Effects of dietary oils on fatty acid composition and lipid peroxidation of brain membranes (myelin and synaptosomes) in rats

Serafina Salvati, Lorenzo Malvezzi Campeggi, Paola Corcos Benedetti, Maurizio Di Felice, Vincenzo Gentile, Mirella Nardini, and Gianni Tomassi

Istituto Superiore di Sanità, Roma, Istituto Nazionale della Nutrizione, Roma, and Università della Tuscia, Viterbo, Rome, Italy

The effects of dietary oils on the fatty acid composition and lipid peroxidation of myelin and synaptosomes isolated from rat brain were studied. The animals were fed diets rich (15% wt/wt) in either long chain n-3 fatty acids (fish oil), n-6 fatty acids (soybean oil), or saturated fatty acids (coconut oil) for 6 weeks. Fish oil led to a significant increase in the n-3 fatty acid percentage in both membranes with a concomitant decrease in n-6 fatty acids percentage in myelin but not in synaptosomes. Soybean and coconut oils influenced less strictly the brain membranes' fatty acid composition. A higher peroxidation rate and a lower concentration of vitamin E was observed in brain membranes of the fish oil group compared with the coconut group, with intermediate values in the soybean oil group. However, no differences among the experimental animals were observed in thiobarbituric acid reacting substances (TBARS) of brain membranes, although in serum TBARS concentrations increased in proportion to dietary PUFA content, suggesting that the brain is more protected in vivo by oxidative challenge. The data also indicate that dietary oils may differently affect the fatty acid composition of isolated brain membranes, with synaptosomes being more susceptible to oxidative stress than myelin membranes.

Keywords: dietary polyunsaturated fatty acids; myelin; synaptosomes; membranes fatty acids; lipid peroxidation; rat brain

Introduction

Dietary fatty acids influence brain membrane fatty acid composition, depending on the chemical nature of the fatty acid source.[1-5] Recent studies[6-8] have shown that saturated (SFA) and monounsaturated long-chain fatty acids (MUFA) in whole rat brain and in brain phospholipids are insensitive to changes in the dietary content of n-6 or n-3 fatty acids, while brain PUFA are more susceptible to dietary changes.

Brain subcellular fractions are characterized by different fatty acid compositions; for example, synaptosomes are rich in PUFA, while myelin is rich in SFA and MUFA. The specific fatty acid distribution in brain membranes indicates its role in regulating the fluidity and functional activity of these structures. Changes in fatty acid composition lead to biochemical and functional alterations, such as lipid-protein interaction, protein distribution, membrane permeability, ion transport, etc.[9-11] Furthermore, elevating the concentration of PUFA in brain lipids could make them particularly susceptible to oxidative damage, considering the high oxygen consumption of this organ. However, differences in the susceptibility to oxidative stress related to the fatty acid composition of the various brain subcellular membranes have not yet been established.

The aim of the current study is to determine the extent to which brain myelin and synaptosomes regulate fatty acid composition, lipid peroxidation, and protein

This research was supported by National Research Council of Italy, special project RAISA, subproject number 4, paper n. 655 and special project INVECCHIAMENTO, subproject number 2, paper 242.
Address reprint requests to Dr. S. Salvati, Neurochemistry Section, Lab. Metabolism and Pathological Biochemistry, Istituto Superiore di Sanità, V. le Regina Elena, 299 - 00161 Roma, Italy.
Received July 27, 1992; accepted October 9, 1992.

Table 1 Fatty acid composition of the dietary lipids (%)

Fatty acid	Fish oil	Soybean oil	Coconut oil
8:0	0.2	—	1.3
10:0	—	—	4.0
12:0	0.1	—	43.7
14:0	6.5	0.2	19.4
16:0	17.4	11.0	11.3
16:1 (n-7)	10.1	0.2	—
18:0	3.9	3.7	4.4
18:1 (n-9)	14.8	21.2	12.3
18:2 (n-6)	1.7	54.1	3.3
18:3 (n-6)	1.0	0.4	—
18:3 (n-3)	0.3	7.2	—
18:4 (n-3)	4.0	—	—
20:0	—	0.4	—
20:1 (n-9)	2.4	0.5	—
20:4 (n-6)	1.1	0.5	—
20:5 (n-3)	19.0	—	—
22:0	—	0.4	—
22:1 (n-9)	1.9	—	—
22:5 (n-3)	2.4	—	—
24:0	—	0.3	—
22:6 (n-3)	12.0	—	—
24:1 (n-9)	1.2	—	—
ΣSFA	28.2	15.8	84.1
ΣMUFA	30.4	21.8	12.3
ΣPUFA	41.6	62.2	3.3
Σn-3	37.7	7.2	—
Σn-6	3.9	55.0	3.3
Σn-3/Σn-6	9.7	0.1	—
U.I.	237	155	19

