Fish Oil Affects Blood Pressure and the Plasma Lipid Profile in Healthy Danish Infants[1]

Camilla T. Damsgaard,[2] Lene Schack-Nielsen, Kim F. Michaelsen, Maj-Britt Fruekilde,* Ole Hels, and Lotte Lauritzen

*Department of Human Nutrition, LMC Centre for Advanced Food Studies, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark and *The Biochemistry and Nutrition Group, BioCentrum, The Technical University of Denmark, Lyngby, Denmark*

ABSTRACT Animal and epidemiologic studies indicate that early nutrition has lasting effects on metabolism and cardiovascular disease risk. In adults, (n-3) long-chain PUFA (LCPUFA) from fish oils improve blood pressure, the lipid profile, and possibly cardiovascular disease mortality. This randomized trial is the first to investigate the effects of fish oil on blood pressure and the lipid profile in infancy. Healthy term 9-mo old infants ($n = 83$) were randomly assigned to 5 mL fish oil daily or no fish oil for 3 mo and to 2 different milk types. Before and after the intervention, blood pressure was measured with an oscillometric device, and blood was sampled for analysis of erythrocyte fatty acid composition and the plasma lipid profile. This paper examines the effects of the fish oil supplement, with adjustment for the effects of the milk intervention when relevant. The fish oil intervention increased erythrocyte (n-3) LCPUFA content ($P < 0.001$). At 12 mo, infants administered fish oil had a lower systolic blood pressure [adjusted mean difference (95% CI)] 6.3 mm Hg (0.9, 11.7) ($P = 0.02$), a 0.51 mmol/L (0.07, 0.95) higher plasma total cholesterol ($P = 0.02$), and a 0.52 mmol/L (0.02,1.01) higher LDL cholesterol ($P = 0.04$) than infants not administered fish oil. Plasma triacylglycerol was inversely associated with the erythrocyte content of eicosapentaenoic acid ($r = 0.34$, $P < 0.01$), a biomarker of fish oil dose. The observed effects of fish oil are in accordance with findings in adults. The long-term health implications warrant further investigation. J. Nutr. 136: 94–99, 2006.

KEY WORDS: • *blood pressure* • *cholesterol* • *fish oil* • *infants* • *programming*

Animal studies have shown that nutrition in fetal life and infancy can induce lifetime effects on blood pressure, cholesterol metabolism, and atherosclerosis. Whether such programming or metabolic imprinting exists in humans has been debated (1). Breast-feeding has been associated with a more favorable blood pressure and lipid profile in adulthood (2–4). Breast milk is a rich source of (n-3) long-chain PUFA (LCPUFA)[3] (5) and the results from 2 recently published studies suggest that (n-3) LCPUFA supplementation in early life is associated with a lower blood pressure later in life (6,7). Observational studies show that blood pressure and cholesterol values track from childhood to adulthood (8,9). Early diet-induced changes in these risk factors may therefore affect later health.

Fatty fish and fish oils are the major dietary sources of (n-3) LCPUFA, primarily eicosapentaenoic acid [EPA, 20:5(n-3)], and docosahexaenoic acid [DHA, 22:6(n-3)]. Fish oil supplementation has been associated with beneficial effects on a number of risk factors in adults such as reductions in blood pressure (10) and blood triacylglycerol (TAG) concentrations and a possible lowering of cardiovascular disease (CVD) mortality (11,12).

Late infancy is a vulnerable period of rapid growth and a transition from a milk-based diet to the family diet. Studies concerning how diet in this period of life affects immediate and later health are rare. Some have observed positive associations between saturated fat intake and blood cholesterol concentrations in infancy (13,14). In most studies, however, it has been impossible to separate the effects of (n-3) LCPUFA from the effects of other fats or changes in energy intake (13–17). The consumption of (n-3) LCPUFA is likely to be limited after cessation of breast-feeding. In most Nordic countries, children have traditionally been given a spoonful of cod liver oil daily, and this regimen is still recommended for infants in Norway, although little is known about its effects.

SUBJECTS AND METHODS

Design. The present study had a randomized 2 × 2 factorial design in which infants were randomly assigned to receive 5 mL fish oil

[1] Funded by the Danish Research and Development Programme for Food and Technology.

[2] To whom correspondence should be addressed. Email: ctd@kvl.dk.

