Reversal of docosahexaenoic acid deficiency in the rat brain, retina, liver, and serum

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Abstract   The loss of docosahexaenoic acid (DHA) from the retina or brain has been associated with a loss in nervous-system function in experimental animals, as well as in human infants fed vegetable oil-based formulas. The reversibility of the loss of DHA and the compensation by an increase in the n-6 docosapentaenoic acid (DPAn-6) was studied in young adult rats. Long-Evans rats were subjected to a very low level of n-3 fatty acids through two generations. The F2 generation, n-3-deficient animals at 7 weeks of age were provided a repletion diet containing both α-linolenate and DHA. A separate group of F2 generation rats had been maintained on an n-3-adequate diet of the same composition. Tissues from the brain, retina, liver, and serum were collected on weeks 0, 1, 2, 4, and 8 from both groups of animals. The concentrations of DHA, DPAn-6, and other fatty acids were determined and the rate of recovery and length of time needed to complete DHA recovery were determined for each tissue. The DHA level in the brain at 1 and 2 weeks after diet reversal was only partially recovered, rising to approximately 20% and 35%, respectively, of the n-3-adequate group level. Full recovery was not obtained until 8 weeks after initiation of the repletion diet.▪ Although the initial rate of retinal DHA accretion was greater than that of brain DHA, the half-time for DHA recovery was only marginally greater. On the other hand, the levels of DHA in the serum and liver were approximately 90% and 100% replaced, respectively, within 2 weeks of diet reversal. A consideration of the total amounts and time courses of DHA repleted in the nervous system compared with the liver and circulation suggests that transport-related processes may limit the rate of DHA repletion in the retina and brain.—Moriguchi, T., J. Loewke, M. Garrison, J. N. Catalan, N. Salem, Jr. **Reversal of docosahexaenoic acid deficiency in the rat brain, retina, liver, and serum.** *J. Lipid Res.* 2001. 42: 419–427.

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The n-3 fatty acid deficiency syndrome (1) typically involves the feeding of a diet low in n-3 fatty acid sources during early development, resulting in a low level of brain docosahexaenoic acid (22:6n-3, DHA) (2–6). When the level of brain or retinal DHA falls, there are functional consequences. For example, rats exhibit abnormal electroretinograms (7) and poorer performance in Y-maze (8), brightness discrimination (9), shock avoidance (10), olfactory discrimination (11), and spatial learning (12–15) tasks. Cats (16) and rhesus (17, 18) also exhibit abnormal electroretinograms associated with lower levels of DHA. In addition, primates have reduced visual acuity and impaired recovery after dark adaptation (19).

The deficiency of n-3 fatty acids is not limited to experimental animals. Infants who receive formulas with vegetable oil sources of essential fatty acids (EFA) also have lower levels of brain DHA, with a compensatory increase in docosapentaenoic acid (22:5n-6, DPAn-6) (20–22). Randomized, placebo-controlled studies of the effects on neural function of long chain polyunsaturate supplementation of infant formula have demonstrated a benefit for various functional measures, including visual acuity (23–26), cognitive tests (27, 28), visual recognition memory (29, 30), and a means-end problem-solving test (31). Taken together, these studies indicate that the lack of preformed DHA in the infant diet leads to losses in nervous system DHA that, in turn, leads to losses in brain and retinal functions.

Clearly then, strategies to promote the recovery of organ DHA, particularly in the brain and retina, are required. It is thus critical to provide clear descriptions of the time course of recovery in the nervous system, its relationship to peripheral measures of EFA status, and the time course of functional recovery. There have been some previous attempts to define some of these parameters. Youyou et al. fed sunflower oil as the source of EFAs to dams and their offspring (32). When the F2 generation animals were 15 days old, the diet was changed to a soy-

based one with a higher level of α-linolenic acid (18:3n-3, LNA). They studied the subsequent increase in brain DHA and decrease in DPAn-6 in various subcellular fractions over the subsequent 75 days. Their data indicated a slow recovery of brain DHA in brain synaptosomes, mitochondrion, myelin, and microsomes, with nearly complete recovery by 75 days after diet reversal. A similar time course was observed for DHA recovery in sciatic nerve (33) and cerebral capillaries (34) for both young and adult rats. However, neurons, astrocytes, and oligodendrocytes appeared to recover somewhat more slowly than these brain fractions (35). Bourre et al. (35) also described the recoveries of various organs in n-3-deficient adult rats switched to a soy-based diet, and found that testes recovery was slower than that of retina.

Connor, Neuringer, and Lin (36) found in rhesus monkeys that cerebral cortex DHA normalized in about 12 weeks when deficient animals were fed a fish oil-based diet beginning at 10–24 months of age. They observed a rather protracted DHA recovery in plasma and erythrocyte phospholipids that appeared to mimic the brain time course. The authors also observed that feeding rhesus monkeys that were deficient from the prenatal period onward either a soy-based diet or one with supplemental DHA led to normal levels of brain and retinal DHA at autopsy after about 3 years (37). Juvenile monkeys did not recover from either the delays in peak latencies of both rod and cone responses or the impairment in recovery of the dark-adapted response, even after the brain DHA level had been fully repleted during 12–28 weeks of a diet containing fish oil (38). More recently, Weisinger et al. observed that guinea pig retinal DHA could be reduced by feeding a safflower oil-based diet from weaning up to 6 or 16 weeks of age (39). The decrease in DHA was associated with a decrease in electroretinographic amplitudes and in the sensitivity of the receptoral response. Deficient guinea pigs, in which retinal DHA was repleted with treatment for 10 weeks of a canola oil-based diet, exhibited full functional recovery. These studies have concentrated on the repletion rates after addition of dietary LNA, and few data are available on organ repletion with a diet containing both LNA and DHA; i.e., two of the key n-3 fats in a naturalistic diet and in mother's milk (40).

