ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

Vol. 48 N° 4, 1998

Effects of dietary polyunsaturated fatty acids on rat brain plasma membrane fatty acid composition

Ofelia Angulo-Guerrero and R.R. Oliart

Instituto Tecnológico de Veracruz, Departamento de Ingeniería Química y Bioquímica, México

SUMMARY. The effects of four different diets on phospholipid fatty acid composition of rat brain plasma membranes were evaluated. Rats were given a semisynthetic diet in which lipids were supplied by 5% peanut oil (n-3 PUFA deficient diet), cod liver oil (n-6 PUFA deficient diet), partially hydrogenated palm oil (total PUFA deficient diet) or a mixture of peanut and rapeseed oil (control group). Animals fed the total PUFA deficient diet had significantly lower body and brain weights than the control group ($p<0.05$). Lower brain cholesterol and phospholipids also were observed in animals fed the total PUFA deficient diet, whereas the brain of animals fed the n-6 PUFA deficient diet had higher levels of these lipid components than the control group ($p<0.05$). Phosphatidylcholine and phosphatidylethanolamine were mutually replaced in animals fed the n-6 and n-3 PUFA deficient diets, so that the sum of these two membrane constituents was maintained around 77% of total phospholipids. Brain phospholipid fatty acid composition was significantly modified by the diets studied. Thus, despite being a highly protected organ, the fatty acid composition of the brain can be extensively modulated by dietary lipids.
Key words: PUFA deficiency, brain plasma membrane, fatty acid composition.

RESUMEN. Efecto de los ácidos grasos poliinsaturados de la dieta en el perfil de ácidos grasos de la membrana plasmática del cerebro de rata. El efecto de cuatro dietas distintas en la fuente de lipidos fue estudiado en el perfil de ácidos grasos de fosfolipidos de la membrana plasmática del cerebro de ratas. Los animales fueron alimentados con dietas que contenían 5% de uno de los siguientes aceites vegetales: aceite de cacahuate (dieta deficiente en AGPI n-3), aceite de hígado de bacalao (dieta deficiente en AGPI n-6), aceite de palma parcialmente hidrogenado (dieta totalmente deficiente en AGPI) o una mezcla de aceite de cacahuate y de canola (dieta testigo). Los animales que recibieron la dieta totalmente deficiente presentaron peso corporal y cerebral mas bajos que los del grupo testigo ($p<0.05$). Los niveles de colesterol y de fosfolipidos en el cerebro también fueron mas bajos en los animales totalmente deficientes que en los testigo. Por otro lado, estos parámetros fueron mas elevados en el grupo de la dieta deficiente en AGPI n-6 ($p<0.05$). Los niveles de fosfatidilcolina y fosfatidiletanolamina fueron mutuamente substituidas en los animales que recibieron las dietas deficientes en AGPI n-6 y n-3, de tal forma que estos dos fosfolipidos (FL) se mantuvieron alrededor del 77% del total de FL. El perfil de ácidos grasos de los fosfolipidos del cerebro fueron significativamente modificados por las dietas estudiadas. Por lo tanto, a pesar de que el cerebro es un órgano muy protegido, la composición en ácidos grasos puede modificarse a través de los lipidos dietarios.
Palabras clave: Deficiencia en AGPI, membrana plasmática cerebral, lípidos dietarios.

INTRODUCTION

The role of n-6 and n-3 polyunsaturated fatty acids (PUFA) in human nutrition is now generally accepted; however, the specific mechanism of actions are yet to be outlined. As essential membrane components, PUFA may act as modulators, specially in the brain were they are in the second highest concentration after adipose tissue. Since polyunsaturated fatty acids are, with cholesterol, the main components of the lipid matrix of biological membranes, their relative proportions determine to a great extend the biophysical and physiological properties of these membranes. It is well established that liver membrane lipid composition can be modified by dietary lipids (1, 2). However, brain components seem to be very well regulated and apparently are less affected by dietary manipulation.

Polyunsaturated fatty acids are divided into two essential fatty acid groups: n-6 and n-3: linoleic and alpha-linolenic acids are the precursors of these two groups, respectively (3,4). The fundamental role of n-6 PUFA is mediated by arachidonic acid as a precursor of eicosanoid molecules which are involved in a great diversity of functions. N-3 PUFA were considered non-essential until recently, when high concentrations of the members of this family (20:5 and 22:6) were found in brain as well as in retina (5).

The brain is the organ with the second highest concentration of lipids and it is the organ with the second greatest concentration of n-3 PUFA, after the retina (6,7). It has been suggested that the n-3 PUFA play a fundamental role in brain development, especially during the first years of life when the organ is being

completed (8-11). N-3 PUFA deficiencies have been associated with alterations in the electroretinogram, altered learning behavior, reduced visual acuity and greater resistance to neurotoxic agents (12, 13). It is necessary therefore, to ensure that nerve cells receive an adequate supply of these fatty acids during their differentiation and multiplication. Whether the brain takes PUFA from the liver, which is modulated by dietary lipids, or produces its own PUFA from the precursors (supplied by diet anyway) is not known. In any case, dietary lipids certainly influence the supply of essential fatty acids to the brain. Therefore, in this study, the effects of four diets, differing only in PUFA type were evaluated on rat brain plasma membrane phospholipid.

