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Lipid composition and fatty acid profiles of myelin and synaptosomal membranes of rat brain in response to the consumption of different fats

Pyata Srinivasarao, Ayyalasomayajula Vajreswari, Padmini Suryaprakash, Rupalatha, and Kommireddi Narayanareddy*

From the National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania PO, Hyderabad-500 007, India

Experiments were performed to ascertain the effect of dietary fats like safflower, mustard, peanut, and coconut oil, which differ widely in the essential fatty acid components on the distribution patterns of specific lipids such as cholesterol, phospholipids, and cerebrosides and the fatty acid profile of the latter two lipid fractions obtained from myelin and synaptosomal membranes of three anatomical regions of the rat brain (cerebrum, cerebellum, and brain stem). Weanling male CFY rats received diets adequate in all essential nutrients but varied with respect to the nature of dietary fat. The fat was fed at the 20% level in the diet for 16 weeks.

Myelin and synaptosomal membranes from cerebrum, cerebellum, and brain stem of these rat brains were prepared using discontinuous sucrose density gradient ultracentrifugation. Cholesterol to phospholipid molar ratio remained constant in the synaptosomal membranes in cerebrum and brainstem regions irrespective of the dietary fat treatment, except in the cerebellar region of coconut and mustard oil-fed animals, which showed significantly higher ratio. The cerebroside content of myelin membranes obtained from cerebra and cerebella of these animals were significantly lower compared with those of groundnut and safflower oil-fed rats.

Fatty acid compositions of myelin and cerebroside fractions of these membranes were determined. Mustard oil feeding resulted in the enrichment of synaptosomal phospholipid fraction with docosahexaenoic (22:6 n-3) acid (DHA) in the cerebral and cerebellar regions. On the contrary, myelin phospholipid fractions of these regions of mustard oil-fed group were characterized by a lower degree of unsaturation. Lignoceric (24:0) and nervonic (24:1 n-9) acids, the marker fatty acids of myelin, were significantly lower in the brain regions of coconut and mustard oil-fed animals. Results indicate that the brain can adapt to essential fatty acid deficiency in response to coconut oil feeding by retaining high concentrations of polyunsaturated fatty acid in its membranes. On the other hand, dietary fats rich in linoleic (18:2 n-6) acid (safflower) or linolenic acid (18:3 n-3) (mustard oil) significantly elevated the polyunsaturated fatty acid content. In addition, they also altered the long chain fatty acids (like lignoceric and nervonic acids) of a stable membrane, like myelin. (J. Nutr. Biochem. 8: 527–534, 1997) © *Elsevier Science Inc. 1997*

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Introduction

The brain is an unique organ characterized by the presence of high concentration of complex lipids, which, in turn, determine the structural and functional properties of its cellular and subcellular membranes including myelin.[1–3]

During the early development of brain, there is a rapid accretion of polyunsaturated fatty acids (PUFA) with predominance of those with higher chain length of 20 and 22 carbons, especially arachidonic (20:4 n-6) and cervonic (22:6 n-3) acids in the phospholipids of neuronal membranes such as myelin and synaptosomes.[4,5] These very long-chain PUFAs are synthesized from two essential fatty acids, linoleic (18:2 n-6) and α-linolenic (18:3 n-3) acids of dietary origin.[6,7] These long-chain fatty acids modify the

Address correspondence and reprint requests to P. Srinivasarao, DD, National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania PO, Hyderabad-500 007, India.
*Present address, K. Narayanareddy, Dr. Reddy's Research Foundation, Bollaram Road, Miyabur, Hyderabad-138, India.
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655 Avenue of the Americas, New York, NY 10010
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Table 1 Diet composition

Ingredient	Diet composition (g/100 g diet)
Casein	20.00
DL-methionine	0.30
Cellulose	5.00
Oil	20.00
Mineral mixture	4.00*
Vitamin mixture	0.10†
Choline chloride	0.20
Starch	50.40

Note: Diet was prepared as per AIN standards for nutrients (*J. Nutr.* **107,** 1340–1348, 1977 and *J. Nutr.* **110,** 1726, 1980).
*USP XVII.
†AIN standard.

structure and composition of membranes and thereby determine their fluidity. This, in turn, alters the activities of membrane-bound enzymes, binding of ligands to their receptors, cellular and molecular interactions, and the transport of nutrients in various organs and the brain in particular.[1,2,8,9] Further, very long-chain fatty acids such as arachidonic (20:4 n-6) and cervonic (22:6 n-3) acid influence certain electrophysiological properties and learning behavior.[10,11,12]

Unlike synaptosomal membranes, myelin membranes of brain are characterized by the presence of long-chain saturated and monounsaturated fatty acids, especially lignoceric (24:0) and nervonic (24:1) acids.[13] In fact, the accumulation of these marker fatty acids of cerebrosides determines the process of myelin maturation.[14,15]

The available literature also suggests significant alterations in the distribution of fatty acids and functional properties of membranes of various peripheral tissues and immature or developing brain in response to dietary fat.[9,16,17]

However, there are not many studies wherein the effects of dietary fat on brain membranes of various anatomical regions (differing in their myelin content) of young adult animals (where the influence of maternal fatty acid status is less predominant) have been investigated. Therefore, a systematic study has been undertaken to assess the impact of most commonly consumed dietary fats of India (coconut, groundnut, safflower, and mustard oil) that differ remarkably with respect to their unsaturated fatty acid content and the proportion of two essential fatty acids, on the maintenance of lipid and fatty acid compositions of myelin and synaptosomal membranes from different anatomical regions of the adult rat brain.

