

Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk¹⁻³

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ABSTRACT

Background: Flaxseed oil is a rich source of 18:3n-3 (α -linolenic acid, or ALA), which is ultimately converted to 22:6n-3 (docosahexaenoic acid, or DHA), a fatty acid important for the development of the infant brain and retina.

Objective: The objective of this study was to determine the effect of flaxseed oil supplementation on the breast-milk, plasma, and erythrocyte contents of DHA and other n-3 fatty acids in lactating women.

Design: Seven women took 20 g flaxseed oil (10.7 g ALA) daily for 4 wk. Breast-milk and blood samples were collected weekly before, during, and after supplementation and were analyzed for fatty acid composition.

Results: Breast milk, plasma, and erythrocyte ALA increased significantly over time ($P < 0.001$) and after 2 and 4 wk of supplementation ($P < 0.05$). Over time, 20:5n-3 (eicosapentaenoic acid, or EPA) increased significantly in breast milk ($P = 0.004$) and in plasma ($P < 0.001$). In addition, plasma EPA increased significantly ($P < 0.05$) after 2 and 4 wk of supplementation. There were significant increases over time in breast-milk 22:5n-3 (docosapentaenoic acid, or DPA) ($P < 0.02$), plasma DPA ($P < 0.001$), and erythrocyte DPA ($P < 0.01$). No significant changes were observed in breast-milk, plasma, or erythrocyte DHA contents after flaxseed oil supplementation.

Conclusions: Dietary flaxseed oil increased the breast-milk, plasma, and erythrocyte contents of the n-3 fatty acids ALA, EPA, and DPA but had no effect on breast-milk, plasma, or erythrocyte DHA contents. *Am J Clin Nutr* 2003;77:226-33.

KEY WORDS Fatty acids, breast milk, human milk, α -linolenic acid, ALA, docosahexaenoic acid, DHA, eicosapentaenoic acid, EPA, docosapentaenoic acid, DPA, long-chain polyunsaturated fatty acids, arachidonic acid, flaxseed oil

INTRODUCTION

Fifty percent of the energy in human milk is supplied by fat, which is necessary to provide energy for the rapid growth of the newborn infant. Fat also supplies n-3 and n-6 essential fatty acids needed to complete the development of the brain, retina, and other organs including the skin (1-4). The fatty acids of human milk are derived from 3 sources: mobilization of endogenous stores of fatty acids, de novo synthesis of fatty acids by the liver or breast tissue, and the diet (3, 5-9). The n-3 fatty acid, 22:6n-3 (docosahexaenoic acid, or DHA) and the n-6 fatty acid, 20:4n-6 (arachidonic acid, or AA) are stored in adipose tissue and can be

secreted into breast milk after mobilization. Dietary sources can supply DHA and AA directly, or DHA and AA can be synthesized from their precursors, which are α -linolenic acid (ALA) and linoleic acid, respectively.

The fatty acid composition of human milk reflects the type of dietary fat consumed by the mother, in both the short term and the long term (1, 10-13, and WE Connor and LF Hatcher, unpublished observations, 1987). The n-3 fatty acids are of particular interest because of their role in the development of the infant's brain and retina (1-3). Harris et al (10) reported dose-dependent increases in breast-milk DHA in women taking fish oil supplements for 1-4 wk. Helland et al (12) reported that 14 d of supplementation with cod liver oil increased breast-milk DHA and 20:5n-3 (eicosapentaenoic acid, or EPA). Francois et al (11) studied the effects of 6 dietary fats, including menhaden oil and herring oil, on breast-milk fatty acids for up to 6 d after ingestion of a single, fat-rich meal. Some fatty acids increased in breast milk within 6 h after the meal, probably as a result of transfer from circulating chylomicrons. These fatty acids peaked in breast milk between 10 and 24 h and remained significantly elevated for up to 3 d.

The effects of 10 different fat supplements on the fatty acid composition of human milk were measured (WE Connor and LF Hatcher, unpublished observations, 1987). After daily supplementation with 40 g flaxseed oil (a good source of the n-3 fatty acid, ALA) for 10 d in 3 lactating women, breast-milk ALA increased significantly, as expected. However, DHA did not increase in breast milk.

