Maternal Fish Oil Supplementation in Lactation: Effect on Visual Acuity and n-3 Fatty Acid Content of Infant Erythrocytes

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ABSTRACT: Studies on formula-fed infants indicate a beneficial effect of dietary DHA on visual acuity. Cross-sectional studies have shown an association between breast-milk DHA levels and visual acuity in breast-fed infants. The objective in this study was to evaluate the biochemical and functional effects of fish oil (FO) supplements in lactating mothers. In this double-blinded randomized trial, Danish mothers with habitual fish intake below the 50th percentile of the Danish National Birth Cohort were randomized to microencapsulated FO [1.3 g/d long-chain n-3 FA (n-3 LCPUFA)] or olive oil (OO). The intervention started within a week after delivery and lasted 4 mon. Mothers with habitual high fish intake and their infants were included as a reference group. Ninety-seven infants completed the trial (44 OO-group, 53 FO-group) and 47 reference infants were followed up. The primary outcome measures were: DHA content of milk samples (0, 2, and 4 mon postnatal) and of infant red blood cell (RBC) membranes (4 mon postnatal), and infant visual acuity (measured by swept visual evoked potential at 2 and 4 mon of age). FO supplementation gave rise to a threefold increase in the DHA content of the 4-mon milk samples ($P < 0.001$). DHA in infant RBC reflected milk contents ($r = 0.564$, $P < 0.001$) and was increased by almost 50% ($P < 0.001$). Infant visual acuity was not significantly different in the randomized groups but was positively associated at 4 mon with infant RBC-DHA ($P = 0.004$, multiple regression). We concluded that maternal FO supplementation during lactation did not enhance visual acuity of the infants who completed the intervention. However, the results showed that infants with higher RBC levels of n-3 LCPUFA had a better visual acuity at 4 mon of age, suggesting that n-3 LCPUFA may influence visual maturation.

Paper no. L9338 in *Lipids 39,* 195–206 (March 2004).

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Abbreviations BMI, body mass index; DNBC, Danish National Birth Cohort; FA%, percentage of total FA; FFQ, Food Frequency Questionnaire; FO, fish oil; HF-group, reference group, the members of which consumed quantities of fish above the 74th percentile; LCPUFA, long-chain PUFA; logMAR, the logarithm of the minimal angle of resolution; MUFA, monounsaturated FA; OO, olive oil; RBC, red blood cells; SFA, saturated FA; SWEEP-VEP, swept visual evoked potential; VEP, visual evoked potential.

Membranes of the brain and retina contain uniquely high levels of long-chain PUFA (LCPUFA), especially DHA (22:6n-3). Formula-fed infants, who do not receive exogenously preformed LCPUFA, have lower levels of DHA in the membranes of the central nervous system (1,2). A meta-analysis has shown an approximately three-point higher IQ in breast-fed infants compared to formula-fed infants (3). Furthermore, studies on formula-fed infants have indicated beneficial effects of dietary DHA on visual acuity (4–6).

The DHA level of human milk varies by more than a factor of 10 among Danish mothers (7). This variation is caused primarily by differences in maternal fish intake. Intake of fish or marine oils has an acute effect on the DHA content of breast milk (8,9). Fish oil (FO) supplementation of the lactating mother will effectively increase breast-milk DHA levels (10–13).

At present there is insufficient scientific evidence to decide whether the variation in DHA level in breast milk has functional implications for the breast-fed infant. In small cross-sectional studies, associations between milk or blood levels of DHA and visual acuity or cognitive abilities in breast-fed infants have been observed (7,14). However, supplementing lactating mothers with n-3 LCPUFA has, so far, been shown to have no or only limited functional effects on infant visual acuity and mental development (15–17).

The primary aim of the present study was to examine whether FO supplementation of lactating mothers confers an advantage in the acuity performance of breast-fed infants above that provided by the habitual maternal diet. The secondary aim was to investigate how maternal FO supplementation influences the n-3 LCPUFA content of breast milk and how this in turn affects FA composition in infant erythrocytes (RBC). The relationship between infant RBC n-3 LCPUFA and visual acuity was also characterized. The study was designed as a double-blind intervention study, randomizing mothers with habitual fish intakes at levels less than values for the 50th percentile of the Danish National Birth Cohort (DNBC) to microencapsulated FO (1.5 g/d of n-3 LCPUFA) or olive oil (OO), starting in the first week after delivery and

lasting for 4 mon. A reference group of mothers with a high habitual fish intake and their infants was also included.

MATERIALS AND METHODS

Participants. The study protocol was approved by the Scientific-Ethical Committees for Copenhagen and Frederiksberg (KF 01-300/98), and all participants gave written consent to participate after the study had been explained to them.

Participants were selected from among women recruited for the ongoing DNBC (18). Women were recruited for the DNBC at the first antenatal visit to the general practitioner. If they consented to participate, they were interviewed by telephone twice during pregnancy. In the 25th week of gestation they were mailed a comprehensive self-administered, semiquantitative 300-item Food Frequency Questionnaire (FFQ), which questioned them about their diet for the 4 wk prior to completion of the questionnaire. An earlier version of the questionnaire has been validated (19). About 75% of the participants returned the questionnaire. The FFQ contained questions about fish intake for lunch (in Denmark as topping on bread) and dinner. Using assumptions of portion sizes and the nutrient content in foods (determined from the Danish Food tables by The Danish Food Agency, which contained comprehensive information on LCPUFA), we estimated consumption of the average daily intake of n-3 LCPUFA in grams using the program FoodCalc (www.foodcalc.dk). This provided the basis for a sampling of women with an expected low and high habitual consumption of n-3 LCPUFA.

During the December 1998 to November 1999 period, 11,179 women, countrywide, were recruited for DNBC. Only those pregnant Danish women living in the greater Copenhagen area who had a fish intake below the 50th percentile of the DNBC population (<0.4 g n-3 LCPUFA/d; the distribution in the DNBC was positively skewed, the 25th percentile being 0.3 g/d) were eligible to enter the present intervention trial. Women with a fish intake above the 74th percentile (>0.8 n-3 LCPUFA/d) were recruited for the reference group (HF-group). The average fish intake in the two subgroups of women eligible for the present study (<0.4 and >0.8 g n-3 LCPUFA/d) were 12.3 ± 8.2 and 55.2 ± 26.7 g/d, respectively. Over a period of 9 mon, 1473 women were invited in nine rounds (919 and 554 with fish intake <50th percentile and >74th percentile, respectively). Of these, 273 women responded to the invitation, of which 211 fit the other inclusion criteria and were recruited in their 8th month of gestation (147 and 64 with fish intake <50th and >74th percentile, respectively). Based on the previously observed association between milk DHA levels and infant visual acuity (7), the expected change in milk DHA in this intervention should result in a difference in visual acuity at 4 mon of 0.07 times the logarithm of the minimal angle of resolution (logMAR) (equivalent to 0.75 SD). We calculated that we needed 40 infants in each group in order to detect a difference of 0.75 SD in visual acuity (power = 80%, level of significance = 0.05). To allow for exclusions of infants not meeting our inclusion criteria and for dropouts of participants, our aim was to recruit 60 infants for each of the intervention groups.

