Will dietary ω-3 fatty acids change the composition of human milk?[1-3]

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ABSTRACT The most abundant long-chain polyunsaturated fatty acid in brain and retinal lipids is docosahexaenoic acid (DHA, C22:6ω3). It becomes incorporated into nerve tissues mostly in utero and during the 1st yr of life. DHA is derived in humans either preformed in the diet or by hepatic synthesis from dietary linolenic acid (C18:3ω3). Since human milk contains DHA, this study was designed to see if increased dietary DHA would be reflected in a higher DHA content in human milk. Eight lactating women were given supplements of a fish oil concentrate rich in ω-3 fatty acids, including DHA (11% of fatty acids). Six women took 5 g/day of fish oil for 28 days; five women consumed 10 g/day for 14 days; and one woman consumed 47 g/day for 8 days. Each intake level of fish oil produced significant dose-dependent increases in the DHA content of milk and plasma. Base-line DHA levels in milk were 0.1 ± 0.06% of total fatty acids. Five g/day of fish oil raised the levels to 0.5 ± 0.1% ($p < 0.001$); 10 g/day raised DHA levels to 0.8 ± 0.1% ($p < 0.001$); and 47 g/day produced DHA levels of 4.8%. The results of this study indicated that relatively low intakes of dietary DHA significantly elevated milk DHA content. This would clearly elevate the infant's DHA intake and might have implications for brain and retinal development. *Am J Clin Nutr* 1984;40:780–785.

KEY WORDS Human milk, dietary fats, ω-3 fatty acids, docosahexaenoic acid, eicosapentaenoic acid, polyunsaturated fatty acids, brain development

Introduction

The role of dietary factors in the pre- and postnatal development of the human brain has received increasing attention in recent years. One of the most abundant components of the brain's structural lipids (which themselves comprise 70% of the solids of gray matter) is the long-chain, polyunsaturated fatty acid docosahexaenoic acid (C22:6ω3, DHA) (1). Retina and sperm also contain large amounts of DHA (2–4). DHA is primarily derived via hepatic synthesis from the essential fatty acid linolenic acid (C18:3ω3) which must be obtained from foods. However, performed (dietary) DHA is incorporated into brain phospholipids much more rapidly than DHA which must be synthesized from linolenic acid (5, 6).

Linolenic acid is present in only small amounts in many diets because it is primarily found in low fat green leafy vegetables associated with the chloroplast and in small amounts in many vegetable oils (4). In addition, the presence in Western diets of large quantities of the other essential fatty acid, linoleic acid (C18:2ω6, derived from vegetable oils) can inhibit the transformation of linolenic acid into DHA by the liver (7). Thus, the possibility of suboptimal DHA nutriture is not remote. Since experiments in rats and infant rhesus monkeys have shown that a gestational deficiency in these fatty acids can impair visual and/or mental function in the offspring (5, 8–10), it is important to understand the link between

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dietary ω-3 fatty acids and brain/retinal function.

A few past studies with human infants have examined the influence of diet on plasma DHA levels (11, 12). Human milk contains small, but significant amounts of DHA (less than 1% total fatty acids), whereas some infant formulas, which are usually based on cows' milk or vegetable oil, contain virtually no DHA (11, 13). Some formulas containing soy bean oil do, however, contain appreciable levels of linolenic acid (up to 8.5%) (12, 14). When these formulas were compared to human milk in their ability to elevate the level of DHA in the plasma phospholipids, human milk was found to be significantly more effective even though its total ω-3 content was less (12). This implied that human milk DHA levels may be influenced more by small amounts of dietary DHA than large amounts of dietary linolenic acid (13).

No studies to date have examined whether directly supplementing the maternal diet with DHA would increase the content of DHA in human milk and thus increase the infant's intake of this important fatty acid. The present study was designed to examine that question. Marine organisms are the only natural dietary sources of DHA. This is because ocean phytoplankton, which form the base of the marine food chain, synthesize DHA. Thus, higher marine organisms such as fish become ultimately enriched with these fatty acids. For this reason, a commercial preparation of fish oil was used in this study to supply DHA.

Methods

Eight lactating women were recruited for these studies. Seven were healthy volunteers, averaging 3.5 months postpartum (range 2 to 7 months). The eighth woman was participating in another protocol which involved the consumption of relatively large amounts of fish oil. She had obstructive atherosclerosis of the carotid artery requiring endartectomy but was otherwise healthy. She had been breast-feeding her child for 2 yr. Informed consent was obtained from each participant before entry into the study which had been approved by our Committee on Human Research.