MATERIALS AND METHODS

Experimental animals

Wistar rats were given a semisynthetic diet in which lipids (5% w/w) were supplied as a mixture of peanut and rapeseed oil (control), peanut oil (n-3 PUFA deficient), cod liver oil (n-6 PUFA deficient) or partially hydrogenated palm oil (total PUFA deficient). The composition of the diets and the fatty acid content of dietary lipids are presented in Tables 1 and 2. Diets were supplied to animals from weaning until the age of 90 d when animals were sacrified by decapitation. Brain was removed and immediately utilized for membrane purification. The experimental proptocols of this study were as reported previously (14).

TABLE 1
Diet composition (g/kg)

Composition %	Group 1 Control	Group 2 n-3 PUFA Deficient	Group 3 n-6 PUFA Deficient	Group 4 Total PUFA Deficient
Casein	22.0	22.0	22.0	22.0
DL methionine	0.16	0.16	0.16	0.16
Cellulose	2.0	2.0	2.0	2.0
Starch	43.90	43.90	43.90	43.90
Sucrose	21.94	21.94	21.94	21.94
Vitamin mixture[a]	1.0	1.0	1.0	1.0
Mineral mixture[b]	4.0	4.0	4.0	4.0
Hydrogenated palm oil	-	-	-	5.0
Peanut oil	2.5	5.0	-	-
Cod liver oil	-	-	5.0	-
Rapeseed oil	2.5	-	-	-

a. Total vitamin supplement, United States Biochemical corp. Cleveland, OH.

b. Composition g/100 g: $CaHPO_4.2H_2O$, 38.0; K_2HPO_4, 24.0; $CaCO_3$,18.0; NaCl, 6.9; MgO, 2.0; $MgSO_4.7H_2O$, 9.0; $FeSO_4.7H_2O$, 0.086; $ZnSO_4.H_2O$, 0.5; $MnSO_4.H_2O$, 0.5; $CuSO4.5H_2O$,0.1; NaF, 0.08; $CrK(SO_4)_2.H2O$, 0.05; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.002; KI, 0.004; $CoCO_3$, 0.002; $NaSeO_3.5H_2O$, 0.002.

TABLE 2
Fatty acid composition of dietary lipids

Fatty Acids (%)	Group 1 Control	Group 2 n-3 PUFA Deficient	Group 3 n-6 PUFA Deficient	Group 4 Total PUFA Deficient
Fatty acids/ 100 g of diet:				
n-6PUFA (mg)	930.6	935.3	136.3	84.6
n-3PUFA (mg)	188.0	4.7	1113.9	-
n-6 PUFA/n-3 PUFA	5.0	199.0	0.1	-

Control diet = mixture of peanut and rapeseed oil, n-3 PUFA deficient diet = peanut oil, n-6 PUFA deficient diet = cod liver oil, total deficient diet = hydrogenated palm oil.

Membrane purification

Brain plasma membranes were separated by a modified method (15). Briefly, 2g. of brain were homogenized in 10 mL of cold buffer (sucrose, 0.32M; Hepes/KOH, 5mM; EGTA, 0.5 mM; pH=7.4) with a Teflon potter homogenizer (Thomas C) using 15 up and down strokes at 1 000 rpm. The homogenate was filtered through a tissue and centrifuged at 10 000 rpm (12 000 xg) for 10 minutes in a Beckman JA20 centrifuge. The supernatant, diluted to 75% with Percoll, was then placed at the bottom of the tube followed by a step Percoll gradient (30, 25, 18, 10 and 0% v/v; Pharmacia) and centrifuged at 20 000 rpm (48 000 xg) for 4 minutes at 4°C. The plasma membrane fraction was collected at the 10%-0% interface. This was diluted with a Percoll solution (75%, v/v) to a ratio of 2 mL of Percoll per 5 mL of membrane fraction. Seven mL of this membrane fraction was placed again at the bottom of the tube followed by a step gradient (18%, 10%, 0% v/v) and centrifuged as before. Protein was measured by the Bradford technic (16).

Plasma membrane lipid analysis

Total lipids from plasma membrane were extracted by the Folch et al. procedure (17) in the presence of butyl hydroxytoluene (BHT) at a 0.02% (w/v). Cholesterol was assayed enzymatically by the Wolff's method (18). Total phospholipids were determined by measuring the total phosphorus as described by Bartlett et al. (19). Since the mass content of phosphorus in phospholipids is about 1/25, phosphorus levels were converted into phospholipid content by multiplying the phosphorus concentration by 25. After total lipid extraction, phospholipid classes from plasma membranes were separated by high pressure liquid chromatography (Beckman 332 silica capillary column coated with zorbax 5 m). Transesterification of phospholipid fatty acids was achieved using BF3-methanol (10% w/v) reagent at 90oC for 20 min. (20). Fatty acid methyl esters (FAME) were extracted using hexane and analyzed by gas chromatography using a Carlo Erba 4180 model with automated on column injection, flame ionization detector and a silica capillary column fused with carbowax 52. Hydrogen was used as the carrier gas. Injection and detector temperatures were 154°C and 250°C,

respectively. The column temperature was programmed to rise from 54°C to 220°C at a rate of 3°C/min. FAME peaks were identified by comparison with authentic standards (SIGMA)

Statistical methods

Results were analyzed statistically by standard analysis of variance (ANOVA). A probability level of $p < 0.05$ was used to indicate statistical significance.

