Dietary effects on brain fatty acid composition: the reversibility of n-3 fatty acid deficiency and turnover of docosahexaenoic acid in the brain, erythrocytes, and plasma of rhesus monkeys[1]

William E. Connor,[2] Martha Neuringer, and Don S. Lin

Division of Endocrinology, Metabolism, and Clinical Nutrition, Department of Medicine, The Oregon Health Sciences University, Portland, OR 97201, and Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006

Abstract Rhesus monkeys given pre- and postnatal diets deficient in n-3 essential fatty acids develop low levels of docosahexaenoic acid (22:6 n-3, DHA) in the cerebral cortex and retina and impaired visual function. This highly polyunsaturated fatty acid is an important component of retinal photoreceptors and brain synaptic membranes. To study the turnover of polyunsaturated fatty acids in the brain and the reversibility of n-3 fatty acid deficiency, we fed five deficient juvenile rhesus monkeys a fish oil diet rich in DHA and other n-3 fatty acids for up to 129 weeks. The results of serial biopsy samples of the cerebral cortex indicated that the changes of brain fatty acid composition began as early as 1 week after fish oil feeding and stabilized at 12 weeks. The DHA content of the phosphatidylethanolamine of the frontal cortex increased progressively from 3.9 ± 1.2 to 28.4 ± 1.7 percent of total fatty acids. The n-6 fatty acid, 22:5, abnormally high in the cerebral cortex of n-3 deficient monkeys, decreased reciprocally from 16.2 ± 3.1 to 1.6 ± 0.4%. The half-life ($t_{1/2}$) of DHA in brain phosphatidylethanolamine was estimated to be 21 days. The fatty acids of other phospholipids in the brain (phosphatidylcholine, -serine, and -inositol) showed similar changes. The DHA content of plasma and erythrocyte phospholipids also increased greatly, with estimated half-lives of 29 and 21 days, respectively. We conclude that monkey cerebral cortex with an abnormal fatty acid composition produced by dietary n-3 fatty acid deficiency has a remarkable capacity to change its fatty acid content after dietary fish oil, both to increase 22:6 n-3 and to decrease 22:5 n-6 fatty acids. The biochemical evidence of n-3 fatty acid deficiency was completely corrected. These data imply a greater lability of the fatty acids of the phospholipids of the cerebral cortex than has been hitherto appreciated. —**Connor, W. E., M. Neuringer, and D. S. Lin.** Dietary effects on brain fatty acid composition: the reversibility on n-3 fatty acid deficiency and turnover of docosahexaenoic acid in the brain, erythrocytes, and plasma of rhesus monkeys. *J. Lipid Res.* 1990. **31:** 237-247.

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Membrane lipids constitute 50-60% of the solid matter in the brain (2), and phospholipids are quantitatively the most significant component of membrane lipids (3). A major proportion of brain phospholipids contain long chain polyunsaturated fatty acids of the two essential fatty acid classes, n-6 and n-3 (4, 5). These fatty acids usually occupy the *sn*-2 position of brain phospholipid molecules. Normally, docosahexaenoic acid (22:6 n-3, DHA) is the predominant polyunsaturated fatty acid in the phospholipids of the cerebral cortex and retina. The primate brain gradually accumulates its full complement of DHA during intrauterine life and during the first year after birth (6, 7). DHA or its precursor n-3 fatty acids must be provided in the diet of the mother and infant for normal brain and retinal development.

In our previous reports, we have shown that infant rhesus monkeys born from mothers fed in n-3 fatty acid-deficient diet and then also fed a deficient diet after birth developed low levels of n-3 fatty acids in the brain and retina and impairment in visual function (8-10). The specific biochemical markers of the n-3-deficient state were a marked decline in the DHA of the cerebral cortex and a compensatory increase in n-6 fatty acids, especially docosapentaenoic acid (22:5 n-6). Thus, the sum total of the n-3 and n-6 fatty acids remained similar, about 50% of the fatty acids in phosphatidylethanolamine and phospha-

Abbreviations: EPA, eicosapentaenoic acid; PE, phosphatidylethanolamine; PS, phosphatidylserine; DHA, docosahexaenoic acid.

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[2]To whom reprint requests should be addressed at: Department of Medicine L465, The Oregon Health Sciences University, Portland, OR 97201.

tidylserine, indicating the existence of mechanisms in the brain to conserve polyunsaturation of membrane phospholipids as much as possible, despite the n-3-deficient state.

In the present study, juvenile rhesus monkeys who had developed n-3 fatty acid deficiency since intrauterine life were repleted with a fish oil diet rich in the n-3 fatty acids, DHA and 20:5 n-3 (eicosapentaenoic acid, EPA). Fatty acid compositions were determined for the lipid classes of plasma and erythrocytes and for the phospholipid classes of frontal cortex samples obtained from serial biopsies and at the time of autopsy. From these analyses, the half-lives of DHA and EPA in the phospholipids of plasma, erythrocytes, and cerebral cortex were estimated. The deficient brain rapidly regained a normal or even supernormal content of DHA with a reciprocal decline in n-6 fatty acids, demonstrating that the fatty acids of the gray matter of the brain turn over with relative rapidity under the circumstances of these experiments.

METHODS

Five adult female rhesus monkeys were fed a semipurified diet low in n-3 fatty acids for at least 2 months before conception and throughout pregnancy. The resulting infants were fed a liquid semipurified diet, similarly deficient in n-3 fatty acids, from birth until 10-24 months of age. The detailed composition of these semipurified diets (11) is shown in **Table 1**. Safflower oil was used as the sole fat source for the deficient diet because it has a very low content of linolenic acid (18:3 n-3) and a very high ratio of n-6 to n-3 fatty acids (**Table 2**).

Biochemical n-3 deficiency, electroretinographic abnormalities, and visual acuity loss were established, as described previously, in the monkeys before repletion (8-10). Beginning at 10 to 24 months of age, the five juvenile monkeys were then given the same semipurified diet with fish oil replacing 80% of the safflower oil as the fat source. The remaining 20% safflower oil provided ample amounts of essential n-6 fatty acids as linoleic acid (18:2 n-6) (4.5% of calories).

TABLE 1. Composition of the semi-purified diets for the monkeys (both dam and offspring)[a]

Ingredients	g/100 g	Percent of Calories
Lactose	30.0	54.9
Dextrose	30.0	
Fat-free casein hydrolysate	17.0	15.1
Oils (safflower, fish oil, or soy oil; see Table 2 for details)	15.0	30.0
Salt mix[b]	4.0	
Vitamin mix[c]	2.5	
Carrageenan	0.5	
Tween 80	1.4	
Taurine	0.05	

[a]One hundred g of this mixture was thoroughly blended with 557 ml of water to form a liquid diet.

