

Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and maternal and infant plasma phospholipids¹⁻⁴

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ABSTRACT To determine whether docosahexaenoic acid (DHA) supplementation of breast-feeding mothers increases the DHA contents of breast milk and infant plasma phospholipids (PPs), breast-feeding women were randomly assigned to 3 DHA-supplementation groups (170–260 mg/d) or a control group. Group 1 ($n = 6$) consumed an algae-produced high-DHA triacylglycerol; group 2 ($n = 6$) consumed high-DHA eggs; group 3 ($n = 6$) consumed a high-DHA, low-eicosapentaenoic acid marine oil; and group 4 ($n = 6$) received no supplementation. From before to after supplementation (2 and 8 wk postpartum), mean (\pm SD) maternal PP DHA increased in groups 1, 2, and 3 by 1.20 ± 0.53 , 0.63 ± 0.82 , and 0.76 ± 0.35 mol% of fatty acids, respectively (23–41%), but decreased in group 4 by 0.44 ± 0.34 mol% (15%). Breast-milk DHA of groups 1, 2, and 3 increased by 0.21 ± 0.16 , 0.07 ± 0.11 , and 0.12 ± 0.07 mol%, respectively (32–91%) but decreased in group 4 by 0.03 ± 0.04 mol% (17%). Mean infant PP DHA in groups 1, 2, and 3 increased by 1.63 ± 0.79 , 0.40 ± 1.0 , and 0.98 ± 0.61 mol%, respectively (11–42%), but only by 0.18 ± 0.74 mol% (5%) in group 4. Correlations between the DHA contents of maternal plasma and breast milk and of milk and infant PPs were significant. Breast-milk and maternal and infant PP 22:5n-6 concentrations were lowest in group 2. DHA supplementation increases the plasma and breast-milk DHA concentrations of lactating women, resulting in higher PP DHA concentrations in infants. *Am J Clin Nutr* 2000; 71(suppl):292S–9S.

KEY WORDS Lactation, breast-feeding, infant nutrition, maternal nutrition, docosahexaenoic acid, polyunsaturated fatty acids, breast-fed infants, infant plasma phospholipids, maternal plasma phospholipids, human-milk phospholipids, breast-milk phospholipids

INTRODUCTION

Docosahexaenoic acid (DHA) is an important component of the structural lipids of brain and retinal cell membranes (1–4). It is present in breast milk but not in most infant formulas (5–21). Plasma, erythrocyte, and brain DHA concentrations are higher in breast-fed than in formula-fed infants (5, 6, 9, 13, 14, 16–18). In addition, some studies have shown more optimal indexes of visual function and neurodevelopmental status in breast-fed than in formula-fed infants (14, 22–24). This combination of findings

has focused attention on the role of this fatty acid in infant development. The further observations that supplementation of infant formulas with DHA results in higher plasma and erythrocyte DHA concentrations (8–11, 15), transient if not permanent improvement in visual function (11, 12), and perhaps a more optimal neurodevelopmental outcome (25) have led many to advocate supplementation of infant formulas, particularly those for preterm infants, with DHA.

Despite the current enthusiasm for supplementation of infant formulas with DHA (16, 26–30), there are several reasons for caution. In one study, supplementation of preterm formula with fish oil conferred a transient beneficial effect on visual function but adversely affected growth and some indexes of neurodevelopmental status (19, 20). Because the lower rates of growth were associated with low plasma and erythrocyte contents of arachidonic acid (AA; 20:4n-6), it was speculated that the marine-oil supplement either inhibited conversion of linoleic acid (LA; 18:2n-6) to AA or that its high content of eicosapentaenoic acid (EPA; 20:5n-3) competed with AA for incorporation into tissue phospholipids, conversion to eicosanoids, or both (10, 15, 21).

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This finding suggests that formulas should be supplemented with AA as well as DHA. Data indicating how much AA, DHA, or both should be added to formulas are not available, and therefore the amounts of these fatty acids found in breast milk have been suggested as reasonable. However, the amounts of these fatty acids in breast milk are variable, particularly DHA (5, 31). Further, the amount of DHA in the breast milk of US women is lower, on average, than that in milk of women from most other countries, particularly areas in which fish consumption is high [eg, Surinam (32), St Lucia (33), Malaysia (34), Dominica (35), and Curacao (32)]. In contrast, the AA concentration in the breast milk of US women is less variable and tends to be comparable to or even higher than that in other populations (5, 31, 36, 37).

