JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

J. Agric. Food Chem. **2007,** *55,* 6191–6197 **6191**

Stereospecific Analysis of Phospholipid Classes in Skeletal Muscle from Rats Fed Different Fat Sources

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The fatty acid (FA) and dimethylacetal profiles of the *sn*-1 and *sn*-2 positions of different phospholipid (PL) classes from skeletal muscle of rats as affected by dietary FA profiles were studied. Rats were fed either a control diet, an olive oil-enriched diet, or a sunflower oil-enriched diet. The FA composition of both positions of the studied PL classes was affected by diet to different extents. The FA composition of the *sn*-2 position of phosphatidylserine was the most influenced by diet, while phosphatidylinositol was less affected by dietary modification. The FA profile of phosphatidylcholine reflected consumed FA better than any other studied PL. Thus, olive oil rats showed higher oleic acid (C18:1 n-9) contents in both positions of phosphatidylcholine, and sunflower oil rats had higher proportions of arachidonic acid (C20:4 n-6) in the *sn*-1 position of this PL class. Dimethylacetals were scarcely affected by diet, and only the dimethylacetal composition of phosphatidylethanolamine showed significant modifications.

KEYWORDS: Phospholipid classes; *sn*-1 and *sn*-2 positions; skeletal muscle; fatty acids; dimetylacetals; dietary fatty acids

INTRODUCTION

Phospholipids (PLs) are the key components of all biological membranes. Each tissue exhibits its own pattern of PL classes; phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) are the more representative classes in mammal skeletal muscle (*1*). Each PL class shows a pattern of molecular species containing different combinations of acyl and alkyl chains in different proportions (*1*). It is generally accepted that the fatty acid (FA) composition of biomembrane PLs can be altered due to nutritional or environmental factors (*2*). In fact, numerous studies have demonstrated that changes in dietary FA composition may alter the FA composition of membrane PLs of different tissues (*3*−*6*). Several works have shown that the FA composition of muscle PLs from pig, beef, or chicken is strongly influenced by the FA composition of dietary fat (*7*−*9*). Moreover, the effect of feeding diets showing a different FA composition on the FA profile of individual PL classes from different animal tissues was shown by Tejeda (*10*) and Sánchez and Lutz (*11*). Similarly, the proportions of dimethylacetals (DMA) in different PL classes have been shown to be influenced by dietary FA composition (*10*, *12*).

The FA and DMA profiles of the *sn*-1 and *sn*-2 positions in PLs from animal muscle may have several important consequences, both in vivo and in the derived foodstuffs. There is a preferential esterification of the *sn*-2 position of PLs with polyunsaturated fatty acids (PUFA), aimed to protect those FAs more prone to oxidation against oxidative damage (*13*). Furthermore, it seems that the vinyl ether linkage in the *sn*-1 position of plasmalogens also plays a certain antioxidative role (*14*). On the other hand, the content of certain FAs in the *sn*-2 position of some PL classes has important consequences for the formation of different eicosanoids (*15*).

However, only a few studies have dealt with the influence of dietary FA composition on the FA distribution in the *sn*-1 and *sn*-2 positions of different PL classes in skeletal muscle. The effect of feeding diets enriched with either linoleic acid (C18:2 n-6) or linolenic acid (C18:3 n-3) on the positional distribution of FAs in liver PC was analyzed by Pudelkewicz et al. (*16*). Hvattum et al. (*17*) and Barceló-Coblijn et al. (*12*) studied the influence of diets with different FA profiles on the relative distribution of molecular species of PL classes from salmon kidney and rat brain, respectively. Recently, Pacetti et al. (*18*) showed the effect of feeding laying hens with refined seal blubber oil on regiospecific distribution of FAs in PC and PE of the produced eggs.

The purpose of the present study was to investigate the effects of feeding diets enriched in different oils on the FA and DMA composition of the *sn*-1 and *sn*-2 positions of PC, PE, PS, and PI from the *Longissimus dorsi* muscles of rats.

MATERIALS AND METHODS

Experiment, Diets, Animals, and Sampling. This study was carried out with 18 male Wistar rats that were randomly assigned to three groups at live weights of 48.3 ± 1.2 g. The control (C) group was fed

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10.1021/jf071354d CCC: $37.00 © 2007 American Chemical Society
Published on Web 06/20/2007

Table 1. Chemical Composition and FA Profile (% of Total FAME Detected) of Diets Supplied to the Studied Animals

	diets		
	C	SO	OO
chemical composition			
dry matter (%)	90.9	92.9	92.4
fat (% DM)[a]	3.3	10.4	10.0
crude fiber (% DM)	3.5	3.0	3.0
ash (% DM)	5.9	3.0	3.0
crude protein (% DM)	16.8	17.5	17.7
NFE[b] (% DM)	67.6	63.7	63.6
FAs			
C16:0	19.6	6.4	11.3
C16:1	0.9	0.2	0.9
C18:0	6.6	3.7	3.6
C18:1 n-9 + C18:1 n-7	30.8	27.3	78.1
C18:2 n-6	39.2	62.3	5.5
C18:3 n-3	2.7	0.1	0.6
ΣSFA[c]	26.3	10.1	14.9
ΣMUFA[d]	31.8	27.5	79.0
ΣPUFA[e]	42.0	62.4	6.1

[a] DM, dry matter. [b] NFE, nitrogen free extractives. [c] ΣSFA, sum of saturated FAMEs detected. [d] ΣMUFA, sum of MUFAs detected. [e] ΣPUFA, sum of PUFAs detected.

