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

Vol. 45 N° 4, 1995

Polyunsaturated fatty acid deficiencies: effects on hepatic plasma membrane fatty acid composition and enzyme activity

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SUMMARY. Research on dietary polyunsaturated fatty acids (PUFA), on the activity of 5'nucleotidase and adenylate cyclase are largely contradictory due, mostly, to the absence of adequate control group. In this study, four different diets have been evaluated on the 5'nucleotidase and adenylate cyclase activities in rat liver plasma membranes. Wistar rats were given a semysinthetic diet in which lipids were supplied by 5% of either peanut oil (n-3 PUFA deficient diet), cod liver oil (n-6 PUFA deficient diet) partially hydrogenated palm oil (total PUFA deficient diet) and a mixture of peanut and rapeseed oil (control group). Liver plasma membranes were separated by using a Percoll gradient in a Beckman JA 20 centrifuge. 5'nucleotidase and adenylate cyclase activities were measured in a liquid scintilation detector by following the degradation of ^{3}HAMP (adenosine monophosphate) and production of ^{3}HcAMP (cyclic adenosine monophosphate) respectively. Animals fed the total PUFA deficient diet exhibited significant lower body weight and lower liver weight than did the control group. Low cholesterol concentrations were observed in animals deficient either in n-3 or total PUFA in relation to the control group. All dietary deficiencies studies provoked reduced phospholipid levels. Phosphatidylcholine and phosphatidylethanolamine were not modified whatever the deficiency studied. Phospholipids fatty acid composition was significantly modified by the diets studied. The specific activity of 5'nucleotidase in hepatic plasma membrane was independent of dietary PUFA. The catalytic unit of adenylate cyclase complex in totally deficient animals was augmented. The unit of the enzyme stimulated by the guanydyl imidodiphosphate (GppNHp) in n-3 PUFA deficient animals was augmented and reduced in animals receiving the n-6 PUFA deficient diet. In conclusion, our results show that each dietary PUFA deficiency modifies differently the proportions of phospholipid classes and their fatty acid composition. The mechanisms responsible for these modification remain to be elucidated. However, the phospholipid fatty acid changes did not influence the 5'nucleotidase activity except in the case of extreme excess which concerns more toxicology than nutritional modifications. Finally, the catalytic unit (Forskoline+GDPßs) of adenylate cyclase complex and the regulatory unit (GppNHp) may be sensitive to alterations in PUFA composition.

RESUMEN. Deficiencias de ácidos grasos poliinsaturados: Efectos sobre la composición lipídica y actividades enzimáticas hepáticas. Los resultados de los trabajos realizados sobre los efectos de los ácidos grasos poliinsaturados (AGPI) dietarios en las actividades de la 5'nucleotidasa y adenilato ciclasa son contradictorios debido principalmente a las diferentes dietas usadas como testigo. En este trabajo se evaluaron 4 dietas diferentes sobre la actividad de la 5'nucleotidasa y de la adenilato ciclasa de la membrana plasmática hepática de rata. Ratas Wistar recibieron una dieta semisintética con 5% de aceite de cacahuate (Dieta deficiente en AGPI n-3), aceite de hígado de bacalao (Dieta deficiente en AGPI n-6), aceite de palma parcialmente hidrogenada (Dieta totalmente deficiente en AGPI) y una mezcla de aceite de cacahuate y colza (Dieta testigo). La membrana plasmática se obtuvo por centrifugación con gradiente de Percoll. Las actividades de la 5'nucleotidasa y adenilato ciclasa se midieron por centelleo líquido siguiendo la degradación de ^{3}HAMP (adenosina monofosfato) y producción de ^{3}HAMPc (adenosina monofosfato cíclico). Los resultados muestran que los animales alimentados con la dieta deficiente en AGPI totales presentaron pesos corporales y peso de hígado menores que los animales testigo. Se observaron niveles de colesterol menores en los animales deficientes en AGPI n-3 y en AGPI totales. Todos los tratamientos dietarios redujeron los niveles de fosfolípidos. La dieta no modificó las proporciones de fosfatidilcolina y fosfatidiletanolamina. La composición lipídica de los fosfolípidos fue drásticamente modificada por las diferentes deficiencias dietarias en AGPI. La actividad específica de la 5'nucleotidasa de la membrana plasmática hepática no se modificó por las diferentes deficiencias dietarias estudiadas. Sin embargo, la actividad catalítica (Forscolina+GDPßs) del complejo adenilato ciclasa aumentó en los animales totalmente deficientes en AGPI. La unidad de la enzima catalizada por el GppNHP en los animales que recibieron la dieta deficiente en AGPI n-3 aumentó y disminuyó en los animales deficientes en AGPI n-6. En conclusión, estos resultados muestran que las deficiencias dietarias en AGPI modifican en forma diferente las proporciones de las clases de fosfolípidos y su composición lipídica. Los mecanismos responsables de estas modificaciones no han sido elucidados. Sin embargo, los cambios en la composición en ácidos grasos no modificó la actividad de la 5'nucleotidasa. Finalmente, la unidad catalítica (Forscolina+GDPßs) y la unidad regulatoria (GppNHp) del complejo adenilato ciclasa pueden ser sensibles a las alteraciones en la composición de AGPI.

INTRODUCTION

Plasma membrane proteins (enzymes, receptors, and ionic channels) are directly related to the membrane lipid bilayer. Examples of these are the 5'nucleotidase, adenylate cyclase and Na-K-ATPPase. The 5'nucleotidase catalyzes the dephosphorylation of adenosine 5'monophosphate (5'AMP). Adenylate cyclase is directly related to the cellular communication and as such plays a fundamental role in regulating cellular metabolism via the cyclic adenosine monophosphate (cAMP).

