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Incorporation of Fatty Acids into Tissue Phospholipids in Mice Fed Diets Rich in n-9 and n-6 Fatty Acids*

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The aim of this study was to investigate the phospholipid fatty acid profile and dietary fatty acids effects in the liver, lung, spleen and submandibular gland mice tissues. Palmitic (C16:0) and stearic (C18:0) fatty acids were major saturates in all the tissues studied. Oleic acid (C18:1n-9) was the major monounsaturated in the phospholipid fraction of all tissues. Docosahexaenoic acid (C22:6n-3) was the major polyunsaturated in the liver and lung, arachidonic acid (C20:4n-6) was the major polyunsaturated in the spleen, and linoleic acid (C18:2n-6) in the submandibular gland tissue samples. Presented data support the hypothesis that corn oil diet induced changes increase linoleic acid (C18:2n-6), and olive oil increases the oleic acid (C18:1n-9) content in total phospholipid fatty acid composition in the majority of the examined tissues. Liver tissue was most affected by the applied diet.

Keywords
olive oil
corn oil
diet
fatty acid phospholipid composition

INTRODUCTION

Lipid and carbohydrate homeostasis in higher organisms is under the control of integrated systems that have the capacity to respond rapidly to metabolic changes. Fatty acids (FAs)[1] are energy-rich molecules that play important metabolic roles. They are also an integral part of cells as membrane components, which can influence fluidity and receptor or channel functions. Multiple factors influence the incorporation of dietary FAs into membrane phospholipids (PLs), including growth, type of tissue, differing energy levels in the diet, and weight loss or gain. Lipid metabolism can be regulated by FAs at three different levels; they interact with enzymes to affect their activity, they interact with nuclear transcription factors to modulate gene expression and finally they can affect mRNA stability and thus regulate the expression of enzymes.[2] The corresponding change in the amount of specific proteins is an adaptive process that the cells develop in response to variations in FA concentration in the vicinity of target tissues.[3,4,5,6]

Most studies rely on current or retrospective reports on dietary intake for determining dietary FA intake. In general, the structures of dietary FAs are important; the basic structure with specific diversity determined by the chain length and the degree of unsaturation and FAs

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which are rapidly metabolized. Saturated (SFA) and monounsaturated (MUFA) FAs are obtained either from the diet or by complete synthesis from acetyl CoA. In contrast, two major classes of polyunsaturated FAs (PUFAs), n-3 (or ω3) and n-6 (or ω6), cannot be synthesized in humans and are essential components of the diet. This explains why the FA composition of the tissue lipids, including membrane PLs, can be modified by PUFAs in the diet. n-3 and n-6 PUFAs may have biologically opposite properties, probably because they give rise to different eicosanoid products. For instance, when model animals with a propensity to develop tumors are fed diets containing a large proportion of n-6 PUFAs, tumor formation is favored, whereas diets with a similar proportion of n-3 PUFAs are somewhat protective.[1] Besides genetic predisposition, diet is also an important risk factor for various diseases. Diets rich in PUFAs such as linoleic acid (C18:2n-6) diminish stearoyl-CoA desaturase (SCD) activity.[7] Consequences of regulating the SCD by PUFAs and cholesterol may be relevant to lipoprotein metabolism.[8] Oleic (C18:1n-9) and palmitoleic (C16:1n-7) FAs are the major MUFAs in fat depots and membrane PLs. To date, dietary trials have demonstrated the major role of C18:1n-9 in primary and secondary prevention of lipid metabolic disorders such as coronary heart disease (CHD).[9] The ratio of stearic acid/oleic acid (C18:0/C18:1n-9) is one of the factors influencing membrane fluidity and cell-cell interaction.[8,10] Abnormal alteration of this ratio has been shown to play a role in several physiological and disease states, including diabetes, cardiovascular disease, obesity, hypertension, neurological diseases, immune disorders, cancer, and aging.[11–21]

The aim of this work was to determine the PLs FA profile of various tissues and the modulation of these profiles by dietary fats. For the present study, we used the n-9 diet (fed virgin olive oil, FOO) and n-6 diet (fed corn oil, FCO) for determining diet variability and control of tissue variability. Male Balb/c mice were used to study the incorporation of FAs into PLs, taken from the liver, spleen, submandibular gland and lung after a period of 3 weeks of dietary intervention.

EXPERIMENTAL

Chemicals and Reagents

All the used chemicals and reference compounds for gas chromatography (GC) were purchased from Sigma (St. Louis, MO, USA) and were of the highest reagent grade available. The compound used for the formulation of diets, the extra-virgin olive oil sample was obtained from an individual producer and the refined corn oil sample was obtained from Oleifico Zucchi S.p.A, Cremona, Italy.

The solid-phase extraction cartridges, NH_2 (Bond Elut, 3 ml volume, 500 mg sorbents) used for the lipid classes separation were purchased from Varian (Harbor City, CA, USA).