a commercial rat chow manufactured by Panlab Laboratories (Barcelona, Spain). The other groups were fed two different fat-enriched diets: an olive oil (OO) (Fedeoliva S.A., Jaen; Spain)-enriched diet and a sunflower oil (SO) (Koipesol S.A., Jaen)-enriched diet. These two fat-enriched diets were essentially AIN93G diets (*19*) except for the total fat content, which was increased from 7 to 10% (by weight) at the expense of carbohydrates. Chemical and FA compositions of the diets are summarized in **Table 1**. All rats were fed ad libitum for 8 weeks. Animals were allocated in boxes in a temperature-controlled room (22 ± 1 °C) and kept on a 12 h light/dark cycle. The protocols were approved by the Ethical Committee of the University of Granada, and animals were handled according to the guidelines for care and use of laboratory animals of the Spanish Society for Laboratory Animal Sciences. Rats were fasted overnight and sacrificed by severance of the vertebral column. Their *Longissimus dorsi* muscles were dissected and stored at −80 °C until analysis.

Intramuscular Fat Extraction. Samples were ground using a commercial grinder immediately before fat extraction. Intramuscular total lipids were extracted with chloroform/methanol (1:2, v/v) with 0.05% butylated hydroxytoluene (BHT), according to the method described by Bligh and Dyer (*20*).

Isolation of PLs. Lipid extracts were separated into lipid classes in NH_2-aminopropyl minicolumns (500 mg) from Varian (Harbor City, CA), following the method described by Ruiz et al. (*21*). Briefly, minicolumns were activated with 7.5 mL of hexane. Twenty milligrams of lipids dissolved in 150 μL of hexane:chloroform:methanol (95:3:2, v/v/v) was loaded onto the column. Neutral lipids were eluted with 5 mL of chloroform and free FA with 5 mL of diethyl ether:acetic acid (98:2, v/v). In this way, minicolumns retained the PLs.

Fractionation of PLs. Retained PLs were further separated into PL classes in the same minicolumn in which they had been retained, following the method used for muscle PLs fractionation into PC, PE, PS, and PI described by Pérez-Palacios et al. (*22*). PC, PE, PS, and PI were eluted with 30 mL of acetonitrile:*n*-propanol (2:1, v/v), 10 mL of methanol, 7.5 mL of isopropanol:3 N methanolic HCl (4:1, v/v), and 17.5 mL of chloroform:methanol:37% HCl (200:100:1, v/v/v), respectively. The vaccum was adjusted to generate a flow of 1 mL/min. The correct separation of PL classes was ensured by analyzing PL classes by thin-layer chromatography (TLC) (*22*).

PL Classes Hydrolysis with Phospholipase A_2. A stock solution of 1 mg of phospholipase A_2 from *Apis mellifera* (1225 units/mg; Sigma-Aldrich) in 23.1 mL of 1 M tris buffer (pH 8.9) containing calcium chloride (4 mM) was made. Hydrolysis was performed by adding 300 μL of the phospholipase A_2 stock solution to each PL-eluted class dissolved in 6 mL of diethyl ether with BHT (0.05%) (*23*). The mixture was incubated for 3 h in a N_2 atmosphere with continuous stirring at 25 °C. Thereafter, it was washed with methanol:chloroform (2:1, v/v) and dried with anhydrous sodium sulfate (*24*). The reaction mixture was separated in NH_2-aminopropyl minicolumns (500 mg), following the method previously described (*21*) with small modifications. Briefly, columns were activated with 7.5 mL of hexane. The sample, dissolved in 3 mL of diethylether:acetic acid (98:2, v/v), was added to the colum. Free FAs (position *sn*-2 in the original PL) were eluted with 2.5 mL of diethylether:acetic acid (98:2, v/v), and lysophospholipids (position *sn*-1 in the original PL) were eluted in two fractions, the first one with 2.5 mL of 0.05 M sodium acetate in methanol:chloroform (6:1, v/v) and the second with 2.5 mL of methanol (*25*). The correct separation of free FAs and lysophospholipids was ensured by TLC (*26*).

Fatty Acid Methyl Esters (FAMEs) and DMA Preparation and Analysis. FAMEs from acyl chains and DMAs from alkenyl chains were prepared by acidic trans-esterification in the presence of sulfuric acid (5% sulfuric acid in methanol) (*27*). FAMEs were analyzed by gas chromatography, using a Hewlett-Packard HP-5890-II gas chromatograph, equipped with a flame ionization detector (FID). Separation was carried out on a polyethyleneglycol capillary column (60 m long, 0.32 mm id, and 0.25 μm film thickness) (Supelcowax-10, Supelco, Bellafonte, PA). Oven temperature programming started at 180 °C. Inmediately, it was raised 4 °C min^{-1} to 200 °C, held for 35 min at 200 °C, increased again at 5 °C min^{-1} to 250 °C, and held for the last 11 min at 250 °C. Injector and detector temperatures were 250 °C. The carrier gas was nitrogen at a flow rate of 0.8 mL/min. Individual FAME peaks were identified by comparing their retention times with those of standards (Sigma, St. Louis, MO). To confirm identification, selected samples were subjected to gas chromatography coupled to mass spectrometry (GC-MS) in a HP5890GC series II gas chromatograph (Hewlett-Packard) coupled to a mass selective detector (HP-5971 A, Hewlett-Packard). FAs and DMAs were separated using the same column as that used for GC-FID, with helium operating at 41.3 KPa of column head pressure, resulting in a flow of 1.45 mL min^{-1} at 180 °C. The injector and oven program temperatures were the same as for the GC-FID analysis. The transfer line to the mass spectrometer was maintained at 280 °C. The mass spectra were obtained by electronic impact at 70 eV, a multiplier voltage of 1756 V, and collecting data at a rate of 1 scan s^{-1} over the m/z range of 30−500. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST/EPA/NIH and Wiley libraries.

