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Heart Phospholipid Content and Fatty Acid Composition in the Rat After Feeding Different Lipid Supplemented Diets

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Summary

The heart phospholipid content and fatty acid composition were examined in adult rats after four weeks of feeding lipid-supplemented diets (20 g % w/w) containing sunflower oil-lard (1:1) mixture (SL group) or margarine (M group). Our results showed a decreased cardiolipin content and distribution in both experimental groups and an increased lysophosphatidylcholine and phosphatidylcholine content and distribution in the SL group with a tendency to lower phosphatidylcholine/phospatidylethanolamine ratio in both experimental groups. In the SL group, the content of saturated fatty acids was higher and that of monounsaturated fatty acids was lower than in the control group. The M group showed inverse results. The content of saturated fatty acids was lower and that of monounsaturated was higher than in the control group. Polyunsaturated n-6 fatty acids were decreased in both experimental groups and n-3 fatty acids were increased in the M group. Feeding lipid-supplemented diets reduced n-6/n-3 and 20:4/22:6 ratios in the M group. The polyunsaturated/saturated fatty acid ratio was lower in the SL and higher in indicating the M group than in the control group. Our results are in agreement with the other reports indicating that the heart is sensitive to diet-induced lipid alterations.

Key words
Heart – Phospholipids – Fatty acids – Dietary lipids

Introduction

The phospholipid (PL) class, its fatty acid composition and cholesterol content in biomembranes are basic determinants of the physical properties of membranes. They have been shown to influence a wide variety of membrane-dependent functions, such as membrane transport, enzyme activity and receptor function (Simopoulos 1992).

There is a considerable body of evidence that links a number of disease processes of the cardiovascular system with the type of dietary fat intake (Dupont *et al.* 1996). Dietary fats can modulate the composition of the fatty acid moiety of membrane phospholipid in tissues. The alterations in this lipid species are of special interest as the functional and pathological consequences can be correlated (McMurchie 1988). This phenomenon is of major importance in the heart since this organ is very sensitive to diet-induced lipid alterations (Abeywardena *et al.* 1987, Pan and Storlien 1992, Grynberg *et al.* 1995).

While many experiments have been designed to examine the influence of certain, rarely used sources of dietary fats in our population (Innis and Clandinin 1981, Charnock *et al.* 1985a, Elshafei 1992, Reig *et al.* 1993), the aim of our project was to study the effect of those dietary fats mostly used in our population. As such, the aim of the present study was to investigate the possible biochemical alterations in the heart phospholipid class distribution and fatty acid profile in rats fed diets supplemented (20 g % w/w) with margarine or a sunflower oil-lard (1:1) mixture.

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Materials and Methods

Diet and animals

Male Wistar rats aged 8 weeks with body weight of 200 g were divided into three groups of 6 animals each. Control group (C group) was fed a standard diet (Veterinarski zavod, Subotica) containing (w/w) 17.2 % protein, 60.9 % carbohydrate, 3.7 % fat with a polyunsaturated/saturated (P/S) fatty acid ratio of 1.3, 5.6 % fibre, and an adequate amount of vitamins and minerals (ash 7.6 %). One gram represented an estimated metabolizable energy of 14.9 kJ with 10 % being derived from fat. The second group (SL) was fed a standard diet supplemented with 20 g % w/w sunflower oil (Vital, Vrbas) and lard (Slavija, Beograd) (1:1) mixture (prepared in our laboratory) containing a fatty acid spectrum and P/S ratio (1.3) similar to the control diet. The SL diet had the following composition: protein 13.7 %, fat 23.7 %, carbohydrate 48.7 %, fibre 4.5 %, and an adequate amount of vitamins and minerals (ash 6.1 %). One gram represented an estimated metabolizable energy of 19.7 kJ with 46 % being derived from fat. The third group (M) was fed a diet identical to the SL diet but the sunflower oil-lard mixture was replaced with margarine (Vital, Vrbas) (20 g % w/w) providing a P/S ratio of 0.95. The fatty acid composition of each diet is given in Table 1.

The rats were maintained on a 12 h light/dark cycle for 4 weeks and had free access to water. Food was restricted to 18 g/day in the control group while the experimental groups were fed *ad libitum*. After the feeding period, all animals were fasted overnight and sacrificed during i.p. nembutal anaesthesia. The heart was rapidly excised and weighted samples were flushed with nitrogen and afterwards stored at −20 °C until further processing (for two weeks at the most).

Table 1. Food fatty acid composition (mol %).