[3] Abbreviations used: ANCOVA, analysis of covariance; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DBP, diastolic blood pressure; E%, percentage of energy intake; EPA, eicosapentaenoic acid; FA%, area percentage of total fatty acids; +FO, fish oil supplemented; −FO, not fish oil supplemented; LCPUFA, long-chain PUFA; MAP, mean arterial pressure; RBC, erythrocyte; SBP, systolic blood pressure; TAG, triacylglycerol.

Manuscript received 3 August 2005. Initial review completed 7 September 2005. Revision accepted 28 September 2005.

daily (+FO group) or no supplement (−FO group) from 9 to 12 mo of age. To study the effects of recommending fish oil, no control oil was used. Infants were also randomly assigned to drink either cow's milk or standard infant formula [no LCPUFA, 18:2(n-6) and 18:3(n-3) in a ratio of ~8:1]. Randomization was done within clusters of 12 by drawing notes from one envelope for each intervention. The study was approved by the Ethical Committee of the Municipalities of Frederiksberg and Copenhagen (J.no. KF 02-014/03).

Recruitment and subjects. Participants were recruited from May to October 2003 by random extraction of infants resident in the County of Copenhagen or Frederiksberg from the National Danish Civil Registry. Mailed invitations were sent to parents of 7- to 8-mo-old infants. The inclusion criteria were singleton infants born ≥ 37 wk of gestation, with birth weight > 2500 g and ≥ the 5th percentile for gestational age (18), a 5-min Apgar score ≥ 7, no major complications at birth or in fetal life, and no chronic diseases. Only infants with a daily consumption of cow's milk or infant formula were included.

Only 2.6% of the invited families participated in the study (**Fig. 1**). The dropout rate after randomization did not differ between the intervention groups ($P = 0.149$). Reasons for dropout were lack of time ($n = 5$) and infant unwillingness to consume the allotted milk type ($n = 2$) or fish oil ($n = 1$) or to be physically examined ($n = 1$). Two families gave no reasons for withdrawal. Dropout infants did not differ in birth or breast-feeding characteristics from those who finished the study, but they were 1.6 cm shorter at 9 mo (95% CI 3.01, 0.21, $P = 0.039$).

Study protocol. All interviews and examinations were performed at the Department of Human Nutrition. Parents who agreed to the principle of randomization and whose infants met the inclusion criteria were invited to an individual introduction visit. The study was explained to them orally and written consent was obtained from the infant's parent or guardian. An interview on diet, growth, and health of the infant was performed, and instructions on food recording were given. The infants were examined before the start of the intervention at 9 mo ± 3 wk and at the end of the intervention 3 mo ± 3 wk later. At both visits, we measured anthropometry and blood pressure, and took a blood sample. The infant's diet was recorded by the parents for 7 d before each examination visit.

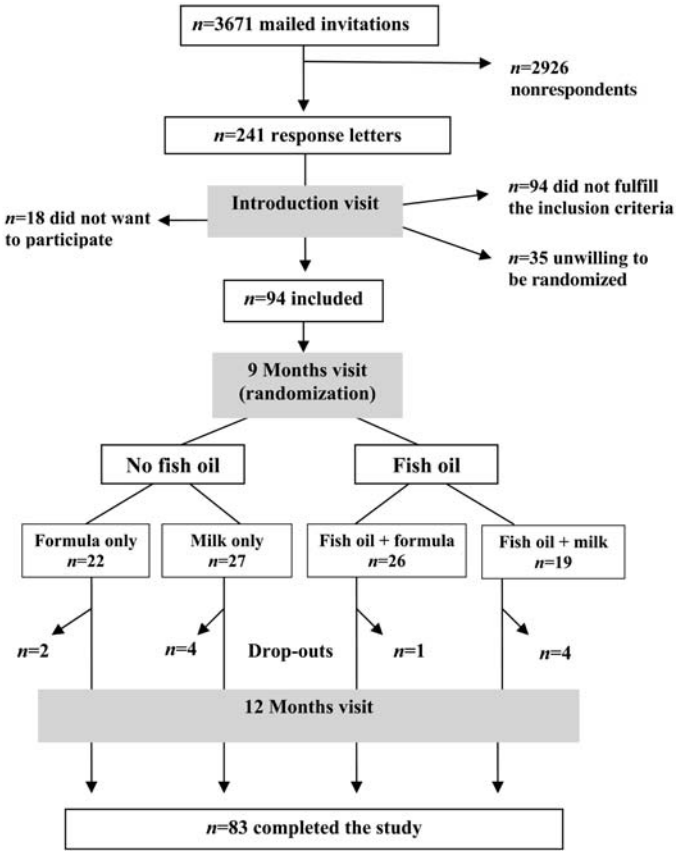

FIGURE 1 Recruitment and participation of infants. Not all outcome variables were successfully measured in all infants.

