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Docosahexaenoic Acid Is the Preferred Dietary n-3 Fatty Acid for the Development of the Brain and Retina[1]

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ABSTRACT. The metabolism of individual dietary n-3 fatty acids was studied in n-3 fatty acid-deficient newly hatched chicks. Laying hens were fed the n-6 fatty acid, ethyl linoleate, as the only source of polyunsaturated fat. Chicks were then fed the n-3-deficient hens' diet, or one of three other diets supplemented with the ethyl ester of 18:3n-3, 20:5n-3 [eicosapentaenoic acid (EPA)], or 22:6n-3 [docosahexaenoic acid (DHA)] at 0.44% of calories. At the end of 0, 1, 2, and 3 wk, the fatty acid composition of the brain, retina, liver, and serum was determined. Dietary EPA and DHA were equally effective at raising levels of DHA in the brain and retina. Dietary 18:3 was relatively ineffective in restoring brain and retina DHA. In the n-3-deficient chicks fed EPA or DHA, levels of DHA recovered to control values in both the brain and retina by 3 wk. Very little EPA accumulated in the brain or retina of chicks fed EPA. Hepatic synthesis of DHA from EPA appeared low, suggesting that the brain and retina synthesized the DHA that accumulated rapidly in these tissues after the feeding of EPA. The δ-4-desaturase enzyme was apparently very active, then, in the brain and retina. Retroconversion of dietary 22:6 to 22:5 and 20:5 was evident in the serum, liver, and retina but not in the brain. Thus, it was possible to study the relative metabolism and especially the interconversion of n-3 fatty acids in a environment uncomplicated by existing stores of these essential fatty acids. This study would suggest that 18:3 as the sole source of n-3 fatty acids in the diets of animals, including the human infant, may not be adequate for the biochemical development of the brain and retina and that dietary DHA is the preferred fatty acid of the n-3 series. (*Pediatr Res* 27: 89–97, 1990)

Abbreviations

DHA, docosahexaenoic acid
EPA, eicosapentaenoic acid

The n-3 family of polyunsaturated fatty acids has become the focus of much interest because of evidence that these compounds are antiatherogenic (2, 3) and may also be essential nutrients. Deficiencies of n-3 fatty acids in the diet lead to disturbances in behavior, deficient vision, and abnormal electroretinograms (4–8). DHA (22:6n-3), a 22 carbon n-3 fatty acid, is especially concentrated in the brain and retina. The need of these nervous tissues for DHA, and the fact that this 22 carbon fatty acid can be synthesized from the precursors α-linolenic acid (18:3n-3) and EPA, has given rise to the question of which fatty acid in the n-3 series would most rapidly be taken up by the brain and retina. Is DHA the preferred fatty acid or would the body synthesize DHA from EPA or linolenic acid, such that brain and retinal DHA accumulate as readily from these precursors in the diet as from dietary DHA?

The purpose of this experiment was to produce n-3-deficient newly hatched chicks and then to replete their brains and retinas by feeding one of three different n-3 fatty acids: DHA, EPA or α-linolenic acid. Such an experiment would test the relative ability of the brain, retina, and liver to synthesize DHA from precursors and would reveal the relative retroconversion of the longer chain fatty acids into shorter chain fatty acids. Such information would provide a tissue benchmark for estimating the ideal fatty acid composition of infant formulas which currently provide only α-linolenic acid.

MATERIALS AND METHODS

Design and diets. n-3 fatty acid-deficient eggs were produced by the feeding of an n-3 fatty acid-free diet to laying hens of the white leghorn variety for 2 mo. This diet consisted of a casein/dextrose-based fat-free basal mix (9) supplemented with 10% fat by weight, including 5% of calories from ethyl linoleate (18:2n-6), to prevent n-6 essential fatty acid deficiency, with the balance of the fat from hydrogenated coconut oil. Control hens received commercial food for laying hens. Eggs were collected and incubated as previously described (9).

Four groups of newly hatched n-3 fatty acid-deficient chicks were fed an n-3 fatty acid-deficient diet or one of three repletion diets for 1, 2, or 3 wk. The deficient diet consisted of a casein/dextrose-based fat-free basal mix for chicks supplemented with 5% fat by weight (Table 1), including 3% of calories from ethyl linoleate and the balance of the fat from hydrogenated coconut oil. The repletion diets consisted of the same n-3 fatty acid deficient diet, but supplemented with 0.44% of calories from ethyl esters of either 18:3n-3, EPA, or DHA. For the 18:3n-3 diet, which was prepared using ethyl esters from soybean oil, the amount of added ethyl linoleate was reduced, to compensate for the 18:2n-6 present in the soybean oil. A group of control chicks received a commercial chick starter containing 3% fat. All semipurified diets contained 0.02% t-butylhydroquinone and

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Table 1. *Composition of diet fed to newly hatched chicks*

	g/100g
Dextrose monohydrate	62.33
Fat free casein	26.32
L-Arginine HCl	1.26
DL-Methionine	0.42
Cellulose	3.16
Salt mix*	6.11
Vitamin mix†	0.41
Fat‡	5.00

* Salt mix (per 100 g diet): 3.6 g $CaHPO_4 \cdot 2\ H_2O$, 0.87 g potassium citrate $\cdot H_2O$, 0.79 g $CaCO_3$, 0.54 g NaCl, 0.15 g MgO, 95 mg ferric citrate, 33 mg $MnSO_4 \cdot H_2O$, 19 mg $ZnCO_3$, 6.3 mg $CuSO_4 \cdot 5\ H_2O$, 3.1 mg $CrK(SO_4)_2 \cdot 12\ H_2O$, 0.95 mg KIO_3, 0.26 mg $Na_2MoO_4 \cdot 2\ H_2O$, $Na_2SeO_3 \cdot 5\ H_2O$.

† Vitamin mix (per 100 g diet): 375 mg choline bitartrate, 6.3 mg niacin, 5.3 mg vitamin B_{12}, 3.2 mg calcium pantothenate, 2.1 mg thiamin $\cdot$ HCl, 1.7 mg menadione sodium bisulfite complex, 1.6 mg pyridoxine $\cdot$ HCl, 1.6 mg riboflavin, 0.4 mg folic acid, 50 μg biotin, 10.5 mg vitamin E acetate (500 U/g), 2.1 mg vitamin A palmitate (500,000 U/g), 0.6 mg vitamin D_3 (500,000 U/g).