Fish oil is a rich source of DHA, and lactating women who eat fish on a regular basis have much higher quantities of DHA in breast milk than do mothers who do not eat much fish (14-16). However, some lactating women are vegetarians, and others do not like fish or have limited access to fish. Flaxseed oil is rich in ALA, the precursor fatty acid in the synthetic steps that ultimately result in DHA. Studies have shown that human adults convert very

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TABLE 1
Fatty acid composition of flaxseed oil¹

Fatty acid	Content of flaxseed oil % by wt of total fatty acids
12:0	0.0
14:0	0.1
16:0	5.4
18:0	3.6
20:0	0.0
ΣSFAs ²	9.1
18:1n-9	0.0
20:1n-9	20.5
22:1n-11	0.0
ΣMUFAs ³	20.6
18:2n-6	15.2
20:3n-6	0.1
20:4n-6	0.1
Σn-6 ⁴	15.8
18:3n-3	53.6
18:4n-3	0.02
20:4n-3	0.02
20:5n-3	0.0
22:5n-3	0.0
22:6n-3	0.0
Σn-3 ⁵	53.7

¹ \bar{x} .²Total saturated fatty acids (SFAs), calculated by adding 8:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, and 24:0.³Total monounsaturated fatty acids (MUFAs), calculated by adding 14:1n-5, 16:1n-7, *trans* 18:1n-9, 18:1n-9 plus 18:1n-7, 20:1n-9, 22:1n-11, 22:1n-9, and 24:1n-9.⁴Total n-6 fatty acids, calculated by adding 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6, 22:3n-6, 22:4n-6, and 22:5n-6.⁵Total n-3 fatty acids, calculated by adding 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

little ALA to EPA or DHA (17–19); this is also the case with laying hens (20). Our hypothesis was that supplementation of longer duration with ALA would increase the synthesis and amount of DHA in breast milk because infants require this fatty acid for brain and retinal development, and perhaps there is some signaling that enhances DHA concentrations.

SUBJECTS AND METHODS

Subjects

Nine healthy lactating women aged 28–39 y enrolled in this study. The subjects were recruited between 2 and 11 mo postpartum through the Oregon Health and Science University campus newsletter, e-mail announcements, word of mouth, and direct contact with study investigators. The women remained in the study for 10 wk, which included a 2-wk washout period at baseline (for subjects to stabilize their dietary intake of ALA and other n-3 fatty acids), a 4-wk flaxseed oil supplementation period, and a 4-wk postsupplementation period. One participant stopped taking the supplements immediately because of side effects, and another subject never collected the baseline breast-milk samples; therefore, 7 women completed the study. Informed consent was obtained from each subject. The study was approved by the

Oregon Health and Science University Institutional Review Board, Committee on Human Research.

Dietary assessment

To monitor dietary consistency, maternal diets were assessed 3 times: at baseline, after 4 wk of flaxseed oil supplementation, and after the 4-wk postsupplementation period. Subjects were advised not to change their current dietary intake of foods and oils containing ALA and other n-3 fatty acids. An eating habits questionnaire, the Diet Habit Survey (21), was used to assess dietary intakes of cholesterol, saturated fat, total fat, carbohydrate, and fish; it was scored by a trained dietitian. This questionnaire was developed by the nutrition staff in the Section of Clinical Nutrition and Lipid Metabolism, Department of Medicine at Oregon Health and Science University. For each subject, the scores obtained included the Cholesterol-Saturated Fat Index, Carbohydrate Score, Fish Score, and Total Score.

Flaxseed oil supplementation

During the 4-wk supplementation period, subjects took 20 g flaxseed oil daily (Spectrum Essentials Veg-Omega 3 Cold Pressed Organic Flax Oil; Spectrum Naturals Inc, Petaluma, CA). The supplements provided a total of 10.7 g ALA/d. Subjects were instructed to take the supplements 3 times/d (total of 20 capsules/d; each capsule contained 1 g flaxseed oil). The fatty acid composition of the flaxseed oil used in this study is reported in **Table 1**. As indicated, flaxseed oil is rich in ALA (53.6% of fatty acids). Another polyunsaturated fatty acid of interest was linoleic acid (15.2% of the total fatty acids in flaxseed oil). The flaxseed oil contained no EPA, 22:5n-3 (docosapentaenoic acid, or DPA), or DHA. The fat in the supplements was 69.5% polyunsaturated fatty acids, 9.1% saturated fatty acids, and 20.6% monounsaturated fatty acids.

Breast-milk collection and analysis

A total of 10 breast-milk samples were collected from each subject at the following time points: 1 sample at study entry, 1 sample after a 2-wk washout period (the baseline sample), 4 samples at weekly intervals during the 4-wk supplementation period, and 4 samples at weekly intervals during the 4-wk postsupplementation period. During the supplementation period, subjects were instructed to collect their milk samples in the morning, before taking the supplements.

Subjects were instructed to collect mid-feeding milk samples by nursing until the first breast was partially emptied, switching to the second breast, and then expressing the milk from the partially emptied first breast into a 5-cc plastic vial, either manually or by using a breast pump. Immediately after collection, the milk samples were placed upright in the subject's freezer (to prevent leakage and contamination). The samples were stored frozen until the next study appointment, when they were transported on ice to the Clinical Research Center. There, the samples were immediately placed in a freezer (-40 °F) until subsequent analysis.