Fish oil supplements. Five bottles of 105 mL fish oil (Eskimo-3®, Cardinova, a kind gift from Anjo A/S), were provided to children in the +FO group. The parents were asked to give their infant a daily dose of 5 mL fish oil. The fish oil brand was found by the Danish Consumer Council to have a low content of environmental contaminants; it contained 352 g/L (n-3) LCPUFA (60% EPA and 40% DHA) and 3 g/L cholesterol. Parents were asked to keep open fish oil bottles refrigerated, to return remaining bottles, and to report any spill of the oil. These reports were obtained from 34 of the 39 families in the +FO group. The mean fish oil consumption was estimated to be 3.3 mL/d (range 0.8–5.0 mL/d), corresponding to 924 mg/d (n-3) LCPUFA. None of the infants consumed any kind of fish oil before the start of the intervention and none of the infants in the −FO group consumed fish oil during the intervention. The erythrocyte (RBC) content of (n-3) LCPUFA is a biomarker of (n-3) LCPUFA consumption (5) and was used as a measure of compliance.

Measurements and blood samples. The weight of naked infants lying on a pediatric infant scale (Sartorius IP 65, Bie & Berntsen AS) was successfully measured in all infants at 9 and 12 mo. Recumbent length was measured using a standard wooden measuring board. Length measures were performed in triplicate in 82 infants (99%) at 9 and 12 mo and in 81 infants (98%) on both occasions. Results are given as means.

Arterial blood pressure was determined with an automated oscillometric device (model 506N, Criticare Systems) during cuff inflation as previously described (19). Blood pressure was measured in 73 (88%) and 69 infants (83%) at 9 and 12 mo, respectively, and in 64 infants (77%) on both occasions. Missing blood pressure data were due to infants moving or becoming irritated. Blood pressure was recorded in triplicate in 26% of the measured infants, 48% were measured in duplicate, and 26% were based on single measurements. Means or single values are reported. Median intra-individual CV for the blood pressure variables were 6–10%, with slightly higher variation in diastolic blood pressure (DBP) and mean arterial pressure (MAP) than in systolic blood pressure (SBP).

A 4-mL venous blood sample was taken from the forearm (3 mL in test tubes containing lithium-heparin and 1 mL with EDTA). The time without food before blood sampling was 2.5 h (range 0.5–5.0 h), except for 1 infant at 12 mo who went without food for 13 h overnight. Local anesthesia of the skin was given using the EMLA patch (Astra Zeneca AB) if the parents wished. Blood sampling was successful in 59 (71%) and 63 infants (76%) at 9 and 12 mo, respectively, and in 49 infants (59%) on both occasions. Samples were kept on ice and RBC and plasma were separated as previously described (20). Samples were frozen at −80°C and RBC was analyzed within 5.5 mo after sampling as described in (20). In brief, RBC were hemolyzed in redistilled water and the lipids extracted by the Folch procedure (21). The extracted lipids were transmethylated with BF_3 in methanolic NaOH (22) and the resulting FAME were separated by GLC on a HP-6890 gas chromatograph (Hewlett-Packard) equipped with a flame ionization detector and a SP2380 capillary column (60 m, i.d. 0.25 mm, and film thickness 0.2 μm, Supelco). The relative content of specific fatty acids is expressed as an area percentage (FA%), which is approximately equal to a weight percentage. Two of the 9-mo samples were excluded in accordance with the principles described in (20) because their content of saturated and monounsaturated fatty acids deviated >3 SD from the mean of all samples. Therefore, RBC fatty acid determination was successful in 56 infants (67%) at 9 mo, 57 infants (69%) at 12 mo, and in 44 (53%) infants at both 9 and 12 mo.

Concentrations of total, LDL, and HDL cholesterol and TAG in heparinized plasma were determined by an automated enzymatic colorimetric principle described in (23) with test kits from Roche Diagnostics (#2016630, 03038777, 3030074, and 2016648) on Cobas Mira (Roche Diagnostic System). The 9- and 12-mo samples from each infant were analyzed on the same day. Analytical intraserial variations (expressed as CV) were as follows: total cholesterol 0.7%, LDL cholesterol 2.4%, HDL cholesterol 2.6%, and TAG 1.9%. Sufficient blood to analyze plasma lipid profile was obtained from 54 (65%) and

53 infants (64%) at 9 and 12 mo, respectively, and from 41 infants (49%) on both occasions.