‡ Ethyl linoleate, hydrogenated coconut oil, and an n-3 fatty acid (see text).

Table 2. *Fatty acid composition (wt%) of control, n-3 fatty acid-deficient, and repletion diets*

	Control diet	n-3 deficient	Deficient + 18:3	Deficient + 20:5	Deficient + 22:6
12:0	0.4	27.9	27.7	32.8	36.5
14:0	1.3	20.0	10.8	14.3	15.6
16:0	23.0	10.7	10.8	7.3	7.4
18:0	7.9	9.4	7.3	8.6	7.7
Total sat.	34.8	68.3	60.2	66.4	70.5
18:1n-9	33.2	0.5	9.6	0.1	0.3
Total mono.	36.7	0.8	9.8	0.1	0.6
18:2n-6	25.9	30.9	26.5	29.6	24.2
20:4n-6				0.2	
Total n-6	25.9	30.9	26.5	29.8	24.3
18:3n-3	1.3		3.5		
20:5n-3				3.6	0.03
22:5n-3					0.26
22:6n-3					3.7
Total n-3	1.3		3.5	3.6	4.0
Ratio n-6/n-3	19.9	High	7.6	8.3	6.1

were handled so as to minimize oxidation of the polyunsaturated fat (9). There was no significant difference in the growth of chicks on the various experimental diets (data not shown), and growth was comparable to that reported for white leghorn chicks (10). The fatty acid composition of the control and experimental diets fed to the chicks is shown in Table 2.

At the designated time periods, chicks were killed, lipids extracted, and the fatty acid composition of the brain, retina, liver, and serum determined as previously described (9). Fatty acid values are reported as percent of total fatty acids, by weight. Complete fatty acid composition data are available from the authors on request.

t-Butylhydroquinone was purchased from Eastman Kodak, Rochester NY. Ethyl esters of EPA and DHA were the gift of Prof. Akira Kumagai (Toyama City, Japan), and the hydrogenated coconut oil was donated by Palmco., Inc., (Portland, OR). Ethyl linoleate was obtained from NuChekPrep (Elysian, MN). Fat-free basal mixes for chicks and laying hens were obtained from Teklad, Madison, WI. Ethyl esters of soybean oil were synthesized by refluxing 60 g of oil in 4% H_2SO_4 in ethanol (vol/vol) for 90 min, followed by extraction with hexane and washing with 2% $NaHCO_3$ (11). This preparation showed only one spot, corresponding to fatty acid ester, after silica gel thin-layer chromatography with hexane:$CHCl_3$:ethyl ether:acetic acid (80:10:10:1.5). The fatty acid composition was identical to that of soybean oil and the yield was quantitative.

Statistical analyses. Comparison of the effect of the diets on tissue levels of DHA, total n-3 fatty acids, and 22:5n-6 was done using two-way analysis of variance ("Stata" statistics package from The Computing Resource Center, Los Angeles, CA). The data were first log transformed, because the raw data failed the Bartlett test for unequal variances. Differences between individual means were detected by use of the appropriate *t*-statistic (12), using the BonFerroni inequality (13) to control the overall α-level.

RESULTS

Brain and retina. The brain and retinal DHA of n-3 fatty acid-deficient chicks increased rapidly after the addition of either EPA or DHA to the diet (Tables 3 and 4). Levels of DHA rose to values of control chicks or higher by 3 wk of refeeding with either of these fatty acids. These changes indicated that the chick was able to use EPA efficiently as a substrate to synthesize DHA. However, dietary 18:3n-3 was only one-third as effective as either EPA or DHA in increasing levels of DHA in these tissues. The repletion of DHA led also to a reduction of 22:5n-6, a fatty acid that accumulates in n-3 deficiency. This decrease was significantly slower in the 18:3 diet group. A graphical representation of the relative effectiveness of the three repletion diets in increasing brain and retinal DHA, and lowering brain 22:5n-6, is shown in Figures 1–3.

Very little EPA accumulated in the brain or retina in any of the diet groups, with the exception of the retina of 1-wk-old chicks fed the EPA diet. This amounted, however, to only 0.4% of total fatty acids, and decreased rapidly by wk 2 and 3. Some EPA was formed in the retina as result of retroconversion of DHA in the DHA-fed chicks. In fact, a nontrivial fraction of the total n-3 fatty acids in the retina of 3-wk-old chicks fed the DHA diet consisted of retroconversion products of DHA (*i.e.* 22:5n-3, EPA, and 18:3n-3). In contrast, these retroconversion products were generally not found in the brain.

Levels of arachidonic acid, 20:4n-6, were not affected by either the deficiency of n-3 fatty acids or the rapid assimilation of DHA into the brain and retina. Previous studies had shown a pronounced lowering of tissue 20:4n-6 in response to dietary DHA and EPA, albeit at high doses fed to animals already possessing normal stores of n-3 fatty acids (14–18).

Serum and liver. The similarity in the effects of dietary EPA and DHA in the brain and retina did not extend to the serum (Table 5). In fact, for the first 2 wk most of the total n-3 fatty acids in the serum of chicks fed EPA remained as EPA. Serum levels of DHA were much lower in the EPA group at all time periods than in those animals fed DHA directly. In the 18:3-fed group, levels of DHA in the serum were lower still. Total n-3 fatty acids in this diet group were also substantially lower than total serum n-3 fatty acids in the chicks fed either EPA or DHA.