In this experiment, rats were made n-3-deficient using a safflower oil-based diet. The offspring of these rats (F2 generation) were weaned to the same diet until 7 weeks of age. This age was chosen because the brain weight has nearly reached its adult maximal value at this stage of development. They were then given a safflower oil-based diet supplemented with both LNA and DHA. The values of 2.6 and 1.3 weight% of LNA and DHA, respectively, with a greater level of LNA than DHA (2:1 ratio) were chosen because this represents a physiological situation applicable to humans. At various times over the subsequent 8 weeks, animals were sacrificed and the fatty acyl composition of brain, retina, liver, and serum was determined in order to determine the time courses for EFA repletion in the various compartments. A reference point for the fatty acyl composition of these recovering animals was provided by

animals in the F2 generation that had been maintained on the n-3-adequate diet.

METHODS

Animals and diets

All animal procedures were approved by the National Institute on Alcohol Abuse & Alcoholism (NIAAA) Animal Care and Use Committee. Female Long-Evans rats were commercially obtained at 3 weeks of age and randomly assigned to either an n-3-adequate (n-3-Adq) or n-3-deficient (n-3-Def) diet. They were maintained within the NIAAA animal facility under conventional conditions with controlled temperature (23 ± 1°C) and a 12-h light cycle (0600–1800). At 11 weeks of age, they were mated with 12-week-old males of the same strain. Their litters were culled to 10 pups and the pups were weaned onto the same diet as their mothers. When the n-3-Def offspring were 7 weeks old, they were switched to the n-3-Adq diet. Groups of animals (n = 20 for each diet and n = 4 at each time point) were subsequently sacrificed after 0, 1, 2, 4, and 8 weeks of this repletion diet. Each animal within a particular group was from a different litter, thus the litter was used as the basis for the number of animals. Animals were killed by decapitation, and the liver, brain, and retina were rapidly dissected. Truncal blood was collected, transferred to a plastic centrifuge tube, and spun in a refrigerated tabletop

TABLE 1. Composition of experimental diets[a]

Ingredient	Amount	
	n-3 Def	n-3 Adq
	g/100 g diet	
Casein, vitamin free	20	20
Carbohydrate	60	60
Cornstarch	15	15
Sucrose	10	10
Dextrose	19.9	19.9
Maltose-dextrin	15	15
Cellulose	5	5
Salt mix	3.5	3.5
Vitamin mix	1	1
L-Cystine	0.3	0.3
Choline bitartrate	0.25	0.25
TBHQ	0.002	0.002
Fat	10	10
Hydrogenated coconut oil	8.1	7.45
Safflower oil	1.9	1.77
Flaxseed oil	0	0.48
DHASCO	0	0.3
Fatty acid composition[b]		
Saturates	80.9	75.6
Monounsaturates	4.0	4.8
18:2n-6	15.1	15.7
18:3n-3	0.04	2.6
20:4n-6	nd	0.02
22:6n-3	nd	1.3
n-6/n-3	378	4.1
18:2n-6/18:3n-3	377	6.2

TBHQ, *t*-butylhydroquinone.

[a] The two experimental diets, an n-3 fatty acid-adequate diet (n-3-Adq) and an n-3 fatty acid-deficient diet (n-3-Def), were based on the AIN-93 (41) formulation with modifications to obtain the extremely low basal level of n-3 fatty acids required for this study (see 11, 15). nd, not detected.

[b] The 20:5n-3 and 22:5n-3 fatty acids were less than 0.01%.

centrifuge at 2,300 *g* for 15 min at 4°C. An aliquot of the upper phase (serum) was transferred to another tube. The serum and tissue samples were frozen at −80°C prior to lipid extraction.

The diets used were modeled after the AIN-93G diet recommendations for rodents (41). However, the fat sources were altered to provide for the low n-3 fatty acid content required as a basal composition. The basal fat ingredients used were hydrogenated coconut and safflower oils for the n-3-Def diet (**Table 1**). In the n-3-Adq diet, a small amount of flaxseed oil and DHASCO (42 ± 1% of fatty acids as DHA) (Martek Biosciences, Columbia, MD) were added to supply LNA and DHA, respectively. These diets were custom prepared and pelleted using low heat conditions (25°C), then stored at 4°C to prevent lipid oxidation (Dyets, Inc, Bethlehem, PA) and the fatty acid distributions of the entire diet quality assured within our own laboratory. The content of the key fatty acid variables of the two diets, LNA and DHA, were 0.04 and nondetectable in the n-3-Def diet, but were 2.6% and 1.3% of total fatty acids, respectively, in the n-3-Adq diet (Table 1). Diet and water were provided ad libitum.