Fatty acid	C group	SL group	M group
16:0	19.62	16.84	9.24
16:1	0.62	0.80	0.10
18:0	7.31	8.77	7.27
18:1	36.53	40.00	66.34
18:2	32.32	30.88	14.98
18:3	1.63	1.20	0.43
20:4	0.41	0.34	0.57
SFA	26.93	25.60	16.81
MUFA	37.15	40.79	66.44
PUFA	34.35	32.41	15.97
PUFA/SFA	1.30	1.30	0.95

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

Analytical procedures

Heart lipids were extracted with a chloroform-methanol mixture (2:1 v/v) by procedures described previously (Ristic *et al.* 1995). During the extraction procedure, lipids were protected against oxidation by addition of 10 mg/100 ml butylated hydroxytoluene to the solvents. Total lipids were determined gravimetrically and total PL by the method of Zilversmith and Davis (1950). One aliquot (containing 60 mg lipid phosphorus) from the heart lipid extract was spotted on thin layer glass plates precoated with a 0.25 mm layer of silica H and florisil (9:1). PL were separated by a two-dimensional thin-layer chromatography (TLC) system: 1) chloroform-methanol - 20 % ammonia (70 : 25 : 5, v/v) and 2) chloroform - acetone - methanol - acetic acid - water (70:17, 5:12, 5:10, 4:4, v/v) into the following fractions: phosphatidylserine (PS), phosphatidylinositol (PI), sphingophospholipids (SPL), lysophosphatidylcholine (LPC), phosphatidylcholine (PC), phosphatidyl-ethanolamine (PE), cardiolipin (CAR). The PL fractions were aspirated from the plates and analyzed for their phosphorus content (Kostic *et al.* 1972).

Heart lipids were fractionated by TLC using hexane-diethylether-acetic acid (87:12:1, v/v) as a solvent. The PL fractions were scraped into glass tubes and transmethylated with 2 M NaOH-methanol (heated at 85 °C for 1 h) and 1 M sulfuric acid-

methanol solution (heated at 85 °C for 2 h). The fatty acid methyl esters were then analyzed by gas liquid chromatography using a Varian GC (model 3400, Varian Associates) as previously described (Ristic *et al.* 1995).

Individual fatty acid methyl esters in the sample were identified from the retention times of authentic standards (Sigma Chemical Co.) and/or polyunsaturated fatty acids (PUFA)-2 mixture (Supelco, Bellefonte). Peak areas were determined with a Varian 4290 integrator, and the results were expressed as percentages of total identified fatty acids.

Statistical analyses

All results are expressed as means ± S.D. The effect of the dietary fat treatment was examined using one-way ANOVA. If a significant effect of the diet was identified, differences between individual diet were compared by Student's t-test.

Table 2. Energy intake and growth of experimental animals.

	C group	SL group	M group
Food (g/day)	18	21.57±3.48	21.48±2.94
Average energy intake (kJ/day)	267.64	424.13±67.96**	422.82±57.91**
Energy derived from fat (kJ/day)	26.76	198.87±33.36**	194.50±26.64**
Initial body mass (g)	190±29	191±23	205±28
Final body mass (g)	276±6	418±48**	424±43**
Daily weight gain (g)	2.85±0.32	7.52±1.08**	7.08±0.56**

*Data are means ± S.D. Significantly different from the controls: **p<0.01.*

Results and Discussion

Table 2 illustrates the energy intake and growth of experimental animals. Even though the experimental groups were fed *ad libitum*, there was no significant difference in food intake when compared with the control group. In group C, food was restricted to 18 g per day (267 kJ) that is considered to meet the daily energy needs of rats and is also sufficient for normal growth and development. *Ad libitum* feeding in the SL and M groups resulted in hyperenergetic food intake and obesity in the experimental animals. Average daily energy intake and percentage of energy derived from fat were significantly higher in both the SL and M groups than in the C group. Final body weights and daily body weight gain were also significantly higher in both the SL and M groups than in the control group.

The wet weight of the heart was higher in both experimental groups but, when calculated as percentage of body weight, the relative heart weight in the M group was lower than in the C group. Total heart lipids increased in both experimental groups but this increase was significant only in the M group. Total heart phospholipids showed no change (Table 3).

Table 3. Effect of diets supplemented with different lipids on heart weight, total heart lipids and phospholipids.

	Controls	SL group	M group
Heart weight (g)	0.91±0.06	1.30±0.20*	1.19±0.09**
Relative heart weight (g/100 g/b.w	0.33±0.02	0.31±0.03	0.28±0.02*
Total lipids (mg/g)	54.2±10.6	66.3±11.3	79.2±14.9*
Total phospholipids (mg/g)	16.3±1.1	16.3±2.34	15.3±1.1

*Data are means ± S.D. Significantly different from the controls: *p<0.05, **p<0.001.*

Table 4. Heart content of phospholipid fractions (mg phosphorus/g) tissue and distribution (%) after feeding diets supplemented with different lipids

