CME

Effects of Maternal Ω-3 Supplementation on Fatty Acids and on Visual and Cognitive Development

†Jose A. Hurtado, ‡Carmen Iznaola, *Manuela Peña, ‡Josefa Ruíz, ||Luis Peña-Quintana, #Naroa Kajarabille, §Yessica Rodriguez-Santana, **Pablo Sanjurjo, **Luis Aldámiz-Echevarría, #Julio Ochoa, and ††Federico Lara-Villoslada, on Behalf of the NUGELA Group

ABSTRACT

Objectives: The aim of the present study was to elucidate whether a dairy drink enriched with ω-3 long-chain polyunsaturated fatty acid (LC-PUFA) could have an impact on the lipid profile of the mother and the newborn, and also whether this intervention could affect the newborns' visual and cognitive development.

Methods: A total of 110 pregnant women were randomly assigned to one of the following intervention groups: control group (n = 54), taking 400 mL/day of the control dairy drink, and supplemented group (fish oil [FO]) (n = 56), taking 400 mL/day of the fish oil–enriched dairy drink (including ~400 mg eicosapentaenoic acid–docosahexaenoic acid [DHA]/day). During the study, the mothers' diets were supervised by a nutritionist to encourage compliance with present recommendations of FA intake. Blood fatty acid profiles were determined in the mother's (at enrollment, at delivery, and at 2.5 and 4 months) and newborn (at delivery and at 2.5 months) placenta and breast milk (colostrum and at 1, 2, and 4 months). Pattern reversal visual evoked potentials (VEPs) (at 2.5 and 7.5 months) and Bayley test (at 12 months) were recorded.

Results: DHA percentage was higher in plasma, erythrocyte membranes, and breast milk samples from the FO group. The ratio of nervonic acid was also higher in plasma and erythrocyte lipids of the mother and newborn's blood samples from the FO group. No differences were observed in the Bayley test. No differences were observed in VEPs between both groups. We observed a shorter latency, however, in the lower visual angle (7.5′) in the boys of the supplemented group.

Conclusions: Omega-3 LC-PUFA dietary supplement during pregnancy and lactation influenced the mother and newborn's fatty acid profile and nervonic acid content but did not show effects on visual and cognitive/psychomotor development.

Key Words: docosahexaenoic acid, fatty acid profile, lactation, nervonic acid, pregnancy, visual evoked potentials

(*JPGN* 2015;61: 472–480)

What Is Known

- Docosahexaenoic acid has an important role in the visual and cognitive development.
- Deficiencies of docosahexaenoic acid provoke alterations in this development.
- Supplementation with ω-3 increases plasma levels.

What Is New

- Maternal supplementation with ω-3 during gestation in a population already following the recommended dietary intake of fish oil, showed a major effect on docosahexaenoic acid levels in plasma and erythrocyte membranes of mothers and infants and in breast milk.
- The supplementation also influenced on the levels of nervonic acid.
- A shorter latency in the lower visual angle in the supplemented boys was observed.

Received September 22, 2014; accepted May 7, 2015.

From the *Department of Neonatology, Hospital Materno Infantil Virgen de las Nieves, Granada, the †Programa de Doctorado de Pediatría y Puericultura, University of Granada, the ‡Department of Neurophysiology, Hospital Materno Infantil Virgen de las Nieves, Granada, the §Gastroenterology and Pediatric Nutrition Unit, Complejo Hospitalario Universitario Insular Materno-Infantil, Las Palmas, the ||Department of Clinical Sciences, Universidad de Las Palmas de Gran Canaria, the #Department of Physiology, Institute of Nutrition and Food Technology ''José MataixVerdú,'' University of Granada, Granada, the **Department of Pediatrics, Hospital de Cruces, Barakaldo, and the ††Research and Development Department, Lactalis Puleva, Granada, Spain.

Address correspondence and reprint requests to Julio J. Ochoa, Institute of Nutrition and Food Technology ''José MataixVerdú,'' University of Granada, Biomedical Research Centre, Health Sciences Technological Park, Avenida del Conocimiento s/n, Armilla, 18071 Granada, Spain (e-mail: jjoh@ugr.es).

This article has been developed as a Journal CME Activity by NASPGHAN. Visit *http://www.naspghan.org/content/59/en/Continuing-Medical-Education-CME* to view instructions, documentation, and the complete necessary steps to receive CME credit for reading this article.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (*www.jpgn.org*).

Members of the NUGELA group are J. Álvarez, F. Andrade, J. Diaz-Castro, M. Exposito, S. González, S. Henriquez, J.A. Hurtado, C. Iznaola, N. Kajarabille, M.C. Lara, F. Lara-Villoslada, S. López, M. López-Frías, E. Machín, J.A. Malo, L. Moltó, J. Moreno, J.J. Ochoa, L. Peña-Quintana, M. Peña, J.A. Prieo, O. Ramírez, C. Rodríguez, Y. Rodriguez-Santana, J. Romero, J. Ruíz, I. Sebastián, O. Soldado, P. Sanjurjo, A. Valero, and M Villanueva.

www.clinicaltrials.gov registration number: NCT01947426.

F.L.-V. is an employee of Lactalis Puleva. The other authors report no conflicts of interest.

DOI: 10.1097/MPG.0000000000000864

It is a well-known fact that the total fatty acid concentrations increase in maternal plasma during pregnancy (1). The level of docosahexaenoic acid (DHA, 22:6 n-3) increases more than other polyunsaturated fatty acids (PUFA). This increase is probably because of an enhanced mobilization of DHA from the maternal adipose tissue in a process partially regulated by the placenta (2).

DHA is highly concentrated in retinal photoreceptors and neuronal cell membranes where DHA has putative roles related to visual function, including signal transduction and neurotransmission (3–5). A high accumulation of DHA in infants' brain and retina has been reported during the third trimester of pregnancy (4–6). This has awakened interest for the connection between maternal DHA intake and the optimum central nervous system development.

Results from observational studies have suggested that there is a positive association between DHA status in cord plasma and visual, cognitive, and motor development during the first year of life (7–9). A positive association has also been reported between the mother's fish consumption during pregnancy and the offspring's cognitive performance (10). Intervention studies with DHA supplements during pregnancy and/or lactation have shown controversial results (8,9,11). Some have shown a better visual (4,7) and/or cognitive development (7,12), and others have shown no effect (8,13) or even negative ones (9,11).

The existing controversies can be attributed to the confounding background factors of the studies and the methodological differences between them. These factors include gestational age, newborn's sex, mother's diet (including DHA intake from foods such as fish), timing (gestation, lactation, or both periods, which is less common) (10,11), dosage form of the supplementation (mainly by capsules), and so on.

