ARTICLE

Effect of Fish Oil on the Fatty Acid Composition of Human Milk and Maternal and Infant Erythrocytes[1]

Robin A. Henderson[2], Robert G. Jensen*, Carol J. Lammi-Keefe, Ann M. Ferris and Kenneth R. Dardick[3]
Department of Nutritional Sciences, The University of Connecticut, Storrs, Connecticut 06269-4017

To examine the effect of fish oil supplementation on the fatty acid (FA) composition of human milk and maternal and infant erythrocytes, five lactating women were supplemented with 6 g of fish oil daily for 21 d. Usual maternal diets contained 1,147 mg of total n-3 FA, with 120 mg from very long-chain (>C_{18}) n-3 FA. Supplementation increased dietary levels to 3,092 mg of total n-3 FA and 2,006 mg of very long-chain n-3 FA. Milk samples were collected daily, prior to fish oil ingestion, and at 4-h intervals on days 1, 7, 14 and 21. Milk n-3 FA content increased within 8 h and reached steady state levels within one week. The n-6 fatty acid content decreased. Erythrocyte eicosapentaenoic acid content increased from 0.24% to 1.4% ($P < 0.01$) in mothers and from 0.11% to 0.70% ($P < 0.05$) in infants. Docosapentaenoic acid increased from 1.4% to 2.2% ($P < 0.05$) in mothers and from 0.30% to 0.78% ($P < 0.01$) in infants. There was no significant change in docosahexaenoic acid or n-6 fatty acid content. Maternal platelet aggregation responses were variable. No differences in milk or plasma tocopherol levels were noted.
Lipids 27, 863-869 (1992).

Animals are unable to synthesize n-6 and n-3 fatty acids and are dependent on dietary sources. Linoleic (18:2n-6) and linolenic (18:3n-3) acids can be converted into their respective families of very long-chain polyunsaturated fatty acids (VLC-PUFA). However, because both fatty acid classes are elongated and desaturated *via* the same enzyme system, the presence of high levels of 18:2n-6 relative to 18:3n-3 may limit the formation of n-3 VLC-PUFA (1,2). The acids of interest derived from 18:3n-3 are eicosapentaenoic (EPA, 20:5n-3), docosapentaenoic (DPA, 22:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids.

Supplementation with 18:3n-3 has been shown to be less effective than fish oil for increasing tissue n-3 VLC-PUFA content (3), and preferential incorporation of dietary VLC-PUFA over their precursors into rat liver and brain has been demonstrated (4). These findings raise the possibility that preformed n-3 VLC-PUFA from the diet are an important source of n-3 VLC-PUFA for tissues.

There is increasing evidence that the n-3 VLC-PUFA have an important role in the structure and function of biological membranes, particularly in brain and retinal tissues. In animals, retinal depletion of DHA has been shown to result in altered electroretinogram response (5-7) and decreased visual acuity (8). Decreased learning ability of rats fed 18:3n-3 deficient diets has been demonstrated, corresponding with the brain DHA depletion (9,10). Visual and neurologic changes attributed to n-3 PUFA deficiency have been reported (11,12) in humans, but it is difficult to separate n-3 PUFA deficiency from other nutrient deficiencies in these studies.

Ascertaining the effect of diet on the fatty acid composition of brain and retinal tissues in humans must be accomplished using indirect markers such as erythrocytes and plasma. Based upon studies in rats and piglets which show that neural and erythrocyte tissues have similar relative effects from dietary modification of n-3 PUFA (13,14), it is likely that human erythrocytes are appropriate indicators of brain n-3 VLC-PUFA composition.

In addition to the potential role of DHA in tissue membranes, the n-3 VLC-PUFA EPA is a precursor for eicosanoids, which are potent metabolic effectors associated with inflammation and platelet aggregation (15,16).

The potential importance of the n-3 VLC-PUFA during perinatal growth and development has stimulated research into the effects of infant feeding practices on the fatty acid composition of infant tissues. Human milk contains small amounts of n-3 VLC-PUFA in addition to 18:3n-3, whereas commercial infant formulas contain only 18:3n-3. Differences between the plasma and erythrocyte fatty acid profiles of breast-fed and formula-fed infants have been observed (17-20). Fish oil supplementation of formula fed to preterm infants resulted in erythrocyte phospholipid DHA levels more similar to those of infants fed human milk (21). The requirement for n-3 VLC-PUFA for optimal infant health and development has not been determined, although recommendations have been made (22).

Maternal dietary fatty acid composition affects the fatty acid composition of breast milk with the PUFA content responding markedly (23-26). The n-3 VLC-PUFA content of human milk can be increased above the levels observed in women consuming a Western omnivorian diet. Inuit women consume high levels of dietary n-3 VLC-PUFA and had significantly higher levels of EPA and DHA in their milk compared to Vancouver controls (27). Harris *et al.* (28) supplemented the usual intake of breast-feeding mothers with fish oil, and dose dependent increases in the n-3 PUFA content of the milk were observed. The effect of high levels of n-3 fatty acids in human milk on infant tissues has not been determined.

A potential side effect of increased consumption of fish and fish oil is that it may increase the need for vitamin E.

[1]Based on a paper presented at the Symposium on Milk Lipids held at the AOCS Annual Meeting, Baltimore, MD, April 1990.
Scientific Contribution No. 1420, Storrs Agricultural Experiment Station, University of Connecticut, Storrs, CT 06269-4017.

[2]Current address: Johns Hopkins Hospital, Department of Pediatrics, 600 N. Wolfe St., Brady 301, Baltimore, MD 21205.

*To whom correspondence should be addressed at Department of Nutritional Sciences, U-17, 3624 Horsebarn Hill Rd. Ext., University of Connecticut, Storrs, CT 06269-4017.

[3]Current address: Mansfield Family Practice, Mansfield Professional Park, Storrs, CT 06269-4017.