ΣSFA = saturated fatty acid; ΣMFA = monounsaturated fatty acid; ΣPUFA = polyunsaturated fatty acid; U.I. = unsaturation index.

distribution by analyzing the effects of fish oil, soybean oil, and coconut oil (rich in n-3 PUFA, n-6 PUFA, and SFA respectively) on the above parameters.

Methods and materials

Animals and diets

Three groups of six 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 in a temperature- and light-controlled room (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%, choline chloride 0.2%, but differing in oil source for 6 weeks. Group 1 received fish oil (MaxEPA, a kind gift of Seven Seas Limited, Hull, England); group 2, soybean oil; and group 3, coconut oil.

The diets were prepared weekly and stored at 4° C under nitrogen and 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 exsanguinated brains were removed and weighed.

Diet analysis

Samples of experimental diets were analyzed for fatty acid composition by gas liquid chromatography (GLC) (*Table 1*).[12] The unsaturation index was calculated by multiplying the number of double bonds by the percentage composition of individual fatty acids and summing the values.

Tocopherol content was determined by high pressure liquid chromatography according to Mc Murray et al.,[13] and peroxide number was assayed by iodimetric titration.[14]

Serum analysis

Vitamin A and E levels were assayed according to Bieri.[15] The lipid peroxide level was measured by the thiobarbituric acid reacting substances (TBARS) assay of Yagi.[16] TBARS activity was expressed as MDA equivalent using freshly prepared malonaldehyde-tetramethyl acetal solution as a standard.

Subcellular fractions preparation and analysis

Myelin was isolated according to Norton and Poduslo[17] and synaptosomes were prepared by Percoll density gradient as reported by Nagy and Delgado.[18] The purity of both subcellular fractions was checked by electron microscopy. The membranes were submitted to an osmotic shock with bidistilled water, pelleted by centrifugation, lyophilized, and stored at −20° C.

Lipids were extracted from membranes with 20 volumes of chloroform-methanol 2:1 (vol/vol) according to Folch et al.,[19] and the fatty acid composition determined by GLC.[12]

Vitamin E content was determined after saponification of freeze-dried samples, suspended in 50 mmol/L Tris-HCl buffer pH 7.4 according to Buttris and Diplock.[20] Lipid peroxide level of brain membranes was determined on freeze-dried samples resuspended in bidistilled water as TBARS, according to the assay procedure described for lipid peroxide level in animal tissue.[21] TBARS activity was expressed as malondialdehyde equivalents.

Measurements of oxygen uptake during lipid peroxidation

Myelin or synaptosomal suspension (0.2 mg protein/mL) were incubated for 2 min at 30° C in 50 mmol/L Tris-HCl/150 mmol/L KCl pH 7.4, containing 500 μmol/L ADP, 200 μmol/L ascorbate followed by the addition of 5 μmol/L ferric chloride.[22] Oxygen uptake was measured polarographically employing a Clark electrode (YSI, Yellow Spring Instruments Co. Inc., Yellow Spring, OH, USA) with a Gilson 5/611 oxigraph. Ambient oxygen concentration in the buffer (30° C) was 270 μmol/L.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Purified myelin was partially delipidated with diethyl ether/ethanol (3:2 vol/vol) and solubilized with 1% sodium dodecyl sulfate. Proteins were separated by 12% (wt/vol) SDS-polyacrylamide gel electrophoresis. The gels were stained with Coomassie Blue and destained and the proteins identified by coelectrophoresis with low molecular weight protein standards (Bio-Rad Laboratories, Richmond, CA USA) (MW operating range 10–100 kDa).[23]

Protein determination

The protein concentration was determined according to Lowry et al.[24] using bovine serum albumin as standard.