Lipid extraction, transmethylation, and gas chromatography

Tissue samples were thawed, weighed, and homogenized in methanol-hexane and methylated in acetyl chloride according to the method of Lepage and Roy (42). Varying amounts of the internal standards methyl docosatrienoate (22:3n-3) for brain, retina, and liver, and methyl tricosanoate (23:0) for serum, were added to each sample to compensate for differences in tissue weight and lipid concentration (279 μg/g brain, 73 μg/whole retina, 200 μg/0.5 g liver, and 20 μg/100 μl serum). As an aid in preventing lipid oxidation during the procedures, 50 μg/ml of butylated hydroxytoluene was added in the methanol. The hexane extracts were concentrated to a small volume with a stream of nitrogen and transferred to microvials for gas chromatographic (GC) injection.

Fatty acid methyl esters were analyzed with an HP-5890B gas chromatograph equipped with a flame ionization detector (Hewlett-Packard, Palo Alto, CA) and a fused silica capillary column (DB-FFAP, 30 m × 0.25 mm i.d. × 0.25 μm film thickness,

TABLE 2. The total fatty acyl composition of the brain and the retina in seven-week-old second generation n-3-deficient rats (wt% of total fatty acids)

Fatty Acid	Brain Def (n = 4)	Brain Adq (n = 4)	Retina Def (n = 4)	Retina Adq (n = 4)
10:0	0.03 ± 0.001[c]	0.08 ± 0.001	0.02 ± 0.01	0.04 ± 0.001
12:0	0.02 ± 0.001	0.05 ± 0.02	0.04 ± 0.004	0.04 ± 0.005
14:0	0.23 ± 0.02	0.26 ± 0.01	0.27 ± 0.02	0.28 ± 0.02
16:0 DMA	2.46 ± 0.02	2.40 ± 0.04	0.57 ± 0.09	0.71 ± 0.03
16:0	16.83 ± 0.24	15.99 ± 0.36	14.02 ± 0.15	14.34 ± 0.52
18:0 DMA	4.01 ± 0.03	4.06 ± 0.07	1.44 ± 0.20[a]	1.94 ± 0.03
18:0	19.36 ± 0.12	18.90 ± 0.17	21.17 ± 0.10	20.56 ± 0.48
20:0	0.67 ± 0.03	0.67 ± 0.04	0.37 ± 0.01	0.39 ± 0.04
22:0	0.70 ± 0.04	0.71 ± 0.04	0.23 ± 0.01	0.23 ± 0.06
23:0	0.15 ± 0.01	0.08 ± 0.05	0.02 ± 0.003	0.02 ± 0.003
24:0	1.35 ± 0.08	1.37 ± 0.08	0.27 ± 0.03	0.31 ± 0.08
Total saturates	45.82 ± 0.19[a]	44.57 ± 0.34	38.41 ± 0.35	38.86 ± 0.95
12:1	0.05 ± 0.003[c]	0.13 ± 0.01	0.07 ± 0.01	0.08 ± 0.005
14:1	0.03 ± 0.001	0.06 ± 0.02	0.07 ± 0.01	0.08 ± 0.005
16:1n-7	0.33 ± 0.01	0.35 ± 0.02	0.38 ± 0.01	0.35 ± 0.02
18:1n-9	13.96 ± 0.19[a]	15.27 ± 0.32	6.29 ± 0.07	6.66 ± 0.26
18:1 DMA	1.59 ± 0.04	1.61 ± 0.08	0.24 ± 0.03	0.31 ± 0.02
18:1n-7	3.37 ± 0.02[a]	3.22 ± 0.25	1.85 ± 0.04[b]	1.57 ± 0.04
20:1n-9	1.37 ± 0.08	1.48 ± 0.11	0.19 ± 0.01	0.19 ± 0.01
22:1n-9	0.18 ± 0.01	0.18 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
24:1n-9	1.94 ± 0.12	1.93 ± 0.14	0.25 ± 0.03[a]	0.11 ± 0.04
Total monosaturates	22.80 ± 0.42	24.21 ± 0.68	9.35 ± 0.10	9.37 ± 0.28
18:2n-6	0.52 ± 0.02[c]	0.76 ± 0.01	0.60 ± 0.03[a]	0.84 ± 0.06
18:3n-6	0.05 ± 0.0005[c]	nd	0.05 ± 0.004	0.04 ± 0.003
20:2n-6	0.16 ± 0.004	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.005
20:3n-6	0.36 ± 0.01[c]	0.54 ± 0.01	0.18 ± 0.01[c]	0.25 ± 0.01
20:4n-6	9.61 ± 0.07[c]	7.92 ± 0.20	9.83 ± 0.24[b]	8.37 ± 0.14
22:4n-6	3.52 ± 0.05[c]	2.03 ± 0.05	2.95 ± 0.04[c]	1.29 ± 0.05
22:5n-6	10.38 ± 0.22[c]	0.18 ± 0.01	25.01 ± 0.79[c]	0.24 ± 0.03
Total n-6	24.59 ± 0.24[c]	11.58 ± 0.23	38.77 ± 0.62[c]	11.19 ± 0.19
22:5n-3	nd[a]	0.17 ± 0.06	0.08 ± 0.01[c]	0.59 ± 0.04
22:6n-3	1.91 ± 0.05[c]	12.36 ± 0.32	5.40 ± 0.32[c]	32.05 ± 0.08
Total n-3	1.99 ± 0.05[c]	12.53 ± 0.34	5.56 ± 0.31[c]	32.90 ± 1.07
22:5n-6/22:6n-3	5.43 ± 0.16[c]	0.01 ± 0.001	4.63 ± 0.15[c]	0.01 ± 0.001
22:5n-6 + 22:6n-3	12.29 ± 0.24	12.54 ± 0.32	30.41 ± 1.10	32.29 ± 1.09
n-6 + n-3	26.58 ± 0.27[b]	24.11 ± 0.53	44.33 ± 0.91	44.09 ± 1.01
Total fatty acids μg/mg wet wt	25.17 ± 0.50	25.73 ± 0.82	18.88 ± 0.74	17.29 ± 1.75