Interestingly, a substantial fraction of the dietary DHA was retroconverted, presumably in the liver, as evidenced by the

Table 3. *Brain fatty acid composition (wt%) after refeeding of n-3-deficient chicks with 18:3, 20:5, or 22:6 for 1, 2, and 3 wk*

	Control diet				Deficient diet*				Deficient diet + 18:3			Deficient diet + EPA			Deficient diet + DHA		
	Wk				Wk				Wk			Wk			Wk		
	0	1	2	3	0	1	2	3	1	2	3	1	2	3	1	2	3
16:0	32.4 ± 2.6	28.5 ± 2.5	25.4 ± 1.2	24.4 ± 0.8	32.2 ± 4.7	32.4 ± 4.0	28.4 ± 0.6	25.8 ± 3.1	28.6 ± 1.4	28.6 ± 2.7	29.0 ± 4.0	31.3 ± 3.4	27.7 ± 2.2	24.8 ± 2.3	30.8 ± 2.9	28.1 ± 2.7	25.4 ± 3.2
18:0	19.5 ± 1.0	19.7 ± 0.7	17.8 ± 2.7	18.8 ± 1.1	16.9 ± 0.5	16.7 ± 0.6	16.4 ± 4.6	17.2 ± 0.4	15.9 ± 1.0	17.7 ± 2.4	18.8 ± 1.3	16.4 ± 0.3	16.8 ± 4.0	17.3 ± 0.9	16.8 ± 2.8	19.4 ± 2.2	17.3 ± 1.1
Total sat.	52.6 ± 3.1	49.3 ± 2.5	46.4 ± 2.4	44.2 ± 0.2	50.9 ± 5.0	51.4 ± 5.4	47.6 ± 2.3	43.9 ± 3.2	45.6 ± 1.6	45.2 ± 0.6	48.6 ± 4.5	49.9 ± 3.5	45.2 ± 3.9	42.7 ± 3.1	49.3 ± 2.3	48.4 ± 2.6	43.2 ± 4.1
16:1n-7	1.4 ± 0.7	1.5 ± 0.8	1.4 ± 0.1	1.2 ± 0.1	1.5 ± 0.2	2.0 ± 0.6	1.8 ± 0.3	1.1 ± 0.1	1.9 ± 0.5	1.8 ± 0.2	1.5 ± 0.3	1.3 ± 0.2	1.3 ± 0.3	1.2 ± 0.4	1.6 ± 0.4	1.1 ± 0.3	1.0 ± 0.2
18:1n-9	16.2 ± 1.1	20.1 ± 0.3	22.3 ± 0.7	23.8 ± 0.4	15.6 ± 0.2	19.2 ± 1.1	19.5 ± 1.0	24.4 ± 0.9	21.0 ± 0.6	22.3 ± 0.6	21.5 ± 3.0	19.0 ± 0.2	20.2 ± 0.8	21.2 ± 1.2	19.7 ± 0.9	20.1 ± 1.1	23.4 ± 4.2
Total mono.	18.4 ± 2.1	22.5 ± 0.7	25.8 ± 2.0	25.8 ± 0.2	17.7 ± 0.0	21.8 ± 1.5	22.4 ± 1.4	26.4 ± 0.9	23.7 ± 0.8	25.0 ± 0.5	23.9 ± 3.4	21.0 ± 0.1	21.9 ± 1.0	23.2 ± 1.2	22.3 ± 1.1	23.7 ± 4.3	22.8 ± 2.4
20:3n-9	0.2 ± 0.1	1.7 ± 0.1	2.8 ± 0.3	2.5 ± 0.3	0.2 ± 0.0	1.5 ± 0.5	1.5 ± 0.3	1.8 ± 0.8	1.0 ± 0.2	1.5 ± 0.5	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.7 ± 0.4	0.8 ± 0.5	0.7 ± 0.2	0.7 ± 0.3
18:2n-6	1.4 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	1.5 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.2	0.7 ± 0.1
20:4n-6	9.3 ± 1.1	8.8 ± 0.8	8.4 ± 0.9	9.6 ± 0.4	10.8 ± 1.0	9.8 ± 0.9	11.6 ± 2.2	11.2 ± 1.1	11.6 ± 0.2	10.2 ± 0.8	10.0 ± 1.8	11.3 ± 1.3	10.1 ± 1.7	10.6 ± 0.6	9.4 ± 0.8	10.2 ± 1.0	11.1 ± 1.5
22:4n-6	2.3 ± 0.5	2.4 ± 0.3	2.2 ± 0.2	2.6 ± 0.1	3.1 ± 0.5	2.6 ± 0.6	3.2 ± 0.3	3.4 ± 0.2	3.4 ± 0.2	2.8 ± 0.2	2.9 ± 0.3	3.2 ± 0.7	2.9 ± 0.5	3.4 ± 0.1	2.8 ± 0.7	2.6 ± 0.7	3.1 ± 0.4
22:5n-6	3.1 ± 0.5	3.8 ± 0.6	3.4 ± 0.6	4.4 ± 0.2	12.4 ± 2.5	10.6 ± 2.1	11.6 ± 0.9	9.8 ± 1.2	11.6 ± 0.7	8.3 ± 0.1	7.4 ± 1.3†	10.2 ± 1.5	7.1 ± 1.6	6.6 ± 2.0	9.4 ± 2.0	8.1 ± 2.3	5.0 ± 1.1
Total n-6	16.9 ± 1.9	16.5 ± 1.6	15.8 ± 1.1	18.2 ± 0.5	28.8 ± 4.3	24.6 ± 3.9	28.2 ± 3.0	27.0 ± 2.0	28.5 ± 1.2	23.2 ± 1.1	22.0 ± 3.3	25.5 ± 3.5	20.4 ± 5.2	21.7 ± 1.8	23.7 ± 2.9	21.0 ± 5.6	20.4 ± 2.6
18:3n-3									0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1						
20:5n-3										0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1			
22:5n-3	0.4 ± 0.1	0.2 ± 0.1	0.7 ± 0.5	0.2 ± 0.1					0.1 ± 0.1	0.7 ± 0.1	0.8 ± 0.3	0.4 ± 0.1	1.0 ± 0.4	1.3 ± 0.0	0.1 ± 0.1		
22:6n-3	10.5 ± 2.5	8.5 ± 0.9	8.3 ± 1.7	8.3 ± 0.4	1.8 ± 0.5	0.6 ± 0.2	0.9 ± 0.6	0.8 ± 0.3	0.7 ± 0.0‡	1.8 ± 0.4‡	3.5 ± 0.7‡	2.0 ± 0.3	5.9 ± 1.1	9.8 ± 3.3	2.2 ± 0.6	5.8 ± 1.6	12.8 ± 3.3
Total n-3	10.7 ± 1.5	8.8 ± 0.9	8.6 ± 1.4	8.5 ± 0.4	1.8 ± 0.5	0.6 ± 0.2	0.9 ± 0.6	0.8 ± 0.3	0.9 ± 0.1‡	2.7 ± 0.4‡	4.3 ± 0.9‡	2.5 ± 0.2	7.1 ± 1.1	10.8 ± 2.8	2.5 ± 0.7	5.8 ± 1.6	12.9 ± 3.2
n	3	3	3	3	3	3	3	3	3	3	4	3	5	3	4	4	5

* The zero time point applies also to the three repletion diets.
† Different from the respective time points for the 20:5 and 22:6 diets, $p = 0.045$.
‡ Different from the respective time points for the 20:5 and 22:6 diets, $p < 0.001$.