For analysis, the milk samples were thawed and shaken vigorously. The fatty acids of breast milk and the flaxseed oil were saponified in alcoholic KOH and extracted into hexane. Fatty acid methyl esters were prepared with 12% BF₃ in methanol. They were analyzed by gas-liquid chromatography, as described by Anderson et al (20) and Anderson (22), on a Perkin-Elmer instrument (model Sigma 3B; Perkin-Elmer, Norwalk, CT) equipped with a hydrogen flame ionization detector and a 30-m SP-2330 fused silica capillary column with a 0.25-mm internal diameter and a 0.2-μm film thickness (Supelco, Bellefonte, PA).

Plasma and erythrocyte fatty acids

For analyzing the plasma and erythrocyte fatty acid profiles, 10 mL blood was drawn into tubes containing EDTA on 5 occasions: at the initial visit, after a 2-wk washout period (baseline sample), after 2 and 4 wk of supplementation with flaxseed oil, and 4 wk after the supplementation ended.

Plasma and erythrocytes were separated immediately by centrifugation at $1000 \times g$ for 10 min at 20 °C. The erythrocytes were washed twice with saline and the lipids of the erythrocytes were extracted with chloroform and isopropanol by using the procedure of Rose and Oklander (23); the use of isopropanol in place of methanol avoids extracting the heme pigment. Aliquots of both erythrocytes and plasma were saponified with 6% ethanolic KOH for 1 h at 37 °C. Fatty acids were extracted with hexane, acidified to remove the sterols, and extracted with hexane again. Lipids were liberated directly by incubation with ethanolic KOH. Methyl esters of the fatty acids were prepared by heating in 14% boron trifluoride-methanol (BF_3/MeOH) for 10 min at 100 °C in tightly sealed tubes with polytetrafluoroethylene-coated screw caps (24). All solvent evaporation was carried out under a gentle stream of nitrogen to reduce lipid peroxidation.

Fatty acid methyl esters were analyzed with gas-liquid chromatography on an instrument equipped with a hydrogen flame ionization detector (Perkin-Elmer model Sigma 3B) and a 30-m SP-2330 fused silica capillary column (Supelco, Bellefonte, PA). The temperatures of the column, detector, and injection ports were 195 °C, 250 °C, and 250 °C, respectively. Helium was used as the carrier gas; the inlet pressure was 80 psi. The split ratio was 1:170. The retention time and area of each peak were measured with an HP-3390 integrator (Hewlett-Packard, Palo Alto, CA); a computer (HP85; Hewlett Packard) was used to identify and quantify each individual fatty acid by percentage. A mixture of fatty acid standards was run daily.

Statistical methods

The data are reported as means \pm SDs. Statistical significance was defined as $P < 0.05$. One-way repeated-measures analysis of variance was used to test for significant differences between baseline values and values at other time points throughout the study for breast-milk, plasma, and erythrocyte fatty acids. The Bonferroni t test and Dunnett's method were then used to determine which time points were significantly different from baseline (25). If the normality test failed, the data were log transformed and used for the analysis. Paired t tests (25) were performed to compare initial and final scores on the Diet Habit Survey. Because the Carbohydrate Score data failed the normality test, a Mann-Whitney rank-sum test was used to analyze these data. SIGMASTAT for WINDOWS, version 2.0 (Jandel Scientific, San Rafael, CA) was used for all statistical computations.

RESULTS

Subjects' diets

The diets of the subjects remained constant during the study. There were no significant differences in their Cholesterol-Saturated Fat Index, Carbohydrate Score, Fish Score, or Total Score between the beginning of the study and the end of the study. The macronutrient composition of their diets remained stable, as did their fish intake. Subjects consumed 2–3 servings of fish/mo on average; the fish consumed consisted primarily of white fish (eg, tuna,

halibut, and snapper). The subjects consumed $\approx 28\%$ of their energy as fat, 9% as saturated fat, and 58% as carbohydrate. Subjects consumed < 250 mg cholesterol/d and 2600 mg Na/d on average.

Changes in breast-milk fatty acid composition during flaxseed oil supplementation

At baseline, the total fatty acid composition of the milk samples ($n = 7$) was 40.0% saturated fatty acids, 40.5% monounsaturated fatty acids, and 16.8% polyunsaturated fatty acids (Table 2). The polyunsaturated fatty acids consisted of $13.3 \pm 3.0\%$ linoleic acid, $1.0 \pm 0.3\%$ ALA, $0.1 \pm 0.0\%$ EPA, $0.1 \pm 0.2\%$ DPA, and $0.2 \pm 0.1\%$ DHA.