Abbreviations: ADP, adenosine phosphate; ANOVA, analysis of variance; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid(s); GLC, gas-liquid chromatography; HPLC, high-pressure liquid chromatography; P/S, polyunsaturated/saturated ratio; RDA, recommended daily allowance; VLC-PUFA, very long-chain polyunsaturated fatty acid(s) (longer than C_{18}).

In animals, fish oil supplementation has been shown to induce tissue vitamin E deficiency, even with excess dietary supply (29). Hartog *et al.* (30) found no adverse effects of fish oil supplementation on tissues at about twice the vitamin E dosage reported above. Vitamin E supplementation led to decreased susceptibility to *in vitro* peroxidation in tissues from rats fed diets supplemented with menhaden oil (31). It is not known whether humans are susceptible to disturbances in vitamin E metabolism due to increased n-3 PUFA consumption. However, in populations ingesting high levels of n-3 fatty acids, vitamin E deficiency is usually not seen (32).

The goal of the present research was to provide data on the changes in the fatty acid composition of breast milk and maternal and infant erythrocytes during maternal fish oil supplementation at a level equivalent to consuming a 4-oz portion of salmon daily. In addition, maternal dietary intake, milk and plasma vitamin E levels and maternal platelet aggregation response were measured. This information may help determine the optimum intake of n-3 fatty acids during infancy and maternal dietary requirements during lactation.

MATERIALS AND METHODS

Study design and data collection. Five lactating women and their infants were recruited for the study. All subjects signed an informed consent form, and the study had human subjects' approval from The University of Connecticut.

The supplementation period was 21 d, between 2 and 5 wk *postpartum.* None of the subjects were consuming any medications which might alter platelet aggregation. Infants received breast milk as their sole source of nutrient intake from birth and throughout the study. Mothers and infants were weighed weekly.

Mothers supplemented their usual diets with six 1-g capsules of Bio-EFA (PGE Technology, Cambridge, MA) daily. Capsules were consumed once daily at approximately the same time each day. Each capsule contained 180 mg EPA and 120 mg DHA, confirmed by gas-liquid chromatography (GLC), and 1 mg of vitamin E. This provided 1,080 mg EPA, 720 mg DHA and six mg of vitamin E per day.

Dietary intake was evaluated by collecting food records for six days (33,34), which were divided into three randomly assigned 48-h periods during the study. At least one weekend day was included. Forms with instructions to record all food and drink consumed and a 2-dimensional visual aid for estimating portion sizes of food and beverages were provided (cited in 35). Overall nutrient composition (Massachusetts Nutrient Data Bank, University of Massachusetts, Amherst, MA), α-tocopherol content (36), and the fatty acid composition of the diets were calculated (37).

Milk samples were collected immediately prior to ingestion of fish oil capsules each day. On days 1 (baseline), 7, 14 and 21, samples were also collected at 4, 8, 12 and 16 h postsupplementation. All samples were collected using an Egnell electric breast pump (Egnell Inc., Cary, IL). Mothers did not use any creams or oils on their breasts during the study. The pump was held to one breast until completely emptied, and an aliquot was taken for analysis. Samples were placed in the home freezer and stored for a maximum of 3 d, transported on dry ice, then stored at −70°C until analysis.

Blood was collected by a physician on days 1 and 21. Maternal blood was sampled by brachial arm venipuncture, and infant blood by heel prick.

Sample analysis. Total lipid was extracted from human milk using a modified Folch procedure (38). Total lipid weight was determined gravimetrically. Lipids were converted to methyl esters for analysis by GLC (39). Fatty acid methyl esters were dissolved in heptane to achieve a concentration of approximately 35 g/L for GLC analysis.

GLC analysis was performed on a Varian Vista 6000 gas chromatograph (Varian Associates, Sunnyvale, CA) using a Supelcowax 10 fused silica capillary column (30 m × 0.34 mm (Supelco Inc., Bellefonte, PA). Temperature programming was as follows: 180°C for 20 min, then increased 5°C/min to the final temperature of 200°C, and held for 20 min.

Quantification was based on relative peak areas as determined by a Hewlett-Packard 3390A integrator (Hewlett-Packard Co., Avondale, PA). Peak identification was made based on relative retention times of two external standard mixtures, PUFA1 and PUFA2 (Supelco).

Blood for the analysis of erythrocyte fatty acid composition was collected and prepared according to the method of Rose and Oklander (40), and fatty acids were methylated for GLC analysis as described above.

Blood for the measurement of platelet aggregation was collected separately into citrated vacutainer tubes. Platelet-rich plasma was obtained by centrifugation at 120 × *g* for 10 min. Platelet-poor plasma was obtained by centrifuging the remaining blood at 1,500 × *g* for 15 min. Platelet-rich and platelet-poor plasmas were prepared within one hour, and aggregation response was measured within 6 h of sample collection. Platelet aggregation response to 1, 2 and 4 μM concentrations of adenosine diphosphate (ADP) was measured in platelet-rich plasma using a Chronolog-Lumi Aggregometer (Chronolog, Philadelphia, PA).

Milk lipid for tocopherol analysis was extracted by the modified Folch prcoedure described above and dissolved in hexane for high-performance liquid chromatography (HPLC). Plasma lipids were extracted by the method of Driscoll *et al.* (41). Lipid weight was determined gravimetrically. Instrumentation for HPLC analysis and methods for quantification of vitamin E in both milk and plasma samples were as described by Lammi-Keefe (42).

Statistical analysis. Erythrocyte fatty acid composition, tocopherol levels in milk and plasma and platelet aggregation response were analyzed by a paired T-test (43). Components in milk samples taken at 4-h intervals during the day were analyzed by repeated measures analysis of variance (ANOVA) (44).