Table 4. *Retinal fatty acid composition (wt%) after refeeding of n-3-deficient chicks with 18:3, 20:5, or 22:6 for 1, 2, and 3 wk*

	Control diet				Deficient diet*				Deficient diet + 18:3			Deficient diet + 20:5			Deficient + 22:6		
	Wk				Wk				Wk			Wk			Wk		
	0	1	2	3	0	1	2	3	1	2	3	1	2	3	1	2	3
16:0	28.6 ± 3.1	29.1 ± 5.3	30.6 ± 1.0	21.8 ± 3.1	33.3 ± 0.1	33.2 ± 2.4	29.5 ± 3.3	25.6 ± 2.3	26.6 ± 2.8	30.3 ± 3.5	29.8 ± 2.9	24.4 ± 6.2	23.5 ± 6.1	27.0 ± 3.7	26.0 ± 4.0	24.2 ± 1.8	25.8 ± 4.1
18:0	20.2 ± 1.9	23.2 ± 1.0	23.1 ± 0.9	24.0 ± 2.7	22.7 ± 5.9	20.4 ± 0.2	21.4 ± 1.3	26.2 ± 1.1	19.9 ± 6.2	21.2 ± 0.6	21.2 ± 1.2	25.4 ± 1.2	26.8 ± 9.5	22.6 ± 3.7	19.7 ± 0.9	21.9 ± 1.5	20.3 ± 0.7
Total sat.	52.3 ± 3.6	53.7 ± 6.5	55.6 ± 0.3	46.3 ± 6.5	55.9 ± 0.5	56.1 ± 3.0	53.9 ± 3.9	52.4 ± 1.5	47.4 ± 4.3	53.0 ± 3.5	54.0 ± 2.7	52.7 ± 7.9	54.1 ± 9.6	52.2 ± 3.4	50.8 ± 5.9	49.1 ± 2.5	48.0 ± 4.5
16:1n-7	2.8 ± 0.8	2.9 ± 1.0	2.4 ± 0.7	1.8 ± 0.2	3.9 ± 0.5	3.5 ± 0.5	3.2 ± 0.6	1.4 ± 0.1	3.7 ± 2.2	2.6 ± 0.8	3.1 ± 0.4	1.6 ± 0.3	2.0 ± 1.6	2.6 ± 1.1	2.6 ± 0.2	2.1 ± 0.3	2.4 ± 0.9
18:1n-9	13.5 ± 1.4	15.8 ± 0.6	15.6 ± 0.2	18.3 ± 2.4	14.2 ± 0.6	16.2 ± 2.0	15.7 ± 1.7	15.4 ± 0.4	20.3 ± 8.9	15.1 ± 0.9	14.5 ± 1.5	16.2 ± 2.5	15.0 ± 2.4	13.5 ± 0.5	15.1 ± 1.6	15.5 ± 1.1	14.4 ± 1.0
Total mono.	16.9 ± 2.0	19.3 ± 1.3	18.8 ± 0.3	20.6 ± 2.2	18.5 ± 0.8	20.3 ± 1.5	19.5 ± 2.3	17.7 ± 0.5	24.6 ± 11.2	18.2 ± 0.9	18.3 ± 1.8	18.0 ± 3.0	17.8 ± 3.5	16.6 ± 1.1	23.6 ± 4.7	18.4 ± 0.8	17.3 ± 1.8
20:3n-9	0.1 ± 0.1	0.4 ± 0.3	0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.2	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
18:2n-6	2.2 ± 1.9	0.8 ± 0.1	1.4 ± 0.2	2.6 ± 0.6	1.6 ± 0.3	1.7 ± 0.6	1.6 ± 0.3	0.8 ± 0.1	3.2 ± 3.5	1.1 ± 0.5	1.0 ± 0.2	2.4 ± 2.1	1.2 ± 0.6	0.9 ± 0.2	4.6 ± 3.4	4.4 ± 3.4	3.3 ± 3.4
20:4n-6	9.3 ± 1.9	9.5 ± 2.8	8.8 ± 1.0	9.4 ± 0.2	8.2 ± 0.8	7.9 ± 0.2	8.2 ± 0.1	10.1 ± 0.3	7.9 ± 3.2	8.6 ± 1.7	8.2 ± 1.1	9.7 ± 1.4	8.6 ± 3.2	9.8 ± 2.1	7.0 ± 1.0	7.7 ± 1.1	7.7 ± 2.2
22:4n-6	1.8 ± 0.6	1.5 ± 0.2	2.0 ± 0.5	2.4 ± 0.2	2.5 ± 0.2	1.9 ± 1.1	2.9 ± 0.5	3.0 ± 0.1	2.9 ± 1.3	3.3 ± 0.5	2.8 ± 0.6	2.8 ± 0.8	2.4 ± 0.8	2.9 ± 0.5	2.6 ± 0.6	2.9 ± 0.3	2.3 ± 0.7
22:5n-6	3.5 ± 1.3	4.4 ± 2.3	4.7 ± 0.6	4.8 ± 2.2	10.2 ± 0.4	11.3 ± 1.4	11.5 ± 1.2	12.7 ± 0.5	12.0 ± 6.0	11.7 ± 1.6	9.8 ± 1.6	12.4 ± 4.7	9.0 ± 3.4	5.7 ± 2.4	9.9 ± 1.2	9.6 ± 1.9	5.8 ± 2.4
Total n-6	16.9 ± 2.2	16.4 ± 4.7	15.4 ± 1.1	20.4 ± 2.9	23.1 ± 1.3	22.8 ± 2.3	24.8 ± 2.1	27.3 ± 0.7	26.9 ± 7.3	25.6 ± 3.3	22.8 ± 2.5	27.2 ± 8.5	21.7 ± 7.8	20.0 ± 3.6	24.7 ± 5.4	25.6 ± 2.5	19.9 ± 3.0
18:3n-3									0.2 ± 0.3	0.1 ± 0.0	0.1 ± 0.1			0.0 ± 0.0		0.2 ± 0.1	0.2 ± 0.2
20:5n-3												0.4 ± 0.3	0.1 ± 0.1	0.0 ± 0.1			0.2 ± 0.2
22:5n-3									0.1 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.2 ± 0.2	0.6 ± 0.3	0.4 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	0.3 ± 0.2
22:6n-3	12.9 ± 3.3	9.6 ± 2.6	7.5 ± 2.6	12.3 ± 2.2	1.6 ± 0.0	0.5 ± 0.3	0.8 ± 0.3	1.2 ± 0.8	0.6 ± 0.3†	2.1 ± 0.6†	3.8 ± 0.6†	1.9 ± 0.9	4.7 ± 1.7	10.7 ± 2.6	2.9 ± 0.8	5.4 ± 0.4	11.2 ± 3.1
Total n-3	12.9 ± 3.3	9.7 ± 2.5	7.5 ± 2.6	12.3 ± 2.2	1.8 ± 0.3	0.5 ± 0.3	0.8 ± 0.3	1.2 ± 0.8	0.8 ± 0.1‡	2.6 ± 0.7‡	4.5 ± 0.7‡	2.2 ± 0.7	5.3 ± 2.0	11.2 ± 2.4	2.9 ± 0.9	5.9 ± 0.4	11.7 ± 3.2
n	3	3	3	3	3	3	3	3	3	3	4	3	5	3	4	4	5