During supplementation with flaxseed oil, significant changes were observed in the fatty acid composition of the milk (Table 2). Breast-milk ALA increased significantly over time, from 1.0% of fatty acids at baseline to 6.8% of fatty acids after 1 wk of flaxseed oil supplementation. ALA remained elevated at the 2-wk and 4-wk time points. After 4 wk of supplementation, ALA peaked at 7.7% of fatty acids; it then returned to near baseline values (1.9% of fatty acids) as early as 1 wk after subjects discontinued supplementation. As expected, breast-milk monounsaturated fatty acids decreased significantly, from 40.5% of total fatty acids at baseline to 34.0% after 4 wk of supplementation.

The EPA content of breast milk increased significantly over time with flaxseed oil supplementation, from 0.08% at baseline to 0.14% after 1 wk, 0.13% after 2 wk, and 0.11% after 4 wk of supplementation. However, none of these time points were significantly different from baseline; only the trend was significant. Also, breast-milk DPA increased significantly over time during flaxseed oil supplementation, from 0.19% at baseline to 0.20% after 2 wk of supplementation. The value then decreased to 0.17% after 4 wk of supplementation. As with EPA, none of these time points were significantly different from baseline; only the trend was significant. Breast-milk DHA content did not increase over time or at any time points. It remained constant at ≈ 0.1 – 0.2% throughout the supplementation and postsupplementation periods.

At baseline, breast-milk linoleic acid accounted for 13.3% of total fatty acids. Although linoleic acid makes up 15.2% of the fatty acids in flaxseed oil, breast-milk linoleic acid did not change significantly during flaxseed oil supplementation. The ratio of $n-6$ to $n-3$ fatty acids in breast milk also decreased significantly, from 9.5 at baseline to 1.9 after 4 wk of supplementation. The breast-milk content of *trans* fatty acids did not change significantly during supplementation with flaxseed oil, remaining at $\approx 3\%$ of total fatty acids.

Changes in plasma fatty acid composition during flaxseed oil supplementation

During supplementation with flaxseed oil, there were significant changes from baseline in the fatty acid composition of maternal plasma (Table 3). Plasma ALA increased significantly over time and after both 2 wk and 4 wk of supplementation, from 0.6% at baseline to 2.8% at 2 wk and 3.4% at 4 wk. ALA returned to near baseline values (0.7% of total fatty acids) by 4 wk postsupplementation.

Plasma EPA increased significantly over time, from 0.7% at baseline to 1.5% after 2 wk and 1.6% after 4 wk of supplementation; it returned to 0.7% at 4 wk postsupplementation. Plasma DPA increased significantly over time, from 0.5% at baseline to 0.8% after both 2 wk and 4 wk of supplementation; it returned to 0.6% by 4 wk postsupplementation. Plasma DHA remained fairly

TABLE 2

Breast-milk fatty acid composition at baseline, after 2 and 4 wk of flaxseed oil supplementation, and at 4 wk postsupplementation¹

Fatty acid	Baseline (n = 7)	After 2 wk of supplementation (n = 7)	After 4 wk of supplementation (n = 4)	4 wk postsupplementation (n = 5)	P
% by wt of total fatty acids					
8:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	NS
10:0	1.1 ± 0.3	1.1 ± 0.2	1.2 ± 0.2	1.0 ± 0.5	NS
12:0	4.9 ± 1.3 ^a	5.7 ± 1.6 ^b	5.8 ± 0.9 ^b	5.0 ± 1.5 ^a	0.04
14:0	6.2 ± 1.8	6.4 ± 2.4	6.7 ± 1.3	6.8 ± 2.0	NS
16:0	19.8 ± 1.3	16.5 ± 2.3	17.8 ± 2.5	18.9 ± 1.6	NS
18:0	7.2 ± 1.2	6.0 ± 1.3	6.9 ± 0.7	7.1 ± 1.5	NS
20:0	0.06 ± 0.04	0.13 ± 0.06	0.10 ± 0.03	0.11 ± 0.03	0.04
ΣSFAs ²	40.0 ± 4.8	36.6 ± 5.9	39.5 ± 3.2	40.2 ± 6.4	NS
18:1n-9	35.0 ± 3.3 ^a	31.3 ± 3.4 ^a	27.9 ± 1.7 ^b	33.1 ± 3.9 ^a	0.01
<i>trans</i> 18:1n-9	2.6 ± 1.1	2.2 ± 0.9	3.1 ± 0.7	2.6 ± 1.1	NS
20:1n-9	0.7 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	NS
22:1n-11	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	NS
ΣMUFAs ³	40.5 ± 3.1 ^a	36.7 ± 3.5 ^a	34.0 ± 2.3 ^b	39.1 ± 3.7 ^a	0.03
18:2n-6	13.3 ± 3.0	15.5 ± 2.8	15.0 ± 1.2	15.1 ± 4.9	NS
20:3n-6	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	NS
20:4n-6	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	NS
Σn-6 ⁴	14.6 ± 3.0	16.9 ± 2.9	16.2 ± 1.2	16.4 ± 4.9	NS
18:3n-3	1.0 ± 0.3 ^a	7.3 ± 1.5 ^b	7.7 ± 1.0 ^b	1.4 ± 0.5 ^a	<0.001
20:5n-3	0.08 ± 0.03	0.13 ± 0.03	0.11 ± 0.01	0.07 ± 0.02	0.004
22:5n-3	0.19 ± 0.05	0.20 ± 0.04	0.17 ± 0.02	0.13 ± 0.02	<0.02
22:6n-3	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	NS
Σn-3 ⁵	1.6 ± 0.3 ^a	7.9 ± 1.5 ^b	8.2 ± 1.0 ^b	1.8 ± 0.4 ^a	<0.001
n-6:n-3	9.5 ± 1.4 ^a	2.2 ± 0.5 ^b	1.9 ± 0.2 ^b	9.1 ± 0.8 ^a	<0.001
ΣPUFAs ⁶	16.8 ± 3.3 ^a	25.2 ± 3.7 ^b	25.0 ± 2.0 ^b	17.4 ± 5.6 ^a	<0.001