Numerous factors affect the fatty acid content of breast milk (36–55), but maternal dietary DHA intake appears to be a major determinant. In a trial reported in 1992, a 3-wk period of maternal fish-oil supplementation (6 g·kg⁻¹·d⁻¹) doubled the milk DHA content; however, milk EPA content increased 6-fold, or >10 SD from the baseline concentration (46). The EPA content of erythrocyte phospholipids of the infants receiving this milk also increased 6-fold (10 SDs from mean control values) during this 3-wk period, whereas the erythrocyte phospholipid DHA content increased by only 35%. In view of the potential adverse effects of EPA, any potential beneficial effect of fish-oil supplementation on milk DHA content might be outweighed by the concurrent increase in milk EPA content. Therefore, we evaluated the effects of 3 alternative maternal DHA supplements on breast-milk DHA content and on the fatty acid content of maternal and infant plasma phospholipids. The objectives were to 1) compare the efficacy of the different supplements for increasing milk DHA content; 2) determine the effects of the supplements on milk contents of other fatty acids; and 3) determine the relations between the DHA content of maternal plasma phospholipids and that of milk, as well as between milk and infant plasma phospholipid DHA content.

SUBJECTS AND METHODS

Subjects and study design

Twenty-six pregnant women who planned to breast-feed exclusively for ≥8 wk were recruited during the last trimester of pregnancy or at the time of delivery and were randomly assigned to 1 of 4 groups. From 2 wk until 8 wk postpartum, those assigned to group 1 (*n* = 7) received an algae-produced triacylglycerol with a high DHA content (DHASCO; Martek Biosciences Corporation, Columbia, MD); those assigned to group 2 (*n* = 6) received eggs (2/d) with a high DHA content (The Country Hen, Hubbardston, MA); those assigned to group 3 (*n* = 6) received a low-EPA, high-DHA fish oil (ROPUFA '30' n-3 INF OIL; Hoffman-LaRoche, Parsippany, NJ); and those assigned to group 4 (control group, *n* = 7) received regular eggs (2/d). Exclusion criteria included maternal age at time of delivery of <19 or >35 y, maternal diabetes, maternal history suggestive of egg allergy, infant gestational age <37 wk, and infant birth weight <2500 or >4200 g.

The fatty acid composition of each of the supplements is shown in Table 1. Women assigned to groups 1, 2, and 3 received <230, 170, and 260 mg DHA/d, respectively, from the supplements; women assigned to group 4 received <35 mg DHA/d from the 2 regular eggs. Mother-infant pairs were dropped from the study if the infant's intake of formula, other foods, or both exceeded 25% of total intake at any time during

TABLE 1

Mole percentages of selected fatty acids in docosahexaenoic acid (DHA; 22:6n-3) supplements

Fatty acid	Algal DHA	High-DHA eggs	Low-EPA fish oil
<i>mol%</i>			
12:0	6.2	—	—
14:0	17.9	—	4.5
16:0	15.3	23.2	24.9
18:0	1.0	8.2	6.7
16:1n-7	1.5	5.6	6.5
18:1n-7	—	1.7	2.5
18:1n-9	11.1	32.5	13.7
18:2n-6	0.7	21.6	2.2
20:4n-6	—	1.5	1.5
18:3n-3	—	1.2	0.7
20:5n-3	—	0.15	4.8
22:5n-3	—	0.27	1.1
22:6n-3	44.9	3.3	26.0

the 6-wk supplementation period. At 2, 5, and 8 wk postpartum (ie, just before starting supplementation and after 3 and 6 wk of supplementation, respectively), maternal blood and milk samples as well as infant blood samples were collected for determination of fatty acid composition as described below. Infant weight, length, and head circumference were measured at the same times. The study was approved by the Institutional Review Board for Human Subject Research for Baylor College of Medicine and Affiliated Hospitals. Written, informed consent was obtained from all subjects before enrollment.

