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Effect of dietary oils on brain enzymatic activities (2′-3′-cyclic nucleotide 3′-phosphodiesterase and acetylcholinesterase) and muscarinic receptor sites in growing rats

Serafina Salvati, Lucilla Attorri, Maurizio Di Felice,* Lorenzo Malvezzi Campeggi, Annita Pintor,† Florindo Tiburzi,† and Gianni Tomassi‡

*Department of Metabolism and Pathological Biochemistry, Istituto Superiore di Sanità, Roma, Italy; *Istituto Nazionale della Nutrizione, Roma, Italy; †Department of Pharmacology, Istituto Superiore di Sanità, Roma, Italy; and ‡Università della Tuscia, Viterbo, Italy*

Dietary fats affect the fatty acid composition of brain membranes, but the consequences of the fatty acid changes on brain functional activities are poorly understood and not yet established. In order to investigate the effects of diet-induced changes on myelin deposition and on the cholinergic system, the present experiment was designed. Three groups of 10 male Sprague–Dawley rats (initial weight 100 ± 5 g) were fed for 6 weeks diets containing 15% (wt/wt) fish oil (FO), soybean oil (SO), and coconut oil (CO) rich in n-3 fatty acids (38%), n-6 fatty acids (55%), and saturated fatty acids (84%), respectively. The 2′-3′-cyclic nucleotide 3′-phosphodiesterase activity (CNPase) in the whole brain and in the cerebral cortex and the acetylcholinesterase activity (AChE) were determined along with the density and affinity of muscarinic receptor sites. The CNPase activity was significantly higher in the SO-fed group than in the other two groups (62.5 versus 47.0 and 54.4 μmol/hr/mg of protein), and the activity was correlated positively with Σ n-6 and negatively with Σ n-3. The AChE activity, the density, and the affinity of the receptor muscarinic receptor sites were not statistically different among the three groups. The results indicate the favorable effect of soybean oil and the adverse effect of fish oil on myelin deposition and the absence of effect of dietary oils on the cholinergic system. (J. Nutr. Biochem. 7:113–117, 1996.)

Keywords: dietary polyunsaturated fatty acids; CNPase activity; AChE activity; muscarinic receptors; rat brain

Introduction

It is well known that dietary oils influence the fatty acid composition of brain membranes.[1–4] The magnitude of the dietary effects depends on the chemical nature of the fatty acid source and on the experimental condition, with particular reference to the level of the ingested lipid.

The experiment by Chaudiere et al.[5] in rats showed that when dietary n-3 fatty acids are fed at low levels (corresponding to 10 g for human adults) brain membrane composition remains unaltered without changes in brain antioxidant activity (vitamin E concentration and glutathione peroxidase and catalase activities). However, experiments of the same group on rats fed high levels of dietary fish oil (FO) demonstrated that the brain fatty acid composition is strongly affected by the diet, indicating possible adverse effects of the therapeutic use of FO.[6] High levels of long-chain n-3 fatty acids have raised the question of possible unwanted physiological effects, such as delayed blood clotting and lipid peroxidation.

Recently, our study[7] showed that diets rich (15% wt/wt) in either long-chain n-3 fatty acids (FO), n-6 fatty acids (soybean oil [SO]), or saturated fatty acids (coconut oil [CO]) affect the fatty acid composition of myelin and syn-

Address reprint requests to Dr. S. Salvati at Neurochemistry Section, Department of Metabolism and Pathological Biochemistry, Istituto Superiore di Sanità, V. le Regina Elena, 299, 00161 Roma, Italy.
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655 Avenue of the Americas, New York, NY 10010

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Table 1 Fatty acid composition of oils (%)

Fatty acid	Fish oil	Soybean oil	Coconut oil
10:0	—	—	4.0
12:0	0.1	—	43.7
14:0	6.3	0.2	19.4
16:0	17.0	10.9	11.3
16:1 (n-7)	9.8	0.2	—
18:0	3.8	3.7	4.4
18:1 (n-9)	14.3	21.2	12.3
18:2 (n-6)	1.6	54.1	3.3
18:3 (n-3)	0.3	7.2	—
18:4 (n-3)	3.9	—	—
20:1 (n-9)	2.3	0.4	—
20:4 (n-6)	1.1	0.5	—
20:5 (n-3)	18.5	—	—
22:5 (n-3)	2.3	—	—
22:6 (n-3)	11.7	—	—
24:1 (n-9)	1.0	—	—
ΣSFA	27.2	15.0	78.4
ΣMUFA	27.4	25.3	12.3
ΣPUFA	39.4	61.8	3.3
Σn-3	36.7	7.2	—
Σn-6	2.7	54.6	3.3
Σn-3/n-6	16.3	0.1	—
UI	226	154	19

ΣSFA = saturated fatty acid; ΣMUFA = monounsaturated fatty acid; ΣPUFA = polyunsaturated fatty acid; UI = unsaturation index.

aptosomes of growing rats. Moreover, the data indicate that the membranes rich in n-3 fatty acids are more susceptible to oxidative stress.