Methods and materials

Dietary oils viz, coconut, groundnut (peanut), safflower, and mustard oils were bought locally in sealed containers. Fatty acid methylester standards were purchased from Nu-Chek (Elysian, MN, USA).

Solvents of Analar grade were used. All fine chemicals were purchased from Sigma Chemical Co. (St. Louis, MO USA).

Table 2 Fatty acid composition of oils

Fatty acids	CO	GNO	SO	MO
8:0	4.4	—	—	—
10:0	6.8	—	—	—
12:0	36.7	—	—	—
14:0	26.5	—	0.1	—
16:0	10.5	12.3	2.9	2.2
18:0	3.1	4.2	2.1	1.3
18:1	9.0	38.6	15.6	10.4
18:2	3.0	28.1	79.4	15.3
18:3	—	2.2	—	14.3
20:0	—	1.8	—	—
20:1	—	—	—	3.0
22:0	—	2.8	—	—
22:1	—	—	—	53.4
n-6	3.0	38.1	79.4	15.3
n-3	—	2.2	—	14.3
n-6/n-3	—	17.3	—	1.07

CO, coconut oil; GNO, groundnut oil, SO, safflower oil; MO, mustard oil. Data are expressed as % distribution of fatty acid methyl esters.

Animals and experimental design

Male weanling rats of CFY strain were divided into four groups and were caged individually. Each group consisting of 12 animals received casein-based semisynthetic diet, adequate with respect to all essential nutrients except the source of dietary fat (groundnut, coconut, safflower, or mustard oil), which was fed at the level of 20% w/w in the diet. The composition of the diet and fatty acid composition of the oils used are given in *Tables 1* and *2*, respectively.The rats were fed different oil-based diets for 16 weeks and were killed by decapitation. The major anatomical regions of the brain (i.e., cerebrum, cerebellum, and brain stem) were quickly removed and kept on ice until membrane fractionation was performed. Two cerebra, three cerebella, or three brain stems were pooled before membrane fractionation to represent a single sample for analysis.

Membrane fractionation

Myelin and synaptosomal membranes were prepared by using the procedure of Whittaker and Barker (1972)[18] with minor modifications. Basically, this procedure involved separation of crude mitochondrial fraction from the sucrose homogenate of brain tissue by differential centrifugation followed by separation of this crude fraction into myelin, synaptosomes, and mitochondria by discontinuous (0.32/0.8/1.2 M) sucrose density gradient centrifugation.

Fractions obtained from sucrose gradient were diluted to isotonocity, centrifuged, and resuspended in known volumes of 0.32 M sucrose. These fractions were later purified by repeating the above centrifugation twice followed by characterization by respective marker enzyme assays: ouabain-sensitive Na, $^{+}K^{+}$-ATPase[19] (EC 3.6.1.3) for synaptosomes and cyclic 2′, 3′-nucleotide-3 phosphohydrolase (EC 2.5.1.3) for myelin.[20] Synaptosomal fraction showed 3- to 4-fold enrichment in Na^{+}, K^{+}-ATPase activity, whereas myelin was enriched by 2-fold in 2′, 3′ CNPase activity. Further, cerebroside concentration was nearly doubled in myelin fraction in terms of total myelin membrane lipids. The protein content of the membranes was estimated by the method of Lowry et al.[21]

Analysis of lipids

Total lipids were extracted from each membrane fraction by the method of Folch et al. (1957).[22] Membranes were suspended in 9

Table 3 Body weight and brain weights of rats fed various oils

Group	Body weights	Brain weights	Brain weight/100g body weight
Groundnut oil (12)	508.7 ± 8.45[a]	2.1 ± 0.02[a]	0.42 ± 0.01[a]
Coconut oil (6)	480.7 ± 11.49[ab]	2.2 ± 0.02[ab]	0.45 ± 0.01[b]
Safflower oil (12)	460.5 ± 12.02[b]	2.2 ± 0.02[b]	0.50 ± 0.01[b]
Mustard oil (12)	370.9 ± 10.13[c]	2.0 ± 0.02[c]	0.54 ± 0.01[c]

Values in vertical rows not showing a common superscript are significantly different from each other at 5% level ($P < 0.05$) by ANOVA.
Numbers in the parentheses indicate the number of observations.

vol of water, and the homogeneous suspension was extracted with 40 vol of $CHCl_3$:CH_30H) (2:1 vol/vol) containing 0.02% (wt/vol) butylated hydroxytoluene, washed with aqueous KCl (0.8%), and centrifuged at 1000 g for 5 min. The organic layer was removed quantitatively and dried under a stream of nitrogen.