* The zero time point applies also to the three repletion diets.
† Different from respective time points for 20:5 and 22:6 diets, $p = 0.015$.
‡ Different from respective time points for 20:5 and 22:6 diets, $p = 0.005$.

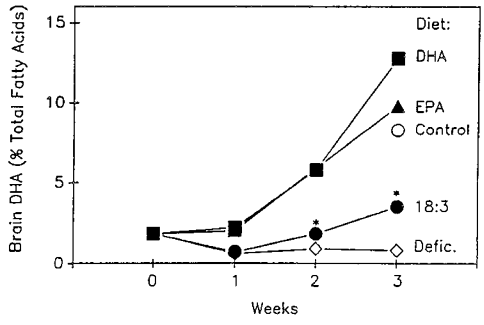

Fig. 1. Repletion of brain DHA after the refeeding of n-3-deficient chicks with 18:3, EPA, or DHA (*different from the other two repletion diets at $p < 0.001$).

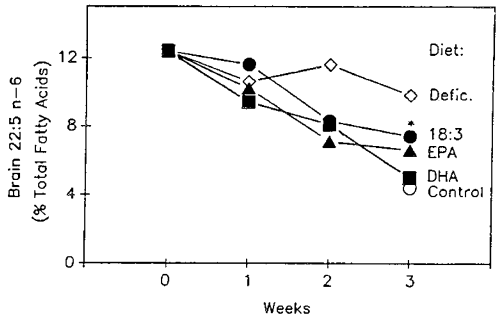

Fig. 2. Decrease in brain 22:5n-6 after the refeeding of n-3-deficient chicks with 18:3, EPA, or DHA (*different from the other two repletion diets at $p = 0.045$).

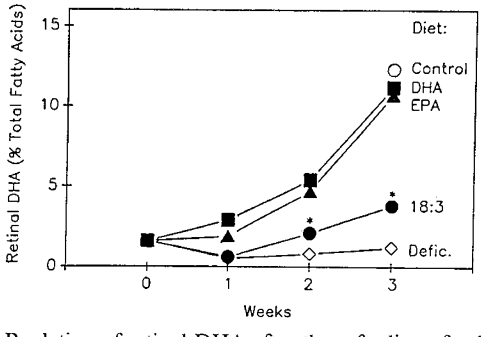

Fig. 3. Repletion of retinal DHA after the refeeding of n-3-deficient chicks with 18:3, EPA, or DHA (*different from the other two repletion diets at $p = 0.015$).

relatively large amount of EPA that was present in the serum of DHA-fed chicks at 3 wk of age. A small amount of 22:5n-3, an intermediate between DHA and EPA, was also detectable.

A similar fatty acid pattern was seen in the livers of the repleted chicks (Table 6). The amount of DHA formed from EPA was especially low at wk 1–2, relative to the amount of DHA that accumulated when DHA was fed directly. Not until wk 3 was any significant synthesis of DHA from dietary EPA apparent in the liver. The amount of DHA in the livers of chicks fed 18:3 was quite low at 3 wk, relative to the chicks fed EPA, and was comparable to levels reported by Rogel and Watkins (19) in the livers of 3-wk-old chicks fed a similar diet. The changes in serum and liver DHA levels are shown graphically in Figures 4–5.

DISCUSSION

Only the very long-chain and highly polyunsaturated fatty acids, DHA and EPA, provided prompt correction of the n-3-deficient state in the brain and retina of the chick. This correction was complete after 3 wk of feeding. Linolenic acid (18:3 n-3) did not correct at all for 2 wk of feeding and only minimally after 3 wk. The slow recovery of nervous tissue DHA levels in n-3 fatty acid deficient rats refed with 18:3 has also been noted by Bourre and coworkers (20–23), who found that complete recovery took 2½ to 3 mo in rats. One possible explanation for the slow recovery with dietary 18:3, as compared with EPA and DHA, could be the fact that 18:3 is positioned before the rate limiting step in the formation of 22:6, namely the δ-6 desaturation of 18:3 to 18:4. However, we think this is not the best explanation, because serum levels of total n-3 fatty acids in our chicks fed 18:3 were much lower than in chicks fed EPA or DHA. This observation would argue that there was a partial diversion of 18:3 to oxidative pathways. In this regard, Leyton *et al.* (24) found that dietary 18:3n-3 was oxidized in rats to a greater extent than other unsaturated fatty acids. This relative selectivity against 18:3n-3 is probably due to the position of the terminal double bond, and not to a special susceptibility of 18 carbon trienoic fatty acids, because 18:3n-6 was oxidized to far lesser extent than 18:3n-3 (24).

Nevertheless, the chicks in our study were able to desaturate 18:3n-3 to some extent, as evidenced by the fact that most of the n-3 fatty acids in the serum of 18:3-fed chicks were present as desaturation-elongation products of 18:3. Other animals, for instance, rats and monkeys, are also able to use 18:3 to produce DHA (16, 20, 25, 26).

