Slow Recovery of the Fatty Acid Composition of Sciatic Nerve in Rats Fed a Diet Initially Low in n-3 Fatty Acids

J.M. Bourre[a,*], A. Youyou[a], G. Durand[b] and G. Pascal[b]

[a]INSERM U-26, Hopital Fernand Widal, Paris, France and [b]INRA-CNRZ, Jouy-en-Josas, France

The sciatic nerve of rats fed sunflower oil (6 mg 18:3-n-3/100 g of diet) presented dramatic alterations in the long chain polyunsaturated fatty acids in comparison with those fed soy oil (130 mg 18:3n-3/100 g of diet). In both 15-day-old and 60-day-old animals fed sunflower oil, 22:6n-3 (cervonic acid) was four-fold less, 22:5n-6 was 10-fold greater; adrenic acid (22:4n-6) was slightly greater and arachidonic acid (20:4n-6) was close to that in rats fed soy oil. The percentage distribution of total polyunsaturated fatty acids as well as the individual saturated and monounsaturated fatty acids were the same in both groups.

When the sunflower oil–fed animals were switched to a soy oil–containing diet for either 15 or 60 days, the percentage distribution of 22:6n-3 increased slowly to reach the control value 2.5 months later. Conversely 22:5n-6 decreased slowly. The decay of 22:5n-6 was more rapid than the increase of 22:6n-3.

Lipids 22, 535–538 (1987).

The fluidity of the lipid environment appears to modulate the activity of membrane-bound enzymes; this fluidity is controlled by essential polyunsaturated fatty acids (PUFA). Increased PUFA, particularly arachidonic acid, were found in endoneurial phosphatidylethanolamine of both developing and regenerating rat sciatic nerve, suggesting a close association between PUFA and peripheral nerve myelination (1). Alterations of PUFA during damage paralleled changes in phospholipid fatty acid composition (2).

The effect of essential fatty acid (EFA) deficiency on rat peripheral nerve myelin has been previously analyzed (3). After 8 mo on the deficient diet, 20:3n-9 was found in the major myelin phospholipids. The level of 18:1n-9 was increased and the levels of 18:2n-6 (linoleic acid), 20:4n-6 (arachidonic acid) and 22:4n-6 (adrenic acid) were decreased. The ratio of 20:4n-6 to 20:3n-9 was clearly depressed by an EFA deficiency (3).

No significant differences in morphology, histology, rate of axonal transport or conduction velocity of peripheral nerve were demonstrated between EFA deficient and control rats (4). Therefore, it would appear that dietary EFA deficiency in postweaned rats can induce fatty acid alterations in peripheral nerve myelin without resulting in detectable changes in function or structure. An excellent review of the lipid composition of normal and degenerating nerve has been recently published (5).

The essentiality of PUFA for the central nervous system has been extensively studied (6–14). The specific role of n-3 fatty acids has been recently reexamined. Interestingly, a pathogenesis of deficiency in linolenic acid has

*To whom correspondence should be sent at INSERM Unite 26, Hopital Fernand Widal, 200, rue du Faubourg St-Denis, 75475 Paris Cedex 10, France.

TABLE 1

Diet Composition (g/kg)

	Soy oil diet	Sunflower oil diet
Casein delipidated[a]	220	220
DL-methionine	1.6	1.6
Cellulose	20	20
Starch	459.7	463.4
Saccharose	230	230
Oil	18.7	15.0
Vitamin mixture[a]	10	10
Mineral mixture[b]	40	40

[a]United States Biochemical Corp. (Cleveland, OH). The vitamin mixture used is the vitamin diet for fortification mixture.

[b]Composition of the mineral mixture/100 g: $CaHPO_4$, 2 H_2O, 38.0; K_2HPO_4, 24.0; $CaCO_3$, 18.1; NaCl, 7.0; MgO, 2.0; MgSO4, 7 H_2O, 9; $FeSO_4$, 7 H_2O, 0.7; $ZnSO_4$, 7 H_2O, 0.5; $MnSO_4$, H_2O, 0.5; $CuSO_4$, 5 H_2O, 0.1; NaF, 0.1; $Al_2(SO_4)_3$ K_2SO_4, 24 H_2O, 0.02; KI, 0.008; $CoCO_3$, 0.008; Na_2SeO_3, 5 H_2O, 0.001.