Cerebrosides and phospholipids were separated by TLC on silica gel H plates using solvent system comprising chloroform: methanol (100:20) and were visualized by brief exposure to iodine vapours. Phospholipids remained at the origin with the above solvent system. Spots corresponding to authentic cerebrosides standard ($Rf = 0.5$) were scraped into screw cap tubes and suspended in chloroform:methanol (2:1 vol/vol). Cerebrosides were eluted with 15 mL of chloroform: methanol (2:1 v/v) followed by 5 mL diethyl ether, whereas phospholipids were eluted with solvent system, comprising chloroform, methanol, ammonia, and water (65:35:5:5). After elution, these fractions were analyzed for galactose and phosphorus contents, respectively.[23–25] The fatty acids of phospholipid fraction were transmethylated using methanolic NaOH,[26] whereas those of the cerebrosides were transmethylated using methanolic HCl.[27] Methylesters of fatty acids were analyzed by gas chromatography (Varian Model 3700) equipped with a flame-ionization detector and an electronic integrator (Model 4270 Varian). Stainless steel column (12' × 1/8") packed with 10% silar 10C coated on chromosorb W 80–100 mesh (Supelco Inc. Bellefonte PA) was used with nitrogen (20 mL/min) as carrier gas. The column, injector, and detector temperatures were 180°C, 200°C, and 220°C, respectively.

Fatty acids were identified by comparison with authentic standard mixture (GLC-68B) obtained from Nu-Chek preparation (Elysian MN USA). All data were analyzed statistically by analysis of variance (ANOVA) and the level of significance was expressed at 5%.

Results

Morphometric measurements

Body weights of the animals fed various dietary fats showed the following trend GNO > CO > SO > MO (*Table 3*), whereas the brain weights of the animals showed altogether a different trend SO > CO > GNO > MO. However, when the brain weights were expressed as a ratio of body weight, mustard oil-fed animals had the highest value followed by those of safflower, coconut, and groundnut oil groups.

Alterations in lipid to protein ratio and lipid classes of synaptosomal and myelin membranes

The relative distribution of cholesterol to phospholipid and total lipids to proteins in synaptosomal membrane are depicted in *Table 4*. A higher ratio of lipid to protein was observed for myelin compared with synaptosomal membranes amongst different regions. In general, brain stem exhibited relatively higher value in this regard. Whereas safflower oil-fed animals had higher ratios of lipid to protein in synaptosomal membranes of cerebral region; myelin membranes of this region had higher value in response to mustard oil feeding. There were no significant

Table 4 Effect of dietary fat on synaptosomal membrane lipid composition of various regions of the brain

Group	Brain regions	mgs Lipid/mg protein (mgs/mg)	μmol Cholesterol/ mg/lipid (C)	μmol Pi/mg lipid (P)	C/P (μmol cholesterol/μmol Pi)
Groundnut oil	CB	0.9 ± 0.31[a]	0.3 ± 0.02[a]	0.2 ± 0.02[a]	1.1 ± 0.10[a]
	CL	1.5 ± 0.46[a]	0.1 ± 0.01[a]	0.20 ± 0.04[a]	0.4 ± 0.01[a]
	BS	2.5 ± 0.65[a]	0.1 ± 0.01[a]	0.1 ± 0.02[a]	0.6 ± 0.08[a]
Coconut oil	CB	1.5 ± 0.31[a]	0.3 ± 0.03[a]	0.3 ± 0.03[a]	1.3 ± 0.07[a]
	CL	1.5 ± 0.15[b]	0.2 ± 0.01[b]	0.1 ± 0.02[a]	1.2 ± 0.19[bc]
	BS	1.4 ± 0.34[a]	0.1 ± 0.02[a]	0.2 ± 0.04[ab]	0.5 ± 0.02[a]
Safflower oil	CB	2.3 ± 0.21[b]	0.3 ± 0.04[a]	0.3 ± 0.03[a]	1.3 ± 0.20[c]
	CL	1.6 ± 0.28[a]	0.3 ± 0.02[c]	0.2 ± 0.02[a]	1.3 ± 0.09[c]
	BS	3.1 ± 0.66[a]	0.1 ± 0.02[a]	0.2 ± 0.04[ab]	0.7 ± 0.08[a]
Mustard oil	CB	1.5 ± 0.08[a]	0.5 ± 0.03[b]	0.2 ± 0.03[a]	2.3 ± 0.23[b]
	CL	1.6 ± 0.40[a]	0.1 ± 0.02[b]	0.2 ± 0.04[a]	0.9 ± 0.06[b]
	BS	3.3 ± 1.71[a]	0.1 ± 0.03[b]	0.3 ± 0.02[b]	0.4 ± 0.23[a]

Values are mean ± SEM. Number of observations n > 3 pooled samples. Two cerebra, three cerebella, or three brain stems were pooled to represent one sample in each case.
Comparisons are made among the various regions of four groups and values in the vertical rows not showing a common superscript are significantly different from each other at 5% level ($P < 0.05$) by ANOVA.