Research on dietary polyunsaturated fatty acids (PUFA) on the activity of 5'nucleotidase and adenylate cyclase from hepatic plasma membrane began in 1970(1). Total dietary PUFA deficiency in the rat reduced the activities of 5'nucleotidase and adenylate cyclase activities from hepatic plasma membrane (2,3). However, the studies regarding the dietary n-3 and n-6 PUFA deficiencies are largely contradictory. Some authors observed increased activity of 5'nucleotidase in hepatic plasma membrane (4), cardiac sarcolema (5) and intestinal brush border membrane (6) whereas Zuniga and Kinsella (7) and Baracca et al (8) showed no effect on this activity by a diet rich in PUFA. Morson and Clandinin (9) reported no relationship between n-6 dietary PUFA and adenylate cyclase activity. However, for Leee and Ham (10) the adenylate cyclase activity is directly related to dietary n-6 PUFA. For some groups, when dietary n-3 PUFA increase, 5'nucleotidase and adenylate cyclase activities in liver and heart plasma membrane increase as well (11, 12, 13). Although there is a vast volume of literature associated with the biological significance of polyunsaturated fatty acids, the results of these research work are largely contradictory due mostly to the absence of similarities in the control group. This factor was taken into account in our study in which the n-6 and n-3 dietary PUFA families were analyzed independently as well as the total PUFA deficiency in relation to the control group that contains both PUFA families (n-6+n-3). Therefore, in this study, four different diets, in which PUFA type was the only modifying factor, have been evaluated on the lipid composition and the 5'nucleotidase and adenylate cyclase activities in rat liver plasma membranes.

MATERIALS AND METHODS

Experimental animals: Wistar rats were given a semysinthetic diet in which lipids were supplied by 5% of oil (Table 1). Two weeks before mating at 10 weeks of age, four groups were constituted. The control group was fed a purified diet in which lipid supply consisted of peanut and repeseed oil mixture (Group 1). The n-3 PUFA deficient group was given a purified diet which contained peanut oil as lipid source (Group 2). The n-6 PUFA deficient diet was obtained by using cod liver oil as lipid source (Group 3). The total PUFA deficient group was fed a purified diet inwhich lipid supply consisted of partially hydrogenated palm oil (Group 4). The fatty acid composition of dietary lipids is presented in Table 2. The deficient and the control group were fed their respective diets for two generations. Only the males of the second generation were used for this experiment. After delivery females were caged individually and the litters were equalized to 10 pups each. Then animals were killed by decapitation at adult age (90 days).

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: $CaHPO_4$ $2H_4$ O, 38.0; K_2 HPO_4 24.0; $CaCO_3$, 18.0; NaCl, 6.9; Mg0. 2.0; $MgSO_4$ $7H_20$, 9.0; $FeSO_4$. $7H_2$ O,, 0.086; $ZnSO_4$ H_2 O, 0.5; $MnSO_4$ H_2 O, 0.5; $CuSO_4$ $5H_2$ O, 01; NaF, 0.08; $CrK(SO_4)_2$ H_2 O, 0.05; $(NH_4)_6$ Mo_7 O_{24} $4H_2$ O, 0.002; KI, 0.004; $CoCO_3$, 0.002; $NaSeO_3$ $5H_2$ O, 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
16:0	10.1	11.9	10.7	51.5
18:0	2.3	2.9	1.6	8.4
Σ Saturated	14.9	18.2	20.2	63.1
18:1 n-9	53.3	56.1	19.1	28.0
18:1 n-7	6.0	3.1	4.1	4.9
Σ Monounsaturated	61.3	61.8	53.2	35.1
18:2 n-6	19.8	19.9	2.2	1.8
Σ PUFA n-6	19.8	19.9	2.9	1.8
18:3 n-3	4.0	0.1	1.2	—
20:5 n-3	—	—	8.5	—
22:5 n-3	—	—	2.4	—
22:6 n-3	—	—	9.0	—
Σ PUFA n-3	4.0	0.1	23.7	—
Σ Total PUFA	23.8	20.0	26.6	1.8
n-6/n-3	5.0	199.0	0.1	—
Fatty acids/100 g of diet:				
n-6 PUFA (mg)	930.6	935.3	136.3	84.6
N-3 PUFA (mg)	188.0	4.7	1113.9	—

Note: Lipid fatty acid composition was analyzed by gas chromatography of fatty acid methyl esters in the following conditions: Carlo Erba Chromatograph model 4180, automated injection using flame ionization detector, silica capillary column fused with carbowax 52.
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: Liver plasma membranes were separated by the method of Epping and Bygrave (14). Briefly, 10 g of liver were homogenized with 15 ml of cold buffer using a Teflon Potter homogenizer (Thomas C) using 15 up and down strokes at 2.000 rpm. The homogenate was filtered through a tissue and centrifuged at 10.000 rpm (12.000 x g) for 10 minutes in a Beckman JA20 centrifuge. The supernatan, 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, St. Quintin Yvelines, France) and centrrifuged at 20.000 rpm (48.000 x g) for 4 minutes at 4 °C. The plasma membrane fraction was collected at the 10%-0% interface. This fraction was measured and incubated at 37 °C for 10 minutes. Then it 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 under the conditions mentioned above. The plasma membranes obtained were immediately used for enzyme activity measurements. Protein analysis was done by the Bradford (15) technique.

Plasma membrane lipid analysis: Total lipids from plasma membrane were extracted by the Folch et al (16) procedure in the presence of butyl hydroxytoluene (BHT) at a 0.02% (wt/v). Cholesterol was assayed enzymatically by the Wolff's method (17). Total phospholipids were determined by measuring the total phosphorus as described by Bartlett et al (18).

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 BF_3-methanol (10% wt/v) reagent at 90 °C for 20 min (19). Fatty acid methyl esters (FAME) were extractedd using hexane and analyzed by gas chromatography using a Carlo Erba 4180 model with automated 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 standars (SIGMA, St. Louis, MO, U.S.A.).

Enzyme activity: 5'Nucleotidase (E.C.3.1.3.5) assay 5'nucleotidase activity of liver plasma membrane was determined by measuring the degradation of ^{3}HAMP (adenosine monophosphate) in a liquid scintillation detector (Packard model TRI-CARB 1500) (20). The assay was carried at 37 °C during 60 min. in an incubation mixture containing Tris/HCl 50 mM, $MgCl_2$ 180 mM, AMP(Na) 254 mM and (^{3}H) AMP 15 Ci/nmole, with 500 ng of protein. The enzyme activity was calculated taking into account the isotope specific activity ($33x10^6$ dpm corresponding to 1 nmole of (^{3}H)AMP hydrolyzed).

