manually expressed 3 mL colostrum within the first week after delivery and 2 mL breast milk 8 wk after delivery. Samples were stored at −80 °C until the milk fatty acids were analyzed.

Plasma fatty acid analysis

Initial total lipid extraction was performed according to the method of Kolarovic and Fournier (22). Briefly, 500 μL plasma with 500 μL water was mixed by vortex for 30 s with 100 μL internal standard containing 0.857 mg heptadecanoic acid/mL as phospholipid dissolved in chloroform. Four mL of a mix of hexane and 2-propanol (at 3:2) containing 25 mg butylated hydroxytoluene (BHT)/L was added. After thorough mixing and centrifugation (10 min, 4 °C, 1500 × *g*), the organic layer was

transferred into another glass tube. The extraction was repeated 3 times with pure hexane. The pooled extracts were dried under vacuum and dissolved in 200 μL hexane:methyl-*tert*-butyl-ether:acetic acid (100:3:0.3, vol:vol:vol). Phospholipids were isolated by liquid chromatography with the use of aminopropyl columns (Sep Pak Cartridges; Waters, Milford, MA) as described by Agren et al (23). Phospholipid fractions obtained from the columns were taken to dryness under vacuum, and 100 μL chloroform was added to each tube. Fatty acid methyl esters were formed according to the method of Lepage et al (24). A gas chromatograph (model HP-5890 Series II; Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector was used for quantification of fatty acid methyl esters. Chromatography was performed by using a capillary column of 60-m length, 0.32-mm internal diameter, and 20-μm film thickness (Sp 2330 FS; Supelco Inc, Bellefonte, Palo Alto, CA). The injector with a split-to-splitless ratio of 29:1 and the detector were maintained at 250 and 275 °C, respectively, and nitrogen was used as carrier gas. DHA and EPA proportions were calculated as percentages by weight (% by wt) of total detected fatty acids with 14–24 C-atoms.

Power calculation and statistical analyses

Using a statistical model of a 2-factor analysis of variance (ANOVA) (4 intervention groups, 3 countries), we achieved a statistical power of 80% to detect 0.8% DHA difference between any of the supplements if 300 pregnancies are studied (error level of 0.05). This seemed a reasonable compromise, especially because interactions between diet and center are detectable with 80% power if they contribute >22% to overall variation. Thus, we aimed for a sample of 100 pregnant women to complete the clinical trial in each of the 3 study centers (corresponding to 75 subjects per dietary group and 25 cases per cell). Calculating a dropout rate of 33% for compliance, giving birth at the obstetrical unit of the study center, and postnatal follow-up of the infant, we assumed that 150 women should to be recruited at each study center.

Baseline characteristics were compared among the 4 intervention groups by using the Kruskal-Wallis test for continuous data and a chi-square test for ordinal data. In the same manner, baseline characteristics were compared among the 3 countries. Crude means and SDs of DHA and EPA were calculated for the 4 intervention groups.

To compare the effects of supplementation with time, a 3-factor repeated-measures ANOVA was performed with the factors FO and MTHF (as between-subject factors) and time (as within-subject factor) with 3 timepoints (gestation weeks 2 and 30 and at delivery). For adjustment, maternal baseline DHA and EPA (gestation week 20) were included as covariates. Cord blood DHA was analyzed separately in a 2-factor analysis (FO and MTHF) and adjusted for maternal DHA baseline proportions (gestation week 20) as covariate. In a secondary analysis, results were adjusted for the study centers (Spain, Germany, and Hungary). If significant effects on DHA and EPA proportions were observed over time, single-timepoint comparisons in gestation week 30 and at delivery with baseline values were tested, with Bonferroni correction as adjustment for multiple comparisons. Spearman's rho was calculated to analyze the effect of baseline maternal DHA on follow-up maternal DHA proportions. Significance was set at $\alpha = 0.05$. All computations were performed with SPSS statistical software (version 12.0; SPSS Inc, Chicago, IL).