Infant diet was recorded by the parents for 7 consecutive days using a precoded dietary record developed for children. Intakes were recorded in household measures or portion sizes estimated from a portion size photo series, as described in (19). Nutritional calculations were made with GIES software (version 0.993B, the Danish Institute for Food and Veterinary Research). A total of 82 families completed the food records at both 9 and 12 mo.

Statistics. Data were analyzed with the Statistical Package for the Social Sciences software (version 12.0; SPSS). Results are given as means ± SEM, or SD for the descriptive variables. Outcome variables were modeled in 2-way ANOVA with fish oil intervention and milk intervention as fixed factors. The milk intervention was kept in the models if it had a significant effect. This was the case for the dietary data, RBC linoleic acid content, and plasma total and LDL cholesterol concentrations at 12 mo. Comparisons of 9- and 12-mo values within each group were done using paired *t* test or 1-way χ^2 test for frequency variables.

To adjust for other covariates or confounding variables, further analyses were performed in extended ANOVAs and analyses of covariance (ANCOVA). Gender, age, BMI, and breast-feeding duration were tested as covariates in both the blood pressure and lipid profile models. Moreover, blood pressure cuff size, outdoor temperature, and protein intake were tested in the blood pressure models, and fasting time, energy content of last meal, and saturated fat intake were tested in the lipid profile models. Covariates that affected the outcome ($P < 0.10$) or that changed the estimated effects of fish oil >20% were kept in the model. All final models were adjusted for baseline values.

Significant effects of the fish oil intervention on blood pressure and lipid profile were further investigated by Pearson's Product Moment correlation and multiple linear regression analyses to test whether a dose-dependent relation existed between RBC EPA or DHA content and the outcome variables. Possible covariates were tested individually in multiple regression analysis and included in the final models using the same criteria as in ANOVA and ANCOVA. Significance was established at $P < 0.05$.

RESULTS

Growth and diet. The only significant difference in baseline characteristics between the +FO group and the −FO group was a lower occurrence of breast-feeding from 9 to 12 mo in the +FO group (**Table 1**). Although weight and height at 12 mo and changes in weight, length, and BMI from 9 to 12 mo did not differ between the groups (data not shown), the +FO group had a significantly higher BMI than the −FO group at 12 mo (17.4 ± 1.2 vs. 16.9 ± 1.0 kg/m^2, $P = 0.04$). This was due primarily to a slightly ($P = 0.18$) higher BMI at baseline (Table 1). The +FO group had a slightly lower intake of saturated fat than the −FO group at 12 mo [16 ± 4 vs. 18 ± 3% of energy intake (E%), $P = 0.03$], but changes in diet from 9 to 12 mo did not differ between the groups (data not shown). When energy and fat contained in the fish oil were included, the +FO group still had a lower intake of saturated fat and also a higher intake of polyunsaturated fat at 12 mo (6 ± 2 vs. 4 ± 1 E% in the −FO group, $P < 0.001$). The milk intervention strongly affected dietary macronutrient composition at 12 mo, with higher relative intakes of protein, saturated fat, and cholesterol ($P < 0.001$) and lower intakes of monounsaturated fat ($P = 0.01$) and polyunsaturated fat ($P < 0.001$) in the cow's milk group.

Reliable data on dietary (n-3) PUFA consumption could not be obtained due to limitations in the food composition tables from the Danish Food Agency. A rough estimate of the daily intake of (n-3) LCPUFA was made from the recorded intake of fish, which was 7 ± 7 g/d at 9 mo and 8 ± 11 g/d at 12 mo. Assuming that the fish consumption of the infants consisted of 50% fatty fish and 50% lean fish, this corresponds to a daily supply of ~90 mg (n-3) LCPUFA from fish, or 10% of the estimated mean consumption of (n-3) LCPUFA from the fish oil supplement in the +FO group.

TABLE 1

Baseline characteristics of infants in the fish oil intervention groups[1]