Statistical Analysis. An individual animal was the experimental unit for analysis of all data. The effect of diet on the FA and DMA composition in each relative position (*sn*-1 and *sn*-2) within each PL class (PC, PE, PS, and PI) was compared by one-way analysis of variance (ANOVA) using the General Linear Model of SPSS (v.12.0). When a significant effect ($p < 0.05$) was detected, paired comparisons between means were conducted using the Tukey's test.

RESULTS

Chemical composition and FA profiles of diets are shown in **Table 1**. Diets enriched in SO and OO showed higher levels of fat (10.4 and 10.0%, respectively) than the control rat chow (3.3%). The latter diet showed the highest levels of saturated FAs (SFA), primarily palmitic acid (C16:0) (19.6 vs 6.4 and 11.3% in C, SO, and OO diets, respectively) and stearic acid (C18:0) (6.6 vs 3.7 and 3.6% in C, SO, and OO, respectively). The highest values for monounsaturated FAs (MUFA) were found in OO diets (79.0 vs 31.8 and 27.5% in OO, C, and SO, respectively). Levels of PUFA were higher in the SO-enriched diet than in the C and OO ones, primarily due to the higher linoleic acid (C18:2 n-6) content (62.3 vs 39.2 and 5.5%, in SO, C, and OO diets, respectively). However, the C diet contained the highest proportion of linolenic acid (C18:3 n-3) (2.7 vs 0.1 and 0.6% in C, SO, and OO diets, respectively).

Results from the ANOVA comparing the proportion of individual FAMEs and DMAs from the *sn*-1 and *sn*-2 positions

Table 2. FA and DMA Composition (% Total FAME and DMA Detected) in the *sn*-1 and *sn*-2 Positions of PC of *Longissimus dorsi* from Rats Fed with Different Diets[a]

FA	feeding groups			SEM[b]	*p*
	C	SO	OO		
		sn-1 position			
C14:0	0.9	1.0	0.2	0.13	0.098
C14:1	1.8 a	1.0 ab	0.3 b	0.24	0.058
C16:0 DMA	5.9	8.7	0.8	1.97	0.426
C16:1 DMA	2.6	7.8	<0.1	1.56	0.184
C16:0	35.8	27.2	35.6	3.91	0.595
C16:1	0.9	0.5	0.7	0.07	0.130
C17:0	0.7	0.7	0.5	0.14	0.830
C17:1	ND[c]	ND	ND		
C18:0 DMA	<0.1	ND	ND	0.01	0.489
C18:1 n-9 DMA	<0.1	ND	ND	0.01	0.489
C18:1 n-7 DMA	<0.1	ND	ND	0.007	0.489
C18:0	17.3	12.9	14.8	1.19	0.295
C18:1 n-9	13.1 ab	7.9 b	18.6 a	1.31	0.002
C18:1 n-7	2.4	1.9	3.1	0.21	0.164
C18:2 n-6	10.3	8.9	9.7	1.28	0.894
C18:3 n-3	<0.1	1.2	0.6	0.3	0.144
C20:4 n-6	5.6 b	17.7 a	10.4 ab	2.07	0.010
C22:5 n-3	0.9	0.5	0.5	0.13	0.530
C24:0	0.9	0.3	0.3	0.16	0.228
C22:6 n-3	<0.1 b	0.2b	1.4 a	0.15	0.000
C24:1 n-9	0.5	0.2	0.1	0.09	0.300
ΣSFA[d]	55.8	42.3	51.7	3.69	0.250
ΣMUFA[e]	18.8 ab	11.2 b	22.9 a	1.55	0.010
ΣPUFA[f]	16.8	27.4	22.1	2.66	0.197
Σn-6[g]	15.9	26.8	21.1	2.64	0.168
Σn-3[h]	0.9	1.9	2.0	0.33	0.307
ΣDMA[i]	8.6	16.4	0.9	3.39	0.272
		sn-2 position			
C14:0	0.7	1.7	0.5	0.56	0.647
C14:1	1.7	2.7	1.00	0.89	0.793
C16:0	6.7	9.9	7.4	0.96	0.319
C16:1	1.1	0.4	0.7	0.12	0.023
C17:0	0.2	0.5	0.3	0.16	0.726
C17:1	<0.1	<0.1	0.1	0.02	0.768
C18:0	5.2	7.0	8.0	0.45	0.070
C18:1 n-9	6.4 b	8.0 b	16.0 a	1.17	0.007
C18:1 n-7	3.0	2.0	3.2	0.28	0.173
C18:2 n-6	20.0	7.4	15.5	2.40	0.034
C18:3 n-3	0.3	0.5	0.7	0.06	0.118
C20:4 n-6	49.9	55.4	42.8	2.93	0.349
C22:5 n-3	1.5	1.0	0.4	0.22	0.304
C24:0	0.8	1.7	0.7	0.25	0.235
C22:6 n-3	1.2 ab	0.2 b	2.2 a	0.25	0.002
C24:1 n-9	<0.1	0.2	<0.1	0.06	0.437
ΣSFA	14.6	21.7	17.2	1.79	0.198
ΣMUFA	12.5	13.9	21.2	1.52	0.163
ΣPUFA	72.6	63.9	61.0	1.97	0.043
Σn-6	69.9	62.8	58.3	1.88	0.059
Σn-3	3.0	1.6	3.4	0.31	0.047

[a] Means with different letters within each relative position differ significantly ($P < 0.05$). [b] SEM, standar error of the mean. [c] ND, not detected. [d] ΣSFA, sum of saturated FAMEs detected. [e] ΣMUFA, sum of MUFAs detected. [f] ΣPUFA, sum of PUFAs detected. [g] Σn-6, sum of n-6 FAMEs detected. [h] Σn-3, sum of n-3 FAMEs detected. [i] ΣDMA, sum of DMA detected.

of PC, PE, PS, and PI of *Longissimus dorsi* of rats fed diets with different fat sources are shown in **Tables 2−5**, respectively.