Table 5 Myelin lipid composition of various brain regions of rats fed different edible oils

Group	Brain regions	μmol Galactose/ mg lipid	μmol PL/ mg lipid	μmol gal/μmol PL	Lipid/protein (mgs/mg)
Groundnut oil	CB	0.2 ± 0.02[a]	0.3 ± 0.02[ac]	0.7 ± 0.08[a]	1.2 ± 0.12[a]
	CL	0.1 ± 0.01[a]	0.1 ± 0.02[a]	0.9 ± 0.01[a]	1.7 ± 0.24[a]
	BS	0.3 ± 0.06[a]	0.3 ± 0.07[a]	0.8 ± 0.05[a]	2.7 ± 0.45[a]
Coconut oil	CB	0.3 ± 0.01[ab]	0.8 ± 0.07[b]	0.3 ± 0.04[b]	2.1 ± 0.28[ab]
	CL	0.3 ± 0.04[b]	0.7 ± 0.10[b]	0.4 ± 0.03[b]	1.9 ± 0.63[a]
	BS	0.2 ± 0.01[a]	0.3 ± 0.05[a]	0.9 ± 0.07[a]	3.1 ± 0.11[a]
Safflower oil	CB	0.5 ± 0.05[c]	0.2 ± 0.02[ac]	1.9 ± 0.15[c]	1.9 ± 0.27[ab]
	CL	0.4 ± 0.08[b]	0.4 ± 0.08[b]	0.9 ± 0.14[a]	2.5 ± 0.36[a]
	BS	0.3 ± 0.03[a]	0.3 ± 0.03[a]	1.0 ± 0.12[a]	2.2 ± 0.43[a]
Mustard oil	CB	0.2 ± 0.04[a]	0.4 ± 0.09[a]	0.4 ± 0.04[b]	2.7 ± 0.45[b]
	CL	0.3 ± 0.04[b]	0.5 ± 0.07[b]	0.6 ± 0.02[b]	2.2 ± 0.57[a]
	BS	0.3 ± 0.07[a]	0.5 ± 0.08[b]	0.5 ± 0.10[b]	2.5 ± 0.45[a]

Values are mean ± SEM. Number of observations $n > 3$ pooled samples.
Comparisons are made among the various regions of four groups and values in the vertical rows not showing a common superscript are significantly different from each other at 5% level ($P < 0.05$) by ANOVA.

changes in phospholipid content of synaptosomal membranes among the regions of various groups except in brain stem, (MO > CO > SO > GNO). The relative increase in cholesterol resulted in elevated molar ratio of cholesterol to phospholipids in the cerebral and cerebellar regions of mustard oil group compared with other groups.

Altogether different trends were observed with respect to lipids in the myelin from various brain regions due to the feeding of these oils (*Table 5*). Cerebroside content (expressed as gal/mg lipid or gal/mg PL) was highest in the safflower oil-fed group followed by groundnut, mustard, and coconut oils in the important regions of the brain, namely cerebrum and cerebellum. In the brain stem, the concentration of myelin cerebrosides was higher in the safflower and coconut oil-fed animals compared with groundnut and mustard oil groups. Phospholipid content was highest in the coconut oil-fed rat cerebra.

Fatty acid composition of phospholipids of synaptosomal membranes

Unlike phospholipids of myelin membranes, synaptosomal membranes were enriched in PUFAs of longer chain length and of n-6 and n-3 series. Higher amounts of linolenic (18:3) acid in the mustard oil were reflected in increased levels of docosahexaenoic acid (22:6, n-3) in synaptosomal membranes of mustard oil-fed group (*Table 6*). Higher concentration of linoleic (18:2) acid in safflower and groundnut oil resulted in higher ratio of long-chain n-6/n-3 fatty acids in synaptosomal membranes of safflower and groundnut oil-fed groups compared with those reared on coconut or mustard oil (*Table 6*). Despite low levels of linoleic and linolenic acids in the diet, the synaptosomal membranes of rats fed coconut oil, however, had higher ratios of arachidonic to linoleic (20:4/18:2) and docosa-

Table 6 Effect of dietary fat on synaptosomal phospholipid fatty acid composition of various brain regions