TABLE I. Fatty acid composition of the used diets and dietary oils expressed in percents[(a)]

Fatty acid	Control diet	Olive oil	FOO diet	Corn oil	FCO diet
C14:0	tr.	tr.	tr.	0.01	tr.
C16:0	18.42	11.36	14.01	13.63	15.43
C18:0	2.83	2.66	2.72	1.44	1.96
C20:0	tr.	0.43	0.27	0.14	0.09
C22:0	tr.	tr.	tr.	0.05	0.03
C24:0	tr.	0.09	0.06	0.07	0.04
ΣSFA	21.25	14.54	17.06	15.49	17.55
C16:1n-7	0.85	0.93	0.90	0.06	0.36
C18:1n-9	21.19	74.48	54.50	25.37	23.80
C20:1n-9	tr.	0.38	0.24	0.97	0.61
C22:1n-9	tr.	tr.	tr.	0.76	tr.
ΣMUFA	22.04	75.79	55.63	27.16	24.77
C18:2n-6	51.80	9.00	25.05	57.06	55.09
Σn-6	51.80	9.00	25.05	57.06	55.09
C18:3n-3	4.90	0.67	2.26	0.30	2.03
Σn-3	4.90	0.67	2.26	0.30	2.03
PUFA	56.70	9.67	27.31	57.36	57.11
n-6/n-3	10.57	13.43	11.10	190.20	27.20
C18:0/C18:1n-9	0.13	0.04	0.05	0.06	0.08

[(a)] FOO, fed olive oil; FCO, fed corn oil; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Diets and Animals

The stock standard diet (pellet, type 4RF21 GLP, Mucedola, Settimo Milanese, Italy) was used as the control diet in all experiments. The control diet already contained 3 % soybean oil as the fat source. Olive oil (FOO) and corn oil (FCO) were added to the stock standard diet, in the quantity of 5 g / 100 g (5 g of oil to 100 g of standard diet pellets). The total fat content was 3 g in the control, and 8 g in FOO and FCO diets. Diets were freshly prepared once a week by the addition of appropriate amounts of oils, gases with N_2 and were stored at 0–4 °C to minimize fatty acid degradation. The FA composition of dietary oils and total fats in the diets were measured by GC. The composition of FAs in the used dietary oils and in the diets containing the mentioned oils (as particular FA fractions expressed in percents) is given in Table 1.

Male Balb/c mice (Medical Faculty, Rijeka, Croatia), aged 2–3 months and weighing at least 25 to 30 g, were acclimated for 1 week in a temperature (21–23 °C) and humidity controlled facility on a 12-hour light/dark cycle. After the acclimatization period, the animals were divided into 3 treatment groups of 6 mice each as follows: Group 1 (Control) control diet; Group 2 (FOO) 5 g/100 g olive oil fed mice; Group 3 (FCO) 5 g/100 g corn oil fed mice. At the end of the feeding period, after 3 weeks, the animals were killed and a portion of each tissue (spleen, lung, submandibular glands and liver) was removed with plastic instru-

ments, washed several times with saline solution (0.9 % NaCl) to remove blood, immediately weighed and stored at –80 °C until the analysis. Body weight and food intake were monitored during the study.

Lipid Analyses

The FA composition of the dietary oil was determined according to the modified EC Regulation 2568/91[22] by GC analysis within 5 % coefficient of variance. Oils, which were subjected to analysis, were heated under reflux with 2 M methanol-hydrochloric acid at 100 °C for four hours. The obtained methyl esters were extracted with petroleum ether, passed through anhydrous sodium sulphate and evaporated in a rotating evaporator to dryness. Total lipids were extracted from pellets containing 5 g/100 g olive and corn oil with hexane and then evaporated in a rotating evaporator to dryness. The residue, which was subjected to analysis, was heated under reflux with 2 M methanol-hydrochloric acid at 100 °C for four hours. The obtained methyl esters were extracted with petroleum ether, passed through anhydrous sodium sulphate and evaporated in a rotating evaporator to dryness. Test portions, in the form of fatty acid methyl esters (FAMEs), were performed in duplicate and 1 μl of each sample solute in hexane was injected.

The PL FA composition in the examined tissues was determined according to Giacometti *et al*.[23]

Total lipids were extracted, according to Folch *et al*.,[24] from tissues with chloroform-methanol (CM, vol. ratio 2:1) containing 0.01 % butylated hydroxytoluene (BHT) as antioxidant. Lipid extracts were fractionated and purified by solid-phase extraction (SPE) and polar lipids were separated on the NH_2 column.[23] FAs of the polar lipids were transmethylated with methanol-n-hexane-sulphuric acid (75:25:1) at 90 °C for 90 min, extracted in petrolether and analyzed by GC. GC analyses of FAMEs were carried out using an Autosystem XL from Perkin-Elmer with flame-ionization detection (FID). Chromatography software from Perkin-Elmer Nelson (Turbochrom 4, rev. 4.1.) was used for data acquisition from the FID. Hydrogen was obtained with a Claind hydrogen generator. An SP-2330 capillary column (Supelco, Bellefonte, PA, USA), 30 m x 0.32 mm I.D., 0.2 mm film thickness, was used. Helium was used as the carrier gas with split injection (100:1). The analyses were carried out in programmed temperature mode from 140 to 220 °C, at 5 °C min^{-1} and then isothermal for 25 min. The detector temperature was 350 °C and injector temperature was 300 °C. The results were expressed as percentage of particular fatty acids in polar lipid fractions.

Statistical Analyses

GC data were evaluated with the Statistica software package for Windows 2001 by Stat soft, Inc. Statistical analysis was performed using the nonparametric Kruskal-Wallis one-way ANOVA by Ranks (variables for which the multiple independent groups were compared) and the Mann-Whitney U-test (variables for which the two groups were compared) to assess significant differences between the groups (tissues) and diets. A linear regression analysis using the least square method was used for the correlation determination of individual chemical parameters in all tissues. Statistical significance was assumed at $p < 0.05$ and data are reported as mean ± SD.