Dietary 18:3 might be expected to be an even less efficient precursor for DHA in animals with low δ-6 desaturase activity. In this light, there is doubt about the ability of the adult human to desaturate and elongate 18:3n-3 and EPA to any appreciable extent (27–29). Even vegans, whose plasma is low in DHA, were not able to increase levels of DHA when fed a diet containing large amounts of 18:3n-3 for 3 wk (30). Thus, it is possible that the human organism could have an even stronger preference than the chick for dietary DHA over dietary 18:3 as the ultimate source of tissue DHA.

This has possible implications for infant feeding practices. Human milk contains both 18:3 and DHA as the "natural" source of n-3 fatty acids for the developing infant. It does not contain any EPA. However, infant formulas have 18:3 as the only source of the n-3 essential fatty acids. They contain no DHA or EPA. Some infant formulas have little 18:3 and a high n-6/n-3 ratio which has produced n-3 fatty acid deficiency in monkeys, both biochemically and clinically (4). Our results in chicks with brain and tissue fatty acid composition correlate very well with the blood findings of Carlson *et al.* (31, 32) in human infants. They determined that formula feeding produces a less than optimal concentration of DHA in erythrocyte membranes as compared with human milk or formula supplemented with EPA and DHA. DHA is apparently so important for development that a laying hen fed an n-3 fatty acid-deficient diet for many months (9) still passed DHA to the chick (through the yolk) at a quantity similar to that found in human milk, namely 0.2% of fatty acids. There are no tissue data from human infants comparable to the data from chicks presented in our report.

The near equivalence of dietary EPA and DHA in restoring levels of DHA in the brain and retina of n-3 fatty acid chicks was unexpected. It has been suggested that the brain receives much of its complement of DHA "preformed" from the circulation via the liver (33, 34), although the rat brain, and especially the developing rat brain, has a substantial capacity for the synthesis of DHA from precursor molecules (35, 36). Our results showed that DHA was formed from dietary EPA and accumulated in the brain to nearly the same extent as the DHA when fed directly in the diet. This occurred despite the lower level of

Table 5. *Serum fatty acid composition (wt%) after refeeding of n-3-deficient chicks with 18:3, 20:5, or 22:6 for 1, 2, and 3 wk*

	Control diet				Deficient diet*				Deficient diet + 18:3			Deficient diet + 20:5			Deficient diet + 22:6		
	Wk				Wk				Wk			Wk			Wk		
	0	1	2	3	0	1	2	3	1	2	3	1	2	3	1	2	3
16:0	24.1 ± 3.4	30.6 ± 2.4	23.6 ± 5.0	25.0 ± 1.3	22.9 ± 2.4	25.5 ± 2.8	23.1 ± 1.1	23.4 ± 3.0	26.0 ± 2.8	25.2 ± 0.6	23.6 ± 4.9	26.3 ± 1.3	24.1 ± 4.0	25.3 ± 1.3	25.9 ± 4.2	28.4 ± 0.6	22.5 ± 2.0
18:0	11.3 ± 0.4	12.3 ± 1.0	14.8 ± 0.7	14.2 ± 1.4	12.1 ± 0.6	13.4 ± 1.0	15.5 ± 1.4	15.9 ± 1.0	8.9 ± 1.1	16.6 ± 0.7	16.2 ± 1.0	11.1 ± 1.8	16.0 ± 0.4	16.5 ± 0.7	14.4 ± 3.0	15.4 ± 0.3	17.1 ± 1.0
Total sat.	36.2 ± 3.3	43.8 ± 2.1	39.2 ± 4.9	39.7 ± 0.5	37.8 ± 2.2	44.1 ± 2.5	41.8 ± 3.1	41.3 ± 3.2	37.3 ± 2.1	43.3 ± 1.5	40.5 ± 3.4	38.7 ± 2.6	42.9 ± 5.4	45.3 ± 2.0	43.7 ± 2.6	48.3 ± 0.8	42.3 ± 3.1
16:1n-7	1.6 ± 0.5	3.8 ± 0.8	2.2 ± 0.5	2.5 ± 0.2	1.6 ± 0.3	4.4 ± 1.4	3.9 ± 0.8	3.5 ± 0.4	7.9 ± 0.7	3.6 ± 0.6	2.7 ± 0.6	5.5 ± 0.8	3.8 ± 0.5	3.0 ± 0.4	4.5 ± 1.3	3.8 ± 0.3	2.6 ± 0.3
18:1n-9	26.2 ± 1.1	27.8 ± 1.5	30.3 ± 4.2	28.7 ± 0.6	22.6 ± 1.4	23.3 ± 1.8	27.3 ± 2.6	26.4 ± 4.1	29.8 ± 0.6	27.0 ± 2.0	24.5 ± 1.0	26.4 ± 0.5	25.3 ± 2.8	19.3 ± 2.2	22.3 ± 1.3	19.8 ± 1.9	21.5 ± 2.3
Total mono.	28.0 ± 1.2	35.2 ± 7.9	33.9 ± 4.2	31.7 ± 0.6	24.7 ± 1.3	28.3 ± 1.9	31.6 ± 3.4	30.4 ± 4.6	37.9 ± 0.7	30.9 ± 2.5	27.6 ± 1.5	32.1 ± 0.9	29.6 ± 3.1	22.4 ± 1.9	27.1 ± 2.6	24.3 ± 2.2	26.0 ± 2.8
20:3n-9	0.2 ± 0.0	0.7 ± 0.2	1.9 ± 0.6	1.4 ± 0.3	0.2 ± 0.0	1.2 ± 0.5	1.0 ± 0.1	0.9 ± 0.2	0.8 ± 0.2	1.0 ± 0.1	0.8 ± 0.2	0.6 ± 0.2	0.7 ± 0.1	0.6 ± 0.2	0.7 ± 0.2	0.7 ± 0.1	0.8 ± 0.2
18:2n-6	24.4 ± 2.2	16.1 ± 1.6	14.6 ± 1.2	15.1 ± 0.6	22.5 ± 1.2	16.5 ± 1.1	14.8 ± 0.9	17.1 ± 2.4	15.0 ± 1.2	14.6 ± 0.5	17.0 ± 2.6	15.3 ± 1.0	13.0 ± 0.7	15.1 ± 0.9	14.8 ± 1.5	14.4 ± 1.7	15.7 ± 0.7
20:4n-6	8.7 ± 2.6	3.8 ± 1.1	6.9 ± 0.4	7.2 ± 0.8	9.7 ± 0.8	5.8 ± 1.0	7.4 ± 3.0	6.4 ± 3.1	4.6 ± 0.8	4.8 ± 1.1	6.9 ± 1.2	6.2 ± 0.8	5.3 ± 0.2	7.2 ± 0.3	6.4 ± 2.1	6.4 ± 0.4	6.8 ± 1.8
22:4n-6	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.3 ± 0.2	0.4 ± 0.3	0.3 ± 0.0	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.3	0.7 ± 0.3	0.5 ± 0.3	0.3 ± 0.1
22:5n-6	0.6 ± 0.5	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	2.8 ± 0.5	1.6 ± 0.4	0.8 ± 0.2	0.7 ± 0.4	1.2 ± 0.4	1.4 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	0.2 ± 0.1	0.3 ± 0.3	0.9 ± 0.2	0.9 ± 0.3	0.2 ± 0.1
Total n-6	33.8 ± 3.3	21.9 ± 2.9	24.3 ± 1.5	25.3 ± 0.2	35.6 ± 1.3	25.9 ± 3.1	25.3 ± 2.5	26.7 ± 5.9	22.9 ± 2.2	22.2 ± 1.4	27.5 ± 3.1	24.2 ± 0.7	20.3 ± 1.0	24.4 ± 0.5	24.6 ± 3.6	22.9 ± 2.8	25.0 ± 2.1
18:3n-3	0.2 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0					0.9 ± 0.1	0.5 ± 0.1	0.6 ± 0.2						
20:5n-3	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1					0.3 ± 0.0	0.5 ± 0.1	0.7 ± 0.1	3.4 ± 0.8	4.4 ± 1.4	4.4 ± 1.2	0.6 ± 0.2	0.8 ± 0.2	1.8 ± 0.2
22:5n-3	0.1 ± 0.0	0.1 ± 0.1	0.3 ± 0.3	0.2 ± 0.2					0.1 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.3	0.9 ± 0.3			0.2 ± 0.1
22:6n-3	1.2 ± 0.5	0.5 ± 0.2	0.8 ± 0.1	1.2 ± 0.2	0.2 ± 0.0				0.3 ± 0.2†	0.8 ± 0.2†	1.7 ± 0.0†‡	0.6 ± 0.3	1.5 ± 0.7	3.0 ± 0.6	3.6 ± 1.6‡	3.8 ± 0.7‡	5.7 ± 0.9‡
Total n-3	1.6 ± 0.6	1.1 ± 0.2	1.5 ± 0.1	1.9 ± 0.4	0.2 ± 0.0				1.5 ± 0.2§	2.3 ± 0.1§	3.4 ± 0.1§	4.3 ± 1.1	6.1 ± 1.3	7.7 ± 1.3	4.2 ± 1.6	4.6 ± 0.9	7.7 ± 0.8
n	3	3	3	3	3	3	3	3	3	3	4	3	4	3	4	3	5