PC. The *sn*-1 position of PC from rats fed an OO-enriched diet had higher levels of oleic acid (C18:1 n-9) and docosahexaenoic acid (DHA) (C22:6 n-3) (18.6 and 1.4%, respectively) than the C group (13.1 and less than 0.1%, respectively) and the SO group (7.9 and 0.2%, respectively) (**Table 2**). The proportion of arachidonic acid (C20:4 n-6) in the *sn*-1 position of PC also showed differences among the three experimental groups ($P = 0.010$), the higher one being that of the SO group (17.7%), followed by the OO (10.4%) and by the C groups (5.6%). SFA and PUFA did not show statistical differences among the three experimental groups. However, the proportion of MUFA was significantly affected by diet ($P = 0.010$), being higher in the OO group (22.9%) than in the SO (12.0%) and C groups (18.8%).

Similarly to the *sn*-1 position, in the *sn*-2 position of PC, oleic acid (C18:1 n-9) and DHA (C22:6 n-3) levels were affected by diet ($P = 0.007$ and $P = 0.002$, respectively), being higher in the OO group (16.0 and 2.2%, respectively) than in the C group (6.44 and 1.2%, respectively) and the SO group (8.0 and 0.2%, respectively). However, no differences were found for SFA and MUFA levels in the *sn*-2 position of PC among the three experimental groups. Curiously, the linoleic acid (C18:2 n-6) content in the *sn*-2 position of PC was significantly affected by diet ($P = 0.034$), the lowest level corresponding to animals from the SO group, followed by those of the OO and C groups (7.4, 15.5, and 20.0%, respectively). As a consequence, the effect of feeding diets with different fat sources was significant for PUFA ($P = 0.043$), although the Tukey's test did not detect significant differences between groups.

PE. Levels of SFA and MUFA in the *sn*-1 position of PE were influenced by the considered experimental diets ($P < 0.001$ and $P = 0.019$, respectively), being lower in the OO group (40.7 and 11.6%, respectively) than in the C (54.8 and 15.9%, respectively) and SO groups (49.8 and 18.3%, respectively) (**Table 3**). These results were the consequence of lower proportions of stearic acid (C18:0) and oleic acid (C18:1 n-9) in the OO group than in the other two experimental groups. The level of PUFA in the *sn*-1 position of PE did not show significant differences among experimental groups, even though rats from the group OO had nearly double contents than those from the group SO (22.4 vs 11.8%). The proportion of docosapentaenoic acid (EPA) (C22:5 n-3) and DHA (C22:6 n-3) showed statistical differences among experimental groups in the *sn*-1 position of PE ($P < 0.001$ and $P = 0.019$, respectively), being higher in the OO group (1.4 and 9.1%, respectively) than in the C group (0.42 and less than 0.1%, respectively) and the SO group (0.3 and 2.3%, respectively). As a consequence, the level of total n-3 FAs was higher ($P < 0.001$) in the OO group (10.6%) than in groups C and SO (0.5 and 4.0%, respectively).

PE was the only PL class in which diet influenced the DMA content. Proportions of octadecenaldimethylacetals (18:1 n-9 DMA, 18:1 n-7 DMA) and total DMAs were significantly affected by diet ($P = 0.040$, $P = 0.006$, and $P = 0.049$, respectively), being higher in rats from the OO group (6.8, 4.8, and 24.3%, respectively) than in the C (3.5, 2.7, and 17.3%, respectively) and SO groups (2.6, 1.2, and 12.6%, respectively).

The FA composition in the *sn*-2 position of PE only showed significant differences for the level of DHA (C22:6 n-3) ($P < 0.001$), which was higher in the OO group than in the C and SO groups (15.8, 9.8, and 1.9%, respectively). Similarly to the *sn*-1 position of PE and because of differences in the proportion of DHA (C22:6 n-3), the level of total n-3 PUFA was affected by diet ($P < 0.001$), being higher in the OO group than in the C and SO groups (16.9, 11.6, and 3.2%, respectively). Proportions of SFA, MUFA, and PUFA in the *sn*-2 position of PE did not show statistical differences among the three experimental groups of rats.

PS. The effect of feeding diets with different added oils on the FA pattern of the *sn*-1 position of PS was only significant for minor FAs, such as heptadecanoic acid (C17:0), heptadecenoic acid (C17:1 n-7), docosapentaenoic acid (C22:5 n-3), nervonic acid (C24:1 n-9), and DHA (C22:6 n-3) (**Table 4**). Contrarily, the FA composition of the *sn*-2 position of PS was

Table 3. FA and DMA Composition (% Total FAME and DMA Detected) in the *sn*-1 and *sn*-2 Positions of PE of *Longissimus dorsi* from Rats Fed with Different Diets[a]