RESULTS

Study participants

Three hundred fifteen women completed the recruitment information sheet and consented to participate in the study (Figure 1). Four women did not meet the inclusion criteria: 2 women weighed >92 kg, 1 of whom used commercial FO preparations; and 2 women regularly consumed FO preparations. Thus, 311 pregnant women were enrolled. Forty-one women were excluded from the analyses because they did not complete the study (dropout rate: 13.18%), which left 270 participants who completed the study. Reasons for dropping out were noncompliance ($n = 2$), relocation ($n = 1$), aversion to or bad taste of the supplement ($n = 9$), and the loss of contact ($n = 2$). In the remaining 17 cases, the reasons for dropping out are not known. Two hundred forty-three maternal blood samples could be drawn at delivery, and cord blood samples were obtained in 220 cases. All but 2 participants were white; the exceptions were 1 Asian woman and 1 woman of noncategorized race-ethnicity. Both of these women belonged to the placebo group. At study entry, the German participants were significantly older than the participants at the other 2 centers, and the German women had a significantly lower mean body mass index (BMI; in kg/m^2) than did the Spanish women. Smoking was significantly ($P < 0.001$) more frequent in the Spanish women than in the German or Hungarian women. Further significant differences among the women from the different countries included gravidity risks, rural versus urban living area, residence, pet keeping, job training of the father, and parental allergies (data not shown). However, after allocation into the 4 different study arms, the proportion of women assigned to each dietary group did not differ significantly across the 3 countries. No significant differences in the baseline characteristics were observed between the groups (**Table 3**). In particular, maternal plasma DHA, EPA, and folate baseline proportions did not differ significantly among the 4 intervention groups (**Table 4**).

After supplementation, the length of gestation did not differ significantly among the 4 intervention groups ($P = 0.390$, Kruskal-Wallis). At delivery, no significant differences in maternal or fetal complications and birth outcomes were observed among the intervention groups (data not shown). Safety parameters did not differ among the intervention groups. Maternal postpartum depression was evaluated by the Edinburgh Postnatal Depression Scale score at delivery. Neither categorized scores (scores ≥ 10 compared with scores < 10, chi-square test = 0.717) nor the analyses of continuous score data ($P < 0.635$, Kruskal-Wallis test) found any differences among the intervention groups.

Effects of FO and MTHF supplementation on maternal and cord blood DHA and EPA

Maternal baseline percentages of DHA and EPA within total fatty acids did not differ significantly among the 4 intervention groups (Table 4). Crude means of DHA and EPA proportions according to the 4 intervention groups at 3 timepoints are summarized in Table 4. FO supplementation significantly increased maternal DHA during the supplementation period, as did MTHF

TABLE 3
Study population after random assignment intervention groups at study entry[1]