In addition, most of the available studies report only DHA plasma levels (14,15), providing only information about the mother's diet during the previous days. Therefore, those changes would not reflect faithfully the modifications in the lipid profile of other tissues such as placenta, erythrocyte, or even in the mother's milk, which reflects prolonged intake (16). It is important to obtain the maximum information about the lipid profiles in both mother and newborn, as well as the possible influence of fish oil supplementation on these parameters. Furthermore, there is still a lack of knowledge regarding the effects on the complete lipid profile including other important fatty acids such as arachidonic and nervonic acids (17,18), which could be modified by the fish oil supplementation and be partially responsible for its effects.

In light of these considerations, the purpose of this randomized controlled trial (RCT) was to evaluate the influence of a dairy drink supplemented with ω-3 long-chain polyunsaturated fatty acid (LC-PUFA) taken by mothers following a balanced controlled diet, during the last trimester of gestation and lactation period on the lipid profile of the mother and the newborn. As a secondary outcome, we also aimed to analyze whether this fish oil intervention could affect the visual and cognitive development of the term newborn.

METHODS

Subjects

The present study involved a group of healthy term infants from mothers enrolled in a registered, double-blind, controlled, and randomized trial, lasting from the 28th week of gestation to the end of lactation. A total of 110 volunteers were recruited from 2 hospitals, ''Hospital Materno-Infantil'' (Granada, Spain) and ''Hospital Universitario Materno-Infantil'' (Las Palmas de Gran Canaria, Spain), between June 2009 and August 2010. The flowchart for participant enrollment and dropouts is shown in Figure 1. The inclusion criteria for the mother-child pair were: no presence of diseases that may affect the normal development of pregnancy or lactation, singleton gestation, normal course of pregnancy, body mass index of 18 to 30 kg/m^2 at the start of pregnancy, weight gain of 8 to 12 kg since pregnancy onset, no intake of DHA supplements during pregnancy, term birth, spontaneous vaginal delivery, appropriate weight for gestational age, Apgar index $\geq$7 at 1st and fifth minute of life, normal monitoring results, and breast-feeding of the neonate. The study was approved by the bioethical committee on research involving human subjects at both hospitals. Written informed consent was obtained from each participant after a detailed explanation of the study, and participants were free to withdraw from the study at any time without any consequence for their further care.

Study Design and Intervention

Mothers were randomly assigned to one of the following intervention groups following a computer generated unpredictable sequence. Control group (CT): 400 mL/day (in 2 doses of 200 mL) of the control dairy drink; fish oil (FO) group: 400 mL/day (in 2 doses of 200 mL) of fish oil–enriched dairy drink. Table 1 shows detailed information on the composition of the dairy drinks used during the intervention. With respect to eicosapentaenoic acid (EPA) and DHA contents, 100 mL of the dairy drink supplemented with fish oil provided 18 mg of EPA and 80 mg of DHA (that means supplementation of 392 mg of LC-PUFA per day in the FO group). The dairy products were distributed in identical white packaging without any indication reflecting the type of product that it contains (double blind); therefore, trial investigators and participants were unaware of the treatment allocation. These dairy drinks are not commercial products and were specifically produced for the trial. The dietetic intervention began in the 28th week of pregnancy and finished in the fourth month of lactation.

Mother's Diet

Diet is an important confounder in this kind of trials. To minimize this confounder, the mother's diet was supervised during the intervention period using the following procedure: the mother's nutritional assessment began with a nutritional survey performed by a nutritionist. Maternal dietary intake was assessed using a validated 110-item food frequency questionnaire (FFQ) (19,20), specially designed to assess the n-3 LC-PUFA consumption, together with a 72-hour diet record and information about food and physical activity habits of every mother. Based on this information, the nutritionist developed a recommended diet based on a 1-month system of type menus with exchangeable contents within every group of nutrients. The compliance with the suggested diet was supervised/monitored by the nutritionist after 1 month and after delivery. Present dietary recommendations were highlighted, especially those relating to the weekly fish consumption (2/3 portions per week as daily sources of EPA + DHA) (21,22). From an ethical point of view, it was important to ensure a sufficient DHA intake in women of both groups because it is a recommendation made by several recognized institutions from Europe and the rest of the world (21,22). The analysis of the nutritional intake was performed using Nutriber version 1.1.1 software (Funiber, Barcelona, Spain).

Blood Sampling

Blood samples (5 mL) were obtained from mothers at the moment of the enrollment (28th week of pregnancy), at delivery, at