Table 2 TBARS, Vitamin A, and Vitamin E concentrations of experimental rats levels in serum

Diet	TBARS (nmol MDA/mL)	Vit.A (ng/mL)	Vit. E (μg/mL)
Fish oil	4.2 ± 0.5[a]	342 ± 61	2.64 ± 0.5[a]
Soybean oil	1.6 ± 0.3[b]	479 ± 79	3.18 ± 0.9[a]
Coconut oil	1.1 ± 0.2[b]	445 ± 121	4.86 ± 0.8[b]
P	0.0001	n.s.	0.001

Values represent the mean ± S.D. of six animals of each dietary group. Values with different superscript are significantly different by ANOVA (Scheffe F test).

Statistical analysis

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

Results

The serum concentrations of TBARS, vitamin A, and vitamin E are reported in *Table 2*. The TBARS value in animals fed fish oil was 2.5 and four times higher than the values for rats fed soybean and coconut oils, respectively, while in the same group vitamin E concentrations were significantly lower than in the other two groups. Vitamin A levels showed no significant differences.

In *Table 3* the myelin fatty acid pattern of the three experimental groups is reported. The total SFA, the total MUFA, and the total PUFA were not affected by dietary fatty acids. The percentage of total n-3 fatty acids and the ratio n-3:n-6 were significantly higher in the fish oil group than in the other groups, even though the total PUFA percentage was not altered for the decrease of 18:2, 20:2, and 20:4 fatty acids of n-6 series (data not shown).

The fatty acid pattern of synaptosomes from animals fed different dietary lipid sources is shown in *Table 4*. The lowest unsaturation index (U.I.) was found with the coconut oil diet and the highest with the fish oil diet, with an intermediate value for the soybean oil diet. Furthermore, the total n-3 fatty acid percentage was higher in the fish oil group than in the coconut group, while the total SFA were higher in the latter group.

The values of the major myelin proteins, expressed as a percentage of total dye binding capacity, are reported in *Table 5*. The small basic protein (BPs) and the proteolipid protein (PLP) showed significant differences among the three groups. The percentage of BPs decreased with the increase of dietary U.I. and n-3:n-6 ratio. The opposite was observed with the PLP percentage. No differences were found for the values of the other proteins.

TBARS and vitamin E values of myelin and synaptosomes from the rats of the three experimental groups are reported in *Table 6*. TBARS concentration was not affected by the diet, while vitamin E concentration decreased in both subcellular fractions with the increase of U.I. of the dietary fat.

In *Table 7* the rate of lipid peroxidation of myelin and synaptosomes from the experimental groups is reported. The peroxidation rate in the fish oil group was significantly higher than in the coconut oil group in both subcellular fractions with intermediate values for the soybean oil group.

Discussion

Data on serum TBARS concentration clearly indicate that dietary lipids with a high unsaturation level (fish oil) induce an oxidative stress. This effect is very likely the cause of the concomitant low concentration of both circulating vitamin A and vitamin E levels, with the vitamin E level being the most sensitive and earliest indicator of the oxidative stress. Other studies[8] were unable to show any changes in serum content of both vitamin A and vitamin E in rats in response to dietary fish oil. This discrepancy could be due to the different experimental conditions; in fact, the diets contained a lower percentage of lipid and a higher level of vitamin E than the diets used in our experiment.

Dietary lipids affected brain membrane fatty acid composition of both myelin and synaptosomes, although the magnitude of the response varied. In myelin, the most important changes were observed in the total n-3 fatty acid percentage, which was twice as high in the fish oil group than in the soybean and coconut oil groups. However, the n-6 percentage decreased (mainly due to a decrease of arachidonic acid), probably compensating for the total degree of unsaturation.