	Group				
	Placebo (n = 72)	FO (n = 69)	5-MTHF (n = 65)	FO and 5-MTHF (n = 64)	P
Study center [n (%)]					0.965
Spain	38 (53)	38 (55)	35 (54)	36 (56)	
Germany	20 (28)	18 (26)	14 (22)	16 (25)	
Hungary	14 (19)	13 (19)	16 (25)	12 (19)	
Age (y)	31.1 (18.4–40.3)[2]	31.1 (18.8–40.8)	30.6 (20.1–41.1)	31.1 (21.5–40.1)	0.476
BMI (kg/m^2)	24.1 (18.5–35.5)	25.2 (18.5–35.2)	24.6 (18.9–39.1)	24.9 (19.5–32.4)	0.403
Gravidity [n (%)]					0.621
<2	32 (45)	34 (50)	23 (35)	26 (41)	
2	25 (35)	19 (28)	25 (39)	26 (41)	
>2	15 (21)	16 (23)	17 (26)	12 (19)	
Parity [n (%)]					0.855
<2	65 (90)	61 (88)	56 (86)	56 (88)	
2	7 (10)	7 (10)	7 (11)	6 (9)	
>2	0 (0)	1 (1)	2 (3)	2 (3)	
Gravidity risks [n (%)][3]					0.447
0	28 (39)	19 (27)	18 (28)	20 (31)	
1	30 (42)	29 (42)	26 (40)	27 (38)	
2	12 (17)	15 (22)	16 (25)	11 (17)	
3	2 (3)	6 (9)	5 (8)	9 (14)	
Smoking [n (%)]					0.555
Yes	5 (7)	9 (13)	8 (12)	9 (14)	
No	67 (93)	60 (87)	57 (88)	55 (86)	
Living status [n (%)]					0.778
Single	3 (4)	3 (4)	3 (5)	1 (7)	
In partnership	69 (96)	66 (96)	62 (95)	63 (98)	
Job training of father					0.310
n[4]	70	68	64	62	
None [n (%)]	33 (47)	27 (40)	29 (45)	17 (27)	
Apprenticeship [n (%)]	10 (14)	14 (21)	14 (22)	19 (31)	
Master's degree [n (%)]	3 (4)	6 (9)	6 (9)	5 (8)	
University degree [n (%)]	24 (34)	20 (29)	15 (23)	21 (34)	
Other [n (%)]	0 (0)	1 (2)	0 (0)	0 (0)	
Gestational week					0.390
Median	39.6	39.3	40.0	39.7	
Maternal baseline plasma folate					0.317
n	71	69	65	64	
Concentration (ng/mL)	13.7 (1.9–189.7)	11.4 (3.1–41.1)	10.8 (2.9–121.1)	11.5 (2.2–57.6)	

[1] Differ among intervention groups were tested by using the Kruskal-Wallis and the chi-square test for continuous and ordinal data, respectively.
[2] Median; range in parentheses (all such values).
[3] The numbers of different risks, as detailed in Subjects and Methods.
[4] Numbers of subjects are indicated only when they are less than the total number of subjects in each group.

supplementation, albeit to a lesser extent. The FO supplementation × MTHF supplementation × time interaction for maternal DHA was not significant; however, the trial may have been underpowered to detect significant interactions. Similarly, FO supplementation significantly increased EPA over time (Table 4).

In contrast with its effect on DHA, the interaction of MTHF supplementation had no significant effect on EPA values over time. The inclusion of the 3 study centers as potential confounders of DHA or EPA proportions did not change the results.

In a secondary analysis, we compared the fatty acid proportions at the single timepoints with the respective baseline values. FO supplementation was associated with greater plasma DHA proportions at both gestation week 30 and delivery, whereas MTHF did not show significant single-timepoint effects after Bonferroni adjustment.

To analyze cord blood DHA, 2-factor ANOVA was used with FO and MTHF as main effects and maternal DHA baseline proportions as covariate. Cord blood DHA was significantly higher in both FO intervention groups (± MTHF) than in groups receiving placebo or MTHF alone (**Table 5**). No significant FO suppelementation × MTHF supplementation interaction for cord blood DHA was observed. EPA was not evaluated in cord blood because of the high number of values below the detection limit.

We next asked how the maternal DHA baseline status is related to cord blood DHA proportions. For this purpose, only the placebo group was considered. Maternal blood DHA at gestation week 20 correlated significantly ($P < 0.05$) with cord blood DHA. The correlation between maternal and cord blood DHA proportions was still present at delivery (**Figure 2**).

TABLE 4
Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) proportions in maternal plasma[1]

	DHA				EPA			
Time	Placebo group (n = 72)	FO group[2] (n = 69)	5-MTHF group[3] (n = 65)	FO and 5-MHTF group[2–4] (n = 64)	Placebo group (n = 72)	FO group[5] (n = 69)	5-MTHF group[6] (n = 65)	FO and 5-MHTF group[5–7] (n = 72)
Gestation week 20, baseline (% by wt)	5.95 ± 1.60[8]	5.75 ± 1.35	5.68 ± 1.14	5.89 ± 1.38	0.28 ± 0.38	0.18 ± 0.21	0.17 ± 0.20	0.22 ± 0.31
n	66	66	63	60	66	66	63	60
Gestation week 30 (% by wt)	5.46 ± 1.21	7.49 ± 2.01	5.65 ± 1.01	8.00 ± 1.52	0.24 ± 0.18	0.53 ± 0.25	0.22 ± 0.19	0.53 ± 0.25
n	65	66	59	61	65	66	59	61
Delivery (% by wt)	5.44 ± 1.50	7.26 ± 1.72	5.46 ± 1.46	7.57 ± 1.64	0.22 ± 0.17	0.37 ± 0.20	0.25 ± 0.19	0.43 ± 0.25
n	65	61	62	55	65	61	62	55