RESULTS

Body and brain weight

Animals fed diets containing partially hydrogenated palm oil had significantly ($p < 0.05$) lower body (294 ± 18 g) and brain weights (1.73 ± 0.08 g) at 90 days of age than did animals fed the control diet (372 ± 14 g, 1.97 ± 0.04 g) (Table 3). Protein per g of brain was around 21 mg in all groups.

TABLE 3
Dietary fatty acid effects on body and brain weight

	Diets			
	Control	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
Body weight (g)	372±14[a]	355±33[a]	378±27[a]	294±18[b]
Brain weight (g)	1.97±0.04[a]	1.98±0.09[a]	1.97±0.05[a]	1.73±0.08[b]
Protein/brain (mg/g)	21.8±1.6[a]	20.5±1.6[a]	21.3±1.7[a]	21.2±1.9[a]

Results are the mean ±standard deviation of 10 animals in each group
Control diet = mixture of peanut and rapeseed oil, n-3 PUFA deficient diet = peanut oil, n-6 PUFA deficient diet = cod liver oil, total deficient diet = hydrogenated palm oil.
Means with different letter are statistically different ($p < 0.05$).

Membrane lipid composition

Cholesterol and phospholipids

Lower cholesterol and phospholipids were observed in brain membranes of animals fed the total PUFA deficient diet than in those of the control group (350±70; 490± 20 µg/g protein) (Table 4).

TABLE 4
Dietary fatty acid effects on brain membrane lipid composition

Parameters (µg/mg)	Diets			
	Control	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
Cholesterol/Protein	480±60[a]	480±50[a]	550±10[b]	350±70[b]
Phospholipids/Protein	530±50[a]	510±30[ab]	580±10[c]	490±20[b]
CHL/PL	900±10[a]	930±90a[b]	940±20[b]	700±160[c]

Same as Table 3.

The content of these lipids in brain membranes of animals fed the n-3 PUFA deficient diet did not differ from the content of the control group. However, cholesterol and phospholipid levels were higher in membranes from animals fed the n-6 PUFA deficient diet (550± 10; 580± 10 µg/g protein) than the levels of the control group (480± 60 ; 530± 50 µg/g protein).

Phospholipid classes

The major membrane phospholipid, phosphatidylethanolamine, was lower in animals fed both the n-6 (45.1± 5.3%) and n-3 PUFA deficient diets (41.2± 2.6%) than observed in controls (51.1± 6.3%). This was compensated by higher contents of phosphatidylcholine in the n-3 and n-6 PUFA deficient animals in comparison to the control group (Table 5). Phosphatidylserine content of membranes did not difer among groups but phosphatidylinositol was higher in animals fed the total PUFA deficient diet.

TABLE 5
Dietary fatty acid effects on brain membrane phospholipid classes

Phospholipids (%)	Diets			
	Control	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
Phosphatidylethanolamine	51.1±6.3[a]	41.2±2.6[c]	45.1±5.3[bc]	47.6±5.4[b]
Phosphatidylcholine	28.1±3.6[a]	35.7±3.1[b]	34.9±2.9[b]	27.7±2.6[a]
Sphingomyelin	11.1±2.7[a]	14.0±2.8[b]	11.0±2.8[a]	12.8±1.9[ab]
Phosphatidylserine	6.9±1.5[ab]	6.3±1.2[b]	6.3±1.4[b]	8.4±1.1[a]
Phosphatidylinositol	2.8±0.5[a]	2.8±0.3[a]	2.7±0.4[a]	3.5±0.6[b]

Same as Table 3.

Phospholipid fatty acid composition

Phosphatidylcholine

In general, the levels of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) in phosphatidylcholine did not differ among groups (Table 6). However, both n-6 and n-3 PUFA were reciprocally replaced by fatty acids of the other family in the n-6 and n-3 PUFA groups. In fact, a lower level of n-6 PUFA (5.1 vs 8.6) was observed in animals fed the diet rich in n-3 PUFA. This observation comes primarily from the lower arachidonic acid level (3.3 vs 6.1). Similarly, n-3 PUFA were lower (1.4 vs 3.7) in membranes PC from animals fed the diet rich in n-6 PUFA. N-3 fatty acids were higher in membrane PC of animals fed the n-6 PUFA deficient diet (4.7 vs 3.7) due primarily to the lower content ofeicosapentaenoic acid. Total (n-6 + n-3) PUFA were maintained for the n-3 PUFA (12.0) and total PUFA (11.1) deficient groups but reduced for the n-6 PUFA deficient group (9.4). The fatty acid modifications mentioned above were reflected by a high n-6/n-3 ratio in the n-3 PUFA deficient lot and total PUFA deficient animals and a reduced n-6/n-3 ratio in the n-6 PUFA deficient animals. The 22:5 n-6/22:6n-3 ratio of phospholipid membrane considered as an index of n-3 PUFA deficiency, if superior to 1, was 1.75 in animals deficient in this n-3 PUFA

family. It was therefore, possible to produce an n-3 PUFA deficiency in this phospholipid. The replacement of n-3 by n-6 PUFAs in phospholipid membranes is sufficient to maintain the fluidity but the physiological functions of each fatty acid family are independent and can not be interchanged.