Adenylate Cyclase (E.C. 4.6.1.1.) assay : Adenylate cyclase activity of liver plasma membrane was determined according to Salesse R. (21) by measurement of labeled cyclic (^{3}H) AMP generated from the substrate ATP in the presence of µg protein. The reaction was carried out at 37 °C during 20 min. The incubation mixture contained creatine phosphate (100 mM), creatine phosphokinase (375 U/ml), myokinase (850 ug/ml), ATP 0.1M, adenosine deaminase (1000 U/ml), isobutylmethylxanthine (200 mg/200 µl DMSO). Basal adenylate cyclase activity was mesured in the presence of GTP (10 mM) and stimulated adenylate cyclase activity was determined replacing GTP by gucagon ($3.3\ 10^{-5}$ to $3.3\ 10^{-10}$ M), GppNHp (10mM), forskoline (300 mM) and forskoline + GDP ßs fixing it to me protein kinase (4 mg/ml) in the presence of saturated ^{3}H cAMP (2.2 pmoles/assay). The cyclic AMP produced was measured by liquid scintillation after separation fromo the incubating mixture.

Statistical methods: Results were analyzed statistically by standard analysis of variance (ANOVA) using a probability level of (p) <0.05

RESULTS

Body and liver weight (Table 3)*:* Animals fed diets containing partially hydrogenated palm oil exhibited statistically significant lower body weight (247 ± 35 g) and lower liver weight (11.7 ± 1.1 g) than did the control group (303 ± 37 g, 14.8 ± 0.9 g). N-3 PUFA and n-6 PUFA deficient animals presented reduced body weight, this reduction however, was not statistically different. Liver weight (12.7 ± 0.5) was also significantly reduced by the n-3 PUFA deficiency.

TABLE 3
DIETARY FATTY ACID EFFECTS ON BODY AND LIVER WEIGHT

PUFA	DIETS CONTROL	n-3 PUFA Deficient	n-6 PUFA Deficient	Total Deficient
Body weight	302.0±37.0^a	275.0±36.0^{ab}	281.0±45.0^{ab}	247.0±35^b
Liver weight	14.8±0.9^a	12.7±0.5^c	14.7±1.7^a	11.7±1.1^b

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.

Membrane lipid composition

Cholesterol and phospholipids (Table 4)

Statistically low cholesterol was observed in animals deficient in either n-3 PUFA or total PUFA in relation to the control group. Animals receiving the n-6 PUFA deficient diet showed no difference in cholesterol level. Phospholipid levels were statistically reduced by all dietary PUFA deficiencies studied.

TABLE 4
DIETARY FATTY ACID EFFECTS ON MEMBRANE LIPID COMPOSITION

Parameters (μg/mg)	DIETS CONTROL	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
Cholesterol/ Protein	63.2±3.6^{a}	44.8±11.7^{b}	56.3±16.4^{ab}	35.4±11.6^{b}
Phospholipids/ Protein	466.0±48.0^{a}	258.0±55.0^{b}	212.0±16.0^{b}	214.0±26.0^{b}
CHL/PL	135.0±16.0^{a}	181.0±51.0^{ab}	270.0±92.0^{b}	180.0±34.0^{b}

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.

Phospholipid classes (Table 5)

The major membrane phospholipid, phosphatidylcholine, was not modified in any group. Phosphatidylethanolamine remained slightly higher in the n-3 PUFA-deficient animals in comparison to the other groups, this difference, however, is not statistically significant. Phosphatidylserine, a minor phospholipid, was either reduced (17%) by the total PUFA deficient diet or augmented (20%) by the individual n-6 and n-3 PUFA deficient diets. The level of phosphatidylinositol was reduced in animals receiving the total PUFA deficient (35%) and the n-3 PUFA (20%) deficient diets. The diet containing the n-6 PUFA provoked a diminished level of sphingomyelin whereas the diet containing either n-3 PUFA or the two of the families produced an increase in sphingomyelin. This was reflected as a 3 fold increase of sphingomyelin level in n-6 PUFA-deficient animals than in either n-3 PUFA and (n-6+n-3) PUFA deficient animals.

Table 5
Dietary fatty acid effects on membrane phospholipid classes

Phospholipids (μg/mg)	CONTROL	DIETS n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
Phosphatidylcholine	56.7±3.3a	55.7±5.8^{a}	53.4±4.9^{a}	57.9±8.2^{a}
Phosphatidyle-thanolamine	22.3±4.0^{ab}	25.0±3.2^{a}	20.0±2.1^{b}	23.0±5.4^{ab}
Phosphatidylserine	7.8±1.5^{ab}	9.4±2.4^{a}	9.6±2.9^{a}	6.5±1.9^{b}
Phosphatidylimositol	8.4±1.4^{ac}	7.0±0.7^{ab}	9.4±2.1^{c}	6.2±1.7^{b}
Sphingomyelin	4.8±0.8^{a}	2.9±0.7^{c}	7.6±2.6^{b}	6.4±1.3^{b}

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.

Phospholipid fatty acid composition

Phosphatidylcholine (Table 6)

In general, the levels of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) in phosphatidylcholine were not significantly modified by the diets studied.

However, both n-6 and n-3 PUFA families were reciprocally replaced by the respective fatty acid family. In fact, it was observed a reduced level of n-6 PUFA in animals receiving the diet rich in n-3 PUFA and the diet deficient in all PUFA. This observation comes primarily from the reduction of arachidonic acid level. Similarly, n-3 PUFA were reduced 50% by the diet rich in n-6 PUFA and by the total PUFA deficient diet. This n-3 PUFA family was elevated 3 fold in PC from animals receiving the n-6 PUFA deficient diet due primarily to the high level of EPA. Total (n-6 + n-3) PUFA were maintained for the n-3 PUFA deficient group but reduced for the n-6 PUFA and (n-6+n-3) PUFA groups.

The fatty acid modification mentioned above were reflected by a high n-6/n-3 ratio in the n-3 PUFA deficient animals and a pronounced n-6/n-3 ratio reduction in the n-6 PUFA deficient animals, whereas the ratio was maintained by the total PUFA deficient group.

