Molecular Species of Phosphoglycerides in Liver Microsomes of Rats Fed a Fat-Free Diet

Horacio A. Garda[1]*, Ana M. Bernasconi, M. Alejandra Tricerri, and Rodolfo R. Brenner[1]

Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CONICET-UNLP, Facultad de Ciencias Médicas, 1900-La Plata, Argentina

ABSTRACT: The influence of a fat-free diet on the molecular species composition of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) of rat liver microsomes was studied by using reversed-phase high-pressure liquid chromatography. In the three phosphoglyceride classes analyzed, the fat-free diet produced a large decrease in the 18:0/20:4n-6 species but less important changes were found in the 16:0/20:4n-6 species. In PC, the most abundant phosphoglyceride class of rat liver microsomes, the fall in the 18:0/20:4n-6 species was counterbalanced mainly by an enhancement in the 16:0/18:1n-9 species although it was not evident in PE. In PI, the decrease in the 18:0/20:4n-6 species was counterbalanced by an increase in the 18:0/20:3n-9 species. Fluorescence polarization measurements of 1,6-diphenyl-1,3,5-hexatriene in liposomes of 16:0/18:1n-9-, 18:0/18:1n-9-, 16:0/20:4n-6-, and 18:0/20:4n-6-PC indicated that the change in the saturated fatty acid in the *sn*-1 position accompanying the replacement of 20:4n-6 by 18:1n-9 could be very important for a homeoviscous compensation, maintaining the membrane physical properties without large alterations in spite of the essential fatty acid deficiency due to the fat-free diet.
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The major function of essential fatty acids (EFA) is to be precursors of biooxygenated derivatives such as eicosanoids and docosanoids. However, only a small fraction of the requirements of these acids in the mammal diet can be explained by this role (1). Since large amounts of these acids and their derivatives are present in membrane phospholipids, a structurally specific role in the membranes has been also attributed to these acids (2,3). Although some authors have minimized the importance of EFA for the structural properties of membranes (4), others have suggested that they could modify the conformational freedom of the acyl chains of lipid bilayers with participation in lipid–protein interactions and membrane function (5,6). The influence of unsaturation on the bilayer structural and dynamic properties is strongly dependent on the position of double bonds (7–9). Since only EFA and their derivatives are able to place double bonds in the deep part of the membrane leaflet (3,10,11), their deficiency could result in altered transversal double-bond distribution and physical properties of the membranes.

EFA-deficient and fat-free diets modify the fatty acid composition of several membranes in different species (3,10–17). Although it depends to some extent on the particular membrane, tissue and species, these diets produce, in general, a fall in the n-6 fatty acids 18:2 and 20:4 and an increase in the nonessential 18:1n-9 fatty acid. Appreciable amounts of the nonessential fatty acid 20:3n-9, which is present only in trace amounts in EFA-sufficient animals, appear as a consequence of EFA or fat-free diets in rat (10,12,13) but not in guinea pig liver microsomes (14–17).

Several studies using spectroscopic methods such as fluorescence (14,16,17) or electron spin resonance (3,6,15) have indicated that EFA or fat-free diets affect the physical properties of different membranes, such as guinea pig liver microsomes (14–17) or piglet jejunum brush border membranes (3,6). This is attributed to changes in the fatty acid composition of membrane lipids, since no changes are observed either in the phospholipid class distribution or in the relative content of cholesterol, phospholipid, and protein. In some membranes, however, as is the case for rat erythrocyte (10) and liver microsomal (10,13) membranes, in spite of large changes in fatty acid composition, membrane lipid order and dynamics remain unaltered, suggesting that some homeoviscous compensation could occur (10). As already shown in artificial membranes of synthetic phosphatidylcholines (PC), the influence of an unsaturated fatty acid in the *sn*-2 position on the viscotropic properties is largely dependent on the saturated one in *sn*-1 (9). Thus, to understand the mechanism of this homeoviscous compensation, it was necessary to study the changes in the phospholipid molecular species resulting from a fat-free diet. This should supply more detailed information than the fatty acid compositional changes.