Variable	No fish oil	Fish oil
Gender, M:F, *n*	23:21 (44)	18:21 (39)
Age, *mo*	9.1 ± 0.3 (44)	9.1 ± 0.3 (39)
Weight, *kg*	9106 ± 779 (44)	9270 ± 901 (39)
Length, *cm*	72.4 ± 2.3 (43)	72.3 ± 2.1 (39)
BMI, *kg/m^2*	17.3 ± 1.3 (43)	17.7 ± 1.3 (39)
Duration of full breast-feeding, *mo*	4.1 ± 1.6 (43)	4.1 ± 1.5 (38)
Duration of any breast-feeding, *mo*	7.5 ± 3.4 (35)	6.2 ± 3.4 (32)
Any breast-feeding 9–12 mo, yes:no, *n*	24:20 (44)	11:24* (35)
Energy intake,[2] *kJ/d*	3521 ± 1224 (44)	3276 ± 996 (38)
Protein intake,[2] *E%*	12 ± 2 (44)	12 ± 2 (38)
Carbohydrate intake,[2] *E%*	50 ± 4 (44)	50 ± 5 (38)
Fat intake,[2] *E%*	38 ± 4 (44)	38 ± 5 (38)
Saturated fatty acids, *E%*	16 ± 3 (44)	15 ± 5 (38)
Monounsaturated fatty acids, *E%*	12 ± 2 (44)	12 ± 7 (38)
Polyunsaturated fatty acids, *E%*	5 ± 1 (44)	5 ± 2 (38)
Cholesterol, *mg/d*	82 ± 40 (44)	67 ± 43 (38)

[1] Values are means ± SD (*n*) or ratios (*n*). Comparisons were done with ANOVA and with 1-way χ^2 tests for frequencies.
[2] Values do not include contributions from breast-feeding.
*Different from infants not administered fish oil, $P < 0.05$.

Erythrocyte membrane fatty acid composition. The RBC fatty acid composition did not differ between the fish oil intervention groups at baseline. During the intervention, the RBC content of EPA and DHA in the +FO group increased from 0.6 ± 0.1 to 3.2 ± 0.2 FA% and from 5.5 ± 0.3 to 7.5 ± 0.2 FA% ($P < 0.001$), respectively, and that of linoleic acid and arachidonic acid decreased from 10.7 ± 0.2 to 9.2 ± 0.2 FA% ($P < 0.001$) and from 16.3 ± 0.3 to 13.7 ± 0.3 FA% ($P < 0.05$), respectively. There were no significant changes in the −FO group, apart from a slight increase in EPA from 0.5 ± 0.1 to 0.7 ± 0.2 FA% ($P < 0.001$). Infants randomly assigned to formula had a 1.4 FA% higher RBC content of linoleic acid at 12 mo (95% CI 0.8, 1.9) ($P < 0.05$) than infants randomly assigned to cow's milk.

The estimated daily intake of fish oil was significantly linearly associated with RBC EPA at 12 mo (**Fig. 2**). There was also a significant positive association between estimated fish oil intake and RBC DHA ($r = 0.53$, $P < 0.001$, $n = 53$), which deviated slightly from linearity as it leveled off at higher fish oil doses (data not shown). Therefore, RBC EPA content was used as the primary biomarker of fish oil dose.

Blood pressure. SBP was significantly affected by the fish oil intervention (**Table 2**) with mean changes of −5.6 ± 3.1 mm Hg ($P = 0.06$) in the +FO group and 2.4 ± 2.8 mm Hg ($P = 0.43$) in the −FO group. The resulting group difference of 4.7 mm Hg (95% CI 0.0, 9.4) was increased to 6.3 mm Hg (95% CI 0.9, 11.7) after relevant adjustment. DBP and MAP did not differ between the groups either with or without adjustment (Table 2). The milk intervention did not affect any of the blood pressure variables.

SBP was not significantly linearly associated with RBC EPA at 12 mo, either with or without adjustment, indicating no significant dose-response relation (data not shown). SBP was,

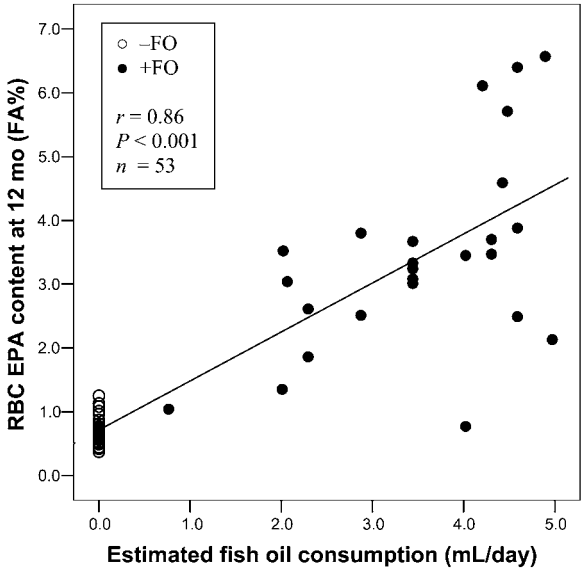

FIGURE 2 Correlation between estimated fish oil consumption and erythrocyte EPA content at 12 mo in infants with or without fish oil administration for 3 mo.

however, significantly negatively associated with RBC DHA content ($\beta = -1.81$, $P = 0.05$, $n = 47$). Adjustment for cuff size, breast-feeding duration, and SBP at 9 mo slightly increased the regression coefficient, but reduced sample size and the level of significance ($\beta = -1.91$, $P = 0.07$, $n = 34$), because of missing values for the covariates.

