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Fatty acid composition of microsomal phospholipids in rats fed different oils and antioxidant vitamins supplement

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The aim of the study was to examine effects of feeding diets containing different oils and antioxidant vitamins supplementation on the fatty acid composition of hepatic microsomal phospholipids in the rat. Forty-eight male Sprague Dawley rats were fed for 20 days diets containing 15% corn, olive, fish, or hazelnut oil, without or with the addition of β*-carotene (30 mg/kg) and dl-*α*-tocopherylacetate (500 mg/kg),* n = *6 per group. Microsomal phospholipids of rats fed corn oil exhibited higher content of linoleic, arachidonic, and docosatetraenoic acids (*$P < 0.05$*). Compared with corn oil (control group), olive oil intake increased palmitic, oleic, and vaccenic acids in phosphatidylcholine and phosphatidylserine + phosphatidylinositol, and vaccenic acid in phosphatidylethanolamine (*$P < 0.05$*). Fish oil intake increased the level of n-3 long-chain polyunsaturated fatty acids, and decreased arachidonic acid in all phospholipids (*$P < 0.05$*). On the other hand, the intake of hazelnut oil increased palmitoleic and oleic acids in phosphatidylcholine and phosphatidylethanolamine, and palmitoleic and vaccenic acids in phosphatidylserine + phosphatidylinositol (*$P < 0.05$*). Vitamin supplementation increased microsomal retinol in rats fed corn oil, and* α*-tocopherol in all groups fed vegetable oils (*$P < 0.05$*). The intake of vitamins supplement induced slight modifications of the fatty acid profile of microsomal phospholipids: a higher level of monounsaturated fatty acids, mainly oleic acid, with a reduction of polyunsaturated fatty acids was observed. These results manifest that the composition of microsomes is affected by the fatty acid profile of dietary oils and the intake of antioxidant vitamins supplements.* (J. Nutr. Biochem. 9:155–163, 1998) © *Elsevier Science Inc. 1998*

Keywords: liver microsomes; phospholipids; fatty acids; α-tocopherol; β-carotene; and dietary oils

Introduction

In hepatocytes, the microsomal fraction has a lipid content of nearly 30 to 40% dry weight, mainly in the form of phospholipids (PLs).[1] Microsomes play important roles in various essential cellular functions, such as the control of endogenous lipid metabolism, (e.g., fatty acid elongation and desaturation), the biotrasformation of xenobiotics (drugs and environmental chemicals), and the regulation of intracellular calcium balance, among others. Both n-3 and n-6 polyunsaturated fatty acids (PUFAs) compete for the microsomal desaturase systems, and several studies have provided evidence supporting that the intake of marine oils induces an increase of the biosynthesis and the incorporation of long-chain n-3 PUFA into microsomal PLs, along with a decrease of arachidonic acid content.[2,3] The oxidative metabolism of drugs that occurs in hepatic microsomes is highly dependent on the type of dietary fat, as it affects the structure and functional properties of membranes.[4–6] Consumption of diets rich in PUFA is associated with increased susceptibility to oxidative attack and, consequently, membrane lipids may undergo oxidation. Free radical-mediated reactions modify membrane structure and biological functions by inducing a series of changes in the physical and chemical properties of membranes, such as the degree of unsaturation, fluidity, cytochrome P-450 content, and enzyme activities.[7–11] Lipoxidation may be reduced by endogenous or exogenous antioxidants, such as α-tocopherol and β-carotene, which exhibit a complementary action.[12] In previous studies, we have observed an interaction of the type of oil ingested and the intake of antioxidant vitamin

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supplement on the vascular smooth muscle reactivity in the rat.[13] Therefore, the objective of this study was to determine whether the intake of vitamin supplement (a mixture of dl-α-tocopherylacetate and β-carotene), in addition to different oils intake, affects the fatty acid composition of hepatic microsomal phospholipids in rats.

Methods and materials

Animals and diets

Forty-eight male Sprague Dawley rats (Universidad de Valparaíso Breeding Unit) weighing ca 100 g were divided into four groups of 12 rats each. The animals were housed individually in wire-bottomed cages and maintained in temperature-controlled room (21 ± 2°C) with controlled relative humidity (40 to 50%) and light/dark cycles (12 hr). Rats were randomly fed for 20 days one of four semisynthetic diets containing 15% (wt/wt) of one of four different oils: group C, fed corn oil (Mazola), as a source of n-6 PUFA (namely 18:2n-6), taken as the control group; group O, fed olive oil (Cánepa), which has an intrinsec resistance to lipoxidation and a particular fatty acid composition with a high content of 18:1n-9; group H, fed hazelnut oil (ACENAT), which has a high content of monounsaturated fatty acids, although differing from O; and group F, fed fish oil (Pesquera Quintero), obtained as a subproduct in the fish-meal industry. Oil was included in the diet as the only lipid source, without or with the addition of the vitamin mixture (V) of β-carotene (30 mg/kg) and dl-α-tocopheryl-acetate (500 mg/kg) (both vitamins kindly supplied by Roche, Santiago), $n = 6$ per group. Feed and water were provided ad libitum, as described earlier.[13] The overall composition of the experimental semipurified diet is as follows (g/kg): 200 casein, 3 DL-methionine, 35 AIN-76 mineral mix, 10 AIN-76 vitamin mix, 2 choline chloride, 50 cellulose, 203 corn starch, 347 sucrose, 150 oil. At the end of the feeding period, rats were killed between 08.30 and 09.30 am and livers were quickly dissected, rinsed in ice-cold physiological saline, blotted, and weighed.