TABLE 2

Fatty Acid Composition (mg %) of Dietary Lipids

Fatty acids	Sunflower oil diet	Soy oil diet
14:0	0.3	0.3
16:0	6.4	10.1
17:0	tr	0.2
18:0	3.9	5.6
20:0	0.3	0.4
22:0	0.7	0.5
Total saturated	11.6	17.2
16:1	0.2	tr
18:1	21.4	21.4
20:1	0.2	0.3
Total monounsaturated	21.6	21.7
18:2 n-6	66.4	53.5
18:3 n-3	0.4	7.4
Fatty acids (mg/100 g) of diet		
18:2 n-6	936.0	940.0
18:3 n-3	6.0	130.0
n-3/n-6	0.006	0.14

tr, Traces. Dietary lipid fatty acid composition was analyzed by gas chromatography of methyl esters under the following conditions: Packard model 427 gas chromatograph; glass capillary column; stationary phase FFAP; gas pressure vector H_2, 0.6 bar; temperature, 190 C; detection by flame ionization.

been described in the monkey (15) and in man (16). A syndrome of modern society has been put forward as a deficiency in acids of the n-3 series (17).

Our previous studies have shown that a diet lacking in α-linolenic acid (sunflower or peanut oil) can modify the PUFA composition in all the cellular and subcellular fractions of brain lipids examined. The percentage distribution of total PUFA remains unchanged, the sharp drop in 22:6n-3 being counterbalanced by an increase in 22:5n-6 (18,19). With a change from a diet containing sunflower oil to one containing soy oil, the uptake of n-3 fatty acids is remarkably slow, e.g., it takes several months before the cerebral organelles recover a normal quantity of cervonic acid (22:6n-3) (20).

However, little attention has been paid to n-3 PUFA in the peripheral nerve. This work was undertaken to determine (i) the specific alterations of peripheral nerve fatty acids in rats fed a diet rich in n-6 fatty acids but deficient in n-3 fatty acids and (ii) to assess the speed of the recovery of the n-3 fatty acid composition induced when a diet containing n-3 fatty acids is reintroduced.

Our preliminary results have shown that when rats are fed a diet deficient in n-3 fatty acids (peanut or sunflower oil), both peripheral nerve and muscle contain reduced amounts of n-3 fatty acids; more specifically, 22:6n-3 is replaced by 22:5n-6 (21).

MATERIALS AND METHODS

Animals. During three generations, female Wistar rats

TABLE 3

Fatty Acid Percentage Distribution (mg %) of Sciatic Nerve from Young and Adult Rats Fed a Diet Containing Either Soy Oil or Sunflower Oil

	Sunflower oil		Soy oil	
Fatty acid	15 days	60 days	15 days	60 days
Saturated	46.0	40.5	45.3	40.8
14:0	4.0	0.9	4.0	1.5
15:0	0.1	0.1	0.1	0.2
16:0	28.2	23.1	27.1	27.8
17:0	0.2	0.2	0.2	0.2
18:0	8.4	8.2	8.4	6.3
20:0	0.9	1.5	0.9	0.8
22:0	1.8	3.2	1.8	1.9
23:0	0.3	0.4	0.3	0.2
24:0	2.0	2.6	2.2	1.7
25:0	0.1	0.3	0.3	0.2
Monounsaturated	37.6	47.6	39.0	49.0
16:1n-9	0.8	0.8	0.8	0.4
16:1n-7	2.0	3.2	2.1	8.4
18:1n-9	26.1	32.2	28.0	33.1
18:1n-7	2.7	3.1	2.7	3.1
20:1n-9	0.6	0.8	0.6	0.4
20:1n-7	0.7	1.0	0.8	0.8
22:1n-9	0.3	0.5	0.3	0.2
22:1n-7	0.3	0.4	0.2	0.2
24:1n-9	3.7	5.0	3.6	2.2
24:1n-7	0.4	0.6	0.4	0.2
n-9	31.5	39.3	33.8	36.3
n-7	6.1	8.3	6.2	12.7
Polyunsaturated	14.7	11.4	15.1	10.0
18:2n-6	2.9	2.8	3.0	5.0
20:3n-6	0.9	0.4	0.9	0.4
20:4n-6	6.8	3.8	6.5	2.0
22:4n-6	2.2	2.3	1.5	0.7
22:5n-6	1.1	1.0	0.2	0.1
24:4n-6	0.1	0.2	0.2	0.1
24:5n-6	0.3	0.4	0.2	tr
n-6	14.3	10.9	12.5	8.3
18:3n-3	tr	0.2	0.2	0.4
22:5n-3	tr		0.5	0.2
22:6n-3	0.4	0.3	1.4	1.1
n-3	0.4	0.5	2.1	1.7
n-3/n-6	0.03	0.05	0.17	0.21

In each experiment, fatty acid determination was performed in triplicate on 8 pooled nerves. At least 3 experiments were performed.