Breast-milk and blood-sample collections

Mothers and infants were admitted to the Metabolic Research Unit of the Children's Nutrition Research Center at 2, 5, and 8 wk postpartum for determination of 24-h milk intake and for collection of milk and blood samples. At each feeding during this 24-h period, the infant was offered one breast and the contents of the other breast were expressed with an electrical pump (Egnell, Inc, Cary, IL), alternating breasts at each successive feeding. Milk collected by pump was weighed and, after thorough mixing, an aliquot (a fixed percentage of the volume collected) was obtained for addition to similar aliquots obtained at other feedings to prepare a proportional daily aliquot for analysis. The remainder of the expressed milk was offered to the infant or was frozen for later use. Each aliquot was refrigerated until all aliquots for the 24-h period were obtained. These were then mixed and frozen immediately at 270 °C for subsequent analysis.

Total daily milk output was considered to be the sum of the volume collected by pump and the amount consumed by the infant as estimated from the difference between pre- and post-feeding weights (56). The total daily DHA intake of the infants was determined from the DHA concentration of the proportional aliquot and the 24-h milk intake. Blood samples from mothers and infants were obtained by venipuncture. Plasma was separated by centrifugation (2450 × *g* for 10 min at 20 °C) and frozen at 270 °C until analysis.

Plasma and breast-milk fatty acids

To determine the fatty acid patterns of maternal and infant plasma phospholipid fractions, plasma lipids were extracted by the method of Bligh and Dyer (57) and the phospholipid fractions

TABLE 2
Characteristics of mothers who completed the study¹

	Age at delivery	Parity	Weight ²	Height ²
	<i>y</i>	<i>n</i>	<i>kg</i>	<i>cm</i>
Group 1 (algal DHA)	29 ± 3	1.5 ± 0.5	69 ± 16	165 ± 4
Group 2 (eggs)	29 ± 4	1.5 ± 0.5	71 ± 14	162 ± 8
Group 3 (fish oil)	30 ± 5	1.7 ± 0.5	70 ± 17	160 ± 8
Group 4 (control)	29 ± 5	1.2 ± 0.4	80 ± 22	165 ± 7

¹ $\bar{x} \pm \text{SD}$; *n* = 6 per group. There were no significant differences among groups in any of these variables.

²Measured at 2 wk postpartum.

of each were separated by 1-dimensional thin-layer chromatography (Silica Gel 60; Sigma-Aldrich, St Louis) using hexane, diethyl ether, and glacial acetic acid (70:35:1 by vol). Methyl esters of the component fatty acids of the plasma phospholipid fraction were prepared with boron trifluoride-methanol (58) and quantified by gas-liquid chromatography (Varian 3500; Varian, Inc, Palo Alto, CA) on a DB-225 capillary column (J & W Scientific, Folsom, CA; 59). The fatty acid patterns of the extracted total lipids of breast milk and the supplements were determined by using the same methods. The amount of each fatty acid was expressed as the mole percentage of total fatty acid content.

Data analysis

All data were expressed as group means ± SDs. The statistical significance of differences in the characteristics of mothers and infants, baseline fatty acid contents, and changes in fatty acid contents from 2 wk (baseline) to 8 wk postpartum among groups were tested by analysis of variance. These analyses were followed, if indicated, by post hoc pairwise comparisons using the Tukey multiple comparison procedure (MINITAB for Windows NT, release 11; Minitab Inc, State College, PA). A probability of 5% was assumed to represent statistical significance. Correlations between selected outcome variables were determined by regression analysis.

RESULTS

Subjects

Twenty-four of the enrolled mother-infant pairs completed the study (*n* = 6 for each group); 1 mother assigned to group 1 (algal DHA) and 1 assigned to group 4 (control) did not produce milk sufficient to satisfy 75% of their infants' needs. Compliance with supplement ingestion (including that of the women who were

required to consume 2 eggs/d) was excellent; no complaints about any of the supplement regimens were expressed. Clinical characteristics of the mothers and infants who completed the study are summarized in **Tables 2** and **3**. There were no significant differences in these clinical variables among the groups. The ethnic distribution within the groups was as follows: group 1, 6 whites; group 2, 1 Asian American, 1 African American, and 4 whites; group 3, 1 African American, 4 whites, and 1 Hispanic; and group 4, 5 whites and 1 Hispanic.

Plasma and breast-milk fatty acids

The mean mole percentage of selected *n*-3 and *n*-6 polyunsaturated fatty acids in maternal and infant plasma phospholipids and breast milk at baseline (2 wk postpartum) and after 6 wk of supplementation (8 wk postpartum) are shown in **Tables 4**, **5**, and **6**. Fatty acid contents at 5 wk postpartum (after 3 wk of supplementation) differed minimally from those at the end of the supplementation period, and therefore are not shown. Changes in maternal and infant plasma phospholipid and milk DHA contents from baseline to 8 wk postpartum were greater in all supplemented groups, although differences in the change of milk and infant plasma DHA content between some supplemented groups and the control group were not all significant.

