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Alteration in Fatty Acid Composition of Adult Rat Brain Capillaries and Choroid Plexus Induced by a Diet Deficient in n-3 Fatty Acids: Slow Recovery After Substitution with a Nondeficient Diet

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Abstract: Wistar rats were fed for three generations with a semisynthetic diet containing either 1.5% sunflower oil (940 mg% of C18:2n-6, 6 mg% of C18:3n-3) or 1.9% soya oil (940 mg% of C18:2n-6, 130 mg% of C18:3n-3). At 60 days of age, the male offspring of the third generation were killed. The fatty acyl composition of isolated capillaries and choroid plexus was determined. The major changes noted in the fatty acid profile of isolated capillaries were a reduction (threefold) in the level of docosahexaenoic acid and, consequently, a fourfold increase in docosapentaenoic acid in sunflower oil-fed animals. The total percentage of polyunsaturated fatty acids was close to that in the soya oil-fed rats, but the ratio of n-3/n-6 fatty acids was reduced by threefold. In the choroid plexus, the C22:6n-3 content was also reduced, but by 2.6-fold, whereas the C22:5n-6 content was increased by 2.3-fold and the ratio of n-3/n-6 fatty acids was reduced by 2.4-fold. When the diet of sunflower oil-fed rats was replaced with a diet containing soya oil at 60 days of age, the recovery in content of n-6 and n-3 fatty acids started immediately after diet substitution; it progressed slowly to reach normal values after 2 months for C22:6n-5 and 2.5 months for C22:6n-3. The recovery in altered fatty acids of choroid plexus was also immediate and very fast. Recovery in content of C22:5n-6 and C22:6n-3 was complete by 46 days after diet substitution.
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Essential fatty acid deficiency has been shown to alter the fatty acid composition of lipids in whole brain (Mohrhauer and Holman, 1963; Rathbone, 1965; Galli et al., 1970; Alling et al., 1974; Trapp and Bernsohn, 1977; Tinoco et al., 1978; Crawford et al., 1981) and isolated subcellular fractions (Sun, 1972; Sun and Sun, 1974; Karlsson, 1975; Selivonchick and Roots, 1979; Matheson et al., 1980). Most of these studies were performed using a diet deficient in both dietary linoleic (C18:2n-6) and linolenic acid (C18:3n-3). The specific role of linolenic acid has not been well studied. Deprivation of this fatty acid during pregnancy and during the life span of the progeny caused learning impairment in rats (Lamptey and Walker, 1976). Bourre et al. (1984) have shown that linolenic acid deficiency causes a reduction in content of polyunsaturated fatty acids of the n-3 series in neurons, astrocytes, oligodendrocytes, and brain fractions such as myelin and synaptosomes. Moreover, the recovery in levels of the altered fatty acids in brain subcellular fractions and cells after supplementation with an optimal level of C18:3n-3 was very slow (Youyou et al., 1985).

With the elaboration of a procedure by which a fraction enriched in functionally and morphologically intact cerebral microvessels could be obtained, direct biochemical investigation of the intraparenchymal microvasculature has become possible (Joó, 1985).

In this work, we have determined the effect of dietary linolenic acid deficiency on the fatty acid composition of rat brain capillaries and choroid plexus and the recovery in content of altered fatty acids after substitution of a nondeficient diet.

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MATERIALS AND METHODS

Animals

Female Wistar rats were maintained on two semisynthetic diets differing in type of oil used: one group received 1.5% sunflower oil (940 mg% linoleic acid and 6 mg% linolenic acid), and the other received 1.9% soya oil (940 mg% linoleic acid and 130 mg% linolenic acid) through three generations. The male offspring of the third generation were fed diets identical to those of the dams. At 60 days of age, these rats (third generation) were killed by decapitation. The forebrain was removed, cleared of surface vessels, and prepared for microvessel isolation. For recovery studies, half of the rats receiving the sunflower oil diet were switch to the soya oil diet at 60 days of age.