Fatty acid methyl esters from 10:0 to 24:1n-9 were analyzed. 18:3n-3 and 20:5n-3 were not detected (nd) in the nervous system (i.e.,<0.01%). Each parameter is presented as the mean ± SEM for four rats. DMA, a dimethylacetal derivative of a plasmalogenic phospholipid.

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ versus n-3-Adq group (Student's *t*-test).

J & W Scientific, Folsom, CA). The detector and injector temperatures were set to 250°C. The oven temperature program began at 130°C and increased to 175°C at the rate of 4°C/min, then at the rate of 1°C/min to 210°C, and finally increased at the rate of 30°C/min to 245°C, with a final hold for 15 min. Hydrogen was used as carrier gas at a linear velocity of 50 cm/s. A custom-mixed, 30-component, quantitative methyl ester standard containing 10–24 carbons and 0–6 double bonds was used for assignment of retention times and to ensure accurate quantification (Nu Chek Prep 462, Elysian, MN). Fatty acid data were expressed as % of total peak area, which corresponded to weight% to within 5%, as demonstrated by quantitative standard mixtures. Internal standards were used to calculate tissue fatty acid concentrations.

Data are expressed as the mean ± the standard error of the mean (SEM). Curves were fit to the data for Figs. 1–4 with the use of regression analysis and the time at which there is 50% ($T_{1/2}$) recovery using the Sigma Plot program (SPSS Science Inc., Chicago, IL).

RESULTS

Study design

This study investigates the time course of reversal of fatty acid compositional differences induced by an n-3-Def diet over two generations. Adult rats 7 weeks of age were used because the brain has reached its adult weight by that age. The mean body (n-3-Adq, 261 ± 13 g; n-3-Def, 277 ± 16 g) and brain (n-3-Adq, 1.95 ± 0.06 g; n-3-Def, 1.95 ± 0.03 g) weights of the animals raised on the two diets were not significantly different when measured at 7 weeks of age. The only variable in this study was the addition of 2.6% of fat as LNA from flaxseed oil and 1.3% of fat as DHA (DHASCO); these fats were substituted for saturated fats. The n-3-Def diet was very effective in inducing n-3 fatty acid deficiency in the nervous system (**Table 2**). In the brain, DHA was 12.4% in the n-3-Adq group but only 1.9% in the n-3-Def group, a decrease of 85%. Similarly, the retinal DHA level was 32% of total fatty acids in the n-3-Adq group but only 5.4% in the n-3-Def group, representing a decrease of 83%. In the brain, DPAn-6 had increased from the n-3-Adq level of 0.18% of total fatty acids to 10.38% in the n-3-Def group. Similarly, in the retina, DPAn-6 was only 0.24% in the n-3-Adq animals but rose to 25% in the n-3-Def case (Table 2). The ratios of DPAn–6/DHA were drastically altered by this dietary treatment, resulting in an approximately 500-fold change in the nervous system.

Serum

When the adult n-3-Def rat diet was reversed, the DHA level in serum rapidly rose to that of the animals in the n-3-Adq group (**Fig. 1A**). After one week, the DHA level reached 73% of the reference level and after two weeks, 89%. Continued small increases in serum DHA were noted after 4 weeks (93% of reference) up to 8 weeks (106%). The $T_{1/2}$ for DHA recovery was calculated to be 0.54 weeks. There was a reciprocal decrease in serum DPAn-6 during this time as well (Fig. 1B). Nearly all of the decrease was observed within the first week, when the
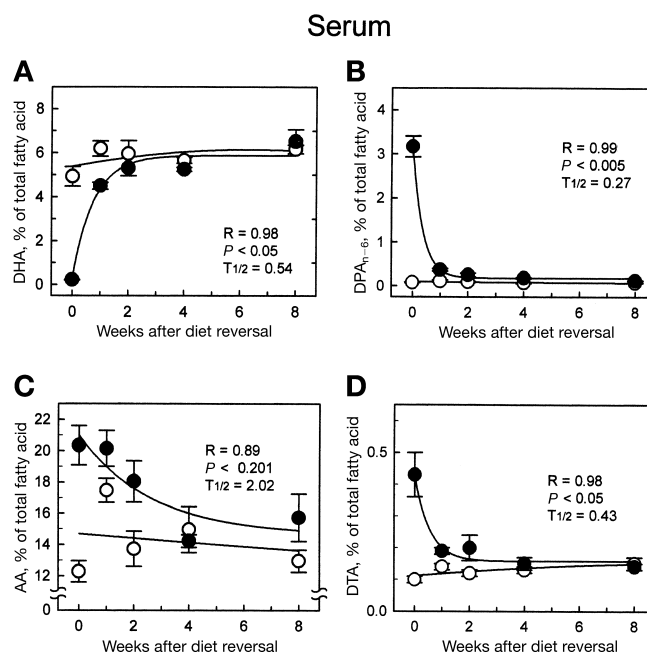
Serum