RESULTS

Animals fed different experimental diets gained comparable amounts of weight during the experimental period, but there were no statistical differences in weight between the experimental groups.

The FA composition of the diets and of dietary oils is shown in Table I. FA analyses of dietary oils showed that corn oil mainly contained C18:2n-6 (57.06 %) with an n-6/n-3 FA ratio approximately 190:1, consisting of total n-6 and n-3 levels of 57.06 and 0.3 %, respectively. When corn oil was added into the control diet, which contained 3 % of soybean oil, the diet based on corn oil (FCO) mainly contained C18:2n-6 (55.09 %) with an n-6/n-3 ratio approximately 27.2:1, consisting of total n-6 and n-3 levels of 55.09 and 2.03 %, respectively. Analysis of the dietary olive oil showed that it mainly contained C18:1n-9 (74.48 %), and the supplemented olive oil diet (FOO) with this oil contained 54.5 % oleic acid. The n-6/n-3 ratio was 13.43:1 in the dietary olive oil and 11.1:1 in the diet supplemented with olive oil (FOO). The theoretical content of triacylglycerols with ECN42 (ECN, equivalent carbon number)[22] of the dietary olive oil (ECN42 = 0.55) and the blends of dietary olive oil and fat in the control diet (ECN42 = 3.12) showed different amounts in trilinoleine. Larger differences were recorded in total n-6 and n-3 of 25.05 and 2.26 % in the FOO diet than in dietary olive oil (where 9 and 0.67 % were found). The level of the n-6 fatty acid in the FCO diet was twice and n-6/n-3 ratio more than twice higher than in the FOO diet. In contrast, the C18:1n-9 proportion in the FOO was more than twice higher than in the FCO.

Palmitic (C16:0) and C18:0 FAs were the major saturates in the polar lipids of all studied tissues. C18:1n-9 was the major monounsaturated, docosahexaenoic acid (C22:6n-3) was the major polyunsaturated in the liver and lung, arachidonic acid (C20:4n-6) was the major polyunsaturated in the spleen, and linoleic acid (C18:2n-6) in the submandibular gland tissue samples in mice fed the control diet. The C18:1n-9 proportion increased significantly in the FOO diet and linoleic acid (C18:2n-6) in the FCO diet compared to the control in all tissues studied. The n-9 and n-6 dietary treatments changed FAs proportions, especially C18:2n-6 and C22:6n-3.

Differences in the FA composition of the total PLs in the mice liver tissue samples in the control and after being fed diets supplemented with 5 g/100 g olive oil (FOO) and 5 g/100 g corn oil (FCO) are shown in Table II. As shown in Table II, significant differences were ob-

TABLE II. Fatty acid composition of the total phospholipids in the mice liver samples, expressed in percents, in the control group and after feeding diets supplemented with 5 g of olive oil (FOO) and 5 g of corn oil (FCO) to 100 g of standard diet pellets[a],[b]

Fatty acid	Control group	FOO group	FCO group
C18:2n-6	13.68 ± 1.23	13.86 ± 1.32	17.56 ± 1.10*
C20:2n-6	0.22 ± 0.11	0.18 ± 0.02	0.35 ± 0.02*
C20:3n-6	1.10 ± 0.41	1.53 ± 0.15	1.53 ± 0.15
C20:4n-6	11.02 ± 0.54	10.70 ± 1.17	13.36 ± 0.66*
Σn-6	26.02 ± 1.23	26.27 ± 0.33	32.81 ± 0.77*
C18:3n-3	0.45 ± 0.48	0.37 ± 0.09	0.36 ± 0.03
C20:5n-3	1.09 ± 0.39	0.69 ± 0.08*	0.46 ± 0.06*
C22:6n-3	20.50 ± 1.57	15.15 ± 1.02*	14.74 ± 1.46*
Σn-3	22.04 ± 1.85	16.21 ± 1.06*	15.55 ± 1.46*
ΣPUFA	48.06 ± 1.95	42.48 ± 0.98*	48.36 ± 1.15
C14:0	0.05 ± 0.03	0.06 ± 0.02	0.07 ± 0.02
C16:0	24.90 ± 0.50	25.33 ± 1.93	23.37 ± 0.52*
C18:0	15.98 ± 1.99	16.34 ± 2.53	19.35 ± 0.53*
C20:0	0.31 ± 0.15	0.57 ± 0.13*	0.56 ± 0.08*
C22:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C24:0	0.31 ± 0.27	0.18 ± 0.08	0.13 ± 0.04
ΣSFA	41.55 ± 2.38	42.49 ± 1.09	43.48 ± 0.72
C16:1n-7	0.86 ± 0.21	1.05 ± 0.39	0.68 ± 0.08
C18:1n-9	9.54 ± 1.44	14.06 ± 0.46*	7.48 ± 0.50*
C20:1n-9	0.00	0.00 ± 0.00	0.00 ± 0.00
ΣMUFA	10.40 ± 1.61	15.11 ± 0.75*	8.16 ± 0.55*
ΣUFA	58.45 ± 2.38	57.59 ± 1.04	56.52 ± 0.72
PUFA/MUFA	4.71 ± 0.71	2.82 ± 0.17*	5.95 ± 0.51*
PUFA/SFA	1.16 ± 0.11	1.00 ± 0.05*	1.11 ± 0.04
C20:4n-6/C22:6n-3	0.54 ± 0.05	0.70 ± 0.03*	0.92 ± 0.12*
C18:0/C18:1	1.72 ± 0.41	1.16 ± 0.19*	2.60 ± 0.20*
n-6/n-3	1.19 ± 0.13	1.63 ± 0.12*	2.13 ± 0.23*
C16:0/C16:1	31.43 ± 7.72	26.65 ± 8.43	34.64 ± 4.01

[a] Mean ± SD; calculated on the basis of peak areas; * significantly different from the control (p<0.05).
[b] Abbreviations: PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid.