[b]Hegsted IV salt mix; each gram contained 299.7 mg $CaCO_3$, 74.39 mg $CaHPO_4$, 0.3 mg $CuSO_4 \cdot H_2O$, 27.47 mg $FeC_6H_5O_7 \cdot 5H_2O$, 101.9 mg $MgSO_4 \cdot 7H_2O$, 4.99 mg $MnSO_4 \cdot {}^4H_2O$, 0.799 mg KI, 322.22 mg K_2HPO_4, 167.35 mg NaCl, and 0.249 mg $ZnCl_2$.

[c]Each gram of vitamin mix contained 0.625 mg retinyl acetate, 5 mg α-tocopherol, 25 mg ascorbic acid, 50 mg myo-inositol, 250 mg choline chloride, 2 mg menaquinone, 2.45 mg niacin, 0.5 mg riboflavin, 0.5 mg thiamine, 0.5 mg pyridoxine, 1.5 mg calcium pantothenate, 10 μg biotin, 50 μg folic acid, 1 μg vitamin B_{12}, ergocalciferol, and 661.86 mg dextrose (11).

TABLE 2. Fatty acid composition of the experimental diets

Fatty Acids	Percent of Total Fatty Acids in Deficient Diet (Safflower Oil)	Repletion Diet (Fish Oil)[a]	Control Diet (Soy Oil)
16:0	7.1	14.6	10.7
18:0	2.5	2.9	4.2
18:1 n-9	13.3	10.2	23.7
18:2 n-6	76.0	16.3	53.1
Total n-6	76.5	18.9	53.4
18:3 n-3	0.3	0.5	7.7
20:5 n-3	0	13.4	0
22:6 n-3	0	9.0	0
Total n-3	0.3	28.1	7.7
n-6/n-3	255.0	0.7	7.0

[a]Consists of a 4:1 mixture of fish oil (San Omega^R or MaxEPA^R) and safflower oil.

Two monkeys began receiving the fish oil diet at 10–11 months of age and two at 23–24 months. As the acceptability of the fish oil diet was unknown, these monkeys were started on the fish oil diet at on-fourth the full dose and increased to the full dose over 9–12 days. It was found that these monkeys accepted the fish oil diet well. The fifth monkey, therefore, was fed the full-strength fish oil diet from the first day starting at 17 months of age. The first four monkeys consumed fish oil from Japan (San Omega^{R}, Nippon Oil & Fat Co., Ltd., Tokyo, Japan) for 9–15 weeks and were then changed to MaxEPA^{R} (Seven Sea Limited, Hull, England); the fifth monkey received MaxEPA^{R} throughout. The fatty acid compositions of these two oils are similar, with San Omega oil having a slightly higher n–3 fatty acid content. Both of these oils have a similar low content of linoleic acid (0.7–1.1%). To provide ample linoleic acid, safflower oil (4.5% of kcal) was added with fish oil in the repletion diet. The n–3 fatty acid content was 46.6 and 34.2% for San Omega and MaxEPA oil, respectively. Two major n–3 fatty acids were EPA and and DHA. San Omega oil contains 24.4% EPA 12.3% DHA. MaxEPA oil has 16.4% EPA and 11.0% DHA. The content of other n–3 fatty acids was similar in these two oils: 18:3 n–3, 0.7–1.1%; 18:4 n–3, 2.4–5.2%; 20:4 n–3, 0.9–1.1%, and 22:5 n–3, 2.5–2.8%. The fatty acid composition of the repletion diet is shown in Table 2.

Serial samples of plasma and erythrocytes were collected just before and at 2, 4, 6, 8, 12, 16, 24, and 28 weeks after initiation of fish oil feeding. Biopsies of frontal cerebral cortex were obtained before and up to 28 weeks after the initiation of fish oil feeding. Four such biopsies were obtained sequentially from each monkey. In the first two monkeys, biopsies were obtained at 0, 12, 20, and 28 weeks of fish oil feeding; at 0, 8, 16, and 24 weeks in the second two monkeys; and at 1, 3, 6, and 12 weeks in the fifth monkey. Each biopsy consisted of a 15–30 mg sample of prefrontal cortex gray matter obtained through a small burr hole in the frontal bone of the skull under thiamylal anesthesia (25 mg/kg). No behavioral or neurological changes were noted after the biopsies. The monkeys were killed 43–129 weeks after initiating fish oil feeding. The first two monkeys who started fish oil feeding at 10–11 months of age were killed at 27–28 months of age after 73 weeks of fish oil feeding. The other two monkeys who started fish oil feeding at 23–24 months of age were killed at 33–36 months of age after 43–52 weeks of fish oil feeding. The fifth monkey who started fish oil feeding at 17 months of age was killed at 47 months of age after 129 weeks of fish oil feeding. Additional samples of frontal cortex were obtained at the time of autopsy. The cortical tissue adjacent to the biopsy sites was carefully examined and no overall damage was observed.

For comparison, previously reported data are also included in Figs. 1–3 for a group of 22-month-old juvenile monkeys fed the same semipurified diets but with soybean oil (control) or safflower oil (deficient) as the only fat source (8–10). The safflower oil diet was the same as that used in the present study. The soy oil diet contained a moderate but sufficient amount of n–3 fatty acid from linolenic acid (8% of total fatty acids) and had an n–6 to n–3 ratio roughly similar to that of Purina laboratory monkey chow. The brain phospholipid fatty acid composition of monkeys fed the soy oil diet was similar to that of monkeys fed Purina laboratory monkey chow. We therefore, chose monkeys fed soybean oil diet as having "normal" values for the comparisons with monkeys fed the n–3-deficient and the fish oil repletion diets.

The lipids of plasma were extracted by the procedure of Bligh and Dyer (12). Freshly separated plasma was vortexed with chloroform–methanol 1:1 to precipitate the plasma proteins, and the supernatant was mixed with chloroform and water to form aqueous and chloroform phases. The lipids in the chloroform phase were then collected. Erythrocytes were washed three times with saline. The lipids of erythrocytes were extracted by the procedure of Rose and Oklander (13), using chloroform and isopropanol, because the use of isopropanol in place of methanol avoids attracting heme pigment. The biopsies of frontal cortex were extracted by the method of Folch, Lees, and Sloane Stanley (14). The brain tissue was homogenized with chloroform–methanol 2:1 and the insoluble material was filtered. The lipid extract was washed with 0.58% NaCl. Butylated hydroxytoluene (5 mg/100 ml) was added to all lipid extracts as an antioxidant (15).