Group	Brain regions	20:4	20:4/18:2	22:4 (n6)	22:5 (n6)	22:6 (n3)	22:6/18:3	Total n6	Ratio n6/n3
Groundnut oil	CB	10.4 ± 0.59[a]	5.0 ± 0.83[a]	3.4 ± 0.36[a]	5.3 ± 0.82[a]	2.4 ± 0.45[a]	2.9 ± 1.09[a]	19.1 ± 1.61[a]	9.4 ± 1.54[a]
	CL	7.7 ± 0.75[a]	2.8 ± 0.71[a]	1.9 ± 0.05[a]	3.3 ± 0.35[a]	2.2 ± 0.10[a]	2.8 ± 0.12[a]	12.8 ± 0.35[a]	5.8 ± 0.43[a]
	BS	8.8 ± 1.79[ab]	2.9 ± 1.65[a]	3.5 ± 0.38[a]	5.4 ± 0.75[a]	4.1 ± 0.42[a]	4.0 ± 2.53[a]	17.7 ± 2.26[a]	4.4 ± 0.79[a]
Coconut oil	CB	14.3 ± 1.10[b]	36.8 ± 14.9[b]	1.5 ± 0.30[b]	3.1 ± 1.20[ab]	4.0 ± 1.39[a]	12.5 ± 5.81[b]	17.4 ± 2.12[ab]	5.6 ± 1.99[b]
	CL	9.9 ± 1.01[a]	40.4 ± 2.94[b]	2.2 ± 0.33[a]	5.2 ± 0.39[b]	9.4 ± 0.88[b]	17.2 ± 2.43[b]	17.2 ± 1.15[a]	1.9 ± 0.28[b]
	BS	10.2 ± 0.80[a]	6.9 ± 2.37[a]	2.4 ± 0.65[ab]	4.2 ± 0.53[ab]	7.9 ± 0.96[b]	3.3 ± 0.85[a]	16.8 ± 1.31[ab]	2.2 ± 0.25[bc]
Safflower oil	CB	10.1 ± 0.68[a]	5.4 ± 1.03[a]	3.5 ± 0.30[a]	5.6 ± 0.86[ac]	2.2 ± 0.50[a]	3.0 ± 1.52[a]	19.2 ± 1.74[a]	9.2 ± 1.06[ab]
	CL	7.2 ± 0.59[a]	2.2 ± 0.18[a]	2.9 ± 0.51[a]	6.8 ± 0.73[b]	3.1 ± 0.32[a]	1.1 ± 0.12[a]	14.6 ± 2.31[a]	4.9 ± 0.76[a]
	BS	8.5 ± 0.98[ab]	4.7 ± 0.66[a]	2.4 ± 0.33[ab]	3.1 ± 0.34[b]	4.1 ± 0.65[a]	1.4 ± 0.31[a]	13.2 ± 1.0[b]	3.5 ± 0.51[ab]
Mustard oil	CB	10.1 ± 0.40[a]	15.2 ± 1.94[a]	2.9 ± 0.24[a]	1.0 ± 0.62[b]	12.9 ± 1.02[b]	15.0 ± 2.18[b]	13.5 ± 0.74[b]	1.1 ± 0.09[c]
	CL	9.1 ± 0.60[a]	7.8 ± 0.64[c]	4.5 ± 2.48[a]	3.1 ± 0.60[a]	13.8 ± 2.06[c]	11.3 ± 2.89[b]	14.9 ± 4.09[a]	1.1 ± 0.39[b]
	BS	6.2 ± 0.43[b]	3.1 ± 0.61[a]	1.8 ± 0.28[b]	ND	10.2 ± 1.03[b]	3.3 ± 1.07[a]	8.0 ± 0.46[c]	0.8 ± 0.10[bc]

Values are mean ± SEM. Number of observations $n \geqslant 3$ pooled samples. ND = not detectable.
Comparisons are made among the various regions of four groups and values in the vertical rows not showing a common superscript are significantly different from each other at 5% level ($P < 0.05$) by ANOVA.
Total n-6 and n-6/n-3 include only fatty acids of chain length ⩾ 20 carbons.

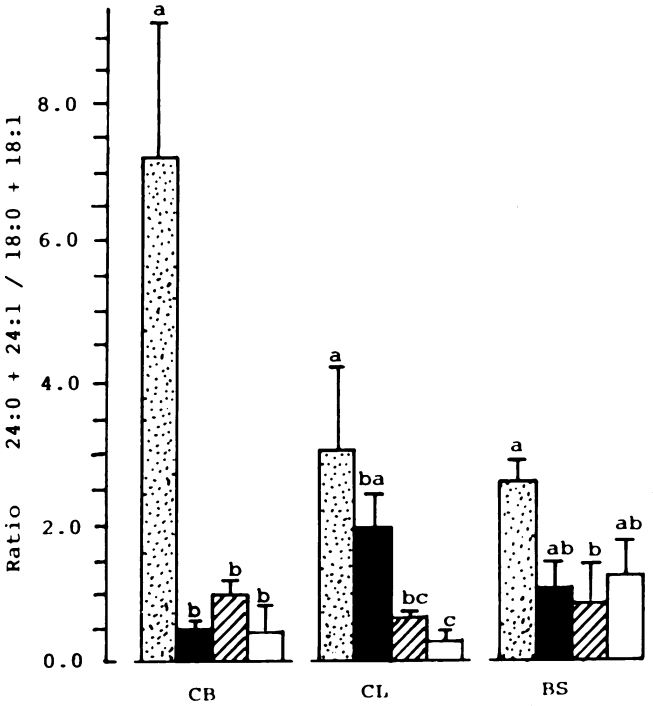

Figure 1 Ratio of lignoceric and nervonic acid to stearic and oleic acids in myelin membranes of brain regions of groups fed groundnut oil ▨, coconut oil ■, safflower oil ▨, mustard oil □. CB, cerebrum; CL, cerebellum; BS, brain stem. Results are the means ± SEM of three or more observations from pooled samples (2 cerebra or 3 cerebella or 3 brainstems were pooled to represent one sample in each case). Statistical evaluation of the data was done by ANOVA. Means in a panel with different superscripts are significantly different ($P < 0.05$). Comparisons are made among the groups for the same region.

