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Maternal Fish Oil Supplementation in Lactation and Growth during the First 2.5 Years of Life

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ABSTRACT

Fish oil addition to infant formulas has raised concern on whether increased intake of *n*-3 long-chain polyunsaturated fatty acid (*n*-3LCPUFA) affects infant growth. The objective of this study was to determine whether maternal fish oil supplementation during 0–4 mo of lactation influences growth in infancy and early childhood. In a randomized, blinded intervention trial, lactating Danish mothers with a fish intake below the population median were randomized to 4.5 g/d fish oil or olive oil. A reference group of 53 mothers with a fish intake in the highest quartile of the population and their infants were included in the study. Head circumference, weight, length, skinfold thickness, and waist circumference of children were measured at 2, 4, and 9 mo and at 2.5 y. One hundred children completed the intervention trial, and 72 were followed up at 2.5 y together with 29 from the reference group. Growth in weight, length, and head circumference did not differ between the randomized groups up to 9 mo, but at 2.5 y, body composition differed significantly. Children in the fish oil group had larger waist circumference body mass index (BMI; 0.6 kg/m^2; $p = 0.022$), and head circumference compared with those in the olive oil group. Adjusted for sex, ponderal index at birth and current energy intake, BMI at 2.5 y was associated with docosahexaenoic acid in maternal erythrocytes after the intervention. In conclusion, the *n*-3LCPUFA intake of lactating mothers may be important for growth of young children. The long-term effect on weight and BMI remains to be investigated. (***Pediatr Res* 58: 235–242, 2005**)

Abbreviations

BMI, body mass index
DHA, docosahexaenoic acid
DNBC, Danish National Birth Cohort
LCPUFA, long-chain polyunsaturated fatty acid
RBC, red blood cell

There has been some concern on whether increasing *n*-3 long-chain polyunsaturated fatty acids (LCPUFA) intake might affect infant growth as early studies of supplying formula-fed premature infants with *n*-3LCPUFA from fish oil showed a negative effect on growth (weight, length, and head circumference) (1). In contrast, none of the *n*-3LCPUFA intervention studies in term infants have found any effect on growth (1,2). A recent study indicated that addition of docosahexaenoic acid (DHA; 22:6*n*-3) to infant formulas in combination with arachidonic acid may enhance weight gain in premature infants (3).

The relative content of DHA in human milk varies by more than a factor of 10 both within and between populations (4,5). 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 (6,7). It is possible to increase effectively breast milk content of DHA by supplementing lactating mothers with fish oil (8–11). At present, there is insufficient scientific evidence to decide whether the variation in the concentration of DHA in breast milk has functional implications for the breast-fed infant (12). Randomized trials in which lactating mothers have been supplemented with *n*-3LCPUFA so far have not shown any effects on infant weight, length, head circumference, or body composition (13,14).

We performed a randomized, double-blinded trial in which lactating mothers were supplemented with fish oil for 4 mo and infant development was followed for 2.5 years. The aim of the trial was to study the effect of maternal fish oil consumption on infant development (visual acuity, cognitive function, and growth). Results on the effects on the fatty acid composition of breast milk and infant red blood cells (RBCs) and visual acuity has been published (15). In this article, we present data on infant growth.

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METHODS

Ethics. The study was conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983. The protocols for the intervention trial and follow-up study were approved by the Scientific-Ethical Committees for Copenhagen and Frederiksberg (KF 01-300/98 and KF 01-183/01). Both parents of all participating children gave written consent to participate after the study had been explained to them orally as well as in writing.

Participants. Participants were selected among women who were recruited for the Danish National Birth Cohort (DNBC) (16). The diet of all the DNBC participants was determined in the 25th week of gestation by a comprehensive 300-item food frequency questionnaire that questioned them about their diet of the month before completion of the questionnaire. Consumption of *n*-3LCPUFA in grams per day was estimated using assumptions of portion sizes and nutrient content in foods (Food Tables produced by The Danish Food Agency). From April 1999 to February 2000, 919 pregnant women, all from the greater Copenhagen area, who were in their eighth month of gestation and had a fish intake below the median (<0.40 g/d *n*-3LCPUFA) were invited to participate in the trial. We also invited 554 women with a fish intake in the upper quartile (>0.82 g/d *n*-3LCPUFA) to participate in the study as a high fish intake reference group. We got a positive response from 273 women, 211 of whom fit the other inclusion criteria (147 with an intake below the population median and 64 with an intake in the upper quartile). The other inclusion criteria were that the women had to have an uncomplicated pregnancy, body mass index (BMI) <30 kg/m^2, no metabolic disorders, and an intention to breastfeed for at least 4 mo. Furthermore, to participate in the study, the newborns had to be healthy (no admission to a neonatal department), term (37–43 wk of gestation), singleton infants with normal weight for gestation (17) and an Apgar score >7 at 5 min after delivery. We also required that the mothers began to take the supplements within 2 wk after birth. A total of 122 and 53 of the women in the below-median and an upper-quartile groups, respectively, fulfilled all of these criteria. The group sizes in the randomized trial were based on a power calculation for infant visual acuity, as explained in a previous paper (15). The characteristics of the participants are given in Table 1.