The lipid extracts of plasma and erythrocytes were separated into four major classes (phospholipids, free fatty acids, triglycerides and cholesteryl esters) by thin-layer chromatography (16). They were chromatographed on silica gel G plates (500 microns, Analtech, Newark, Del). The solvent system was hexane-chloroform-ethyl ether-acetic acid 80:10:10:1. The phospholipid species in the frontal cortex extracts were separated by a different thin-layer chromatography system (17), using pre-coated silica gel 60 plates (EM Science, Gibbstown, NJ) and a solvent system of methyl acetate–n-propanol–chloroform–methanol–0.25% aqueous KCl 25:25:25:10:9. The fatty acids in each lipid class or phospholipid class were transmethylated with boron trifluoride–methanol (18).

Methyl esters of fatty acids were analyzed by gas–liquid chromatography (19) on an instrument equipped with a hydrogen flame ionization detector (Perkin-Elmer Model Sigma 3B, Norwalk, CT) and a 30-meter SP-2330 fused silica capillary column (Supelco, Bellefonte, PA). Temperatures of column, detector, and injection port were 195° 250°, 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 by an HP-3390 integrator, and a computer (HP85, Hewlett Packard, Palo Alto, CA) identified and quantified each individual fatty acid. A mixture of fatty

acid standards was run daily.

The half-life was defined as the time required to accumulate half of the final steady-state concentration or decay to half of the original concentration of a given phospholipid fatty acid. Fish oil feeding increased or decreased many essential fatty acids in tissue pool steadily until they reached a new steady state (constant concentration). As shown in Figs. 1-3, these fatty acids in various tissues reached a new steady state 12 weeks after fish oil feeding. From the fatty acid compositions of phospholipids of serial samples of plasma, erythrocytes, and cerebral cortex, the accumulation and decay curves of several key polyunsaturated fatty acids were plotted on semilog paper. These included the changes of DHA and 22:5 n-6 in cerebral cortex, the changes of linoleic acid (18:2 n-6), arachidonic acid (20:4 n-6), EPA, and DHA in erythrocytes, and changes of linoleic acid, EPA, and DHA in plasma. From these graphs, the half-lives ($t_{1/2}$) of these fatty acids were then estimated (20). This approach only provided a gross estimate of turnover times. More accurate turnover data would, of course, have been desirable but was not possible to obtain given the limitations of our experiments. It also should be borne in mind that the half-lives of these fatty acids only represented their turnover under these experimental conditions: n-3 fatty acid-deficient monkeys repleted with fish oil.

The unsaturation index of each tissue was calculated by multiplying the number of double bonds by the percent composition of the fatty acid in the lipid class and summating the values (21).

The weight percent of key fatty acid before and after fish oil feeding were compared by the paired *t* test (22).

TABLE 3. Major fatty acids of the plasmas of monkeys fed safflower oil followed by fish oil feeding

Fatty Acids	Safflower Oil	Fish Oil
	% of total fatty acids, mean ± SD	
16:0	12.6 ± 1.4	17.2 ± 2.3
18:0	10.3 ± 0.7	10.7 ± 1.1
Total saturated[a]	24.8 ± 1.8	31.0 ± 2.4
16:1 n-7	0.5 ± 0.1	3.9 ± 0.4[c]
18:1 n-9	7.4 ± 0.1	12.1 ± 1.8
Total monounsaturated[a]	8.8 ± 0.4	17.8 ± 2.2[d]
18:2 n-6	54.3 ± 3.0	9.2 ± 4.5[e]
20:4 n-6	6.4 ± 1.3	5.0 ± 0.4
22:4 n-6	0.8 ± 0.2	0.2 ± 0.1[b]
22:5 n-6	0.5 ± 0.1	0.4 ± 0.1
Total n-6[a]	65.4 ± 1.6	15.5 ± 3.8[e]
18:3 n-3	0	0.2 ± 0.1[e]
20:5 n-3	0	22.1 ± 2.2[e]
22:5 n-3	0	2.1 ± 0.3[e]
22:6 n-3	0.1 ± 0.1	8.3 ± 1.0[e]
Total n-3[a]	0.1 ± 0.1	33.6 ± 3.6[e]
Total polyunsaturated[a]	65.6 ± 1.6	50.1 ± 5.6[b]
Unsaturation index	149	230

[a] A few other fatty acids of this class are included in the total.
As determined by paired *t* test (safflower oil vs. fish oil): [b] $P < 0.025$; [c] $P < 0.01$; [d] $P < 0.005$; [e] $P < 0.001$.

RESULTS

Plasma

Table 3 depicts the major fatty acids in the plasma of five juvenile monkeys before fish oil feeding and at the time of the last brain biopsy (after 12-28 weeks of fish oil feeding). Changing from the n-3 fatty acid-deficient diet (safflower oil) to the n-3-rich diet (fish oil) increased the total plasma n-3 fatty acids greatly, from 0.1 to 33.6% of total fatty acids. EPA, which was especially high in the fish oil, contributed the major increase, from zero to 22.1%, and represented 66% of the total n-3 fatty acid increase. DHA increased from 0.1 to 8.3% and 22:5 n-3 from zero to 2.1%. A major reciprocal decrease occurred in the n-6 fatty acid linoleic acid which was reduced from 54.3 to 9.2% of total fatty acids while total n-6 fatty acids fell from 65.4 to 15.5%. The change in arachidonic acid, however, was relatively small, from 6.4 to 5.0%. The unsaturation index of the plasma lipids (21) increased from 149 in the safflower oil-fed monkeys to 230 after fish oil feeding. From the analyses of serial plasma phospholipid samples, the changes (increase or decrease) of the three major fatty acids as function of time were plotted (**Fig. 1**). These changes reached a new steady state after 12 weeks of the fish oil feeding and remained constant until autopsy.

Table 4 presents the fatty acid composition of the four plasma lipid classes of these monkeys before and after fish oil feeding. These lipid classes showed a similar dietary influence on fatty acid content. In the phospholipid fraction, the n-3 fatty acids increased from 0.4% to 33.8% in the fish oil diet. EPA increased from zero to 19.0%, accounting for 55% of the total increase, while DHA increased from zero to 11.3%, or 35% of the total increase. Linoleic acid reciprocally decreased from 36.4 to 5.0% and total n-6 fatty acids from 48.0 to 11.8% of total fatty acids. In cholesteryl esters, n-3 fatty acids increased from 0.2% to 35.7%. An increase in EPA from zero to 30.8% accounted for 86 percent of the increase, a much greater proportion than in phospholipids, whereas DHA only increased from zero to 3.8%. The decline in n-6 fatty acids from 76.9 to 23.4% was largely accounted for by a decrease in linoleic acid from 73.2 to 16.9%. Similar changes were seen in the triglycerides and free fatty acid fractions. The unsaturation indexes of all lipid classes were increased by fish oil feeding.