Fig. 1. Alteration in long chain polyunsaturate composition in serum after reversal of dietary n-3 deficiency. Seven-week-old rats who were fed an n-3-deficient diet for two generations were switched to an n-3-adequate diet (●) at 0 week, and the acyl composition measured at various time points (n = 4 rats per time point). The open circles (○) represent the reference point, animals maintained on the n-3-adequate diet for two generations. The time at which half-maximal recovery was obtained ($T_{1/2}$) was estimated by a regression model and the parameters thus obtained are indicated on the graphs; the units for the $T_{1/2}$ values are given in weeks. The R values represent the correlation coefficients for the fitted curves.

level fell from 3.2% to 0.37%. The n-3-Adq level was 0.07–0.11%, and this value was reached gradually over 8 weeks. The $T_{1/2}$ for DPAn-6 decline was 0.27 weeks. The initial marked loss of both eicosapentaenoate (EPA, 20:5n3) and docosapentaenoate n-3 (DPAn-3, 22:5n3) was partially reversed after 1 week of the repletion diet, and had nearly reached the level of the n-3-Adq group after 2 weeks (data not shown). There were also apparent initial reductions in the levels of serum arachidonic acid (AA, 20:4n6) and docosatetraenoic acid (DTA, 22:4n6) in the n-3-Def animals that recovered with a time course similar to that of DPAn-6 (Fig. 1C and D).

Liver

The liver was the most rapidly recovering of all tissues examined in this study. After one week, the level of liver DHA had fully recovered when the n-3-Def animals were switched to the n-3-Adq diet (**Fig. 2A**). Liver DPAn-6 followed much the same pattern, with 88% of its decrease coming within the first week after the n-3-Adq diet was fed (Fig. 2B). The $T_{1/2}$ for liver DHA and DPAn-6 were only 0.30 and 0.32 weeks, respectively. However, the AA and DTA recovered somewhat more slowly (Fig. 2C and D). In the case of DTA, for example, the $T_{1/2}$ was 1.44 weeks, several times more than that for the DPAn-6. The AA appeared to have a time course similar
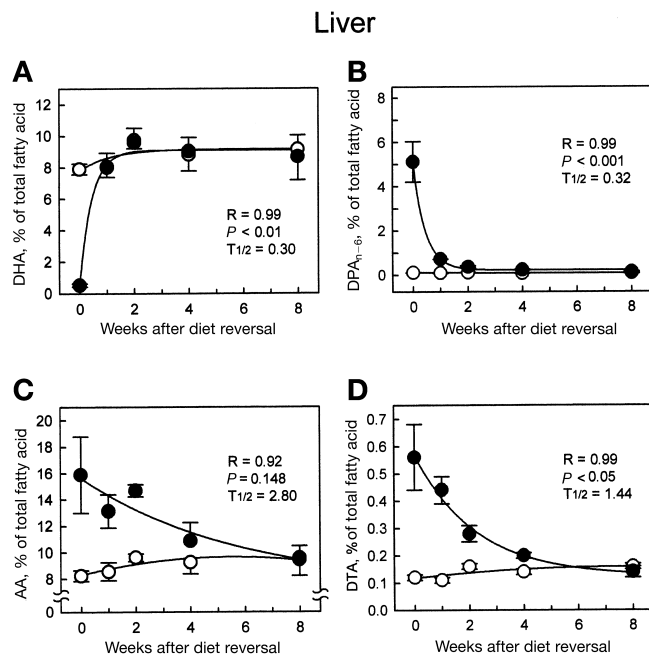
Liver

Brain

Fig. 2. Alteration in long chain polyunsaturate composition in liver after reversal of dietary n-3 deficiency. See footnotes to Fig. 1.

Fig. 3. Alteration in long chain polyunsaturate composition in brain after reversal of dietary n-3 deficiency. See footnotes to Fig. 1.

to that of DTA. Within the first week on the repletion diet, the liver DPAn-3 had fully recovered, but EPA required 2 weeks to achieve the level of the n-3-Adq group.

Brain

The brain exhibited a much slower time course for DHA recovery in comparison with the peripheral tissues examined (**Fig. 3A**). There was only a 19% recovery in brain DHA within the first week, and only 35% after 2 weeks. Even after 8 weeks of diet reversal there was incomplete recovery; brain DHA reached only 80% of that of the n-3-Adq group level. There was a reciprocal increase in DPAn-6, albeit slower than DHA recovery; it decreased only 14% within the first week and 34% after 2 weeks. After 8 weeks, it had fallen to 1.5% of total fatty acids, whereas the n-3-Adq level was 0.2% (Fig. 3B). The $T_{1/2}$ values for DHA and DPAn-6 were 2.9 and 3.3 weeks, respectively. The AA recovery had a time course similar to that of DHA ($T_{1/2}$ was 2.5, Fig. 3C), while DTA recovery was rather slow, with a $T_{1/2}$ value of 3.7 (Fig. 3D). The DPAn-3 recovery was about half completed within the first week of the repletion diet, but it did not appear to be completely recovered until the 8-week time point.