The other inclusion criteria were that the recruited women had to have an uncomplicated pregnancy, prepregnancy body mass index (BMI) < 30 kg/m^2, and an absence of metabolic disorders. In addition, the participants were included in the study only if they, at the time of recruiting, had the intention to breast-feed for at least 4 mon. The newborns had to be healthy (no admission to a neonatal department), term (37–43 wks of gestation), singleton infants with normal weight for gestation (20) and have an Apgar score >7 at 5 min after delivery. Furthermore, we required that they be started on the supplements within 2 wks after birth. None of the recruited women took any types of oil supplements besides the ones given in the study. The participants were not given any dietary instructions. One hundred twenty-two and 53 women in the low- and high-fish-intake groups, respectively, fulfilled these criteria (see flow diagram of trial in Fig. 1 and characteristics of the participants in Table 1).

After birth, the women with fish intakes below the 50th percentile were randomly assigned to a supplementation group by a randomization schedule prepared by a person uninvolved in the study. Random block-wise allocation to the supplement groups was applied in blocks of two in five strata according to mean parental education (grouped in five categories according to the Official Danish Classification of Educations from 1994). Sixty-two and 60 women were allocated to the experimental group (FO group) and the control group (OO group), respectively. Investigators and families were blinded to the randomization until all data had been analyzed.

Supplements. For the first 4 mon of lactation, the experimental group was given 17 g/d of deodorized microencapsulated FO powder, containing 4.5 g of FO and 1.5 g of n-3 LCPUFA. The supplement dose aimed at making the total n-3 LCPUFA intake in the experimental group equivalent to the habitual intake of the women in the population with the highest fish intake (above the 90th percentile). The control group was given a similar amount of microencapsulated OO. To supply individual n-3 LCPUFA in relative amounts similar to the average Danish fish consumption, we used a 1:2 mixture of two different oils: a standard FO and a tuna oil with a low content of EPA (20:5n-3) (Dry n-3™ 18:12 and Dry n-3™ 5:25, respectively, from BASF Health and Nutrition A/S, Ballerup, Denmark) (see Table 2). The supplied FO and OO were microencapsulated and added to müsli bars (produced by Halo Foods Ltd., Tywyn Gwynedd, Wales, United Kingdom). The participants were instructed to consume two 35-g müsli bars daily, which supplied altogether 285 kcal and the intended doses oil. During the study period we ran out of bars and had to give the supplements in homemade cookies or as capsules; these alternatives were also offered if any of the women disliked the müsli bars. The homemade cookies were made at the department by kitchen personnel not otherwise involved in the study and contained the exact same amounts of microencapsulated oils as the müsli bars. Thus, with respect to formulation as well as blinding of mothers and investigators,

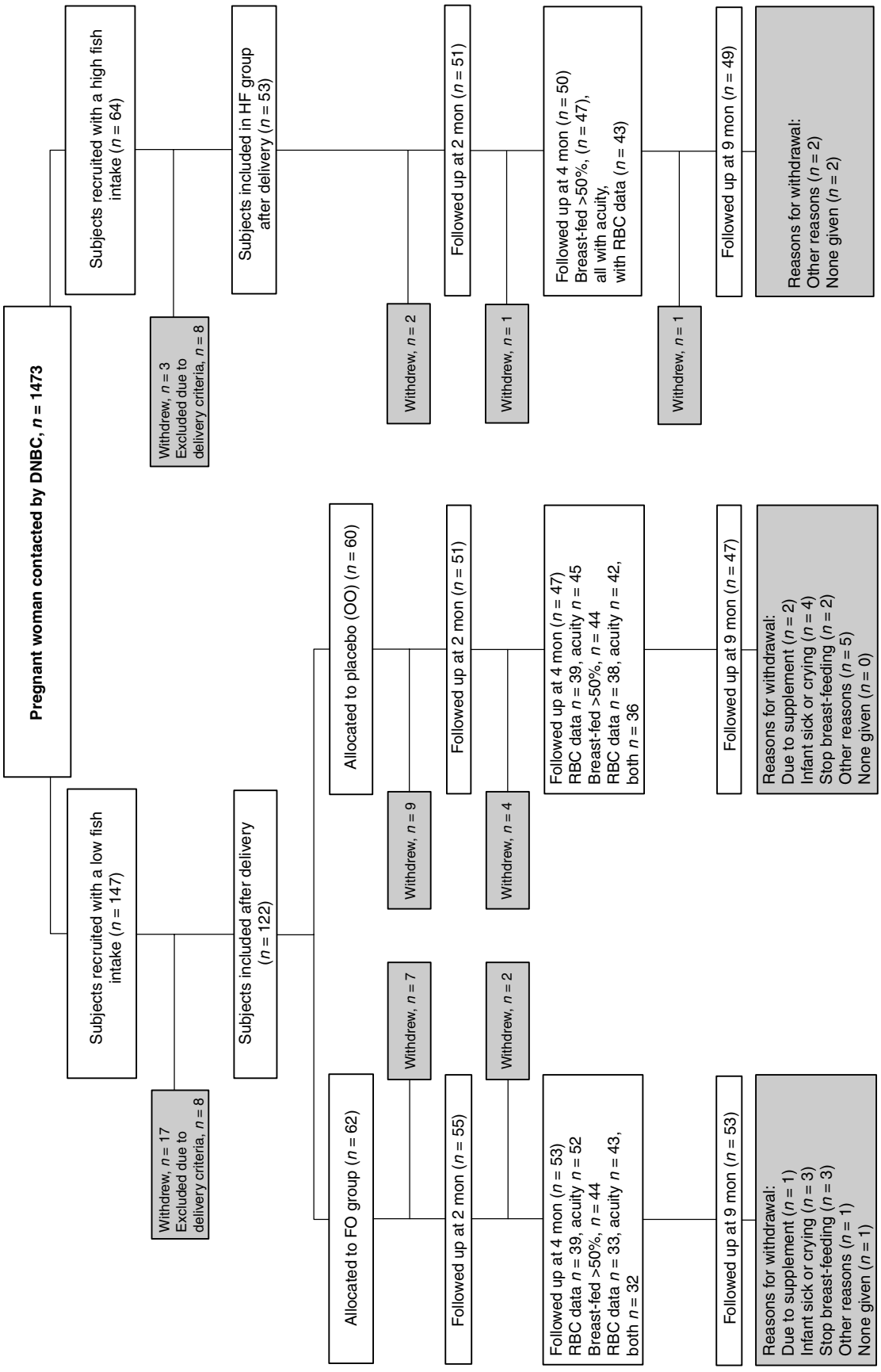

FIG 1. Trial profile summarizing participant flow, numbers of randomization assignments, interventions, and follow-up examinations for all groups. DNBC, Danish National Birth Cohort; FO, fish oil; OO; olive oil; HF group, group eating high amounts of fish; RBC, red blood cell.

TABLE 1
Characteristics of Study Infants and Parents[a]