To determine if there was a plateauing, after an initial rise in the n-3 PUFA content of baseline milk samples, a two-step procedure in SAS was used (45). First, a spline model was fitted through the data to characterize the shape of the curve and to determine the approximate location of the knot, the point at which no further increase was observed. For data to the right of the knot, the hypothesis that there was no trend in the data was tested using repeated measures univariate ANOVA. The significance of the linear, quadratic and cubic components of the orthagonal polynomial contrasts generated by the SAS statement summary were analyzed.

RESULTS

Five mother-infant pairs participated in the study. All subjects who volunteered completed the study, and no deleterious side effects were reported. Infants were full term, between 37–42 wk gestation, and gained weight normally (mean 35 g per day) during the study. Maternal weight for all subjects remained constant within 1 kg.

Maternal diet analysis. Nutrient composition was analyzed for each of the 6 d of food record data collected, and an average intake calculated. The average intake of α-tocopherol for subjects during this study was 1.9 mg (range 0.8–5.1 mg). Subjects consumed an average of 34% of their calories as fat with a polyunsaturated to saturated fat (P/S) ratio of 0.4 and 376 mg cholesterol per day.

Average daily intake of n-3 fatty acids, the n-6/n-3 ratio and the change in dietary fatty acid composition when fish oil was supplemented are shown in Table 1. The study population consumed an average of 1,148 mg of dietary n-3 PUFA with 121 mg from the n-3 VLC-PUFA. Fish oil supplementation tripled dietary intake of n-3 PUFA to an average of 3,093 mg. Over 15 times more n-3 VLC-PUFA, 2,007 mg, was consumed. Total n-6/n-3 PUFA ratio decreased from 9 to 3.

Daily measurement of the fatty acid composition of human milk. The content of individual fatty acids in human milk before and after the study period is presented in Table 2. No significant changes in 18:2n-6 or 18:3n-3 were noted. Increases were: total n-3 VLC-PUFA 162%, EPA 525%, DPA 143% and DHA 89%. Total n-6 VLC-PUFA levels decreased 30%, and the ratio of n-6/n-3 VLC-PUFA decreased 275%. The ratio of total n-6/n-3 fatty acids decreased 45%. The P/S ratio of the milk was similar to that of the maternal diet and was not affected by the supplement.

Mean total milk lipid levels (±SD) for the 5 subjects were 2.74 ± 1.06 g/dL on day 1, and 2.10 ± 0.83 g/dL on day 21, not significantly different.

Analysis of the daily changes in fatty acid composition during the study are presented in Figure 1. There was a significant linear increase ($P = 0.001$) in total saturated fatty acid content and a quadratic decrease in total monounsaturated fatty acid content ($P = 0.02$) during the study (data not shown). The LC n-3 PUFA in human milk were best fit to a cubic-constant spline model. The equation for this model is as follows:

$$\text{Wt\%} = \text{BO} + \text{B1Z} + \text{B2U} + \text{E} \qquad [1]$$

where $z = [(\text{Day-6}) - (\text{Day-6})_+]$, $U = [(\text{Day-6})^2 - (\text{day-6})_+^2]$, $w = [(\text{Day-6})^3 - (\text{Day-6})_+^3]$.

Figure 1a–d shows the plot of EPA, DPA and DHA, and total n-3 VLC-PUFA contents using this model. For EPA, the knot was estimated to be at day 6.2, with correlation coefficient (r/2) of 0.38, and parameter estimates B/O = 0.4424, B/1 = 0.1357, B/2 = 0.0578 and B/3 = 0.0090. For DPA, the knot was estimated to be at day 6.6, with r/2 of 0.43 and parameter estimates B/O = 0.3463, B/1 = 0.3463, B/1 = 0.0711, B/2 = 0.0208 and B/3 = 0.0078. For DHA, the knot was estimated to be at day 6.3, with r/2 of 0.22, and parameter estimates B/O = 0.6604, B/1 = 0.1974, B/2 = 0.0851 and B/3 = 0.0115. For total n-3 VLC-PUFA (EPA + DPA + DHA) content, the knot was estimated to be at day 6.3, with r/2 of 0.35, and parameter estimates B/O = 1.446, B/1 = 0.4450, B/2 = 0.1858 and B/3 = 0.0263. Univariate analysis supported the hypothesis that there was no significant change to the right of the knot.

Figure 1E is the plot of total n-6 VLC-PUFA (20:2 + 20:3 + 20:4 + 22:4) content during the study. The decline in n-6 fatty acid content was best depicted using a cubic-linear spline model because levels continued to decrease significantly to the right of the knot ($P = 0.004$). Nonlinear estimation of the knot was day 6.5, with r/2 of 0.30. The equation for the cubic-linear model is:

$$\text{wt\%} = \text{B/O} + \text{B/1 (Day L)} + \text{B/2U} + \text{b/3W} + \text{E} \qquad [2]$$

TABLE 1

Dietary Intake of n-3 Fatty Acids[a]

	18:3n-3 (mg/d)	20:5n-3 (mg/d)	22:5n-3 (mg/d)	22:6n-3 (mg/d)	n-6/n-3 ratio (mg/d)
Mean ± SD	1027 ± 352	31 ± 42	7 ± 3	82 ± 106	9 ± 2
Range	760–1574	4–103	4–10	18–267	6–11
+FO	1086	1120	22	864	3

[a]Values are based on 6 d of food record analysis. +FO denotes increased intake with fish oil supplementation. SD, standard deviation.