¹ $\bar{x} \pm$ SD. Values in the same row with different superscript letters are significantly different, $P < 0.05$.²Total saturated fatty acids (SFAs) include 8:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, and 24:0.³Total monounsaturated fatty acids (MUFAs) include 14:1n-5, 16:1n-7, *trans* 18:1n-9, 18:1n-9 plus 18:1n-7, 20:1n-9, 22:1n-11, 22:1n-9, and 24:1n-9.⁴Total n-6 fatty acids, calculated by adding 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6, 22:3n-6, 22:4n-6, and 22:5n-6.⁵Total n-3 fatty acids, calculated by adding 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3.⁶Total PUFAs include total n-6, total n-3, and other PUFAs (16:2n-4, 16:4n-1, 20:3n-9, and 22:3n-9).

constant at ≈ 1.2 – 1.3% of total fatty acids throughout the supplementation and postsupplementation periods. The plasma ratio of n-6 to n-3 fatty acids decreased significantly from 13.0 at baseline to 6.8 after 4 wk of supplementation.

Plasma total monounsaturated fatty acids decreased significantly from 24.5% of total fatty acids at baseline to 21.1% of total fatty acids after 4 wk of supplementation. Plasma *trans* fatty acids remained fairly constant, at $\approx 1\%$ of total fatty acids, after supplementation with flaxseed oil.

Changes in erythrocyte fatty acid composition during flaxseed oil supplementation

After supplementation with flaxseed oil, similar changes were observed in the fatty acid composition of maternal erythrocytes (Table 4). Erythrocyte ALA increased significantly over time and was significantly increased after both 2 and 4 wk of supplementation; the values were 0.1% at baseline, 0.6% at 2 wk, and 0.7% at 4 wk. Erythrocyte EPA increased significantly over time, from 0.6% at baseline to 1.0% after 2 wk and 0.7% after 4 wk of supplementation; it returned to 0.8% at 4 wk postsupplementation. Erythrocyte DPA increased significantly over time, from 2.4% at baseline to 2.7% after both 2 wk and 4 wk of supplementation. Erythrocyte DHA did not change significantly, remaining at $\approx 3.5\%$ throughout the supplementation and postsupplementation periods.

Erythrocyte linoleic acid did not change significantly over time or at any time points. Erythrocyte total monounsaturated fatty acids decreased from 18.6% at baseline to 18.0% after 4 wk of supplementation; this decrease was not significant. The erythrocyte ratio of n-6 to n-3 fatty acids decreased from 5.1 at baseline to 4.1 after 4 wk of supplementation. Erythrocyte *trans* fatty acids did not change significantly after supplementation with flaxseed oil, remaining at $\approx 1\%$ of total fatty acids.

DISCUSSION

In this study of 7 lactating women, ALA, EPA, and DPA increased in breast milk, plasma, and erythrocytes after flaxseed oil supplementation. These results were different from what we observed in 7 nonlactating control subjects supplemented with flaxseed oil (26). In that previous study, subjects ranged in age from 24 to 54 y and included 3 men and 4 women; all the women were premenopausal. The subjects received 15 g flaxseed oil (11 g ALA) daily for 12 wk, which did not increase EPA, DPA, DHA, or total n-3 fatty acids in plasma or erythrocytes. However, in the present study of lactating women, we measured significant increases in milk and plasma EPA and milk, plasma, and erythrocyte DPA. This finding supports our hypothesis that lactating women synthesized some of the longer-chain n-3 fatty acids, whereas nonlactating control subjects did not.