hexaenoic (DHA) to linolenic (22:6/18:3) acids in the various regions (*Table 6*). Irrespective of the dietary fat fed, cerebra in general, showed higher accumulation of arachidonic acid compared with two other regions, namely cerebellum and brain stem.

Distribution of cerebroside fatty acids of myelin membranes

The fatty acid analysis of myelin cerebrosides of various regions displayed certain interesting trends with respect to long-chain and medium-chain fatty acids such as myelin-specific lignoceric (24:0) and nervonic (24:1 n-9) acids and their precursor fatty acids, namely stearic (18:0) and oleic (18:1 n-9) acids (*Figure 1*). A distinctly lower concentration of these long-chain fatty acids (24:0 + 24:1) was evident in the cerebra and cerebella of mustard oil-fed group. Somewhat lower levels of these two fatty acids were also seen in the cerebra of coconut oil-fed rats. The relative decrease in these long-chain fatty acid species was associated with concomitant increase in the medium-chain fatty acid species, especially 18:0 + 18:1 in specific regions of the brain. Groundnut oil, a dietary fat having higher proportion of oleic acid resulted in higher levels of long-chain fatty acids and with concomitant decrease in levels of oleic acid in myelin cerebrosides. In contrast, coconut oil, safflower, or mustard oil with lower levels of oleic acid showed increased accretion of oleic acid in cerebrosides (individual data not shown but indirectly indicated in *Figure 1*).

Fatty acid profiles of phospholipids in myelin membranes

Phospholipids of myelin membranes were rich in saturated fatty acids and monoenoic fatty acids (oleic 18:1 n-9 acid) compared with PUFAs of 20 and 22 carbon chain length (*Table 7*). Oleic acid was the predominant fatty acid of this brain membrane irrespective of the source of dietary fat. A distinct trend with respect to the accretion of PUFAs in specific brain regions, and the type of dietary fat consumed was observed (*Table 8*). The concentration of total n-6 PUFA, as well as the ratio of long-chain n6/n3, increased because of feeding of safflower oil rich in linoleic acid. This varied in different regions, the order being cerebrum > cerebellum > brain stem. However, exactly the opposite trend was observed in response to feeding mustard oil (brain stem > cerebellum > cerebrum). Such regional variation in

Table 7 Distribution pattern of fatty acid species in total lipids of synaptosomal membranes of brain regions of rats fed different oil diets (expressed as percentage of total fatty acid methyl esters)

Group	Brain regions	Saturates	Monoenes	Dienes	Trienes	Polyenes*
Groundnut oil	CB	53.4 ± 1.96^{a}	21.7 ± 0.85^{ab}	12.1 ± 0.5^{a}	1.3 ± 0.33^{a}	23.8 ± 3.14^{a}
	CL	59.6 ± 2.30^{a}	21.9 ± 2.4^{a}	12.9 ± 0.45^{a}	0.8 ± 0.01^{a}	15.0 ± 0.25^{a}
	BS	47.5 ± 2.71^{a}	23.8 ± 1.53^{a}	4.7 ± 1.86^{a}	2.1 ± 0.82^{a}	26.9 ± 3.82^{a}
Coconut oil	CB	50.6 ± 3.18^{a}	23.8 ± 2.27^{a}	10.4 ± 0.18^{b}	0.4 ± 0.15^{bc}	21.4 ± 3.38^{a}
	CL	47.8 ± 1.46^{b}	24.80 ± 0.87^{a}	10.2 ± 0.04^{b}	0.6 ± 0.07^{a}	24.3 ± 2.67^{b}
	BS	41.9 ± 1.39^{a}	29.1 ± 2.34^{a}	1.3 ± 0.55^{ab}	2.8 ± 0.52^{a}	25.9 ± 1.26^{a}
Safflower oil	CB	53.8 ± 2.21^{a}	21.5 ± 0.76^{ab}	12.1 ± 0.5^{a}	1.2 ± 0.38^{ac}	21.4 ± 2.19^{a}
	CL	56.2 ± 2.52^{a}	22.8 ± 0.57^{a}	2.5 ± 0.14^{a}	1.1 ± 0.21^{a}	17.6 ± 2.44^{ab}
	BS	50.2 ± 1.68^{ab}	28.2 ± 1.03^{a}	1.9 ± 0.15^{ab}	3.2 ± 0.58^{a}	6.80 ± 1.39^{b}
Mustard oil	CB	54.1 ± 1.59^{a}	21.0 ± 0.48^{b}	10.7 ± 0.09^{ab}	1.0 ± 0.26^{ac}	26.5 ± 1.40^{a}
	CL	47.8 ± 3.03^{b}	21.1 ± 0.85^{a}	11.2 ± 0.03^{b}	1.2 ± 0.11^{a}	27.4 ± 3.01^{b}
	BS	47.1 ± 1.36^{a}	29.7 ± 1.67^{a}	2.2 ± 0.61^{a}	3.5 ± 1.13^{a}	17.5 ± 1.05^{b}