Intervention. After birth, the women with a fish intake <50th percentile were randomly allocated to supplementation with fish or olive oil in blocks of two in five strata according to mean parental education. Parental education was assessed by a simple questionnaire and scored from 1 to 8 (1 = primary school and 8 = PhD degree from a university) according to the Official Danish Classification of Education from 1994. Investigators and families were blinded to the randomization throughout the first year of life of the infants. Fish oil as well as olive oil supplements were given as microencapsulated oils concealed in two müsli bars (produced by Halo Foods Ltd., Tywyn Gwynedd, Wales, UK) daily for the first 4 mo of lactation. The fish oil group received 17 g/d microencapsulated fish oils (a mixture of "Dry *n-3* 18:12" and "Dry *n-3* 5:25 from BASF Health and Nutrition A/S, Ballerup, Denmark) that provided 4.5 g of fish oil [containing 1.5 g of *n*-3LCPUFA hereof 0.62 g eicosapentaenoic acid (EPA, 20:5*n*-3) and 0.79 g DHA), which is equivalent to the habitual intake of the women in the DNBC population with the highest fish intake (>90th percentile)]. The olive oil group was given a similar amount of microencapsulated olive oil. As an alternative, women were offered the supplements in homemade cookies or as capsules, if they disliked the müsli bars. The homemade cookies were made in the department kitchen with microencapsulated oils in amounts similar to the müsli bars. To obtain an approximately similar supplementation (with respect to amounts and fatty acid composition) with capsules, the olive oil group was given four 1000-mg olive oil capsules and the fish oil group was given six 500-mg capsules plus one 1000-mg capsule with a high and low concentration of DHA, respectively (all capsules were a gift from Lupe/ProNova Biocare, Lysaker, Norway). These 4 g of fish oil supplied 1.4 g of *n*-3LCPUFA (hereof 0.36 g EPA and 0.99 g DHA). The distribution of müsli bars, cookies, and capsules among participants in the fish oil and olive oil groups were not different among the groups: 10% of the women got their entire supplementation as capsules, and 60% of the women had only müsli bars or cookies. The lower amount of oil supplied by the capsules was taken into account in the calculation of overall supplement compliance, which is expressed as the percentage of oil taken relative to the intended dose of two müsli bars per day. The overall self-reported compliance in both groups was 93% (median, 1st–3rd quartiles 86–97%; $n = 99$) during the 4-mo supplementation period. One hundred mothers completed the intervention trial period of 4 mo, and 50 mothers from the high fish reference group remained in the study for the initial 4-mo period [a full report on dropouts in given by Lauritzen *et al.* (15)]. The mean *n*-3LCPUFA intake of the three groups during the intervention period (habitual intake plus supplement adjusted for compliance) is given in Table 1.

A total of 107 mothers complied with the criterion for exclusive breastfeeding for 4 mo. However, mothers, who did not fulfill this criterion were not excluded from the trial or analysis. For infants who were not exclusively breast-fed at 4 mo of age, we estimated to which extent breast milk covered their energy needs from the amount of formula and complementary food ingested. Breast milk covered >90% of the intake for 16 of the nonexclusively breast-fed infants, 75–90% for nine of the infants, 50–75% for three infants, and <50% for 15 infants. The median degree of breastfeeding in the three groups is given in Table 1. Most of the infants who were breast-fed <50% during the 4-mo period were from the fish oil group (nine *versus* three from both olive oil and high fish). Degree of breastfeeding was taken into account in the analysis. 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 fatty acid ratio of ~10.

Fatty acid analysis. Ten-milliliter blood samples were taken by venipuncture on the mother at recruiting and at the end of the 4-mo intervention period. Blood samples were collected in ice-cold EDTA-conditioned tubes. Immediately after sampling, RBCs were separated from plasma and leukocytes and washed three times in physiologic saline. The isolated packed RBCs were reconstituted 1:1 in physiologic saline with 1 mM EDTA and 0.005% BHT and kept at −80°C for a maximum of 8 mo before they were analyzed, as previously described (15). The relative amounts of identified fatty acids are given as a percentage of the overall identified FAME area.

Follow-up visits. A 9-mo follow-up visit was an integrated part of the intervention study. All children except one from the high fish group were followed up at 9 mo of age. When the children were 2.5 y of age, all 150 families were invited to participate in the follow-up visit. This follow-up visit was carried out from November 2001 to September 2002 at the Department of Human Nutrition (Frederiksberg, Denmark). Of the 150 families who completed the initial 4-mo period, 11 were lost to follow-up, six had moved far away from the area, 15 did not wish to attend the follow-up examination for various personal reasons, and 13 did not give any reason for the lack of participation. A total of 105 families agreed to participate in the 2.5-y follow-up visit. Four of the children were uncooperative during the examination, leaving 101 children with 2.5-y follow-up data. The follow-up rate in the randomized groups and the high fish group was 72 and 58%, respectively. With respect to weight, length, and head circumference at birth, those who participated did not differ from those who did not, but there was a slight overrepresentation of boys in the follow-up group ($p = 0.253$ in χ^2 test). Four of the