TABLE 2

Fatty Acid Composition of Human Milk Before and After Supplementation with Fish Oil[a]

Fatty acids	Day 1	Day 21
12:0	4.8 ± 1.9	5.4 ± 2.0
14:0	5.3 ± 1.5	6.5 ± 1.6
16:0	20.6 ± 2.3	21.2 ± 3.0
16:1	2.9 ± 0.7	2.8 ± 0.7
18:0	6.5 ± 1.6	.8 ± 1.5
18:1	38.6 ± 3.1	35.0 ± 2.5
18:2n-6	13.0 ± 1.7	12.5 ± 3.0
18:3n-6	0.15 ± 0.07	0.15 ± 0.12
18:3n-3	0.77 ± 0.12	0.76 ± 0.23
20:1	0.67 ± 0.20	0.73 ± 0.11
20:2n-6	0.39 ± 0.12	0.23 ± 0.05
20:3n-6	0.55 ± 0.16	0.45 ± 0.26
20:4n-6	0.67 ± 0.11	0.52 ± 0.10
20:5n-3	0.08 ± 0.04	0.50 ± 0.12[b]
22:1	0.20 ± 0.06	0.18 ± 0.05
22:4n-6	0.24 ± 0.16	0.21 ± 0.15
22:5n-3	0.14 ± 0.05	0.34 ± 0.08[b]
22:6n-3	0.37 ± 0.26	0.70 ± 0.12[b]
Total polyunsaturated	16.5 ± 1.6	16.5 ± 3.2
Total saturated	37.2 ± 3.6	39.9 ± 3.8[b]
P/S ratio	0.45 ± 0.08	0.42 ± 0.12
Total n-6	15.0 ± 1.5	14.1 ± 2.9
Total n-3	1.4 ± 0.4	2.3 ± 0.5
n-6/n-3 ratio	11.0 ± 2.7	6.1 ± 1.0
Total monounsaturated	42.4 ± 2.9	38.6 ± 2.7[b]
Total n-6 VLC-PUFA	1.85 ± 0.34	1.42 ± 0.29[b]
Total n-3 VLC-PUFA	0.59 ± 0.35	1.55 ± 0.29[b]
n-3/n-3 VLC-PUFA ratio	3.60 ± 1.10	0.96 ± 0.30[b]

[a]Values are daily mean wt% ± SD (n = 5). P/S, polyunsaturated/saturated ratio. VLC-PUFA, very long-chain-polyunsaturated fatty acids.

[b]Values are significantly different by nonlinear regression analysis.

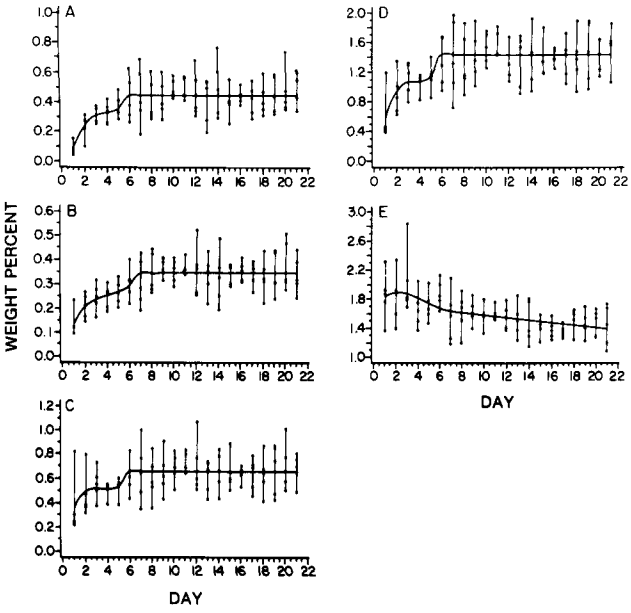

FIG. 1. Fatty acids in human milk during fish oil supplementation, plot of spline fit over raw data. A. Eicosapentaenoic acid (EPA), cubic-constant spline, assumed knot 6; B. Docosapentaenoic acid (DPA), cubic-constant spline, assumed knot 7; C. Docosahexaenoic acid (DHA), cubic-constant spline, assumed knot 6; D. Total n-3 very long-chain polyunsaturated fatty acid (VLC-PUFA), cubic-constant spline, assumed knot 6; E. Total n-6 VLC-PUFA, cubic-linear spline, assumed knot 7.

where Day L = (day-7), U = $[(\text{Day } 7)^2 - (\text{day-}7)_+^2]$, w = $[(\text{Day-}7)^3 - (\text{Day-}7)_+^3]$. Parameter estimates for this model are B/O = 1.635, B/1 = 0.0179, B/2 = 0.0260 and B/3 = 0.0039.

n-3 PUFA composition of milk sampled at four-hour intervals. Figure 2 represents the change in total n-3 LC-PUFA content of milk measured at 4-h intervals on days 1, 7, 14 and 21. Time 0 is the baseline level prior to taking the fish oil capsules for that day. Samples were then taken at 4, 8, 12 and 16 h postsupplementation. On day 1, LC n-3 PUFA content of the milk was significantly increased ($P = 0.0001$) by 8 h postsupplementation, and peak increases were observed at 12 h. The increase in milk LC n-3 PUFA resulting from fish oil ingestion continued on days 7 ($P = 0.02$), 14 ($P = 0.007$) and 21 ($P = 0.001$).

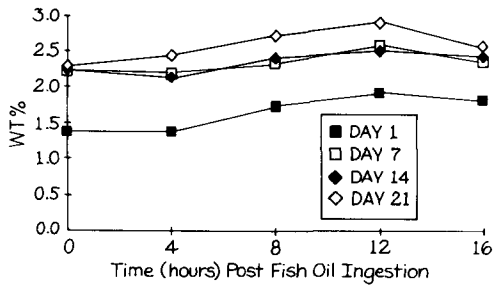

FIG. 2. Total n-3 polyunsaturated fatty acids (PUFA) content of human milk measured at four-hour intervals postsupplementation. Curves represent mean values (n = 5).

Erythrocyte fatty acid composition. The change in fatty acid composition of maternal erythrocytes is shown in Table 3. Significant increases were observed for EPA (583%) and DPA (150%) and for total n-3 PUFA (148%) contents. The n-6/n-3 fatty acid ratio was significantly decreased (77%) after fish oil supplementation. Although there was a trend toward increased DHA content (124%), this was not statistically significant. No significant changes were observed for the other fatty acids analyzed.