	Olive oil supplement	Fish oil supplement	High fish
Included subjects (n)	60	62	53
Gender (*n*, male/female)	28:32	37:25	26:27
Cesarian deliveries (%)	10.0	9.7	5.7
Gestational age (wk)	40.1 ± 1.2	40.1 ± 1.1	40.2 ± 1.2
Birth weight (kg)	3.56 ± 0.41	3.60 ± 0.45	3.65 ± 0.44
Birth length (cm)[b]	52 ± 2	52 ± 2	53 ± 2
Head circumference at 1 wk (cm)	35.7 ± 1.5	36.1 ± 1.3	36.2 ± 1.6
Apgar score at 5 min[b]	9.9 ± 0.5	9.9 ± 0.3	10.0 ± 0.1
Siblings (*n*)[b]	0.6 ± 0.8	0.5 ± 0.7	0.7 ± 0.7
Maternal age (yr)	30.2 ± 4.1	29.6 ± 4.3[a]	31.9 ± 4.1
Maternal height (m)[b]	1.69 ± 0.06	1.67 ± 0.05[b,d]	1.71 ± 0.06
Pregravida BMI (kg/m^2)[b]	22.5 ± 2.7	22.5 ± 2.8	22.4 ± 3.0
Weight increase during pregnancy (kg)[c]	13.7 ± 5.1	13.4 ± 5.0	13.2 ± 4.0
Maternal smokers (%)	6.7	11.3	15.1
Maternal education score[b,d]	5.4 ± 1.2	5.3 ± 1.2	5.4 ± 1.3
Paternal height (m)[b]	1.80 ± 0.08[b]	1.82 ± 0.08	1.84 ± 0.06
Paternal smokers (%)	21.7	32.2	35.8
Paternal education score[b,d]	5.3 ± 1.3	5.3 ± 1.2	5.3 ± 1.6
Habitual n-6 PUFA intake (g/d)[b]	9.0 ± 3.3[c]	9.1 ± 3.6[c]	11.2 ± 3.8
Habitual n-3 LCPUFA intake (g/d)[b]	0.3 ± 0.3[c]	0.3 ± 0.3[c]	1.1 ± 0.6
Subjects completing 4 mon (*n*)	47	53	50
n-3 LCPUFA intake in lactation (g/d)[b]	0.3 ± 0.2[c]	1.5 ± 0.3[c,f]	0.9 ± 0.4[f]
Compliance (% taken of intended dose)	87 ± 9	88 ± 9	—
Exclusively breast-fed at 4 mon (%)	74.5	62.3	78.0
Estimated breast-milk intake during the 4 mon (% of total intake)[b,e]	93 ± 19	86 ± 28	93 ± 20
Infants breast-fed <50% (%)	6.4	17.0	6.0

[a]Data given as mean ± SD.
[b]Statistical comparison by nonparametric tests (Kruskal–Wallis and Mann–Whitney U-test). Nominal data tested with χ^2-test and all other data (not indicated by [b]) are compared by ANOVA and Bonferroni post hoc test. Superscript letters indicate the level of significance for statistical comparisons with the high fish group: a, $P < 0.05$; b, $P < 0.01$; or c, $P < 0.001$, or the OO-group: d, $P < 0.05$; e, $P < 0.01$; or f, $P < 0.001$.
[c]Gestational week 36.4 ± 1.54.
[d]Educational scores according to the Official Danish Classification of Educations from 1994. Highest rank (7) is equivalent to >18 yr of education, (6) 17–18 yr, (5) 15–16 yr, (4) 13–14 yr, (3) 11–12 yrs, (2) 10 yr, and (1) <10 yr of education.
[e]Estimated breast-milk intake as a percentage of total dietary intake assessed by their intake of infant formula and solid foods. BMI, body mass index; LCPUFA, long-chain PUFA; OO, olive oil.

cookies were similar to bars. For an approximately similar supplementation with capsules, the control group was given four 1000-mg OO capsules, and the experimental group was given six 500-mg low-EPA FO capsules plus one 1000-mg standard FO capsule (all capsules were a gift from Lupe/ProNova Biocare, Lysaker, Norway). Owing to the nonidentical appearance of the capsules for the two groups, a person who was not otherwise involved in the project handled the capsules in order to avoid breaking the blinding of the investigators. The distribution of müsli bars, cookies, and capsules among participants in the FO and OO groups was identical. Only 10% of the women got their entire supplementation as capsules, whereas 60% of the women only had müsli bars or cookies. The lower oil content in the capsules was taken into account in the calculation of overall supplement compliance. The overall self-reported compliance in both groups was on average 88% of the allocated number of müsli bars (SD = 9%, $n = 99$) during the entire 4 mon supplementation period.

TABLE 2
FA Composition[a] of Microencapsulated Oils Used for the Intervention

	Olive oil	Fish oil
Total SFA	13.6	30.2
Total MUFA	70.9	21.4
18:2n-6	7.4	1.3
20:4n-6	—	1.7
Total n-6 PUFA	7.4	4.0
18:3n-3	0.6	0.5
20:5n-3	—	10.0
22:5n-3	—	1.7
22:6n-3	—	22.8
Total n-3 PUFA	0.6	38.3

[a]FA values are based on GC analysis and are expressed as a percentage of total FA. SFA, saturated FA; MUFA, monounsaturated FA.

Protocol. At the pre-enrollment visit (in week 36.4 ± 1.5 of gestation, 4.0 ± 1.9 wk before birth, $n = 175$), demographic and social information was collected, including parental

education and a 10-mL blood sample for baseline analysis of the RBC FA composition. Within a week following the birth, the parents forwarded to us information about the delivery. Shortly thereafter, we visited the mothers in their homes [(9 ± 3 d after birth (n = 175)], gave them the supplements for the first two months of the intervention period (supplements for the last two months were dispensed at the 2-mon visit), measured the head circumference of the infant, and collected a breast-milk sample for baseline analysis of the milk FA composition.

Mothers and infants were assessed at 2 and 4 mon of age at the Research Department of Human Nutrition. At each assessment the infant was weighed and its length was measured. The mother delivered a breast-milk sample and was interviewed about her fish intake the previous day and for the past month. Fish intake during the intervention period was assessed with a fish frequency questionnaire similar to that used in the DNBC, and intake of n-3 LCPUFA was estimated in the same way as in the DNBC. The visual acuity of the infant was measured by swept visual evoked potential (SWEEP-VEP).

One hundred infant-mother pairs completed the first 4 mon of the intervention trial, and 50 mother-infant pairs from the HF-group were followed up at 4 mon (see Fig. 1). One hundred seven mothers complied with the criterion for exclusive breast-feeding for 4 mon. However, mothers who did not fulfill this criterion were not excluded from the trial or the analysis. Complementary food was introduced in the diet of five of the infants between 3 and 4 mon of age (mean age for complementary food introduction in the entire group of infants was 4.8 ± 1.0 mon). For infants not exclusively breast-fed at the end of the intervention, we estimated to what extent breast-milk covered their energy needs from the amount of formula and complementary food ingested. Thus, for 16 infants, breast-milk covered >90% of the intake, 75–90% for 9 infants, 50–75% for 3 infants, and 15 infants were estimated to be breast-fed <50%. Most of the infants who were breast-fed less than 50% during the 4-mon period were from the FO-group (nine vs. three from both the OO- and HF-group). The degree of breast-feeding was taken into account in the analysis of the outcome. The FA composition of RBC in infants breast-fed <50% is given separately, and visual acuity was analyzed as both intention-to-treat and for the mainly breast-fed alone. At the time of the study there were no LCPUFA-containing infant formulas on the Danish market, and the three most-used formulas had an n-6/n-3 FA ratio of approximately 10.

The mothers were asked to collect milk samples immediately after nursing their baby during the afternoon on the day before the visit at 1 wk and 2 and 4 mon. Milk samples (2–5 mL) were stored in the home at 5°C until they were collected no later than 30 h after expression [previous results had shown that this does not affect the FA composition of the breast-milk as compared to that in immediately frozen samples (Lauritzen, L., unpublished data)]. To 2-mL aliquots of the milk samples were then added 2 drops of 0.01% BHT from Sigma (St. Louis, MO), and aliquots were frozen at –80°C. All milk samples were analyzed within 1 yr after they had been taken.

From 4-mon-old infants, we collected a 500-μL blood sample by heel-prick, and from their mothers a 10-mL blood sample by venipuncture. All blood samples were collected in ice-cold EDTA-conditioned tubes. Immediately after sampling, RBC were separated from plasma and leukocytes and washed thrice in physiological saline. The isolated packed RBC were reconstituted 1:1 in physiological saline with 1 mM EDTA and 0.005% BHT and kept at –80°C until they were analyzed (maximum storage time was 8 mon).