This study shows that the largest change evoked by a fat-free diet in rat liver microsomes is the replacement of

[1]These authors are members of the Carrera del Investigador Científico, CONICET, Argentina.

*To whom correspondence should be addressed at INIBIOLP, Facultad de Ciencias Médicas, Calle 60 y 120, 1900-La Plata, Argentina.

Abbreviations: DPH, 1,6-Diphenyl-1,3,5-hexatriene; EFA, essential fatty acids; HPLC, high-performance liquid chromatography; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; UV, ultraviolet.

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18:0/20:4n-6-PC by 16:0/18:1n-9-PC. Fluorescence anisotropy measurements of 1,6-diphenyl-1,3,5-hexatriene (DPH) in liposomes of these PC in comparison with 16:0/20:4n-6- and 18:0/18:1n-9-PC suggest that the replacement in the *sn*-1 position of 18:0 by 16:0 could be very important for the homeoviscous compensation observed in liver microsomes of rats fed on the fat-free diet.

EXPERIMENTAL PROCEDURES

Materials. DPH was obtained from Aldrich Chemical Co. (Milwaukee, WI). A stock solution of 2.0 mM in tetrahydrofuran was prepared. The 16:0/18:1n-9-, 18:0/18:1n-9-, 16:0/20:4n-6-, and 18:0/20:4n-6-PC (dissolved in chloroform) were obtained from Avanti Polar Lipids (Alabaster, AL).

Animals and diets. After weaning, male Wistar rats were fed either a control diet: 52% starch, 19% casein, 23% sunflower oil plus minerals (4%) and vitamins (2%) (18) or a fat-free diet: 66% starch, 28% casein, minerals (4%) and vitamins (2%). The rats were killed by decapitation either one or three months later, and the livers were excised.

Microsome preparation. Livers were homogenized in 0.25 M sucrose, 1 mM EDTA, pH 7.0. Microsomes were obtained by centrifugation as previously described (19) and stored at −80°C. The protein content of microsomal preparations was measured by the method of Lowry *et al*. (20).

Extraction and separation of lipid fractions. Total microsomal lipids were extracted according to the procedure of Folch *et al*. (21) with chloroform/methanol 2:1 (vol/vol). Glycerophospholipid classes were separated by one-dimensional thin-layer chromatography on 20×20 cm plates coated with a mixture of silica gel H–florisil 9:1 (w/w) with a double development solvent system (22): chloroform/methanol/30% ammonium hydroxide/water 140:50:7:3 (by vol) in the first run and chloroform/methanol/acetic acid/water 320:40:8:3 (by vol) in the second run. The extreme ends of the plates were sprayed with an iodine solution to visualize the spots. The unexposed part of the bands corresponding to PC, phosphatidylethanolamine (PE), and phosphatidylinositol (PI) were scraped off and extracted with methanol/chloroform 2:1 (vol/vol).

Fatty acid composition analysis. After evaporation of the solvent under vacuum, the total lipid extracts and the different phospholipid fractions were transesterified by heating at 64° for 3 h in 10% boron trifluoride in methanol (from Sigma Chemical Co., St. Louis, MO). The resulting methyl esters were separated on a column of 10% SP 2330 on 100–120 mesh Chromosorb WAW (Supelco, Bellefonte, PA) using a Hewlett-Packard 5840 A gas–liquid chromatograph (Palo Alto, CA). The fatty acids were identified by comparison of their relative retention times with those of reference standards.