TABLE 3

Maternal plasma fatty acid composition at baseline, after 2 and 4 wk of flaxseed oil supplementation, and at 4 wk postsupplementation¹

Fatty acid	Baseline (n = 8)	After 2 wk of supplementation (n = 8)	After 4 wk of supplementation (n = 6)	4 wk postsupplementation (n = 6)	P
% by wt of total fatty acids					
12:0	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.3	0.1 ± 0.1	NS
14:0	0.9 ± 0.4	1.4 ± 0.7	1.3 ± 0.9	1.1 ± 0.5	NS
16:0	19.3 ± 1.1	19.0 ± 1.7	18.8 ± 0.9	19.6 ± 1.7	NS
18:0	7.4 ± 0.8	7.5 ± 0.6	8.0 ± 0.7	7.2 ± 0.5	NS
20:0	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.0 ± 0.0	NS
ΣSFAs ²	28.7 ± 1.3	29.4 ± 2.7	29.3 ± 1.7	28.9 ± 2.1	NS
18:1n-9	20.4 ± 2.7	17.6 ± 2.7	17.5 ± 1.2	20.3 ± 2.9	0.004
<i>trans</i> 18:1n-9	1.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	0.9 ± 0.3	NS
20:1n-9	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	NS
22:1n-11	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS
ΣMUFAs ³	24.5 ± 2.9	21.3 ± 2.2	21.1 ± 1.7	25.4 ± 6.0	0.02
18:2n-6	30.9 ± 2.6	31.9 ± 3.5	32.1 ± 2.2	31.2 ± 4.6	NS
20:3n-6	1.6 ± 0.3	1.1 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	0.001
20:4n-6	7.5 ± 1.8	6.5 ± 1.4	6.2 ± 1.4	7.3 ± 1.3	0.001
Σn-6 ⁴	41.3 ± 3.7	40.6 ± 4.1	41.0 ± 2.5	38.7 ± 11.3	NS
18:3n-3	0.6 ± 0.2 ^a	2.8 ± 0.9 ^b	3.4 ± 1.2 ^b	0.7 ± 0.1 ^a	<0.001
20:5n-3	0.7 ± 0.2 ^a	1.5 ± 0.3 ^b	1.6 ± 0.4 ^b	0.7 ± 0.1 ^a	<0.001
22:5n-3	0.5 ± 0.1 ^a	0.8 ± 0.1 ^b	0.8 ± 0.1 ^b	0.6 ± 0.1 ^a	<0.001
22:6n-3	1.3 ± 0.4	1.3 ± 0.4	1.2 ± 0.3	1.3 ± 0.3	NS
Σn-3 ⁵	3.3 ± 0.5 ^a	6.5 ± 1.0 ^b	7.1 ± 1.4 ^b	3.3 ± 0.4 ^a	0.001
n-6:n-3	13.0 ± 2.8 ^a	6.4 ± 1.3 ^b	6.8 ± 1.7 ^b	12.6 ± 2.8 ^a	<0.001
ΣPUFAs ⁶	45.2 ± 3.4	47.7 ± 3.9	48.2 ± 2.2	45.3 ± 5.3	NS

¹ $\bar{x} \pm$ SD. Values in the same row with different superscript letters are significantly different, $P < 0.05$.²Total saturated fatty acids (SFAs) include 8:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, and 24:0.³Total monounsaturated fatty acids (MUFAs) include 14:1n-5, 16:1n-7, *trans* 18:1n-9, 18:1n-9 plus 18:1n-7, 20:1n-9, 22:1n-11, 22:1n-9, and 24:1n-9.⁴Total n-6 fatty acids, calculated by adding 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6, 22:3n-6, 22:4n-6, and 22:5n-6.⁵Total n-3 fatty acids, calculated by adding 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3.⁶Total polyunsaturated fatty acids (PUFAs) include total n-6, total n-3, and other PUFAs (16:2n-4, 16:4n-1, 20:3n-9, and 22:3n-9).

Contrary to our hypothesis, DHA did not increase in breast milk, plasma, or erythrocytes. There are several possible reasons for the lack of increase in breast-milk DHA concentrations after ALA consumption.

One possible reason is that there is very limited conversion of ALA to DHA in human adults (11, 17–19). Pawlosky et al (27) recently quantified the inefficiency of the conversion of ALA to DHA. Only 0.2% of the plasma ALA was available for synthesis of EPA. Of this, 63% was available for synthesis of DPA, of which 37% was accessible for production of DHA. Thus, <0.05% of the plasma ALA was available for synthesis of DHA. Even so, long-term vegetarians with virtually no dietary DHA intake have DHA in their plasma phospholipids (18). In studies of vegans who ate no foods of animal origin (including fish), DHA was still found in erythrocyte and breast-milk fatty acids (16, 28). These data suggest that conversion of ALA to DHA must occur in humans. Likewise, infant monkeys fed prenatal and postnatal diets containing only ALA from soy oil had DHA in the blood, brain, retina, and other organs, indicating that synthesis of DHA from ALA did indeed occur in the monkeys as well (29). Similar results were found in human infants fed formulas containing ALA but no DHA; DHA was found in their blood, although the concentration was lower than that in infants fed human milk, which contains DHA as well as ALA (30). The failure of conversion of ALA to DHA in the present study may have resulted from limited conversion in combination with a relatively short duration of supplementation (4 wk).