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 1.8 in animal deficient in this n-3 PUFA family. The presence of 20:3 n-9 (5% of total FA) in animals deficient intotal PUFA is a sign of this kind of deficiency.

Phosphatidylethanolamine (Table 7)

The level of palmitic acid in phosphatidylethanolamine was significantly reduced by the total PUFA and the n-6 PUFA deficiencies, whereas the n-3 PUFA deficiency lead to a high level of this fatty acid. The n-6 and total deficiencies produced high levels of oleic acid (18:1 n-9). The n-6 PUFA deficient diet produced a significant reduction of 20:4 n-6, high level of the 20:5 n-3 and a reduction of the n-6/n-3 ratio.

The n-3 PUFA deficient diet produced a reduction of 22:6 n-3, high n-6/n-3 ratio, a 22:5 n-6/22:6 n-3 ratio of 1.83, indicating that the n-3 PUFA deficiency was achieved.

TABLE 6
EFFECT OF DIETARY LIPIDS ON MEMBRANE PHOSPHATIDYLCHOLINE FATTY ACID COMPOSITION

FATTY ACIDS (%)	DIETS			
	CONTROL	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
14:0	1.5±0.3^{a}	1.5±0.5^{a}	1.4±0.5^{a}	1.4±0.1^{a}
16:0	23.6±1.3^{ac}	25.8±1.6^{ab}	29.0±2.7^{b}	22.7±1.4^{c}
18:0	18.1±1.5^{ab}	17.7±2.4^{a}	16.2±2.9^{a}	18.1±1.9^{b}
ΣSFA	44.7±3.1^{a}	46.5±1.1^{a}	48.7±1.8^{a}	47.8±1.8^{a}
16:1 n-7	2.5±0.3^{ab}	3.2±0.9^{a}	2.0±0.3^{b}	6.1±1.4^{c}
18:1 n-9	9.0±1.6^{a}	10.7±1.8^{a}	12.7±2.8^{b}	16.2±0.8^{b}
18:1 n-7	2.6±0.6^{ab}	1.7±0.9^{a}	2.7±0.4^{ab}	2.9±0.1^{b}
ΣMUFA	15.8±1.6^{a}	16.3±1.9^{a}	18.7±4.0^{a}	26.1±0.8^{b}
18:2 n-6	6.6±0.9^{a}	5.9±0.7^{ac}	4.7±1.1^{bc}	5.2±0.3^{b}
20:3 n-6	0.4±0.1^{a}	0.5±0.2^{ac}	0.6±0.1^{bc}	1.0±0.2^{d}
20:4 n-6	22.9±2.0^{a}	23.7±2.1^{a}	5.4±1.0^{b}	7.5±1.3^{c}
22:5 n-6	1.3±0.9^{a}	2.6±0.4^{b}	0.1±0.0^{c}	1.2±0.1^{a}
ΣPUFA n-6	33.1±2.0^{a}	34.2±2.4^{a}	13.7±3.1^{b}	17.9±2.4^{b}
18:3 n-3	0.3±0.1^{a}	0.1±0.0^{b}	0.2±0.1^{b}	0.2±0.0^{b}
20:5 n-3	0.4±0.1^{a}	0.3±0.2^{a}	8.9±2.9^{b}	0.6±0.3^{a}
22:5 n-3	0.5±0.1^{a}	1.0±0.5^{ab}	1.2±0.3^{b}	0.2±0.0^{c}
22:6 n-3	5.2±0.4^{a}	1.4±0.4^{b}	8.5±2.3^{c}	2.2±0.4^{d}
ΣPUFA n-3	6.4±0.6^{a}	3.0±1.0^{b}	18.9±5.3^{c}	3.2±0.4^{b}
Σn-6+n-3	39.5±2.5^{a}	37.0±3.3^{ab}	32.6±5.0^{b}	21.2±2.4^{c}
20:3 n-9	—	—	—	5.0±0.7^{a}
n-6/n-3	5.2±0.3^{a}	12.2±4.0^{b}	0.7±0.4^{c}	5.5±1.2^{a}
22:5 n-6/22: 6n-3	0.04±0.01^{d}	1.80±0.60^{b}	0.02±0.01^{c}	0.5±0.0^{a}

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.

TABLE 7
EFFECT OF DIETARY LIPIDS ON MEMBRANE PHOSPHATIDYLETHANOLAMINE FATTY ACID COMPOSITION

FATTY ACIDS (%)	DIETS			
	CONTROL	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
14:0	4.0±1.0^{a}	2.3±0.8^{b}	6.5±0.9^{c}	3.8±1.8^{a}
16:0	30.8±4.3^{a}	38.3±3.8^{b}	24.4±3.2^{c}	24.1±2.1^{c}
18:0	16.1±0.8^{a}	15.3±2.4^{a}	16.9±1.8^{a}	16.7±4.4^{a}
ΣSFA	53.8±5.8^{ab}	60.0±2.9^{b}	53.9±4.1^{a}	46.7±2.9^{a}
16:1 n-7	3.9±1.5^{a}	4.8±0.7^{a}	1.7±0.5^{b}	6.4±1.9^{a}
18:1 n-9	7.0±1.6^{a}	7.1±0.4^{a}	10.8±0.8^{b}	13.9±1.0^{c}
18:1 n-7	1.5±0.2^{a}	7.2±0.0^{b}	2.2±0.4^{c}	2.1±0.8^{ac}
ΣMUFA	14.3±3.9^{ab}	13.0±0.7^{a}	17.0±0.8^{b}	24.4±2.5^{c}
18:2 n-6	4.9±0.9^{a}	2.8±0.5^{b}	4.7±5^{ab}	3.8±1.2^{ab}
20:3 n-6	0.2±0.0^{a}	0.5±0.2^{b}	0.2±0.4^{c}	0.7±0.0^{b}
20:4 n-6	16.5±3.3^{a}	15.3±3.6^{a}	3.0±0.7^{b}	11.9±2.5^{a}
22:5 n-6	0.9±0.2^{a}	2.8±1.3^{bd}	0.2±0.1^{c}	1.8±0.2^{d}
ΣPUFA n-6	23.8±5.4^{a}	24.0±3.5^{a}	10.9±1.4^{b}	21.8±4.8^{a}
18:3 n-3	0.5±0.1^{a}	0.2±0.1^{b}	0.4±0.1^{a}	0.2±0.0^{b}
20:4 n-3	—	0.1±0.0^{b}	6.5±1.0^{c}	0.4±0.1^{d}
20:5 n-3	0.9±0.2^{a}	0.3±0.1^{b}	2.0±0.4^{c}	0.3±0.1^{b}
22:6 n-3	5.3±1.3^{a}	1.5±0.7^{b}	8.0±1.2^{c}	3.2±0.2^{d}
ΣPUFA n-3	8.1±3.8	3.0±1.0^{b}	18.0±2.6^{c}	4.0±0.6^{ab}
Σn-6+n-3	31.9±4.3^{a}	26.9±2.8^{a}	29.1±3.6^{a}	26.8±3.7^{a}
20:3 n-9	—	—	—	3.1±1.3^{a}
n-6/n-3	3.2±0.7^{a}	12.0±2.6^{b}	0.6±0.1^{c}	4.6±1.5^{a}
22:5 n-6/22: 6n-3	0.08±0.03^{a}	1.83±0.52^{b}	0.02±0.01^{c}	0.4±0.2^{a}