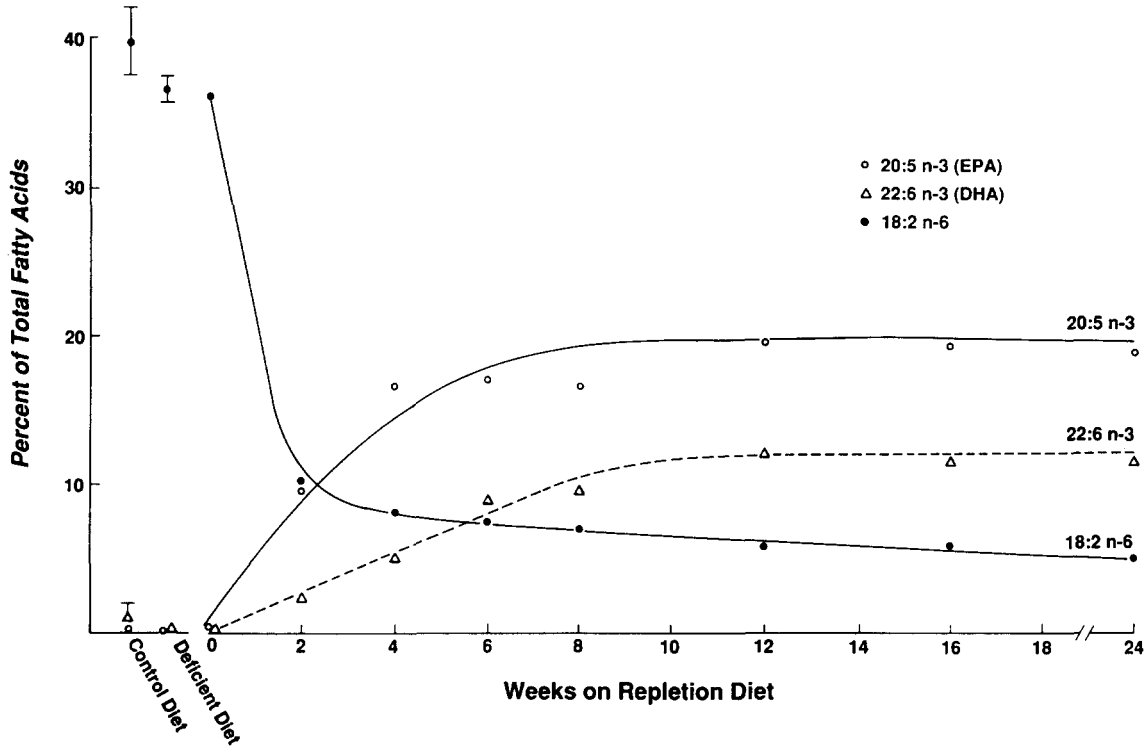

Fig. 1. The time course of mean fatty acid changes in plasma phospholipids after the feeding of fish oil. For standard deviations, see Table 4. Note that reciprocal changes of the two major n-3 (EPA and DHA) and the major n-6 (18:2) polyunsaturated fatty acids occurred as n-3 fatty acids increased and n-6 fatty acids decreased. The concentrations of these fatty acids in the plasma phospholipids of monkeys fed the control soybean oil and safflower oil diet from our previous study (10) are given for comparison. Expressed as percentage of total fatty acids, DHA in control monkeys was 1.1 ± 0.7%; EPA 0.2 ± 0.1%; 18:2 n-6, 39.6 ± 2.3%. In deficient monkeys, DHA was 0%; EPA 0%; 18:2 n-6 36.7 ± 0.7%.

TABLE 4. Major fatty acids of the plasma lipid clases of juvenile monkeys fed safflower oil followed by fish oil feeding

	Phospholipids		Cholesterol Esters		Triglyceride		Free Fatty Acids	
Fatty Acids	Safflower Oil	Fish Oil	Safflower Oil	Fish Oil	Safflower Oil	Fish Oil	Safflower Oil	Fish Oil
	% of total fatty acids, mean ± SD							
16:0	17.1 ± 1.9	19.8 ± 1.0[a]	6.7 ± 1.3	15.1 ± 1.9[e]	10.8 ± 4.7	6.6 ± 4.4	14.4 ± 6.2	14.0 ± 4.9
18:0	23.8 ± 2.0	18.5 ± 0.9[d]	1.3 ± 0.2	1.5 ± 0.2	4.3 ± 1.6	2.7 ± 0.8	8.6 ± 1.3	8.5 ± 1.7
Total saturated[f]	42.5 ± 3.2	40.7 ± 2.1	8.9 ± 1.6	18.4 ± 2.2[e]	17.9 ± 6.2	11.5 ± 6.0	25.5 ± 5.7	22.8 ± 7.1
16:1 n-7	0.4 ± 0.2	1.9 ± 0.5[d]	0.9 ± 0.3	3.5 ± 0.2[e]	1.4 ± 1.0	4.5 ± 1.3[e]	1.7 ± 1.0	4.2 ± 1.0[d]
18:1 n-9	6.5 ± 1.0	7.7 ± 1.3	11.6 ± 2.5	16.8 ± 2.9[c]	15.3 ± 3.3	12.6 ± 1.8	11.9 ± 1.2	16.1 ± 0.9[e]
Total monounsaturated[f]	8.3 ± 1.3	12.6 ± 1.7[c]	13.1 ± 2.2	21.4 ± 3.2[d]	17.6 ± 3.7	19.8 ± 3.3	15.3 ± 2.3	26.5 ± 8.5[a]
18:2 n-6	36.4 ± 2.8	5.0 ± 3.2[e]	73.2 ± 2.4	16.9 ± 10.8[e]	58.2 ± 10.3	9.3 ± 5.5[e]	52.9 ± 7.0	10.2 ± 3.7[e]
20:4 n-6	6.1 ± 2.3	5.6 ± 0.5	2.8 ± 0.7	5.8 ± 0.9[c]	1.3 ± 0.7	2.2 ± 0.7	1.8 ± 0.8	1.8 ± 0.8
22:4 n-6	0.4 ± 0.2	0.2 ± 0.1	0	0.1 ± 0.1	0.3 ± 0.3	0.9 ± 0.3[d]	0.2 ± 0.3	tr
22:5 n-6	0.3 ± 0.2	0.1 ± 0.1	0	tr	0	0.3 ± 0.2[b]	0.2 ± 0.4	tr
Total n-6[f]	48.0 ± 4.1	11.8 ± 3.6[e]	76.9 ± 2.1	23.4 ± 10.0[e]	62.1 ± 9.5	13.6 ± 4.5[e]	56.9 ± 6.4	12.9 ± 3.9[e]
18:3 n-3	tr	tr	0	0.2 ± 0.1	0.1 ± 0.1	0.6 ± 0.6	0	0.8 ± 0.2
20:5 n-3	tr	19.0 ± 2.0[e]	0	30.8 ± 4.9[e]	tr	34.0 ± 10.2[e]	0.1 ± 0.1	15.4 ± 2.9[e]
22:5 n-3	tr	3.1 ± 0.3[e]	0	0.3 ± 0.1[e]	0	3.8 ± 0.5[e]	0	1.9 ± 1.2[e]
22:6 n-3	tr	11.3 ± 0.7[e]	0	3.8 ± 0.8[e]	0	13.3 ± 4.2[e]	0.3 ± 0.4	13.5 ± 2.5[e]
Total n-3[f]	0.4 ± 0.3	33.8 ± 1.3[e]	0.2 ± 0.1	35.7 ± 6.4[e]	0.7 ± 0.7	33.0 ± 14.2[e]	0.5 ± 0.4	33.8 ± 3.0[e]
Total polyunsaturated	48.3 ± 4.3	45.6 ± 3.7	77.1 ± 2.0	59.1 ± 4.6[e]	62.8 ± 9.1	66.6 ± 10.0	55.1 ± 7.7	46.7 ± 5.9[a]
Unsaturation index	109	225	171	258	141	323	132	225