Phospholipid molecular species analysis. The analysis of phospholipid molecular species was essentially made as described by Patton *et al*. (23) with some modifications. PC, PE, and PI molecular species were separated on a 4×250 mm Bio-Sil ODS-5S column (Bio-Rad, Richmond, CA), eluted with 50 mM choline chloride in methanol/water/acetonitrile 90:7.5:8 (vol/vol) at a flow rate of 1.5 mL/min using a Merck-Hitachi L-6200 pump (Darmstadt, Germany). Detection was performed by absorption at 205 nm using a Merck-Hitachi L-4200 ultraviolet–visible (UV-VIS) detector. For the identification and quantification of the molecular species, 400 µg of each phosphoglyceride were injected onto the column. Individual peaks were collected and mixed with 40 µg of di-20:1(n-9)-PC used as internal standard. They were extracted with chloroform and after the evaporation of the solvent under vacuum; they were transesterified as described above for the fatty acid analysis. Gas–liquid chromatography (GLC) analysis of each high-performance liquid chromatography (HPLC) peak made possible the identification of the fatty acids which were present in each molecular species. The relative GLC–flame-ionization detection (FID) response with respect to the internal standard 20:1 made possible the quantification of each molecular species. Relative response factors for the HPLC UV absorption detection were calculated for each molecular species of phosphoglyceride. These factors were used to calculate the composition in molecular species of the individual samples directly from the HPLC chromatograms obtained by injecting 200-µg phosphoglyceride samples.

Fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene. Multilamellar liposomes of 16:0/18:1-, 18:0/18:1-, 16:0/20:4, and 18:0/20:4-PC were prepared in 20 mM sodium phosphate pH 7.1 using the procedure described by Kutchai *et al*. (24). For labeling with DPH, 2 mL of a 2.5 µM probe dispersion in 20 mM phosphate buffer pH 7.1 were mixed with the same volume of liposome preparations, agitated, and incubated at 40°C for 30 min. All procedures were carried out under N_2 atmosphere. For background corrections, unlabeled blanks with the same concentration of liposomes were also prepared (25).

Steady-state anisotropy was measured at 20°C in an SLM 4800 C spectrofluorometer (SLM Instruments, Inc., Urbana, IL) as described (26). The excitation wavelength was 361 nm, and the emission was observed through a sharp cut-off filter (Schott KV 389; Glass Technologies, Inc., Duryea, PA).

RESULTS

Table 1 shows the effect of a 1-mon treatment with a fat-free diet on the fatty acid composition of rat liver microsomes. This is in agreement with our previous findings (10,13). The fat-free diet resulted in a highly significant decrease in the n-6 acids 18:2 and 20:4. With respect to the minor n-3 acids, although a significant redistribution was produced by the treatment, the total amount was not changed. The decrease in n-6 acids was counterbalanced by a rise in the nonessential n-9 monoenoics 16:1 and 18:1 and the trienoic 20:3n-9. An increase in 16:0 was compensated by a decline in 18:0, and no net change in the total saturated acid content occurred. In this way, the ratio of unsaturated to saturated acyl chains was not

TABLE 1
Effect of a Fat-Free Diet on the Fatty Acid Composition of the Total Lipids and the Major Phosphoglyceride Classes of Rat Liver Microsomes