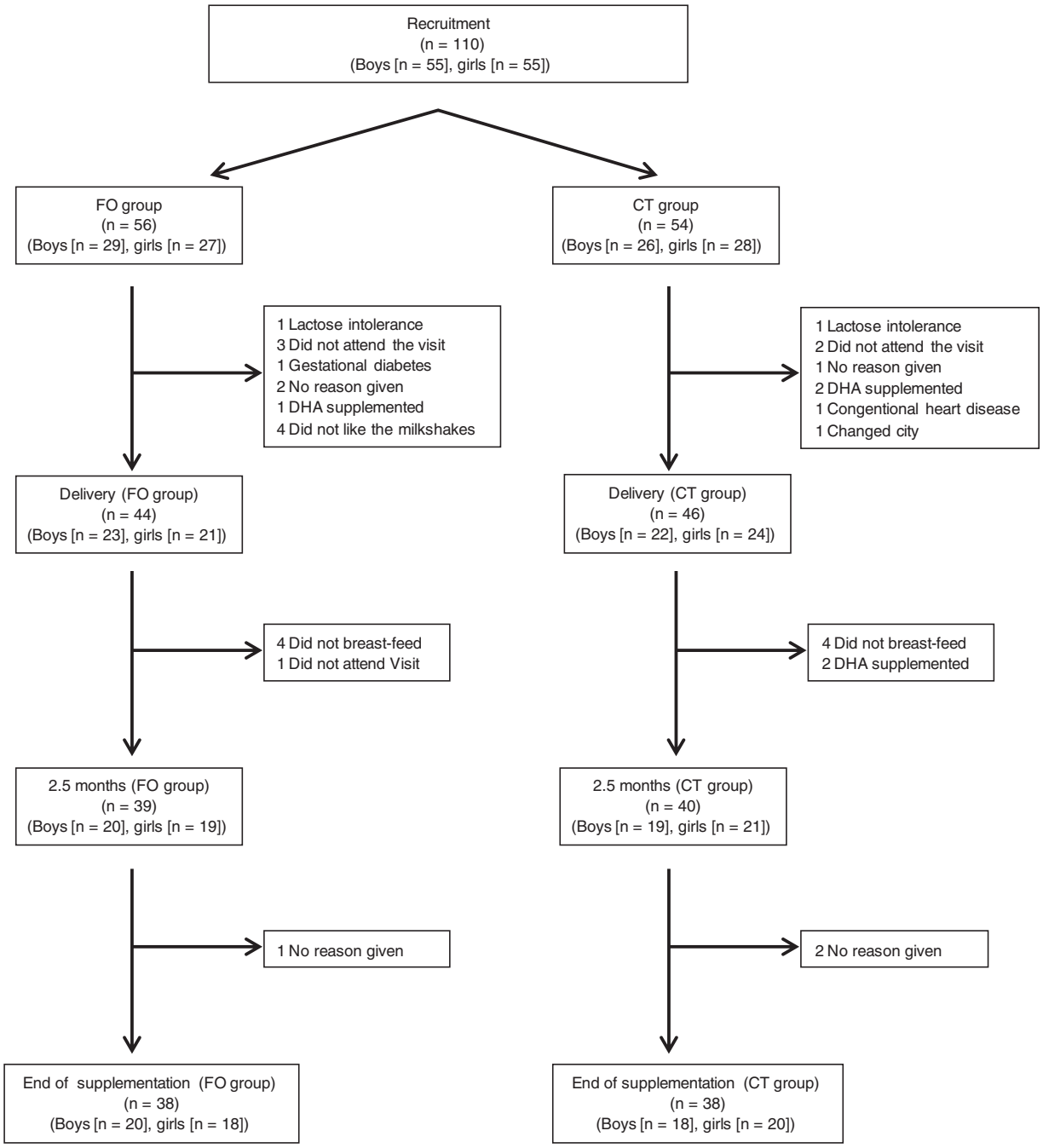

FIGURE 1. Flowchart showing participant progress and dropouts in the study. CT = control group; DHA = docosahexaenoic acid; FO = fish oil.

2.5 months postpartum, and at the end of the dietary intervention (4 months postpartum). Immediately after the delivery, blood samples were collected from the umbilical vein and arteries, and at 2.5 months of life a sample of blood from all of the neonates was obtained. Blood samples were centrifuged at 1750*g* for 10 minutes at 4°C in a Beckman GS-6R refrigerated centrifuge (Beckman, Fullerton, CA) to separate plasma and red blood cells. Erythrocyte cytosolic and membrane fractions were prepared by differential centrifugation according to the method of Hanahan and Ekholm (23). Plasma samples and the final fractions of red blood cells were aliquoted and stored at −80°C until analysis.

Mother's Milk and Placenta Collection

Mothers' milk was obtained after delivery (colostrum) and at the first, second, and fourth month of breast-feeding. A $2 \times 2 \times 2$ cm sample of placental cotyledons (from the central part) was also

TABLE 1. Composition of the dairy drinks used during the study

	Control drink	Fish oil drink
Energy, kcal-kJ/100 mL	58–145	58–145
Protein, g/100 mL	3.7	3.7
Carbohydrates, g/100 mL	6.7	6.7
Fats, g/100 mL	1.8	1.8
Saturated, g/100 mL	0.36	0.32
Monounsaturated, g/100 mL	1.10	1.06
Polyunsaturated, g/100 mL	0.34	0.42
EPA, mg/100 mL	—	18
DHA, mg/100mL	—	80
Folic acid, μg/100 mL	80	80
Vitamin B_{12}, μg/100 mL	0.4	0.4
Vitamin C, mg/100 mL	9.0	9.0
Vitamin D, μg/100 mL	0.75	0.75
Vitamin E, mg/100 mL	1.5	1.5
Calcium, mg/100 mL	160	160
Iron, mg/100mL	2.2	2.2
Iodine, μg/100mL	23.0	23.0

DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.

obtained, excluding placental membranes. Afterwards, placenta samples were subjected to multiple washings to eliminate any remaining blood, with a chilled ice-cold 0.9 NaCl solution with 0.1% butylhydroxytoluene (BHT) and 1 mmol/L EDTA.

FA Analysis in Mother's Milk, Placenta, Plasma, and Erythrocytes

For placenta, lipids were extracted according to a slightly modified Folch procedure (24). Placenta lipids were fractionated using thin layer chromatography with a solvent composed of n-heptane/diisopropyl ether/acetic acid (70:30:2, vol/vol/vol). Bands were detected by spraying the plates with an 0.1% (wt/vol) solution of 2′,7′-dichlorofluorescein in 95% methanol and looking at the dried plate under ultraviolet light. The phospholipid bands were scraped immediately. The fatty acid profile of placental phospholipids, human milk, plasma, and erythrocyte membrane was determined by gas-liquid chromatography as described by Lepage and Roy (25). Samples were precisely weighed in glass tubes and dissolved in 2 mL of methanol/benzene (4:1, vol/vol); 100 mL of the fatty acid 23:0 (0.4 mg/dL) and 9 mmol/L of BHT were added to the samples as internal standard and antioxidant, respectively. The FA methyl esters were separated and quantified on a Hewlett Packard (Santa Clara, CA) GC 5890 gas chromatograph using a flame ionization detector oil capillary column SP 2330 (30 m × 0.25 mm, 0.20 μm) (Supelco Company, Bellefonte, PA). Nitrogen was used as carrier gas under a pressure of 0.5 bars. Individual fatty acids were identified by comparison with a mixture of fatty acid methyl esters (Sigma, St Louis, MO). Values reported are expressed as percentages (%) of total fatty acids determined (C12; C14; C15; C16; C16:1n-9; C18, C18:1 n-9; C18:2 n-6; C18:3 n-3; C18:3 n-6; C20:4 n-6; C24:1 n-9; EPA [C20: 5 n-3], docosapentaenoic acid [C22:5 n-3], DHA).

12-Month Neurodevelopment Assessment

Bayley Scales of Infant Development, second edition (BSID-II) were obtained in 61 healthy infants at 12 months (29 infants from CT group and 32 infants from FO group). The Mental Development Index (MDI) of the BSID-II evaluates several aspects related to cognitive development, and the Psychomotor Development Index (PDI) of the BSID-II evaluates the control of the gross muscle groups including movements associated with standing, walking, running, and jumping, among others. The test was performed by a pediatric neurologist together with a neonatologist, and the same team of professionals attended every baby when 1 year old. The MDI and PDI scores are standardized to a mean of 100 with a standard deviation of 15 (range 50–150) (26).

Visual Evoked Potential

Binocular visual evoked potentials (VEPs) were obtained in 60 healthy infants at 2.5 months (29 from the CT group and 31 from the FO group) and in 53 infants at 7.5 months (28 from the CT group and 25 from the FO group). Two caps of 2 different sizes (38–42 and 42–46 cm, respectively) with electrodes placed according to the 10-20 system (27) were used for both exploration moments (Electro-Cap International, Eaton, OH). The electrodes have been Fz as reference; O1, Oz, and O2 as actives (Oz is on inion, O1 is 3 cm on the left, and O2 is 3 cm on the right); Cz as ground electrode (28).

VEPs were obtained in a quiet room in mesopic conditions while the participants were aware and alert and at the same height of the stimulation screen. Factors such as crying, sleep, cold, heat, and so on were avoided. Two researchers were necessary (to control the computer and to control the fixation of the baby, respectively). If the baby did not keep attention, then the test stopped and only began when attention came back. If somnolence was present, the baby slept while another baby was being assessed.