Preparation of microsomes

Livers were homogenized in 0.25 M sucrose, 0.05 M phosphate buffer (pH 7.4) using a Potter Elvejhem homogenizer. The homogenates were centrifuged for 20 min at 10,000 × g (Sorvall centrifuge), and the resulting supernatant was centrifuged for 60 min at 105,000 × g (Beckman Model L2 65B centrifuge, Columbia, MD USA). The microsomal pellet was washed with sucrose/phosphate buffer and resuspended in 0.1M Tris buffer (pH 7.4).[14] All microsomal preparations were done at 0 to 4°C.

Fatty acid and vitamin analysis

Lipids were extracted from microsomes according to Folch et al.[15] Microsomal phospholipids (PLs) were separated by thin layer chromatography in Silica Gel 60 PF250 (Merck, Darmstadt) plates (20 × 20 cm) impregnated with 1 mM sodium carbonate, using chloroform: methanol: acetic acid: water (25:15:4:2) as mobile phase.[16] Plates were dried under N_2 for 30 min and PL classes were revealed with 0.01% (w/w) Rhodamin 6G solution in methanol under UV light. The fractions obtained were: lysophosphatidylcholine, sphingomyelin, phosphatidylcholine (PC), phosphatidylethanolamine (PE) and a mixture of phosphatidylserine + phosphatidylinositol (PSI). The last three fractions were obtained in amounts suitable to analyze their fatty acid composition by capillary gas-liquid chromatography. The identification of PLs was made against authentic standards (PC, PE, PS, PI were purchased from Sigma Chemical, St. Louis, MO USA). The PL fractions were scraped off the plates and kept in tubes containing 1 mL methanol and 10 μg/mL *t*-butyl-hydroxytoluene (BHT), under a stream of N_2, until analysis. Fatty acid methyl esters (FAME) of each PL class were prepared using 14% BF_3-methanol by heating at 100°C for 30 min.[17]

GC separation of FAME was performed on a Model 5890 A gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector, using a 25 m × 0.20 mm I.D. fused silica column coated with 25 μm HP-FFAP. The oven was temperature-programmed. The identities of the peaks were established by spiking with reference compounds (Sigma). Microsomal α-tocopherol and retinol contents were determined by high performance liquid chromatography (Merck-Hitachi) with UV-vis detection at 290 nm, using a Lichrosorb RP-18 (Merck, Darmstadt) column and methanol: water (96:4) as the mobile phase.[18] External standards of (±)α-tocopherol and all-*trans* retinol (Sigma) were used for the quantitative analysis of vitamins. All solvents used were added BHT (10 μg/mL) to prevent oxidation.

Statistical analysis

Data are expressed as mean ± SEM. Analysis of variance (ANOVA) followed by a least significance test were used to determine whether there were significant differences among the groups. Nonpaired Student's *t* test was used to evaluate the effect of vitamin supplementation (V) for each oil. *P* values less than 0.05 were considered significant.

Table 1 Fatty acid composition of dietary oils (wt % of total fatty acids)

Fatty acid	Corn oil	Olive oil	Fish oil	Hazelnut oil
14:0	—	—	6.7	—
14:1	—	—	0.3	—
15:0	—	—	0.8	—
16:0	11.2	13.5	18.5	2.2
16:1	—	1.4	7.7	25.2
17:0	—	—	1.6	—
17:1	—	—	0.9	—
18:0	2.2	1.8	4.6	0.6
18:1n-9	26.8	69.1	12.9	36.8
18:1n-7	0.6	2.9	3.3	6.0
18:2n-6	56.9	9.6	1.3	7.9
18:3n-3	1.0	0.7	0.8	—
20:0	0.4	0.4	—	1.5
20:1	0.3	0.4	2.5	2.9
20:2	—	—	0.4	5.9
20:4n-6	—	—	1.1	—
20:5n-3	—	—	10.7	—
21:0	—	—	1.9	—
22:1	—	—	0.5	1.6
22:2	—	—	—	6.7
22:4n-6	—	—	0.4	—
22:5n-6	—	—	0.5	—
22:5n-3	—	—	2.6	—
22:6n-3	—	—	13.0	0.3
24:0	—	—	2.4	—
24:1	—	—	0.3	—
Others	0.6	0.2	4.3	2.7
n-3/n-6	0.02	0.07	9.4	0.04

n-3/n-6 indicates the total n-3 fatty acid/total n-6 fatty acid ratio.
—, not detected.