Note that the maternal and infant plasma-phospholipid and breast-milk contents of 22:5*n*-6 were significantly lower in group 2 (high-DHA eggs) than in the other groups at the end of the study period. No other significant differences among groups in *n*-3 or *n*-6 fatty acid concentrations were observed at 8 wk postpartum. Total saturated and total monounsaturated fatty acids did not differ among the groups at any time (data not shown).

Correlations between the long-chain *n*-3 and *n*-6 polyunsaturated fatty acid contents of maternal plasma phospholipids and milk, as well as between milk and infant plasma phospholipids, are shown in **Figures 1** and **2**. There were significant correlations between the contents of all long-chain (containing >18 carbons) *n*-3 and *n*-6 polyunsaturated fatty acids in maternal plasma phospholipids and the contents of these fatty acids in milk. The correlation between maternal plasma phospholipid DHA and milk DHA was particularly strong. Similar, although weaker, correlations were observed between the contents of these fatty acids in milk and those in infant plasma phospholipids.

DISCUSSION

This is the first study to evaluate the effects of more than one form of maternal DHA supplementation on the DHA content of breast milk. All 3 forms of DHA supplementation studied

TABLE 3
Characteristics of infants who completed the study¹

	Weight at birth	Gestational age	Weight	
			2 wk of age	8 wk of age
			<i>g</i>	<i>g</i>
Group 1 (algal DHA)	3509 ± 560	40 ± 1	3941 ± 481	5405 ± 836
Group 2 (eggs)	3402 ± 456	40 ± 1	3564 ± 369	5158 ± 398
Group 3 (fish oil)	3390 ± 609	39 ± 1	3704 ± 760	5513 ± 851
Group 4 (control)	3799 ± 382	40 ± 1	3866 ± 307	5101 ± 827

¹ $\bar{x} \pm \text{SD}$; *n* = 6 per group. There were no significant differences among the groups in any of these variables.

TABLE 4
Fatty acid composition of maternal plasma phospholipids¹

	2 wk postpartum ²	8 wk postpartum ³
<i>mol% of total fatty acids</i>		
18:3n-3		
Group 1	0.25 ± 0.04	0.22 ± 0.06
Group 2	0.21 ± 0.08	0.25 ± 0.02
Group 3	0.28 ± 0.05	0.27 ± 0.08
Group 4	0.26 ± 0.10	0.23 ± 0.07
20:5n-3		
Group 1	0.53 ± 0.22 ^{a,b}	0.62 ± 0.41
Group 2	0.34 ± 0.13 ^a	0.55 ± 0.22
Group 3	0.63 ± 0.15 ^b	0.78 ± 0.22
Group 4	0.49 ± 0.13 ^{a,b}	0.54 ± 0.10
22:6n-3		
Group 1	2.90 ± 0.49	4.10 ± 0.54 ^a
Group 2	2.78 ± 0.52	3.41 ± 0.63 ^a
Group 3	2.95 ± 0.67	3.71 ± 0.58 ^a
Group 4	2.90 ± 0.65	2.46 ± 0.35 ^b
18:2n-6		
Group 1	23.2 ± 3.7	23.4 ± 3.1
Group 2	22.8 ± 3.1	24.0 ± 3.6
Group 3	22.4 ± 0.9	24.0 ± 3.2
Group 4	22.8 ± 3.8	22.7 ± 3.5
20:4n-6		
Group 1	11.3 ± 1.9	11.4 ± 1.5
Group 2	12.3 ± 2.6	11.2 ± 1.8
Group 3	11.3 ± 1.6	12.0 ± 1.8
Group 4	11.3 ± 1.4	12.4 ± 2.0
22:5n-6		
Group 1	0.64 ± 0.14	0.60 ± 0.15 ^a
Group 2	0.67 ± 0.11	0.41 ± 0.09 ^b
Group 3	0.62 ± 0.14	0.64 ± 0.14 ^{a,c}
Group 4	0.53 ± 0.19	0.70 ± 0.11 ^c

¹ $\bar{x} \pm$ SD. DHA, docosahexaenoic acid. Group 1 (*n* = 6) consumed algal DHA; group 2 (*n* = 6) consumed high-DHA eggs; group 3 (*n* = 6) consumed fish oil; group 4 (*n* = 6; control) consumed 2 regular eggs.