FA analysis. Lipids from 1-mL aliquots of the milk samples were extracted according to Bligh and Dyer (21). Samples of 150 and 500 μL, respectively, of infant and maternal reconstituted RBC were hemolyzed in redistilled water, and the lipids were extracted by the Folch procedure (22). The extracted RBC lipids were methylated with BF_3 in methanolic NaOH, and milk lipids were methylated with KOH in methanol (23). The resulting FAME were extracted with heptane.

FAME from milk as well as from RBC were separated by G-LC on an HP-6890 Series II chromatograph (Hewlett-Packard Inc., Waldbronn, Germany) equipped with an FID and SP2380 capillary columns (30 and 60 m, respectively; i.d. 0.32 and 0.25 mm, respectively; and film thickness 0.2 μm; Supelco Inc., Bellefonte, PA). The milk FAME were injected using split mode (1:49) at 250°C. Initially, the oven temperature was set to 80°C for 3 min and then increased in three steps—to 110°C at 30°C/min, to 208°C at 3°C/min, and to 240°C at 50°C/min—and finally held at 240°C for 10 min before cool-down and injection of a new sample (total run time 46 min). Helium was used as carrier gas at a constant flow of 2 mL/min (pressure 10.7 psi, velocity 35 cm/s). All peaks from lauric acid (12:0) to DHA, except that of BHT, were integrated. RBC FAME-injections were run in split mode with a split ratio of 1:11. Injector and detector temperatures were 270°C. Initial oven temperature was 70°C for 0.5 min, and temperature programming was as follows: 15°C/min to 160°C, 1.5°C/min to 200°C, which was maintained for 15 min followed by a rate of 30°C/min to 225°C, which was maintained for 10 min. The carrier gas was helium at a constant flow of 1.2 mL/min (pressure was 25.0 psi).

The FAME peaks of the resulting chromatograms were tentatively identified from retention times of commercial standards (Nu-Chek-Prep Inc., Elysian, MN) as previously described (8). More than 97 and 99% of the chromatogram areas were identified in the milk- and RBC-FAME analyses, respectively. The FA composition of all samples was determined in duplicate, and in series of 8–18 samples. Each series contained a blank and a reference sample. The whole series was rejected if major FA in the reference sample deviated by more than 2 SD from the previously established mean values. The individual sample was reanalyzed if the relative difference (difference/mean) of major FA in the duplicates was appreciably increased relative to the typical deviation for that particular FA. The interassay variation (CV%) for DHA in milk and RBC was around 5%. The relative amounts of identified FA are given as a percentage of the overall identified FAME area (FA%).

Blood samples were obtained from 147 infants. Eighteen of the infant RBC samples were partly coagulated and thus excluded. An additional eight infant RBC samples had a higher relative content of saturated FA (SFA) and monounsaturated FA (MUFA) and a lower content of PUFA (n-6 and n-3 FA) than in the coagulated samples. All these samples deviated in SFA, MUFA, and PUFA content with >2 SD from the mean of all uncoagulated samples, being on average 58 ± 3, 27 ± 1, and 15 ± 4 (13 ± 3 n-6 PUFA and 1.9 ± 0.9 n-3 PUFA) FA%, respectively. These samples were also excluded, leaving 121 (81%) infants with successful determinations of RBC FA composition. Unfortunately, infants with excluded, coagulated, and missing RBC samples were not balanced between the groups, and the RBC FA analyses resulted in fewer successful determinations in the FO-group (74 vs. 83 and 86% in the OO-group and HF-group, respectively).

SWEEP-VEP visual acuity determination. Binocular visual acuity was assessed by SWEEP-VEP using the NuDiva system (24) equipped with an M2400 high-resolution monochrome monitor (Dotronix, Eau Claire, WI). Infants sitting on their parent's lap were presented with vertical sine-wave gratings at 80% contrast at a mean luminance of 47.6 cd. The gratings were contrast-reversed at a rate of 6.0 Hz, and the spatial frequency of the gratings was increased in 10 linear steps during the 10-s trial. Viewing distance and range of spatial frequencies depended on the age of the subject (for two 1-mon-olds it was 70 cm and 1.70–0.47 times the logMAR; for 4-mon-olds, 100 cm and 1.48–0.27 logMAR) (25). The infant's attention was attracted to the screen by small toys or bells, and trials were interrupted if the infant's gaze moved off the stimuli. Visual evoked potentials (VEP) were recorded with gold EEG electrodes attached to the scalp at five recording points (26). The EEG was amplified (gain 10.000–20.000) and Fourier-transformed to isolate the VEP. Visual acuity was estimated by extrapolating the VEP amplitude at 12 Hz vs. spatial frequency to zero amplitude (27). The signals from the individual trials and averages for each of the five channels were scored automatically by the NuDiva system (27) and checked manually for errors by one trained observer.

We aimed at five trials per session (more if the first five trials did not give 10 successful extrapolations), but in a few cases it was not possible to reach these predefined goals while the infant was attentive. The interassay variation of the SWEEP-VEP assessment of infant visual acuity is 23% (25). Visual acuity is given as the average of all obtained thresholds expressed as logMAR (the lower the logMAR, the better the acuity).

Statistical analysis. Nominal data were compared by χ^2-test for homogeneity. For other types of data, statistical group comparisons were performed with one-way ANOVA and a Bonferroni *post hoc* test or linear regression analysis and Pearson's correlation unless otherwise stated. Alternatively, nonparametric statistics (Kruskall–Wallis test, Mann–Whitney U-test, and Kendall's τ correlation) were applied if data did not agree with a Gaussian distribution (tested by the Kolmogorov–Smirnov test) with equal variances (Levene's test) or if data were in ordinal scale. Parametric statistical methods were used for results on visual acuity and RBC FA composition, whereas nonparametric tests had to be applied for most of the milk FA. All multiple linear regression analyses (milk-DHA vs. maternal n-3 LCPUFA intake, infant RBC-DHA vs. milk-DHA or maternal n-3 LCPUFA intake, and visual acuity vs. infant RBC-DHA or maternal n-3 LCPUFA intake) were performed as parametric analyses with initial inclusion of the explanatory factors maternal BMI, smoking, parity, and infant gestational age. If not significant, these factors were subsequently dropped from the model.

All statistical analyses were performed by SPSS (version 10.0, SPSS Inc., Chicago, IL). Quantitative results from separate groups are generally summarized as a mean ± SD.

RESULTS

The self-reported compliance in the two randomized groups was comparable, as were most of the subject characteristics (Table 1). The intervention resulted in an increase in the estimated total intake of n-3 LCPUFA in the FO-group from 0.3 to 1.5 g/d, equivalent to the highest habitual intakes in the population. Fish intake (expressed as intake of n-3 LCPUFA) in the OO-group remained low during lactation.

FA composition of breast milk. The DHA concentration of a milk sample taken 1 wk after delivery before the start of the intervention was associated with the estimated habitual n-3 LCPUFA intake (from fish) of the mother (r = 0.314, $P < 0.001$, n = 171). The DHA content of milk from women with high fish intake was on average 1.4 times as high as in the milk of those with the lower fish consumption (Table 3).