were fed with a semisynthetic diet containing 1.5% sunflower oil (6 mg % α-linolenic acid). Two weeks before mating, one group (2/3 of the animals) was continued on that diet, while the other group was changed to a diet in which sunflower oil was replaced by 1.9% soy oil (130 mg % α-linolenic acid). Both oils and diets contained ca. 940 mg n-6 fatty acids. Since animals fed either diet ate similar amounts of food, they ate the same amount of n-6 fatty acids. Soy oil–fed rats received ca. 22 times more n-3 fatty acids than animals fed sunflower oil. The composition of the oils and the diets is shown in Tables 1 and 2. Under these experimental conditions, the α-linolenic acid deficiency (sunflower oil–fed rats) had no effect on fecundity (% of pregnant females), fertility (number of pups/litter), pup birth weight, food intake and weight of pregnant or lactating females, or pup growth during suckling. However, this deficiency did cause abnormally high rates of perinatal mortality from birth to postpartum day 3 (22). Three days after delivery the litters (fourth generation) were adjusted to 10 animals. After weaning, the young rats received the same diet as their mother. At 15 days of age, half the animals fed sunflower oil were fed soy oil from then on. In these animals, the n-3–deficient diet was therefore substituted by an n-3–normal diet. The same procedure was performed on other animals at 60 days. Thus, we determined the speed of recovery of fatty acid composition in young and in adult animals. Only male animals were used throughout. The sciatic nerves were very carefully dissected to avoid any contamination by adipose tissue.

Analytical methodology. The sciatic nerve was dissected outward, and lipids were extracted by sonication in chloroform/methanol (2:1, v/v) (23,24) and methylated (25). Fatty acid methyl esters were separated by gas liquid chromatography (GLC) on an open tubular capillary column coated with FFAP (0.30 mm in diameter, 45 m long), using a flame ionization detector. Identification of fatty acids was performed with commercial standards by the means of relative retention times. Areas were calculated with an ICAP integrator (LTT, Paris, France).

RESULTS AND DISCUSSION

Fatty acid analysis (Table 3). The sciatic nerve of sunflower oil–fed animals (6 mg % n-3 fatty acids) presented dramatic alterations in the very long chain polyunsaturated fatty acids in comparison to soy oil–fed (130 mg % n-3 fatty acids) animals. The n-3/n-6 ratio was six- and four-fold less in 15- and 60-day-old animals fed sunflower oil. In both 15-day-old and 60-day-old animals, 22:6n-3 was ca. five- and four-fold less, respectively; conversely, 22:5n-6 was 10-fold greater in sunflower oil–fed animals (22:4n-6 was greater; 20:4n-6 was close to normal). The percentage distribution of PUFA to total fatty acids was normal in sunflower oil–fed animals, as were individual saturated and monounsaturated fatty acids.

In agreement with Yao (30), we found that during development 20:4n-6 and 22:6n-3 decreased and 18:1n-9 increased. The values we obtained compared favorably with previously published results (26–32).

A control group was obtained by feeding animals with regular diet (standard chow). No significant difference was found between this group and animals fed with soy oil.

This study was performed on whole nerve, and contami-

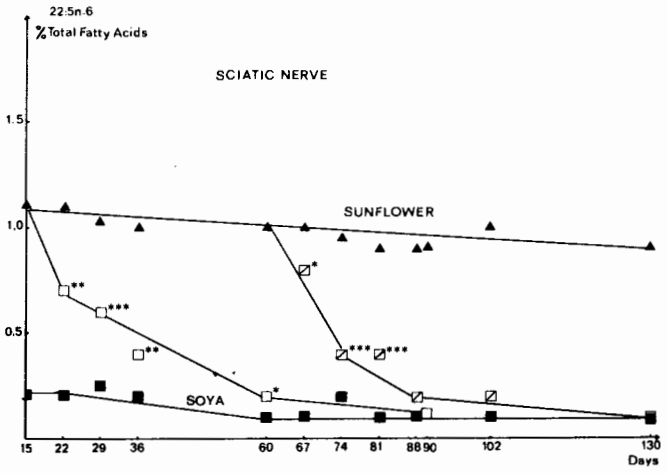

FIG. 1. Recovery of fatty acid composition (mg %) as measured by the amount of cervonic acid (22:6n-3) in animals fed soy oil, sunflower oil or soy oil replacing sunflower oil in either 15-day-old or 60-day-old rats. Dietary lipid fatty acid composition was analyzed by gas chromatography of methyl esters under the following conditions: Packard model 427 gas chromatograph; glass capillary column; stationary phase FFAP; gas pressure vector H_2, 0.6 bar; temperature, 190 C; detection by flame ionization. Solid squares, soy-fed animals; triangles, sunflower-fed animals; open squares, animals initially fed sunflower oil, then fed soy oil when 15 days old; hatched squares, animals initially fed sunflower oil, then fed soy oil when 60 days old. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.001$; 552 animals were used. Values at each time point represent mean for at least 4 samples. Each sample consisted of sciatic nerves from 4 rats.