	Total lipids		Phosphatidylcholine		Phosphatidylethanolamine		Phosphatidylinositol	
Fatty acid	Control diet (% area)[a]	Fat-free diet (% area)	Control diet (% area)	Fat-free diet (% area)	Control diet (% area)	Fat-free diet (% area)	Control diet (% area)	Fat-free diet (% area)
16:0	14.7 ± 0.4	21.7 ± 2.4**	19.6 ± 3.0	23.9 ± 3.5*	13.4 ± 1.2	20.0 ± 3.4**	9.0 ± 2.6	10.7 ± 2.6[ns]
16:1n-7	0.5 ± 0.3	6.9 ± 0.9***	0.4 ± 0.4	6.3 ± 1.3***	n.d.[c]	2.5 ± 0.8**	0.5 ± 1.2	0.6 ± 0.6[ns]
18:0	18.6 ± 0.3	13.1 ± 2.1**	18.6 ± 2.3	11.8 ± 2.3**	21.4 ± 1.8	14.9 ± 1.5***	37.6 ± 2.6	37.8 ± 4.9[ns]
18:1n-9	12.6 ± 0.7	22.6 ± 1.8***	11.4 ± 1.0	22.5 ± 2.4***	10.6 ± 1.4	17.4 ± 2.2***	6.6 ± 2.0	8.1 ± 3.8[ns]
18:2n-6	16.5 ± 0.8	4.7 ± 0.5***	14.5 ± 1.6	6.1 ± 0.6***	10.3 ± 1.2	3.3 ± 0.5***	5.4 ± 1.5	1.6 ± 0.6**
20:3n-9	1.1 ± 0.2	4.1 ± 0.8**	1.8 ± 0.5	3.8 ± 1.4*	1.7 ± 0.6	2.5 ± 0.8ns	1.3 ± 0.9	11.7 ± 1.7***
20:3n-6	0.9 ± 0.1	2.7 ± 0.4***	0.7 ± 0.3	2.8 ± 0.7**	0.7 ± 0.4	1.5 ± 0.5*	0.5 ± 0.2	1.9 ± 1.2*
20:4n-6	25.1 ± 0.8	14.9 ± 1.1***	23.9 ± 3.9	13.7 ± 1.4**	24.6 ± 0.7	21.5 ± 0.8***	34.7 ± 1.7	21.5 ± 3.7***
22:4n-6	2.8 ± 0.5	1.4 ± 0.4*	1.8 ± 0.6	0.8 ± 0.5*	4.0 ± 0.9	1.2 ± 0.6***	1.1 ± 0.7	0.9 ± 0.3[ns]
22:4n-3[b]	4.6 ± 0.8	3.9 ± 0.8[ns]	4.5 ± 0.9	4.1 ± 0.9[ns]	8.3 ± 1.4	6.6 ± 1.7[ns]	2.1 ± 1.2	3.2 ± 1.5[ns]
22:5n-3	0.6 ± 0.2	0.9 ± 0.4[ns]	0.7 ± 0.4	0.7 ± 0.3[ns]	1.2 ± 0.6	1.8 ± 1.7[ns]	0.5 ± 0.7	0.4 ± 0.3[ns]
22:6n-3	1.8 ± 0.4	3.0 ± 0.4**	2.1 ± 1.3	3.3 ± 1.2[ns]	3.6 ± 1.1	6.7 ± 2.2*	0.7 ± 0.6	1.5 ± 0.4*
U/S[d]	1.8 ± 0.2	1.7 ± 0.4[ns]	1.5 ± 0.4	1.6 ± 0.4[ns]	1.7 ± 0.3	1.6 ± 0.4[ns]	1.1 ± 0.3	1.0 ± 0.4[ns]

[a]Data are the mean of five animals for each group ± standard deviation. [b]Contains also 22:5n-6. [c]Nondetectable amount. [d]Molar ratio of unsaturated to saturated fatty acids; [ns]not significant difference; *$P < 0.1$; **$P < 0.01$; *** $P < 0.001$.

significantly altered. After 3 mon of the fat-free diet treatment, the same changes were basically found in the fatty acid composition (not shown).

Table 1 also shows the effect of a 1-mon treatment with a fat-free diet on the fatty acid composition of the three major phosphoglyceride classes of rat liver microsomes: PC, PE, and PI. Although microsomes contain minor phospholipid classes such as phosphatidylserine and sphingomyelin, PC, PE and PI make more than 80% of the microsomal lipids. The changes in the fatty acid composition produced by the fat-free diet were different in each one of these phospholipid classes. A decrease in 18:2n-6 was produced in all the phospholipid classes, but there was no large decrease in 20:4n-6 in the PE fraction as a result of fat deprivation. The highest increase in monoenoic acids was found in the PC fraction. In the PI fraction containing the largest amount of 20:4n-6 but the smallest amount of 18:2n-6, the fat-free diet produced the highest increase in the nonessential trienoic 20:3n-9 and no increase in monoenoic acids. As in the total microsomal lipids, the 3-mon treatment resulted in similar changes in the fatty acid composition of each phospholipid class (not shown) as those observed for the 1 mon-treatment.

Figure 1 shows typical chromatograms obtained for the HPLC separation of the molecular species of PC, PE, and PI from liver microsomes of rats fed on a control or a fat-free diet. In the PC and PE fractions of fat-fed animals, eight major molecular species were observed. Two new species containing 20:3n-9 became detectable in animals fed the fat-free diet. Only two major molecular species (16:0/20:4n-6 and 18:0/20:4n-6) were observed in the PI fraction of control animals, and 18:0/20:3n-9 became observable in the animals fed the fat-free diet.