A second possible reason for the lack of increase in breast-milk DHA is that DHA may not have increased because of competitive enzyme inhibition. The Δ^6 enzyme is required for 2 steps of the DHA synthesis pathway (Figure 1). The first step is the conversion of 18:3n-3 (ALA) to 18:4n-3. The second step is the conversion of 24:5n-3 to 24:6n-3, with subsequent retroconversion to 22:6n-3. An excess of the substrate ALA (from the flaxseed oil) might provide for synthesis of some fatty acids (eg, EPA) in the initial phases of the synthetic pathway to DHA, but the excess of substrate might have suppressed the final Δ^6 -desaturase step needed to produce 24:6n-3 from 24:5n-3 later in the pathway.

A third possible reason for a lack of increase in breast-milk DHA is that high dietary DHA intakes can suppress the conversion of ALA to DHA, but in the present study, this was unlikely because the subjects did not appear to have high DHA intakes. The average amount of DHA in the milk of these American women was much lower (\approx 0.2% of total fatty acids) than the amounts found in the milk of women throughout the world, especially in China, where the concentrations are 4 or 5 times those found in the United States. These high concentrations have been correlated with the consumption of large amounts of fish (14). Even in Cuba, breast-milk concentrations of DHA are double those reported in the milk of US women (0.4% of total fatty acids versus 0.2%, respectively). Cuban lactating women generally consume 1 serving of fish each day, in comparison with our study subjects, who consumed 2–3 servings of fish/mo; this probably

TABLE 4
Maternal erythrocyte fatty acid composition at baseline, after 2 and 4 wk of flaxseed oil supplementation, and at 4 wk postsupplementation¹

Fatty acid	Baseline (n = 8)	After 2 wk of supplementation (n = 8)	After 4 wk of supplementation (n = 6)	4 wk postsupplementation (n = 6)	P
% by wt of total fatty acids					
12:0	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	NS
14:0	0.4 ± 0.2	0.5 ± 0.1	0.4 ± 0.2	0.4 ± 0.1	NS
16:0	19.6 ± 1.0	19.1 ± 0.8	19.2 ± 1.3	19.8 ± 1.2	NS
18:0	15.9 ± 0.7	15.9 ± 0.8	16.2 ± 1.2	17.2 ± 1.6	NS
20:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	NS
ΣSFAs ²	39.3 ± 1.1	38.5 ± 1.0	39.4 ± 1.3	39.4 ± 1.3	NS
18:1n-9	15.8 ± 1.1	15.4 ± 1.0	14.9 ± 1.0	15.7 ± 1.2	NS
<i>trans</i> 18:1n-9	1.0 ± 0.2	1.1 ± 0.3	1.3 ± 0.2	1.1 ± 0.1	NS
20:1n-9	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	NS
22:1n-11	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	NS
ΣMUFAs ³	18.6 ± 0.9	18.0 ± 1.4	18.0 ± 1.0	18.6 ± 1.0	NS
18:2n-6	11.6 ± 1.2	11.6 ± 0.7	11.7 ± 0.8	11.6 ± 0.8	NS
20:3n-6	1.7 ± 0.4	1.5 ± 0.3	1.4 ± 0.4	1.6 ± 0.1	0.001
20:4n-6	14.8 ± 1.1	14.9 ± 1.0	14.0 ± 1.4	14.0 ± 1.4	0.04
Σn-6 ⁴	32.7 ± 1.8	32.7 ± 1.1	31.6 ± 2.2	32.6 ± 1.1	NS
18:3n-3	0.1 ± 0.0 ^a	0.6 ± 0.1 ^b	0.7 ± 0.2 ^b	0.2 ± 0.1 ^a	<0.001
20:5n-3	0.6 ± 0.2 ^a	1.0 ± 0.2 ^b	0.7 ± 0.2 ^a	0.8 ± 0.2 ^a	<0.001
22:5n-3	2.4 ± 0.4	2.7 ± 0.4	2.7 ± 0.3	2.8 ± 0.4	<0.001
22:6n-3	3.5 ± 0.9	3.7 ± 1.1	3.3 ± 0.9	3.3 ± 1.1	NS
Σn-3 ⁵	6.7 ± 1.3 ^a	8.1 ± 1.4 ^b	7.9 ± 1.2 ^b	7.1 ± 1.5 ^a	<0.001
n-6:n-3	5.1 ± 1.1 ^a	4.2 ± 0.8 ^b	4.1 ± 0.8 ^b	4.7 ± 1.0 ^a	<0.001
ΣPUFAs ⁶	40.3 ± 1.6	41.8 ± 1.2	40.5 ± 1.7	40.5 ± 1.5	NS

¹ $\bar{x} \pm SD$. Values in the same row with different superscript letters are significantly different, $P < 0.05$.