FA	feeding groups			SEM	p
	C	SO	OO		
		sn-1 position			
C14:0	2.2	2.4	1.7	0.210	0.537
C14:1	2.5	2.8	1.8	0.220	0.348
C16:0 DMA	8.8	5.8	10.7	0.910	0.109
C16:1 DMA	1.5	2.3	1.2	0.350	0.501
C16:0	8.8	9.4	7.2	0.440	0.188
C16:1	0.4	0.6	0.6	0.080	0.632
C17:0	0.6	0.7	0.3	0.090	0.257
C17:1	0.9	1.2	0.8	0.170	0.583
C18:0 DMA	0.8	0.6	1.2	0.110	0.150
C18:1 n-9 DMA	3.5 ab	2.6 b	6.8 a	0.570	0.040
C18:1 n-7 DMA	2.7 ab	1.2 b	4.8 a	0.460	0.006
C18:0	39.7 a	36.1 ab	29.7 b	1.390	0.018
C18:1 n-9	9.8 ab	11.5 a	6.8 b	0.700	0.040
C18:1 n-7	1.8	1.9	1.2	0.110	0.085
C18:2 n-6	5.6	6.2	3.4	0.630	0.298
C18:3 n-3	0.1	1.4	<0.1	0.290	0.095
C20:4 n-6	5.7	8.6	8.4	1.300	0.605
C22:5 n-3	0.4 b	0.3 b	1.4 a	0.130	0.000
C24:0	3.4	0.7	1.6	0.450	0.029
C22:6 n-3	<0.1 b	2.3 b	9.1 a	0.945	0.000
C24:1 n-9	0.2	0.1	0.1	0.030	0.561
ΣSFA	54.8 a	49.8 a	40.7 b	1.620	0.000
ΣMUFA	15.9 ab	18.4 a	11.6 b	0.950	0.019
ΣPUFA	11.8	17.4	22.4	2.070	0.152
Σn-6	11.3	14.9	12.2	1.610	0.633
Σn-3	0.5 c	4.0 b	10.6 a	1.020	0.000
ΣDMA	17.3 b	12.6 ab	24.3 a	1.830	0.049
		sn-2 position			
C14:0	0.9	1.2	0.8	0.300	0.582
C14:1	1.8	1.9	1.3	0.170	0.753
C16:0	6.5	9.6	6.9	0.990	0.357
C16:1	1.1	2.0	0.3	3.680	0.302
C17:0	0.3	1.0	0.3	0.190	0.614
C17:1	<0.1	0.4	<0.1	0.070	0.450
C18:0	8.1	8.6	11.7	0.760	0.150
C18:1 n-9	7.8	13.0	8.0	2.740	0.687
C18:1 n-7	1.1	2.3	1.2	0.250	0.261
C18:2 n-6	7.8	4.9	5.6	0.620	0.052
C18:3 n-3	0.3	0.5	0.4	0.070	0.547
C20:4 n-6	47.4	48.5	43.2	5.340	0.916
C22:5 n-3	1.5	0.8	0.7	0.180	0.156
C24:0	4.5 a	1.8 b	3.1 a	0.450	0.008
C22:6 n-3	9.8 a	1.9 b	15.8 a	1.630	0.000
C24:1 n-9	<0.1	<0.1	<0.1	0.010	0.230
ΣSFA	21.1	23.3	23.3	1.640	0.771
ΣMUFA	12.0	19.1	10.9	4.650	0.488
ΣPUFA	66.5	56.3	65.3	5.690	0.576
Σn-6	55.3	53.6	48.8	5.190	0.873
Σn-3	11.6 a	3.2 b	16.9 a		

[a] For footnotes and abbreviations, see **Table 2**.

Table 4. FA and DMA Composition (% Total FAME and DMA Detected) in the *sn*-1 and *sn*-2 Positions of PS of *Longissimus dorsi* from Rats Fed with Differents Diets[a]

FA	feeding groups			SEM	*p*
	C	SO	OO		
		sn-1 position			
C14:0	1.4	1.6	1.6	0.080	0.493
C14:1	3.7	3.1	3.6	0.270	0.619
C16:0 DMA	3.3	4.4	0.7	1.210	0.631
C16:1 DMA	2.0	3.9	0.4	0.910	0.430
C16:0	15.0	17.0	20.3	0.940	0.156
C16:1	1.3	1.5	0.9	0.140	0.413
C17:0	0.4 b	<0.1 b	1.0 a	0.110	0.002
C17:1	0.2 ab	<0.1 b	0.7 a	0.096	0.023
C18:0 DMA	ND	ND	ND		
C18:1 n-9 DMA	ND	ND	ND		
C18:1 n-7 DMA	ND	ND	ND		
C18:0	27.8	24.0	36.2	2.450	0.275
C18:1 n-9	19.2	21.0.	13.7	1.260	0.158
C18:1 n-7	2.9	2.8	2.2	0.170	0.457
C18:2 n-6	11.9	10.5	10.5	1.330	0.892
C18:3 n-3	ND	2.2	0.4	0.470	0.083
C20:4 n-6	6.4	5.2	4.7	1.010	0.822
C22:5 n-3	1.1 a	0.3 ab	<0.1 b	0.180	0.014
C24:0	2.2	1.7	ND	0.450	0.283
C22:6 n-3	<0.1 b	0.7 ab	1.0 a	0.150	0.013
C24:1 n-9	0.9	0.2	0.2	0.150	0.029
ΣSFA	46.8	44.8	59.8	2.530	0.128
ΣMUFA	28.4	28.8	22.4	1.620	0.424
ΣPUFA	19.4	16.6	16.3	2.100	0.815
Σn-6	18.3	15.7	15.2	2.170	0.844
Σn-3	1.1	3.1	1.4	0.470	0.133
ΣDMA	5.3	7.5	1.1	1.840	0.557
		sn-2 position			
C14:0	0.8	1.8	1.0	0.320	0.391
C14:1	1.6	1.0	1.4	0.150	0.187
C16:0	8.7	7.8	10.2	0.440	0.126
C16:1	1.0	0.5	0.5	0.090	0.028
C17:0	0.4	0.2	0.4	0.050	0.407
C17:1	<0.1	<0.1	0.1	0.020	0.705
C18:0	15.9 b	9.2 c	20.4 a	1.280	0.000
C18:1 n-9	14.3	13.5	13.9	0.760	0.902
C18:1 n-7	2.0	1.4	1.8	0.150	0.151
C18:2 n-6	10.8 a	5.3 b	9.2 ab	0.890	0.009
C18:3 n-3	0.3 b	0.5 a	0.4 ab	0.033	0.024
C20:4 n-6	40.0 b	55.5 a	36.3 b	2.990	0.008
C22:5 n-3	1.5 a	0.6 b	0.7 b	0.130	0.001
C24:0	0.7	1.1	0.5	0.150	0.373
C22:6 n-3	1.0 b	0.6 b	2.2 a	0.220	0.015
C24:1 n-9	<0.1	<0.1	0.1	0.014	0.111
ΣSFA	27.2 a	20.6 b	33.2 a	1.520	0.001
ΣMUFA	19.2	16.9	18.0	0.880	0.530
ΣPUFA	53.3 ab	62.0 a	48.5 b	2.080	0.020
Σn-6	50.8 ab	60.8 a	45.5 b	2.200	0.012
Σn-3	2.7 ab	1.7 b	3.4 a	0.260	0.019