Table 2 shows changes produced by the fat-free diet on the composition in molecular species of the major phosphoglyceride classes from rat liver microsomes. The two major molecular species in PC, PE, and PI were those containing arachidonic acid (16:0/20:4n-6 and 18:0/20:4n-6). The fat-free diet produced a marked decrease in the 18:0/20:4n-6 species of the three phosphoglycerides analyzed. However, in the case of 16:0/20:4n-6, the fat-free diet evoked only a small decrease in the PC fraction, a small increase in the PE fraction and no significant change in the PI fraction. A second major change produced by the fat-free diet was a large increase in 16:0/18:1n-9-PC. The amount of 16:0/18:1n-9 species of PE, however, was not significantly changed by the treatment. The nonessential polyenoic 20:3n-9 fatty acid, which appeared as a consequence of the fat deficiency, was found in two molecular species of PC and PE (16:0/20:3n-9 and 18:0/20:3n-9) but in only one species of PI (18:0/20:3n-9). Although the fat-free diet produced a significant decrease in the content of the 18:2n-6 fatty acid in PC and PE (see Table 1), no significant changes were observed in the 16:0/18:2n-6 and 18:0/18:2n-6 species of these phosphoglycerides. This fact could be due to the presence of other minor molecular species which were not detected by the HPLC analysis.

In accordance with previous studies (19), the fat-free diet did not evoke significant changes in the phospholipid class distribution and in the lipid/protein or cholesterol/phospholipid ratios of rat liver microsomes (not shown). The PC, PE, and PI contents of both groups of liver microsomes were 0.32, 0.15, and 0.055 μmol/mg of protein, respectively. The amount of each phosphoglyceride molecular species (in nmol/mg of protein) was calculated and is given in Table 2. It shows that the major change produced by the fat-free diet was the decrease in 18:0/20:4n-6 PC, which was mainly replaced by 16:0/18:1n-9.

It was our interest to know if the change in the saturated fatty acid in *sn*-1 (18:0 by 16:0) which accompanies the change in the unsaturated one in *sn*-2 (20:4n-6 by 18:1n-9) could play some role in the compensation observed in the fat-deficient condition. For this reason, steady-state fluorescence anisotropy of DPH in multilamellar liposomes of 18:0/20:4