Lipid profile. The plasma total and LDL cholesterol concentrations were significantly higher in the +FO group than in the −FO group at 12 mo (**Table 3**). Adjustment increased the estimated group differences to 0.51 mmol/L (95% CI 0.07, 0.95) in total cholesterol and 0.52 mmol/L (95% CI 0.02, 1.01) in LDL cholesterol. The cow's milk group had a higher total cholesterol concentration than the formula group at 12 mo

TABLE 2

Blood pressure of infants at 9 and 12 mo of age with or without fish oil administration[1]

Variable	No fish oil	Fish oil	*P*
	mm Hg		
Systolic blood pressure			
9 mo	106.6 ± 2.0 (41)	109.9 ± 2.2 (32)	0.27
12 mo	108.8 ± 1.7 (35)	104.1 ± 1.7 (34)	0.05
12 mo adj.[2]	106.7 ± 2.2 (27)	100.4 ± 2.4 (26)	0.02
Diabetic blood pressure			
9 mo	63.7 ± 1.7 (41)	64.9 ± 1.9 (32)	0.65
12 mo	63.4 ± 1.8 (35)	61.4 ± 1.8 (34)	0.43
12 mo adj.[3]	60.8 ± 2.0 (33)	57.8 ± 2.2 (29)	0.23
Mean arterial pressure			
9 mo	79.6 ± 1.5 (41)	80.3 ± 1.8 (32)	0.76
12 mo	79.1 ± 1.5 (35)	78.8 ± 1.6 (34)	0.91
12 mo adj.[4]	76.8 ± 1.8 (33)	75.9 ± 2.0 (29)	0.69

[1] Values are means ± SEM (*n*). Comparisons were done using ANOVA or ANCOVA.
[2] Adjusted for age, blood pressure cuff size, breast-feeding, and 9-mo values.
[3] Adjusted for cuff size, outdoor temperature, and 9-mo values.
[4] Adjusted for gender, cuff size, outdoor temperature, and 9-mo values.

TABLE 3

Plasma lipid concentrations in infants at 9 and 12 mo of age with or without fish oil administration[1]

Variable	No fish oil	Fish oil	*P*
	mmol/L		
Total cholesterol			
9 mo	4.16 ± 0.17 (26)	4.16 ± 0.13 (28)	0.99
12 mo[2]	4.28 ± 0.13 (26)	4.65 ± 0.12 (27)	0.04
12 mo adj.[3]	4.20 ± 0.15 (20)	4.71 ± 0.15 (21)	0.02
LDL cholesterol			
9 mo	2.71 ± 0.18 (26)	2.62 ± 0.12 (28)	0.67
12 mo[2]	2.84 ± 0.14 (26)	3.32 ± 0.14 (27)	0.02
12 mo adj.[4]	2.84 ± 0.16 (20)	3.35 ± 0.16 (21)	0.04
HDL cholesterol			
9 mo	1.19 ± 0.05 (26)	1.23 ± 0.06 (28)	0.64
12 mo	1.21 ± 0.06 (26)	1.21 ± 0.05 (27)	0.98
12 mo adj.[5]	1.11 ± 0.06 (20)	1.21 ± 0.06 (21)	0.20
TAG			
9 mo	1.46 ± 0.13 (26)	1.71 ± 0.13 (28)	0.17
12 mo	1.38 ± 0.09 (26)	1.30 ± 0.09 (27)	0.52
12 mo adj.[6]	1.50 ± 0.09 (19)	1.29 ± 0.09 (20)	0.10

[1] Values are means ± SEM (*n*). Comparisons were done using ANOVA or ANCOVA.
[2] Adjusted for milk intervention.
[3] Adjusted for milk intervention, age, and total cholesterol at 9 mo.
[4] Adjusted for milk intervention, gender, and LDL cholesterol at 9 mo.
[5] Adjusted for gender and HDL cholesterol at 9 mo.
[6] Adjusted for gender, age, energy content of last meal, and TAG at 9 mo.