The FO intervention increased the relative content of DHA in the breast-milk at 2 and 4 mon of lactation (Table 3). The contents of all other n-3 LCPUFA also were increased in the milk of the FO-group, and the overall ratio of n-6 to n-3 FA was approximately 30% lower than that in the OO-group. The content of n-3 FA in the FO-group was similar regardless of supplement form (bars, cookies, or capsules; data not shown). The relative content of individual n-6 FA, including arachidonic acid, in the breast milk from the FO-supplemented women was not significantly lower than in the OO-group. The relatively higher content of n-3 LCPUFA corresponded to a relatively lower content of MUFA, primarily oleic acid. Overall, the differences in the milk FA composition between the FO- and OO-groups reflected the differences in FA composition of the supplements (Table 2). Mead acid (20:3n-9) was not detected in most of the milk, but very low levels (<0.05 FA%) were observed in 2% of the samples. Docosapentaenoic acid of the n-6 family (22:5n-6) was found in approximately half of the samples (47%) with a mean levels of 0.05 ± 0.05 FA%, more often in the FO-group than in the OO-group (data not shown).

The DHA concentration of a breast-milk sample after 4 mon of lactation also was associated with the estimated maternal intake of n-3 LCPUFA from the diet and the

TABLE 3
FA Composition[a] of Breast Milk from Mothers with a High Fish Intake and from Mothers with a Low Fish Intake, Who Were Supplemented with Olive Oil (OO) or Fish Oil (FO) During the First 4 mon of Lactation

	1 wk			2 mon			4 mon		
	OO	FO	High fish	OO	FO	High fish	OO	FO	High fish
n	57	60	52	51	50	48	45	46	47
Total SFA	42.74 ± 4.25	42.50 ± 4.64	43.35 ± 3.13	42.50 ± 4.22	44.80 ± 5.31^{d}	43.17 ± 3.74	42.71 ± 4.13	43.62 ± 4.61	42.51 ± 3.93
Total MUFA	42.58 ± 3.20	42.32 ± 3.14	41.60 ± 3.42	42.51 ± 3.45	39.15 ± 3.91^{a,f}	41.17 ± 3.06	42.38 ± 3.47	39.88 ± 3.96^{b,e}	42.29 ± 3.12
18:2n-6	9.74 ± 1.46	10.04 ± 1.98	9.73 ± 1.55	10.18 ± 1.59	10.06 ± 2.06	10.16 ± 1.42	11.09 ± 2.51	11.26 ± 2.36	10.67 ± 1.89
20:2n-6	0.40 ± 0.10	0.44 ± 0.10^{b,d}	0.39 ± 0.09	0.23 ± 0.04	0.23 ± 0.05	0.21 ± 0.06	0.22 ± 0.05	0.23 ± 0.04	0.22 ± 0.04
20:3n-6	0.46 ± 0.11	0.48 ± 0.14	0.47 ± 0.12	0.40 ± 0.06	0.38 ± 0.08	0.42 ± 0.08	0.33 ± 0.06	0.30 ± 0.07	0.33 ± 0.07
20:4n-6	0.74 ± 0.13	0.76 ± 0.16^{a}	0.70 ± 0.13	0.43 ± 0.09^{a}	0.44 ± 0.09^{a}	0.51 ± 0.20	0.48 ± 0.10	0.51 ± 0.09	0.50 ± 0.07
22:4n-6	0.18 ± 0.08	0.20 ± 0.08	0.17 ± 0.07	0.08 ± 0.07^{a}	0.12 ± 0.09^{e}	0.12 ± 0.09	0.08 ± 0.04	0.08 ± 0.03	0.07 ± 0.04
Total n-6 PUFA	11.71 ± 1.53	12.12 ± 2.10	11.67 ± 1.61	11.39 ± 1.63	11.31 ± 2.14	11.50 ± 1.51	12.36 ± 2.55	12.59 ± 2.46	11.99 ± 1.96
18:3n-3	1.11 ± 0.35	1.09 ± 0.38	1.19 ± 0.35	1.31 ± 0.33	1.29 ± 0.35	1.37 ± 0.35	1.25 ± 0.43^{b}	1.31 ± 0.47	1.50 ± 0.52
20:5n-3	0.17 ± 0.07^{c}	0.17 ± 0.05^{c}	0.23 ± 0.09	0.08 ± 0.08^{c}	0.28 ± 0.13^{c,f}	0.16 ± 0.12	0.13 ± 0.07^{c}	0.30 ± 0.14^{b,f}	0.22 ± 0.09
22:5n-3	0.25 ± 0.10^{c}	0.25 ± 0.08^{b}	0.31 ± 0.09	0.17 ± 0.10^{b}	0.29 ± 0.11^{b,f}	0.23 ± 0.11	0.22 ± 0.06^{c}	0.29 ± 0.10^{f}	0.28 ± 0.08
22:6n-3	0.67 ± 0.28^{c}	0.66 ± 0.23^{c}	0.91 ± 0.31	0.30 ± 0.36^{c}	1.16 ± 0.45^{c,f}	0.60 ± 0.30	0.41 ± 0.20^{c}	1.34 ± 0.67^{c,f}	0.74 ± 0.33
Total n-3 PUFA	2.21 ± 0.55^{c}	2.18 ± 0.51^{c}	2.64 ± 0.60	1.99 ± 0.53^{b}	3.16 ± 0.81^{c,f}	2.47 ± 0.76	2.00 ± 0.56^{c}	3.24 ± 0.95^{b,f}	2.74 ± 0.67
n-6/n-3	5.52 ± 1.21^{c}	5.74 ± 1.22^{c}	4.58 ± 0.99	6.06 ± 1.57^{c}	3.78 ± 1.12^{c,f}	5.00 ± 1.30	6.51 ± 1.77^{c}	4.22 ± 1.80^{a,f}	4.64 ± 1.43

[a]Data are given as mean ± SD. Individual FA are given as a percentage of total FA. Superscript letters indicate the level of significance for statistical comparisons with the high fish group: a, $P < 0.05$; b, $P < 0.01$; or c, $P < 0.001$, and the OO-group; d, $P < 0.05$; e, $P < 0.01$; or f, $P < 0.001$. For other abbreviations see Table 2.

FO-supplement (Kendall's τ; $r = 0.556$, $P < 0.001$, $n = 138$). The n-3 LCPUFA intake from the FO supplement, the estimated mean fish intake during lactation, and the estimated intake of fish within 24 h of milk sampling (all in g/d) were the only factors that were significantly associated with the DHA concentration in the milk sample (dependent variable log-transformed to approximate a Gaussian distribution, all with *P*-values of, at the most, 0.001). Maternal BMI, smoking, parity, and infant gestational age also were included in the analysis, but all were excluded in an automated backward multiple regression analysis, leaving only the estimated n-3 LCPUFA intake factors as significant determinants [with regression coefficients (*b*) of 0.38 ± 0.03, 0.25 ± 0.04, and 0.09 ± 0.02 per gram of n-3 LCPUFA from the FO supplement, habitual fish intake, and acute fish intake, respectively]. The model that included only these three significant factors explained 59% of the overall variance. In the FO-group, maternal BMI had a significant negative effect on the DHA level in milk after control for compliance (data not shown).

Acute influence from dietary n-3 LCPUFA accounted for some of the difference in the DHA content of milk at 2 and 4 mon of lactation between the FO- and HF-groups (Table 3). All milk samples in the FO-group were acutely affected by dietary n-3 LCPUFA as a result of the daily supplements. The DHA content of milk in the HF-group was 1.2 ± 0.4 FA% ($n = 6$) if the mother had eaten >1 g n-3 LCPUFA within 24 h before milk sampling.