[a] For footnotes and abbreviations, see **Table 2**.

strongly affected by the FA profile of diets. Thus, the proportion of stearic acid (C18:0) showed significant differences ($P < 0.001$) among the three experimental groups of rats, being higher in the OO group than in the C and SO groups (20.4, 15.9, and 9.1%, repsectively). Consequently, the level of SFA was also significantly affected by diet ($P = 0.001$), the OO group showing higher levels (33.2%) than C and SO groups (27.2 and 20.6%, respectively).

The proportion of arachidonic acid (C20:4 n-6) in the *sn*-2 position of PS was significantly affected by the type of experimental diet ($P = 0.008$). As a consequence, PUFA levels in the *sn*-2 position of PS also showed significant differences between the three experimental groups ($P = 0.020$). The proportions of both variables were higher in the SO group of rats (55.5 and 62.0%, respectively) than in the C (40.0 and 53.3%, respectively) and OO groups (36.3 and 48.5%, respectively). Contrarily, the level of linoleic acid (C18:2 n-6) was also affected by diet ($P = 0.009$) but lower in the SO group (5.3%) than in the OO and C groups (9.2 and 10.8%, repsectively). The influence of oil-enriched diets on the level of DHA (C22:6 n-3) in the *sn*-2 position of PS was similar to that in the *sn*-2 position of PC and PE, showing differences ($P = 0.015$) among experimental groups, the OO group having higher levels (2.2%) than the C and SO groups (1.0 and 0.6%, repsectively). Consequently, the proportion of total n-3 FAs was higher in the *sn*-2 position of PS of the OO group (3.4%) than in the other two groups (2.7 and 1.7% in C and SO groups, respectively). Levels of other minor FAs, such as palmitoleic (C16:1 n-7),

Table 5. FA and DMA Composition (% Total FAME and DMA Detected) in the *sn*-1 and *sn*-2 Positions of PI of *Longissimus dorsi* from Rats Fed with Differents Diets[a]

FA	feeding groups			SEM	*p*
	C	SO	OO		
		sn-1 position			
C14:0	1.6	2.7	1.8	0.370	0.457
C14:1	4.8	2.1	4.2	0.490	0.043
C16:0 DMA	4.6	11.2	0.1	2.220	0.183
C16:1 DMA	6.8	6.9	0.6	1.270	0.114
C16:0	19.2	22.1	20.7	1.840	0.825
C16:1	0.7	6.7	1.0	1.670	0.289
C17:0	ND	ND	ND		
C17:1	ND	ND	ND		
C18:0 DMA	ND	ND	ND		
C18:1 n-9 DMA	ND	ND	ND		
C18:1 n-7 DMA	ND	ND	ND		
C18:0	23.9	18.0	32.7	2.580	0.112
C18:1 n-9	22.3	14.3	17.3	1.740	0.133
C18:1 n-7	2.4	1.3	1.8	0.190	0.039
C18:2 n-6	7.4	3.5	13.2	1.720	0.115
C18:3 n-3	<0.1 b	3.1 a	2.2 ab	0.540	0.024
C20:4 n-6	4.5	6.5	2.3	1.340	0.565
C22:5 n-3	<0.1	<0.1		0.020	0.768
C24:0	0.5	0.2	1.0	0.260	0.550
C22:6 n-3	0.2	<0.1		0.051	0.270
C24:1 n-9	0.2	0.1	0.5	0.070	0.090
ΣSFA	45.8	35.0	56.7	4.240	0.170
ΣMUFA	30.5	19.8	24.8	2.510	0.180
ΣPUFA	12.2	8.1	15.5	1.840	0.348
Σn-6	11.9	8.0	15.5	1.830	0.340
Σn-3	0.4	2.6	2.2	0.800	0.113
ΣDMA	11.4	14.5	0.8	3.140	0.286
		sn-2 position			
C14:0	1.1	2.3	1.4	0.240	0.078
C14:1	2.5 ab	1.1 b	2.6 a	0.270	0.022
C16:0	7.5	7.5	9.8	0.420	0.067
C16:1	1.7 a	0.5 b	0.4 b	0.190	0.000
C17:0	0.4 a	<0.1 b	0.5 a	0.050	0.002
C17:1	<0.1 ab	<0.1 b	<0.1 a	0.010	0.025
C18:0	9.4 b	8.8 b	12.8 a	0.610	0.029
C18:1 n-9	7.7	10.1	8.3	0.560	0.175
C18:1 n-7	0.6	1.1	0.7	0.090	0.031
C18:2 n-6	3.2	4.5	2.5	0.410	0.174
C18:3 n-3	0.5	0.6	0.7	0.040	0.120
C20:4 n-6	61.1	60.0	57.9	2.030	0.853
C22:5 n-3	1.9 a	1.2 ab	0.8 b	0.190	0.037
C24:0	1.1	1.3	0.5	0.250	0.540
C22:6 n-3	<0.1	<0.1	<0.1	0.010	0.343
C24:1 n-9	<0.1	<0.1	<0.1	0.010	0.505
ΣSFA	20.3	20.9	25.8	1.260	0.228
ΣMUFA	12.8	13.0	12.2	0.640	0.915
ΣPUFA	66.4	65.8	61.2	1.760	0.503
Σn-6	64.3	64.5	60.3	1.780	0.652
Σn-3	2.5	1.9	1.6	0.170	0.065