Diet habit questionnaires were given to the women to determine their habitual intake of fish and fish oil. Finding that DHA intake from these sources was negligible, the volunteers were instructed to continue eating their usual home diets supplemented with either 5 or 10 g (as capsules) of MaxEPA (Marfleet Refining Co, Hull, England), a commercially available fish oil. This intake provided 0.5 or 1.0 g of DHA daily (1.4 and 2.8 g intake of total ω-3 fatty acids) (**Table 1**).

Six women took the 5-g supplements daily for 4 wk. Five women subsequently took 10 g/day for 2 wk after a 4-wk washout period. Milk and plasma samples were taken at the beginning, the midpoint and the conclusion of each supplementation phase. Samples were also taken 2 wk after fish oil cessation in the 4-wk study, and 1 wk after cessation in the 2-wk study. The single subject who was involved in the endarterectomy protocol consumed 47 g of fish oil for 8 days. This provided 6.1 g/day of DHA.

Fasting blood samples were collected in standard EDTA (0.1 g/dl) tubes. Milk samples were hand expressed by the mothers and subsequently analyzed for lipid class fatty acids according to previously described methods (15). Plasma and milk lipids were first extracted with chloroform/methanol (2:1). The milk lipids (primarily triglycerides) were methylated according to Morrison and Smith (16). The plasma samples were separated into lipid classes (cholesterol esters, triglycerides, free fatty acids, and phospholipids) by thin-layer chromatography. Subsequently, they were methylated as above. Fatty acid compositions were determined using a Perkin-Elmer Sigma 3B gas chromatograph (Norwalk, CT) fitted with a SP-2330, 30-m glass capillary column (Supelco, Bellefonte, PA). Column conditions were: oven temperature 200°C, detector and injector, 250°C; carrier gas, helium; column pressure, 30 psi; split ratio, about 1:100. The peak areas were determined with a Hewlett-Packard 3390 A integrator (Palo Alto, CA). Fatty acids were identified by relative retention times determined with known standards (Supelco and Nuchek Prep, Elysian, MN).

The data were evaluated statistically by the "Student's" paired t test for comparisons between base-line and treatment values for the same women. Comparisons between treatment groups were made with the unpaired t test using standard computer programs.

Results

The content of ω-3 fatty acids in the milk increased rapidly and in a dose-related manner when the dietary fish oil was given (**Fig 1**, Table 1). The levels of the long-chain, ω-3 fatty acids rose from 0.2 ± 0.06 to 1.1 ± 0.3% ($p < 0.01$) after 4 wk of 5 g of fish oil per day and to 1.7 ± 0.3% ($p < 0.01$) after 2 wk of 10 g/day. Levels at 2 and 1 wk, respectively, on these two supplementation schedules were not different from the values at 4 and 2 wk (Fig 1). In addition, the ω-3 levels after 2 wk of the 5 and 10 g/day

[4] The nonsaponifiable fraction of MaxEPA contained cholesterol and trace amounts of vitamins A and D. Vitamin E was added to the oil at 1 mg/ml to retard oxidation.

TABLE 1
The effects of dietary fish oil on the content of ω-3 fatty acids in human milk

Fatty acids	Dietary fish oil	Base-line	4 wk at 5 g/day	2 wk at 10 g/day	8 days at 47 g/day
10:0	Tr*	Tr	1.0 ± 0.4	Tr	1.2
12:0	0.1	4.2 ± 1.3	6.3 ± 4.1	6.0 ± 1.5	7.4
14:0	7.4	5.9 ± 0.7	6.4 ± 1.9	7.4 ± 2.0	9.6
16:0	16.8	22.8 ± 1.6	21.1 ± 1.8	21.5 ± 2.0	18.4
16:1ω7	9.5	2.5 ± 0.6	2.1 ± 0.3	2.5 ± 0.7	2.7
18:0	3.3	8.2 ± 1.2	7.5 ± 0.5	7.1 ± 1.4	5.3
18:1ω9	15.0	32.6 ± 3.3	27.5 ± 3.2	29.6 ± 4.6	25.1
18:2ω6	1.2	15.3 ± 3.3	18.2 ± 4.3	15.8 ± 3.5	15.2
18:3ω3	0.5	0.8 ± 0.5	1.0 ± 0.8	1.0 ± 0.2	0.8
20:1ω9	2.3	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5
20:3ω6	0.2	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1
20:4ω6	Tr	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4
20:5ω3 (eicosapentaenoic acid)	17.1	Tr	0.3 ± 0.15	0.5 ± 0.2	1.8
22:5ω3	2.6	Tr	0.2 ± 0.09	0.36 ± 0.1	0.8
22:6ω3 (DHA)	10.7	0.1 ± 0.1	0.5 ± 0.09	0.83 ± 0.17	1.9
Other	13.3†	6.4	6.8	6.4	8.8
Total ω-3 ≥ C20	31	0.24 ± 0.06	1.05 ± 0.32	1.7 ± 0.43	4.5