FA composition of infant RBC. The FO-intervention exerted a pronounced effect on the FA composition of RBC of the mainly breast-fed infants at 4 mon (Table 4), and the levels of all n-3 FA were similar in all FO-supplement subgroups. The relative content of DHA in RBC membranes in the FO-group compared with the control group equaled an increase of almost 50%. In the FO-group, RBC-DHA and total n-3 PUFA were also significantly higher than in the HF-group. The ratio of n-6 to n-3 PUFA in RBC from infants in the FO-group was lower than that in the OO-groups, not only because of a high content of n-3 PUFA but also because of a low relative content of arachidonic acid and other n-6 LCPUFA. The differences in RBC FA composition between the FO- and OO-groups did not reflect the differences in supplement FA compositions to the same extent as the milk. The n-3 FA content in RBC of infants with an estimated low degree of breast-feeding (<50% of total intake) was significantly lower than that of the other infants, and the level of 20:3n-9 and the 22:5n-6/22:6n-3 and n-6/n-3 PUFA ratios were significantly higher (data not shown). The difference is probably attenuated by the fact that the majority of these infants were from the FO-group (6 out of 7). RBC levels of 20:3n-9 in infants in the FO-group were significantly higher than those in the OO-group when infants breast-fed <50% were excluded from the analysis.

Maternal RBC-DHA was 8.9 ± 1.3 FA% in the FO-group compared with 5.5 ± 1.0 FA% in the control group (t = –14.656, $P < 0.001$, $n = 98$). There was a strong association between maternal and infant RBC-DHA levels at 4 mon given that the degree of breast-feeding was >50% ($r_{(0,0)} = 0.961 \pm 1.550$, $P < 0.001$, $n = 113$). The DHA content of infant RBC was also

TABLE 4
FA Composition of Infant Erythrocytes at 4 mon of Age in the FO- and OO-Supplemented Groups and the Reference Group[a] (high fish)

	OO	FO	High fish
n	39	39	43
Total SFA	42.07 ± 2.79	41.99 ± 2.03	43.02 ± 3.03
Total MUFA	17.67 ± 2.18	17.16 ± 2.15	17.33 ± 1.85
Total PUFA	39.54 ± 4.76	40.19 ± 3.46	38.93 ± 4.59
20:3n-9	0.07 ± 0.06	0.05 ± 0.06	0.06 ± 0.06
18:2n-6	8.55 ± 0.85	8.43 ± 1.10	8.11 ± 0.89
20:3n-6	1.92 ± 0.36	1.73 ± 0.32	1.81 ± 0.33
20:4n-6	16.18 ± 2.38	14.86 ± 1.67[d]	15.40 ± 2.20
22:4n-6	2.92 ± 0.59[c]	2.30 ± 0.45[f]	2.45 ± 0.53
22:5n-6	0.60 ± 0.19[c]	0.52 ± 0.10[a,d]	0.42 ± 0.14
Total n-6 PUFA	30.49 ± 3.01[b]	28.17 ± 2.16[e]	28.51 ± 3.07
20:5n-3	0.54 ± 0.24[b]	1.21 ± 0.58[b,f]	0.88 ± 0.37
22:5n-3	2.06 ± 0.49	1.96 ± 0.36	2.04 ± 0.40
22:6n-3	6.29 ± 1.71[a]	8.72 ± 2.40[b,f]	7.33 ± 1.55
Total n-3 PUFA	8.98 ± 2.31[a]	11.97 ± 3.03[a,f]	10.36 ± 2.14
n-6/n-3	3.64 ± 1.02[b]	2.65 ± 1.31[f]	2.86 ± 0.58
22:5n-6/22:6n-3	0.10 ± 0.04[b]	0.07 ± 0.06[c,f]	0.06 ± 0.02

[a]Individual FA are given as a percentage of total FA in erythrocyte membranes. Data are given as mean ± SD. Superscript letters indicate the level of significance for statistical comparisons with the high fish group: a, $P < 0.05$; b, $P < 0.01$; or c, $P < 0.001$, or the OO-group: d, $P < 0.05$; e, $P < 0.01$; or f, $P < 0.001$.

associated with the DHA content of breast milk (Kendall's τ, $r = 0.416$, $P < 0.001$, $n = 115$), as well as with the estimated maternal n-3 LCPUFA intake during lactation (Kendall's τ, $r = 0.550$, $P < 0.001$, $n = 138$). The association between breast-milk DHA and DHA in infant RBC seemed to follow a saturation curve. Linear regression analysis between the log-transformed DHA concentration of the milk and infant RBC-DHA showed that infant DHA intake as assessed by a single milk sample alone explained approximately 30% of the variation in infant RBC-DHA (Fig. 2). However, more variation was accounted for in a multiple regression analysis with the DHA level of infant RBC vs. the two factors that contribute to the maternal n-3 LCPUFA intake during lactation, that from fish ($b = 1.64 \pm 0.33$ per g/d, $P < 0.001$), and that from the FO-supplement ($b = 2.99 \pm 0.33$ in FO relative to OO, $P < 0.001$), and the extent to which it was transferred to the infant *via* breast milk expressed as the degree of breast-feeding ($b = 0.06 \pm 0.01$ per % increase in estimated breast milk intake, $P < 0.001$). These factors explained 46% of the variation.

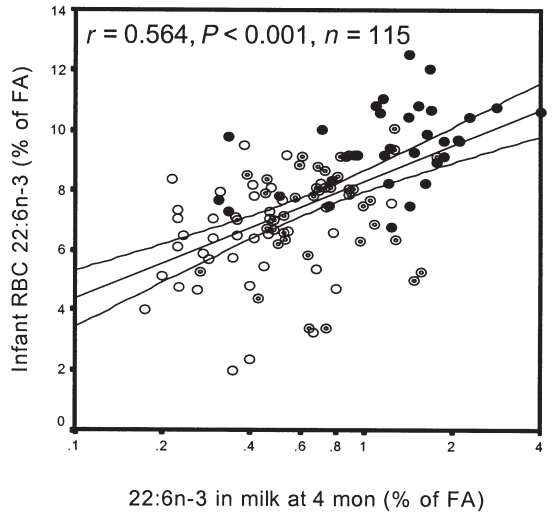

FIG. 2. Association between DHA in infant RBC and a breast-milk sample at 4 mon of age. Symbols indicate whether the mother was supplemented with fish oil (●) or olive oil (○) during the 4 mon of lactation or whether she had a habitual high intake of fish (⊙). The linear regression line is drawn in the plot with 95% confidence intervals. All data were included in the figure. For abbreviation see Figure 1.

Visual acuity of the infants. SWEEP-VEP acuity was determined successfully in 85 and 98% of the 2- and 4-mon-old infants, respectively. Infants with successful SWEEP-VEP acuity determinations were given an average of 5.9 ± 1.9 and 4.8 ± 1.4 trials at the 2- and 4-mon assessment, respectively, which resulted in an average of 12.4 ± 6.8 and 18.1 ± 6.6 scores per session ($n = 133$ and 147), respectively. There was no effect of FO supplementation on visual acuity of the infants at either 2 and 4 mon of age, neither in the intention-to-treat analysis of all infants, those who successfully completed both SWEEP-VEP examinations (Table 5), nor in infants breast-fed more than 50% (data not shown). Furthermore, there was no significant difference between groups in the increase in visual acuity from 2 to 4 mon (data not shown).