TABLE III. Fatty acid composition of the total phospholipids in the mice lung samples, expressed in percents, in the control group and after feeding diets supplemented with 5 g of olive oil (FOO) and 5 g of corn oil (FCO) to 100 g of standard diet[a],[b]

Fatty acid	Control group	FOO group	FCO group
C18:2n-6	6.80 ± 0.36	6.99 ± 1.06	8.83 ± 0.59*
C20:2n-6	0.44 ± 0.05	0.38 ± 0.10	0.64 ± 0.10*
C20:3n-6	0.58 ± 0.10	0.77 ± 0.18	0.74 ± 0.07*
C20:4n-6	7.10 ± 0.35	7.61 ± 1.99	6.82 ± 1.22
Σn-6	14.92 ± 0.54	15.75 ± 2.58	17.03 ± 1.71*
C18:3n-3	0.37 ± 0.21	0.54 ± 0.13	0.36 ± 0.10
C20:5n-3	1.49 ± 1.13	0.57 ± 0.02*	0.48 ± 0.05*
C22:6n-3	8.94 ± 0.48	9.96 ± 2.32	8.18 ± 2.35
Σn-3	10.79 ± 1.17	11.07 ± 2.25	9.02 ± 2.35
ΣPUFA	25.71 ± 1.36	26.81 ± 4.66	26.05 ± 3.37
C14:0	1.00 ± 0.09	0.99 ± 0.30	1.18 ± 0.29
C16:0	48.86 ± 1.21	43.51 ± 4.29*	48.97 ± 3.80
C18:0	8.93 ± 0.34	9.99 ± 1.53	9.53 ± 0.49
C20:0	0.23 ± 0.09	0.23 ± 0.12	0.28 ± 0.26
C22:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C24:0	0.33 ± 0.15	0.40 ± 0.11	1.12 ± 0.97
ΣSFA	59.35 ± 1.29	55.13 ± 4.44	61.08 ± 3.66
C16:1n-7	4.09 ± 0.05	3.65 ± 0.84	3.33 ± 0.57
C18:1n-9	10.85 ± 0.67	14.41 ± 1.82*	9.53 ± 0.90*
C20:1n-9	0.00	0.00 ± 0.00	0.00 ± 0.00
ΣMUFA	14.94 ± 0.72	18.06 ± 2.61*	12.86 ± 1.17*
ΣUFA	40.65 ± 1.29	44.87 ± 4.44	38.92 ± 3.66
PUFA/MUFA	1.73 ± 0.15	1.52 ± 0.37	2.04 ± 0.31
PUFA/SFA	0.43 ± 0.03	0.49 ± 0.12	0.43 ± 0.08
C20:4n-6/C22:6n-3	0.80 ± 0.06	0.76 ± 0.06	0.88 ± 0.25
C18:0/C18:1	0.83 ± 0.06	0.71 ± 0.18	1.01 ± 0.11*
n-6/n-3	1.39 ± 0.15	1.44 ± 0.11	1.99 ± 0.50*
C16:0/C16:1	11.95 ± 0.38	12.42 ± 2.94	14.97 ± 2.20*

[a] Mean ± SD, calculated on the basis of peak areas; * significantly different from the control (p<0.05).
[b] Abbreviations: PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid.

served in the FA composition of PL fraction in the liver tissue samples (p = 0.0032). Compared to the control, significant differences in the liver tissues after feeding FOO diets were found in the n-3 PUFAs contents such as C20:5 and C22:6, in the C20:0 content as SFA and in the C18:1n-9 content as MUFA, also in PUFA/MUFA, PUFA/SFA, C20:4n-6/C22:6n-3, C18:0/C18:1 and n-6/n-3 ratios. After feeding the FCO diet, significantly different from the control, in the mice liver PL FAs there were n-6 PUFAs such as C18:2, C20:2 and C20:4; n-3 PUFAs such as C20:5 and C22:6; SFAs such as C16:0, C18:0 and C20:0 and oleic acid as MUFAs. These differences were reflected in PUFA/MUFA, C20:4n-6/C22:6n-3 and C18:0/C18:1 ratios in the liver.

No significant differences in the total PL FAs and diets studied were found in the lung, spleen and submandibular gland samples in the studied diets. The differences in the PL FAs in the mice lung tissue samples in the control and after feeding diets supplemented with 5 g of olive oil (FOO) and 5 g of corn oil (FCO) to 100 g of standard diet are shown in Table III. After feeding the studied diets, more significant differences in the lung tissues were found in the FCO diet (n-6 PUFAs such as C18:2, C20:2 and C20:3; n-3 PUFAs such as C20:5; C18:1n-9 as MUFA; also in C18:0/C18:1, n-6/ n-3 and C16:0/C16:1 ratios) compared to the control. Comparable results relating to the used diet were obtained in C18:1n-9 and C20:5n-3, where C18:1n-9 was significantly elevated (p = 0.002) in the FOO diet, and suppressed (p = 0.041) in the FCO diet. Increases in C16:0/C16:1 and C18:0/C18:1 ratios occurred in the FCO diet in the lung tissue.