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.

The total (n-6+n-3) PUFA-deficiency produced a high level of 20:3 n-9 (3.1%), however, neither the n-6/n-3 ratio nor the sum of total PUFA (n-6+n-3) were modified. In phosphatidylethanolamine the sum of PUFA was not modified by the deficiencies studied here.

Phophatidylserine (Table 8)

The deficiencies studied presented no effects on the sum of saturated and monounsaturated fatty acids in the composition of phosphatidylserine. The n-6 PUFA deficient diet produced a significant reduction of the level of n-6 PUFA (20:4), a high level of n-3 PUFA and no effects on the sum of total PUFA (n-6+n-3). The n-3 PUFA deficient diet produced a high level of n-6 PUFA in PS (20:4 and 22:5), reduced levels of n-3 PUFA and a high n-6/n-3 ratio. Eicosatrienoic acid (20:3 n-9) was present as well in phosphatidylserine from animals receiving the diet deficient in total PUFA.

TABLE 8
EFFECT OF DIETARY LIPIDS ON MEMBRANE PHOSPHATIDYLSERINE FATTY ACID COMPOSITION

FATTY ACIDS (%)	DIETS			
	CONTROL	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
14:0	3.8±1.0^{a}	2.3±0.6^{b}	3.4±0.9^{a}	2.2±0.4^{b}
16:0	25.9±1.5^{a}	23.8±2.1^{a}	24.4±2.7^{a}	26.9±5.4^{a}
18:0	21.2±3.3^{a}	21.8±3.4^{a}	21.6±1.7^{a}	19.7±4.3^{a}
ΣSFA	56.8±2.8^{a}	51.9±3.3^{a}	53.7±2.6^{a}	52.7±4.6^{a}
16:1 n-7	6.3±1.2^{a}	4.4±1.4^{a}	1.7±0.5^{b}	8.9±2.8^{a}
18:1 n-9	10.7±0.5^{a}	13.4±1.9^{b}	11.5±3.1^{ab}	13.9±1.6^{b}
18:1 n-7	1.2±0.0^{a}	0.2±0.1^{b}	1.6±0.4^{a}	0.0±0.0^{b}
ΣMUFA	19.8±1.5^{a}	20.0±2.6^{a}	21.0±1.1^{a}	23.1±2.2^{a}
18:2 n-6	3.4±0.4^{a}	3.4±1.1^{a}	3.5±1.0^{a}	3.4±0.1^{a}
22:6 n-6	3.3±1.5^{ab}	2.5±0.5^{a}	5.3±1.0^{b}	2.9±0.4^{a}
20:3 n-6	0.4±0.1^{a}	1.3±0.9^{b}	0.4±0.1^{a}	0.7±0.0^{c}
20:4 n-6	11.2±1.4^{a}	14.3±2.5^{a}	3.2±0.8^{b}	7.4±1.9^{c}
22:5 n-6	0.4±0.2^{a}	2.9±0.9^{b}	0.2±0.1^{a}	1.8±0.2^{c}
ΣPUFA n-6	18.5±1.3^{a}	24.7±3.2^{b}	13.8±1.6^{c}	17.6±5.4^{abc}
18:3 n-3	0.3±0.1^{a}	—	1.1±0.5^{b}	0.5±0.2^{ab}
20:5 n-3	0.5±0.1^{a}	0.2±0.1^{a}	2.6±0.6^{c}	0.4±0.1^{a}
22:5 n-3	0.4±0.0^{a}	0.1±0.0^{b}	1.1±0.4^{c}	0.1±0.0^{b}
22:6 n-3	3.5±0.6^{a}	1.7±0.4^{b}	6.5±1.1^{c}	2.6±0.8^{ab}
ΣPUFA n-3	4.9±0.3^{a}	3.4±1.0^{b}	11.5±1.8^{c}	3.9±1.5^{ab}
Σn-6+n-3	22.4±1.5^{a}	28.11±2.7^{b}	25.3±2.5^{ab}	21.5±2.7^{a}
20:3 n-9	—	—	—	2.7±0.4^{a}
n-6/n-3	3.6±0.2^{a}	10.2±3.3^{b}	1.2±0.2^{c}	4.5±1.8^{a}
22:5 n-6/22: 6n-3	0.10±0.03^{a}	1.64±0.53^{b}	0.02±0.01^{c}	0.5±0.2^{a}

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.

Phosphatydilinositol (Table 9)

The level of palmitic acid (16:0) in phosphatidylnositol was significantly higher (2 fold) in animals fed a total PUFA deficient diet as compared to animals taking the control diet. However, the level of oleic acid (18:1 n-9) was reduced by the total PUFA deficient diet. Even though, the sum of n-6 PUFA in Pl was not modified by the diet deficient in n-3 PUFA, the level of 20:4 n-6 was reduced 31%. This was compensated by the high level of 22:5 n-3. The n-6 PUFA deficient diet, however, produced a reduction of the level of this family. The levels of the n-3 PUFA family in PI was high in the lot deficient in n-6 PUFA and low in the n-3 PUFA and total PUFA lots. The sum of PUFA was strikingly reduced by the total PUFA-deficient diet.