Table 3 Fatty acid pattern of the myelin from rats fed the experimental diets (%)

Fatty acid	Fish oil	Soybean oil	Coconut oil	*P*
ΣSFA	35.04 ± 0.81	35.33 ± 0.63	36.59 ± 1.11	n.s.
ΣMUFA	46.65 ± 1.40	46.96 ± 1.07	47.71 ± 0.76	n.s.
ΣPUFA	17.90 ± 1.94	17.41 ± 1.39	15.53 ± 0.48	n.s.
Σn-3	6.54 ± 0.84[a]	3.70 ± 0.90[b]	2.89 ± 0.18[b]	0.0001
Σn-6	11.36 ± 1.47[a]	13.71 ± 1.12[b]	12.65 ± 0.47[a,b]	0.0150
Σn-3/Σn-6	0.57 ± 0.08[a]	0.27 ± 0.07[b]	0.23 ± 0.17[b]	0.0001
U.I.	124 ± 7[a]	118 ± 5[ab]	113 ± 2[b]	0.0141

Values represent the mean ± S.D. of six animals of each dietary group. Values with different superscript are significantly different by ANOVA (Scheffe F test). For the explanation of symbols see *Table 1*.

Table 4 Fatty acid pattern of synaptosomes from rats fed the experimental diets (%)

Fatty acid	Fish oil	Soybean oil	Coconut oil	*P*
ΣSFA	41.61 ± 2.15[a]	45.21 ± 0.95[b]	46.77 ± 2.08[b]	0.0027
ΣMUFA	18.95 ± 1.11[a]	18.37 ± 0.16[a]	22.92 ± 1.99[b]	0.0017
ΣPUFA	39.35 ± 2.99[a]	36.47 ± 1.10[a]	30.32 ± 0.94[b]	0.0001
Σn-3	20.35 ± 1.72[a]	18.00 ± 0.40[b]	14.17 ± 1.00[c]	0.0001
Σn-6	19.00 ± 2.08[a]	18.47 ± 0.85[a,b]	16.15 ± 1.02[b]	0.0350
Σn-3/Σn-6	1.07 ± 0.12[a]	0.97 ± 0.04[a,b]	0.88 ± 0.10[b]	0.0307
U.I.	210 ± 13[a]	193 ± 4[a]	167 ± 4[b]	0.0001

Values represent the mean ± S.D. of six animals of each dietary group. Values with different superscript are significantly different by ANOVA (Scheffe F test). For the explanation of symbols see *Table 1.*

Table 5 Myelin protein composition from experimental rats (percent of dye-binding capacity)

Protein	Fish oil	Soybean oil	Coconut oil	*P*
BPs	43.13 ± 2.35[a]	47.33 ± 1.70[b]	51.87 ± 0.23[c]	0.0012
18.5 kDa	7.43 ± 1.13	6.45 ± 1.07	7.43 ± 3.04	n.s.
21.5 kDa	16.00 ± 2.40	15.20 ± 2.24	17.77 ± 4.36	n.s.
PLP	25.23 ± 1.91[a]	23.75 ± 4.35[a]	15.57 ± 6.64[b]	0.0286
HMV	8.30 ± 0.59	7.08 ± 1.10	7.10 ± 1.30	n.s.

Values represent the mean ± S.D. of four animals of each dietary group. Values with different superscript are significantly different by ANOVA (Scheffe F test). BPs, small Basic protein; PLP, proteolipid protein; HMW, high molecular weight.

Table 6 TBARS and Vitamin E values of myelin and synaptosomes from rats fed the experimental diets

	Myelin		Synaptosomes	
Diet	TBARS (nmol MDA/mg protein)	Vit. E (ng/mg protein)	TBARS (nmol MDA/mg protein)	Vit. E (ng/mg protein)
Fish oil	0.48 ± 0.05	217.8 ± 32.5[a]	12.7 ± 3.2	173.2 ± 63.3
Soybean oil	0.48 ± 0.05	282.2 ± 76.2[a,b]	11.0 ± 0.7	157.0 ± 8.5
Coconut oil	0.44 ± 0.06	346.3 ± 60.4[b]	8.00 ± 2.3	278.3 ± 57.4
P	n.s.	0.0399	n.s.	n.s.

Values represent the mean ± S.D. of four animals of each dietary group. Values with different superscript are significantly different by ANOVA (Scheffe F test).