Signals were registered and filtered by a Dantec Keypoint (NATUS, Pleasanton, CA), a 3-channel amplifier was used (O1-Fz, Oz-Fz, and O2-Fz). The signal was filtered (1–100 Hz) and a 20 to 50 μV per division was used. Notch filter was on, the sweep speed was 500 milliseconds, and the artifact reject was manual. The impedance was below 5 kΩ. The luminance was ~80 to 100 cd/m^2.

At least 30 responses were obtained for each visual angle, beginning with an angle of visual stimulation of 2° continuing with subtending visual angles of 1°, 30, 15, and 7.5 minutes of arc (2°, 1°, 30′, 15′, and 7.5′). Two or more trials at each stimulus were recorded to ensure reliability and reproducibility of the waveform. The latencies were expressed in milliseconds using P1 or P100 as a reference value.

Statistical Analysis

On the basis of our main aim and according to the results previously reported in the scientific literature (9,14,20), to detect a difference in DHA levels in mother's plasma of 2.26, with a standard deviation of 1.43, using a power of 87%, and a significance level of 0.05, we needed 45 mothers per group. This amount is increased by 20%, taking into account possible dropouts, so the final sample size was 55 per group. Before any statistical analysis, all of the variables were checked for normality and homogeneous variance using Kolmogorov-Smirnov and Levene tests, respectively. Categorical variables were compared using χ^2 tests. Differences between group and sex were tested using nonparametric tests (Mann-Whitney *U* test with nonpaired samples and Wilcoxon with paired samples [comparative analysis between time and angle for the same child]), when the DHA responses were not normally distributed, or using *t* test for independent samples and dependent samples when the DHA responses were normally distributed. Finally, the groups were also combined to determine connections between individual fatty acid proportions in several compartments and VEP using Pearson correlation. All of the statistical analyses were performed using SPSS software version 20.0 (IBM SPSS Statistics, Armonk, NY).

TABLE 2. Characteristics of the mothers and their neonates

	CT group	FO group	P
Mothers			
Age, y	29.9 ± 4.7	30.5 ± 4.8	0.409
Weight, kg	73.2 ± 12.3	71.7 ± 11.5	0.265
Length, cm	164.2 ± 6.5	163.2 ± 6.3	0.236
BMI	27.2 ± 4.6	26.9 ± 4.1	0.167
Parity			
Uni, %	68.2	64.4	
Multi, %	31.8	35.6	
Weight gaining	7.0 ± 2.8	7.4 ± 2.8	0.240
SBP, mmHg	110.5 ± 12.3	107.2 ± 8.7	0.085
DBP, mmHg	65.5 ± 8.1	64.3 ± 6.5	0.216
Hemoglobin, g/L	11.7 ± 1.0	11.8 ± 0.8	0.243
Hematocrit, %	34.4 ± 2.5	34.4 ± 2.5	0.493
Glucose, g/dL	101.2 ± 29.2	102.5 ± 34.0	0.423
Cholesterol	257.3 ± 39.2	253.2 ± 41.1	0.319
Triglycerides	169.2 ± 72.5	157.7 ± 42.2	0.182
Neonates			
Gestational age, wk	39.6 ± 1.5	39.3 ± 1.8	0.193
Sex			
Boys, %	51.9	47.9	
Girls, %	48.1	52.1	
Apgar 1	8.6 ± 0.8	8.7 ± 0.7	0.238
Apgar 2	8.9 ± 0.5	9.0 ± 0.4	0.221
Weight, kg	3.2 ± 0.5	3.3 ± 0.5	0.225
Height, cm	50.2 ± 2.7	50.0 ± 2.5	0.436
Cranial perimeter, cm	34.1 ± 1.4	34.0 ± 1.5	0.183
Weight gaining first year	6.6 ± 0.9	6.3 ± 0.9	0.103
Height increase first year	25.3 ± 2.6	24.7 ± 2.4	0.197
Cranial perimeter increase first year	11.92 ± 2.0	12.6 ± 6.3	0.497

Values are mean ± SD. Mothers (CT [n = 54], FO [n = 56]); neonates (CT [n = 46], FO [n = 44]). CT = control group; FO = fish oil; DBP = diastolic blood pressure; SBP = systolic blood pressure.

RESULTS

General Characteristics

As shown in Table 2, no statistically significant differences were found in the age, height, weight, and biochemical parameters of the volunteers participating in the study. Concerning newborns, there were no statistically significant differences in anthropometric parameters between the groups. The dropout percentage was similar in both groups (32% in the FO group and 29% in the CT group) (Fig. 1).

Nutritional Assessment

The analysis of both questionnaires (110-item FFQ and 72-hour diet record) did not show significant differences between the groups (data not shown). No statistical differences were observed in the 3 surveys performed on the mothers during the study.

Fatty Acids in Placenta Phospholipids

Owing to the high number of fatty acids assessed and the lipid index obtained, the tables appearing in the present study show only some of the fatty acids and the most representative indexes.

As shown in Table 3, placental concentrations of oleic acid (C18:1 n-9), linoleic acid (C18:2 n-6), DHA, and total n-3 PUFAs were significantly higher ($P < 0.05$) in the FO group compared with the CT group. No statistically significant differences were observed for any other of the fatty acids analyzed.

TABLE 3. Fatty acid composition in phospholipids of placenta

	CT group	FO group	P
C18:1n-9	7.49 ± 0.72*	7.95 ± 0.92	0.016
C18:2n-6	7.38 ± 0.97*	7.98 ± 1.25	0.023
C18:3n-3	0.019 ± 0.01	0.022 ± 0.01	0.091
C24:1 n-9	2.03 ± 0.51	1.92 ± 0.50	0.136
EPA	1.07 ± 0.34	1.08 ± 0.26	0.423
DHA	4.32 ± 0.78*	4.66 ± 0.76	0.037
SFA	49.03 ± 4.17	48.61 ± 3.50	0.355
MUFA	11.74 ± 1.16	12.02 ± 1.09	0.211
n-6 PUFA	33.63 ± 3.63	33.39 ± 3.09	0.381
n-3 PUFA	5.49 ± 0.97*	5.87 ± 0.80	0.035

Results are expressed as percentages (%) of total fatty acids determined. Values are mean ± SD. CT (n = 46); FO (n = 44). CT = control group; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; FO = fish oil; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acids.

* Statistically significant differences between groups ($P < 0.05$).

FA Composition in Mother's Plasma and Erythrocyte

The most relevant fatty acids are shown in Table 4, whereas the rest of the fatty acids, because of the high number of data, appear as supplementary material (*http://links.lww.com/MPG/A485*). There were no statistically significant differences between groups in the lipid profile either in plasma or in erythrocytes at the recruitment stage of the study. In contrast, at delivery, plasmatic concentrations of oleic acid, nervonic acid, DHA, and total n-3 PUFA were significantly higher in the FO group. Similar differences were observed in the fatty acid profile of erythrocyte membranes. In addition, the percentage of nervonic acid was also significantly higher in the FO group at delivery and at 4 months.