As determined by paired *t* test (safflower oil vs fish oil): [a]$P < 0.05$; [b]$P < 0.0025$; [c]$P < 0.01$; [d]$P < 0.005$; [e]$P < 0.001$.
[f]A few other fatty acids of this class are included in the total.

Erythrocytes

The major fatty acids of the phospholipids of erythrocytes before fish oil feeding and at the time of the last cortical biopsy (after 12–28 weeks of fish oil feeding) are displayed in **Table 5**. Total n-3 fatty acids increased from 1.3% to 30.9% of total fatty acids after the fish oil diet. EPA and DHA had similar increases, from 0.2 to 14.0% for EPA and from 0.2 to 13.4% for DHA. Total n-6 fatty acids decreased from 53.4 to 13.4%. Unlike plasma, erythrocytes showed a significant decrease in arachidonic acid, from 18.4 to 5.5%, as well as in linoleic acid, from 24.0 to 6.9%. The changing concentrations of the four major fatty acids (EPA, DHA, linoleic, and arachidonic acids) affected by the fish oil feeding are illustrated in **Fig. 2**. The fatty acid composition of erythrocytes reached a new steady state by 12 weeks after fish oil feeding and remained constant until autopsy. The unsaturation index of erythrocytes increased from 165 on the deficient diet to 220 after fish oil consumption.

Cerebral cortex

Dramatic changes in the fatty acids of the gray matter of frontal cortex could be detected within 1 week after the

TABLE 5. Major fatty acids of erythrocyte phospholipids of juvenile monkeys fed safflower oil followed by fish oil feeding

Fatty Acids	Safflower Oil	Fish Oil
	% of total fatty acids, mean ± SD	
16:0	13.0 ± 4.1	19.3 ± 1.5
18:0	17.9 ± 1.1	15.5 ± 1.3
Total saturated[a]	33.5 ± 3.0	37.9 ± 1.6
16:1 n-7	0.8 ± 0.5	1.8 ± 1.0
18:1 n-9	6.4 ± 0.1	10.3 ± 1.1[c]
Total monounsaturated[a]	8.7 ± 0.8	16.5 ± 1.1[d]
18:2 n-6	24.0 ± 1.2	6.9 ± 1.2[e]
20:4 n-6	18.4 ± 2.4	5.5 ± 1.6[d]
22:4 n-6	5.8 ± 0.9	0[e]
22:5 n-6	1.4 ± 0.3	0.1 ± 0.2
Total n-6[a]	53.4 ± 2.8	13.4 ± 2.0[e]
18:3 n-3	0	0.1 ± 0.2
20:5 n-3	0.2 ± 0.2	14.0 ± 0.9[e]
22:5 n-3	0.5 ± 0.3	3.3 ± 0.3[e]
22:6 n-3	0.2 ± 0.2	13.4 ± 2.4[e]
Total n-3[a]	1.3 ± 1.0	30.9 ± 3.6[e]
Total polyunsaturated[a]	54.6 ± 3.5	44.3 ± 4.5[b]
Unsaturation index	165	220

[a]A few other fatty acids of this class are included in the total.
As determined by paired *t* test (safflower oil vs fish oil): [b]$P < 0.025$; [c]$P < 0.01$; [d]$P < 0.005$; [e]$P < 0.001$.