Table 1. *Characteristics of participants in the trial*

	High fish	Fish oil	Olive oil
Age of mother at delivery (years)	31.9 ± 4.1 (52)	29.6 ± 4.3 (62)	30.2 ± 4.1 (60)
Mean parental education (years)**	14, 12–17 (51)	14, 12–18 (55)	14, 12–18 (51)
Maternal pre-pregnancy BMI (kg/m^2)**	22, 19–27 (53)	22, 19–26 (62)	22, 19–27 (60)
Maternal height (m)	1.70 ± 0.06 (53)	1.67 ± 0.05 (62)*	1.69 ± 0.06 (60)
Paternal height (m)**	1.85, 1.75–1.92 (51)	1.83, 1.72–1.90 (62)	1.80, 1.70–1.92 (58)
No of siblings (0:1:>1)	22:26:5	39:18:4	30:24:6
No of smokers in home (0:1:2)	29:21:3	38:18:5	45:13:2
Sex ratio in group (M:F)	26:27	37:25	28:32
Gestation (wks)	40.2 ± 1.2 (53)	40.1 ± 1.1 (62)	40.1 ± 1.2 (60)
Degree of breast-feeding during the 4 month intervention (%)**	100, 85–100 (50)	100, 34–100 (54)	100, 74–100 (48)
Maternal n-3 LCPUFA intake during lactation (g/day)**	0.8, 0.4–1.65 (50)	1.6, 1.23–1.91 (52)	0.3, 0.03–0.51 (47)

* The value for the fish oil group is significantly different from that of the olive oil group ($p < 0.05$) in a Student t-test.

** Values are given as median, 10th–90th percentile (number of subjects included (*n*)), all other values are mean ± SD (*n*).

children declined all anthropometric measurements, two did not want their height measured, and 7–11 would not let us measure one or both of their skinfold thicknesses. One and six refused to have their head and waist circumference measured, respectively. The dietary intake of the 2.5-y-old children was assessed for 7 consecutive days using a precoded dietary questionnaire, as previously described (18).

Anthropometric measurements. The length and weight at birth were taken from the hospital journal. Head circumference was measured at home 9 ± 3 d after birth ($n = 175$). Weight, length, and head circumference were measured at the department three times during infancy, at 2, 4, and 9 mo. Weight in infancy was measured on an electronic scale (Sartorius IP65; Bie & Berntsen, Rødovre, Denmark), which during a period of 15 s measures body weight 40 times and gives the result as a mean of all of these measures. Length in infancy was measured three times on a table with a sliding foot and headboard equipped with an electronic reading device (Force Technology, Brøndby, Denmark). Head circumference was measured three times with a tape measure. At 2.5 y, the height was measured on barefooted children with a resolution of 0.1 cm using a stadiometer. Body weight was measured with a resolution of 0.1 kg on a digital scale (Lindeltronic 8000; Samhall Lavi AB, Kristianstad, Sweden). Waist circumference was measured three times with a regular tape measure. Triceps and subscapular skinfold thicknesses were measured in triplicate with a standard skinfold caliper (Harpenden, Chasmars Ltd, London, UK). Triceps skinfold thickness was measured parallel to the long axis of the arm midway between the shoulder and the elbow with the arm slightly flexed. The subscapular skinfold thickness was measured below the inferior angle of the scapula at a diagonal in the natural cleavage of the skin. Most of the measurements were made by one observer and the remaining by one of two well-trained stand-ins. The mean of all obtained measures of the anthropometric variables was used. Percentage of body fat was calculated from the sum of triceps and subscapular skinfold thicknesses using the equations of Slaughter *et al.* (19), which may not give an optimal estimate (20), but at a sum of skinfold thicknesses ~16 mm as in the present study seems to give a fair estimate of body fat. BMI *z* scores were calculated from Danish growth curves (21).

Statistics. All results are given as mean ± SD, and estimates are given as mean ± SE unless otherwise stated. *T* test or ANOVA combined with Bonferroni *post hoc* tests were used for comparisons between groups for all normally distributed continuous variables. Nominal variables were compared by χ^2 analysis, and variables in the ordinal scale and non–Gauss-distributed variables were compared by a Mann-Whitney *U* test. Multiple regression analyses of observed differences between the randomized groups in head circumference and body composition at 2.5 y of age were performed by a general linear model. These analyses were controlled for sex and head circumference at first visit and ponderal index at birth, respectively. A similar analysis was also performed for the difference in body composition with additional inclusion of the energy intake of the child at 2.5 y of age (kJ/d). Furthermore, both effects were analyzed with the inclusion of a sex-intervention-interaction variable. In parametric comparisons, we verified that the groups had homogeneous variances, and all multiple models were checked for absence of heteroscedasticity by an even scatter in the residuals plots. Pearson analysis of univariate correlation was used to detect possible associations between anthropometric measures and the biochemical effect of the intervention as measured by the DHA content of maternal RBC membranes at the end of the intervention. Associations between anthropometric measures and maternal RBC DHA were also investigated with a multiple general linear model with inclusion of the previously mentioned variables plus the degree of breastfeeding. All data were analyzed using SPSS for Windows 11.0 (Chicago, IL).