* The zero time point applies also to the three repletion diets.
† Different from respective time points for 22:6 diet, $p = 0.02$.
‡ Different from respective time points for 20:5 diet, $p = 0.02$.
§ Different from respective time points for 20:5 and 22:6 diets, $p < 0.001$.

Table 6. *Liver fatty acid composition (wt%) after refeeding of n-3-deficient chicks with 18:3, 20:5, or 22:6 for 1, 2, and 3 wk*

	Deficient + 18:3			Deficient + 20:5			Deficient + 22:6		
	Wk			Wk			Wk		
	1	2	3	1	2	3	1	2	3
16:0	33.3 ± 3.4	28.1 ± 3.8	25.9 ± 1.9	34.9 ± 6.6	32.6 ± 1.1	24.5 ± 2.9	30.9 ± 6.5	30.3 ± 5.5	24.2 ± 3.7
18:0	7.1 ± 0.7	13.6 ± 1.8	14.2 ± 3.1	8.7 ± 0.9	11.0 ± 0.8	17.1 ± 0.9	11.5 ± 5.8	14.0 ± 2.2	14.6 ± 2.7
Total sat.	42.1 ± 3.0	44.0 ± 3.0	42.1 ± 3.7	46.0 ± 8.3	45.3 ± 2.7	42.5 ± 2.8	43.5 ± 4.9	45.1 ± 6.5	39.6 ± 4.3
16:1n-7	11.0 ± 1.5	5.4 ± 1.1	4.6 ± 1.5	7.7 ± 1.7	6.7 ± 0.8	3.2 ± 0.5	6.5 ± 2.6	4.3 ± 1.0	3.7 ± 1.6
18:1n-9	37.5 ± 2.4	34.4 ± 4.1	30.9 ± 4.4	37.7 ± 6.1	32.5 ± 3.7	24.6 ± 2.7	35.3 ± 6.9	31.0 ± 4.0	27.6 ± 4.4
Total mono.	49.3 ± 0.7	40.5 ± 5.2	35.9 ± 4.4	46.1 ± 5.3	39.9 ± 4.4	28.4 ± 3.0	42.4 ± 9.1	35.7 ± 4.2	31.7 ± 5.5
20:3n-9	0.2 ± 0.0	0.5 ± 0.2	0.7 ± 0.4	0.2 ± 0.0	0.4 ± 0.1	0.7 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
18:2n-6	4.3 ± 0.7	6.9 ± 2.7	9.5 ± 0.7	3.9 ± 1.2	5.5 ± 0.8	9.0 ± 0.7	5.8 ± 2.7	7.6 ± 0.3	10.0 ± 1.5
20:4n-6	2.5 ± 1.1	4.5 ± 2.2	6.6 ± 1.1	2.4 ± 0.7	3.6 ± 0.8	8.8 ± 0.6	4.0 ± 2.6	5.4 ± 1.2	7.6 ± 1.9
22:4n-6	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.4 ± 0.5	0.3 ± 0.1	0.0 ± 0.0	0.3 ± 0.2
22:5n-6	0.4 ± 0.4	0.3 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0.4 ± 0.3	0.6 ± 0.4	0.0 ± 0.0	0.1 ± 0.1
Total n-6	8.2 ± 2.3	13.7 ± 5.7	17.5 ± 0.9	6.7 ± 2.9	10.3 ± 1.9	20.4 ± 1.0	11.4 ± 6.3	14.5 ± 1.5	19.9 ± 3.4
18:3n-3	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1						
20:5n-3	0.2 ± 0.1	0.4 ± 0.2	0.7 ± 0.5	0.7 ± 0.2	1.7 ± 0.4	3.0 ± 0.3	0.2 ± 0.1	0.6 ± 0.3	1.3 ± 0.4
22:5n-3		0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	0.6 ± 0.3	1.5 ± 0.4			0.3 ± 0.2
22:6n-3	0.1 ± 0.1*	0.8 ± 0.4*	1.4 ± 0.2*†	0.1 ± 0.1	1.2 ± 0.4	5.1 ± 1.0	2.2 ± 1.5†	3.6 ± 0.9†	6.6 ± 1.7
Total n-3	0.4 ± 0.1‡	1.7 ± 0.7‡	2.6 ± 0.7‡	0.9 ± 0.4§	3.4 ± 0.8	9.5 ± 1.2	2.4 ± 1.6	4.2 ± 1.2	8.1 ± 2.0
n	3	3	4	3	5	3	4	4	5