TABLE 9
EFFECT OF DIETARY LIPIDS ON MEMBRANE PHOSPHATIDYLINOSITOL FATTY ACID COMPOSITION

FATTY ACIDS (%)	DIETS			
	CONTROL	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
14:0	3.9±1.1^{a}	2.9±1.0^{a}	3.7±1.1^{a}	3.5±0.7^{a}
16:0	25.2±5.5^{a}	28.2±7.2^{a}	21.7±4.1^{a}	52.1±10.0^{b}
18:0	20.4±5.2^{a}	17.8±2.4^{a}	18.3±3.0^{a}	13.5±4.7^{a}
ΣSFA	53.6±4.5^{a}	52.2±7.1^{a}	46.2±6.6^{a}	71.9±6.4^{b}
16:1 n-7	4.4±1.0^{a}	6.9±1.4^{b}	6.7±2.2^{ab}	4.7±0.6^{a}
18:1 n-9	9.6±2.8^{ab}	14.8±3.8^{a}	13.5±4.8^{a}	7.3±2.3^{b}
18:1 n-7	1.2±0.3^{a}	0.4±0.1^{b}	1.4±0.8^{a}	1.2±0.4^{a}
ΣMUFA	16.8±3.4^{a}	23.5±3.6^{b}	25.6±3.7^{b}	14.2±3.2^{a}
18:2 n-6	3.6±0.4^{a}	3.8±1.2^{a}	4.0±1.0^{a}	2.2±1.0^{b}
20:2 n-6	1.8±0.5^{a}	0.9±0.4^{b}	3.9±1.0^{c}	2.1±0.3^{a}
20:3 n-6	1.0±0.7^{ab}	2.5±1.4^{a}	0.3±0.0^{b}	0.4±0.3^{b}
20:4 n-6	16.1±2.0^{a}	12.3±2.3^{b}	10.4±2.9^{b}	3.3±1.7^{c}
20:5 n-6	0.5±0.2^{a}	2.0±1.0^{b}	0.9±0.3^{b}	0.3±0.2^{a}
ΣPUFA n-6	26.2±2.3^{a}	22.7±3.1^{ab}	19.0±5.0^{b}	9.0±3.5^{c}
18:3 n-3	0.4±0.2^{a}	0.0±0.1^{ac}	1.5±0.8^{b}	0.2±0.0^{c}
20:5 n-3	1.0±0.7^{a}	0.2±0.1^{b}	0.9±0.1^{a}	0.4±0.1^{c}
22:6 n-3	1.4±0.3^{a}	1.0±0.3^{ac}	5.3±2.3^{b}	0.7±0.3^{c}
ΣPUFA n-3	3.4±0.6^{a}	1.6±0.5^{bd}	9.2±2.7^{c}	2.0±0.3^{d}
Σn-6+n-3	29.6±2.4^{a}	24.3±3.8^{b}	28.2±7.3^{ab}	11.0±3.2^{c}
20:3 n-9	—	—	—	2.9±1.4^{a}
n-6/n-3	7.7±1.7^{a}	11.0±2.1^{b}	2.1±0.5^{c}	4.5±2.7^{c}
22:5 n-6/22:6n-3	0.30±0.10^{a}	1.42±0.60^{b}	0.23±0.08^{a}	0.40±0.20^{a}

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.

Sphingomyelin (Table 10)

The level of this minor phospholipid appears to be significantly influenced by dietary PUFA. These modification in composition were even observed at the level of monounsaturated fatty acids where oleic acid was largely increased by the three types of PUFA deficiencies studied. However, it was the PUFA content which was mostly afected. In fact, the level of arachidonic acid (20:4 n-6) was significantly reduced by the three types of deficiency. This reduction, already observed in PI was not compensated by the levle of 22:5 n-6 in the animals fed the n-6 and total PUFA deficient diet. N-3 PUFA were, as expected, reduced by the n-3 PUFA deficient diet and elevated by the n-6 PUFA deficient diet. It was also observed a 40% reduction in total PUFA whatever the dietary PUFA deficiency analyzed. The n-6 / n-3 ratio, was not modified by the n-3 PUFA deficiency but reduced by the n-6 and total PUFA deficiencies. The 22:5 n-6 / 22:6 n-3 ratio was very high (19 fold) in SPh from n-3 PUFA and total PUFA deficient animals.

TABLE 10
EFFECT OF DIETARY LIPIDS ON MEMBRANE SPHINGOMYELINE FATTY ACID COMPOSITION

FATTY ACIDS (%)	DIETS			
	CONTROL	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
14:0	3.6±0.6^{a}	3.2±1.9^{a}	3.9±1.9^{a}	3.3±0.9^{a}
16:0	30.8±2.4^{a}	33.0±4.0^{ab}	38.1±3.9^{b}	31.0±1.0^{a}
18:0	16.0±4.1^{a}	13.7±2.1^{a}	10.0±1.9^{b}	20.3±3.2^{a}
ΣSFA	54.7±3.8^{a}	53.0±5.6^{a}	57.1±2.6^{a}	58.3±2.4^{a}
16:1 n-7	5.9±1.3	5.8±2.2^{a}	6.3±0.6^{a}	6.8±1.5^{a}
18:1 n-9	11.1±2.4^{a}	19.7±4.2^{b}	19.3±4.8^{b}	15.7±2.0^{b}
18:1 n-7	2.3±0.2^{a}	1.1±0.1^{b}	1.0±0.1^{b}	—
ΣMUFA	19.9±1.0^{a}	31.2±6.0^{b}	27.9±2.5^{b}	23.0±3.1^{a}
18:2 n-6	4.8±1.4^{a}	4.2±1.4^{a}	4.2±0.8^{a}	3.9±0.5^{b}
20:2 n-6	1.8±0.2^{a}	0.7±0.2^{b}	0.6±0.3^{b}	2.0±0.2^{a}
20:3 n-6	0.3±0.1^{a}	1.4±0.6^{b}	0.2±0.0^{a}	0.2±0.1^{a}
20:4 n-6	9.8±1.4^{a}	4.6±0.4^{b}	2.4±0.2^{c}	3.6±1.3^{bc}
20:5 n-6	0.2±0.1^{a}	2.5±1.0^{b}	—	0.7±0.3^{d}
ΣPUFA n-6	21.4±4.5^{a}	13.0±2.3^{b}	8.2±0.7^{c}	12.0±1.2^{b}
18:3 n-3	0.4±0.0^{a}	0.2±0.1^{b}	0.8±0.0^{c}	0.4±0.1^{a}
20:5 n-3	1.2±0.4^{a}	0.6±0.2^{b}	2.2±0.1^{c}	1.8±0.2^{d}
22:5 n-3	0.2±0.0^{a}	0.2±0.1^{a}	0.5±0.1^{b}	0.5±0.3^{b}
22:6 n-3	2.2±0.4^{a}	1.4±0.9^{ab}	3.3±0.6^{c}	0.9±0.2^{b}
ΣPUFA n-3	4.0±0.4^{a}	2.8±0.6^{b}	6.8±0.3^{c}	3.8±0.2^{a}
Σn-6+n-3	25.4±4.6^{a}	15.8±2.0^{b}	15.0±1.0^{b}	15.8±1.1^{b}
20:3 n-9	—	—	—	2.9±1.4^{a}
n-6/n-3	5.5±1.1^{a}	5.2±1.4^{a}	1.6±0.0^{b}	3.2±0.4^{c}
22:5 n-6/22: 6n-3	0.09±0.06^{a}	1.72±0.20^{b}	—	0.70±0.30^{c}