Results

The relative fatty acid composition of dietary oils (*Table 1*) reflects their origin. Olive and hazelnut oils contain a high relative proportion of monounsaturated fatty acids (MUFA):

Table 2 Feed efficiency[1] and relative liver weight[2] of rats fed different dietary oils, without or with vitamin supplement[3]

	V	Diet			
		Corn oil	Olive oil	Fish oil	Hazelnut oil
Feed efficiency (%)	−	42.2 ± 2.2	41.7 ± 0.8	52.1 ± 2.2—a	41.6 ± 3.2
	+	41.6 ± 2.4	39.6 ± 1.2	24.6 ± 2.2*	38.5 ± 1.6
Relative liver weight (g/100 g)	−	5.1 ± 0.2	5.5 ± 0.3—a	5.3 ± 0.4—a	5.8 ± 0.2—b
	+	5.0 ± 0.4	4.9 ± 0.3*	4.4 ± 0.2*	5.7 ± 0.3

V: vitamin supplement (dl-α-tocopherylacetate + β-carotene).
[1]Feed efficiency = g weight gain/g feed consumed.
[2]Relative liver weight = g liver/100 g body weight.
[3]Data are expressed as mean ± SEM ($n = 6$).
*Within a column, values with * are significantly different from the respective nonsupplemented group as determined by Student's t test at $P < 0.05$.
Within a line, values without a common letter are significantly different from the corn oil group at $P < 0.05$ as determined by ANOVA.

73.8% and 72.2%, respectively. The predominant fatty acid in O is 18:1n-9, whereas H contains other MUFA species, namely 16:1, in high amounts (25.2%). The PUFA n-3/n-6 ratio of fish oil was 100 to 400 times higher than the value observed in the vegetable oils used. The feed efficiency (body weight gain/feed intake) and relative liver weight of rats are shown in *Table 2*. Feed efficiency was similar in C, O, and H diets, and was higher in F diet without the addition

Table 3 Fatty acid composition of liver microsomal phospholipids of rats fed diets containing 15% oil for 20 days[1] (wt % of total fatty acids)

Fatty acid	Phospholipid	Corn oil	Olive oil	Fish oil	Hazelnut oil
16:0	PC	22.8 ± 0.6	22.7 ± 1.4	25.8 ± 1.4—a	15.8 ± 0.2—b
	PE	20.6 ± 0.7	19.5 ± 0.3—a	22.6 ± 0.2—b	16.7 ± 0.6—c
	PSI	7.3 ± 0.4	7.5 ± 0.3	11.4 ± 1.0—a	8.0 ± 0.6
16:1	PC	0.7 ± 0.1	1.1 ± 0.2—a	1.7 ± 0.1—b	1.2 ± 0.1—a
	PE	0.8 ± 0.1	0.6 ± 0.1—a	1.4 ± 0.1—b	2.0 ± 0.1—c
	PSI	—	0.8 ± 0.2—a	1.2 ± 0.1—a	1.2 ± 0.2—a
18:0	PC	22.7 ± 0.7	23.6 ± 1.3	17.6 ± 0.6—a	14.0 ± 0.7—b
	PE	21.6 ± 0.8	22.0 ± 0.9	20.5 ± 0.7	13.9 ± 0.8—a
	PSI	40.1 ± 1.6	38.1 ± 1.0	37.4 ± 0.5	32.6 ± 2.1—a
18:1n-9	PC	3.6 ± 0.1	9.7 ± 0.4—a	7.5 ± 0.3—b	8.0 ± 0.3—b
	PE	7.8 ± 0.3	8.8 ± 0.4	6.3 ± 1.0—a	12.6 ± 0.8—b
	PSI	4.7 ± 0.6	8.8 ± 0.6—a	7.6 ± 0.9—a	6.1 ± 0.8
18:1n-7	PC	2.2 ± 0.3	3.2 ± 0.3—a	2.7 ± 0.2—b	2.0 ± 0.1
	PE	2.0 ± 0.2	8.8 ± 0.2—a	1.9 ± 0.2	1.7 ± 0.1
	PSI	—	0.6 ± 0.1—a	0.8 ± 0.1—a	0.7 ± 0.1—a
18:2n-6	PC	12.2 ± 0.5	7.2 ± 0.3—a	2.7 ± 0.2—b	7.1 ± 0.4—a
	PE	7.3 ± 0.4	2.9 ± 0.1—a	1.6 ± 0.2—b	3.6 ± 0.3—a
	PSI	2.5 ± 0.2	1.9 ± 0.2—a	1.4 ± 0.1—b	1.9 ± 0.1—a
20:4n-6	PC	26.4 ± 1.1	22.6 ± 0.5	10.9 ± 0.4—a	20.0 ± 0.6—b
	PE	21.4 ± 1.0	20.3 ± 0.3	8.0 ± 0.5—a	16.7 ± 1.0—b
	PSI	34.1 ± 1.2	27.2 ± 1.0—a	17.1 ± 1.1—b	23.8 ± 1.7—c
20:5n-3	PC	—	—	7.5 ± 0.4—a	—
	PE	—	—	5.2 ± 0.3—a	—
	PSI	—	—	1.7 ± 0.2—a	—
22:4n-6	PC	0.4 ± 0.1	—	—	—
	PE	1.5 ± 0.1	0.5 ± 0.1—a	—	—
	PSI	0.9 ± 0.1	—	—	—
22:5n-3	PC	—	—	1.4 ± 0.1—a	—
	PE	0.5 ± 0.1	—	2.8 ± 0.2—a	—
	PSI	—	—	2.7 ± 0.1—a	—
22:6n-3	PC	3.4 ± 0.2	4.0 ± 0.2—a	12.3 ± 0.4—b	4.7 ± 0.4—c
	PE	7.4 ± 0.5	8.3 ± 0.5	22.4 ± 1.3—a	8.9 ± 0.5
	PSI	2.0 ± 0.1	2.3 ± 0.2	11.4 ± 0.4—a	3.5 ± 0.4—b
DBI	PC	3.6 ± 0.2	3.2 ± 0.1—a	3.9 ± 0.2—b	4.5 ± 0.2—c
	PE	4.1 ± 0.2	3.8 ± 0.1—a	4.6 ± 0.2—b	4.8 ± 0.2—b
	PSI	3.5 ± 0.1	3.1 ± 0.1—a	3.6 ± 0.2	3.2 ± 0.1—a