TABLE 5
Visual Acuity of the Infants at 2 and 4 mon of Age[a]

	OO	FO	High fish
VEP at 2 mon			
Exact age (wk)	8.27 ± 0.46	8.18 ± 0.48	8.32 ± 0.47
n attempted recorded	50	48	49
n with no implemented trials[b]	1	4	0
n with no extrapolations[c]	3	6	4
n successful	46	42	45
Hereof with 4 mon VEP[d]	40	41	45
Mean acuity	0.84 ± 0.08	0.84 ± 0.09	0.82 ± 0.08
VEP at 4 mon			
Exact age (wk)	17.21 ± 0.50	17.09 ± 0.49	17.35 ± 0.66
n attempted recorded	46	52	50
n with no implemented trials[b]	1	0	0
n with no extrapolations[c]	0	0	0
n successful	45	52	50
Hereof with 2 mon VEP[d]	40	41	45
Mean acuity	0.64 ± 0.09	0.62 ± 0.08	0.63 ± 0.09

[a]Visual acuity was measured by swept visual evoked potential. Individual visual acuities were calculated as the mean of all obtained estimates of visual acuity. Data are based on an intention-to-treat analysis including those who were not exclusively breast-fed. The mean visual acuity in each of the supplement groups is expressed as the logarithim of the minimal angle of resolution and given as mean ± SD. None of the values was significantly different from one another.
[b]Too much noise to complete the 10-s trials.
[c]Signal-to-noise ratio too low to make significant extrapolations to threshold. For abbreviations see Table 3.
[d]Mean activities at 2 and 4 mon are given only for infants with successful VEP determinations at both ages.

We measured a clear improvement in visual acuity with age in all groups (Table 5). At the individual level, there was only a weak tendency toward tracking in visual acuity between the two examinations [i.e., infants with a high (or low) visual acuity at 2 mon tended also to have a high (or low) value at 4 mon, Kendall's τ, $r = 0.11$, $P = 0.14$, $n = 81$]. Furthermore, there was no detectable association between visual acuity and the exact age at testing (at 4 mon Kendall's τ, $r = –0.05$, $P = 0.51$, $n = 97$). Maternal prepregnancy BMI was positively associated with infant visual acuity (at 4 mon Kendall's τ, $r = –0.15$, $P = 0.03$, $n = 97$). The degree of breast-feeding was also associated with infant visual acuity at 4 mon of age (inverse to the expected, and based on a limited number of infants breast-fed <50%) (Kendall's τ, $r = 0.15$, $P = 0.04$, $n = 97$). There was no significant association between visual acuity at 2 or 4 mon and maternal smoking habits, level of education, gestational age, birth weight, head circumference, number of siblings, or gender in this homogeneous group of breast-fed infants. Visual acuity was not univariately associated with the estimated maternal n-3 LCPUFA intake (neither habitual intake from fish, total intake, nor FO supplementation) or with the DHA concentration of breast milk (data not shown). Also, there was no univariate association between infant visual acuity at any age and maternal or infant RBC-DHA at 4 mon (Kendall's τ, $r = –0.09$, $P = 0.20$, $n = 145$ and $r = –0.12$, $P = 0.06$, $n = 119$, respectively, at 4 mon).

Figure 3 shows a plot of bivariate association between infant visual acuity at 4 mon of age and infant RBC-DHA at 4 mon. There was no association between these parameters in the randomized part of the study. In the OO-group there was a near-significant association between visual acuity and infant RBC levels of DHA ($r = –0.309$, $P = 0.059$, $n = 38$). There was no detectable association in the FO-group, which in that respect was similar to the reference group (Fig. 3). However, five of the infants (from the FO- and HF-groups) had visual acuities below that of the average 2-mon-old infants, possibly being erroneous determinations (although we lacked clear exclusion criteria).

The association between infant RBC-DHA and visual acuity was explored by a multiple regression analysis. The degree of breast-feeding was included as a confounding factor in this analysis because it was associated with infant RBC-DHA and has also in previous studies been shown to influence visual acuity (5). Maternal prepregnancy BMI, parity, gestational age, and gender were included as potential confounding factors in the analysis; all but gestational age and parity were excluded in the final model as they did not reach statistical significance in the model. The latter three factors were included because they affected most aspects of infant development: Maternal BMI was significantly associated with visual acuity and breast-milk DHA in the FO-group; gestational age and parity could also in theory influence DHA levels in breast milk and infant RBC; and the sex was unequally distributed between the two randomized groups. This multiple regression analysis showed that infant RBC-DHA was a major determinant of visual acuity in the randomized groups (Table 6). A similar multiple regression analysis was performed with maternal n-3 LCPUFA intake divided in an FO-part and a habitual part, but neither of these was significantly associated with infant visual acuity (data not shown). Simultaneous inclusion of infant RBC-DHA and a group variable (FO vs. OO) in the multiple analyses showed no significant effect of the FO supplement.

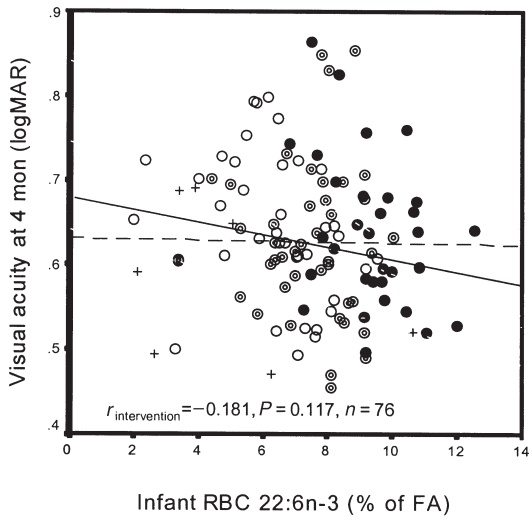

FIG. 3. Association between visual acuity and DHA level in infant RBC at 4 mon of age. Visual acuity, measured as the mean of all thresholds obtained by swept visual evoked potential, is given as the logarithm of the minimal angle of resolution (logMAR). Symbols indicate maternal supplementation with fish oil (●) or olive oil (○) during the 4 mon of lactation, or a habitual high maternal intake of fish (◎) or if the infants had an estimated breast-milk intake of <50% of their diet (+). Regression lines were calculated separately for the high-fish reference (dashed line) and the randomized groups (solid line) (with all infants included irrespective of degree of breast-feeding). For abbreviations see Figure 1.

DISCUSSION

Biochemical outcomes. The FO supplement had a pronounced effect on the DHA content of breast milk, as previously shown (10–13). The effect of FO appeared to be larger (per g) than that caused by the habitual and acute intake of fish. However, this does not necessarily translate into n-3 LCPUFA in FO being more biologically effective than those from fish, as there are some differences to consider. The FO supplement

TABLE 6
Multiple Regression Analysis of Associations with Infant Visual Acuity (logarithm of the minimal angle of resolution)[a]

Factor	Coefficient	*P*
Infant RBC DHA (FA%)	–0.011 ± 0.004	0.008
Degree of breast-feeding (% of nutrition)	0.001 ± 0.000	0.013
Gestational age (wk)	0.024 ± 0.007	0.001
No. of siblings	–0.024 ± 0.011	0.029

[a]The regression was performed on all data for the intervention groups with inclusion of all mentioned factors ($n = 76$). The table gives regression coefficients and *P*-values for significant factors. This model explained 24% (adjusted r^2) of the overall variance in visual acuity.

was taken daily, and in that respect should be considered a habitual as well as an acute dietary factor. Results from our previous study indicated that acute fish intake could exert greater influence than the habitual (8). However, in a study like this, the effects of habitual and acute fish intake are difficult to separate as the probability of obtaining an acutely affected milk sample is higher in women eating more fish owing to the higher frequency of fish intake. Furthermore, calculating the n-3 LCPUFA intake from fish relies on a series of assumptions and uncertainties, whereas the intake of n-3 LCPUFA from FO is known with respect to FA composition as well as dose. We did not determine the total fat intake of the women or their intake of n-6 PUFA, which, *via* dilution and competition, may be an important factor in explaining some of the unaccounted variance observed in the effect of n-3 LCPUFA from fish and FO on milk DHA levels.