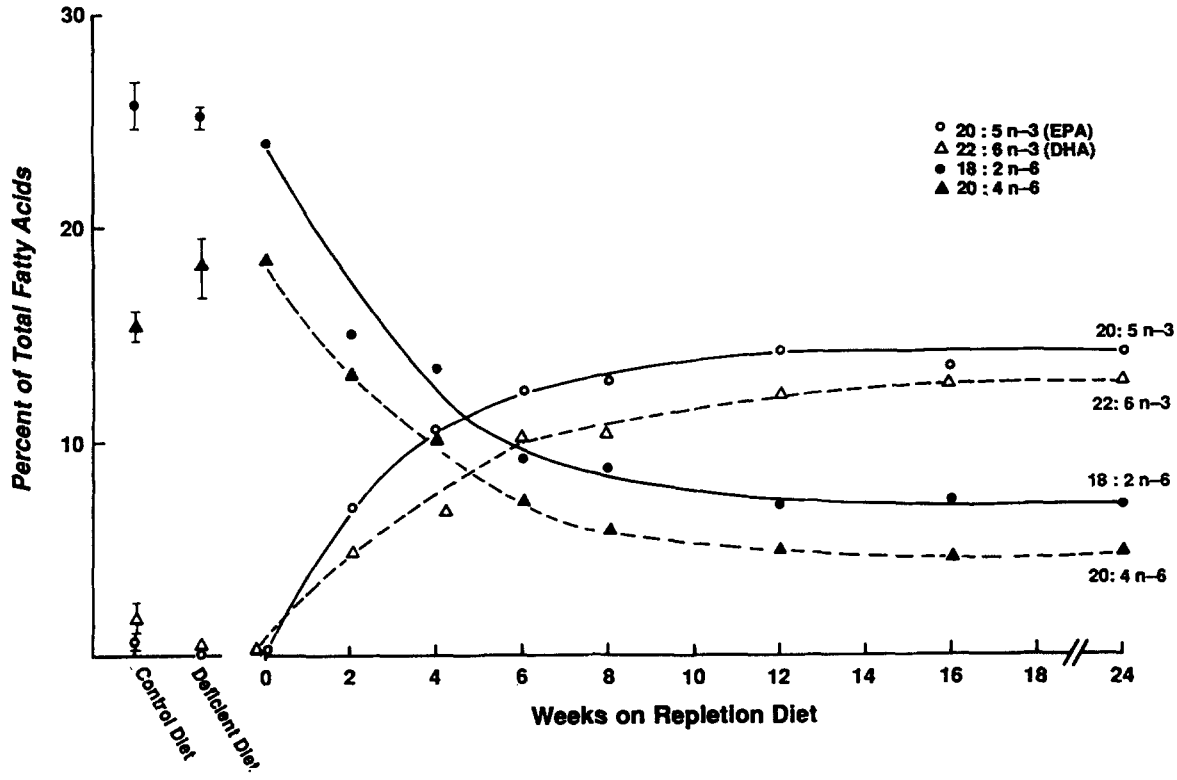

Fig. 2. The time course of mean fatty acid changes in erythrocyte phospholipids after the feeding of fish oil. For standard deviations, see Table 5. Note that the reciprocal changes of the two major n-3 (EPA and DHA) and the two major n-6 (18:2 and 20:4) polyunsaturated fatty acids occurred as n-3 fatty acids increased and n-6 fatty acids decreased. The concentrations of these four fatty acids in the erythrocyte phospholipids of monkeys fed the control soybean oil and the deficient safflower oil diet from our previous study (10) are given for comparison. Expressed as percentage of total fatty acids, DHA in control monkeys as 1.7 ± 0.9%; EPA, 0.5 ± 0.1%; 20:4 n-6, 15.4 ± 0.5%; 18:2 n-6, 25.7 ± 1.0%. In deficient monkeys, DHA was 0.2 ± 0%; EPA, 0%; 20:4 n-6, 18.2 ± 1.3; 18:2 n-6, 25.2 ± 0.4%.

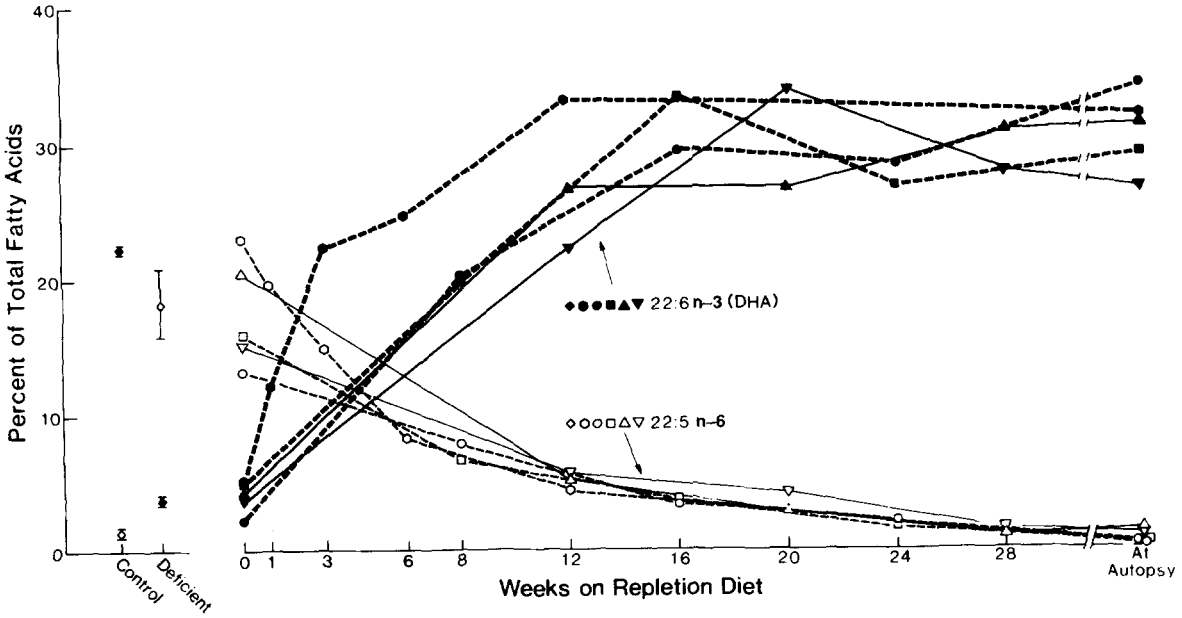

Fig. 3. The time course of fatty acid changes in phosphatidylethanolamine of the cerebral cortex of five juvenile monkeys fed fish oil for 43–129 weeks. As DHA increased, 22:5 n-6 decreased reciprocally. Levels of DHA and 22:5 n-6 in phosphatidylethanolamine of the frontal cortex of monkeys fed control (soybean oil) and deficient diets from a previous study (10) are given for comparison. DHA and 22:5 n-6 in control monkeys were 22.3 ± 0.3 and 1.4 ± 0.3% of total fatty acids, respectively. DHA and 22:5 n-6 in the deficient monkeys were 3.8 ± 0.4 and 18.3 ± 2.5%, respectively.

fish oil diet was given (**Fig. 3**). All four major phospholipid species of the brain underwent extensive remodeling of their constituent fatty acids (**Table 6**). By 12–28 weeks, the total n-3 fatty acids in phosphatidylethanolamine increased from 4.2 to 36.2% of total fatty acids. The major increase was in DHA, from 4.2 to 29.3%, while EPA and 22:5 n-3, another n-3 fatty acid found in fish oil, each increased from zero to about 3%. Total n-6 fatty acids reciprocally decreased from 44.1 to 15.8% of the total fatty acids, with the major reductions occurring in 22:5 n-6,

TABLE 6. Major fatty acids of the phospholipid classes of frontal lobe of the cerebral cortex of juvenile monkeys fed safflower oil followed by fish oil feeding