[1]Data (weight percent) are expressed as mean ± SEM ($n = 6$).
Within a line, values without a common letter are significantly different from corn oil group at $P < 0.05$.
—: not detected. PC: phosphatidylcholine, PE: phosphatidylethanolamine, PSI: phosphatidylserine + phosphatidylinositol. DBI: double bond index = Σ (% unsaturated fatty acids × N° of unsaturations)/% SFA.

of vitamin supplement ($P < 0.05$). On the contrary, the intake of vitamin supplement lowered feed efficiency in this group (F + V) ($P < 0.05$). The liver relative weight was higher in animals fed H, compared with the group fed C. Vitamin supplementation decreased this parameter in rats fed O or F ($P < 0.05$).

Rats were fed for 20 days to obtain a clear-cut effect of dietary fats on liver PL composition,[19] although the PL fatty acid composition has been shown to be modulated by the diet even on a 1-week basis.[20] C intake induced higher incorporation of 18:2n-6 and its metabolites, 20:4n-6 and 22:4n-6, into microsomal PLs compared to all other groups. The increase of linoleic acid was significant in all PLs ($P < 0.05$), and of 20:4n-6 in PSI ($P < 0.05$). Compared with corn oil feeding, O intake increased the levels of MUFA 18:1n-9 in PC and PSI, and 18:1n-7 in PC and PE ($P < 0.05$). As expected, F intake induced an increase of n-3 long-chain 20:5, 22:5, and 22:6, and a reduction of 20:4n-6 level in the three PL fractions analyzed ($P < 0.05$). On the other hand, consumption of H resulted in elevation of the 18:1n-9 in PE ($P < 0.05$). The double bond index, DBI, or Σ (% unsaturated fatty acids $\times$ N° of unsaturations)/% SFA, was higher in the PC and PE fractions of rats fed H versus C ($P < 0.05$). This result is not attributable to a high PUFA content of hazelnut oil, but rather to an artifact because of the high incorporation of SFA into microsomal PLs. The PC fraction of rats fed H showed a SFA content of 30.8 $\pm$ 0.5%, whereas the relative amount of PUFA in this PL was 34.8 $\pm$ 0.5%. As a consequence, in group fed H the DBI for PC was significantly higher ($P < 0.05$) than the calculated values for C, O, and F groups (*Table 3*).

Retinol concentration of fish oil was 12.9 μg/g. In vegetable oils, retinol content was negligible. α-Tocopherol concentrations of the dietary oils were (μg/g): C, 60.5; O, 37.8; F, 6.8; and H, 6.2. As expected, after 20 days of dietary treatment the microsomal retinol level was higher in the group fed F ($P < 0.05$). Meanwhile, the α-tocopherol content was higher in the group fed O, compared with all other groups ($P < 0.05$). After the ingestion of nonsupplemented diets, no correlation was observed between the α-tocopherol content of liver microsomes and their degree of unsaturation, calculated as the DBI (*Table 3*). The intake of vitamin supplement increased microsomal retinol level only in rats fed C + V, while α-tocopherol concentrations were higher in all supplemented groups consuming vegetable oils ($P < 0.05$), and the group fed F + V exhibited a lower content of this vitamin ($P < 0.05$) (*Figure 1*).

Figures 2–4 show the effect of the vitamin supplement intake on the main fatty acids composition of PC, PE, and PSI, respectively, for each dietary treatment. In general terms, a higher amount of MUFA was observed in rat liver microsomal PLs after the consumption of supplemented diets, mainly as 18:1n-9, with a compensatory reduction of the amount of PUFA. However, each PL fraction exhibited a differential response.