* Different from respective time points for 22:6 diet, $p < 0.01$.
† Different from respective time points for 20:5 diet, $p < 0.01$.
‡ Different from respective time points for 20:5 and 22:6 diets, $p = 0.043$.
§ Different from respective time points for 22:6 diet, $p = 0.043$.

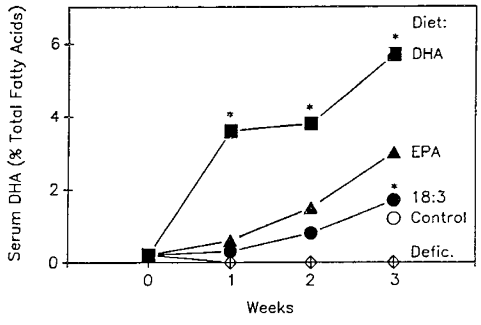

Fig. 4. Serum levels of DHA after the refeeding of n-3-deficient chicks with 18:3, EPA, or DHA (*different from the other two repletion diets at $p = 0.02$).

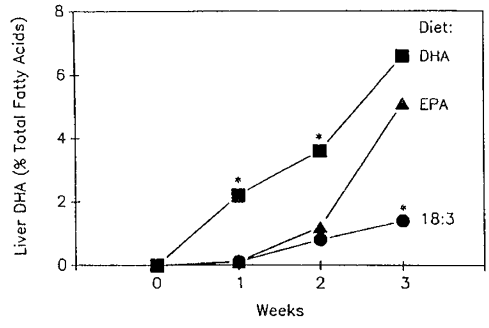

Fig. 5. Liver levels of DHA after the refeeding of n-3-deficient chicks with 18:3, EPA, or DHA (*different from the other two repletion diets at $p < 0.01$).

serum DHA in chicks fed EPA and suggests that the brain and retina themselves were responsible for synthesis of a major part of the DHA that accumulated during the first 2 wk of EPA refeeding. Such synthesis implies that δ-4-desaturase and the fatty acid elongation enzyme(s) were active in these tissues.

By 3 wk of life, levels of DHA were roughly equivalent in the livers of chicks fed either EPA or DHA. This suggests that the liver could have ultimately begun to contribute to the total body pool of DHA by synthesizing DHA from EPA. However, serum levels of DHA were still not equivalent in 3-wk-old chicks fed the EPA and DHA diets. In an earlier study, Edwards and Marion (37) also found evidence of elongation of dietary EPA to 22:5n-3 in chick liver after a few weeks. Unfortunately, it was not possible in that report to distinguish between 22:5n-3 formed from EPA and 22:5n-3 formed by retroconversion of DHA, because the chicks were fed fish oil containing both EPA and DHA. The relative contribution of the liver and brain in this situation is more difficult to ascertain in nondeficient animals. For example, in humans (38) and rats (39), the liver has as high a level of DHA as the developing brain. Perhaps the immature chick liver, unlike the brain and retina, has less δ-4 desaturase activity.

In previous reports using different dietary n-3 fatty acids, the extent to which the dietary fatty acid is incorporated into the brain has varied. For example, in some experiments involving the feeding of large amounts of 18:3 or fish oil to rats (40), chickens (9, 14, 41), and monkeys (41a) the levels of EPA were increased to as much as 2% of total fatty acids in the brain. This fatty acid is usually present at a very low level, if at all, in the brain (26). Other studies have found little effect, however. Bourre

et al. (17) fed cod liver oil (14.5% wt:wt, containing 8.6% of fatty acids as EPA and 9.9% of fatty acids as DHA) to adult rats and found only a negligible change in brain EPA, although brain DHA did increase. Holub and coworkers (18) obtained similar results but found that EPA did increase substantially in the retina. Mice fed 10% fish oil (15) showed a transient rise in brain EPA, which mostly disappeared by 10 d of feeding. Some studies involving the feeding of large amounts of 18:3n-3 also showed little effect on brain EPA levels (42, 43). In the present study, modest amounts (0.44% of calories) of 18:3, EPA, and DHA were fed to n-3 fatty acid-deficient chicks, and generally no more than trace amounts of EPA appeared in the brain and retina.

Inasmuch as stores of n-3 fatty acids in the tissues of the deficient chicks were minimal at the beginning of the repletion feeding, we were able to identify unambiguously the elongation and retroconversion products from each of the three n-3 fatty acids fed. Our finding that substantial amounts of dietary DHA are retroconverted to 22:5n-3 and EPA is in agreement with previous dietary and radiotracer studies (29, 44, 45), and we have reported for the first time that small quantities of dietary EPA and DHA are retroconverted to 18:3 in the retina.

In summary, these findings have possible implications for the feeding of human infants with commercial formula preparations. The sole source of n-3 fatty acids in these preparations is α-linolenic acid (18:3n-3) and even this is at very low levels in some of the powdered formula preparations (W. E. Connor and S. Van Winkle, unpublished observations). However, human milk contains both DHA and 18:3 as sources of the essential n-3 fatty acids (46). In chicks, DHA was the preferred dietary n-3 fatty acid for accumulation of DHA in the brain and retina, raising questions about the importance of dietary DHA in the development of other species, including the human infant.

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