	Phosphatidylethanolamine		Phosphatidylserine		Phosphatidylinositol		Phosphatidylcholine	
Fatty Acids	Safflower Oil	Fish Oil	Safflower Oil	Fish Oil	Safflower Oil	Fish Oil	Safflower Oil	Fish Oil
	% of total fatty acids, mean ± SD							
16:0	5.7 ± 0.9	6.2 ± 1.1	5.4 ± 1.7	3.8 ± 1.6	9.7 ± 9.0	12.9 ± 2.1	48.2 ± 4.8	38.8 ± 5.6
18:0	26.2 ± 3.5	25.1 ± 6.4	36.3 ± 6.4	35.3 ± 1.7	20.4 ± 1.1	22.2 ± 1.6	11.9 ± 1.4	11.7 ± 1.2
Total saturated[a]	35.7 ± 4.9	34.9 ± 5.4	43.8 ± 7.3	41.9 ± 4.6	36.9 ± 8.7	36.6 ± 9.0	62.6 ± 4.2	53.3 ± 6.3
16:1 n-7	2.5 ± 2.0	1.5 ± 1.2	1.1 ± 0.5	0.7 ± 0.4	2.5 ± 1.7	1.9 ± 0.5	1.3 ± 0.8	2.7 ± 1.0
18:1 n-9	6.5 ± 1.0	7.2 ± 0.9	7.9 ± 1.6	7.5 ± 1.2	7.4 ± 3.1	9.2 ± 3.9	20.1 ± 1.6	27.1 ± 3.9
Total monounsaturated[a]	11.3 ± 3.2	10.2 ± 1.8	10.5 ± 1.9	9.2 ± 0.9	12.8 ± 3.4	14.0 ± 3.5	23.2 ± 2.0	31.6 ± 2.9[c]
18:2 n-6	0.8 ± 0.4	0.3 ± 0.1[b]	0.7 ± 0.2	0.3 ± 0.2[c]	2.2 ± 1.6	1.5 ± 1.7	2.2 ± 0.5	0.6 ± 0.1[b]
20:4 n-6	12.8 ± 1.5	8.9 ± 1.9[c]	6.3 ± 2.0	3.0 ± 1.8[b]	17.9 ± 6.0	17.7 ± 3.6	4.9 ± 1.9	4.2 ± 0.8
22:4 n-6	11.5 ± 1.7	3.7 ± 1.1[f]	9.4 ± 3.2	3.0 ± 0.6[c]	10.8 ± 3.1	2.9 ± 1.0[e]	0.8 ± 0.3	0.2 ± 0.1[d]
22:5 n-6	17.5 ± 4.0	2.1 ± 1.3[e]	20.4 ± 2.6	3.5 ± 1.7[c]	10.6 ± 2.7	0.7 ± 0.3[e]	1.2 ± 0.5	0[e]
Total n-6[a]	44.1 ± 6.1	15.8 ± 3.7[f]	38.4 ± 6.9	11.0 ± 3.7[f]	43.2 ± 9.9	24.5 ± 4.8[b]	10.7 ± 3.3	5.9 ± 1.0[b]
18:3 n-3	0	0	0	0	0	0	0	0
20:5 n-3	0	3.1 ± 1.4[f]	0	1.0 ± 0.2[f]	0	3.8 ± 1.8[f]	0	1.5 ± 0.5[f]
22:5 n-3	0	3.5 ± 0.5[f]	0	3.2 ± 0.6[f]	0	3.2 ± 2.2[f]	0	0.5 ± 0.1[f]
22:6 n-3	4.2 ± 1.2	29.3 ± 2.6[f]	4.7 ± 1.8	32.1 ± 4.8[f]	2.8 ± 1.9	10.5 ± 2.1[e]	0.3 ± 0.2	2.9 ± 0.3[e]
Total n-3[a]	4.2 ± 1.2	36.2 ± 3.0[f]	4.8 ± 1.9	36.4 ± 5.4[e]	2.9 ± 1.9	17.8 ± 5.7[e]	0.4 ± 0.2	5.1 ± 1.0[e]
Total polyunsaturated[a]	48.3 ± 6.9	52.3 ± 5.7	43.3 ± 8.3	46.2 ± 5.6	46.1 ± 11.3	42.6 ± 2.8	11.3 ± 3.5	11.1 ± 1.8
Unsaturated index	223	281	205	265	202	201	58	78

[a]A few other fatty acids of this class are included in the total.
As determined by paired *t* test (safflower oil vs fish oil); [b]$P < 0.05$; [c]$P < 0.025$; [d]$P < 0.01$; [e]$P < 0.005$; [f]$P < 0.001$.

from 17.5 to 2.1%, and 22:4 n-6, from 11.5 to 3.7%. There was also a moderate decrease of arachidonic acid from 12.8 to 8.9% of total fatty acids.

In the phosphatidylserine fraction, the total n-3 fatty acids also increased greatly from 4.8 to 36.4% of total fatty acids with DHA increasing from 4.7 to 32.1%. Total n-6 fatty acids decreased from 38.4 to 11.0%. Again, the major and reciprocal decrease was in 22:5 n-6, from 20.4 to 3.5%. In the phosphatidylinositol fraction, total n-3 fatty acids increased from 2.9 to 17.8%, while n-6 fatty acids decreased from 43.2 to 24.5% and 22:5 n-6 decreased from 10.6 to 0.7%. Although the content of n-3 fatty acids in phosphatidylcholine is relatively small even in the normal brain, this fraction also showed an increase from 0.4 to 5.1% after fish oil feeding. Unlike PE and PS, the arachidonic acid levels in PI and PC were not decreased by the fish oil feeding.

In summary, fish oil feeding resulted in reciprocal changes in the levels of n-3 and n-6 fatty acids in the phospholipids of cerebral cortex. The two major 22-carbon n-3 and n-6 fatty acids, DHA and 22:5 n-6, were responsible for the greatest changes. Fig. 3 plots the changes in these two fatty acids in phosphatidylethanolamine, plus the analogous values for juvenile monkeys fed a control (soybean oil) diet and deficient (safflower oil) diet from our previous study (10). In control monkeys the DHA and 22:5 n-6 contents of frontal cortex were 22.3 ± 0.3 and 1.4 ± 0.3%, respectively, whereas in deficient monkeys they were nearly the reverse, 3.8 ± 0.4% DHA and 18.3 ± 2.5% 22:5 n-6 (10). Reflecting the high content of the long chain n-3 fatty acids of fish oil, the DHA content of cerebral cortex in the fish oil monkeys was even higher than in the soybean oil-fed control monkeys (29.3 ± 2.6 vs. 22.3 ± 0.3% $P < 0.025$).

Turnover of fatty acids in various tissues

Using the serial data for the cerebral cortex, plasma, and erythrocytes, we constructed accumulation and decay curves for several key fatty acids in these tissues which provided gross estimates of their turnover times under steady state conditions after fish oil feeding to n-3 fatty acid-deficient monkeys. For cerebral cortex, a steady state was reached after 12 weeks of fish oil feeding for DHA, but 22:5 n-6 took longer to decline to the low levels found in the cortex of control animals. The half-lives of DHA in cerebral phospholipids ranged from 17 to 21 days: 21 days for phosphatidylethanolamine, 21 days for phosphatidylserine, 18 days for phosphatidylinositol, and 17 days for phosphatidylcholine. The corresponding values for 22:5 n-6 in these same phospholipids were 32, 49, 14, and 28 days, respectively. The half-lives ($t_{1/2}$) of linoleic acid, EPA, and DHA in plasma phospholipids were estimated to be 8, 18, and 29 days, respectively. In the phospholipids of erythrocytes, linoleic acid, arachidonic acid, EPA, and DHA had half-lives of 28, 32, 14, and 21 days, respectively.