All are mean ± SEM. Number of observations ⩾ 3 pooled samples * includes fatty acids 20:4 n6; 22:4 n6; 22:5 n3 and 22:6 n3 species.
Comparisons are made among the various regions of four groups and values in the vertical rows not showing a common superscript are significantly different from each other at 5% level ($P < 0.05$) by Analysis of variance.

Table 8 Effect of dietary fat on fatty acids of myelin phospholipid of various brain regions

Group	Brain regions	18:2	18:3	20:4	22:6	20:4/18:2	22:6/18:3	Total long chain n-6	Total long chain n-3	Long chain n-6/n-3
Groundnut oil	CB	2.48 ± 0.68	3.06 ± 0.60	2.16 ± 0.34^{a}	1.13 ± 0.28^{a}	1.09 ± 0.27	0.39 ± 0.10^{a}	4.64 ± 0.79^{a}	4.18 ± 0.79	1.14 ± 0.14^{a}
	CL	4.1 ± 0.40^{a}	2.35 ± 0.78^{a}	2.85 ± 0.95	1.22 ± 0.11	0.66 ± 0.17	0.73 ± 0.34	6.95 ± 1.36^{a}	3.56 ± 0.67	2.26 ± 0.85^{a}
	BS	2.4 ± 0.92	2.2 ± 0.98^{a}	0.95 ± 0.03^{a}	2.35 ± 0.26	0.69 ± 0.25	3.04 ± 1.47	3.35 ± 0.95	4.55 ± 0.72	0.85 ± 0.36^{a}
Coconut oil	CB	1.16 ± 0.18	2.09 ± 0.46	1.86 ± 0.23^{b}	2.9 ± 1.35^{b}	1.83 ± 0.48	1.26 ± 0.35^{ab}	3.015 ± 0.05^{b}	2.05 ± 0.45	1.42 ± 0.45^{b}
	CL	0.97 ± 0.41^{b}	4.61 ± 0.75^{b}	1.36 ± 0.43	2.3 ± 0.74	1.22 ± 0.49	0.49 ± 0.24	3.77 ± 0.61^{b}	3.91 ± 0.75	0.95 ± 0.22^{b}
	BS	1.48 ± 0.22	5.99 ± 0.93^{b}	1.96 ± 0.36^{b}	2.5 ± 0.77	1.79 ± 0.69	0.42 ± 0.23	3.44 ± 0.25	5.99 ± 0.93	0.63 ± 0.09^{ab}
Safflower oil	CB	1.7 ± 0.47	1.85 ± 0.25	3.0 ± 0.14^{a}	3.3 ± 0.31^{b}	1.76 ± 0.32	2.06 ± 0.34^{bc}	9.5 ± 0.56^{ab}	1.85 ± 0.25	5.3 ± 0.74^{b}
	CL	0.97 ± 0.41^{b}	4.61 ± 0.75^{b}	1.36 ± 0.43	2.3 ± 0.74	1.22 ± 0.49	0.49 ± 0.24	7.23 ± 0.99^{b}	4.61 ± 0.75	1.46 ± 0.08^{b}
	BS	0.71 ± 0.14	5.77 ± 0.82^{bc}	0.63 ± 0.31^{a}	2.1 ± 1.51	1.03 ± 0.62	0.48 ± 0.32	6.6 ± 1.72	6.35 ± 0.68	1.13 ± 0.21^{b}
Mustard oil	CB	ND	2.4 ± 0.83	0.81 ± 0.17^{c}	0.8 ± 0.14	—	0.33 ± 0.25	1.49 ± 0.27^{c}	3.20 ± 0.83	0.72 ± 0.12^{c}
	CL	ND	5.89 ± 0.39^{b}	0.81 ± 0.25	1.6 ± 0.32	—	2.29 ± 0.13	2.97 ± 0.44^{b}	7.31 ± 0.39	0.51 ± 0.09^{b}
	BS	ND	5.03 ± 1.39^{ac}	0.76 ± 0.13^{a}	1.2 ± 0.9	—	0.24 ± 0.11	4.39 ± 1.06	6.02 ± 1.39	0.87 ± 0.13^{b}

Values are mean ± SEM. ND, not detectable
Number of observations n ⩾ 3 pooled samples.
Comparisons are made among the various regions of four groups and values in the vertical rows not showing a common superscript are significantly different from each other at 5% level ($P < 0.05$) by ANOVA.
Total n-6 or n-3 refers to PUFA of chain length ⩾ 20 carbons.

the distribution of PUFA of myelin membranes was not seen in the other two groups. Surprisingly coconut oil deficient in linoleic acid did not have any negative effect on the accretion of PUFA of myelin membranes and the values were almost comparable to those of groundnut or safflower oil-fed groups.