(4.65 ± 0.12 vs. 4.29 ± 0.13 mmol/L, $P = 0.05$) and tended to have a higher LDL cholesterol concentration (3.27 ± 0.14 vs. 2.89 ± 0.15 mmol/L, $P = 0.061$). These effects of the milk intervention were not significant, however, after adjustment for other covariates ($P = 0.07$ for total cholesterol and $P = 0.14$ for LDL cholesterol). Paired tests showed a significant decrease in plasma TAG in the +FO group from 9 to 12 mo ($P = 0.04$). TAG was 0.21 mmol/L (95% CI −0.48, 0.05) lower in the +FO group after adjustment, although the difference was not significant (Table 3).

Total and LDL cholesterol concentrations at 12 mo were not significantly linearly associated with RBC EPA or DHA content, either with or without relevant adjustment (data not shown). Plasma TAG concentration was significantly negatively associated with RBC EPA ($\beta = -0.08$, $P = 0.04$, $n = 51$). Adjustment for gender, age, and energy content of the last meal did not affect the regression coefficient (**Fig. 3**). Further adjustment for baseline values slightly decreased sample size and increased the *P*-value ($\beta = -0.07$, $P = 0.06$, $n = 39$) because of incomplete data sets. TAG at 12 mo was not significantly associated with RBC DHA ($P = 0.30$, $n = 51$).

DISCUSSION

This study is the first published randomized trial to investigate the effects of fish oil on blood pressure and the lipid profile in infancy. We had a strong biomarker of fish oil consumption and explored the effects of recommending and providing fish oil. Because fish oil supplements are rarely substituting for other fats in the diet, blinding and provision of control oil would have been inappropriate. The fish oil trial was performed in association with a milk intervention in which consumption of cow's milk significantly increased blood cholesterol values. We did, however, carefully adjust for the

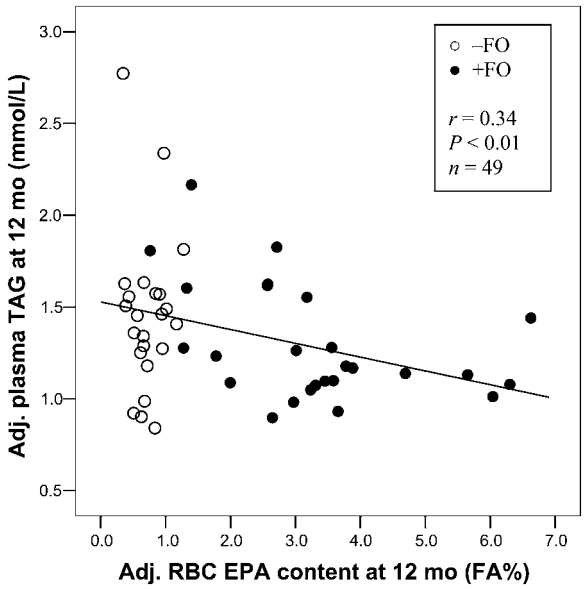

FIGURE 3 The correlation between erythrocyte EPA content and plasma TAG concentrations at 12 mo in infants with or without fish oil administration for 3 mo, adjusted for gender, age, and energy content of the last meal.

milk intervention; this part of the design, therefore, is unlikely to have confounded the results.

Fish oil supplementation consistently reduced SBP in this population of healthy infants, both with and without control for possible confounding. This is in agreement with studies in adults, although the effects are normally modest (1–3 mm Hg) and largest in older, hypertensive adults (10,24). A blood pressure–lowering effect of fish oil in childhood is also in accordance with a recent follow-up study of a randomized trial by Forsyth et al. (7) showing 3 mm Hg lower DBP and MAP in 6-y olds after supplementation with (n-3) LCPUFA in the first 4 mo of life. The long-term effects of (n-3) PUFA observed by Forsyth et al. (7) and by Armitage et al. (6) in rats may indicate that the blood pressure–lowering effects of (n-3) PUFA in infancy may be persistent and still evident in later life.

We did not observe any short-term effects of fish oil on DBP or MAP. We are not aware of any studies reporting the variation in blood pressure measurements in infants. A similar magnitude of variation was observed in a study among 2.5-y-old children (19) and high variation is likely to be a problem in infants. This will reduce power and the ability to detect small blood pressure differences. Thus, the observed group differences in SBP are likely to be actual effects of fish oil and differences in variables such as DBP may also have existed, although they were undetectable.