DISCUSSION

Before 1940 it was generally considered that phospholipids, once laid down in the nervous system of mammals during growth and development, were comparatively static entities. However, later studies using [^{32}P]orthophosphate showed that brain phospholipids as a whole are metabolically active in vivo (23, 24). In the present study, by following the changes in phospholipid fatty acid composition, we have demonstrated for the first time that an n-3 fatty acid-enriched diet can reverse a severe n-3 fatty acid deficiency in the brains of primates. The phospholipid fatty acids of the cerebral cortex of juvenile monkeys are in a dynamic state and are subject to continuous turnover under certain defined conditions.

The observed changes in fatty acid composition could be the result of a complete breakdown and resynthesis of cortical phospholipids or a turnover of only the fatty acids in the *sn*-2 position, which has the higher proportion of polyunsaturated acyl groups. Turnover of fatty acids at the *sn*-2 position is well known, and is commonly referred to as deacylation/reacylation (25, 26). This process could have an important role in maintaining optimal membrane composition without the high energy cost associated with de novo phospholipid synthesis.

The reversibility of n-3 fatty acid deficiency in the monkey cerebral cortex was relatively rapid in our study. Effects of fish oil feeding were seen within 1 week after its initiation. By that time, DHA in the phosphatidylethanolamine of the cerebral cortex had more than doubled. The DHA concentration in phosphatidylethanolamine reached the control value of 22% in 6-12 weeks after fish oil feeding. We and others have demonstrated that the uptake of DHA and other fatty acids occurs within minutes after their intravenous injection bound to albumin (27). Furthermore, DHA is taken up by the brain in preference to other fatty acids (27). In contrast to the rapid incorporation of DHA, 22:5 n-6 only decreased from 23% to 20% during the first week. This asymmetry may indicate that the replacement of 22:5 n-6 from the *sn*-2 position by DHA is not specific.

In the present study, the half-life of DHA of phosphatidylethanolamine in cerebral cortex was similar to the half-lives of DHA in plasma and erythrocyte phospholipids, roughly 21 dyas. These data suggest that the blood-brain barrier present for cholesterol (28, 29) and other substances may not exist for the fatty acids of the plasma phospholipids because of the relatively rapid uptake of plasma DHA into the brain. The mechansims of transport of these fatty acids remain to be investigated.

Similar reversals of biochemical deficiencies of n-3 fatty acids or of total essential fatty acids have been studied in the rodent brain under somewhat different experimental condition (21, 30-34). In recent study by Youyou et al. (33), complete recovery from the n-3 fatty acid deficiency,

as measured by an increase of DHA and a decrease of 22:5 n-6, required 13 weeks as compared to 6-12 weeks in our monkeys. There were major differences between this study and ours which may be responsible for the different recovery rates observed. Most importantly we fed monkeys fish oil, which is high in DHA and EPA, whereas they fed young rats soy oil, which is lower in total n-3 fatty acids, and contains only the precursor of DHA, linolenic acid (18:3 n-3). The in situ biosynthesis of DHA from 18:3 n-3 may be a rate-limiting factor because of low desaturase activity, and also because 18:3 n-3 is oxidized more readily than DHA (35). Dietary 18:3 n-3 is thus less effective in promoting biochemical recovery than dietary DHA or EPA (36). The effects of dietary n-3 fatty acids from fish oil, linseed oil, and soy oil upon the lipid composition of the rat brain have also been reported by several groups of investigators (37-41).

Because of the reciprocal changes of n-3 and n-6 fatty acids with fish oil feeding, the sum total of n-3 and n-6 polyunsaturation of the brain of animals fed n-3-deficient and fish oil diets remained very similar. However, the unsaturation index of phospholipids of the frontal cortex was higher in the fish oil-fed monkeys than in n-3-deficient monkeys. The functional significance of this difference in the unsaturation index is not known. Since phospholipids rich in polyunsaturated fatty acids constitute an integral part of brain and retinal membranes, the degree of unsaturation of these fatty acids may have an important influence on the structure of the membranes and their functions, via changes in biophysical properties and/or the activities of membrane-bound proteins including enzymes, receptors, or transport systems (42).

In our n-3-deficient monkeys, the electroretinograms showed several abnormalities before fish oil was fed (10). After repletion, when the concentration of DHA had been restored to above normal levels, the electroretinograms remained abnormal (43). The reason for the failure of the electroretinogram to improve is unknown but may relate to the time of repletion in the animal's development or to the use of fish oil containing EPA as well as DHA. The use of purified DHA, when it is available in quantity, might be a more physiologic way of repleting n-3 fatty acid-deficient monkeys. As demonstrated in the present experiment, fish oil feeding was able not only to reverse the n-3 fatty acid-deficient state in the brain, but also to increase the n-3 fatty acid content in brain phospholipids above control levels. Furthermore, EPA increased from zero in control monkeys to 3.1% after fish oil feeding and 22:5 n-3 increased from 0.1 to 3.5%. At the same time, arachidonic acid and 22:4 n-6 decreased below control levels. Whether this "overload" of n-3 fatty acids and perhaps unphysiologic reduction of n-6 fatty acids in brain phospholipids was advantageous or detrimental in terms of membrane function is uncertain. Similar considerations would apply to the retina and its functioning.

Several questions are raised by the rapid incorporation of dietary DHA and other n-3 fatty acids from fish oil into phospholipid membranes of the cerebral cortex of juvenile rhesus monkeys. Would primate brains of "normal" fatty acid composition incorporate dietary DHA just as avidly as the brains of n-3-deficient monkeys? This situation is analogous to humans consuming large quantities of fish oil. Do their brains and other tissues change in composition and would this be advantageous? Quantities of EPA in the erythrocytes and cerebral cortex of the fish oil-supplemented monkeys were much higher than is normally the case. These abnormal levels might lead to functional disturbances, but no information is available about this point. Future studies of fish oil feeding to normal adult monkeys may provide answers to these questions, especially if molecular species of fatty acids of the phospholipid classes are determined and the function of the "changed" organs is measured. For example, when we analyzed individual phospholipid molecular species of the brains of monkeys fed different diets, we observed highly significant dietary effects (44).

If DHA turns over as rapidly in the adult normal brain as in the deficient monkey brain, then perhaps the brain should be provided with a constant supply of DHA or other n-3 fatty acids. Ultimatly, dietary sources of n-3 fatty acids would be desirable in both adults and infants (45). Whether the n-3 fatty acid supply from the diet should be as 18:3 n-3 or preformed DHA or both is not completely known. It is possible that ample amounts of DHA could be synthesized from 18:3 n-3 via the desaturation and elongation pathways. However, the active uptake of DHA by the infant rat brain over other fatty acids suggests preference for acquiring preformed DHA directly from the blood (29). In view of the significant impact of diet on brain composition, it will be important in the future to address the question of the appropriate amount and type of dietary n-3 fatty acids for optimal brain development during infancy and for maintenance during adult life (46).

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