* < 0.1%.
† Other fatty acids present in the fish oil were: 14:1, 15:0, 17:0, 19:0, 18:3ω6, 20:0, 18:4ω3, 21:0, 20:3ω9, 22:0, 20:3ω3, 22:1ω11, 20:4ω3, 24:0, 22:4ω6, and 24:1ω9.

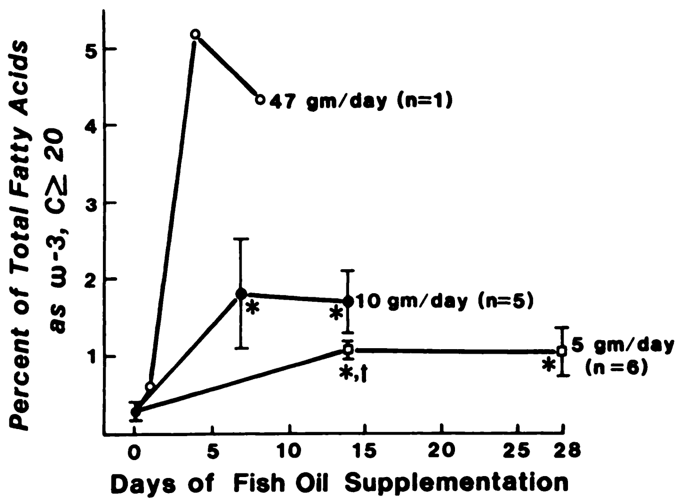

FIG 1. The effects of various intakes of fish oil on the levels of ω-3 fatty acids of chain length C20 and above in human milk. The respective intake levels are indicated adjacent to the curves; the number of subjects is in parentheses. * indicates value different from base-line ω-3 levels, p < 0.001; † indicates that this value is different from the 2-wk value at 10 g/day, p < 0.025.

regimens were significantly different from each other (p < 0.025). In the woman who took 47 g of fish oil per day, her milk contained markedly elevated levels of ω-3 fatty acids (4.8% of total fatty acids) compared to base-line or low dose fish oil.

Two weeks after ending the 5 g/day regimen, the marine ω-3 fatty acids in human milk had returned to almost base-line levels (0.3 ± 0.2%). However, after only 1 wk of washout from the 10 g/day regimen, the ω-3 fatty acids in milk were still significantly elevated over base-line levels (0.7 ± 0.2 versus 0.2 ± 0.06, p < 0.005), although they had fallen significantly below the peak amounts (1.7 ± 0.4, p < 0.005).

Plasma lipid classes became enriched with the marine ω-3 fatty acids (**Table 2**). As with the milk fatty acids, there was a dose-response relationship in the triglyceride and

TABLE 2
ω-3 fatty acids in the plasma lipid classes

	Base-line		4 wk at 5 g/day		2 wk at 10 g/day
Total ω-3 fatty acids ($C \geq 20$)*					
Triglyceride	0.4 ± 0.3	†	3.8 ± 1.9	‡	8.6 ± 4.2
			†		
Cholesteryl esters	1.1 ± 0.4	†	2.9 ± 0.9	‡	4.5 ± 1.2
			†		
Phospholipids	3.1 ± 0.6	†	8.3 ± 2.2		8.0 ± 1.2
			†		
Free fatty acids	0.2 ± 0.2	‡	2.7 ± 2.1		3.9 ± 3.7
			‡		
DHA					
Triglyceride	0.24 ± 0.17	†	1.9 ± 1.0	‡	4.5 ± 2.3
			†		
Cholesteryl esters	0.6 ± 0.3		0.8 ± 0.1		0.9 ± 0.25
Phospholipids	2.5 ± 0.62	†	5.3 ± 1.1		4.4 ± 0.5
			†		
Free fatty acids	0.15 ± 0.1	†	1.0 ± 0.4	‡	1.4 ± 1.7