Retina

The initial rate of DHA recovery in the retina after the n-3-Def group was switched to the n-3-Adq diet was faster than that in the brain. It recovered to 62% of the reference DHA level within 2 weeks (**Fig. 4A**). However, the subsequent recovery rate decreased, as it had only recovered 72% of the DHA by 4 weeks. The retinal DHA was fully recovered after 8 weeks. The decrease in DPAn-6 in the retina also appeared faster than that in the brain. After 2 weeks, 56% of the total DPAn-6 recovery needed to
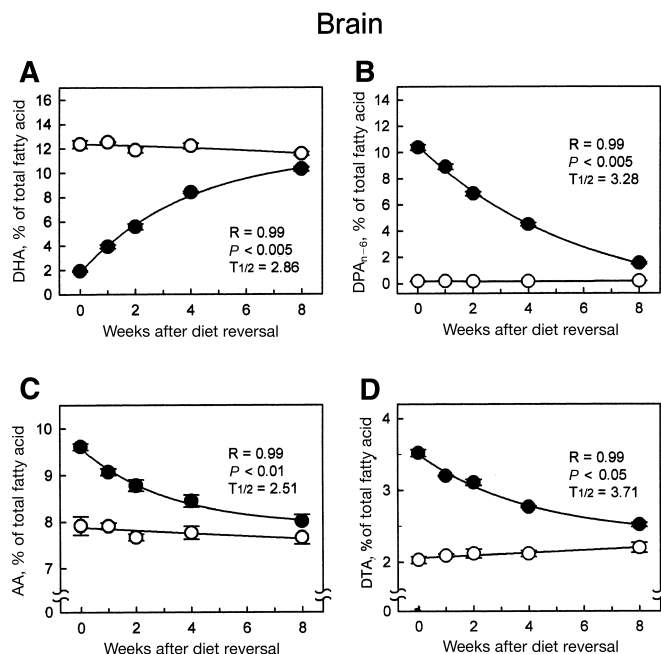
reach the reference level was attained (Fig. 4B). After 4 weeks, the DPAn-6 level had fallen from 25% to 3% of total fatty acids, owing to the feeding of the n-3-Adq diet. The DPAn-6 had fallen by 98% after 8 weeks but remained at a higher level (0.61%) than in the n-3-Adq group (0.23%). The $T_{1/2}$ values for DHA and DPAn-6 were 2.1 and 1.6 weeks, respectively; thus the rate of DPAn-6 decline was faster than the rate of DHA increase. The AA
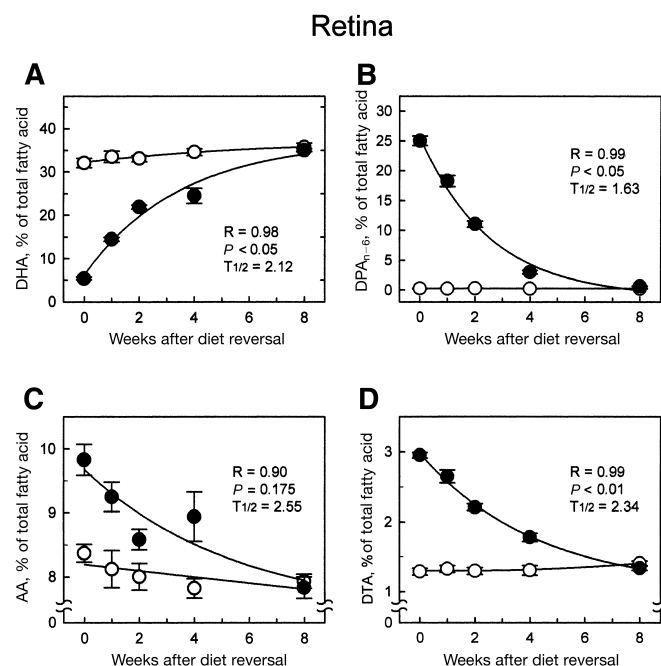
Retina

Fig. 4. Alteration in long chain polyunsaturate composition in retina after reversal of dietary n-3 deficiency. See footnotes to Fig. 1.

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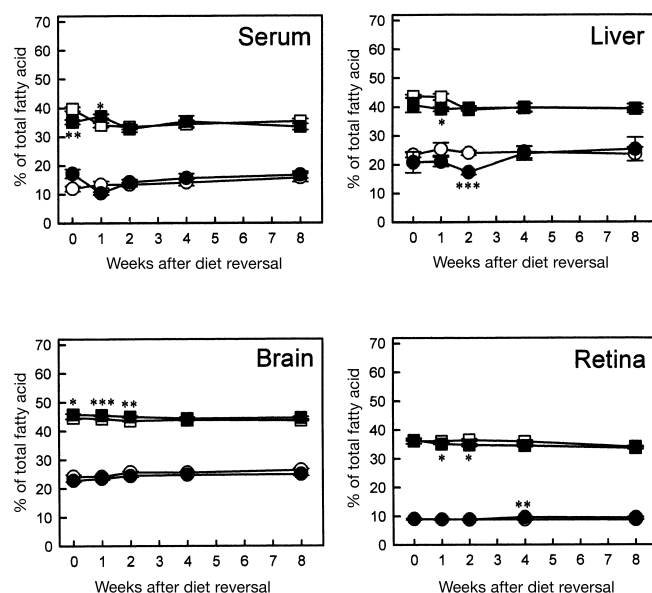
Saturates/Monounsaturates

Fig. 5. Alteration in long chain saturate and monounsaturated fatty acid composition in various tissues after reversal of dietary n-3 deficiency. Seven-week-old rats who were fed an n-3-deficient diet for two generations were switched to an n-3-adequate diet (● monounsaturates, ■ saturates) at 0 week and the acyl composition measured at various time points (n = 4 rats per time point). The open symbols represent the reference point (○ monounsaturates, □ saturates), animals maintained on the n-3-adequate diet for two generations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with adequate diet group (Student's t-test).

and DTA also recovered rather slowly in the retina, with $T_{1/2}$ values of 2.6 and 2.3, respectively. The DPAn-3 recovery was protracted: It reached about half of the n-3-Adq level within 1–2 weeks, but had not completely recovered even after 8 weeks.