Infant RBC-DHA content was also greatly increased by the FO-supplement, in agreement with previous reports (13,15,16). Infant RBC-DHA at 4 mon of age was associated with the current breast-milk DHA content. Owing to fluctuations in milk DHA levels with the acute fish intake of the mother (8), a single milk sample provides only a very rough estimate of infant DHA intake. As in adults (28,29), infant RBC-DHA will reflect the intake of the infant over the past months. This study showed a strong agreement between maternal and infant RBC-DHA levels at 4 mon. This association was stronger than the association between infant RBC-DHA and milk DHA levels. Multiple regression analysis showed that infant RBC-DHA was associated with the estimated maternal n-3 LCPUFA intake during lactation, habitual as well as from the supplement, and the degree of breast-feeding. Thus, infant RBC-DHA at 4 mon seems to reflect infant DHA intake during the lactation period.

Maternal FO-supplementation resulted in a significant decrease in the infant RBC arachidonic acid level. However, the level of arachidonic acid in the RBC of the infants in the FO-group was only about 0.5 SD lower than in the OO-group but was considerably higher than that in infants who were breast-fed less than 50%. In our opinion, this slight decrease in RBC arachidonic acid level should not have any adverse effects on infant development or growth (6).

Functional outcome. We could not show any direct effect of the FO-supplement on infant visual acuity at either 2 or 4 mon of age. However, visual acuity at 4 mon tended to be associated with current infant—as well as maternal—RBC-DHA levels. The association between visual acuity and RBC-DHA was strengthened if controlled for sex, degree of breast-feeding, parity, and maternal BMI, making infant RBC-DHA one of the most significant determinants of infant visual acuity.

Despite the large difference in infant RBC-DHA levels between the OO- and FO-groups, the association between infant RBC-DHA and visual acuity was not reflected in differences in visual acuity between the randomized groups. This could not be explained by accidental differences in other factors (e.g., more primiparous, boys, infants with a low degree of breast-feeding, slightly younger age at testing in the FO-group) nor by possibly erroneously determined low visual acuities (data not shown). Based on the regression line in Figure 3 (or the regression coefficient in Table 6), we estimate that a change in infant RBC-DHA levels from 6.3 to 9.4 FA% should give rise to a difference in visual acuity of roughly around 0.03 logMAR.

However, the SE is about 0.018 logMAR, based on the variations observed in the present study. Therefore, it is not in disagreement with the estimated regression effect of infant RBC-DHA on visual acuity to observe (by chance) no direct intervention effect on visual acuity (i.e., a type II error). The regression has higher power because it also uses the information available in the natural variation in infant RBC-DHA, besides that created by the intervention. The high power of our intervention study for detecting differences in infant RBC-DHA is transformed by the flat regression line to a much lower power for detecting differences in visual acuity (based on post hoc computation). To detect a 0.03 logMAR difference in visual acuity (about 1/3 SD; see Table 5) a study with a power of 80% would require about 140 subjects in each group. The detection of differences in visual acuity of this magnitude is limited by the high CV% of the SWEEP-VEP method (25).

It was not possible to detect an effect of the n-3 LCPUFA of fish oil in multiple regression analyses where this was included in combination with either habitual maternal intake of n-3 LCPUFA, maternal RBC-DHA in pregnancy (as a proxy for the maternal intake of n-3 LCPUFA in the last trimester of pregnancy), or infant RBC-DHA. Infant visual acuity tended to be associated with pre- as well as postintervention RBC levels of DHA of the mothers of the randomized part of the study. This indicates that the association between infant RBC-DHA and visual acuity at 4 mon of age could be secondary to some other association or possibly caused by intrauterine effects of maternal n-3 LCPUFA intake or some dietary or sociocultural factor associated with maternal fish intake. However, the estimated maternal n-3 LCPUFA intake in the OO-group, which contributed most to the association, was not affected by the intervention. Also, there was no significant n-3 LCPUFA-independent effect of fish oil in multiple regression analyses including a group variable (FO vs. OO) and biomarkers of the n-3 LCPUFA intake during lactation (infant or maternal RBC-DHA levels at 4 mon, $P = 0.23$ and $P = 0.068$, respectively). Furthermore, if the observed association had been caused by prenatal factors, we would have expected the regression analysis to give regression coefficients of the same magnitude in the two randomized groups, but with a lower constant in the FO-group. However, the association in the FO-group was like that in the HF-group, indicating that the FO-supplement affected infant visual acuity independent of the RBC level of DHA and that infants in these two groups with respect to visual maturation had optimal levels of RBC-DHA.

The Danish population has a higher fish intake than that in many other countries (30), and this is also reflected in its relatively high RBC DHA content. It is possible that the habitual

fish intake in the randomized trial was close to optimal and that the FO supplementation would have had a larger effect in a population with a lower fish intake. A further limitation of this trial was that not all of the infants were exclusively breast-fed for the entire intervention period. Finally, we aimed at making a perfectly double-blinded trial by adding the supplements in müsli bars and cookies. This was not achieved, however, as some of the subjects were given capsules. Although these subjects were in theory still blinded, it is in most cases possible to taste if one receives FO capsules, which is a limitation in all studies using FO-capsules. This is a potential problem, as one could suspect that the control group might take fish oil if they knew that they were not in the FO-group. However, RBC-DHA and milk DHA (and the SD of these) did not differ between subjects given olive oil as müsli bars and subjects given capsules. Also, RBC-DHA values decreased for all mothers in the OO-group during the intervention.

Two cross-sectional studies have shown an association between visual acuity and breast-milk or infant RBC-DHA levels in subjects with mean milk DHA levels lower than that of the OO-group of this study (both around 0.3 FA%) (7,22). In these studies the observed change in visual acuity at 4 mon of age was between 0 and 0.1 logMAR with changes in milk DHA within the range of 0.1–0.8 FA%. In one of the studies, the association between RBC-DHA and visual acuity was only observed at some of the tested ages (22). Two previous maternal DHA supplementation trials did not show any effects on infant visual acuity in populations with lower baseline levels of milk DHA than the mothers in the present study (16,17). However, one of these trials gave only very small DHA supplements (17) and the other had very few infants with a successful VEP-acuity measure (16). It is therefore our opinion that neither the present nor any of the previous studies provide any solid evidence as to whether the DHA intake of breast-fed infants is of any importance for their visual development.

The estimated maternal n-3 LCPUFA intake during lactation was a strong determinant of the infant RBC-DHA level. Maternal FO supplementation increased the level of DHA and other n-3 LCPUFA in human milk as well as infant RBC. Maternal FO-supplementation during lactation did not enhance visual acuity of those infants who successfully completed the visual assessments. However, the results showed that RBC-DHA was an important determinant of visual acuity. Thus, the results from this study indicate that variation in breast-milk n-3 LCPUFA may have implications for the visual development of breast-fed infants. If this is to be determined conclusively, larger intervention studies in mothers with a very well defined low habitual fish intake are needed.

ACKNOWLEDGMENTS

We gratefully acknowledge dietitian Majken Ege and pediatric nurses Charlotte Mester and Heidi Eismark for collecting the data and technicians Sidsel Abildgaard Jensen, Bettina Sørensen, and Grete Peitersen for performing the FA analyses. This study was financed by FØTEK—The Danish Research and Development Program for Food and Technology and BASF Aktiengesellschaft.

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[Received June 19, 2003; accepted February 26, 2004]