TABLE 6
Effect of dietary lipids on brain membrane phosphatidylcholine fatty acid composition

Fatty acids (%)	Diets: Control	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
16:0	32.7±0.2^{a}	32.4±2.0^{a}	31.4±2.9^{a}	31.8±2.1^{a}
18:0	13.9±0.5^{a}	14.6±0.8^{ab}	15.2±1.1^{ab}	17.0±2.9^{b}
ΣSFA	**48.1±0.3^{a}**	**48.8±1.7^{a}**	**49.7±2.3^{a}**	**50.6±2.7^{a}**
18:1 n-9	27.4±0.4^{a}	27.0±0.4^{a}	29.1±1.6^{b}	25.4±1.6^{a}
18:1 n-7	7.3±0.2^{a}	7.7±0.4^{a}	6.5.±0.5^{b}	7.2±0.5^{a}
20:1 n-9	1.7±0.2^{a}	1.6±0.2^{a}	1.7±0.3^{a}	1.5±0.4^{a}
20:1 n-7	1.0±0.1^{a}	0.9±0.1^{a}	0.9±0.1^{a}	0.9±0.2^{a}
ΣMUFA	**39.6±0.8^{a}**	**39.2±0.7^{a}**	**40.9±1.7^{a}**	**37.7±3.8^{a}**
18:2 n-6	0.7±0.1^{a}	0.8±0.1^{a}	0.6±01^{a}	1.1±0.5^{b}
20:4 n-6	6.1±0.4^{a}	6.2±0.6^{a}	3.3±0.5^{b}	4.2±0.6^{c}
22:4 n-6	0.6±0.1^{a}	0.9±0.2^{b}	0.4±0.2^{ac}	0.2±0.1^{c}
22:5 n-6	0.3±0.1^{a}	2.4±0.4^{b}	0.2±0.1^{a}	1.9±0.6^{c}
ΣPUFA n-6	**8.6±0.9^{a}**	**10.6±1.2^{b}**	**5.1±0.9^{c}**	**8.8±1.2^{a}**
20:5 n-3	0.1±0.2^{a}	- -	0.3±0.1^{b}	0.3±0.1^{b}
22:5 n-3	0.0±0.0^{a}	- -	0.4±0.1^{b}	0.2±0.1^{c}
22:6 n-3	3.1±0.8^{a}	1.4±0.2^{b}	3.7±0.3^{a}	1.7±0.6^{b}
ΣPUFA n-3	**3.7±0.2^{a}**	**1.4±0.2^{b}**	**4.3±0.2^{c}**	**2.3±0.7^{d}**
Σn-6+n-3	11.9±0.6^{a}	12.0±1.4^{a}	9.4±0.9^{b}	11.1±1.3^{a}
n-6/n-3	2.2±0.1^{a}	7.4±0.7^{b}	1.1±0.2^{c}	3.8±1.3^{d}
22:5 n-6/22:6 n-3	0.09±0.02^{a}	1.75±0.18^{b}	0.03±0.01^{c}	0.9±0.2^{d}

Results are the mean ±standard deviation
control diet = mixture of peanut and rapeseed oil, n-3 PUFA deficient diet = peanut oil, n-6 PUFA deficient diet = cod liver oil, total deficient diet = hydrogenated palm oil.
Means with different letter are statistically different ($p < 0.01$).

Phosphatidylethanolamine

The levels of saturated fatty acids and monounsaturated fatty acids in phosphatidyethanolamine did not differ significantly among groups. A slight increase was observed in oleic acid in aminals fed the n-6 PUFA deficient diet (25.4 vs 22.0). The n-6 PUFA deficient diet produced lower level of 20:4 n-6 (8.2 vs 12.1) and 22:4 n-6 (3.0 vs 6.4), higher levels of the n-3 PUFA (22 vs 16) and a half reduction of the n-6/n-3 ratio (0.7 vs 1.3) than those levels found in the control group (Table 7). The n-3 PUFA deficient diet, on the other hand, provoked an important raise of 22:5 n-6 (11.1 vs1.5), a reduction of 22:6 n-3 (6.7 vs 15.9), high n-6/n-3 ratio (4.8 vs 1.3), and a 22:5 n-6/22:6 n-3 ratio of 1.66 in comparison to the control values. The total (n-6+n-3) PUFA- deficiency produced no change in the sum of total PUFA (n-6+n-3). As expected, phosphatidylethanolamine was most resistant to changes in fatty acids. Nevertheless, the n-6 and n-3 PUFA deficiencies were achieved at a lower level than in PC, meaning probably that PE is more important in physiological functions than PC due to its higher concentration in PUFA.