Brain capillary and choroid plexus preparation

Brain capillaries (15 animals per preparation) were isolated by a modification of the method described by Goldstein et al. (1975). Cerebral cortices were placed in ice-cold buffer made of oxygen-saturated Ringer solution containing 1.2 m*M* $MgCl_2$, 15 m*M* HEPES at pH 7.4, and 1% bovine serum albumin (fraction V). The tissue was homogenized in a glass homogenizer with a Teflon pestle (clearance = 0.25 mm) at 390 rpm (20 strokes). The homogenate was centrifuged at 1,000 *g* for 10 min. The pellet was resuspended in cold buffer containing 17.5% (wt/vol) dextran and centrifuged at 4,000 *g* for 15 min. The supernatant, consisting of a mixture of the myelin fraction and some capillaries, was rehomogenized and recentrifuged at 4,000 *g*. The resulting pellet of these two steps, which consisted of free nuclei and capillaries, was resuspended in buffer and then passed through nylon mesh (pore size = 118 μm) under gentle vacuum to eliminate large microvessels. The capillaries were separated from nuclei by passing the suspension through a 1.2- × 1.5-cm column containing glass beads 0.25 mm in diameter. Nuclei were removed by extensive washing with buffer, whereas capillaries remained attached to the glass beads. After resuspension in buffer, capillaries were collected by gentle agitation and subsequent sedimentation of the beads. The capillary suspension was centrifuged at 500 *g* for 5 min. To remove traces of albumin the resulting pellet was washed twice with buffer without serum albumin. The purity of each preparation was verified by phase-contrast microscopy as previously described (Homayoun et al., 1985). The yield was ~3 mg/10 g of cortex.

Choroid plexuses were removed, put in the same buffer as that used for the capillary preparation, washed well with buffer without serum albumin, and stored at −20°C until lipid extraction.

Lipids were extracted from cerebral capillaries and the choroid plexus by sonication in chloroform/methanol (2:1 vol/vol) (Folch et al., 1957; Pollet et al., 1978) and transmethylated according to the method described by Luddy et al. (1959). Fatty acid methyl ester analysis was performed by GLC on an open tubular capillary column coated with FFAP (0.3 mm in diameter, 0.45 m long).

Statistically significant differences were calculated using analysis of variance and Student's *t* test.

RESULTS

Capillaries

The fatty acid composition of cerebral capillaries of rats fed diets containing oils with different levels of n-3 fatty acids is shown in Table 1. In rats fed the diet containing sunflower oil (deficient in n-3 fatty acid), the most important difference was a highly significant ($p < 0.001$) reduction in C22:6n-3 content (threefold). This reduction was accompanied by a fourfold rise in C22:5n-6 content ($p < 0.001$). There was no significant difference in C22:5n-3 levels. The level of arachidonic acid was comparable in both groups. The total amount of polyunsaturated fatty acids (n-3 plus n-6) in sunflower oil-fed rats was close to that in soya oil-fed rats. The ratio of n-3/n-6 fatty acids was significantly decreased in sunflower oil-fed rats (threefold). There was an increase in saturated and monounsaturated fatty acids in sunflower oil-fed animals. This increase was significant for some monoenoic acids (C16:1n-7 and C20:1n-9).

Choroid plexus

In the choroid plexus, dietary deficiency of n-3 fatty acids induced, here again, a marked reduction in C22:6n-3 content (2.6-fold). This reduction was, in part, compensated for by a rise in C22:5n-6 content (2.3-fold). However, this increase was not significantly different from values in soya oil-fed rats. The ratio of n-3/n-6 fatty acids was decreased 2.4-fold.

Recovery in fatty acid levels

The recovery in fatty acid levels was measured essentially by the increase in C22:6n-3 content and the decrease in C22:5n-6 content. Figure 1 shows the recovery in cerebral capillaries. The increase in the level of C22:6n-3 started very early (4 days after diet substitution). Recovery was slow for 2 months; thereafter, levels rose sharply until 2.5 months, becoming comparable to control levels.