²Values with different superscript letters are significantly different from each other, *P* < 0.05 (ANOVA).

³Values with different superscript letters indicate that the change from 2 wk to 8 wk postpartum was significantly different, *P* < 0.05.

increased the DHA concentrations of both maternal plasma phospholipids and milk lipids. Interestingly, both the regular and high-DHA eggs were well accepted by the subjects. Moreover, consumption of 2 eggs/d over the 6-wk period had no adverse effects on total-serum-cholesterol, LDL-cholesterol, HDL-cholesterol, or triacylglycerol concentrations. Serum concentrations of these lipids did not increase in any group and did not differ among groups at the end of the supplementation period (data not shown). The other 2 forms of supplementation were also well accepted. Thus, if the small number of women enrolled in this study was typical of US women in general, it appears that any of the supplements evaluated would be acceptable as DHA supplements for lactating women.

To our knowledge, this is first study in which high-DHA eggs and a low-EPA, high-DHA fish oil were evaluated as maternal supplements for increasing breast-milk DHA concentrations. Makrides et al (60) used the same high-DHA, algae-derived triacylglycerol to assess the effect of DHA supplementation on maternal plasma-phospholipid and breast-milk DHA. In that study, lactating mothers were randomly assigned on postpartum day 5 to receive either

placebo or DHA supplementation ranging from 0.2 to 1.3 g DHA/d. At 12 wk postpartum, fatty acids in breast milk and maternal plasma and erythrocyte phospholipids were measured. Just as we reported here, there was a strong, significant correlation between the DHA content of maternal plasma phospholipids and that of milk lipids. In addition, after controlling for maternal body mass index, there was a strong, dose-dependent effect of dietary DHA intake on milk DHA content, but no effect of DHA intake on breast-milk AA concentrations.

Several studies that measured breast-milk fatty acids after maternal supplementation with standard fish oils have been reported. As mentioned above, Henderson et al (46) measured milk DHA in 5 breast-feeding women before and after they received 6 g fish oil/d for 3 wk, and Harris et al (61) measured milk DHA in 8 lactating women given 3 different amounts (5, 10, and 47 g/d) of fish oil for 8–28 d. In both studies, milk DHA concentrations increased but there was an accompanying several-fold increase in milk EPA concentrations. As discussed previously, EPA may be detrimental to young infants. Thus, we thought that standard fish oil would not be an ideal candidate for supplementation of breast-feeding women.

TABLE 5
Fatty acid composition of breast milk total lipids¹

	2 wk postpartum	8 wk postpartum ²
<i>mol% of total fatty acids</i>		
18:3n-3		
Group 1	1.00 ± 0.18	0.95 ± 0.23
Group 2	1.04 ± 0.17	1.20 ± 0.47
Group 3	0.96 ± 0.21	1.11 ± 0.33
Group 4	1.16 ± 0.27	1.05 ± 0.36
20:5n-3		
Group 1	0.07 ± 0.02	0.07 ± 0.02
Group 2	0.07 ± 0.02	0.06 ± 0.01
Group 3	0.08 ± 0.02	0.09 ± 0.02
Group 4	0.08 ± 0.02	0.07 ± 0.02
22:6n-3		
Group 1	0.23 ± 0.04	0.44 ± 0.17 ^a
Group 2	0.22 ± 0.05	0.29 ± 0.10 ^{a,b}
Group 3	0.27 ± 0.11	0.39 ± 0.07 ^a
Group 4	0.23 ± 0.06	0.19 ± 0.06 ^b
18:2n-6		
Group 1	15.1 ± 1.4	15.2 ± 1.5
Group 2	16.6 ± 3.0	15.9 ± 3.2
Group 3	15.4 ± 2.3	16.1 ± 3.8
Group 4	15.4 ± 3.8	15.2 ± 3.2
20:4n-6		
Group 1	0.58 ± 0.12	0.53 ± 0.08
Group 2	0.61 ± 0.11	0.46 ± 0.06
Group 3	0.60 ± 0.06	0.55 ± 0.04
Group 4	0.59 ± 0.11	0.53 ± 0.14
22:5n-6		
Group 1	0.09 ± 0.02	0.11 ± 0.02 ^a
Group 2	0.09 ± 0.03	0.05 ± 0.01 ^b
Group 3	0.08 ± 0.03	0.12 ± 0.03 ^a
Group 4	0.09 ± 0.03	0.10 ± 0.03 ^a