Changes in the fatty acid composition can affect a variety of membrane-mediated cellular functions such as Na^+,K^+ ATPase,[8,9] acetylcholinesterase activity (AChE, E.C.3.1.1.7.),[10,11] and receptor binding.[12] Moreover, Clanidin et al.[13] have shown an increase of ethanolamine glycerophospholipids (EPL) methyltransferase activity in membranes rich in polyunsaturated fatty acids (PUFAs) resulting in an increase of choline glycerophospholipids (CPL). The higher level of CPL may lead to an increase in the "reservoir" of membrane choline.[14] Such an increase in the membrane choline supply could be reflected in an increase of biosynthesis and release of acetylcholine (ACh) from physiologically active neurons[15] and secondarily in an enhancement of the AChE activity.

In order to investigate the effects of dietary-induced changes in fatty acid membrane composition on myelination and cholinergic function, we measured the 2′-3′-cyclic nucleotide 3′-phosphodiesterase activity (CNPase; E.C.3.1.4.37) in the whole brain, and in the cerebral cortex we studied the AChE activity, density, and affinity of muscarinic receptor sites (mAChRs).

Methods and materials

Animals and diets

Three groups of 10 male albino Sprague–Dawley rats, pathogen-free SPF/COBS (Charles River, Calco, Italy), weighing 100 ± 5 g were individually housed in wire-bottom stainless-steel cages under constant environmental conditions (room temperature 22 ± 1°C, 12 hr light–dark cycle) in a pathogen-free environment.

All groups received the same basal synthetic diet containing casein 20%, dl methionine 0.3%, rice starch 40%, sucrose 17%, oil 15%, fiber 3%, salt mixture (AIN-76) 3.5%, vitamin mixture (AIN-76) 1%, and choline chloride 0.2% for 6 weeks. Group 1 (FO) received fish oil (MAXEPA, the kind gift of Seven Seas Limited, Hull, UK); group 2 (SO), soybean oil; and group 3 (CO), coconut oil. Food intakes in grams per day were 14 ± 1 for FO and 17 ± 2 for the SO and CO groups.

The diets were prepared weekly and stored at 4°C under nitrogen. Vitamin E and selenium contents were measured and equalized, respectively, at 57 IU/kg and 665 μg/kg.

At the end of the experimental period, the animals were sacrificed by decapitation, and the exsanguinated brains were removed and weighed. The brains from five animals were homogenized in a 0.32 M sucrose solution at a 1:10 ratio, and the CNPase activity and protein content were determined. The brains from the other five rats were dissected on ice, and the cerebral cortex was removed. For the determination of the AChE activity, the protein content, and mAChRs, the tissue was homogenized in a 0.32 M sucrose solution at a ratio of 1:10, frozen, and stored at −80°C for up to 4 weeks before analysis.

Diet analysis

Samples of experimental dietary oils were analyzed for fatty acid composition by gas liquid chromatography (GLC) (*Table 1*). The unsaturation index was calculated by multiplying the number of double bonds by the percentage composition of individual fatty acids and summing the values. The tocopherol content was determined by high pressure liquid chromatography (HPLC) according to McMurray et al.[16] and the selenium content by instrumental neutron activation analysis.[17]

Enzymatic activities

The CNPase activity was determined in the whole brain homogenate treated with an equal volume of 1% aqueous Triton X-100 as previously described,[18] and the AChE activity was measured in the cerebral cortex homogenate according to Ellman et al.[19]

Table 2 Brain weight and the protein content of the brain and cerebral cortex of rats fed the experimental diets

Diet	Body weight (g)	Brain weight (g)	Protein (mg/g of brain)	Protein (mg/g of cerebral cortex)
Fish oil	343 ± 14	2.06 ± 0.12	97.3 ± 6.4	105.8 ± 5.5^b
Soybean oil	399 ± 43	2.02 ± 0.13	107.2 ± 12.0^c	125.4 ± 11.4^a
Coconut oil	376 ± 41	2.24 ± 0.12	88.3 ± 1.5^d	101.2 ± 5.6^b

Values represent the mean ± SD of five animals. Values with different superscript are significantly different by ANOVA (Scheffe *F*-test): *c* vs. *d*, $P < 0.01$; *a* vs. *b*, $P < 0.01$.