[1] FO, fish oil; MTHF, methyltetrahydrofolate. Baseline DHA ($P = 0.881$, Kruskal-Wallis) and EPA ($P = 0.619$, Kruskal-Wallis) proportions did not differ significantly among the intervention groups. All further significance was calculated by 3-factor repeated-measures ANOVA with the factors FO and MTHF (as between-subject factors) and time (3 levels for DHA and EPA as within-subject factor). Maternal baseline DHA and EPA proportions (gestation week 20) were included as covariates for adjustment, respectively. The significance of the main effects and their interactions without time was $P < 0.001$ for FO supplementation and $P = 0.041$ for MTHF supplementation on DHA proportions; $P < 0.001$ for FO supplementation and $P = 0.077$ for MTHF supplementation on EPA proportions (main effects); $P = 0.934$ for the interaction of FO with MTHF on DHA proportions; and $P = 0.800$ for the interaction of FO with MTHF on EPA proportions. The FO × MTHF × time interaction was significant for neither DHA nor EPA. Inclusion of the country as potential confounder did not significantly change the results.
[2] FO supplementation × time interaction, $P < 0.001$ (ANOVA).
[3] MTHF supplementation × time interaction, $P = 0.047$ (ANOVA).
[4] FO supplementation × MTHF supplementation × time interaction, $P = 0.927$ (ANOVA).
[5] FO supplementation × time interaction, $P < 0.001$ (ANOVA).
[6] MTHF supplementation × time interaction, $P = 0.081$ (ANOVA).
[7] FO supplementation × MTHF supplementation × time interaction, $P = 0.893$ (ANOVA).
[8] $\bar{x}$ ± SD (all such values).

DISCUSSION

This European trial shows that supplementation of pregnant women from gestation week 22 with an FO preparation containing 0.5 g DHA and 0.15 g EPA is associated with a significant increase in maternal plasma DHA and EPA until delivery. Furthermore, FO supplementation is associated with significantly greater cord plasma DHA proportions. MTHF supplementation also has an enhancing effect on maternal DHA proportions, as shown by 3-factor repeated-measures ANOVA that found an independent association of MTHF with maternal plasma DHA. However, no significant interaction was found between MTHF and FO supplementation at the sample size in this study.

Previous RCTs with n−3 LC-PUFA supplementation in healthy pregnant women showed partly different effects on fetal DHA (25). In one study, 40 pregnant women with allergic disease received FO providing 2 g DHA/d from gestation week 20 until delivery. This increased the DHA proportion in cord blood and also in maternal RBCs at both gestation weeks 30 and 37 (25). Similarly, supplementation of healthy Norwegian women, providing 1.2 g DHA/d and 0.8 g EPA/d from gestation week 18, led to higher DHA proportions in cord plasma than in the control group receiving corn oil. Although maternal plasma DHA proportions were not assessed in that trial, DHA was increased in breast milk, which indicated a replenishment of maternal DHA

TABLE 5
Docosahexaenoic acid (DHA) in cord blood plasma[1]

	DHA proportions			
	Placebo group (n = 72)	FO group[2] (n = 69)	5-MTHF group[3] (n = 65)	FO and 5-MHTF group[2–4] (n = 64)
Cord blood (% by wt)	8.74 ± 2.02[5]	9.90 ± 2.13	8.11 ± 2.06	9.35 ± 2.30
n	56	59	54	51

[1] FO, fish oil; MTHF, methyltetrahydrofolate. Cord blood DHA proportions were analyzed by 2-factor ANOVA with FO and MTHF; maternal baseline DHA (gestation week 20) was included as a covariate. Inclusion of the country as potential confounder did not significantly change the results.
[2] FO supplementation, $P < 0.001$ (ANOVA).
[3] MTHF supplementation, $P = 0.095$ (ANOVA).
[4] FO supplementation × MTHF supplementation interaction for cord blood DHA proportions, $P = 0.689$ (ANOVA).
[5] $\bar{x}$ ± SD (all such values).