FA Composition in Neonate's Plasma and Erythrocyte Membranes

The most relevant fatty acids are shown in Table 4, whereas the rest of the fatty acids, because of the high number of data supplied, appear as supplementary material (*http://links.lww.com/MPG/A486*). DHA and total n-3 PUFA concentrations in cord plasma and in cord erythrocytes were significantly higher in the FO group than in the CT group both in plasma and erythrocyte. In addition, the nervonic acid percentage in cord artery plasma was also significantly higher in the FO group, although in cord erythrocytes the difference was not statistically significant.

At 2.5 months of age, the most relevant difference was again the higher plasmatic and erythrocyte percentage of DHA and total n-3 PUFA in the FO group compared with the CT group ($P < 0.05$). The percentage of nervonic acid was also significantly higher in plasma and erythrocytes from neonates of the FO group compared with those of the CT group.

Fatty Acid Composition in Mother's Milk

As with the previous lipid profiles, the most relevant fatty acids are shown in Table 5, and the rest of them are reported as supplementary material (*http://links.lww.com/MPG/A487*). The percentages of EPA and DHA were significantly higher in the

TABLE 4. Fatty acid compositions in mothers' and neonates' plasma and erythrocyte membranes

	Plasma			Erythrocyte		
	CT group	FO group	*P*	CT group	FO group	*P*
Mother						
M0						
C24:1 n-9	2.25 ± 0.91	2.72 ± 2.44	0.100	7.72 ± 1.77	7.62 ± 1.41	0.257
EPA	0.50 ± 0.33	0.45 ± 0.28	0.193	1.22 ± 1.07	1.42 ± 1.02	0.309
DHA	3.57 ± 1.52	3.64 ± 1.29	0.408	4.48 ± 1.28	4.67 ± 0.77	0.148
n-3 PUFA	5.00 ± 1.84	5.08 ± 1.59	0.414	6.76 ± 2.26	7.02 ± 1.58	0.249
M1						
C24:1 n-9	1.04 ± 0.43*	1.54 ± 1.07	0.016	6.33 ± 1.53*	7.02 ± 1.51	0.040
EPA	0.51 ± 0.48	0.55 ± 0.34	0.358	0.26 ± 0.11	0.51 ± 0.55	0.057
DHA	2.43 ± 0.93*	3.43 ± 1.44	0.002	2.74 ± 2.09*	3.75 ± 1.98	0.038
n-3 PUFA	3.78 ± 1.58*	4.80 ± 1.94	0.020	3.41 ± 2.42*	4.87 ± 2.71	0.022
M2						
C24:1 n-9	2.04 ± 1.23	2.03 ± 1.15	0.488	4.22 ± 0.72	4.24 ± 0.58	0.447
EPA	1.00 ± 0.70*	1.37 ± 0.59	0.020	0.48 ± 0.34*	0.87 ± 0.40	0.001
DHA	2.63 ± 1.56*	3.71 ± 1.56	0.006	3.98 ± 1.25*	5.04 ± 1.14	0.001
n-3 PUFA	4.32 ± 2.31*	5.88 ± 1.79	0.002	5.79 ± 1.73*	7.52 ± 1.73	0.001
M3						
C24:1 n-9	2.39 ± 1.19	2.35 ± 1.24	0.459	4.82 ± 1.35*	5.68 ± 1.74	0.025
EPA	0.72 ± 0.32*	1.05 ± 0.67	0.016	0.54 ± 0.35*	0.82 ± 0.43	0.008
DHA	2.83 ± 1.42*	3.69 ± 1.43	0.017	4.04 ± 1.41*	5.14 ± 1.29	0.002
n-3 PUFA	4.61 ± 1.98*	5.66 ± 2.08	0.034	6.01 ± 2.05*	7.32 ± 2.83	0.028
Neonate						
NOV						
C24:1 n-9	2.43 ± 0.91	2.70 ± 1.28	0.070	5.74 ± 1.55	5.60 ± 1.38	0.361
EPA	0.19 ± 0.01	0.45 ± 0.18	0.040	0.08 ± 0.06*	0.13 ± 0.07	0.005
DHA	4.58 ± 1.51*	5.35 ± 1.46	0.035	4.34 ± 2.42*	5.85 ± 2.20	0.008
n-3 PUFA	4.89 ± 1.29*	5.80 ± 1.74	0.019	5.22 ± 2.58*	6.91 ± 2.71	0.008
NOA						
C24:1 n-9	1.83 ± 0.49*	2.15 ± 0.25	0.041	6.05 ± 0.84	6.07 ± 0.84	0.472
EPA	0.26 ± 0.15	0.40 ± 0.09	0.073	0.07 ± 0.03*	0.12 ± 0.08	0.033
DHA	3.42 ± 0.86*	4.22 ± 1.10	0.042	3.91 ± 2.19*	5.48 ± 2.84	0.024
n-3 PUFA	3.78 ± 1.33*	4.97 ± 1.31	0.024	4.80 ± 2.51*	7.88 ± 3.97	0.002
N1						
C24:1 n-9	1.85 ± 1.16*	2.56 ± 1.60	0.043	1.94 ± 0.71*	2.29 ± 0.64	0.041
EPA	0.28 ± 0.18*	0.49 ± 0.28	0.021	0.23 ± 0.15	0.45 ± 0.48	0.093
DHA	2.69 ± 1.40*	3.69 ± 1.43	0.006	3.67 ± 1.71*	5.18 ± 2.19	0.000
n-3 PUFA	3.70 ± 1.70*	4.80 ± 1.70	0.012	4.75 ± 2.34*	7.16 ± 2.58	0.001

Results are expressed as percentages (%) of total fatty acids determined. Values are mean ± SD. M0: at recruitment; M1: at delivery; M2: at 2.5 months postpartum; M3: at 4 months postpartum; NOV: cord blood from vein; NOA: cord blood from artery; N1: at 2.5 months of life. CT = control group; DH = docosahexaenoic acid; EPA = eicosapentaenoic acid; FO = fish oil; PUFA = polyunsaturated fatty acid.

* Statistically significant differences between groups ($P < 0.05$).

milk of the mothers belonging to the FO group throughout the study. Total n-3 PUFA were also significantly higher in mature milk in the FO group but not in colostrum, in which the difference was not statistically significant. The percentage of nervonic acid was also significantly higher in colostrum but not in mature milk (except for the sample obtained at 2 months).

12-Month Neurodevelopment Assessment

There were no statistically significant differences between the CT and FO groups in the 12-month Bayley score for MDI (102.1 ± 12.2 in the CT group vs 99.9 ± 17.5 in the FO group) or PDI (96.2 ± 15.3 in the CT group vs 94.6 ± 17.5 in the FO group). All of the children were in the range of normal values for the age of 12 months.