For C22:5n-6, the speed of recovery was faster, and the content reached control values after 2 months. The alteration in content of saturated and monounsaturated fatty acids was also corrected by diet substitution (data not shown).

In the choroid plexus (data not shown), the increase in C22:6n-3 content started at day 64 and continued until day 106, when it reached normal values. The same trend was observed for C22:5n-6.

DISCUSSION

The fatty acid profile obtained from cerebral capillaries of soya oil-fed rats (control diet) resembled those reported by others (Selivonchick and Roots, 1979; Matheson et al., 1980; Brown et al., 1984). Previous studies on the effect of essential fatty acid deficiency on cerebral capillaries examined deficiencies in both linoleic and linolenic acids (Selivonchick and Roots, 1979; Matheson et al., 1980). A deficiency in dietary n-3 fatty acid has been shown to alter the fatty acid composition of various organs (Youyou et al., 1988), brain fractions, and brain cells (Bourre et al., 1984). This study shows that similar alterations also occur in cerebral capillaries and the choroid plexus.

TABLE 1. *Fatty acid composition of cerebral capillaries and choroid plexus of rats fed diets containing different levels of n-3 fatty acids*

	Cerebral capillaries		Choroid plexus	
Fatty acid chain length	Soya oil diet (n = 4)	Sunflower oil diet (n = 6)	Soya oil diet (n = 5)	Sunflower oil diet (n = 5)
14:0	0.60 ± 0.06	0.66 ± 0.31	0.20 ± 0.01	0.20 ± 0.02
16:0	16.35 ± 0.73	15.75 ± 0.56	16.46 ± 1.05	18.80 ± 1.30
16:1n-9	0.55 ± 0.05	0.99 ± 0.40	0.33 ± 0.08	0.35 ± 0.09
16:1n-7	1.03 ± 0.09	1.25 ± 0.14[a]	0.78 ± 0.72	0.72 ± 0.14
18:0	15.80 ± 0.66	14.35 ± 0.89	20.00 ± 2.60	23.60 ± 2.70
18:1n-9	17.27 ± 0.72	17.42 ± 1.48	10.74 ± 1.45	11.40 ± 1.30
18:1n-7	4.10 ± 0.30	5.11 ± 0.24	4.50 ± 0.32	5.20 ± 0.16
18:2n-6	4.35 ± 0.33	4.46 ± 0.57	3.00 ± 0.18	2.70 ± 0.16
20:0	1.45 ± 0.03	1.50 ± 0.15	1.90 ± 0.14	2.70 ± 0.25
20:1n-9	1.05 ± 0.10	1.60 ± 0.06[b]	0.58 ± 0.10	1.20 ± 0.20
20:4n-6	18.85 ± 1.40	17.58 ± 2.38	31.60 ± 6.00	20.70 ± 5.20
22:0	1.42 ± 0.13	1.20 ± 0.14	1.78 ± 0.18	2.24 ± 0.23[b]
22:1n-9	0.28 ± 0.09	0.40 ± 0.19	Tr	0.35 ± 0.15
22:4n-6	1.88 ± 0.20	2.28 ± 0.17	0.92 ± 0.11	1.12 ± 0.14
22:5n-6	0.80 ± 0.10	3.42 ± 0.24[c]	0.38 ± 0.02	0.86 ± 0.25
22:5n-3	0.23 ± 0.03	0.25 ± 0.05	0.23 ± 0.03	Tr
22:6n-3	4.85 ± 0.68	1.58 ± 0.25[c]	1.20 ± 0.20	0.47 ± 0.03[b]
24:0	1.50 ± 0.10	1.38 ± 0.8	1.26 ± 0.26	1.36 ± 0.20
24:1n-9	2.65 ± 1.27	2.68 ± 0.27	0.70 ± 0.27	0.60 ± 0.14
n-3 + n-6	30.87 ± 2.46	29.43 ± 2.96	37.20 ± 6.30	25.66 ± 5.7
n-3/n-6	0.19 ± 0.02	0.06 ± 0.01[c]	0.037 ± 0.001	0.015 ± 0.002[c]

Data are mean ± SEM values, expressed as a percentage of the identified fatty acids. The number of times the experiment was repeated is given by n. The sum of the linoleic and linolenic series is given as n-3 + n-6. Tr, trace amount.