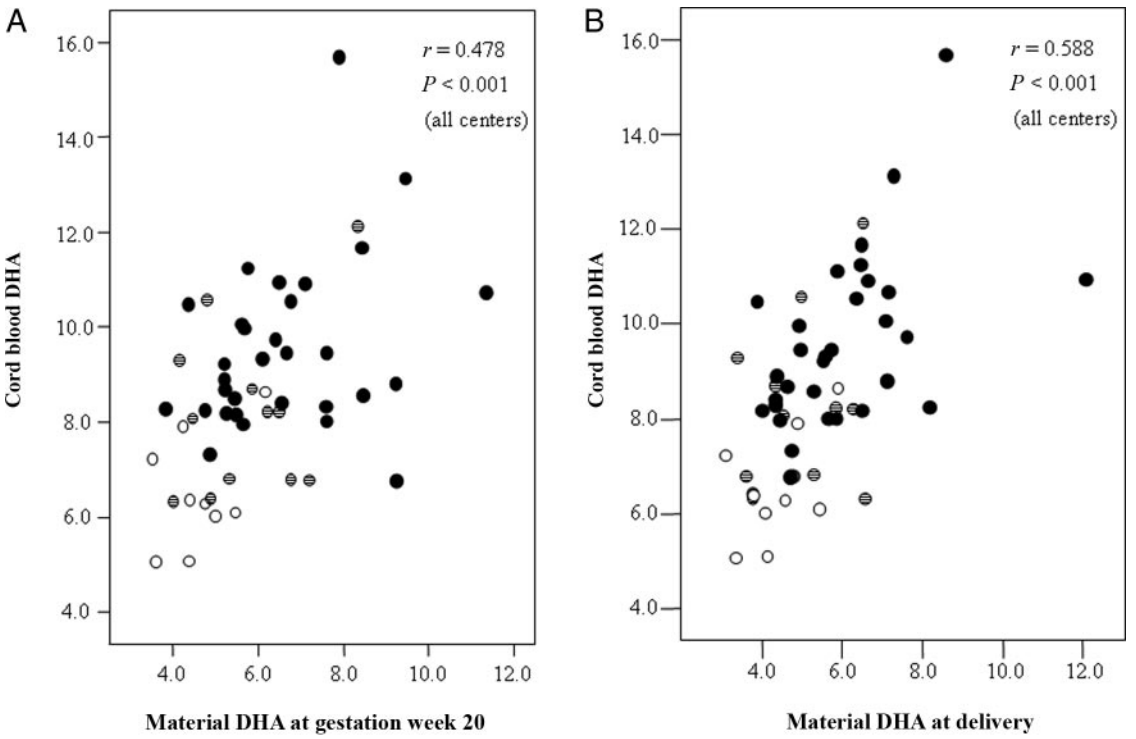

FIGURE 2. A: Correlation of maternal docosahexaenoic acid (DHA) proportions (% by wt) at gestation week 20 (baseline) with cord blood DHA proportions in subjects from the placebo group (⛀, Germany; ●, Spain; ○, Hungary). Correlations were tested with Spearman's rho. The slopes did not differ significantly between the 3 countries. B: Correlation of maternal DHA proportions at delivery with cord blood DHA proportions in subjects from the placebo group. Correlations were tested with Spearman's rho. The slopes did not differ significantly between the 3 countries.