VEP in Neonates at 2.5 and 7.5 Months

Figure 2 shows latencies at 2.5 (A) and 7.5 (B) months of age. There were no statistically significant differences between groups at any of the angles analyzed either at 2.5 months or at 7.5 months. In contrast, when only boys were considered, latency at 7.5 months was significantly shorter in the FO group compared with the CT group in the lowest visual angle (7.5′) (156.6 ± 27.4 milliseconds in boys of the CT group vs 132.5 ± 16.0 milliseconds in boys of the FO group, $P < 0.05$). In addition, when we consider only the FO group, latency was significantly shorter in boys compared with girls in the angles 15′ and 7.5′ at 7.5 months (123.2 ± 10.8 milliseconds in boys vs 146.1 ± 34.8 milliseconds in girls at 15′, $P < 0.05$; 132.5 ± 16.0 milliseconds in boys vs 158.2 ± 38.2 milliseconds in girls at 7.5′, $P < 0.05$).

TABLE 5. Fatty acid composition in mother's milk

	CT group	FO group	*P*
Colostrum			
C24:1 n-9	0.85 ± 0.38*	1.08 ± 0.52	0.030
EPA	0.12 ± 0.05*	0.22 ± 0.13	0.018
DHA	0.67 ± 0.29*	1.03 ± 0.38	0.000
n-3 PUFA	1.58 ± 0.61	2.12 ± 0.84	0.003
1 month's milk			
C24:1 n-9	0.15 ± 0.07	0.14 ± 0.05	0.272
EPA	0.12 ± 0.09*	0.18 ± 0.09	0.025
DHA	0.41 ± 0.17*	0.67 ± 0.26	0.000
n-3 PUFA	1.29 ± 0.47*	1.62 ± 0.58	0.017
2 months' milk			
C24:1 n-9	0.15 ± 0.07*	0.12 ± 0.08	0.009
EPA	0.11 ± 0.06*	0.18 ± 0.09	0.004
DHA	0.37 ± 0.19*	0.80 ± 0.33	0.000
n-3 PUFA	1.17 ± 0.32*	1.79 ± 0.61	0.000
4 months' milk			
C24:1 n-9	0.09 ± 0.05	0.09 ± 0.03	0.365
EPA	0.08 ± 0.04*	0.17 ± 0.11	0.006
DHA	0.32 ± 0.15*	0.65 ± 0.37	0.002
n-3 PUFA	1.23 ± 0.41*	1.72 ± 0.55	0.004
n-3 PUFA	1.23 ± 0.41*	1.72 ± 0.55	

Results are expressed as percentages (%) of total fatty acids determined. Values are mean ± SD. Colostrum (CT [n = 46], FO [n = 44]); 1 month's milk (CT [n = 46], FO [n = 44]); 2 months' milk (CT [n = 40], FO [n = 39]); 4 months' milk (CT [n = 38], FO [n = 38]). CT = control group; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; FO = fish oil; PUFA = polyunsaturated fatty acid.

* Statistically significant differences between groups ($P < 0.05$).

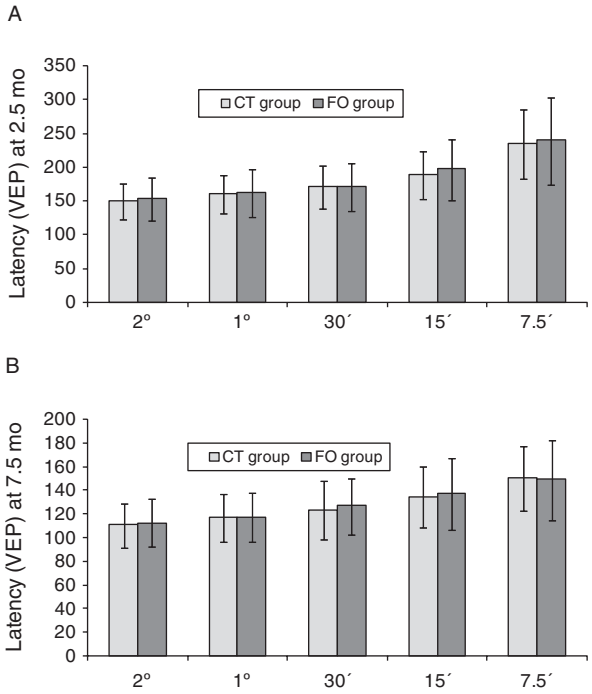

FIGURE 2. Latency (VEP) in neonates at 2.5 months (A) and 7.5 months (B) of age. Values are mean ± SD. Statistical significances: (*) means statistically significant differences between groups ($P < 0.05$). CT = control group; FO = fish oil; VEP = visual evoked potential.

Correlations Between Fatty Acids and VEP Values

We found a negative correlation between mothers' nervonic acid concentration at delivery and VEP latency at 2.5 m regardless of the stimulus angles analyzed ($r = -0.522$, $r = -0.582$, $r = -0.580$, $r = -0.419$, and $r = -0.450$ for 2°, 1°, 30′, 15′, and 7.5′, respectively). In contrast, we did not find any other correlation between fatty acid profile of the different samples analyzed and VEP values.

DISCUSSION

Maternal and Infant Fatty Acid Status

The main finding of the present study was that the daily intake of a dairy drink enriched with ω-3 LC-PUFA (mainly EPA and DHA) during pregnancy and lactation affected both maternal and infant DHA status. In the present study, supplementation began during pregnancy and continued up to the end of lactation. A recent review has reported that only 12% of the intervention studies of the impact of ω-3 LC-PUFA on mother/infant health are focused both on pregnancy and lactation (10). Thus, most studies analyze the effect of supplementation either during pregnancy or during lactation. The World Health Organization/Food and Agriculture Organization clearly states that both, pregnant and lactating women, however, have higher ω-3 LC-PUFA needs, especially for DHA (22).

It has been widely reported that maternal dietary and circulating DHA is an important determinant of fetal blood DHA concentrations (29,30). To our knowledge, this is the first trial to demonstrate that maternal supplementation through a fish oil–enriched drink during pregnancy and lactation does not only affect the FA profiles of the mother and the newborn but also of the cord blood, the placenta, and breast milk. In a previous study, a daily dose of 500 mg of DHA in the form of a milk-based supplement from the 20th week of gestation increased DHA concentrations in maternal plasma and erythrocyte at 30th week and at delivery (14,31). These authors, however, did not report DHA concentrations in human milk or in other tissues.

It has been suggested that the mother's DHA uptake increases during the last trimester of gestation and lactation (2,33). Thus, DHA concentrations have been reported to be higher in the umbilical cord vein than in the mother, suggesting preferential transfer of FA at the end of gestation (33). Our results are in agreement with these findings because they show a decrease in DHA concentrations in maternal plasma at delivery and at 6 months postpartum. In the FO group, DHA concentrations in maternal plasma and erythrocyte were not significantly different between the 28th week of gestation and delivery, thus suggesting that an FO-enriched dairy drink could be useful in maintaining maternal DHA stores during gestation.