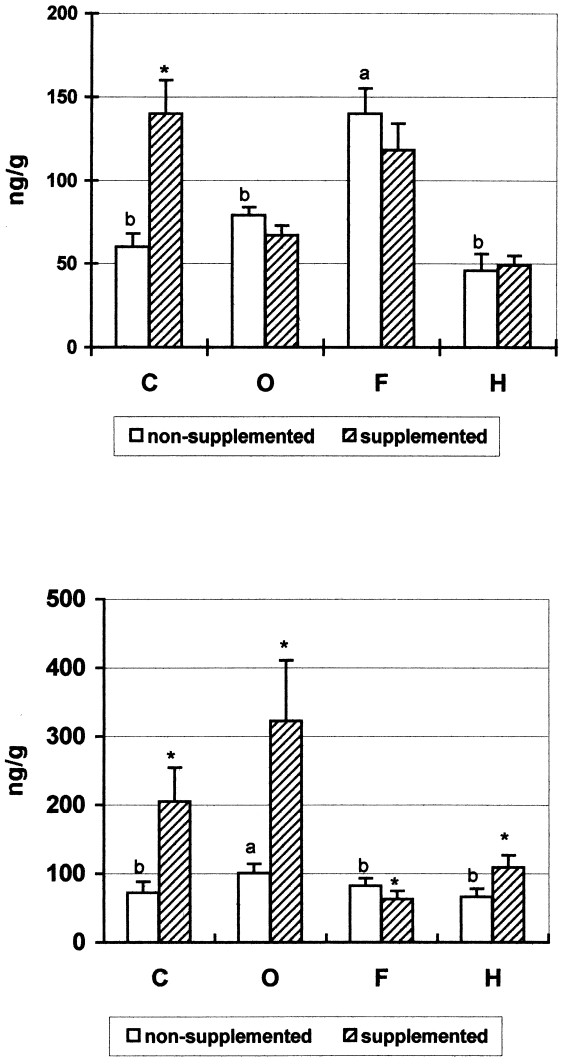

Figure 1 Microsomal content of retinol (top) and α-tocopherol (bottom) of liver microsomes of rats fed different oils, without or with antioxidant vitamins supplement (d,l-α-tocopherylacetate + β-carotene). C: corn oil, O: olive oil, F: fish oil, H: hazelnut oil. All values are expressed as mean + SEM ($n = 6$). Bars with different letters differ significantly ($P < 0.05$). *$P < 0.05$ as compared with nonsupplemented group.

Discussion

In the present study, we compared the effect of the intake of diets containing one of four different dietary oils and an antioxidant vitamins supplement on the composition of liver microsomal PL fractions. As expected, a differential incorporation of fatty acids into liver microsomes induced by dietary lipids was observed. The lack of incorporation of short and medium-chain fatty acids into the microsomal membrane PLs has been widely observed,[21] while long-chain PUFA species, namely 20:4, 22:4 and 22:6, are commonly found in the *sn*-2 position. The composition of liver microsomal PLs is determined by the molecular fatty acid species pattern of the initial synthesis and processes such as acyl remodelling, turnover, interconversion or base-exchange between PL classes and the competition between n-3 and n-6 PUFA for the same desaturases.[22–24] The 20:4n-6 content of liver microsomal PLs may be considered as a pool available for the synthesis of bioactive metabolites,[25] which explains the relatively constant level observed of this PUFA in all groups fed vegetable oils,