TABLE 7
Effect of dietary lipids on brain membrane phosphatidylethanolamine fatty acid composition

Fatty acids (%)	Diets: Control	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
16:0	7.9±1.9^{a}	7.5±0.7^{a}	7.1±0.8^{a}	7.1±0.6^{a}
18:0	15.3±0.7^{a}	16.0±1.4^{a}	14.0±1.3^{a}	15.8±0.6^{a}
ΣSFA	**24.5±2.6^{a}**	**24.9±1.8^{a}**	**22.0±1.8^{a}**	**24.4±0.7^{a}**
18:1 n-9	22.0±1.9^{a}	21.0±0.5^{a}	25.4±1.4^{b}	21.3±0.8^{a}
18:1 n-7	4.6±0.9^{a}	4.6±0.2^{a}	4.9±0.4^{a}	4.6±0.4^{a}
20:1. n-9	5.0±0.5^{a}	5.1±0.7^{a}	5.4±0.7^{a}	4.6±0.4^{a}
20:1 n-7	1.3±0.1^{a}	1.3±0.2^{a}	1.1±0.1^{a}	1.1±0.1^{a}
ΣMUFA	**37.4±3.5^{ab}**	**34.1±1.2^{a}**	**40.4±1.5^{b}**	**34.6±1.5^{a}**
18:2 n-6	0.6±0.2^{a}	0.5±0.2^{a}	0.7±0.2^{a}	0.8±0.2^{a}
20:4 n-6	12.1±1.3^{a}	13.8±0.5^{b}	8.2±0.5^{c}	12.2±0.7^{a}
22:4 n-6	6.4±1.0^{ac}	7.5±1.0^{a}	3.0±0.2^{b}	5.6±0.2^{c}
22:5 n-6	1.5±0.4^{a}	11.1±0.7^{b}	2.6±0.5^{c}	9.4±0.7
ΣPUFA n-6	**21.9±2.4^{a}**	**33.9±1.5^{b}**	**15.6±1.8^{c}**	**28.6±1.4^{d}**
20:5 n-3	0.1±0.0^{a}	- -	0.6±0.1^{b}	0.2±0.0^{c}
22:5 n-3	0.1±0.0^{a}	- -	1.5±0.3^{b}	0.1±0.0^{c}
22:6 n-3	15.9±1.9^{a}	6.7±0.7^{b}	19.6±3.4^{c}	9.2±0.8^{d}
ΣPUFA n-3	**16.2±2.0^{a}**	**7.1±0.8^{b}**	**22.0±3.6^{c}**	**10.0±1.0^{d}**
Σn-6+n-3	38.1±1.8^{a}	41.0±1.1^{b}	37.6±2.4^{a}	38.6±1.4^{a}
n-6/n-3	1.3±0.1^{a}	4.8±0.7^{b}	0.7±0.2^{c}	2.9±0.3^{d}
22:5 n-6/22:6 n-3	0.09±0.0^{a}	1.66±0.24^{b}	0.15±0.04^{c}	1.02±0.15^{d}

Results are the mean ±standard deviation of ten animals in each group control diet = mixture of peanut and rapeseed oil, n-3 PUFA deficient diet = peanut oil, n-6 PUFA deficient diet = cod liver oil, total deficient diet = hydrogenated palm oil.

Phosphatidylserine

Even though stearic acid the major saturated fatty acid was not modified by diet, total saturated fatty acids was slightly reduced by the n-6 PUFA deficient diet (Table 8).

TABLE 8
Effect of dietary lipids on brain membrane phosphatidylserine fatty acid composition

Fatty acids (%)	Diets: Control	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
16:0	6.7± 1.3^{ac}	8.4 ± 1.1^{b}	6.5 ± 0.3^{a}	8.6 ± 1.7^{bc}
18:0	30.5+2.1^{a}	30.1 ± 0.9^{a}	28.0 ± 2.9^{a}	30.0 ± 2.3^{a}
ΣSFA	**41.4 ±2.8^{ab}**	**42.6 ± 1.8^{a}**	**38.1 ± 2.6^{b}**	**40.0±3.6^{ab}**
18:1 n-9	22.2 ± 1.7^{a}	20.3 ± 0.7^{b}	25.7 ± 2.0^{c}	20.0±1.4^{b}
18:1 n-7	2.5 ± 0.3^{a}	3.0 ± 0.6^{a}	2.9 ± 0.3^{a}	2.6 ± 0.6^{a}
20:1 n-9	2.3 ± 0.4^{a}	2.4 ± 0.4^{a}	2.5 ± 0.2^{a}	2.0 ± 0.4^{a}
20:1 n-7	0.6 ± 0.2^{a}	0.6 ± 0.1^{a}	0.1 ± 0.0^{b}	0.7 ± 0.3^{a}
ΣMUFA	**31.4± 1.3^{a}**	**30.6 ± 1.8^{a}**	**36.5 ± 1.9^{b}**	**30.2 ± 1.4^{a}**
18:2 n-6	1.0 ± 0.1^{a}	0.9 ± 0.2^{a}	2.2 ± 0.8^{b}	1.3±0.1^{c}
20:4 n-6	4.8± 0.7^{a}	4.6 ± 0.4^{a}	2.5 ± 0.2^{b}	3.7 ± 0.6^{c}
22:4 n-6	2.7 ± 0.3^{a}	3.0 ± 0.5^{a}	2.6 ± 1.0^{a}	2.3 ± 0.4^{a}
22:5 n-6	2.4 ±0.7^{a}	11.0 ± 2.1^{b}	0.7 ± 0.2^{c}	9.4 ± 2.5^{b}
ΣPUFA n-6	**11.6 ± 0.5^{a}**	**20.7 ± 2.2^{b}**	**8.5 ± 2.0^{c}**	**19.3 ± 3.0^{b}**
18:3 n-3	0.2 ± 0.1^{a}	- -	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}
20:5 n-3	0.3 ± 0.2^{a}	0.2 ± 0.1^{a}	0.3 ± 0.2^{a}	0.3 ± 0.1^{a}
22:5 n-3	0.1 ± 0.0^{a}	0.2 ± 0.1^{a}	0.6 ± 0.3^{b}	0.1 ± 0.0^{a}
22:6 n-3	15.0 ± 3.2^{a}	5.6 ± 0.5^{b}	15.3 ± 2.1^{a}	8.2 ± 1.2^{c}
ΣPUFA n-3	**15.6± 3.5^{a}**	**6.1 ± 0.3^{b}**	**16.9 ± 2.0^{a}**	**8.9 ± 0.9^{c}**
Σn-6+n-3	27.2±3.4^{a}	26.8 ± 2.3^{a}	25.4 ± 3.9^{a}	28.2 ± 3.6^{a}
n-6/n-3	0.7 ± 0.2^{a}	3.4 ± 0.4^{b}	0.5 ± 0.1^{a}	2.2 ± 0.3^{c}
22:5 n-6/22:6 n-3	0.16±0.05^{a}	1.95±0.27^{b}	0.05±0.00^{c}	1.15±0.19^{d}