RESULTS

The intervention resulted in a substantial increase in the content of DHA in the membranes of maternal RBCs (Table 2). The relative DHA content of RBC fatty acid was increased from 7.7 ± 1.1% ($n = 95$) in the last trimester of pregnancy to 8.8 ± 1.2% ($n = 50$) in the fish oil group at the end of the intervention period, whereas the DHA content in the olive oil group decreased to 5.5 ± 1.0% ($n = 45$) during the intervention (fish oil *versus* olive oil: $p < 0.001$). All other *n*-3LCPUFA of RBCs was also affected by the intervention (Table 2). The *n*-3LCPUFA content of breast milk was 2.5-fold increased, and infant RBCs were also increased (15).

The growth (weight, length, and head circumference) of the children from birth to the age of 2.5 y in the three dietary groups is shown in Table 3. Weight and length did not differ between the groups at any time point, but at 2.5 y of age, children in the olive oil group had a significantly smaller head circumference than the children in the two other groups [$p = 0.028$ (fish oil) and $p = 0.010$ (high fish)]. When infants who were breast-fed <50% were excluded from the analysis, the head circumference of the two randomized groups was still

Table 2. *Fatty acid composition of maternal red blood cells in the last trimester of pregnancy and the 4th month of lactation, before and after the intervention trial period, respectively*

	High fish		Fish oil		Olive oil	
	Before trial	After trial	Before trial	After trial	Before trial	After trial
No. of subjects	49		50		45	
Total SFA	39.50 ± 1.24	39.53 ± 0.79[b]	39.34 ± 1.16	40.10 ± 0.89[a]	39.62 ± 1.37	39.95 ± 0.58[a]
Total MUFA	17.48 ± 0.85	17.22 ± 0.80[a,b]	17.36 ± 1.17	16.89 ± 0.94[b]	17.47 ± 0.97	17.45 ± 1.02[a]
20:3n-9	0.01 ± 0.04	0.01 ± 0.04	0.03 ± 0.05	0.01 ± 0.04	0.02 ± 0.04	0.03 ± 0.07
18:2n-6	9.20 ± 0.92[b]	10.44 ± 0.89[a,b]	9.97 ± 0.96[a]	10.32 ± 0.96[b]	9.53 ± 1.00[a,b]	10.84 ± 1.12[a]
20:3n-6	2.00 ± 0.29	1.75 ± 0.32[a]	2.12 ± 0.44	1.55 ± 0.28[b]	2.12 ± 0.33	1.89 ± 0.31[a]
20:4n-6	14.16 ± 1.25	15.05 ± 1.21[b]	14.76 ± 1.45	13.73 ± 1.13[c]	14.79 ± 1.30	15.80 ± 1.09[a]
22:4n-6	2.78 ± 0.46[b]	2.56 ± 0.44[b]	3.22 ± 0.49[a]	2.31 ± 0.46[c]	3.07 ± 0.58[a]	2.99 ± 0.53[a]
22:5n-6	0.55 ± 0.16[b]	0.43 ± 0.13[b]	0.74 ± 0.17[a]	0.52 ± 0.08[a]	0.71 ± 0.17[a]	0.54 ± 0.10[a]
Total n-6 PUFA	29.06 ± 1.89[b]	30.47 ± 1.78[b]	31.17 ± 1.89[a]	28.66 ± 1.64[c]	30.60 ± 2.04[a]	32.29 ± 1.69[a]
18:3n-3	0.23 ± 0.06	0.20 ± 0.08[a]	0.26 ± 0.07	0.14 ± 0.10[b]	0.27 ± 0.07	0.19 ± 0.08[a]
20:5n-3	1.07 ± 0.38[a]	1.72 ± 0.53[b]	0.72 ± 0.22[c]	2.04 ± 0.59[a]	0.81 ± 0.23[b]	1.10 ± 0.31[c]
22:5n-3	2.96 ± 0.36[a]	3.01 ± 0.25[a]	2.72 ± 0.28[b]	2.70 ± 0.25[c]	2.85 ± 0.28[a,b]	2.86 ± 0.26[b]
22:6n-3	8.92 ± 1.27[a]	7.12 ± 1.23[b]	7.72 ± 1.10[b]	8.82 ± 1.21[a]	7.67 ± 1.03[b]	5.45 ± 1.01[c]
Total n-3 PUFA	13.19 ± 1.69[a]	12.10 ± 1.74[b]	11.42 ± 1.38[b]	13.73 ± 1.69[a]	11.59 ± 1.28[b]	9.63 ± 1.27[c]
n-6/n-3	2.41 ± 0.40[b]	2.58 ± 0.47[b]	2.98 ± 0.44[a]	2.13 ± 0.39[c]	2.87 ± 0.44[a]	3.43 ± 0.60[a]

The relative content of fatty acids are given as percentage of all identified fatty acids (area%). The values represent mean ± SD.

Fatty acids are identified by the number of carbon atoms, number of double bonds and position of the last double bond calculated from the methyl-end of the carbon chain.

Group comparisons before and after trial are performed by analysis of variance and individual groups were compared by Bonferroni post hoc tests. Values with different superscripts are significantly different from one another.

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty.