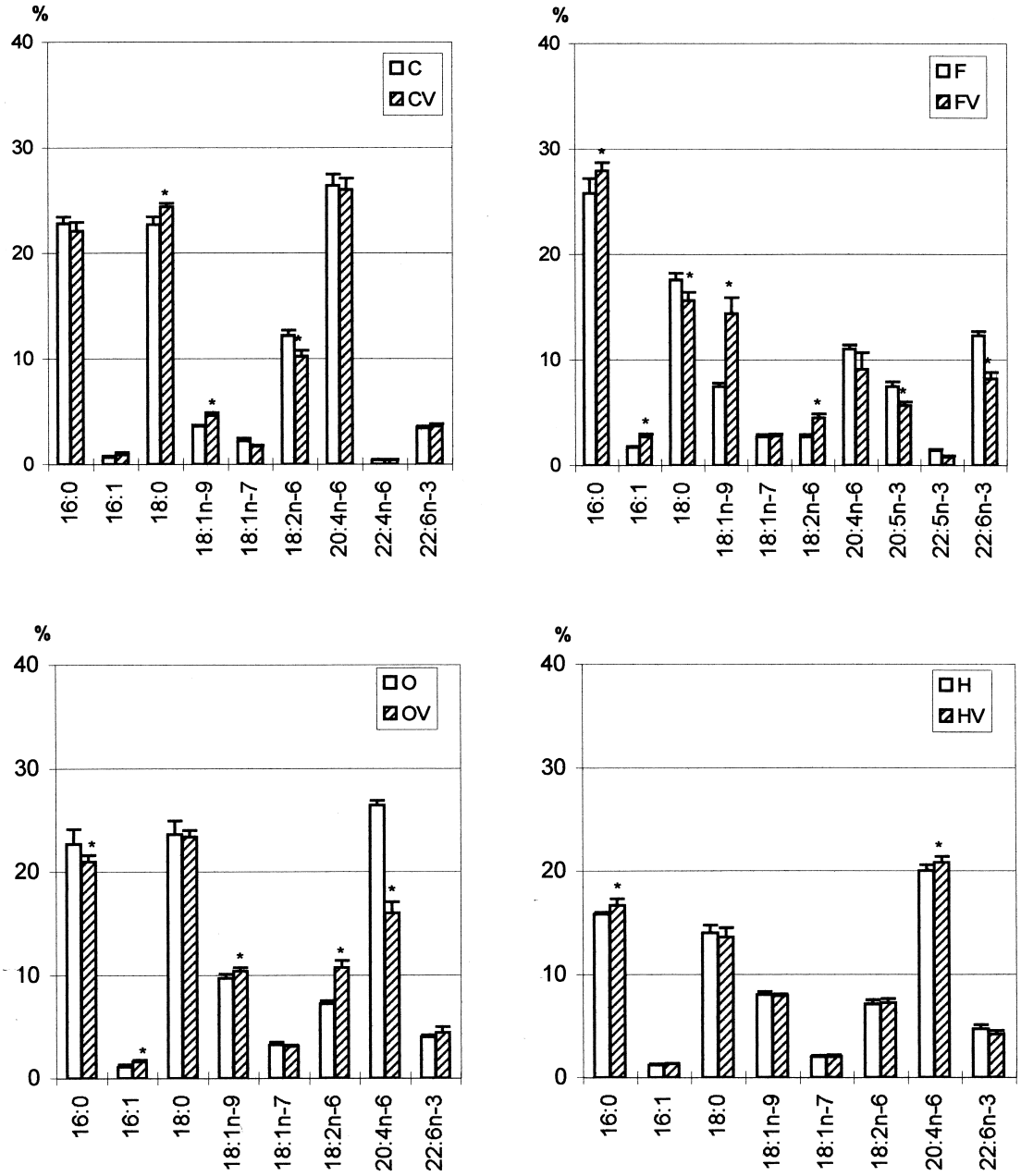

Figure 2 Main fatty acids content of phosphatidylcholine in liver microsomes of rats fed different oils, without or with antioxidant vitamins supplement (V: d,l-α-tocopherylacetate + β-carotene). C: corn oil, O: olive oil, F: fish oil, H: hazelnut oil. All values are expressed as mean + SEM ($n = 6$). *$P <$ 0.05 as compared with nonsupplemented group.

taking into account that the dietary supply of the precursor, 18:2n-6, was lower in rats fed O and H compared with animals fed C (*Table 1*). On the other hand, the 20:4n-6 content of the PL fractions in group fed F was 50–60% lower than the amount observed in group fed C. This finding may be explained by the inhibition of Δ6 and Δ5 desaturases induced by the high supply of n-3 PUFA from F.[26]

All groups exhibited high levels of 18:0 and 20:4n-6 in PSI, compared with other PL fractions ($P < 0.05$). This may reflect the high proportion of the fraction of phosphatidylinositol into the mixture with phosphatidylserine (PSI). Taken individually, PE incorporated a higher amount of 22:6n-3 ($P < 0.05$), whereas PC exhibited the highest amounts of 18:2n-6 in all the PLs analyzed ($P < 0.05$). These results manifest that the incorporation of fatty acids into microsomal PLs is a selective process. These observations are in accordance with Castuma and Brenner[27] and may be explained by specific differences in the acylation process on each individual PL[28] and the functional roles they play into the membrane.

The lipid composition of liver microsomes influences the activity of xenobiotic-metabolizing enzymes, which is determinant of both the intensity and duration of the action of