stores also (5). Lower amounts of DHA (0.2 g) administered to 50 healthy women from the United Kingdom from gestation week 15 had no effect on cord blood fatty acid composition, but the maternal plasma and RBC DHA proportions increased (26). Similar results were obtained in a small trial ($n = 40$) with 0.2 g DHA (27). Approximately 0.133 g DHA/d was provided in eggs from gestation weeks 24 to 28 until delivery to 144 pregnant African American women with low baseline consumption of n−3 LC-PUFAs. Cord plasma and RBC DHA increased significantly, but maternal DHA proportions at delivery did not (28). In another RCT, 23 pregnant Norwegian women received 2.3 g DHA/d from gestation week 30, which increased both neonatal and maternal DHA (10). It appears that not only dosage and duration of maternal supplementation, but also the socioeconomic background and habitual dietary fish consumption of the subjects, explain variations in fetal DHA (29, 30). When DHA stores are low in less well-nourished women, low DHA supplementation may primarily affect the fetal DHA status, because DHA is preferentially transported to the fetus (31, 32). In this situation, low-dose supplementation may not be sufficient to improve maternal stores (28). If maternal stores are high at baseline, there is no need for a further increase of fetal stores, whereas depletion of maternal stores at delivery may be prevented by a small dose of DHA (27).

Compared with the studies cited above, our trial used intermediate amounts of DHA (0.5 g) that increased both fetal and maternal DHA and maternal EPA. The effect occurred across the 3 countries, even though the populations in the 3 study centers had substantial differences with respect to fish consumption (33) and several socioeconomic and pregnancy-related variables.

Mean maternal baseline plasma DHA was 5.88 ± 1.38% by wt, which is higher than that in the studies of Matorras et al (34) ($\bar{x}$ ± SD: 2.78 ± 0.57% in the second trimester), Montgomery et al (26) (median: 1.6% in gestation week 28), or Sanjurjo et al (27) (2.99 ± 0.31% early in the third trimester) but similar to that in the study of van Houwelingen et al (10) (4.7 ± 0.5%). Maternal baseline and cord blood DHA proportions were positively correlated in the placebo group, as previously reported by others (28, 34, 35). Regardless of the decrease in maternal plasma DHA during pregnancy, maternal and cord blood DHA proportions still correlated significantly at delivery.

The association of MTHF with increased maternal plasma DHA indicates a potential relation of DHA proportions with MTHF supplementation. Other studies also indicate a potential synergy between FO and folic acid (18, 19). Explanations for the underlying mechanism are lacking and remain speculative. In vitro studies found that homocysteine-induced trophoblast apoptosis can be reversed by folate (36). Thus, folate may affect placental DHA transfer through improvement of the placental microarchitecture. However, this possibility does not explain the increase in maternal DHA by MTHF in our study. Folate improves the remethylation of homocysteine, which leads to the formation of methionine. Sugiyma et al (37) suggested that methionine stimulates phosphatidylethanolamine methylation, thereby altering the ratio of phosphatidylcholine to phosphatidylethanolamine in liver microsomes. This in turn increases the activity of δ-5 and δ-6 acyl-coenzyme A desaturases. This effect of MTHF was found to be more pronounced on α-linolenic acid desaturation than on that of linoleic acid (17). Other explanations include the prevention of lipid peroxidation or stabilization of

DNA involved in fatty acid metabolism through increased availability of *S*-adenosylmethionine.

The present study did not find obvious differences among the intervention groups with respect to birth weight, birth length, or head circumference, which is in line with some (5, 27, 38) but not all (28, 39, 40) RCTs. The possible long-term implications of higher cord blood DHA proportions for infant development should be assessed further. We conclude from the present study that daily supplementation of pregnant women with 0.5 g DHA and 0.15 g EPA from gestation week 22 until delivery increases maternal and fetal plasma DHA and maternal plasma EPA proportions. The intervention strategy used was effective in enhancing maternal and fetal DHA in pregnant women from 3 European countries with somewhat different fish consumption and baseline DHA stores. The present study further indicates that MTHF supplementation may increase maternal DHA and EPA, and this possibility deserves to be elucidated further in larger trials.

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