Table 3. *Growth of children from birth to 2½ years of age*

		High Fish	Fish Oil	Olive Oil
Age (mo)	1 wk visit# (days) circumference	9, 6–13 (53)	8, 6–14 (62)	9, 6–12 (60)
	2 mo visit# (wks)	8.1, 8.0–9.1 (50)	8.1, 7.6–8.7 (54)	8.3, 7.7–8.7 (51)
	4 mo visit# (wks)	17.3, 16.7–18.3 (50)	17.1, 16.4–17.9 (53)	17.1, 16.7–17.9 (47)
	9 mo visit# (wks)	38.6, 38.0–39.6 (48)	38.5, 38.2–39.2 (53)	38.5, 38.1–39.2 (47)
	2½ y visit (mos)	31.76 ± 1.02 (29)	31.62 ± 0.85 (42)	31.83 ± 0.86 (30)
Head circumference (cm)#	1 week	36.18 ± 1.59 (51)	36.11±1.25 (54)	35.72 ± 1.53 (56)
	2 months	39.68 ± 1.27 (47)	39.70 ± 1.22 (50)	39.28 ± 1.16 (50)
	4 months	42.40 ± 1.38 (45)	42.17 ± 1.16 (45)	41.84 ± 1.12 (46)
	9 months	45.81 ± 1.36 (42)	45.85 ± 1.53 (52)	45.29 ± 1.40 (45)
	2½ years	50.62 ± 1.23 (29)	50.42 ± 1.20 (41)*	49.74 ± 1.34 (30)
Weight (kg)	Birth	3.65 ± 0.44 (53)	3.60 ± 0.45 (62)	3.56 ± 0.41 (60)
	2 months#	5.3, 4.9–6.3 (50)	5.5, 4.7–6.2 (53)	5.4, 4.7–6.6 (51)
	4 months	6.93 ± 0.67 (49)	7.00 ± 0.73 (53)	7.00 ± 0.85 (47)
	9 months	9.15 ± 0.90 (48)	9.47 ± 0.94 (53)	9.19 ± 0.94 (47)
	2½ years	14.18 ± 1.43 (29)	14.16 ± 1.26 (42)	13.71 ± 1.26 (30)
Length/height (cm)	Birth#	53, 50–55 (53)	52, 50–56 (62)	52, 50–56 (60)
	2 months#	59.1, 56.6–60.9 (50)	58.8, 56.5–61.0 (52)	58.7, 55.8–61.3 (51)
	4 months	64.70 ± 1.71 (50)	64.21 ± 2.08 (52)	64.02 ± 2.16 (46)
	9 months	72.75 ± 2.01 (48)	72.66 ± 2.35 (53)	72.15 ± 2.04 (47)
	2½ years (standing)	93.74 ± 2.93 (29)	92.58 ± 3.14 (42)	92.65 ± 3.04 (28)
Ponderal index (kg·m-3)	Birth	25.01 ± 1.97 (53)	24.87 ± 2.16 (62)	25.02 ± 2.07 (60)
BMI (kg·m-2)	Birth#	13.0, 11.7–14.6 (53)	12.9, 11.7–14.4 (62)	13.0, 11.6–14.4 (60)
	2 months (kg·m-2)	15.63 ± 1.36 (50)	15.74 ± 1.24 (52)	15.93 ± 1.37 (51)
	4 months (kg·m-2)	16.57 ± 1.66 (49)	16.93 ± 1.23 (52)	17.04 ± 1.70 (46)
	9 months (kg·m-2)	17.27 ± 1.39 (48)	17.91 ± 1.24 (53)	17.64 ± 1.52 (47)
	2½ years (kg·m-2)	16.11 ± 1.08 (29)	16.51 ± 1.08 (42)*	15.86 ± 1.21 (28)

All values are given as mean ± SD (*n*) or for variables that were not normally distributed (indicated by #) median, 10th–90th percentile (*n*).

* The value of the FO-group is significantly different from that of the OO-group ($p < 0.05$) in Student t-test.

significantly different ($p = 0.038$), being 50.4 ± 1.2 ($n = 32$) and 49.7 ± 1.3 ($n = 29$) cm in the fish oil and olive oil groups, respectively. After control for sex and head circumference at 2 mo of age, the difference between the two randomized groups was found to be 0.5 ± 0.2 cm ($p = 0.019$). Head circumference at 2 mo of age was used because the age at which the 1-wk head circumference measure was taken had a high variation (CV% of 30) and was measured in only 90% of the infants. When only these children were included in the analysis, controlling for head circumference at 1 wk of age and sex, the difference was 0.4 ± 0.3 cm ($p = 0.114$; $n = 65$). No significant interaction between sex and intervention was observed.