Table 7 Peroxidation rate of myelin and synaptosomes of rats fed the experimental diets

Diet	Myelin (nmol O_2/min/mg protein)	Synaptosomes (nmol O_2/min/mg protein)
Fish oil	4.18 ± 0.33[a]	220.5 ± 10.6[a]
Soybean oil	2.90 ± 0.37[b]	195.6 ± 18.0[a,b]
Coconut oil	2.20 ± 0.46[b]	147.0 ± 24.5[b]
P	0.002	0.002

Values represent the mean ± S.D. of six animals of each dietary group. Values with different superscript are significantly different by ANOVA (Scheffe F test).

The differences observed in the relative protein distribution indicate a possible loss of membrane structural integrity and stability because the BPs play an important role in maintaining a compact myelin.[25]

In synaptosomes the changes were much more pronounced in agreement with the findings of Foote et al.[26] on piglets fed formulas supplemented with fish oil during the suckling period. These studies on isolated membranes allowed evaluation of differences in fatty acid composition difficult to show in the whole brain.

Brain membranes showed strong differences in lipid peroxidation status; synaptosomes showed TBARS concentration about 20 times that of myelin. These differences were evident in the peroxidation rate, which was significantly higher in synaptosomes than in myelin. Vitamin E concentrations were of the same order of magnitude in the two membranes, but considering that the protein content in synaptosomes is about twice that of myelin,[27] the total amount of vitamin E in synaptosomes was about double that of myelin membrane. Furthermore, considering that myelin contains more lipid than synaptosomes,[28] the higher lipid peroxidation both in vivo and in vitro of synaptosomes clearly indicates that it is the fatty acid unsaturation degree and not the total amount of lipids that is mainly responsible for the oxidative resistance of the membranes. On the whole,

the data indicate that even though dietary oils do not affect the oxidative status in vivo of brain membranes according to the unchanged endogenous lipid peroxidation values found by Burns et al.[29] in brain of mice fed fish oil enriched diet, the susceptibility to peroxidation increases with n-3 rich diets, probably through a higher consumption of vitamin E.

Acknowledgments

The authors thank Mrs. Rita Rami and Mrs. Pierina Rami for their skillful technical assistance.

References

1 Tahin, Q.S., Blum, M., and Carofoli, E. (1981). The fatty acid composition of subcellular membranes of rat liver, heart and brain: diet-induced modification. *Eur. J. Biochem.* **121,** 5–13

2 Carlson, S.E., Carver, J.D., and House, S.G. (1986). High fat diets varying in ratios of polyunsaturated to saturated fatty acid and linoleic acid: a comparison of rat neural and red cell membrane phospholipids. *J. Nutr.* **116,** 718–725

3 Bourre, J.M., Pascal, G., Masson, M., Dumont, O., and Piciotti, M. (1984). Alterations in the fatty acid composition of rat brain cells (neurons, astrocytes and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n-3 fatty acids. *J. Neurochem.* **43,** 342–348

4 Foot, M., Cruz, T.F., and Clandinin, M.T. (1982). Influence of dietary fat on the lipid composition of rat brain synaptosomal and microsomal membranes. *Biochem. J.* **298,** 631–640

5 Conti, L., Salvati, S., Serlupi-Crescenzi, G., Di Felice, M., Tagliamonte, B., and Tomassi, G. (1980). Influence of dietary lipids on myelinogenesis in the rat: effect of lipids from n-alkane grown yeast on myelin subfraction composition. *Ital. J. Biochem.* **29,** 371–373

6 Bourre, J.M., Bonneil, M., Dumont, O., Piciotti, M., Nalbone, G., and Lafont, H. (1988). High dietary fish oil alters the brain polyunsaturated fatty acid composition. *Biochim. Biophys. Acta* **960,** 458–461

7 Philbrick, D.J., Mahadevappa, V.G., Ackman, R.G., and Holub, B.J. (1987). Ingestion of fish oil or a derived n-3 fatty acid concentrate containing eicosapentaenoic acid (EPA) affects fatty acid compositions of individual phospholipids of rat brain, sciatic nerve and retina. *J. Nutr.* **117,** 1663–1670