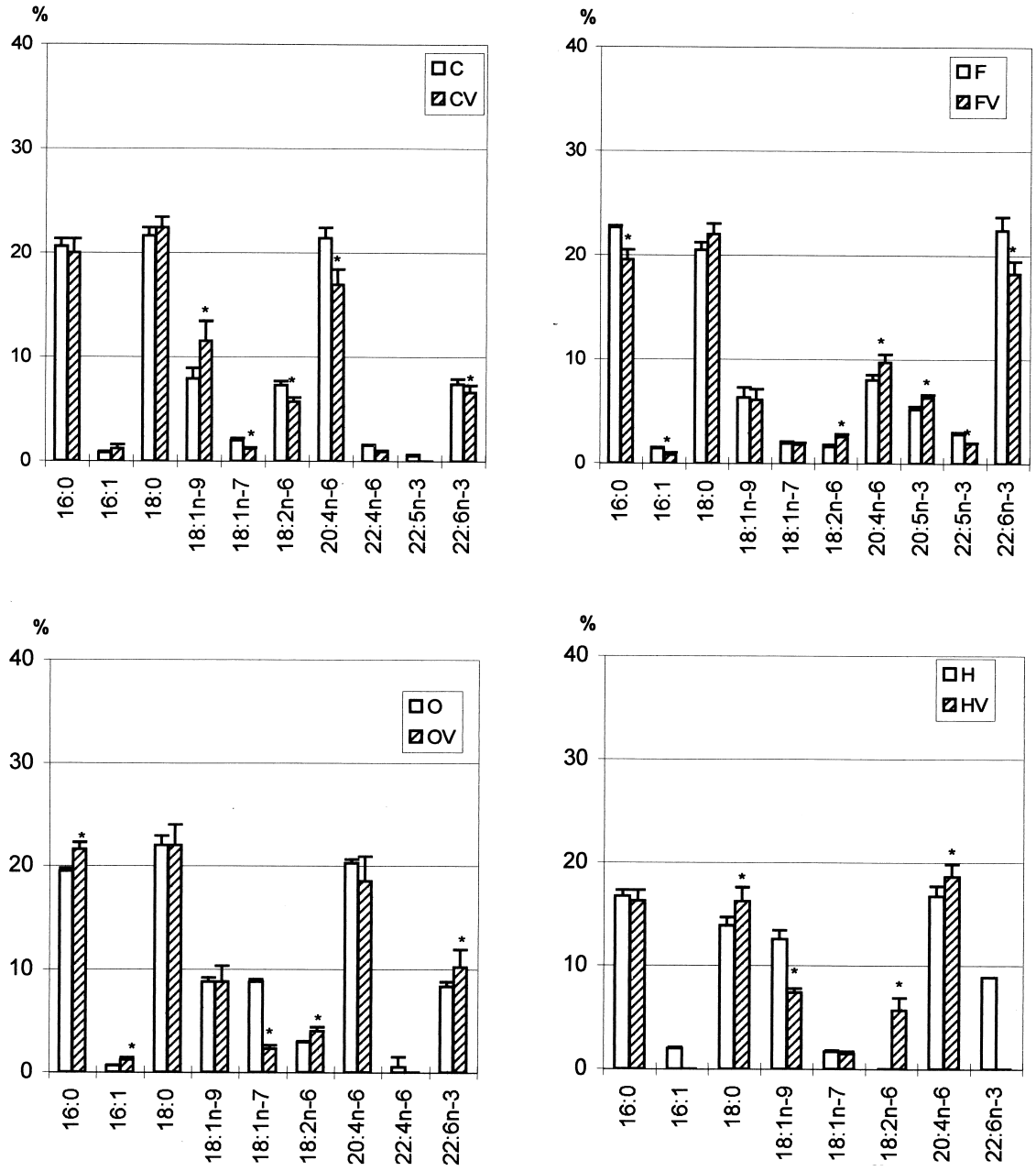

Figure 3 Main fatty acids content of phosphatidylethanolamine in liver microsomes of rats fed different oils, without or with antioxidant vitamins supplement (V: d,l-α-tocopherylacetate + β-carotene). C: corn oil, O: olive oil, F: fish oil, H: hazelnut oil. All values are expressed as mean + SEM ($n = 6$). *$P < 0.05$ as compared with nonsupplemented group.

these foreign compounds, which may become more or less toxic after the biotransformation process. The effects of dietary lipids, mainly in the form of PUFA, on microsomal functions are related with changes in the physicochemical properties of membranes, such as the fluidity of the lipid bilayer.[29] Additionally, the incorporation of highly unsaturated fatty acids in membranes augment their lability to oxidative stress and lipoperoxidation induced by free radicals.[8] The availability of PE and PS, synthesized via PL base exchange, is a requisite for the activity of microsomal cytochrome P-450 metabolizing enzymes. At the same time, both PLs are very sensitive to oxidative stress,[24] and a high amount of antioxidant vitamins, such as the supplement used, was given to prevent membrane damage through a combination of the free radical trapping action of α-tocopherol[30] and the oxygen reactive species trapping effect of retinol.[12]

Although nonsupplemented groups exhibited similar α-tocopherol microsomal content, a different response after vitamins supplement intake was observed: the elevation of the concentration of α-tocopherol was higher in O + V group (3.2 times), followed by the increase in C + V group (2.8 times) and H + V group (1.7 times). The low α-tocopherol content observed in F + V group reveals a