Measures of body composition of the children at 2.5 y are given in Table 4. All measures of body composition at 2.5 y of age showed that the children from the fish oil–supplemented mothers were fatter than those in the olive oil group, although only the differences in BMI and waist circumference were statistically significant. Triceps and subscapular skinfold thicknesses as well as the sum of these skinfold thicknesses and

Table 4. *Body composition of children at 2½ years of age*

		Randomized groups			
	High Fish	Fish Oil	Olive Oil	Non-adjusted mean difference (*p*-value)	Adjusted mean difference (*p, n*)
BMI (kg·m-2)	16.1 ± 1.1 (29)	16.5 ± 1.1 (42)	15.9 ± 1.2 (28)	0.65 ± 0.28 (0.022)	0.80 ± 0.28 (0.006, 64)
Waist circumference (cm)	49.5 ± 3.0 (27)	50.2 ± 2.4 (40)	48.6 ± 2.7 (28)	1.54 ± 0.63 (0.017)	1.80 ± 0.65 (0.007, 62)
Triceps skinfold thickness (mm)	9.3 ± 2.2 (27)	9.7 ± 1.8 (38)	8.9 ± 1.7 (29)	0.79 ± 0.44 (0.076)	1.14 ± 0.44 (0.012, 61)
Subscapular skinfold (mm)*	6.4, 4.1–8.7 (26)	6.5, 4.7–10.0 (35)	6.1, 4.7–7.8 (29)	(0.147)	1.06–1.21 (0.020, 59)
Sum of skinfolds (mm)	15.7 ± 3.6 (26)	16.5 ± 3.3 (35)	15.0 ± 2.6 (29)	1.47 ± 0.76 (0.057)	2.15 ± 0.77 (0.007, 59)
Percent body fat**	15.1 ± 3.2 (26)	15.9 ± 2.9 (35)	14.5 ± 2.5 (29)	1.35 ± 0.69 (0.055)	1.65 ± 0.69 (0.021, 59)
Lean body mass (kg)**	12.0 ± 1.0 (26)	11.9 ± 1.1 (35)	11.7 ± 1.0 (29)	0.15 ± 0.25 (0.553)	—

Values in the groups are given as mean ± SD (*n*) along with the non-adjusted mean difference (± SE) and *p*-values from a Student t-test comparison of the two randomized groups. Last column gives mean difference (± SE) plus *p*-values and *n* from multiple regression analysis controlling for sex, ponderal index at birth and current energy intake (kJ·kg − 1·d − 1).

* Subscapular skinfold thickness was not Gauss-distributed and thus are given as median, 10th–90th percentile (*n*), *p*-value of Mann-Whitney comparison and multiple regression performed on log-transformed values and mean difference (± SE) back-calculated to a range.

** Percent body fat was estimated from the sum of skinfold thicknesses using the equations of Slaughter et al. 19 and lean body mass by subtracting this from body.

percentage of body fat as estimated from the sum of the skinfold thicknesses were also higher, although not significant, but lean body mass did not differ between the groups. The difference in BMI remained when expressed as *z* scores, being 0.1 ± 0.9 in the fish oil group and −0.5 ± 1.0 in the olive oil group ($p = 0.013$). Similar results were obtained when infants who were breast-fed <50% were excluded from the analysis (data not shown). There was no difference between the two groups in body composition at birth or during infancy as judged by ponderal index at birth and BMI values at 2, 4, and 9 mo of age (Table 3). The differences in body composition between the two randomized groups became more evident when the comparisons were controlled for sex and ponderal index at birth and even more so when also controlled for current energy intake (Table 4). No significant interaction was observed between sex and intervention ($p = 0.266$).

BMI, waist circumference, and percentage of body fat at 2.5 y of age were positively associated with the DHA content of maternal RBCs at the end of the intervention period ($r = 0.238$, $p = 0.021$; $r = 0.301$, $p = 0.007$; and $r = 0.264$, $p = 0.035$, respectively). BMI and waist circumference were also significantly associated with the DHA content in a sample of breast milk at 4 mo ($r = 0.209$, $p = 0.015$; and $r = 0.187$, $p = 0.034$). The associations between maternal RBC-DHA and skinfold thicknesses were not significant ($p = 0.053$). All associations between body composition and maternal RBC-DHA were significant after control for sex, ponderal index at birth, current energy intake, and degree of breastfeeding (as shown for BMI in Table 5 and Fig. 1, *p* values for waist circumference, triceps skinfold thickness, and percentage of body fat of 0.002, 0.001, and 0.002, respectively). Age at the 2.5-y examination, maternal smoking, parity, and prepregnancy BMI did not have any significant effect when included in the model alone or in combination and did not result in any major changes in the estimates or levels of significance given in Table 5. In this context, maternal RBC-DHA is used as a measure of intervention compliance, although it also reflects the habitual maternal fish intake. In combination with the degree of breastfeeding during the first 4 mo of lactation, this can also be used as a proxy for how much the intervention influences the child. The DHA content of breast milk is strongly associated with DHA in maternal RBC (6), and DHA in milk as well as maternal RBCs is strongly associated with DHA in infant RBCs (15). Most if not all of the difference between the two randomized groups seems to be explained by this difference in *n*-3LCPUFA intake, as no independent effect of group was evident when included in the model in Table 5. When group was included instead of maternal RBC-DHA, the percentage of the variation explained by the model decreased from 24 to 21%.