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.

Enzyme activies

5'Nucleotidase activity (Table 11)

The specific of 5' nucleotidase in hepatic plasma membrane was independent of dietary PUFA. In fact, its activity varied in a range from 98 to 110 µmol protein/h.

TABLE 11
DIETARY FATTY ACID EFFECTS ON PLASMA MEMBRANE 5'NUCLEOTIDASE ACTIVITY

ACTIVITY	DIETS			
	CONTROL	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
µmolesPO4 mg prot.-h	106.0±18.0^{a}	98.0±13.5^{a}	110.0±15.0^{a}	105.0±19.0^{a}

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.

Adenylate Cyclase (Table 12)

Basal adenylate cyclase activity in hepatic plasma membrane was not modified by the dietary treatments. There was, however, a significant difference in basal activity between total PUFA and n-6 PUFA-deficient animals.

Forskoline and glucagon stimulated adenylate cyclase was not infuenced by dietary PUFA deficiencies. However, GppNHp stimulated activity through the regulatory unit of the enzyme was highly reduced by the n-6 PUFA deficient diet, and augmented by the n-3 PUFA deficient diet. The enzyme activity from the n-6 PUFA deficient animals was reduced as compared to the activity from the total and n-3 PUFA deficient animals. On the other hand, the activity of the enzyme stimulated by the forskoline + GDP ßs, which gives information on the catalytic unit, was augmented by the total PUFA deficient diet.

TABLE 12
DIETARY FATTY ACID EFFECTS ON PLASMA MEMBRANE ADENYLATE CYCLASE ACTIVITY

pmoles cAMP/mg protein minute	DIETS			
	CONTROL	n-3 PUFA Deficient	n-6 PUFA Deficient	Total PUFA Deficient
Basal Stimulated:	11.0±2.5^{ab}	11.0±3.0^{ab}	9.8±1.0^{b}	13.0±2.5^{a}
Glucagon				
3.3 10^{-10}M	62.0±22.0^{a}	72.0±22.0a	60.0±15.0^{a}	61.0±14.0a
3.3 10^{-9}M	80.0±28.0^{a}	76.0±23.0a	72.0±18.0^{a}	71.0±16.0a
3.3 10^{-8}M	91.0±32.0^{a}	79.0±24.0a	51.0±13.0^{a}	76.0±17.0a
3.3 10^{-7}M	103.0±37.0^{a}	87.0±26.0a	82.0±18.0^{a}	89.0±20.0a
3.3 10^{-6}M	139.0±51.0^{a}	140.0±42.0a	146.0±36.0	141.0±33.0a
3.3 10^{-5}M	162.0±60.0^{a}	156.0±46.0a	152.0±38.0^{a}	150±34.0a
GppNHp[1]	150.0±35.0^{a}	196.0±34.0a	92.0±28.0^{c}	191.0±49.0ab
Forskolin	79.0±17.0^{a}	74.0±24.0a	93.0±17.0^{a}	71.0±23.0^{a}
Forskoline + GDPßs[2]	27.0±8.0^{a}	21.0±6.0a	24.0±8.0^{a}	38.0±10.0^{b}

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. 1. Guanydyl-imidoliphosphate, 2. Guanydyl-diphosphate ßs

DISCUSSION

In our study, the levels of cholesterol and phospholipids were reduced by the n-3 and total PUFA deficiencies whereas the n-6 PUFA dietary deficiency reduced only the level of phospholipids. Filier et al (11) found that an excess of n-3 PUFA had no effect on the hepatic plasma membrane phospholipid content in animals. Similarly, Zuniga and Kinsella (12) did not find any correlation between the levels of cholesterol and phospholipids in plasma membrane and the type of lipids in the diet. However, it is important to notice that the control group used in these two studies was a n-3 PUFA-deficient group. Therefore, the comparison was made between two deficiencies (n-6 and n-3 PUFA). As such our results are comparable tho those of Zuniga and Kinsella.

According to our results, the levels of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were not modified by the type of dietary lipids. On the contrary, phosphatidylserine (PS), phosphatidylinositol (PI) and sphingomyeline (SPh) were largely modified by the type of dietary PUFA deficiency. In fact, the total PUFA deficiency diminished the level of PS, and PI and augmented the level of Sph. The n-6 PUFA deficiency provoked high levels of Sph. Finally, the n-3 PUFA deficiency produced low levels of Sph. We found no reports regarding the levels of phospholipid classes in hepatic plasma membranes to compare them with our results. It is very interesting, however, to observe the significance of dietary PUFA on the proportion of minor phospholipid classes. Notice also that the n-6 and n-3 PUFA deficiencies have the same effect on PS but opposite effects on PI and Sph.