Same as Table 7

This reduction was compensated by the high levels of MUFA mainly due to oleic acid, the major fatty acid of this family. Arachidonic acid was lower in animals fed the n-6 PUFA deficient diet and in a less extent by the total PUFA deficient diet than th level of 20:4 n-6 found in the control group (Table 8). N-6 PUFA deficient animals presented significantly ($p < 0.01$) reduced levels of 22:5 n-6 (0.7 vs 2.4). The n-3 PUFA deficient diet produced an extremely higher 22:5 n-6 content in PS (11.0 vs 2.4) and lower 22:6 n-3 (5.6 vs 15.0), producing as consequence a higher n-6/n-3 ratio than the control animals. It was interesting to observe that, in spite of the important modifications in each of the two PUFA families, the sum of n-6 + n-3 was not altered by the diet.

Phosphatidylinositol

The level of palmitic acid and stearic acid was not modified in phosphatidylinositol whatever the diet considered, which has as consequence similar total saturated fatty acids in all groups (Table 9).

TABLE 9
Effect of dietary lipids on brain membrane phosphatidylinositol fatty acid composition

Fatty acids (%)	Diets			
	Control	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
16:0	11.7 ± 1.9^{a}	15.0 ± 4.3^{a}	13.6 ± 1.1^{a}	16.2 ± 3.7^{a}
18:0	25.4 ± 2.2^{a}	24.7 ± 2.5^{a}	23.5 ± 1.6^{a}	23.7 ± 3.2^{a}
ΣSFA	**41.5±3.4^{a}**	**45.2±4.9^{a}**	**41.8±1.0^{a}**	**45.6±6.8^{a}**
18:1 n-9	8.9 ± 1.3	8.3 ± 1.4^{a}	11.5 ± 1.5^{b}	10.4 ± 0.7^{b}
18:1 n-7	2.4 ± 0.2^{a}	2.2 ± 0.3^{a}	2.5 ± 0.3^{a}	2.4 ± 0.5^{a}
20:1 N-9	1.3 ± 0.4^{a}	1.0 ± 0.2^{a}	0.9 ± 0.1^{a}	1.0 ± 0.3^{a}
20:1 N-7	0.5 ± 0.2^{a}	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}
ΣMUFA	**17.5±2.6^{a}**	**16.8±2.9^{a}**	**20.3±2.5^{b}**	**20.5±1.4^{b}**
18:2 n-6	1.4 ± 0.4^{a}	2.4 ± 0.5^{b}	2.0 ± 0.3^{b}	2.2 ± 0.6^{b}
20:4 n-6	31.5 ± 4.2^{a}	28.9 ± 3.4^{ab}	25.2 ± 3.4^{b}	23.1 ± 5.2^{b}
22:4 n-6	1.4 ± 0.4^{a}	1.1 ± 0.2^{a}	0.4 ± 0.2^{b}	0.9 ± 0.4^{c}
22:5 n-6	1.1 ± 0.3^{a}	1.7 ± 0.4^{a}	0.5 ± 0.2^{b}	1.3 ± 0.7^{a}
ΣPUFA n-6	**36.2±3.9^{a}**	**35.0±5.5^{ab}**	**29.9±3.4^{b}**	**28.1±5.7^{b}**
18:3 n-3	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	0.6 ± 0.1^{b}	0.4 ± 0.2^{ab}
20:5 n-3	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	1.5 ± 0.2^{b}	0.4 ± 0.1^{c}
22:5 n-3	0.5 ± 0.2^{a}	0.8 ± 0.2^{a}	1.1 ± 0.2^{b}	0.8 ± 0.2^{a}
22:6 n-3	3.5 ± 0.8^{a}	1.6 ± 0.3^{bc}	4.0 ± 0.4^{a}	2.0 ± 1.0^{c}
ΣPUFA n-3	**4.8±1.0^{a}**	**3.0±0.5^{b}**	**8.0±0.9^{c}**	**4.0±1.2^{ab}**
Σn-6+n-3	41.2±3.8^{a}	38.0±5.1^{ab}	37.9±3.3^{ab}	32.1±6.5^{b}
n-6/n-3	7.5±2.2^{a}	11.5±3.5^{b}	3.7±0.6^{c}	7.0±1.7^{a}
22:5 n-6/22:6 n-3	0.3±0.04^{a}	1.02±0.05^{b}	0.12±0.06^{b}	0.86±0.3^{a}