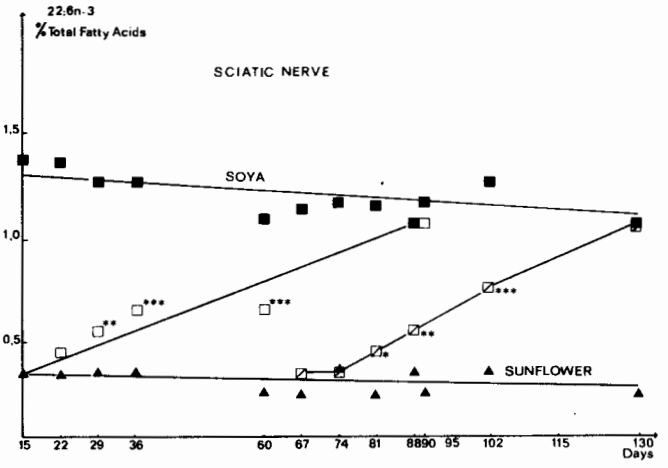

FIG. 2. Recovery of fatty acid composition (mg %) as determined by the amount of 22:5n-6 in animals fed soy oil, sunflower oil or soy oil replacing sunflower oil in either 15-day-old or 60-day-old rats. Dietary lipid fatty acid composition was analyzed by gas chromatography of methyl esters under the following conditions: Packard model 427 gas chromatograph; glass capillary column; stationary phase FFAP; gas pressure vector H_2, 0.6 bar; temperature, 190 C; detection by flame ionization. Solid squares, soy-fed animals; triangles, sunflower-fed animals; open squares, animals initially fed sunflower oil, then fed soy oil when 15 days old; hatched squares, animals initially fed sunflower oil, then fed soy oil when 60 days old. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.001$; 552 animals were used. Values at each time point represent mean for at least 4 samples. Each sample consisted of sciatic nerve from 4 rats.

nation by adipose tissue was carefully eliminated. However, endoneurium, perineurium and epineurium were not separated. Thus, specific alteration in one of these elements is not excluded as they present differences in lipid composition (5,32).

Recovery of altered fatty acid composition. Figure 1 shows changes for 22:6n-3 and Figure 2 for 22:5n-6 after changing the dietary oil from sunflower to soy oil. As no significant differences were found for the other fatty acids, the curves are not shown. However, 22:4n-6 was found to be increased in sunflower oil–fed animals, and the recovery after starting soy oil was slow in 60-day-old animals. In the 15-day-old animals, the decrease was hardly significant, and thus the recovery was difficult to estimate. The high level of 22:4n-6 in sciatic nerve in comparison with brain is to be noted. Sciatic nerves of adult animals fed sunflower oil contained a slightly increased percentage distribution of saturated and monounsaturated very long chain fatty acids. When the sunflower oil–fed animals received a diet containing soy oil at either 15 or 60 days, the percentage distribution of 22:6n-3 increased slowly to reach the control value after 2.5 months (Fig. 1). Conversely, 22:5n-6 decreased slowly throughout (Fig. 2). Interestingly, in adult animals, the recovery did not start from the day on which the diet was changed, but there was a delay of 20 days. In contrast, in young animals, the recovery started from a few days after changing the diet, but the recovery was statistically significant only after 14 days. It is interesting to note that the recovery as measured by the decay of 22:5n-6 was more rapid than with increase of 22:6n-3. This very low speed of recovery of PUFA composition of sciatic nerve after deprivation of n-3 fatty acids was unexpected. We have previously shown (20) that all brain subfractions (myelin, synaptosomes, mitochondria and microsomes) recover very slowly but all at the same speed. Thus, the peripheral nervous system responds to dietary fatty acid changes in a manner similar to the central nervous system.

The slow recovery could be due to the slow turnover of major membranes in the peripheral nervous system. Another explanation could be that the rate-limiting factor is the reduced in situ synthesis of n-3 fatty acid synthesis from α-linolenic acid due to low desaturase activity.

In addition, nervous tissue contains a very minute amount of α-linolenic acid, and studies of brain cells in culture have suggested that the only truly essential fatty acid for these cells is probably 22:6n-3 (33). Thus, another explanation for the low recovery of fatty acid composition could be either a limited transport through the blood-nerve barrier or a restricted synthesis of 22:6n-3 by the liver or reduced dietary origin.

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