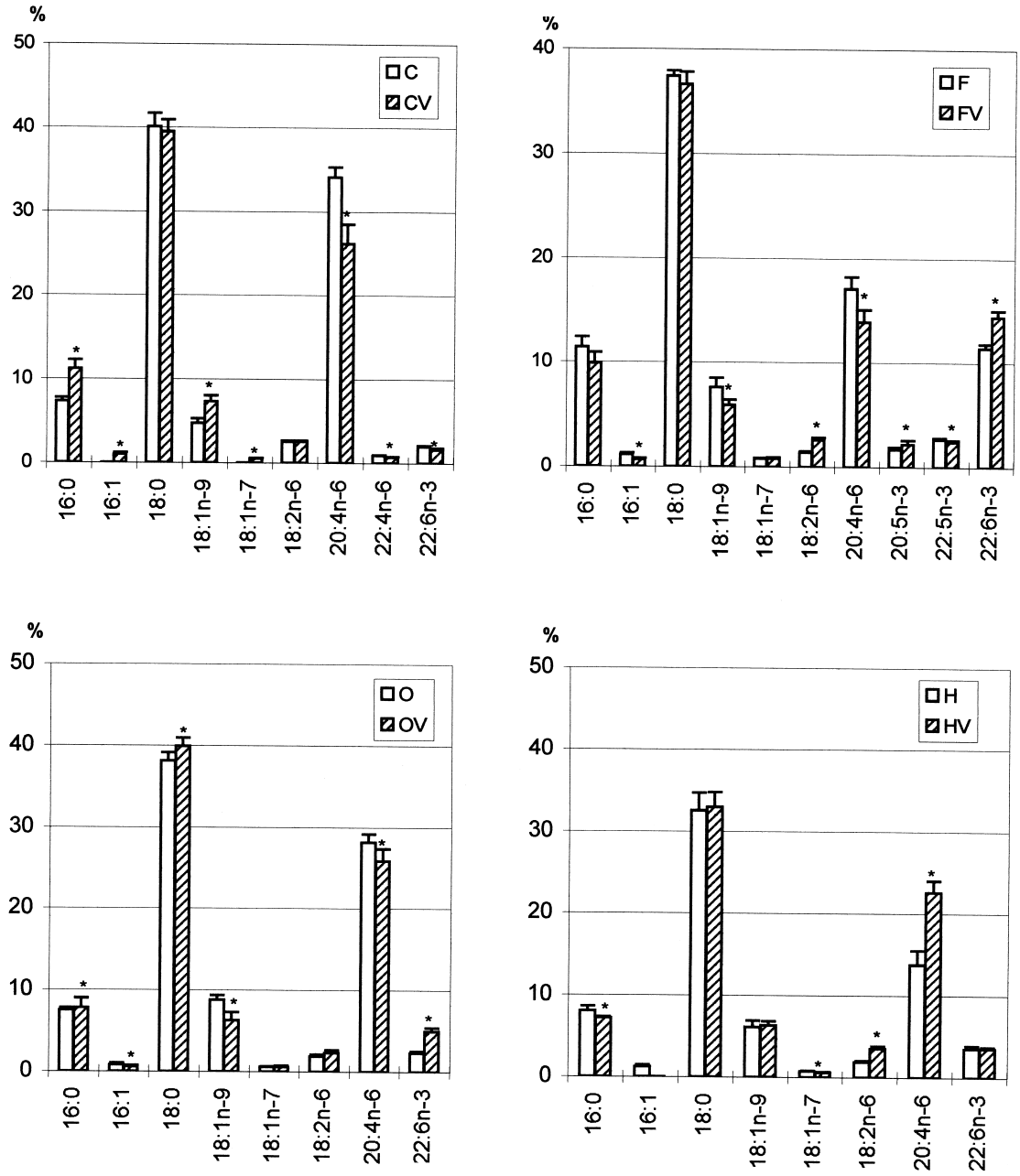

Figure 4 Main fatty acids content of phosphatidylserine + phosphatidylinositol in liver microsomes of rats fed different oils, without or with antioxidant vitamins supplement (V: d,l-α-tocopherylacetate + β-carotene). C: corn oil, O: olive oil, F: fish oil, H: hazelnut oil. All values are expressed as mean + SEM ($n = 6$). $P < 0.05$ as compared with nonsupplemented group.

higher endogenous consumption of this antioxidant, which additionally provides the membrane for its structural and functional stability.[31] The greater unsaturation of this membrane yielding less acceptability with α-tocopherol may also have contributed to this result. As a consequence, in F + V group the liver microsomal content of the vitamin was similar, although slightly lower, to the nonsupplemented F group. Besides, the vitamins supplement increased significantly the microsomal retinol content only in C + V group, where the vitamin level was 2.3 times higher than the value observed in the nonsupplemented C group (140 ± 20 ng/g liver versus 60 ± 8 ng/g liver, respectively). All other animals exhibited similar microsomal retinol values with or without the addition of V, although O + V and F + V groups exhibited a slightly lower content. This reflects that the incorporation and uses of both antioxidant vitamins by liver microsomal membranes is also differential, and depends on the type of fats that constitute the structure of the lipid bilayer. It is noteworthy that microsomal α-tocopherol and retinol concentrations do not correlate with the whole liver content of these vitamins (data not shown). As a consequence, it is not possible to observe a competing effect of these two vitamins in the liver, as proposed by Pellett et al.[32] This explains the lack of correlation of our current results with

those previously observed, under similar experimental conditions, in rat plasma of rats fed the vitamins supplement.[13]

Dietary supplementation with antioxidant vitamins induced slight modifications in the fatty acid profile of hepatic microsomal PLs, revealing that the DBI of each PL series tends to be conserved. Also, a tendency to the increase of the MUFA content of membranes was observed, mainly in rats fed F, with a compensatory decrease of the PUFA. This change may be attributed to a higher release of PUFA from microsomal PLs in these animals, because of the higher availability of these fatty acids, which have not undergone lipoxidation (*Figure 2*). The increase of MUFA may also be partly attributed to an interaction of α-tocopherol with Δ9 desaturase, as described by Fuhrmann and Sallman,[33] who observed an increase of MUFA of the n-9 series after supplementing the diet of growing chicks with this vitamin. However, an increase of 20:4n-6 has also been observed after feeding rats with diets high in n-3 PUFA and vitamin E supplementation.[27]

In summary, our data support the conclusion that, in spite that the amount of certain fatty acids in some rat liver microsomal PL fractions is not affected by the type of dietary fat ingested, the fatty acid relative composition of hepatic microsomal PLs is affected not only by the fatty acid profile of the ingested oil but also by the intake of antioxidant vitamins supplement. A significant meaning of these results is the fact that the enzymatic systems associated with microsomes may be affected by the quality of the fat consumed as well as the intake of vitamins supplement. Taking into account the current growing demand for antioxidant mixtures taken in the form of dietary supplements, the results of this study represent an interesting challenge from the biochemical, pharmacological, and toxicological points of view.

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