Saturated and monounsaturated fatty acids

The time courses for the levels of total saturated and monenoic fatty acids for serum, liver, brain, and retina during n-3 fatty acid repletion are presented in **Fig. 5**. The changes in nonessential fats were small, and only significant at a few time points. In the liver, in particular, there was a slight decline in both saturated and monounsaturated fatty acids in the repletion group.

DISCUSSION

This study defines the time courses for the recovery of DHA and the corresponding declines in n-6 polyunsaturates in second-generation, n-3-Def, adult rats subjected to dietary repletion. The repletion diet contained both LNA and DHA. As may be expected, the recovery of the deficient group to the level of DHA observed in the n-3-Adq control group was rapid for serum and liver. However, the recovery of DHA in the nervous system was slow, as has been observed by others (32–39). The retina took more

than 4 weeks to fully recover; the brain required 8 weeks to reach the levels of DHA found in the n-3-Adq group.

It is instructive to consider the total amounts of DHA (and DPAn-6) in each organ/fluid compartment examined in this study. At the beginning of the experiment, the n-3-Def retinas had a deficit of about 100 μg of DHA, whereas the brain had a deficit of over 5 mg (**Table 3**). Similarly, the serum had a DHA deficit of 1.1 mg and the liver a relatively large deficit of 38 mg. However, after 1 week of consuming the repletion diet to which both LNA and DHA had been added, the liver had accumulated 46 mg of DHA from the circulation. In spite of the great demand of the liver for circulating DHA, the serum had also repleted 1.1 mg of DHA within 1 week. It is remarkable, then, that the retina managed to accumulate only 32 μg of DHA during this period while the brain accumulated less than 1 mg. It appears that even though the serum did not reach its maximal level of DHA within 1 week of dietary repletion, a very large quantity of DHA was deposited in the liver during this time period.

However, these tissues have vastly differing total weights. In an attempt to normalize the data for this factor, the amount of DHA accumulated by each tissue was divided by the weight of each tissue. The results during the first week of repletion are as follows, expressed in the units of g of DHA/mg of tissue: liver, 2.82; retina, 1.24; brain, 0.44; and serum, 0.09. From this perspective, the apparent discrimination against the nervous system for DHA accretion, and particularly the retina, is greatly diminished. In fact, the serum has the lowest amount of recovery per tissue weight. This was largely a reflection of the relatively low plasma content of DHA, and thus the relatively low amount accreted in the first week, notwithstanding the relatively fast plasma $T_{1/2}$ for recovery.

The total amount of DHA deposited in the four tissue compartments examined here during the first week of dietary repletion was about 48.4 mg. Assuming that a 350-g rat consumes about 20 g of this pelleted diet per day, a calculated intake of 25.8 mg of DHA would be consumed daily, or about 181 mg of DHA/week. Therefore, about 27% of the DHA consumed was accreted by these four tissues, with most (96%) of it taken into the liver. Since there is undoubtedly more DHA in other rat tissues (not measured), it appears that the n-3-Def rats accumulated dietary DHA in a fairly efficient manner.

It is perplexing, then, to consider why the circulation did not replete the much smaller "deficits" of the nervous system and, in particular, the relatively small DHA requirement of the retina. Apparently, the uptake and delivery systems of the nervous system are not capable of rapid accumulation of DHA, even when it is available in the circulation. Of course, other possibilities exist; it may be hypothesized, for example, that DHA enters the nervous system but is rapidly catabolized. This appears unlikely, particularly since there is experimental evidence to the contrary. Stinson, Wiegand, and Anderson (43) found that after n-3 deficiency (safflower oil-based diet), the half-life of dog retina DHA was nearly 3-fold higher than that in a linseed oil group. Contreras et al. (44) observed

TABLE 3.   Total quantities of DHA and DPAn-6 in serum, liver, retina, and brain
during the initial phase of n-3 fatty acid repletion[a]