The p values were determined by Student's *t* test: [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$.

A deficiency in C18:3n-3 fatty acid in the diet caused a dramatic decrease in content of the end product of n-3 fatty acids, C22:6n-3, and this reduction was accompanied by an increase in C22:5n-6 content, thus maintaining a nearly normal level of polyunsaturated fatty acids. That a mechanism exists for maintaining a constant level of highly polyunsaturated pentaenes and hexaenes has been suggested

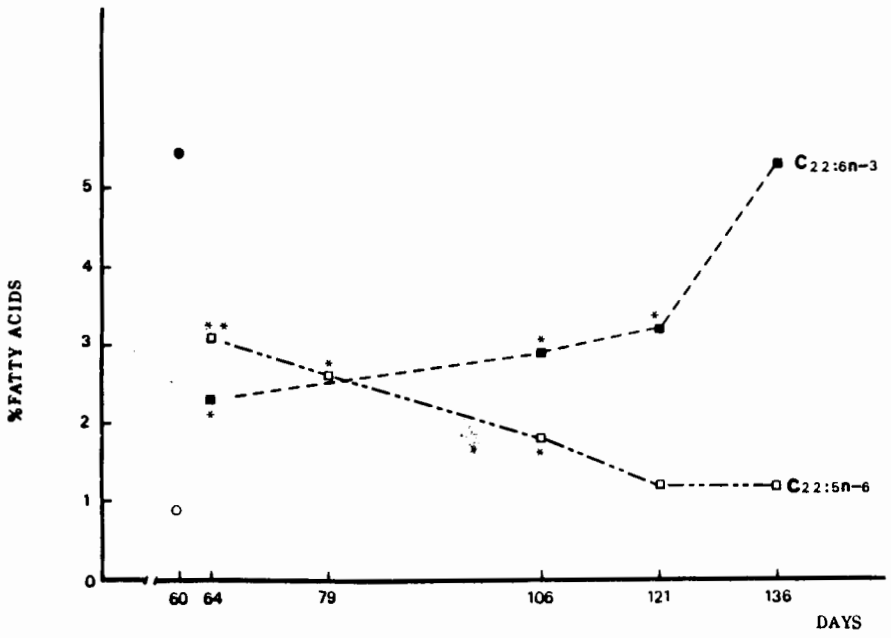

FIG. 1. Recovery in levels of C22:5n-6 (□) and C22:6n-3 (■) in cerebral capillaries. Control values for C22:5n-6 (○) and C22:6n-3 (●) are also given. Each point represents the mean of at least three determinations (15 rats were used for each determination). Values significantly different from control values are indicated: *$p < 0.05$, **$p < 0.01$.

(Trapp and Bernsohn, 1977); a biological requirement may exist for a minimal percentage of fatty acids with double bonds in the 4, 7, 10, 13, and 16 positions (or any combination of these), as these double bonds are common to both C22:5n-6 and C22:6n-3. The recovery in the C22:6n-3 content in cerebral capillaries after replacement of the sunflower oil diet with soya oil was very slow; it took ~2.5 months for the normal value to be reached. Similar results have been obtained for other brain fractions and cells (Youyou et al., 1985). Recovery, as measured by a decrease in C22:5n-6 content, was much faster (2 months). The level of these fatty acids in the choroid plexus reached normal values by 46 days after diet substitution.

It has been shown that alterations in membrane fatty acid composition can modify some cellular enzymatic activities (Solomonson et al., 1976; Engelhard et al., 1976). Moreover, it seems that alterations in essential fatty acids induced by either diet or toxicants (Nixon et al., 1974; Darsi et al., 1976) could have some effect on the permeability of brain capillaries affecting vital transport of the blood–brain barrier. The functional consequences on these regulatory systems remain to be elucidated.

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