As shown in Tables II, III, IV and V, the levels of SFAs and C16:0 were the highest in the lung tissues total PLs in all diets and tissues studied. Although liver and

TABLE IV. Fatty acid composition of the total phospholipids in the mice spleen samples, expressed in percents, in the control group and after feeding diets supplemented with 5 g of olive oil (FOO) and 5 g of corn oil (FCO) to 100 g of standard diet[(a),(b)]

Fatty acids	Control group	FOO group	FCO group
C18:2n-6	8.71 ± 1.22	7.90 ± 0.44	12.10 ± 2.40*
C20:2n-6	0.81 ± 0.09	1.11 ± 0.22*	1.25 ± 0.17*
C20:3n-6	0.86 ± 0.09	0.73 ± 0.28	0.56 ± 0.04*
C20:4n-6	13.97 ± 1.19	11.92 ± 1.53	13.91 ± 1.08
Σn-6	24.35 ± 1.79	21.65 ± 1.43*	27.82 ± 3.19
C18:3n-3	0.73 ± 0.10	1.00 ± 0.25*	0.61 ± 0.12
C20:5n-3	0.83 ± 0.13	0.84 ± 0.19	0.68 ± 0.14
C22:6n-3	10.46 ± 1.75	8.81 ± 1.20	9.92 ± 1.47
Σn-3	12.02 ± 1.74	10.66 ± 1.22	11.21 ± 1.37
ΣPUFA	36.37 ± 2.56	32.31 ± 2.08*	39.02 ± 3.64
C14:0	0.49 ± 0.08	0.51 ± 0.14	0.47 ± 0.08
C16:0	33.86 ± 2.26	34.42 ± 3.95	32.36 ± 3.41
C18:0	13.98 ± 1.65	13.31 ± 2.51	13.35 ± 0.81
C20:0	0.54 ± 0.26	0.33 ± 0.27	0.30 ± 0.08
C22:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C24:0	1.12 ± 0.47	0.78 ± 0.66	0.47 ± 0.18*
ΣSFA	49.99 ± 2.76	49.35 ± 4.14	46.95 ± 3.84
C16:1n-7	1.63 ± 0.46	1.66 ± 0.59	1.20 ± 0.30
C18:1n-9	12.00 ± 0.49	16.68 ± 2.17*	12.73 ± 0.97
C20:1n-9	0.00	0.00 ± 0.00	0.10 ± 0.14
ΣMUFA	13.64 ± 0.76	18.34 ± 2.43*	14.03 ± 0.97
ΣUFA	50.01 ± 2.76	50.65 ± 4.14	53.05 ± 3.84
PUFA/MUFA	2.67 ± 0.22	1.78 ± 0.17*	2.79 ± 0.31
PUFA/SFA	0.73 ± 0.09	0.66 ± 0.10	0.84 ± 0.14
C20:4n-6/C22:6n-3	1.36 ± 0.19	1.37 ± 0.22	1.43 ± 0.23
C18:0/C18:1	1.17 ± 0.15	0.82 ± 0.25*	1.06 ± 0.12
n-6/n-3	2.06 ± 0.33	2.05 ± 0.23	2.51 ± 0.39
C16:0/C16:1	22.60 ± 8.21	24.38 ± 12.68	29.00 ± 10.45

[(a)] Mean ± SD, calculated on the basis of peak areas; * significantly different from the control (p<0.05).
[(b)] Abbreviations: PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid.

TABLE V. Fatty acid composition of the total phospholipids in the mice submandibular gland samples, expressed in percents, in the control group and after feeding diets supplemented with 5 g of olive oil (FOO) and 5 g of corn oil (FCO) to 100 g of standard diet.[(a),(b)]

Fatty acid	Control group	FOO group	FCO group
C18:2n-6	20.69 ± 0.97	19.05 ± 1.34	22.55 ± 1.47*
C20:2n-6	0.70 ± 0.09	0.48 ± 0.34	0.94 ± 0.09*
C20:3n-6	0.85 ± 0.54	0.86 ± 0.30	0.86 ± 0.41
C20:4n-6	10.12 ± 1.24	9.63 ± 1.97	7.88 ± 1.94
Σn-6	32.36 ± 1.47	30.02 ± 1.70	32.23 ± 2.81
C18:3n-3	0.25 ± 0.01	0.63 ± 0.45	1.74 ± 1.38*
C20:5n-3	1.76 ± 0.10	0.75 ± 0.56*	0.88 ± 0.23*
C22:6n-3	8.37 ± 0.67	7.79 ± 1.63	6.91 ± 2.57
Σn-3	10.38 ± 0.72	9.17 ± 1.79	9.53 ± 3.50
ΣPUFA	42.74 ± 1.61	39.19 ± 3.46	41.76 ± 5.77
C14:0	0.27 ± 0.04	0.25 ± 0.05	0.28 ± 0.17
C16:0	28.48 ± 1.29	28.16 ± 1.40	28.60 ± 3.88
C18:0	11.24 ± 0.53	12.63 ± 0.63*	14.28 ± 1.31*
C20:0	0.33 ± 0.06	0.29 ± 0.11	0.28 ± 0.13
C22:0	0.00	0.00 ± 0.00	0.00 ± 0.00
C24:0	0.43 ± 0.18	0.35 ± 0.15	0.56 ± 0.08
ΣSFA	42.74 ± 1.31	41.68 ± 1.90	44.01 ± 5.09
C16:1n-7	1.44 ± 0.15	1.22 ± 0.17	0.91 ± 0.30*
C18:1n-9	13.63 ± 0.33	17.40 ± 1.78*	13.32 ± 2.13
C20:1n-9	0.67 ± 0.06	0.50 ± 0.45	0.00
ΣMUFA	15.75 ± 0.46	19.13 ± 2.19*	14.23 ± 2.37
ΣUFA	58.49 ± 1.59	58.32 ± 1.90	55.99 ± 5.09
PUFA/MUFA	2.72 ± 0.14	2.08 ± 0.39	3.03 ± 0.76
PUFA/SFA	1.05 ± 0.06	0.94 ± 0.12*	0.97 ± 0.22
C20:4n-6/C22:6n-3	1.22 ± 0.18	1.25 ± 0.17	1.30 ± 0.57
C18:0/C18:1	0.83 ± 0.05	0.73 ± 0.08	1.09 ± 0.20*
n-6/n-3	3.13 ± 0.26	3.37 ± 0.59	3.95 ± 1.97
C16:0/C16:1	19.84 ± 1.39	23.36 ± 3.29*	34.40 ± 12.14*