¹ $\bar{x} \pm$ SD. DHA, docosahexaenoic acid. Group 1 (*n* = 6) consumed algal DHA; group 2 (*n* = 6) consumed high-DHA eggs; group 3 (*n* = 6) consumed fish oil; group 4 (*n* = 6; control) consumed 2 regular eggs.

²Values with different superscript letters indicate that the change from 2 wk to 8 wk postpartum was significantly different, *P* < 0.05.

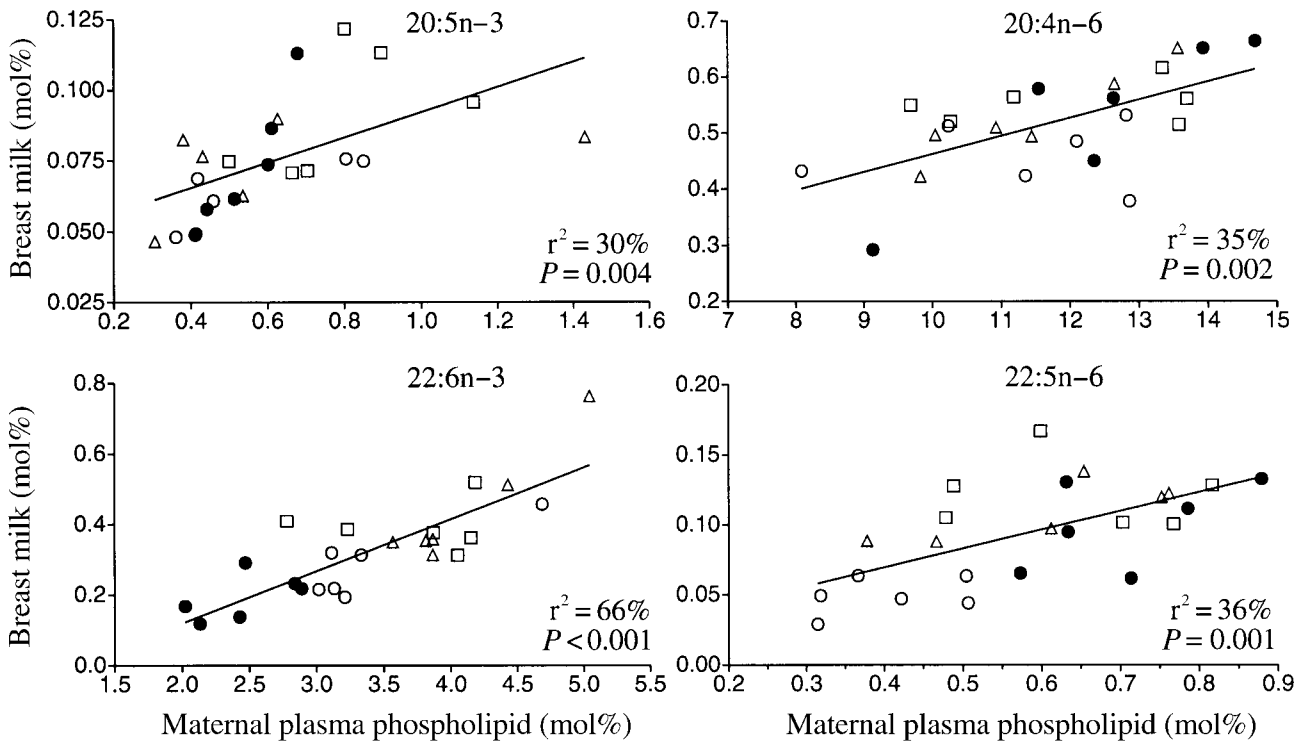


FIGURE 1. Relations between maternal plasma phospholipid and milk contents of selected n-3 and n-6 fatty acids in mole percentage of total fatty acids at 8 wk postpartum. Δ , group 1 [algal docosahexaenoic acid (DHA)]; \circ , group 2 (high-DHA eggs); \square , group 3 (low-eicosapentaenoic-acid fish oil); \bullet , group 4 (control).