* Includes eicosapentaenoic acid (C20:5 ω3) and DHA (C22:6 ω3).
† = $p < 0.005$
‡ = $p < 0.05$

cholesteryl ester fractions but not in the phospholipid or free fatty acid fractions. The fish oil had a negligible effect on the other plasma fatty acids (data not shown). The mothers reported no untoward side effects from the fish oil supplementation other than a mild "fishy" aftertaste during the first 1 to 3 h after taking the supplements. They also reported no change in the nursing behavior of their infants.

Discussion

The nutritional supremacy of human milk over infant formulas is being more firmly established year after year (17). One component of human milk that is not found in any infant formula is the fatty acid DHA. This fatty acid constitutes a major proportion of brain, retina, and sperm fatty acids (1–3) and can be derived only from direct ingestion or by synthesis from dietary linolenic acid. When equal amounts of DHA and linolenic acid are fed to rats, the former produced higher levels of DHA in the plasma and brain lipids than the latter (6). Feeding human infants DHA (in human milk) produced higher plasma DHA levels than feeding 100 times more linolenic acid from a formula (12).

The type of dietary fat given to a lactating mother will rapidly become reflected in the fatty acid composition of her milk (18). This occurs because lactating mammary tissue has a very active lipoprotein lipase which removes triglyceride fatty acids from chylomicrons and very low-density lipoproteins (19). Not surprisingly then, feeding ω-3 fatty acids to lactating rats elevated their milk ω-3 fatty acid levels (20). Before gas chromatography was used for fatty acid identification, Soderhjelm (21) reported that feeding 30 ml of cod liver oil to lactating women elevated their milk "hexaene" fraction. The desirability of having milk enriched with ω-3 fatty acids (DHA), however, is not known.

The importance of dietary ω-3 fatty acids in brain and retinal function has been only recently addressed experimentally. Rats made deficient in linolenic acid have been shown to have impaired electroretinograms (8) and a reduced ability to learn a visual discrimination maze (9). Recent studies in our laboratory in rhesus monkeys have shown that a dietary deficiency of ω-3 fatty acids leads to a marked reduction in plasma DHA levels and significantly reduced visual acuity in the young animals (10). Thus, ω-3 fatty acids appear to have specific functions in the visual process. The possibility that learning ability is also impaired is currently under investigation.

The results of this study indicated that feeding of fish oil rich in DHA to lactating women significantly elevated the levels of DHA in their milk. This, of course, would increase the infant's intake of this important fatty acid and could conceivably influence brain development and growth. The amount of fish oil fed in this study provided 0.5 and 1.0 g of DHA per day. This amount of DHA could be obtained by the daily consumption of 350 to 700 g of a low fat (1% fat) white fish such as cod, snapper, or sole, or by eating 75 to 150 g (about 3 oz) of a high fat fish (10% fat) such as salmon or mackerel. These intakes, particularly 3 oz of fish per day, are not unrealistic or unachievable. It should be noted that most fish oils contain another ω-3 fatty acid called eicosapentaenoic acid (C20:5ω3) in even greater amounts than DHA (Table 1). Eicosapentaenoic acid is a much closer metabolic precursor of DHA than is linolenic acid (7), and thus its presence in the diet may have also contributed to the rise in plasma and milk DHA levels.

Animals, including man, enter into life with the nervous tissue incompletely developed. In particular, the content of both ω-6 and ω-3 fatty acids in brain tissue is considerably lower at birth than it will be later in infancy and very early childhood (22). While precise data are lacking at this time, perhaps up to 50% of the ultimate mass of DHA accumulate after birth. A supply of this fatty acid and the precursor linolenic fatty acid in milk would clearly facilitate the laying down of DHA in the brain tissue. Thus, an adequate amount of ω-3 fatty acids in the diet would be vital during both pregnancy and lactation. Whether supplementation of the diet with fish oil containing DHA during pregnancy would influence brain development and learning ability in the young has not been experimentally addressed. Nevertheless, increasing the intake of DHA or its precursors during this critical period of brain development might well be considered.

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