Same as Table 7

Oleic acid and total MUFA was observed to be higher in PI from n-6 and total PUFA deficient animals than those from the control and the n-3 PUFA deficient animals. Dietary n-6 PUFA and total PUFA deficient diets produced lower levels of arachidonic acid and total n-6 PUFA (Table 9). In PI, the fish oil diet increased significantly ($p < 0.01$). the levels of total n-3 PUFA (8.0 vs 4.8), due primarily to 20:5 and 22:5 n-3. Eventough total (n-6+n-3) PUFA was diminished by the total PUFA deficient diet, the n-6/n-3 ratio was maintained in PI from animals receiving this diet. The latter was, however, increased (11.5 vs 7.5) and reduced (3.7 vs 7.5) by the dietary n-3 and n-6 PUFA deficient diets respectively. It is interesting to note that of all phospholipids analyzed here, phosphatidylinositol presented the highest n-6/n-3 ratios and not necessarily the highest n-3 PUFA levels which was observed in phosphatidylethanolamine.

DISCUSSION

Total PUFA deficiencies have been related to reduced growth in general, the reduction in body weight seems to be a distinctive characteristic of this essential fatty acid families. The mechanism responsible for this reduced weight during PUFA deficiency is through a reduced caloric intake rather than any other physiological mechanism (21).

It has been shown (22,23) a diminished effect of PUFA on cholesterol levels in rat liver and heart tissue. This hipocholesterolemic effect of PUFA was attributed to the reduced HMG-CoA reductase activity in liver microsome, and to increase level of HDL (high density lipoproteins). However, in the literature review, we did not find any report on dietary fatty acid effects on brain membrane cholesterol levels. Apparently, the effects observed in muscle, liver and heart are not necessarily observed in this highly protected organ, where n-3 PUFA had no effect on cholesterol levels and the effects of n-6 and total PUFA deficiencies were inverse.

In our study, the PL proportions were modulated by the dietary fatty acids individually. Brain cells are very rich in polyunsaturated fatty acids which predominate in PE the major phospholipid in brain. In this respect, it was not surprising to find PE to be the most predominant phospholipid in this particular organ. Alsted and Hoy (24) reported similar fatty acid patterns in PE and PS after supplementation with fish oil at a 20 weight % fat diet after two generations. Our results confirmed their results using lower (5%) fat levels in the diet.

The literature review in regard to the effects of dietary PUFA on brain membrane phospholipid (PL) composition does not include the individual phospholipid fatty acid composition but the total phospholipid fatty acid composition are most frequently reported. Alsted & Loyd (24) reported the fatty acid profile of brain subclasses of rats fed n-3 PUFA either from marine oil or vegetable oil. Their conclusions were that, as expected, fish oil demonstrated to be a better source than 18:3 n-3 to incorporate n-3 PUFA into phospholipids in brain. In our study, fish oil increased, in general, the levels of 22:6, 20:5 and 22:5 n-3 and reduced the 20:4 and 22:5 n-6 as reported by the authors above.

Although, in general, n-3 dietary deficiency produced reduced n-3 fatty acids in each phospholipid, compensated by a high incorporation of n-6 fatty acids and the n-6 PUFA dietary deficiency produced a reduced level of n-6 fatty acid compensated by a high level of n-3 PUFA, the degree of fatty acid modification of each phospholipid class was different.

Therefore, it is important to measure not only total phospholipid fatty acid composition but also each phospholipid class in order to have more information as to which PL is most modified by the dietary manipulations, specially when considering physiological changes known to be correlated to a specific phospholipid microenvironment. In this study, PC manifested greater changes than PE, although both were deprived of essential fatty acids. In fact, the 22:5 n-6/22:6n-3 ratio of phospholipid membrane considered as an index of n-3 PUFA deficiency, if superior to 1, was 1.75 in animals deficient in the n-3 PUFA family. It was therefore, possible to produce an n-3 PUFA deficiency in phospholipids. The replacement of n-3 by n-6 PUFAs in phospholipid membranes may be sufficient to maintain the fluidity but the physiological functions of each fatty acid family are independent and can not be interchanged. Therefore, these changes may be related to physiological functions such as: monoaminergic neurotransmission, learning ability and visual acuity.

The role of n-6 and n-3 PUFA in dietary treatments of metabolic disorders was generated from epidemiological studies which support a recommendation to increase the consumption of n-3 PUFA without reducing the intake of n-6 PUFA (arachidonic acid), particularly in communities having low n-3 fatty acid intake, as it is the case of western countries (25,26). In conclusion, the importance of supplying sufficient dietary essential fatty acids (EFA), specially during the growing stage, is manifest in our results since dietary EFA deficiencies are able to change the fatty acid composition of phospholipids in brain.