[(a)] Mean ± SD, calculated on the basis of peak areas; * significantly different from the control (p<0.05)
[(b)] Abbreviations: PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid.

spleen are lymphatic organs, their total PL FAs composition was different. The FCO diet significantly elevated n-6 FAs in the liver while the FOO diet significantly suppressed n-6 FAs in the spleen, especially in the C18:2n-6. A higher content of dietary C18:1n-9 in FOO diets significantly decreased PUFAs (n-6 PUFAs) and increased C18:1n-9 compared to the spleen control. A lower C18:0/C18:1 ratio compared to the control suggests an increase in the SCD activity in the spleen in the FOO group.

The FOO diet significantly decreased C20:5n-3 when C18:0, C18:1n-9, and C16:0/16:1 ratio increased, but did not change the C18:0/C18:1 ratio in the submandibular gland. As shown in Table IV, more differences were found in the FCO diet, where C18:0, C18:2n-6, C20:2n-6 and C18:3n-3 were significantly increased, and C16:1n-7 and C20:5n-3 were decreased. The C16:0/C16:1 and C18:0/C18:1 ratios were significantly increased in the FCO diet.

Comparing all studied tissues (total number of samples was 24 divided into four groups of 6), significant correlations were found between C20:4n-6/C22:6n-3 and n-6/n-3 (r = 0.77 in the control; r = 0.66 in the FOO; r = 0.80 in the FCO), as shown in Figure 1. Significant correlation between C18:0/C18:1n-9 and C16:0/C16:1n-7 ratios in tissues was found only in the control diet.

DISCUSSION

Lipid metabolism is very complex and can be affected by many parameters such as diet, oxidative stress, drugs, *etc*. PLs are major structural components of cells and intracellular membranes in all living organisms. Dietary induced changes in membranes are recognized as functionally important for a specific role of membrane lipids in modulating membrane function. Nutritional balance