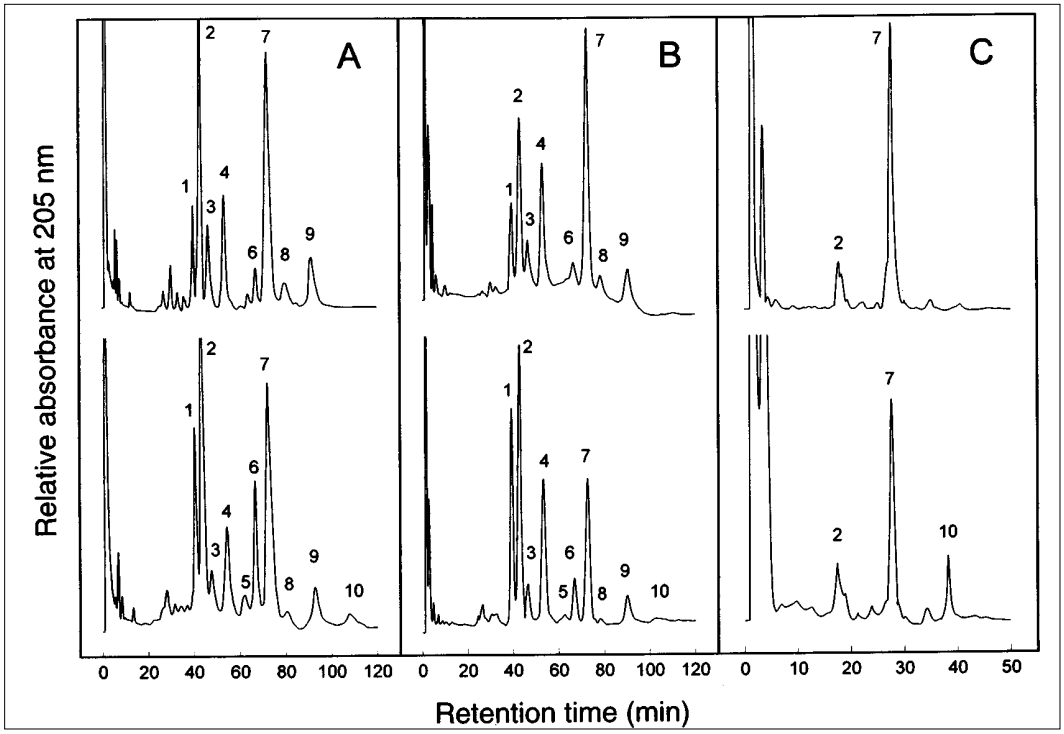

FIG. 1. Reversed-phase high-performance liquid chromatographic separation of molecular species of phosphatidylcholine (A), phosphatidylethanolamine (B), and phosphatidylinositol (C) of liver microsomes from rats fed on a control (top chromatograms) or a fat-free (bottom chromatograms) diet. About 200 µg of the corresponding lipids were chromatographed as described in the Experimental Procedures section. Identified peaks are numbered in sequence of elution: 1, 16:0/22:6n-3; 2, 16:0/20:4n-6; 3, 16:0/18:2n-6; 4, 16:0/22:5n-3; 5, 16:0/20:3n-9; 6, 16:0/18:1n-9; 7, 18:0/20:4n-6; 8, 18:0/18:2n-6; 9, 18:0/22:5n-3; and 10, 18:0/20:3n-9.

n-6-, 18:0/18:1n-9-, 16:0/20:4n-6- and 16:0/18:1n-9 PC, was measured. Figure 2 shows that fluorescence anisotropy was lower in liposomes of the 20:4n-6-containing species compared with those of the 18:1n-9-containing species. Fluorescence anisotropy was higher in liposomes of the 18:0-containing PC than in those of the 16:0-containing ones. The increase in fluorescence anisotropy produced by the change of 20:4n-6 for 18:1n-9 in *sn*-2 was largely compensated by the change of 18:0 for 16:0 in the *sn*-1 position.

DISCUSSION

Differential polarized phase fluorescence studies dealing with probes of different localization in the lipid bilayer, using either two fixed frequencies (10) or multiple modulation frequencies for the excitation (27), indicated that the order and dynamics of the lipid bilayer of rat liver microsomes are not significantly modified by the EFA deficiency produced by fat-free diets. Only a small decrease in the rate of dipolar relaxation at the interfacial level, where phospholipid polar groups are located, was detected using Laurdan as probe (27). These studies suggest that changes in the fatty acid composition produced by fat deficiency would occur in such a way that the lipid bilayer properties are largely compensated.

The present work shows that a fat-free diet affects differentially the composition in molecular species of the main phosphoglyceride classes of rat liver microsomes and that the changes in the unsaturated fatty acids of *sn*-2 are accompanied by changes in the saturated acids in *sn*-1. In the three phosphoglyceride classes analyzed, fat deficiency produced a large decrease in the 18:0/20:4n-6 species but small changes in the 16:0/20:4n-6 species. In PC, the most abundant phosphoglyceride class of rat liver microsomes, the decrease in the 18:0/20:4n-6 species was mainly counterbalanced by an increase in the 16:0/18:1n-9 species. However, in PI, the decrease in the 18:0/20:4n-6 species was counterbalanced by an increase in the 18:0/20:3n-9 species.