Table 3 Effect of the experimental diets on CNPase and AChE activities in the whole brain and cerebral cortex, respectively

Diet	CNPase (μmol/mg of protein/hr)	AChE (nmol/mg of protein/hr)
Fish oil	47.0 ± 2.3[a]	42.2 ± 7.0
Soybean oil	62.5 ± 3.4[b]	38.9 ± 3.0
Coconut oil	54.4 ± 1.7[c]	42.2 ± 7.0

Values represent the mean ± SD of five animals. Values with different superscript are significantly different by ANOVA (Scheffe *F*-test): *b* vs. *c*, $P < 0.001$; *b* vs. *a*, $P < 0.001$; *c* vs. *a*, $P < 0.01$.

Muscarinic receptor binding

Total muscarinic receptor binding in the cerebral cortex homogenates was evaluated using as a specific ligand (-)-[^{3}H]quinuclidinylbenzilate ([^{3}H]QNB) and a rapid filtration method as previously described.[20] The homogenates (250 mL corresponding to 125 to 300 mg of protein) were incubated in 50 mM phosphate buffer, pH 7.4, containing six different concentrations of [^{3}H]QNB (from 0.005 to 1.8 nM) in a final volume of 1 mL (in duplicate). Parallel tubes, which also contained 1 μM atropine sulfate were prepared to determine nonspecific binding. After incubation at 25°C for 2 hr, the reaction was stopped by the addition of 4 mL of the above ice-cold phosphate buffer and immediate filtration through Whatman GF/B filters. The tubes were rinsed twice with an additional 4 mL of buffer, the filters were placed in vials with 5 mL of Filter count (Packard), and, radioactivity was measured in a Packard Tricarb 4640 (Camberra, Australia) at a counting efficiency of 50%. The specific binding was carried out on six points with computer-fitted regression lines (correlation coefficients $r > 0.80$), and the results were expressed as B_{max} in fmol/mg of protein, and K_d (pM). The M_1-AChRs were evaluated with a similar procedure using [^{3}H] pirenzepine ([^{3}H]PZ) as a ligand.[21] Its concentrations were from 0.075 to 11.25 nM, the samples contained 500 to 600 μg of protein, and the incubation time was 1 hr.

Protein determination

The protein concentration was determined according to Lowry et al.[22] using bovine serum albumin as standard.

Statistical analysis

Statistical analysis was performed using one-factor analysis of variance and the Scheffe *t*-test for multiple comparison. Regression analysis was also performed. Differences of $P < 0.05$ were considered statistically significant.

Results

The data on the effects of experimental diets on body weight, brain weight, and protein content of the whole brain and of the cerebral cortex are presented in *Table 2*. The protein concentration of brains is higher in the soybean group than in the other two groups, even though the differences are significant only with the coconut group. In the cerebral cortex the protein content of the soybean group is significantly higher than both the FO and CO groups. However, the body and brain weights are not affected by dietary oils.

Table 3 shows the CNPase and AChE activities in the brain and cerebral cortex, respectively. The CNPase activity in animals fed a soybean diet is higher than the other groups. The activity is positively correlated with Σn-6 fatty acids (mainly with arachidonic acid) and negatively correlated with Σn-3 fatty acids (mainly with docosahexaenoic acid) (*Figure 1*), with higher statistical significance for Σn-6 ($P > 0.001$ versus $P > 0.01$). No correlation was found with Σ saturated fatty acids and Σ monounsaturated fatty acids. No statistically significant differences are present in the AChE activity among the three groups. The values are not correlated with fatty acid composition (data not shown).

The data on cortical mAChRs are presented in *Table 4*. No significant changes in density (B_{max}) and affinity (K_d) for [^{3}H]QNB and [^{3}H]PZ binding are observed among the three groups.

Discussion

Our study indicated that dietary oils may modulate the activity of CNPase without affecting the cholinergic markers. Since the CNPase activity increases in parallel with the rate of the myelinogenesis process, it is considered a useful indicator of myelination.[23] The reduced activity observed in the brains of rats fed a FO diet could be due either to a delay in myelin deposition and/or to instability of its stucture during postweaning growth. These hypotheses seem to be con-