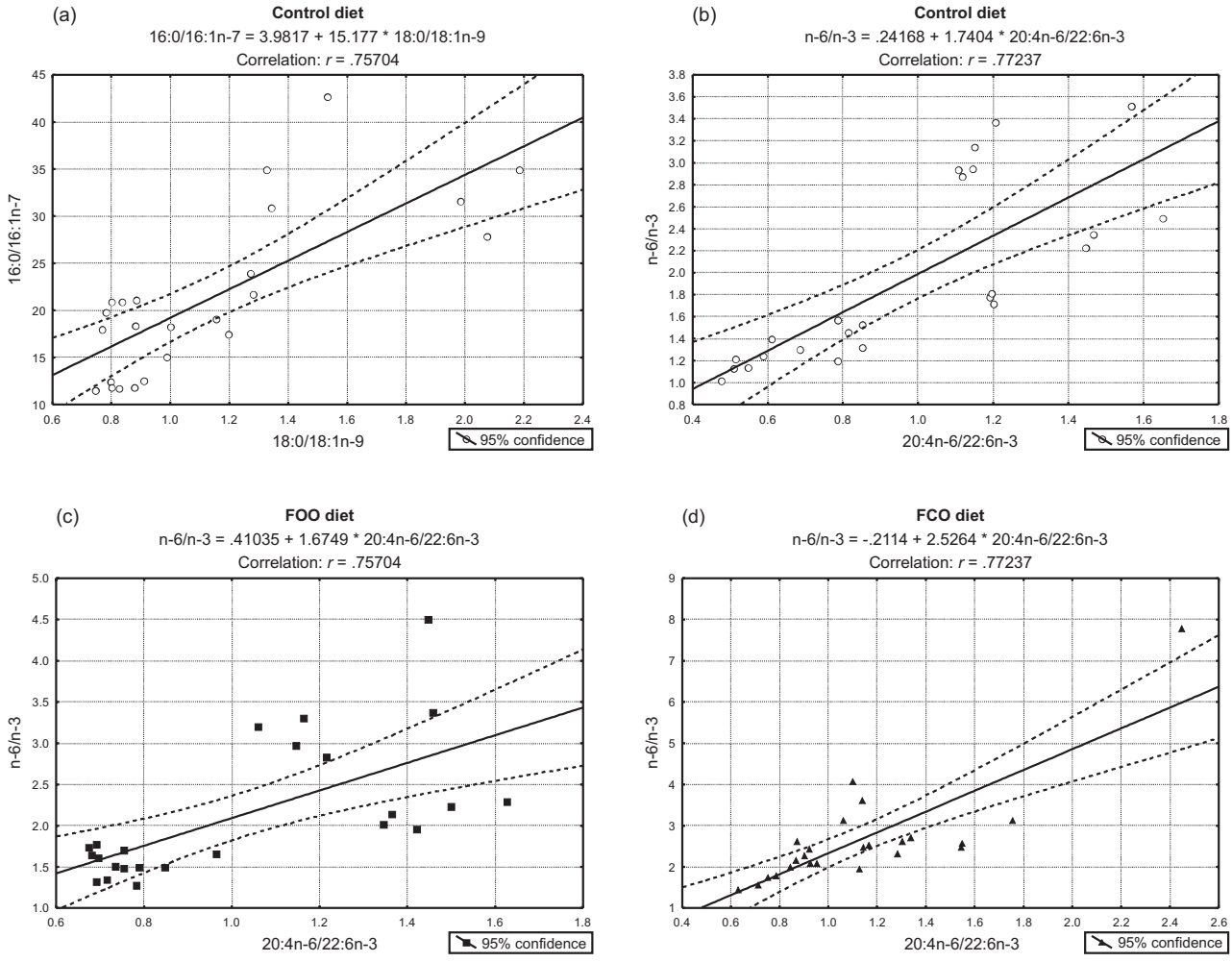

Figure 1. Significant differences in correlation between the C20:4n-6/C22:6n-3 and n-6/n-3 family fatty acid ratio in examined tissues in the control (○), FOO (■) and FCO (▲) diet groups, and between C18:0/C18:1n-9 and C16:0/C16:1n-7 ratio in studied tissues in the control diet group.

between different FAs is a very important objective, but this balance is difficult to achieve because FAs are numerous and their physiological functions are multiple and complex. Imbalanced essential FA ratio has led to an increase of various metabolic disorders affected by diets or diseases. In the altering cellular response, FAs do not act alone. All PUFAs having the 1,4-*cis,cis*-pentadiene structure may undergo peroxidation, which can be achieved by cell lipooxygenases or/and cyclooxygenases, depending on the FA type, affecting the production of arachidonic acid-derived eicosanoids.

The present work was undertaken to study the tissue-specific PL FA profiles and the effects of the dietary lipid source on possible modification in the total PL FA profiles in the examined tissues in mice. We compared the effects of diets in order to study the nutritional balance between FAs in the tissue PLs. Distinctive patterns of FAs were seen in the total PLs in the tissue response to the diets that contained olive and corn oil. Our data suggest that dietary induced changes in PL FA profiles are reflected on the used dietary fat composition and can influence the fatty acid composition in cell membranes only in some tissues, mostly in the liver, affecting individual fatty acids but not modifying Δ6- and Δ9-desaturase activities in all tissues. In the liver and spleen (as lymphatic organs) tissue samples, PUFA content significantly decreased with an increase in C18:1n-9 content, but not in the other studied organs.

Olive oil (n-9 FAs source) and corn oil (n-6 FAs source) contain different types and amounts of minor components, such as plant sterols, polyphenols and squalene, and triacylglycerol (TAG) contents. Contrary to olive oil, corn oil is refined oil, which changes the nature of PUFAs and the obtained derivates have atherogenic properties. Blends of vegetable oils were used to achieve overall diets with 5 g of fat supplements. For example, olive oil, which has been shown not to share unfavorable effects on body weight, may increase triacylglycerols secretion by the liver, depending on the Δ9 desaturase activity. Dietary squalene, contained in olive oil, can be used in the regulation of serum cholesterol concentration, cholesterol synthesis and elimination and

in the kinetics of lipoprotein regulation by downregulated hydroxylmethylglutaryl (HMG)-CoA reductase activity.[25] Olive oil polyphenols can also affect the fatty acid composition in liver tissue.[26]

The strategy of diet induced changes was largely based on the balance of monounsaturated FAs with saturated and polyunsaturated FAs. Feeding laboratory animals diets rich in C18:1n-9 (olive oil) and n-6 fatty acids (corn oil), significant differences in the tissue PL FA composition were found only in liver tissue ($p = 0.0032$). However, the differences existing in individual fatty acid proportions were found in all examined tissues, as can be seen in Tables II, III, IV and V. Compared to the control, MUFAs were significantly elevated and PUFAs were suppressed in the liver and spleen in the diet rich in C18:1n-9. C18:1n-9 significantly differs in the FOO and from that in the control group, as well as PUFA/MUFA, PUFA/SFA, n-6/n-3 and C20:4n-6/C22:6n-3 ratios. C22:6n-3 and C20:4n-6 were decreased when C18:1n-9 was increased in the liver. PUFAs balance is better represented by the ratios between n-3 and n-6 FAs. We found that C18:1n-9 negatively correlated with C20:4n-6 ($r = –0.65$) in all tissues in the control group. According to Ding and Mersmann,[27] the main effect of C18:1n-9 is regulating the gene expression rather than changing the membrane fluidity as a result of changing the membrane FA composition. Our data suggest that these changes were affected by the used diet depending of tissue types.