Since the fat-free diet evoked no change in the phospholipid class distribution and PC is the major lipid class of rat liver microsomes, the replacement of 18:0/20:4n-6 PC by 16:0/18:1n-9 PC was the major change produced by the treatment. These results also show that the increase in the fluorescence steady-state anisotropy of DPH due to the change of 20:4n-6 by 18:1n-9 in the *sn*-2 position of PC was largely compensated by the change of 18:0 by 16:0 in the *sn*-1 position. This fact indicates that the change in the saturated fatty acid in *sn*-1 accompanying the replacement of 20:4n-6 by 18:1n-9 in *sn*-2 could be very important for a homeoviscous compensation, maintaining the membrane physical proper-

TABLE 2
Effect of a Fat-Free Diet on the Composition in Molecular Species of the Major Phosphoglyceride Classes of Rat Liver Microsomes

	Phosphatidylcholine		Phosphatidylethanolamine		Phosphatidylinositol	
Molecular species	Control diet mol%[a] (nmol/mg)[b]	Fat-free diet mol% (nmol/mg)	Control diet mol% (nmol/mg)	Fat-free diet mol% (nmol/mg)	Control diet mol% (nmol/mg)	Fat-free diet mol% (nmol/mg)
16:0/22:6n-3	2.3 ± 0.3 (7.4 ± 1.0)	5.9 ± 1.4** (18.9 ± 4.5)	4.5 ± 0.2 (7.2 ± 0.3)	9.3 ± 1.7** (14.9 ± 2.7)	n.d.	n.d.
16:0/20:4n-6	25.9 ± 2.2 (82.9 ± 7.0)	19.7 ± 3.8* (63.0 ± 12.2)	17.0 ± 2.4 (27.2 ± 3.8)	22.0 ± 3.7* (35.2 ± 5.9)	18.4 ± 8.7 (10.1 ± 4.8)	17.2 ± 6.9[ns] (9.5 ± 3.8)
16:0/18:2n-6	17.8 ± 3.3 (57.0 ± 10.6)	15.0 ± 2.3[ns] (48.0 ± 7.4)	12.6 ± 1.5 (20.2 ± 2.4)	13.8 ± 4.1[ns] (22.1 ± 6.6)	n.d.	n.d.
16:0/22:5n-3	6.2 ± 0.6 (19.8 ± 1.9)	8.1 ± 0.9** (25.9 ± 2.9)	11.7 ± 1.0 (18.7 ± 1.6)	10.2 ± 2.1[ns] (16.3 ± 3.4)	n.d.	n.d.
16:0/20:3n-9	n.d.[c]	5.6 ± 1.5** (17.9 ± 4.8)	n.d.	1.8 ± 2.6[ns] (2.9 ± 4.2)	n.d.	n.d.
16:0/18:1n-9	11.6 ± 3.2 (37.1 ± 10.2)	22.5 ± 1.9*** (72.0 ± 6.1)	15.3 ± 5.0 (24.5 ± 8.0)	18.1 ± 2.7[ns] (29.0 ± 4.3)	n.d.	n.d.
18:0/20:4n-6	27.0 ± 3.3 (86.4 ± 10.6)	10.9 ± 2.8*** (34.9 ± 9.0)	30.1 ± 1.8 (48.2 ± 2.9)	15.0 ± 2.9*** (24.0 ± 4.6)	81.6 ± 8.7 (44.9 ± 4.8)	54.3 ± 5.8*** (29.9 ± 3.2)
18:0/18:2n-6	6.7 ± 1.4 (21.4 ± 4.5)	6.5 ± 3.0[ns] (20.8 ± 9.6)	2.9 ± 0.7 (4.6 ± 1.1)	2.2 ± 2.2[ns] (3.5 ± 3.5)	n.d.	n.d.
18:0/22:5n-3	2.6 ± 0.6 (8.3 ± 1.9)	3.6 ± 1.5[ns] (11.5 ± 4.8)	5.8 ± 1.0 (9.3 ± 1.6)	3.7 ± 0.2** (5.9 ± 0.3)	n.d.	n.d.
18:0/20:3n-9	n.d.	2.2 ± 1.0** (7.0 ± 3.2)	n.d.	3.8 ± 3.7* (6.1 ± 5.9)	n.d.	28.5 ± 8.3** (15.7 ± 4.6)

[a]Data are the mean of five animals for each group ± standard deviation. [b]Absolute amount in nmol/mg of microsomal protein. [c]Nondetectable amount.
[ns]Not significant difference; *$P < 0.1$; **$P < 0.01$; ***$P < 0.001$.