[a] For footnotes and abbreviations, see **Table 2**.

linolenic (C18:3 n-3), and docosapentaenoic acids (C22:5 n-3), also showed significant differences in the *sn*-2 position of PS among experimental groups.

PI. The effect of OO- or SO-enriched diets on the FA profile of the *sn*-1 and *sn*-2 positions of PI (**Table 5**) was significant only for some minor FAs, such as miristoleic (C14:1 n-7), vaccenic (C18:1 n-7), and linoleic acids (C18:3 n-3) in the *sn*-1 position and miristoleic (C14:1 n-7), palmitoleic (C16:1 n-7), heptadecanoic (C17:0), heptacedenoic (C17:1 n-7), vaccenic (C18:1 n-7), and docosapentaenoic acids (C22:5 n-3) in the *sn*-2 position. Moreover, in the *sn*-2 position of PI, the proportion of stearic acid (C18:0) was also affected by diet ($P = 0.029$), with higher values for the OO group (12.8%) than for the C and SO groups (9.4 and 8.8%, respectively).

DISCUSSION

Results from this study suggest that the enrichment of diets with different oils shows a different influence on the FA and DMA profiles of the *sn*-1 and *sn*-2 in skeletal muscle of rats depending on the PL class. The incorporation of FAs into each PL class is a selective process (*28*, *11*), which can be explained by specific differences in the acylation process for each individual PL (*29*). Thus, the FA compositions of the *sn*-1 position of PE and the *sn*-2 position of PS were modified by the considered type of experimental diet to a large extent. Important changes in the FA profile of the *sn*-2 position of PE and in both positions of PC as a consequence of diet were also found. Contrarily, in the *sn*-1 and *sn*-2 postitions of PI, only minor FAs were affected by the type of experimental diet. Williams and Maunder (*30*), studying the effect of feeding diets with a different FA composition on the FA profile of membrane erythrocytes, also found that membrane PI was relatively resistant to modifications. These authors attributed this finding to the role of PI as a second messenger in cell signal transdution mechanisms and also to the fact that the maintenance of the FA composition of PI is an important feature of membrane homoesostatic mechanisms. Recently, Dannenberger et al. (*9*), studying PL classes of beef, also evidenced that among considered PL classes, PI was the less affected by the studied factors. However, PS, which was strongly influenced by diet in our study, is not believed to be involved in cell signaling through the formation of metabolites (*31*).

The FA composition of PC reflected dietary FAs better than that of other PL classes, which could be related to the abundance and situation of PC in membranes. This PL class is the most abundant one in skeletal muscle (*32*), often amounting to almost 50% of the total PLs, and as such, it is obviously the key building block of membrane bilayers in this tissue. Other studies have shown a strong influence of the dietary FA profile on the FA profile of PC; this PL class is more similar to the composition of total PLs than those of PE and PS + PI fractions (*10*).

Only small differences were found in the proportion of total SFA and palmitic (C16:0) and stearic acids (C18:0), despite the fact that differences in the content of such FAs were found in the diet. This is most likely due to the fact that a high proportion of SFA are from de novo synthesis, and only a small proportion of total SFA in skeletal muscle are directly accumulated from dietary FAs (*33*). In fact, other authors have observed that PLs from skeletal muscle of animals fed with diets enriched in OO and SO showed similar proportions of palmitic acid (C16:0); the proportion of this FA is higher only in animals fed diets containing 100 and 83.7% of palmitic acid (C16:0), due to the higher amounts of SFA in such diets (*33*). Nevertheless, total SFA in the *sn*-1 position of PE was reduced due to the use of the studied oil-enriched diets, and total SFA in the *sn*-2 position of PS of animals fed an SO-enriched diet was also strongly reduced. This effect could be due to the lower amount of SFA in such diets but in the case of 00-enriched diet also to an increase in the Δ-9 desaturase activity due to the presence of higher amounts of MUFA, as described by Monahan et al. (*34*).