The ratio n-6/n-3 differs significantly between tissues, with the submandibular gland having the highest ratio value and liver the lowest, as well as the ratio value of C20:4n-6/C22:6n-3. Furthermore, mammals have Δ5 and Δ6-desaturase, which provide PUFAs for incorporation into the membrane and for eicosanoid synthesis. Moreno *et al.*[28] found that olive oil diet reduces C20:4n-6 release and subsequent production of arachidonic acid metabolites by the prostaglandin G/H synthase-2 pathway and induces less oxidative stress in macrophages in rats fed the olive oil than in those fed the fish oil diet.

Liver tissues responded with significant differences. C18:2n-6, C18:0 and C20:4n-6 were significantly elevated, C18:1n-9 and C16:0 were significantly suppressed in the FCO group compared to the control. The highest C20:4n-6/C22:6n-3 ratio in all tissues belonged to the animals fed corn oil. The increase in the C20:4n-6 proportion, like in C20:4n-6/C22:6n-3 ratio, indicated the possibility of eicosanoid production prior to other used diet types. n-6 fatty acids were significantly higher and n-3 were lower in the liver tissue than in the control. Modern Western diet changes the n-6 and n-3 ratio of PUFAs in the diet, thus the relative deficiency of n-3 PUFAs (C15–C20:1) was found.

The highest proportion of C16:0 was found in the lung. Pulmonary surfactant contains C16:0 in the phosphatidylcholine fraction, which is essential for normal lung function because it reduces surface tension at the air-liquid interface of alveolar space.[29] PUFAs were elevated in the lung if the FCO and FCO diets were compared to the control. C18:1n-9 was significantly increased in the FOO and C18:2n-6 in the FCO diet group in the lung. Diets containing C20:5n-3 or C20:5n-3 + C18:2n-6 alter the PUFA composition of pulmonary surfactants in the pigs.[30] Minimal surface tension was elevated by suppressed C18:2n-6 content.[30,31] Analogously, our data suggest a higher minimal surface tension in the lung in the FCO group.

The C18:0/C18:1n-9 and C16:0/C16:1n-7 ratios differ significantly between tissues, with the liver having the highest and lung the lowest ratio value. The C16:0/C16:1n-7 ratio was higher than C18:0/C18:1n-9 ratio in all tissues, which reflected a higher proportion of C16:0. These significantly elevated ratios in the lung and submandibular gland in mice fed corn oil suggested a lower activity of SCD and palmitoyl-CoA desaturase (PCD). The SCD plays an important role in the synthesis of monounsaturated FAs and the construction of membrane PLs and lipid metabolism. Enzymatic activity of SCD was increased by the C16:1n-7 and C18:1n-9 content in TAG[32] like in total PLs in liver tissue presented in our data. In rats, the activity of SCD enzymes is regulated by diets, hormone balance, developmental state, *etc.*[33] Lipoproteins regulation can be possible by modulating the SCD activity.[34,35] Oleic acid could not reduce the transcription of the SCD genes either in *vivo* or in *vitro.*[36,37,38]

The tissue FAs profile plays an important role before and after induced oxidative stress. Diet intervention modified this profile, and in this way reduced dysfunctions in the eicosanoid metabolism and disorders such as cardiovascular and gastrointestinal diseases as well as the incidence of cancer. These disorders have been linked to an imbalanced PUFA intake, related to diets with high n-6 and/or n-3 balance similar to high n-3 diet. The biological plausibility of any diet and health relationship is crucial when deciding whether or not it is worthy of assessment. This work has presented the tissue PL FA profile before induced stress with and without diet intervention, which can be the »starting position« for investigating various diseases and their prevention. The data presented here support the hypothesis that changes induced by diet corn oil increase linoleic acid, whereas olive oil increases the oleic acid content in the total phospholipid fatty acid composition in the majority of the examined tissues. Liver tissue was most affected by the used diet.

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SAŽETAK

Utjecaj prehrane obogaćene n-6 i n-9 masnim kiselinama na ugradnju masnih kiselina u tkivne fosfolipide miša

Jasminka Giacometti, Čedomila Milin, Marin Tota, Mira Ćuk i Biserka Radošević-Stašić

Cilj je ovoga rada bio istražiti sastav masnih kiselina u fosfolipidima jetre, pluća, slezene i submandibularne žlijezde u miša te odrediti utjecaj prehranom unešenih masnih kiselina na sastav masnih kiselina ukupnih fosfolipida. U svim istraživanim tkivima najzastupljenije zasićene kiseline su palmitinska (C16:0) i stearinska (C18:0) kiselina, a oleinska kiselina (C18:1n-9) je najzastupljenija jednostrukonezasićena kiselina. Dokozaheksaenska kiselina (C22:6n-3) je najzastupljenija višestrukonezasićena kiselina u jetri i plućima, arahidonska kiselina (C20:4n-6) u slezeni, a linoleinska kiselina (C18:2n-6) u tkivu submandibularne žlijezde. Dobiveni rezultati podupiru hipotezu da je prehrana obogaćena kukuruznim uljem utjecala na povećanje linoleinske kiseline (C18:2n-6), a prehrana obogaćena maslinovim uljem na povećanje oleinske kiseline (C18:1n-9) u fosfolipidima istraživanih tkiva. Primjenjena prehrana najviše je utjecala na lipide tkiva jetre.