Concerning the fatty acid composition of each phospholipid classes, it was observed that the 85 mg of 18:2 n-6 present in the toal PUFA deficient diet was not sufficient to maintain the level of the sum of PUFA in PC, PI and Sph. Similarly, the quantity of PUFA (1g) present in each dietary specific PUFA deficiency (n-6 or n-3) was supposed to be adequate to maintain, by replacement of one family by the other, the sum of PUFA in membrane phospholipids. Nevertheless, this sum was modified in three phospholipids due to n-3 PUFA deficiency and in two phospholipids due to n-6 PUFA deficiency. These results showed either certain priority among phospholipids or difficulty of replacement between the two PUFA families.

The n-6/n-3 ratio was expected to be systematically reduced by the n-6 PUFA deficiency and increased by the n-3 and total PUFA deficiencies. Results were confirmed in the case of n-6 PUFA deficiency. However, the sphingomyeline found in the group of n-3 PUFA deficient animals had no modification in this ratio and phospholipids from the total deficient group presented ratios totally unexpected. Only a few works have been reported over the past few years on the fatty acid compoosition of phospholipid classes of hepatic plasma membranes. Some authors reported either the fatty acid composition of total phospholipids or the fatty acid composition of PC and PE. These two phospholipids are, however, modified only slightly by diet as reported in this work, whereas the minor phospholipids PS, PI and SPh can be largely modified by diet. Our results are in accordance to those by Brivio-Haugland et al (2) where total deficient diets produced an accumulation of 16:1 n-7 and 20-3 n-9 and a reduction of 18:2 and 20:4 n-6. Zuniga and Kinsella (12) have reported, as in our study, a reduction of the n-6 PUFA family and an augmentation of the n-3 PUFA family. The opposite effect was observed in phospholipids from animals fed the n-3 PUFA deficient diet. However, the fatty acid composition of Sph was not reported in those two studies.

In spite of the large modification in fatty acid composition, the 5'nucleotidase activity was not modified at all. This is probably due to the fact that the 5'nucleotidase activity is favored by a PC membrane environment which was not modified in our study. Our results are in contradiction to those of Monchilova et al (4) and Brivio-Haugland et al. (2) in which the 5'nucleotidase activity was either higher or lower respectively. The authors however did not present the fatty acid composition of diets nor the fatty acid composition of phospholipid classes. Flier et al (11) reported a high 5'nucleotidase activity in hepatic plasma membrane from animals receiving a diet rich in n-3 PUFA in comparison to a saturated diet. Zuniga and kinsella (7) did nor report any effect on enzyme activity by using the same type of diet. However, in 1989, the same authors showed higher 5'nucleotidase activity in animals under a diet rich in n-3 PUFA than in animals receiving a diet rich in n-6 PUFA or totally deficient (12). In conclusion, the few works done on the effects of membrane fatty acid composition are not reliable enough to reject our results which show that the phospholipid fatty acid composition did not influence the 5'nucleotidase activity except in the case of extreme excess which concerns more toxicological than nutritional modifications.

Adenyate cycles complex carries a receptor unit, a catalytic unit and a regulator unit. The effects of dietary PUFA on the three units were analyzed by the effectors 1) glucagon, 2) GppNHp 3)forskoline and 4) forskoline + GDPßs which work respectively on 1) receptor unit, 2) regulator unit, 3) catalytic unit, through the regulator unit and 4) catalytic unit per se.

Basal adenylate cyclase activity was not modified by dietary deficiencies in relation to the control group but it was, however, highly reduced by the n-6 PUFA deficiency in comparison to the activity of the total deficient group.

Dietary PUFA, whatever the type, had no effect on adenylate cyclase activity stimulated by glucagon or forskoline. Therefore, the receptor unit and the catalytic unit modulated through the regulator unit do not appear to be sensible to the PUFA modifcations in hepatic plasma membrane phospholipids. The protein G unit, regulator unit, stimulated by GppNHp, was largely reduced by dietary n-6 PUFA deficiency and augmented by the dietary n-3 PUFA deficiency in comparison to the activity of all other groups. Finally, the

adenylate cyclase activity modulated by the catalytic unit (Forskoline+GDPßs) was significantly increased by the total PUFA deficiency.

Our results are in contradiction to those of Brivio-Haugland et al (2) that showed a reduction in adenylate cyclase activity basal and stimulated by the glucagon in animals receiving a totally PUFA deficient diet. Houslay and Palmer (22) and Calorini et al (28) have reported that the adenylate cyclase activity stimulated by the glucagon can be modulated by the proportions of membrane phsophatidylcholine. In our study, we did not show any modification of either phosphatidylcholine or enzyme activity through glucagon by dietary PUFA.

The research group of Clandinin has shown in rat a direct relationship between adenylate cyclase activity stimulated by glucagon and the content of 18:2 n-6 (23,24) or 18:3 n-3 content (9). However, even though our results are in accordance to those of Clandinin concerning membrane lipid modifications, adenylate cyclse activity stimulated by glucagon were independent of PUFA dietary deficiency.

Finally, Lee and Hamm (10) reported a stimulating effect of n-3 PUFA on NaF and forskoline adenylate cyclase actvity. The conclusions of these authors were that dietary PUFA have a direct incidence on the catalytic and the regulator (protein G) units and have no incidence on the receptor unit due primarily to the unchanged receptor numbers. However these results were not validated by Dax et al (26) whose conclusions were, as ours, that dietary PUFA had no influence on adenylate cyclase activity stimulated by glucagon and forskoline. Very recently, Alam et al 927) studied the reversal effect of diet induced modification on adenylate activity in the heart membrane in chich they showed that the adenylate cyclase activity was only partially restored even though the membrane fatty acid composition was restored to normal. They postulate the need of the enzyme activity for a specific phospholipid environment for instance phosphatidylcholine as put in evidence by Calorini et al (28).

In conclusion, our results show that each dietary PUFA deficiency modifies differently the proportions of phospholipid classes and their fatty acid composition. The mechanism responsible for these modifications remain to be elucidated. The protein G (GppNHp) and the catalytic unit of adenylate cyclase complex appear to be sensitive to alterations in PUFA membrane composition.

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Recibido : 23-01-1995
Aceptado: 01-09-1995