²Total saturated fatty acids (SFAs) include 8:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, and 24:0.

³Total monounsaturated fatty acids (MUFAs) include 14:1n-5, 16:1n-7, *trans* 18:1n-9, 18:1n-9 plus 18:1n-7, 20:1n-9, 22:1n-11, 22:1n-9, and 24:1n-9.

⁴Total n-6 fatty acids, calculated by adding 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6, 22:3n-6, 22:4n-6, and 22:5n-6.

⁵Total n-3 fatty acids, calculated by adding 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

⁶Total polyunsaturated fatty acids (PUFAs) include total n-6, total n-3, and other PUFAs (16:2n-4, 16:4n-1, 20:3n-9, and 22:3n-9).

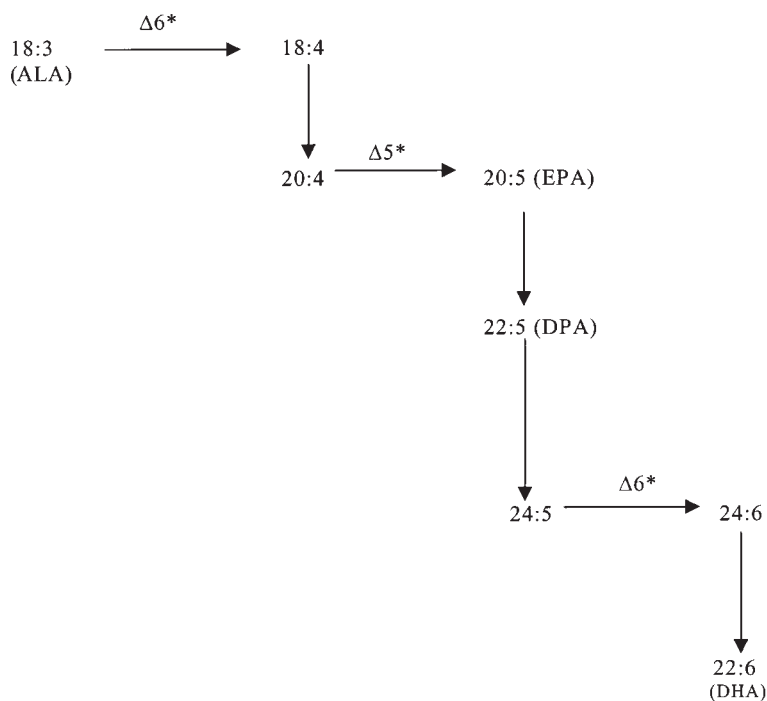



FIGURE 1. The conversion of α -linolenic acid (ALA) to docosahexaenoic acid (DHA) via desaturation and elongation. EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; *, desaturase enzymes.

explains the greater DHA concentrations found in the milk of Cuban mothers (31).

Also of interest is the fact that ALA is known to be catabolized rapidly (32). Our data support the rapid catabolism of ALA (Table 2). One week after the flaxseed oil supplementation was discontinued, ALA concentrations in the blood and breast milk had declined to baseline concentrations. In milk, ALA decreased from 7.7% to <2.0% of total fatty acids. However, the decline in breast-milk DPA concentrations was much slower, with baseline values not reached until 3 wk postsupplementation. For EPA, baseline values were also reached at 3 wk postsupplementation. Similar findings were noted for plasma and erythrocyte ALA, DPA, and EPA, although the declines of their concentrations were much less rapid in the erythrocytes than in the plasma.

Although there were no significant changes in the *trans* fatty acid content of the breast milk, plasma, or erythrocytes, we reported these data because of the potential adverse effects of dietary *trans* fatty acids. These potential adverse effects include reduced serum HDL concentrations (33, 34) and impaired biosynthesis of long-chain polyunsaturated fatty acids (33, 35).

In conclusion, 4 wk of supplementation with flaxseed oil was not an effective way to increase DHA concentrations in maternal plasma, erythrocytes, or breast milk. Thus, flaxseed oil supplementation would not be an adequate method of increasing the availability of DHA for the developing infant. Increasing the maternal intake of DHA from fish or fish oil would still be the most effective way to increase the DHA in breast milk and provide this long-chain polyunsaturated fatty acid that is so critical for infant development (36). 

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