Organ	Week	n-3–Deficient		n-3–Adequate	
		DHA	DPAn-6	DHA	DPAn-6
Serum (*mg*)	0	0.08 ± 0.02	1.13 ± 0.22	1.14 ± 0.18	0.02 ± 0.002
	1	1.22 ± 0.06	0.10 ± 0.01	1.46 ± 0.28	0.03 ± 0.01
	2	1.27 ± 0.13	0.06 ± 0.01	1.21 ± 0.22	0.02 ± 0.003
Liver (*mg*)	0	2.66 ± 0.21	26.73 ± 3.96	40.89 ± 1.68	0.55 ± 0.02
	1	48.94 ± 3.50	4.49 ± 0.68	51.61 ± 2.48	0.58 ± 0.06
	2	46.78 ± 2.75	1.78 ± 0.22	52.11 ± 7.23	0.61 ± 0.09
Brain (*mg*)	0	0.94 ± 0.03	5.09 ± 0.11	6.19 ± 0.25	0.09 ± 0.01
	1	1.85 ± 0.15	4.17 ± 0.16	5.38 ± 0.29	0.08 ± 0.01
	2	2.94 ± 0.15	3.61 ± 0.13	6.27 ± 0.29	0.08 ± 0.01
Retina (μg)	0	25.12 ± 3.98	115.33 ±16.04	123.71 ± 9.65	0.93 ± 0.12
	1	57.33 ± 3.50	72.79 ± 6.49	139.98 ±11.95	0.95 ± 0.03
	2	71.35 ±11.46	35.78 ± 5.56	141.09 ±13.33	1.23 ± 0.16

[a] Each parameter is presented as the mean ± SEM for n = 4 rats. Mean tissue weights for brain, liver, retina, and serum in 8-week-old rats were 2.1 ± 0.05, 14.8 ± 1.64, 0.027 ± 0.0026, and 11.3 ± 0.77 g, respectively, in the n-3-Adq group. The serum volume was calculated by interpolating from the data supplied by McGuill and Rowan (57) for blood volume and assuming that serum comprised 60% of blood volume. A density of 1.026 g/ml was used to convert serum volume into weight.

a 40-fold decrease in DHA incorporation from plasma into the rat brain, as well as a reduction in recycling via deacylation/reacylation reactions in n-3-Def rats. Thus, although the rate of uptake of DHA from the circulation into the brain/retina may be altered during dietary n-3 deficiency, the overall rate of this process appears slow and may be rate-limiting for DHA repletion.

Most previous studies have used repletion diets with only LNA. This study used 2.6% and 1.3% of total fatty acids as LNA and DHA, respectively, with fat representing 10 weight % of the diet. Generally, preformed DHA is more efficient in supporting organ DHA composition than is LNA. This contention is based on several different observations. First, LNA is rapidly metabolized to cholesterol and nonessential fatty acids (45), and much of it is sequestered in the skin/fur (46). Early radiotracer experiments suggested that dietary DHA was a much more efficient source of brain and liver DHA than was its precursor, LNA (47, 48). Recent studies of stable-isotope-labeled LNA metabolism in human infants in vivo indicated rather limited metabolism to DHA (49–52). In tracer studies in which a direct comparison was made of the efficiency of labeled LNA versus DHA incorporation into the DHA of several baboon tissues, Su et al. (53) found that the relative accretion of preformed DHA was 7-fold greater than that of LNA when expressed as a % of dose. In baboon retina and retinal pigment epithelium, Su et al. (54) also observed that preformed DHA was at levels 12- to 15-fold greater than that of LNA-derived DHA. Woods, Ward, and Salem (55) found that even high levels of dietary LNA could not support the same level of brain and retinal DHA found in dam-reared rat pups that were given milk containing preformed DHA. Abedin and Sinclair (56) reported a similar finding in guinea pig brain, where a 1.8% dietary level of DHA supported a 25.5% level of retinal phospholipid DHA, whereas a 7.1% level of dietary LNA led to a retinal phospholipid DHA content of only 16.4%.

On the basis of these observations, it may be expected that the organ DHA recovery would proceed faster in the present study than it did in those where only LNA was added to their repletion diets. In the study of Bourre et al. (35), where soybean oil was used for repletion, the liver took two weeks to return to the control level of DHA. Because the liver in our study had fully recovered its control DHA level after 1 week, with a $T_{1/2}$ of only 0.3 weeks, it appeared that the supply of DHA supported a faster tissue DHA recovery. This was in spite of the more than 2-fold higher level of total n-3 fatty acids in the soybean oil diet used in the Bourre et al. study, in relation to the diet used in the present study (7.4% vs. 3.9%). The retina in the Bourre et al. study reached the DHA control level (soybean control) after 6 weeks, and thus had a similar time course to that observed in the present study. Also, the brain recovery was slower than that described here, as the recovery of neuronal cell DHA was incomplete even after 10 weeks for the soybean-based diet (36). The 10 week time frame observed for rats is similar to the findings of Weisinger et al. (39) for functional recovery of electroretinographic responses in the guinea pig retina. Bourre et al. (33) found that the sciatic nerve had not recovered its control DHA level after 6 weeks but reached the control level after 10 weeks. Brain synaptosomal DHA recovered only after 75 days on a soybean oil-based diet (32).

Another important aspect of the current study is its utility as a model of the permanence of the effects of nutritional deprivation on organ function, in particular on nervous system function. Now that the time course for DHA recovery in the adult rat brain and retina has been defined under a well-defined set of conditions, the degree of reversal of the DHA deficiency by a repletion diet can be correlated with the recovery of rat brain and retinal functions. This work will also facilitate the study of reversal of n-3 deficiency at various stages of brain development. One important question of relevance to infant nutrition is

whether there are critical stages of neurodevelopment in which DHA is required for optimal development, or whether DHA supply later in development will be sufficient. It is clear from autopsy studies of human infants that the brain DHA levels of formula-fed infants are lower than those of breast-fed infants (20–22). Thus, it will be of relevance to know whether there are permanent effects on nervous system function of dietary deprivation of DHA when LNA alone is provided in the infant diet.█

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