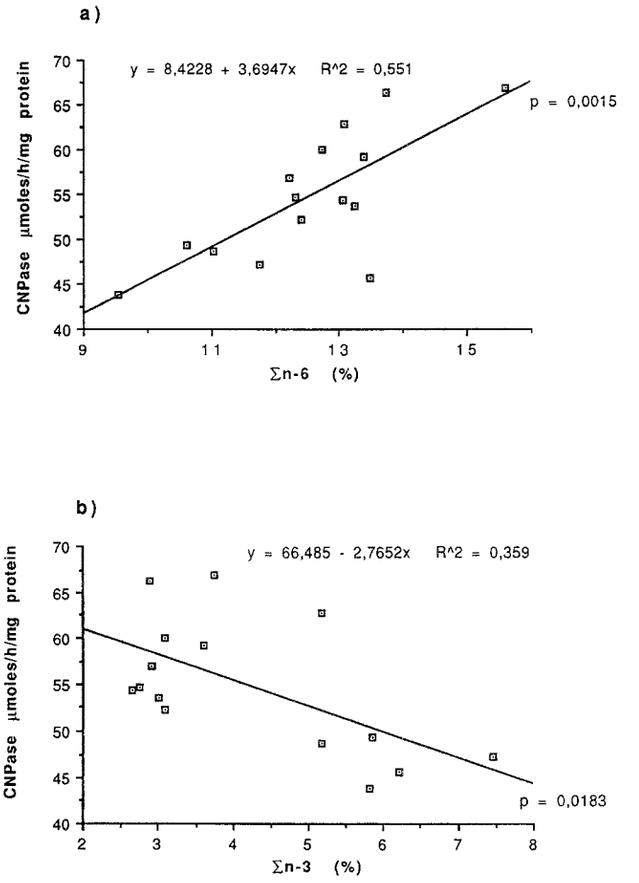

Figure 1 Correlation between CNPase activity and Σn-6 (a) and Σn-3 (b) in the myelin of the experimental groups.

Table 4 Effect of the experimental diets on total and M_1-mAChRs

	Fish oil		Soybean oil		Coconut oil	
	B_{max} (fmol/mg of protein) (pM)	K_d	B_{max} (fmol/mg of protein) (pM)	K_d	B_{max} (fmol/mg of protein) (pM)	K_d
[^{3}H]QNB	1,220 ± 113	100 ± 10.6	1,152 ± 144	103 ± 29	1,075 ± 118	78 ± 20.9
[^{3}H]PZ	745 ± 261.0	6 ± 1.8	794 ± 83.1	8 ± 1.1	874 ± 69.4	7 ± 2.1

Values represent the mean ± SD of five animals.

firmed by the results of our previous paper[7] in which the myelin protein composition showed a decrease of the relative amount of basic proteins (BPs) in the FO group. By analogy to the CNPase activity, the BPs concentration increases with myelin deposition, and it seems to play an important role in membrane structural integrity and stability.[24] It has been demonstrated that deficiency of dietary essential fatty acids during the postnatal period results in a reduction of myelination,[25–28] while it has been shown that microbial dietary lipids[29] rich in odd-chain fatty acids may accelerate the myelinogenesis process.

In the postweaning period, data on the effect on myelin deposition of dietary lipids different in their fatty acid composition are lacking. Our data indicate that the myelinogenesis process is sensitive to dietary treatment also in the postweaning period which in rats corresponds to a decrease of myelin turnover. The decrease of the enzymatic activity observed in the FO group could be due to high levels of n-3 fatty acids that are negatively correlated with the CNPase activity, while in the SO group the increase could be due to the high levels of n-6 fatty acids. These changes in the enzymatic activity could be associated to changes in the physicochemical properties of membranes and/or a direct effect of dietary fatty acid on gene expression.[30]

However, the protein content of the whole brain and of the cerebral cortex is higher in the SO group than in the other two groups. Experiments in vivo on rats showed that an n-3 fatty acid dietary deficiency increases brain protein synthesis.[31] The authors suggest that this effect is due to a deficiency of C 22:6 that could exert an inhibitory role on protein synthesis. On this basis we can explain the low protein content observed in the FO group but not the low values observed in the coconut group. Therefore, we can speculate that a similar inhibitory effect can be played also by saturated fatty acids present in CO as observed by DeWille et al. in developing mice.[32]

The AChE activity is not influenced by dietary oils, even though the synaptosomal membrane fatty acid composition is more susceptible to dietary lipids than myelin. Our results are not in agreement with the experiments by Foot et al.[10] who demonstrated that changes in dietary lipids affect the AChE activity in synaptosomes. However, our data were obtained in different experimental conditions particularly in postweaning rats and with different dietary lipids. The unchanged enzymatic activity together with unchanged binding of muscarinic receptors suggests that the amount of acetylcholine within the synaptic cleft would be at the same level in the three experimental groups in the postweaning period.

On the whole, the results indicate the favorable effect of soybean oil and the adverse effect of FO on myelin deposition and the absence of an effect of dietary oils on the cholinergic system. Feeding high levels of n-3 fatty acids during the growth periods therefore seems inadvisable, not only for the bleeding and immunological problems,[33,34] but also for the normal development of CNS structure. However, large amounts of n-6 fatty acids do not show similar effects on CNS, in agreement with the results of Widdowson[35] on infant formulas rich in linoleic acid, which did not show any short-term adverse effect. On the other hand, the European Scientific Committee for Food also recommends having a ratio of n-6/n-3 fatty acids of 4 in the diet of children and adolescents.[36]

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