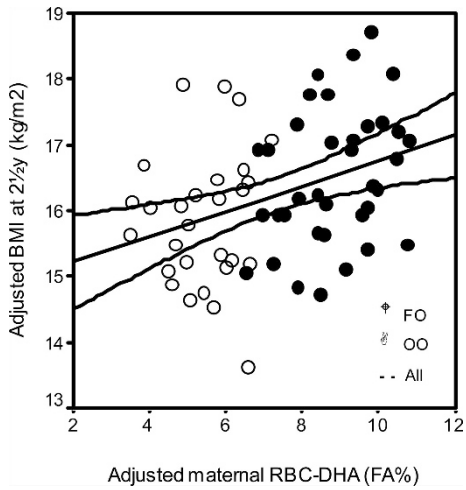

Figure 1. Association between BMI of the child at 2.5 y of age and the content of DHA in maternal RBCs (given as percentage of fatty acids relative to all identified fatty acids) after the 4-mo intervention period during lactation. Both variables have been adjusted for sex, ponderal index at birth, current energy intake of the child, and degree of breastfeeding. ●, fish oil group (FO); ○, olive oil group (OO). The regression line was calculated from all data ($r = 0.362$, $p = 0.003$; $n = 64$).

In the randomized groups, head circumference at 2.5 y of age was also found to be positively associated with maternal RBC-DHA at the end of the intervention ($r = 0.257$, $p = 0.017$; $n = 68$). However, this association was no longer significant (B ± SE = 0.09 ± 0.05, $p = 0.080$), when controlled for sex, head circumference at 2 mo, and the degree of breastfeeding during the intervention period. Together, these parameters explained 63% of the variation in head circumference ($p < 0.001$), with head circumference at 2 mo being the most significant of the included determining factors.

DISCUSSION

Fish oil supplementation of lactating mothers did not affect the weight and length gain of the infants during the first year of life, and in this respect, our study was in agreement with results from previous maternal fish oil–supplementation trials (13,14).

Table 5. *Association between the docosahexaenoic acid (DHA) content of maternal red blood (RBC) after the intervention period and BMI of the child at 2½ years of age in the two randomized groups in a multiple regression analysis controlling for sex, ponderal index at birth, current energy intake and degree of breast-feeding*

	Coefficient	p
Constant	14.701 ± 1.866	<0.001
Gender (boy = 2)	−0.151 ± 0.275	0.585
Ponderal index at birth (kg·m − 3)	0.095 ± 0.063	0.136
Energy intake (kJ·kg − 1·d − 1)	−0.002 ± 0.002	0.286
Estimated energy intake from breast-milk during intervention (%)	−0.013 ± 0.005	0.017
Maternal RBC DHA (% of fatty acids)	0.193 ± 0.065	0.004

The table gives regression coefficients (± SE) and *p*-values for all included factors. The overall model explained 24% of the variation in BMI ($p = 0.005$, $n = 64$).

However, when examined at 2.5 y of age, children from the fish oil group had larger head circumference and higher BMI than those in the olive oil group after adjustment for relevant confounders. Both body composition and head circumference at 2.5 y of age was also positively associated with the biochemical effect of the intervention expressed as the DHA content of maternal RBCs at the end of the intervention.

Systematic review and meta-analysis of all randomized, controlled LCPUFA treatment trials in term and preterm formula-fed infants with growth outcome show no effect of LCPUFA on growth in infancy measured as weight, length, and head circumference (2). Our study, however, is not the first study to show that LCPUFA intake may enhance growth. An association has been observed between the DHA content of breast milk and weight and length gain from birth to 3 mo of age in Chinese infants (22). One of the more recent studies with LCPUFA-enriched formulas has shown higher weight gain in the supplemented groups of preterm infants and a consistently higher weight-to-length ratio from 40 to 57 wk postmenstrual age in comparison with infants who were fed standard infant formula (3). Higher weight gain has also been observed in a term infant LCPUFA-intervention trial but only in male infants (23). Few studies have examined effects on body composition. One study has reported that formula with LCPUFA resulted in a significant decrease in subscapular skinfold thickness in term infants at the age of 1.5 and 3 mo, but this was not evident at 6 and 12 mo and was not accompanied by lower triceps skinfold thickness (24).

Our trial was limited by a relatively large drop-out rate and some minor problems with the blinding. The participants who were given capsules were in theory still blinded but probably could taste whether they received fish oil. The families were informed about which group they were in before the 2.5-y follow-up. However, those who examined the children were in praxis blinded during the follow-up visit. Not all of the infants were exclusively breast-fed for the entire intervention period, which limits the impact of maternal supplementation on infant development. Furthermore, the degree of breastfeeding was not equally distributed in the randomized groups. The randomized groups also differed with respect to sex ratio and maternal height. It is not plausible that the difference in maternal height could explain the observed increase in growth in the fish oil group, as no difference was observed in height of the children or in mean parental height and because mothers in the fish oil group were shorter. Sex and degree of breastfeeding have been shown to be associated with infant growth (25), and both variables would in our study favor increased growth in the fish oil group. However, inclusion of these variables in the multiple regression model did not eliminate the difference between the groups and, thus, do not seem to explain the growth-promoting effect of maternal fish oil supplementation during lactation.