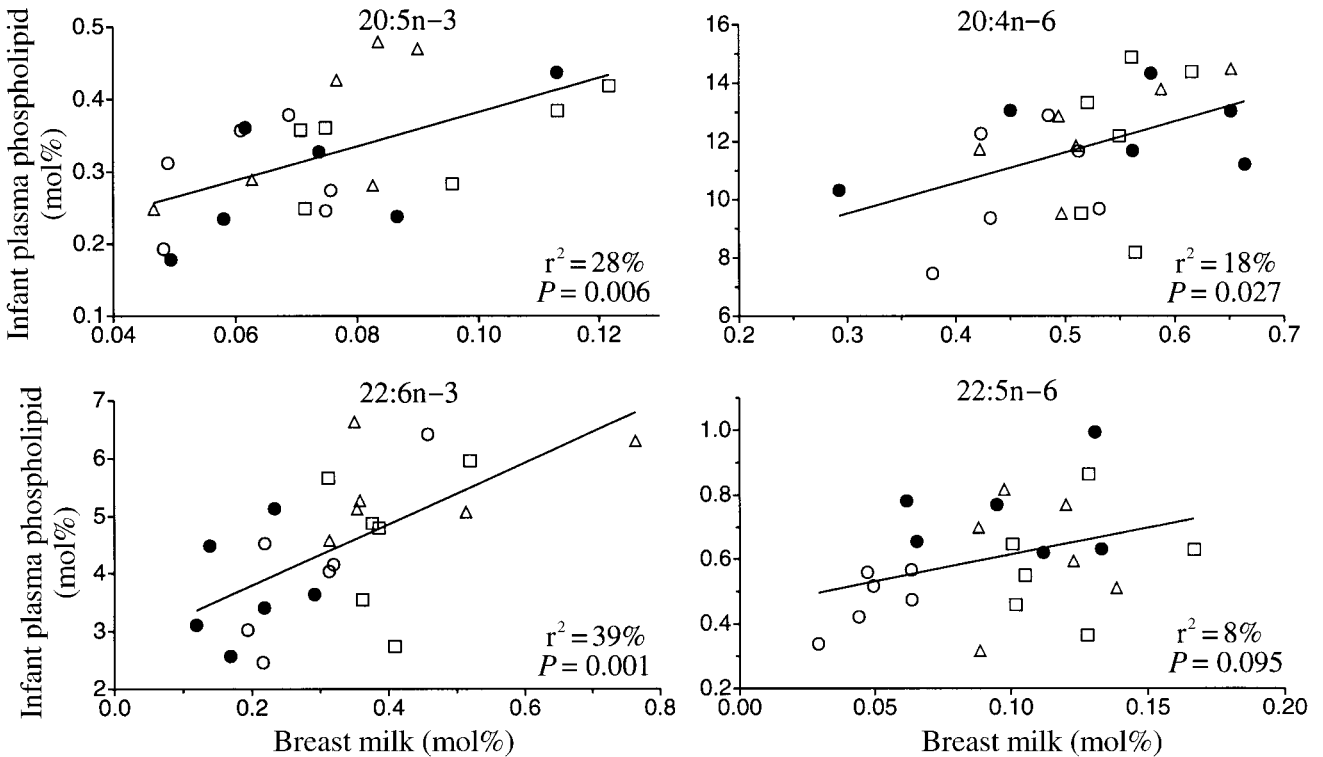


FIGURE 2. Relations between milk and infant plasma phospholipid contents of selected n-3 and n-6 fatty acids in mole percentage of total fatty acids at 8 wk postpartum. Δ , group 1 [algal docosahexaenoic acid (DHA)]; \circ , group 2 (high-DHA eggs); \square , group 3 (low-eicosapentaenoic-acid fish oil); \bullet , group 4 (control).