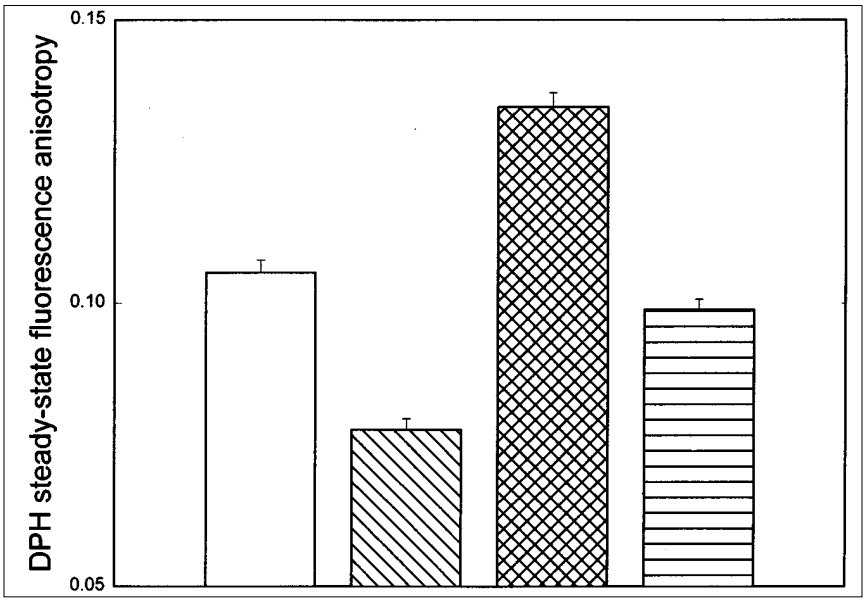

FIG. 2. Steady-state fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) in multilamellar liposomes of 16:0/18:1n-9- (open bar), 16:0/20:4n-6- (diagonally-lined bar), 18:0/18:1n-9- (cross-hatched bar), and 18:0/20:4n-6-phosphatidylcholine (vertically-lined bar).

ties without large alterations under the fat-deficient condition.

Differential polarized phase fluorescence studies using several probes of different localization in the lipid bilayer were previously made in multilamellar liposomes of these PC (9). They showed that order and viscous resistance to motion in the external bilayer region sensed by 2- and 7-(9-anthroyloxy) stearic acids are similar for 16:0/18:1 PC and 18:0/20:4 PC liposomes. However, in 18:0/20:4 PC liposomes, the deep region of the bilayer is somewhat more disordered, allowing a faster motion to 12-(9-anthroyloxy) stearic acid than in the case of 16:0/18:1 PC liposomes (9). This indicates that, although a large compensation in the microsomal membrane viscotropic properties is produced by the replacement of 18:0/20:4n-6 PC for 16:0/18:1n-9 PC, it is not enough to reach the almost exact compensation observed in fat deficiency (10). Table 2 shows that relatively minor but significant changes in other phosphoglyceride molecular species were produced by the fat-free diet in addition to the pronounced changes in 18:0/20:4n-6 PC and 16:0/18:1n-9 PC. Several of these relatively minor changes could help maintain unaltered the dynamic properties of rat liver microsomes in fat deficiency. The influence of the double-bond number and position in *sn*-2 on the acyl chain dynamics at different bilayer depths seems to be complex and seems to depend on the fatty acid in *sn*-1. In order to learn the role of these relatively minor changes for the viscotropic compensation in the fat-free condition, further measurements in liposomes of these individual phosphoglyceride species are necessary.

Another important subject is the metabolic origin of the phosphoglyceride molecular species. The 18:0/20:4n-6 PC is known to originate appreciably by methylation of PE (28), whereas the 16:0/18:1n-6 PC mainly comes from the *de novo* path. Since PE methylation seems to be sensitive to the membrane lipid dynamics (28), an interesting question to resolve in the future is whether the fat-free diet might alter the flux along the different paths.

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