Infant erythrocyte fatty acid composition is shown in Table 4. Supplementation resulted in significantly increased EPA (636%) and DPA (260%) content, decreased n-6/n-3 fatty acid ratio (78%), and no change in DHA content (136%), similar to maternal changes.

Maternal platelet aggregation. Maternal platelet aggregation was measured in response to 1, 2 and 4 μM concentrations of ADP. Similar results were obtained at all ADP concentrations. Although there was no significant difference between mean measurements, some changes in response to fish oil supplementation were noted. Mean total aggregation response (%) to 4 μM ADP was 85 ± 12 on day 0, and 78 ± 3 on day 21. Total aggregation decreased 21% in subject 1, 7% in subject 2, 12% in subject 4, 5% in subject 5, and increased 10% in subject 3. Mean time to reach 50% aggregation was 24 ± 5 s on day 0 and 33 ± 12 s on day 21. Time to reach 50% aggregation doubled after supplementation in subjects 1 and 2, while subjects 3–5 showed little or no change.

Tocopherol content of human milk and plasma. No significant differences were observed in the tocopherol content of human milk or plasma from mothers or infants measured before and after the supplementation period. Mean α- and γ-tocopherol contents of milk and plasma are listed in Table 5.

TABLE 3

Change in Maternal Erythrocyte Fatty Acid Composition After Fish Oil Supplement[a]

Fatty acids	Day 0	Day 21
14:0	1.7 ± 2.4	0.5 ± 0.2
16:0	22.3 ± 2.5	20.4 ± 1.1
16:1	1.0 ± 1.1	0.1 ± 0.1
18:0	13.0 ± 2.2	14.3 ± 1.3
18:1	20.1 ± 6.7	17.2 ± 2.1
18:2n-6	8.2 ± 2.6	9.6 ± 1.2
18:3n-3	0.09 ± 0.06	0.03 ± 0.04
20:1	0.45 ± 0.11	0.43 ± 0.15
20:2n-6	0.45 ± 0.40	0.26 ± 0.06
20:3n-6	1.4 ± 0.54	1.5 ± 0.38
20:4n-6	12.9 ± 5.2	14.6 ± 1.3
20:5n-3	0.24 ± 0.16	1.4 ± 0.37[b]
22:1	0.35 ± 0.34	0.53 ± 0.35
22:4n-6	3.8 ± 1.4	4.1 ± 0.3
22:5n-3	1.4 ± 0.5	2.2 ± 0.3[c]
22:6n-3	4.5 ± 1.7	5.6 ± 1.1
Total PUFA	32.9 ± 11.3	39.2 ± 1.4
Total n-6	26.7 ± 9.2	30.0 ± 0.76
Total n-3	6.2 ± 2.2	9.2 ± 1.6[c]
n-6/n-3 ratio	4.3 ± 0.28	3.3 ± 0.56[b]

[a]Values are mean wt% ± SD (n = 5). PUFA, polyunsaturated fatty acids.

[b]Significantly different from day 0 ($P = 0.05$).

[c]Significantly different from day 0 ($P = 0.01$).

TABLE 4

Change in Infant Erythrocyte Fatty Acid Composition After Fish Oil Supplement[a]

Fatty acids	Day 0	Day 21
14:0	2.6 ± 3.7	0.84 ± 0.53
16:0	23.8 ± 3.1	20.9 ± 0.95
16:1	0.98 ± 1.3	0.16 ± 0.22
18:0	14.2 ± 1.5	15.4 ± 1.5
18:1	18.7 ± 6.0	15.2 ± 1.7
18:2n-6	5.6 ± 1.9	7.9 ± 0.33
18:3n-3	0.11 ± 0.15	—
20:1	0.26 ± 0.15	0.49 ± 0.08
20:2n-6	0.81 ± 0.22	0.58 ± 0.10
20:3n-6	1.7 ± 0.67	1.7 ± 0.21
20:4n-6	13.0 ± 6.2	15.0 ± 1.5
20:5n-3	0.11 ± 0.06	0.70 ± 0.34[b]
22:1	0.49 ± 0.33	0.47 ± 0.44
22:4n-6	3.4 ± 1.7	3.6 ± 0.63
22:5n-3	0.30 ± 0.15	0.78 ± 0.13[c]
22:6n-3	4.5 ± 2.2	6.1 ± 1.2
Total PUFA	29.5 ± 12.6	36.4 ± 3.3
Total n-6	24.5 ± 10.4	28.8 ± 1.9
Total n-3	5.1 ± 2.2	7.6 ± 1.5
n-6/n-3 ratio	5.0 ± 0.55	3.9 ± 0.66[b]

[a]Values are mean wt% ± SD (n = 5). PUFA, polyunsaturated fatty acids.

[b]Significantly different from day 0 ($P = 0.05$).

[c]Significantly different from day 0 ($P = 0.01$).

DISCUSSION

This study assessed the changes in human milk and maternal and infant erythrocytes in response to daily fish oil supplementation.

Analysis of human milk during fish oil supplementation. Fatty acids with chain lengths greater than C_{16} are derived from the diet and mobilized from adipose tissue stores. Because maternal weight remained stable during the study, it is assumed that the majority of milk fatty acids were derived from dietary sources (46).

In milk sampled at 4-h intervals on day 1, the n-3 VLC-PUFA content increased within 8 h after ingestion of the supplement. Peak levels occurred by 12 h and began to decline by 16 h. These results are in agreement with the findings of Hachey *et al.* (47) that peak fatty acid enrichment of breast milk occurred 8 to 10 h after consuming a labeled triglyceride meal.