Our dose-response analyses showed no significant association between RBC EPA and SBP. It may require larger sample sizes if such correlations are to be detected. RBC DHA showed saturation at higher fish oil doses, in accordance with findings in adult supplementation studies (25). Therefore, the indicated negative association between SBP and RBC DHA may point to a dose-response lowering of blood pressure that levels off at high fish oil doses, rather than to a differential physiological effect of EPA and DHA.

A number of mechanisms for the effects of fish oil on blood pressure have been proposed; most involve incorporation of (n-3) LCPUFA into membrane phospholipids. (n-3) LCPUFA incorporation may increase membrane fluidity or elasticity, which may increase erythrocyte deformability and decrease blood viscosity (26) or affect membrane receptors involved in blood pressure regulation (6). A third proposed mechanism involves improved vasodilation through an altered balance between eicosanoids derived from (n-3) and (n-6) PUFA or through an increase in nitric oxide synthesis and release (27).

We observed a consistent increase in infant plasma concentrations of total and LDL cholesterol with fish oil supplementation in both unadjusted and adjusted analyses. The relation between fish oil and lipid profile in infancy has rarely been studied. In a longitudinal study among 103 healthy Icelandic infants, Thorsdottir et al. (28) found that PUFA intake between 9 and 12 mo of age was positively associated with total cholesterol at 12 mo. Furthermore, in accordance with our findings, consumption of cod liver oil was positively associated with total and LDL cholesterol, but only in girls. In a randomized, double-blind, placebo-controlled trial among children and adolescents with familial dyslipidemic disorders, supplementation with DHA (1.2 g/d) for 6 wk elevated total, LDL, and HDL cholesterol (29). Although lipid metabolism of dyslipidemic children may deviate from that of healthy infants, these findings are in general agreement with ours. A recent Cochrane review concluded that fish oil does not affect total or HDL cholesterol in adults, but increases LDL cholesterol concentrations (12). It is difficult to compare the present study with trials in adults, because the regulation of lipid metabolism may be different in infants. Furthermore, control oils of many different compositions have been used in adult studies, whereas in the present study, no control oil was provided. The cholesterol increases we observed may not be caused by (n-3) LCPUFA per se but may be due to an increased intake of fat and cholesterol contained in the fish oil.

A dose-dependent TAG-lowering effect of fish oil was indicated in the present study. This is a well-established effect of fish oil in adults (12,30,31). Inconsistencies between dose-response analyses and group comparisons in the present study may be due to differences in time without food, and energy and fat content of last meal, which have profound effects on plasma TAG. We did, however, try to minimize this random variation by carefully recording and statistically adjusting for these variables. The health implications of a TAG-lowering effect of fish oil in infancy are not known. In adults, the effect is reversible and diminishes a few days after supplementation has ended (32).

Clarke and co-workers (33,34) have shown that ingestion of (n-3) LCPUFA affects lipid metabolism by suppressing hepatic lipogenesis, reducing hepatic TAG output and increasing fatty acid oxidation in the liver and muscle tissue, possibly via interaction with transcription factors that regulate the expression of genes encoding key regulatory proteins of lipid metabolism. The possible effects on cholesterol metabolism are more hypothetical. A lowering of TAG concentrations would be likely to decrease transfer of TAG to LDL and removal of cholesterol from LDL, resulting in larger, more cholesterol-rich LDL particles. Early "high" cholesterol may not be unfavorable for acute and later health. Breast-feeding is associated with higher total and LDL cholesterol concentrations in infancy (15,28,35), and has been inversely associated with cholesterol values later in life (3). The higher cholesterol concentrations of breast-fed infants may be due to (n-3) LCPUFA, cholesterol, or other components in breast milk. It has been proposed that the high cholesterol content of breast milk downregulates cholesterol synthesis later in life (28) and possibly early fish oil supplementation could induce comparable effects.

The varying sample sizes due to missing data are an inevitable consequence of doing research among healthy infants. It

makes interpretation of our results slightly more difficult, but we believe that the varying sample sizes have not confounded the results. Our findings are consistent in the different analyses performed, and consistent with evidence in the literature. Together with the biologically plausible mechanisms presented, this indicates that the observed effects are causally related to fish oil supplementation. It is unknown, however, whether these short-term effects of fish oil are evident later in life and whether they affect long-term health.

ACKNOWLEDGMENTS

We gratefully acknowledge the contribution of all the families and children who participated in the study and Majken Ege Rasmussen, Susan Bjerregaard Nielsen, and Puk Ingemann Holm for collecting the data. We also thank Vivian Julia Anker and Grete Peitersen for technical assistance and blood sample analysis.

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