The results obtained also show a significant increase in DHA concentrations in breast milk (analyzed at any time) in the FO group as compared with the CT group. Higher DHA concentrations in human milk could be responsible for the higher plasma and erythrocyte DHA concentrations found in 2.5-month-old infants. This is based on the fact that human milk was the only dietary source of this fatty acid, and the endogenous synthesis of DHA has been described to be low in humans, especially in infants (34). The DHA increase in breast milk after ω-3 LC-PUFA supplementation during pregnancy has also been previously reported (35), although its impact on infant status is more unknown. These results suggest that ω-3 LC-PUFA supplementation during pregnancy and lactation

is useful in improving the FA profile both in the mother and the newborn.

Another interesting finding of this study is that the placental lipid profile changed after the intervention. Other authors have reported a correlation between alterations in the lipid profile of the placenta and functional alterations in this tissue. Thus, low placental DHA and ω-3 FA levels were more frequent in women suffering from preeclampsia as compared with normotensive women (18). In addition, the transfer of fatty acids across the placenta is of great importance because their synthesis in the fetus is limited. DHA supplementation has shown to have an impact on fatty acid transport through the placenta (21); thus, analyzing the fatty acid profile of the placenta in this type of intervention studies is crucial. Our results show that ω-3 LC-PUFA supplementation through an FO-enriched dairy drink led to an increase in placental DHA and total ω-3 LC-PUFA concentrations. Larqué et al (33) have also demonstrated that the intake of a fish oil supplement (650 mg/day of EPA + DHA) from the 22nd week of gestation increases DHA concentrations in placental phospholipids.

Another important aspect to be considered is the interaction between different FAs. In our case, we emphasize the interaction between ω-3 LC-PUFA and nervonic acid because of its importance for the neuronal development of the newborn (18). Nervonic acid has been associated with brain maturation, and there is a concordance between concentrations of this fatty acid in red cells and brain sphingomyelin (36). In our study, we observed an increase in nervonic acid levels (24:1 n-9) in maternal plasma at delivery and also in cord plasma in the FO group. To our knowledge, this is the first study to report an increase in nervonic acid levels in the maternal and infant erythrocytes and in human milk after ω-3 LC-PUFA supplementation. Little is known about the interaction between ω-3 LC-PUFA and nervonic acid. In a recent report, nervonic acid levels were positively correlated with the total PUFA intake in an adult male cohort (37). More studies are needed to elucidate whether this finding has a rationale or it is only a coincidence.

Pregnancy Outcomes

Several studies have shown that maternal intake of ω-3 LC-PUFA during pregnancy has an impact on some birth outcomes, such as duration of gestation and infant's weight (20). In the present study, no differences were found in pregnancy outcomes between groups regarding the newborn's weight, craneal perimeter, or perinatal development. Our results are in agreement with those reported by a systematic review (8), although the small sample size of this trial limits its statistical power, and conclusions cannot be drawn in this regard.

Visual Acuity

Infant visual acuity was analyzed by VEPs. Our study did not show any effects of maternal supplementation with ω-3 LC-PUFA on infant visual acuity because differences in latency values between the CT and the FO groups were not statistically significant. Two systematic reviews have been recently published about the effects of maternal intake of ω-3 LC-PUFA (8,9). From these 2 reviews, including a total of 28 RCTs, it can be concluded that ω-3 LC-PUFA supplementation during pregnancy and/or lactation has no clear effect on infant visual function. In this aspect, our results are limited by the number of infants evaluated (n = 60 at 2.5 months and n = 53 at 7.5 months).

Nevertheless, we analyzed boys and girls separately because a correlation has been reported between sex and visual acuity (4). Differences have also been found in brain development between boys and girls (38,39). Preterm boys have lower levels of the growth factor insulin-like growth factor-1 than girls (34), the lack of which has been associated with retinopathy and low visual acuity (34). It has been reported that girls have a higher rate of endogenous synthesis of DHA from the precursor α-linolenic acid (C18:3 n-3) (40), which could limit the efficacy of ω-3 LC-PUFA supplementation. Our study shows some differences in the effect of ω-3 LC-PUFA supplementation on boys and girls. In the FO group, boys had a better visual acuity than girls, and in addition ω-3 LC-PUFA supplementation was more effective in boys than in girls. These results are not in agreement with those published by Innis and Friesen (4), who reported a higher visual acuity in girls than in boys and a positive effect of DHA only on girls. These discrepancies could be because of the different study designs, ω-3 LC-PUFA doses, control on the mother's diet, or duration of supplementation. Given that visual acuity was not the main outcome of the present study and the study previously mentioned, sufficiently powered studies should be conducted to examine sex differences in the effect of ω-3 LC-PUFA supplementation on visual acuity.

Neurodevelopment Outcomes

BSID-II results showed that all children were in the range of normal values defined for the age of 12 months. Omega-3 LC-PUFA supplementation had no neurodevelopment effects in the present study. In a recent systematic review, it was concluded that ω-3 LC-PUFA supplementation during pregnancy and/or lactation has no clear and consistent benefits on the neurodevelopment of term infants (10). Only 3 out of 27 of the studies, however, included in that review used a methodology similar to that used in the present study; in fact, one of these studies (41) showed a higher PDI at 30 months of age after DHA supplementation (200 mg/day) during pregnancy and lactation. In our study, the number of infants who were evaluated with BSID-II was very limited (n = 61) as compared with the study mentioned above (n = 160). In addition, neurodevelopment was not the main outcome of the present study, and conclusions cannot be drawn from the results obtained. Furthermore, it has been suggested that the benefits of DHA supplementation during pregnancy and/or lactation on infant visual and cognitive development are greater in case of DHA deficiency (42).

CONCLUSIONS

Maternal supplementation with ω-3 LC-PUFA through a fish oil–enriched dairy drink affected maternal and infant FA profile but has not a clear effect on infants' visual function or neurodevelopment. Our results confirm the previous finding that supplementation increases ω-3 LC-PUFA concentrations. A larger population study should be studied to fully examine the effects of ω-3 LC-PUFA supplementation on the visual and cognitive/psychomotor development of infants. Further studies are needed to elucidate the influence of sex on the efficacy of ω-3 LC-PUFA supplementation and the role of other fatty acids such as nervonic acid on infant visual and cognitive development.

REFERENCES

1. Boyd EM. The lipemia of pregnancy. *J Clin Invest* 1934;13:347–63.
2. Al MD, Van Houwelingen AC, Kester AD, et al. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. *Br J Nutr* 1995;74:55–68.
3. Salem N, Litman B, Kim H-Y, et al. Mechanisms of action of docosahexaenoic acid in the nervous system. *Lipids* 2001;36:945–59.
4. Innis SM, Friesen RW. Essential n-3 fatty acids in pregnant women and early visual acuity maturation in term infants. *Am J Clin Nutr* 2008;87:548–57.