Evidence indicates that the fatty acid composition of human milk does not exhibit diurnal variation (48–50). In the present study, samples taken at 4-h intervals had a significant rise in n-3 VLC-PUFA levels at 8 and 12 h postsupplementation on all days tested (see Fig. 2). It is not clear whether this within-day variation would persist if an equivalent level of n-3 VLC-PUFA was consumed in the form of a fish meal, or what effect smaller doses throughout the day would have on this variation. This observation is important to consider when planning a supplementation trial or comparing results between studies. Consistency in timing of sample collection is necessary for appropriate assessment and analysis of results.

Only one study has previously examined the change in fatty acid composition of human milk during fish oil supplementation. Harris *et al.* (28) supplemented 6 lactating women with 5 g of fish oil per day for 28 d. Samples were taken at only three time points, on days 0, 14 and 28. The authors also studied the effect of higher intakes for shorter time periods, again collecting only baseline, mid, and endpoint milk samples. Dose response-related increases were noted.

In the present study, daily milk samples were collected in order to formulate a model to characterize the change in fatty acid composition over the course of the supplementation period. The n-3 VLC-PUFA content increased most rapidly during the first and second day of supplementation, with smaller daily increases occurring between days 2 and 7. The n-3 VLC-PUFA content remained constant after day 7, at which point daily intake of the supplement served to maintain milk levels. There was a reciprocal decline in the n-6 VLC-PUFA content of the milk as n-3 fatty acid levels increased. Apparently, the synthesis and/or transport of n-6 VLC-PUFA were decreased by increased n-3 VLC-PUFA intake.

There was a decline in monounsaturated fatty acids and a significant increase in saturated fatty acids during the study period. These fatty acids did not exhibit response curves or knot estimates similar to those of the n-6 or n-3 fatty acids. Thus, it is not clear whether these changes were related to the supplementation trial or due to other factors, such as the stage of lactation. There is some evidence that 14:0 is increased, and 18:1 is decreased as lactation progresses (51), although Clark *et al.* (38) found that the fatty acid composition of mature milk remains constant between 2 and 16 wk *postpartum.*

Data from various studies indicate that human milk usually contains 0.03–0.2% EPA, trace–0.5% DPA and

TABLE 5

Tocopherol Status Before and After Fish Oil Supplement[a]

	α-Tocopherol		γ-Tocopherol	
Sample	Day 0	Day 21	Day 0	Day 21
Human milk (μg/L)	1779 ± 804	1592 ± 522	886 ± 290	1032 ± 439
Maternal plasma (μg/mL)	1.50 ± 0.6	1.49 ± 1.1	3.75 ± 1.1	3.48 ± 1.0
Infant plasma (μg/mL)	1.71 ± 1.9	4.17 ± 3.0	1.72 ± 0.3	2.06 ± 0.7

[a]Values are mean ± standard deviation (SD) (n = 5).

0.1-0.6% DHA (17,19,20,52). The present study is the first to calculate the dietary intake of n-3 VLC-PUFA. Our data show that mean breast milk composition from women consuming about 1 g of total n-3 PUFA, with 120 mg from n-3 VLC-PUFA, was 0.08% EPA, 0.14% DPA and 0.37% DHA.

A comparison of the n-3 fatty acid composition of human milk from women supplemented with fish oil (29) to milk from Inuit women naturally consuming increased levels of n-3 VLC-PUFA (28) indicates that supplementation with over 10 g of fish oil is necessary to produce n-3 VLC-PUFA levels comparable to those found in Inuit breast milk. Recommendations to increase fish consumption to this level are impractical in our society, and the optimal level for health benefits and infant development may not need to be this high. Studies regarding the effect of maternal dietary changes on infant tissue fatty acid composition are crucial to determine dietary requirements for lactating women as well as for making appropriate recommendations regarding the composition of infant formulas.

Erythrocyte fatty acid composition. This study was the first to examine the change in fatty acid composition in response to maternal fish oil supplementation. It is interesting to note the large increase in EPA, the doubling of DPA, with no significant change in DHA content (Tables 3 and 4). There was no change in the n-6 fatty acid composition. These findings are in agreement with studies in adults, where fish or fish oil consumption resulted in a much greater increase in EPA than DHA in erythrocyte phospholipids, while 18:2n-6 and 20:4n-6 did not change or decreased only slightly (32).

The high level of DHA found in brain and retinal phospholipids is thought to be important to the physical properties of membranes in these tissues. The membrane content of DHA is tenaciously maintained in the face of deficiency (5). Subjects in this study consumed only 33% more EPA than DHA and a small amount (22 mg) of DPA per day (Table 1). The lack of increase in DHA content, with correspondingly large increases in EPA and DPA, suggests that retroconversion of DHA to EPA and DPA may be occurring. This phenomenon has been demonstrated in platelet phospholipids (53) and is suggesetive of a regulatory mechanism which controls the level of DHA in cell membranes.

Increased tissue content of EPA is thought to be an important modulator of eicosanoid metabolism with potential cardiovascular benefits. The level required to confer such benefits without adverse effect remains uncertain.

Maternal platelet aggregation response. This is the first study to examine platelet aggregation in lactating women. Total aggregation response was similar to the mean of 78 ± 11% observed by Roper *et al.* (54) in 51 females. Some changes in platelet responsiveness were noted, although mean differences were not statistically significant. Decreased aggregation response was expected based on previous studies using similar dosages of fish oil for 3-4 wk (55,56), although Boberg *et al.* (57) also found no significant difference in ADP-induced platelet aggregation response after fish oil supplementation. The clinical relevance of the changes observed in this study remains to be determined.

Tocopherol content of diets, milk and plasma. Although dietary vitamin E was found to be below the recommended daily allowance (RDA) of 12 mg during lactation, only the α-tocopherol content of foods was calculated for this study, thus there was an underestimate of dietary tocopherol intake. As the tocopherol content of foods and daily intakes are widely variable and the available databases are incomplete with regard to the tocopherol content of foods, it is difficult to truly assess the adequacy of vitamin E intake in this study. Others have also found dietary intake to be below the RDA for vitamin E (58,59), although subjects maintained adequate plasma levels of vitamin E (59).