Unfortunately, we did not have proper information about head circumference at birth. We therefore used head circumference measured at the first examination as a control parameter when exploiting the association between intervention and growth in head size. However, the first examination occurred when the infants were 2 mo of age. The increase in head circumference from birth to 2 mo of age could possibly be affected by the intervention, which would result in an underestimation of the effect of the fish oil supplement. The data (Table 3) suggest that this could be the case. The difference in head size, as that in body composition, was observed only at the follow-up visit. No significant differences were detectable at the earlier examinations. From the values in Table 3, it seems as though both differences were increasing throughout the study period, but the power of the study was too small to confirm this.

Breast-fed infants tend to be thinner than those who are formula-fed at 1 y of age (25). It has been hypothesized that breastfeeding protects against obesity (26). The *n*-3LCPUFA intake of breast-fed infants is higher than that of formula-fed infants (12); thus, if intake of *n*-3LCPUFA should in any way play a role in the proposed obesity protection, then one would expect it to be beneficial. The breast milk of the mothers in the present study has relatively low levels of *n*-6PUFA and high levels of *n*-3LCPUFA (12) compared with that in most Western populations (5). The Danish population has a higher fish intake than that in many other countries (27), which is also reflected in relatively high levels of DHA in maternal RBCs. Despite this, we found an effect of fish oil supplementation on body composition that was apparent 2 y after completion of the intervention. During the intervention and at the 9-mo follow-up, there was no difference in BMI between the groups. The late onset of the effect and that it persisted after control for current energy intake could indicate some kind of programming effect. Studies in animals and cell cultures suggest that an increase in *n*-6PUFA promotes adipocyte differentiation and induces adipogenesis, whereas *n*-3PUFA reduces fat deposition (28–30). Massiera *et al.* (30) suggested that high intake of linoleic acid during early life can program later obesity and that this in part may explain the increasing prevalence of obesity with no apparent increase in the intake of fat. The results from our study are opposite of what should be expected from this hypothesis, as we found that a high intake of *n*-3PUFA during lactation resulted in more fat tissue accumulation. However, it should be kept in mind that the hypothesis on the effect of *n*-3 and *n*-6PUFA on fat disposition is based on animal models and cell cultures, and, to our knowledge, there are no data on human infants and children showing these effects.

None of the infants were obese according to the definition of Cole *et al.* (31), but 6% were overweight. The percentage of overweight infants in the fish oil group was twice as high as in the olive oil group (7 and 4%, respectively), and the number of underweight infants (weight-for-age 2 SD) was similarly lower (2 *versus* 7% in the olive oil group), indicating that the whole distribution curve was shifted ~0.05 of a BMI unit (equivalent to 0.05 SD). The increase in BMI seems to be due to an increase in fat mass, as judged from the concomitant increase in skinfold thickness and the estimated percentage of body fat. At the age of 2.5 y, BMI is declining (31) and the thickness of the s.c. fat layer is decreasing. The observed increase in BMI after maternal fish oil supplementation in theory could reflect a delayed decline that could be followed by a delayed adiposity rebound, which is associated with lower risk for obesity later in life (32). Alternatively, the increase in BMI could track and result in an increased risk for overweight and obesity later in

life. It therefore is hard to know whether the observed increase in BMI is potentially beneficial or harmful.

We found a 5-mm difference in head circumference between the two intervention groups. It is not possible to determine whether this is due to a difference in brain size or in bone and skin thickness. The brain grows and accretes large amounts of DHA during the first year of life (33,34). Brain growth (measured as head circumference and estimated brain weight) has been shown to be associated with the level of DHA in infant RBCs (35). Thus, the observed increase in head circumference in theory could be explained by a higher deposition of DHA during the first 4 mo of life as a result of the maternal fish oil supplement's providing an increase in DHA supply. A small Swedish study showed that brain growth in the first months of lactation was positively associated with the arachidonic acid to DHA ratio in breast milk (36). As the ratio of arachidonic acid to DHA in breast milk decreased with fish oil supplementation (15), this is contradictory to what we observed in this study. However, a 5-mm difference in circumference can also be explained by a 0.8-mm difference in thickness of skin or s.c. fat. That difference is of the same size as the difference in triceps or subscapular skinfold thickness found in this study.

Although somewhat controversial, head size in early life has been reported to be associated with developmental scores in later life, especially in infants with low birth weight and disadvantaged children (37). Also among normal children, head size seems to be related to IQ (38,39). However, whether the small difference in head circumference found in the present study has any functional implications is highly speculative.

CONCLUSION

The present study shows that the *n*-3LCPUFA intake of lactating mothers may be a determinant for head size and body composition of breast-fed infants. The long-term effect on weight and BMI remains to be investigated. We plan to follow up on the growth of the children later in childhood and hope that this will give us an indication of the potential long-term implications of our findings.

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L.L. planned and designed the study in collaboration with K.F.M. As primary investigator, L.L. was responsible for data collection, data management, statistical analysis, interpretation, and writing of the manuscript. L.L. and E.M.S. were responsible for analysis of FA composition in maternal RBCs. C.H., K.F.M., and E.M.S. engaged in discussions on data interpretation, contributed to the drafting of the paper, and approved of the paper in its final version. None of the authors has any financial or personal relationships with the company or organization sponsoring the research.

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