Even though the oleic acid (C18:1 n-9) content was much higher in OO-enriched diets, the proportion of this FA in the studied PLs of the OO group was higher only in the *sn*-1 and *sn*-2 positions of PC. Moreover, in the *sn*-1 position of PE, oleic acid (C18:1 n-9) values in the OO group were the lowest. This could be related with the higher levels of C18:1 n-9 DMA found in this specific position, since one of the pathways for the

biosynthesis of plasmalogens involves a desaturation process of the FA sterified in the *sn*-1 position of the analogue PLs (*35*).

The found levels of PUFA showed a variable behavior. Thus, feeding diets enriched in linoleic acid (C18:2 n-6) (SO diet) did not affect the proportion of this FA in the *sn*-1 position of any of the studied PLs. Moreover, in the *sn*-2 position, the linoleic acid (C18:2 n-6) level was lower in PC and PS from animals fed the SO diet. Some of these results could be related with the higher proportion of arachidonic acid (C20:4 n-6) in the *sn*-1 position of PC and in the *sn*-2 position of PS in the SO group. Soriguer et al. (*33*) also found higher values of arachiconic acid (C20:4 n-6) in tissues from rats fed SO. This is due to the pathway for biosynthesis of arachidonic acid (C20:4 n-6), which involves dasaturation and elongation of the dietary linoleic acid (C18:2 n-6) (*35*). However, in our study, the proportion of arachidonic acid (C20:4 n-6) only increased in the *sn*-1 position of PC and in the *sn*-2 position of PE from rats fed with SO diet. Some works have shown a relative constant content of arachidonic acid (C20:4 n-6) of PLs, despite the variation in the linoleic acid (C18:2 n-6) intake (*11*, *36*). The arachidonic acid (C20:4 n-6) content of PLs can be considered as a available pool for the synthesis of bioactive metabolites (*37*), which could explain the relative constant level observed for this FA (*11*).

The proportion of DHA (C22:6 n-3) was strongly influenced by consuming diets with a different FA profile. Soriguer et al. (*33*) found that n-3 PUFA were the most influenced by diet, specially EPA (C20:5 n-3) and DHA (C22:6 n-3). The proportion of DHA (C22:6 n-3) increased in both positions of PC, PE, and PS in the OO group. In a number of feeding studies with OO, an increase in n-3 PUFA has also been observed in total lipids and PLs from different organs and tissues (*38*−*41*). This is most likely due to the lower linoleic acid content (C18:2 n-6) of OO-enriched diets, since the competition between linoleic acid (C18:2 n-6) and linolenic acid (C18:3 n-3) for desaturases may result in lower levels of n-3 PUFA in lipids from animals fed diets with a higher linoleic acid (C18:2 n-6) content (*40*). Moreover, a decrease in Δ6-desaturase activity due to feeding native virgin OO has been reported (*39*). This effect could be responsible for the relative increase in DHA (C22:6 n-3) through a decrease in the conversion of linoleic acid (C18:2 n-6) to arachidonic acid (C20:4 n-6) (*42*).

The DMA content of studied PLs was scarcely affected by diet. Thus, only the DMAs in the *sn*-1 position of PE changed as a consequence of feeding different diets, which could be related to the higher DMA in this PL class as compared to other PL classes (*43*). The increase in the levels of C18:1 n-9 DMA and C18:1 n-7 DMA in the OO group could be an indirect consequence of the pathways for the biosynthesis of plasmalogens previously explained: desaturation of the FA sterified in the *sn*-1 position of the analogue PLs, giving rise to a higher C18:1 DMAs in the animals fed the OO diet. Tejeda (*10*) also found an increase in the C18:1 n-9 DMA level and a decrease in the C16:0 DMA level in PC and PE from pigs fattened on acorns, due to the high proportion of oleic acid (C18:1n-9) and low proportion of palmitic acid (C16:0) in such feeding source (*43*).

Overall, despite the fact that diets showed great differences in the FA profile, the FA and DMA compositions of the different studied PL classes were not so different. Nevertheless, some of the observed changes in the FA and DMA compositions of the *sn*-1 and *sn*-2 positions of studied PL classes as a consequence of diet could have important consequences. In fact, some authors (*14*) have proposed that plasmalogens actively participate in the protection of PUFA in the *sn*-2 position of PLs against oxidative degradation; hence, modifications in the amount of DMAs could have implications both in vivo and in the obtained meat. In addition, found changes in the FA profile of the *sn*-1 and *sn*-2 positions of studied PL classes could also have consequences on membrane functionality and on the susceptibility of PLs to lipid oxidation. Such differences could be related with particular PL and FA characteristics, such as the abundance and situation of the PL in the membrane (in the case of PC), the physiological function of a particular PL (PI, PS), or the role as precursor of several FAs for the biosynthesis of biologically active compounds (C20:4 n-6 or C22:6 n-3).

It can be concluded that dietary FA profiles influence the FA and DMA compositions of the *sn*-1 and *sn*-2 positions of PC, PE, PS, and PI from the *Longissimus dorsi* of rat. This effect was more marked in the *sn*-1 position of PE and in the *sn*-2 position of PS, while the FA profile of both positions in PI was scarcely affected. The FA profile of PC reflects consumed FAs better than the other studied PLs.

ABBREVIATIONS USED

FA, fatty acid; PL, phospholipid; DMA, dimethylacetal; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; C, control; SO, sunflower oil; OO, olive oil; SFA, saturated FA; MUFA, monounsaturated FA; PUFA polyunsaturated FA; FAME, FA methyl ester.

ACKNOWLEDGMENT

We thank Maria Alba Martínez Burgos of the Department of Physiology of the University of Granada for providing the animals of this study, and the Department of Anatomy at the University of Extremadura, where the mucles were dissected.

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Received for review May 9, 2007. Accepted May 16, 2007.

JF071354D