8 Bourre, J.M., Bonneil, M., Dumont, O., Piciotti, M., Calaf, M., Portugal, H., Nalbone, G., and Lafont, H. (1990). Effect of increasing amounts of dietary fish oil on brain and liver fatty acid composition. *Biochim. Biophys. Acta* **1043,** 149–152

9 Spector, A.A. and Yorek, M.A. (1985). Membrane lipid composition and cellular function. *J. Lipid Res.* **26,** 1015–1035

10 Neuringer, M., Anderson, G.J., and Connor, W.E. (1988). The essentiality of n-3 fatty acids for the development and function of the retina and brain. *Ann. Rev. Nutr.* **8,** 517–541

11 Stubbs, C.D. and Smith, A.D. (1984). The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim. Biophys. Acta* **779,** 89–137

12 Metcalfe, L.D. and Schmitz, A.A. (1961). Rapid preparation of fatty acid ester for gas-chromatography analysis. *Anal. Chem.* **33,** 363–369

13 Mc Murray, C., Blauchflower, W.J., and Rice, D.A. (1980). Influence of extraction techniques on determination of α-tocopherol in animal foodstuff. *J. Ass. Off. Anal. Chem.* **63,** 1258–1261

14 Iodine absorption number (1980). Hanus method AOAC Methods n.28018 (W. Horwitz, ed.), p. 440–443 AOAC Washington D.C.

15 Bieri, J.G., Talliver, G.T., and Catignani, L.G. (1979). Simultaneous determination of α-tocopherol and retinol in plasma or red cell by high pressure liquid chromatography. *Am. J. Clin. Nutr.* **32,** 2143–2149

16 Yagi, K. (1982). Assay for serum lipid peroxide level and its clinical significance. In *Lipid peroxide in biology and medicine,* (K. Yagi, ed.), p. 324–340 Academic Press, New York, NY USA

17 Norton, W.T. and Poduslo, S.E. (1973). Myelination in rat brain: method of myelin isolation. *J. Neurochem.* **21,** 749–757

18 Nagy, A. and Delgado-Escueta, A.V. (1984). Rapid preparation of synaptosomes from mammalian brain using nontoxic isoosmotic gradient material (Percoll) *J. Neurochem.* **43,** 1114–1123

19 Folch, J., Lees, M., and Sloan-Stanley, G.H. (1957). A single method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–502

20 Buttris, J.L. and Diplock, T. (1984). High-performance liquid chromatography methods for vitamin E in tissues. In *Methods in enzymology,* (L. Packer, ed.), p. 131–147 Academic Press, New York, NY USA

21 Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95,** 351–358

22 Willis, E.D. (1969). Lipid peroxide formation in microsomes. *Biochem. J.* **113,** 315–324

23 Heukeshoven, J. and Dernick, R. (1988). Increased sensitivity for Coomassie staining of sodium dodecylsulfate-polyacrylamide gels using phast system development unit. *Electrophoresis* **9,** 60–61

24 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275

25 Kirschner, D.A., Ganser, A.L., and Caspar, L.D.D. (1984). Diffraction studies of molecular organization and membrane interaction in myelin. In *Myelin,* (P. Morell, ed.) p. 51–95 Plenum Press, New York, NY USA

26 Foote, K.D., Hrboticky, N., Mackinnon, M.J., and Imis, M.S. (1990). Brain synaptosomal, liver, plasma and red blood cell lipids in piglets fed exclusively on a vegetable-oil containing formula with and without fish-oil supplements. *Am. J. Clin. Nutr.* **51,** 1001–1006

27 Seminario, L.M., Hren, N., and Gòmez, C.J. (1964). Lipid distribution in subcellular fractions of the rat brain. *J. Neurochem.* **11,** 197–209

28 Morell, P., Quarles, R.H., and Norton, W.T. (1989). Formation, structure and biochemistry of myelin. In *Basic Neurochemistry,* (G. Siegel, B. Agranoff, R.W. Albers, and P. Molinoff, eds), p 109–136, Raven Press, New York, NY, USA

29 Burns A., Lin, Y., Gibson, G., and Jamieson, D. (1991). The effect of a fish oil enriched diet on oxygen toxicity and lipid peroxidation in mice. *Biochem. Pharmacol.* **42,** 1353–1360