The tocopherol content of the milk sampled in this study was within the range observed by others (60-62). Maternal plasma α-tocopherol levels were lower than levels observed by others (63,64). This may be a manifestation of the low dietary intake noted in this study. Alternately, data regarding plasma tocopherol status during lactation are lacking, and levels may be different from nonlactating women. Infant plasma tocopherol was also somewhat lower than the 6.89 μg/mL observed in 4-15 d-old infants (65), despite comparable milk levels.

As no changes in tocoperhol levels were noted, this suggests that vitamin E deficiency is not induced by fish oil supplementation. However, fish oil supplementation provided mothers with an additional 6 mg of vitamin E per day, and the significance of this contribution to dietary intake was not determined. Knapp *et al.* (66) also found no change in serum α-tocopherol during fish oil supplementation.

Conclusion. Maternal fish oil supplementation increased the n-3 VLC-PUFA and decreased the n-6 VLC-PUFA contents of human milk. One week was required to reach steady state levels. Increases in milk n-3 VLC-PUFA were observed within 8-h postsupplementation, and a diurnal variation existed throughout the study. Maternal and infant erythrocyte fatty acid compositions were similarly affected with significant increases in EPA and DPA but no change in DHA or n-6 PUFA contents. No differences in mean tocopherol values or maternal platelet aggregation responses were noted.

ACKNOWLEDGMENTS

This work was supported by federal funds made available through the Hatch Act and by NIH contract NO1-HD6-2917.

REFERENCES

1. Dyerberg, J., Bang, H.O., and Aagard, O. (1980) *Lancet 1,* 199.
2. Budowski, P., and Crawford, M.A. (1985) *Proc. Nutr. Soc. 44,* 221-229.
3. Sanders, T.A.B., and Younger, K.M. (1981) *Br. J. Nutr. 45,* 613-616.
4. Sinclair, A.J. (1974) *Lipids 10,* 175-184.
5. Anderson, R.E., Benolken, R.M., Dudley, P.A., Landis, D.J., and Wheeler, T.G. (1974) *Exp. Eye Res. 18,* 205-213.
6. Benolken, R.M., Anderson, R.E., and Wheeler, T.G. (1973) *Science 182,* 1253-1254.
7. Wheeler, T.G., and Benolken, R.M. (1975) *Science 188,* 1312-1314.
8. Neuringer, M., Connor, W.E., Lin, D.S., Barstad, L., and Luck, S. (1986) *Proc. Natl. Acad. Sci. USA 83,* 4021-4025.
9. Lamptey, M.S., and Walker, B.L. (1976) *J. Nutr. 106,* 86-93.
10. Yamamoto, N., Saitoh, M., Moriuchi, A., Nomura, M., and Okuyama, H. (1987) *J. Lipid Res. 28,* 144-151.
11. Holman, R.T., Johnson, S.B., and Hatch, T.F. (1982) *Am. J. Clin. Nutr. 35,* 617-623.
12. Bozian, R.C., and Moussavian, S.N. (1982) *Am. J. Clin. Nutr. 36,* 1253-1262.