REFERENCES

1. McMurphy M. Dietary fatty acids and membrane function. J Nutr Biochem, 1990; 1, 68-79.
2. Angulo MO, Guesnet Ph, Durand G. Effects of alfa-linolenic acid deficiency and age on oxidative phosphorylation and fatty acid composition of rat liver mitochondria. J Nutr Biochem, 1991; 2, 484-491.
3. Burr GO and Burr MM. A new deficiency disease produced by the rigid exclusion of fat from the diet. J Biol Chem, 1929; 82, 2, 345.
4. Burr GO and Burr MM. On the nature and role of the fatty acids essential in nutrition. J Biol Chem, 1930;86, 587-621.
5. Lamptey MS and Walker BL. A possible essential role of dietary linolenic acid in the development of the young rat. J Nutr, 1976;106, 86-93.
6. Neuringer M, Connor W, Lin D, Barstad L. and Luck S. Biochemical and functional effects of prenatal and post-natal omega-3 fatty acids deficiency on retina and brain in rhesus monkeys. Proc. Natl. Acad. Sci. U.S.A. 1986; 83, 285-294.
7. Bourre JM, Bonneil M, Clement M, Dumont O, Durand G, Lafornt H, Nalbone G & Piciotti M. Function of dietary polyunsaturated fatty acids in the nervous system Prostaglandins-Leukot-Essent-Fatty-Acids Jan; 1993;48(1):5-15.
8. Neuringer M, Connor W. N-3 fatty acids in the brain and the retina. Evidence for their essentiality. Nutr Rev, 1986; 44,9 295-294.
9. Carlson S and Salem N. Essentiality of w3 fatty acids in growth and development in infants. In Health effects of omega 3 polyunsaturated fatty acids in deafood, Worl Rev Nutr Diet, ed. Basel Karger, 1991; vol 66, 74-86.
10. Neuringer M, Anderson G, Connor W. The essentiality of n-3 fatty acids for the development and function of the retina and the brain. Ann Rev Nutr, 1993; 8, 517-541, 1988.
11. Jokota A. Relationship between polyunsaurated fatty acid and learning ability in the brain of rat fetus and newborn. Nippon-Sanka-Fujinka-Gakkai-Zashi. 45,1,15-22.
12. Bourre JM, Francois M, Youyou A, Dumont O, Piciotti M, Pascal G & Durand G. The effect of dietary a-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poison and performance of learning tasks in rats. Lipids, 1992;119, 1880-1892.
13. Neuringer M, Connor W, Van Petten C, Barstad L. Dietary omega -3 fatty acid deficiency and visual loss in infant rhesus monkeys. J Clin Invest, 1984; 73, 272-276.
14. Angulo GO. Polyunsaturated fatty acid deficiencies: effects on hepatic plasma membrane fatty acid composition and enzyme activity. Arch Latinoamer Nutr. 1995; 48, 5-15.
15. Epping RJ, and Bygrave FL. A Procedure For The Rapid Isolation From Rat Liver Of Plasma Membrane Exhibiting Ca+2- Transport And Ca+2-AT Pase Activities. Biochem J, 1984; 223, 733-745.
16. Bradford MA Rapid And Sensitive Method For The Quantification Of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye-Binding. Annal Biochem, 1976;72, 248-254.
17. Folch J, Lees M, Sloane-Stanley GH. A Simple Method For The Isolation And Purification Of Total Lipids From Animal Tissues. Biol Chem,1957; 226,497-506.
18. Wolff R. Mise Au Point Sur Le Dosage Enzymatique Des Esters De Cholestérol a Chaines Polyunsaturés Très Longues. Rev Franç. Des Corps Gras, 1985; 32, 3,111-115.
19. Bartlett GR. Phosphorus Essay In Column Chromatography. J Biol Chem,1959; 234, 466-468.
20. Morrison WR. And Smith LM. Preparation of Fatty Acid Methyl Esters And Dimethylacetals From Lipids With Boron Fluoride-Methanol., J Lipid Research, 1964; 5, 600-608.
21. MacDonald RS, Zhang W, Shang JP, Sun GY. Brain neutral lipids and phospholipids are modified by long term feeding of beef tallow vs corn oil diets. J Nutr. 1996;126, 1554-1562.
22. Park BS, Lee NH & Rhee YC. Effect of dietary Omega 3-PUFA and Omega-6 PUFA on redistribution of cholesterol from blood to tissue in rat. Korean J. Animal Nutr. and Feedstuffs 1992(a); 16(4) 176-182.
23. Park BS, Seon KS, Yoon CS, Hwangbo J, Lee HW, Lee NN & Rhee YC. Effect of Omega-PUFA on cholesterol synthesis and Steroid excretion in rats Korean J. Animal Nutr. and Feedstuffs, 1992(b); 16(5) 283-290.
24. Alsted AL & Hoy CE. The fatty acid profile of brain phospholipid subclasses of rats fed n-3 PUFA of marine or vegetable origen: A two generation study. Biochim Biophys. Acta, 1992;1125, (3) 237-244.
25. Uauy-Dagach R and Valenzuela A. Marine oils as a source of omega-3 fatty acids in the diet: how to optimize the health benefits. Prog Food Nutr Sci, 1992; 16, 3,199-243.
26. Collier GR and Sinclair AJ. The Role of N-6 and N-3 fatty acids in the dietary treatment of metabolic disorders. Ann New York Academy of Science, 1993; 683: 322-330.

Recibido: 14-10-1997.
Aceptado: 19-06-1998.