5. Schuchardt JP, Huss M, Stauss-Grabo M, et al. Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children. *Eur J Pediatr* 2010;169:149–64.
6. Smithers LG, Gibson RB, Makrides M. Maternal supplementation with docosahexaenoic acid during pregnancy does not affect early visual development in the infant: a randomized controlled trial. *Am J Clin Nutr* 2011;93:1293–9.
7. Jacobson JL, Jacobson SW, Muckle G, et al. Beneficial effects of a polyunsaturated fatty acid on infant development: evidence from the Inuit of Artic Quebec. *J Pediatr* 2008;152:356–64.
8. Larqué E, Gil-Sánchez A, Prieto-Sanchez MT, et al. Omega 3 fatty acids, gestation and pregnancy outcomes. *Br J Nutr* 2012;107:S77–84.
9. Campoy C, Escolano-Margarit MV, Anjos T, et al. Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. *Br J Nutr* 2012;107:S85–106.
10. Hibbeln JR, Davis JM, Steer C, et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in child hood (ALSPAC study): an observational cohort study. *Lancet* 2007;369:578–85.
11. Rogers LK, Valentine CJ, Keim SA. DHA supplementation: current implications in pregnancy and childhood. *Pharmacol Res* 2013;70:13–9.
12. Helland IB, Smith L, Saarem K, et al. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics* 2003;111:39–44.
13. Strain JJ, Davidson PW, Bonham MP, et al. Associations of maternal long-chain polyunsaturated fatty acids, methyl mercury, and infant development in the Seychelles Child Development Nutrition Study. *Neurotoxicology* 2008;29:776–82.
14. Krauss-Etschmann S, Shadid R, Campoy C, et al. 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. *Am J Clin Nutr* 2007;85:1392–400.
15. Courville AB, Keplinger MR, Judge MP, et al. Plasma or red blood cell phospholipids can be used to assess docosahexaenoic acid status in women during pregnancy. *Nutr Res* 2009;29:151–5.
16. Baylin A, Campos H. The use of fatty acid biomarkers to reflect dietary intake. *Curr Opin Lipidol* 2006;17:22–7.
17. Laasonen M, Erkkilä AT, Isotalo E, et al. Serum lipid fatty acids and temporal processing acuity in children with oral clefts. *Prostaglandins Leukot Essent Fatty Acids* 2006;74:263–70.
18. Amminger GP, Schäfer MR, Klier CM, et al. Decreased nervonic acid levels in erythrocyte membranes predict psychosis in help-seeking ultra-high-risk individuals. *Mol Psychiatry* 2012;17:1150–2.
19. Parra-Cabrera S, Stein AD, Wang M, et al. Dietary intakes of polyunsaturated fatty acids among pregnant Mexican women. *Matern Child Nutr* 2011;7:140–7.
20. Miles EA, Noakes PS, Kremmyda LS, et al. The Salmon in Pregnancy Study: study design, subject characteristics, maternal fish and marine n-3 fatty acid intake, and marine n-3 fatty acid status in maternal and umbilical cord blood. *Am J Clin Nutr* 2011;94:1986S–92S.
21. EFSA Panel on Dietetic Products, Nutrition, and Allergies. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA J* 2010;8:1461.
22. Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition. *Fat and Fatty Acids in Human Nutrition: Report of an Expert Consultation.* Rome, Italy: Food and Agriculture Organization; 2010.
23. Hanahan DJ, Ekholm JE. The preparation of red cell ghosts (membranes). *Methods Enzymol* 1974;31:168–72.
24. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957; 226:497–509.
25. Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. *J Lipid Res* 1986;27:114–20.
26. Wolraich ML, Dworkin PH, Drotar DD. *Developmental-Behavioral Pediatrics: Evidence and Practice.* Philadelphia, PA: Mosby Elsevier; 2008.
27. Guideline thirteen: guidelines for standard electrode position nomenclature. American Electroencephalographic Society. *J Clin Neurophysiol* 1994;11:111–3.
28. Harding GF, Odom JV, Spileers W, et al. Standard for visual evoked potentials 1995. The International Society for Clinical Electrophysiology of Vision. *Vision Res* 1996;36:3567–72.
29. Elias SL, Innis SM. Infant plasma trans, n-6 and n-3 fatty acids and conjugated linoleic acids are related to maternal plasma fatty acids, length of gestation and birthweight and length. *Am J Clin Nutr* 2001;73:807–14.
30. Innis SM. Essential fatty acid transfer and fetal development. *Placenta* 2005;26:S70–5.
31. Campoy C, Escolano-Margarit MV, Ramos R, et al. Effects of prenatal fish-oil and 5-methyltetrahydrofolate supplementation on cognitive development of children at 6.5 y of age. *Am J Clin Nutr* 2011;94 (6 suppl):1880S–8.
32. Deleted in proof.
33. Larqué E, Krauss-Etschmann S, Campoy C, et al. Docosahexaenoic acid supply in pregnancy affects placental expression of fatty acid transport proteins. *Am J Clin Nutr* 2006;84:853–61.
34. Löfqvist C, Engström E, Sigurdsson J, et al. Postnatal head growth deficit among premature infants parallels retinopathy of prematurity and insulin-like growth factor-1 deficit. *Pediatrics* 2006;117:1930–8.
35. Helland IB, Saugstad OD, Saarem K, et al. Supplementation of n-3 fatty acids during pregnancy and lactation reduces maternal plasma lipid levels and provides DHA to the infants. *J Matern Fetal Neonatal Med* 2006;19:397–406.
36. Babin F, Sarda P, Limasset B, et al. Nervonic acid in red blood cell sphingomyelin in premature infants: an index of myelin maturation. *Lipids* 1993;28:627–30.
37. Takkunen M, Agren J, Kuusisto J, et al. Dietary fat in relation to erythrocyte fatty acid composition in men. *Lipids* 2013;48:1093–102.
38. Hintz SR, Kendrick DE, Vohr BR, et al. Gender differences in neurodevelopmental outcomes among extremely preterm, extremely-low-birthweight infants. *Acta Paediatr* 2006;95:1239–48.
39. Marlow N, Hennessy EM, Bracewell MA, et al. Motor and executive function at 6 years of age after extremely preterm birth. *Pediatrics* 2007;120:793–804.
40. Burdge GC, Calder PC. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev* 2005;45:581–97.
41. Jensen CL, Voigt RG, Prager TC, et al. Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. *Am J Clin Nutr* 2005;82:125–32.
42. Makrides M. DHA supplementation during the perinatal period and neurodevelopment: do some babies benefit more than others? *Prostaglandins Leukot Essent Fatty Acids* 2013;88:87–90.