Discussion

These studies were primarily aimed at determining the magnitude of changes in long chain fatty acids (characteristic of mature myelin), polyunsaturated fatty acids, and molar ratios of cholesterol to phospholipids in synaptosomal membranes, in response to feeding of different dietary fats. Dietary fats used in these experiments considerably differ in essential fatty acid components. We have specifically chosen myelin and synaptosomal membranes for our study as these are unique to nervous tissue and any alteration in the microenvironment of these membranes could prove detrimental to the structure and function of the brain.

In recent years the obligatory role of n-6 and n-3 fatty acids in the early neuronal development has been established unequivocally.[28] Although the crucial role played by linoleate in the maintenance of normal growth and reproduction has been known very early, only very recently the essentiality of α-linolenate (by virtue of being a precursor for docosahexaenoic (22:6 n-3) acid (DHA) in the neuronal and retinal development has been demonstrated.

Most of the available literature is confined to the effect of EFA deficiency on developing brain and to the analysis of phospholipids of synaptosomes and myelin membranes.[29] However, in the present study the quantitative and qualitative changes in the cerebroside fraction (a marker lipid for myelin membrane) of various anatomical regions of the brain in response to altered dietary fat treatment were also investigated.

The results on body weights and brain weights clearly show that the feeding of high erucic acid containing mustard oil resulted in higher brain to body weight ratio. Decrease in body weight of these animals might be attributable to reduced food intake. However, the decrease in body weight was higher than the decrease in brain weight, thereby resulting in higher brain to body weight ratio. This observation is corroborative of the fact that the brain is spared, to a great extent, from the onslaught of marginal under-nutrition.

From the data on cholesterol and phospholipids of the synaptosomal membranes, it seems that cholesterol and phospholipid concentrations were higher in coconut, safflower, and mustard oil-fed rat brains as compared with those of groundnut oil groups. However, the ratio of cholesterol to phospholipid remained almost constant in cerebrum and brain stem of different groups. Similar observation has been made with respect to the cardiac membranes of these animals.[30] Cholesterol to phospholipid ratio and lipid to protein ratio are important determinants of membrane fluidity and their function.[31]

Two important characteristics of myelin membranes namely the quantity of cerebrosides expressed as μmol gal/mg lipid and its maturity as assessed by the ratio of 24:0 + 24:1 to 18:0 + 18:1 indicate the existence of regional differences in this regard and also support the view

that the type of dietary fat has a greater effect on the myelin marker fatty acid namely nervonic (24:1) acid content.[32] Both stearic and oleic acids are considered to be precursors for the long chain, and long chain mono-unsaturated fatty acids.[33] Thus, groundnut oil rich in oleic acid might have facilitated the accumulation of 24:1 more easily. In fact, oleic acid itself is considered to be essential for myelination.[34] Another interesting observation of this study was, feeding of other oils with relatively lower concentrations of oleic acid resulted in the accumulation of this acid which was reflected in decreased ratios of 24:0 + 24:1 to 18:0 + 18:1 and long chain fatty acid to medium chain fatty acids.

The remarkable ability of the brain to retain higher concentration of PUFA in its membranes (such as synaptosomal and myelin membrane phospholipids) was evident from the fact that feeding of coconut oil (deficient in both linoleic and linolenic acids) did not result in depletion of very long chain PUFA such as arachidonic and docosahexaenoic acids. These findings are similar to those of Bourre et al. 1990[35] who have observed that diets either deficient in α-linolenic acid or having very low amounts of α-linolenic acid (0.06 g/kg) diet) and linoleic acid effectively maintained the concentration of 22:6 n-3, 20:4 n-6 respectively in the brain.[35,36] However, feeding of oil rich in α-linolenic acid (mustard oil) caused a significant increase in docosahexaenoic (22:6 n-3) acid content of brain synaptosomal but not myelin membranes of various regions. In conclusion, feeding of coconut oil, a dietary fat deficient in essential fatty acids induced an adaptive retention of PUFA and on the other hand the consumption of safflower oil diet rich in linoleic (18:2 n-6) acid or mustard oil diet rich in linolenic (18:3 n-3) did influence the fatty acid profiles of synaptosomal membranes. Further, it is interesting to note a relatively stable membrane like myelin exhibited significant alterations in the concentration of the characteristic long chain fatty acids (lignoceric and nervonic acids) in response to consumption of groundnut oil rich in oleic acid. It is possible that these subtle changes in brain membrane PUFA content and that of long-chain fatty acids might have various functional implications such as, signal transduction and the enzymatic activities or metabolism of brain neurotransmitters.

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