13. Carlson, S.E., Carver, J.D., and House, S.G. (1986) *J. Nutr. 116,* 718–725.
14. Hrboticky, N., MacKinnon, M.J., and Innis, S.M. (1990) *Am. J. Clin. Nutr. 51,* 173–182.
15. Leaf, A., and Weber, P.C. (1988) *N. Engl. J. Med. 318,* 548–557.
16. Salmon, J.A. (1987) *n-3 News 2,* 1–3.
17. Sanders, T.A.B., and Naismith, D.J. (1979) *Br. J. Nutr. 41,* 619–623.
18. Olegard, R., and Svennerholm, L. (1971) *Acta Paediatr. Scand. 60,* 505–511.
19. Putnam, J.C., Carlson, S.E., DeVoe, P.W., and Barness, L.A. (1982) *Am. J. Clin. Nutr. 36,* 106–114.
20. DeLucchi, C., Pita, M.L., Faus, M.J., Periage, J.L., and Gil, A. (1988) *Ann. Nutr. Metab. 32,* 231–239.
21. Carlson, S.E., Rhodes, P.G., Rao, V.S., and Goldgar, D.E. (1987) *Pediatr. Res. 21,* 507–510.
22. Uauy, R. (1990) *J. Pediatr. Gastroenterol. Nutr. 11,* 296–302.
23. Vuori, E., Kiuru, K., Makinen, S.M., Vayrynen, P., Kara, R., and Kuirunen, P. (1982) *Acta. Paediatr. Scand. 71,* 959–963.
24. Mellies, M.J., Ishikawa, T.T., Gartside, P.S., Burton, K., MacGee, J., Allen, K., Steiner, P.M., Brady, D., and Gleuck, C.J. (1979) *Am. J. Clin. Nutr. 32,* 299–303.
25. Finley, D.A., Lonnerdal, B., Dewey, K.G., and Grivetti, L.E. (1985) *Am. J. Clin. Nutr. 41,* 787–800.
26. Sanders, T.A.B., Ellis, F.R., and Dickerson, J.W.T. (1978) *Am. J. Clin. Nutr. 31,* 805–813.
27. Innis, S.M., and Kuhnlein, H.V. (1988) *Early Hum. Dev. 18,* 185–189.
28. Harris, W.S., Connor, W.E., and Lindsey, S. (1984) *Am. J. Clin. Nutr. 40,* 780–785.
29. Ruter, A., Jongbloed, A.W., vanGent, C.M., Danse, L.H.J.C., and Metz, S.H.M. (1978) *Am. J. Clin. Nutr. 31,* 2159–2166.
30. Hartog, J.H., Lamers, J.M.J., Montfuort, A., Becker, A.E., Klompe, M., Morse, H., Tencate, F.J., Vanderweef, L., Hulsman, W.C., Hugonholtz, P.G., and Verdouw, P.D. (1987) *Am. J. Clin. Nutr. 46,* 258–266.
31. Hu, M.L., Frankel, E.N., Leibovitz, B.E., and Tappel, A.L. (1989) *J. Nutr. 119,* 1574–1582.
32. Kinsella, J.E. (1987) *Seafoods and Fish Oils in Human Health and Disease,* Marcel Dekker, New York.
33. Stuff, J.E., Garza, C., O'Brien-Smith, E., Nichols, B.L., and Montandon, C.M. (1983) *Am. J. Clin. Nutr. 37,* 300–306.
34. Beaton, G.H., Milner, J., Corey, P., McGuire, V., Cousins, M., Stewart, E., deRamos, M., Hewitt, D., Gransbach, P.V., Kassim, N., and Little, J.A. (1979) *Am. J. Clin. Nutr. 32,* 2546–2554.
35. Posner, B.M., Borman, C.L., Morgan, J.L., Borden, W.S., and Ohls, J.C. (1982) *Am. J. Clin. Nutr. 36,* 546–553.
36. Pennington, J.A.T., and Church, H.N. (1985) *Food Values of Portions Commonly Used,* J.B. Lippincott Company, Philadelphia.
37. United States Department of Agriculture (1976) *Composition of Foods,* Agricultural Handbook No. 8, Agricultural Research Service, Washington, D.C.
38. Clark, R.M., Ferris, A.M., Fey, N., Brown, P.B., Hundreiser, K.W., and Jensen, R.G. (1982) *J. Pediatr. Gastroenterol. Nutr. 1,* 311–315.
39. Lee, T.W. (1987) *J. Assoc. Off. Anal. Chem. 70,* 702–705.
40. Rose, H.G., and Oklander, M. (1965) *J. Lipid. Res. 6,* 428–431.
41. Driscoll, W.J., Neese, J.W., Bryant, C.C., and Bashor, M.M. (1982) *J. Chromatog. 231,* 439–444.
42. Lammi-Keefe, C.J. (1986) *J. Pediatr. Gastroenterol. Nutr. 5,* 934–937.
43. Frude, N. (1987) *A Guide to SPSS/PC+,* Springer-Verlag, New York.
44. Norusis, M.J. (1988) *SPSS Advanced Statistics V2.0,* SPSS Inc., Chicago.
45. Fruend, R.J., Littell, R.C., and Spector, P.C. (1986) *SAS Systems for Linear Models, 1986 Edition,* SAS Institute, Inc., Cary.
46. Insull, W., Hirsch, J., James, T., and Ahrens, Jr., E.H. (1959) *J. Clin. Invest. 38,* 443–450.
47. Hachey, D.L., Thomas, M.R., Emkin, E.A., Garza, C., Brown-Booth, L., Adlof, R.O., and Klein, P.D. (1987) *J. Lipid Res. 28,* 1158–1192.
48. Guthrie, H.A., Picciano, M.F., and Sheehe, D. (1977) *J. Pediatr. 90,* 39–41.
49. Hall, B. (1979) *Am. J. Clin. Nutr. 32,* 304–312.
50. Harzer, G., Haug, M., Dieterich, I., and Gentner, P.R. (1983) *Am. J. Clin. Nutr. 37,* 612–621.
51. Lauber, E., and Reinhardt, M. (1979) *Am. J. Clin. Nutr. 32,* 1159–1173.
52. Boersma, E.R., Offringa, P.J., Muskiet, F.A.J., Chase, W.M., and Simmons, I.J. (1991) *Am. J. Clin. Nutr. 53,* 1197–1204.
53. vonSchacky, C., and Weber, P.C. (1985) *J. Clin. Invest. 76,* 2446–2450.
54. Roper, P., Drewinko, B., Hasler, D., Johnston, D., Hester, J., and Freireich, E.J. (1979) *Am. J. Clin. Pathol.,* 263–268.
55. Nagakawa, Y., Orimo, H., Harasawa, M., Morita, I., Yashiro, K., and Murota, S. (1983) *Atherosclerosis 47,* 71–75.
56. Lammi-Keefe, C.J., Hammerschmidt, D.E., Weisdorf, D.J., and Jacob, H.S. (1982) *Inflammation 3,* 227–234.
57. Boberg, M., Vessby, B., and Selinus, I. (1986) *Acta Med. Scand. 220,* 153–160.
58. Murphy, S.P., Subar, A.F., and Block, G. (1990) *Am. J. Clin. Nutr. 28,* 756–760.
59. Witting, L.A., and Lee, L. (1975) *Am. J. Clin. Nutr. 28,* 571–576.
60. Kobayashi, H., Kanno, C., Yamauchi, K., and Tsugo, T. (1975) *Biochim. Biophys. Acta 380,* 282–290.
61. Jansson, L., Akesson, B., and Holmberg, L. (1981) *Am. J. Clin. Nutr. 34,* 8–13.
62. Chappell, J.E., Francis, T., and Clandinin, M.T. (1986) *Nutr. Res. 6,* 849–852.
63. Chow, C.K. (1975) *Am. J. Clin. Nutr. 28,* 756–760.
64. Meydani, M., Cohn, J.S., Macauley, J.B., McNamara, J.R., Blumberg, J.B., and Schaefer, E.J. (1989) *J. Nutr. 119,* 1252–1258.
65. Ciccoli, H.Y., Berti, D., and Bracci, R. (1981) *Biol. Neonate 40,* 187–195.
66. Knapp, H.R., Reilly, I.A.G., Allesandrini, P., and FitzGerald, G.A. (1986) *N. Engl. J. Med. 310,* 1209–1212.

[Received November 14, 1991, and in revised form September 11, 1992; Revision accepted September 14, 1992]