TABLE 6
Fatty acid composition of infant plasma phospholipids¹

	2 wk postpartum ²	8 wk postpartum ³
	<i>mol% of total fatty acids</i>	
18:3n-3		
Group 1	0.11 ± 0.02 ^a	0.10 ± 0.04
Group 2	0.09 ± 0.05 ^a	0.18 ± 0.10
Group 3	0.12 ± 0.03 ^{a,b}	0.14 ± 0.08
Group 4	0.18 ± 0.06 ^b	0.13 ± 0.04
20:5n-3		
Group 1	0.28 ± 0.07	0.37 ± 0.10
Group 2	0.24 ± 0.02	0.29 ± 0.07
Group 3	0.34 ± 0.07	0.34 ± 0.06
Group 4	0.32 ± 0.08	0.30 ± 0.10
22:6n-3		
Group 1	3.87 ± 0.81	5.50 ± 0.79 ^a
Group 2	3.70 ± 0.87	4.10 ± 1.37 ^b
Group 3	3.62 ± 0.74	4.60 ± 1.24 ^{a,b}
Group 4	3.55 ± 0.80	3.73 ± 0.94 ^b
18:2n-6		
Group 1	18.6 ± 2.2	20.1 ± 2.5
Group 2	18.4 ± 2.9	23.1 ± 3.7
Group 3	20.4 ± 3.4	22.2 ± 3.5
Group 4	21.0 ± 2.4	21.9 ± 2.4
20:4n-6		
Group 1	14.4 ± 1.5	12.4 ± 1.8
Group 2	14.1 ± 2.3	10.6 ± 2.1
Group 3	13.6 ± 3.2	12.1 ± 2.7
Group 4	13.2 ± 1.0	12.3 ± 1.5
22:5n-6		
Group 1	0.72 ± 0.11	0.62 ± 0.19 ^{a,b}
Group 2	0.75 ± 0.24	0.48 ± 0.09 ^b
Group 3	0.58 ± 0.11	0.59 ± 0.17 ^a
Group 4	0.60 ± 0.06	0.74 ± 0.14 ^a

¹ \bar{x} ± SD. DHA, docosahexaenoic acid. Group 1 (*n* = 6) consumed algal DHA; group 2 (*n* = 6) consumed high-DHA eggs; group 3 (*n* = 6) consumed fish oil; group 4 (*n* = 6; control) consumed 2 regular eggs.

²Values with different superscript letters are significantly different from each other, *P* < 0.05 (ANOVA).

³Values with different superscript letters indicate that the change from 2 wk to 8 wk postpartum was significantly different, *P* < 0.05.

Of major importance is the observation that infants whose mothers received dietary DHA supplements had higher plasma-phospholipid DHA. It is unclear whether the higher plasma-phospholipid DHA of these infants had functional benefits. However, if DHA is important for visual and neurologic development in infancy, the amount of DHA ingested by breast-fed infants, as reflected by higher plasma-phospholipid DHA content, should be important. Alternatively, the minimal amounts of DHA found in breast milk may be sufficient for optimal growth and development.

Maternal DHA supplementation had minimal effects on maternal plasma phospholipid or milk contents of other fatty acids, with the exception of 22:5n-6, which was lower in plasma and milk of women in the high-DHA-egg group. Likewise, the infant plasma-phospholipid contents of other fatty acids did not differ significantly among groups, with the exception of 22:5n-6, which was lower in infants of mothers in the high-DHA-egg group. However, it should be noted that a statistically significant (*P* < 0.05) difference of only <1.6 SD is

detectable with a sample size of only 6 subjects per group. Thus, the possibility that even low-EPA fish oil supplementation, such as that used in this study, might result in higher milk EPA concentrations should be further assessed.

The reasons for and the potential biological significance of the lower 22:5n-6 concentrations in maternal plasma phospholipids, breast milk, and infant plasma phospholipids of the subjects in the high-DHA-egg group are unclear. Generally, 22:5n-6 concentrations are thought to increase if adequate quantities of DHA are not available from either dietary intake or biosynthesis; thus, low concentrations of this fatty acid presumably indicate an adequate long-chain n-3 polyunsaturated fatty acid status. Paradoxically, higher plasma-phospholipid and milk DHA concentrations were observed in the other 2 DHA-supplemented groups.

Finally, the strong positive correlations between maternal plasma-phospholipid DHA, EPA, AA, and 22:5n-6 contents and the contents of these fatty acids in breast milk are striking, especially for DHA (*r*² = 66.2%, *P* < 0.001). This supports other evidence that milk DHA is derived from plasma rather than from *in situ* synthesis in the mammary gland. Thus, supplementation of lactating women with DHA seems to be the most reliable means of increasing breast-milk DHA. However, the crucial question of whether increases in breast-milk DHA concentrations will yield functional benefits for breast-fed infants remains unresolved.

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