PL fraction	Controls		SL group		M group	
	μg/g	%	μg/g	%	μg/g	%
PC×	293.10±18.0	45.01±1.59	288.09±40.4	44.24±1.32	276.47±22.8	45.19±2.29
PE	193.67±52.8	29.08±1.32	202.52±31.2	31.01±2.48	188.74±19.2	30.85±1.68
CAR	99.56±8.0	15.32±0.85	77.29±12.4*	11.87±0.78**	80.45±7.2*	13.15±0.50*
PS	11.11±2.0	1.71±0.22	14.26±2.0*	2.19±0.19*	11.01±2.4	1.80±0.30
PI	16.50±2.8	2.54±0.28	17.25±2.4	2.65±0.06	13.76±3.2	2.25±0.37
SPL	28.59±4.8	4.40±0.64	34.51±7.2	5.30±0.51	26.92±5.6	4.40±0.76
LPC	11.11±2.4	1.71±0.23	15.30±2.4*	2.35±0.29*	11.26±2.0	1.84±0.30
PC/PE	1.51±0.25		1.42±0.14		1.46±0.13	
SPL/PC	0.097±0.011		0.119±0.021		0.097±0.014	

*Data are means ± S.D, #phosphatidylcholine (PC). phosphatidylethanolamine (PE). cardiolipin (CAR), phosphatidylserine (PS). phosphatidylinositol (PI). sphingophospholipids (SPL). lysophosphatidylcholine (LPC), Significantly different from the controls: *p<0.05, **p<0.001.*

The effect of different diets on the content of PL fractions and their distribution is shown in Table 4. Many studies (Kramer 1980, Innis and Claudinin 1981, Charnock *et al.* 1985a) demonstrated that the distribution of major phospholipids (PC, PE and CAR), in the membranes of the rat heart is not altered by changes in dietary lipid intake (sunflower seed oil, tuna fish oil, sheep fat) over a long period. Our results showed a decrease in the CAR content and distribution in both experimental groups and a rise in the PS and LPC content and distribution in the SL group. One can hypothesize that adaptation might have occurred during long-term feeding (9, 12 and 18 weeks) so that the changes in this study were not observed. Cardiolipin is one of the principle phospholipids in the mammalian heart. It is localized primarily in the mitochondria and appears to be essential for the function of several enzymes of oxidative phosphorylation. Cardiolipin is also essential for energy production in the heart (Hatch 1996). Our findings suggest that diets enriched by different fats (sunflower oil-lard mixture and margarine) altered the content of heart cardiolipin and caused changes in the flux of oxidative phosphorylation in the heart. The functional significance of these biochemical changes is not clear. However, a number of possibilities may be considered. If changes in the distribution of membrane phospholipids do occur in response to dietary fat supplementation, it is conceivable that changes in membrane fluidity also occur. Phospholipids can serve as determinants of membrane lipid fluidity either as "fluidizers", (e.g. PC) or "rigidifiers", (e.g. SPL). Thus, the SPL/PE ratio is inversely related to membrane lipid fluidity. Our study also demonstrated a tendency to a lower PC/PE ratio in rats fed supplemented diets both with both sunflower oil-lard or margarine and the tendency to an enhanced SPL/PC ratio in the SL group. These results are in agreement with the findings of Innis and Claudinin (1981).

Membrane fluidity reflects alterations in the phospholipid acyl fatty acid composition. Fatty acid chain length, degree and type of unsaturation are important determinants of membrane fluidity. The saturated fatty acids (mainly 16:0 and 18:0) tend to decrease membrane lipid fluidity. The proportion of saturated fatty acids to unsaturated fatty acids is inversely related to membrane fluidity.

The effect of diets supplemented with different lipids upon the fatty acid content of rat cardiac PL is shown in Table 5. Heart PL of rats fed the diet supplemented with sunflower oil-lard mixture showed a significant increase in the SFA content (18:0) and a decrease in the MUFA (18:1) content. The content of polyunsaturated fatty acids (PUFA) showed no difference but PUFA n-6 were lower because the content of 18:2 n-6 was significantly decreased. The 20:4 n-6 content remained almost unchanged. Some studies of feeding diets supplemented with different lipids showed (Charnock *et al.* 1984, 1985a) that heart phospholipids maintained a relatively constant proportion of 20:4. The PUFA n-3 replaced 18:2 n-6 more effectively than 20:4 n-6 in the total phospholipids.

Table 5. Heart phospholipid fatty acid composition (mol %) after feeding diets supplemented with different lipids

Fatty acid	Control	SL group	M group
16: 0	11.88±1.18	10.60±1.32	8.83±1.03**
18: 0	22.23±2.05	31.67±5.17**	17.33±1.11**
16: 1	0.19±0.30	0.045±0.005	0.043±0.007**
18:1	11.16±0.97	6.95±1.95**	19.33±1.88**
18: 2 n-6	24.97±3.03	16.27±1.82**	15.91±1.21**
20: 3 n-6	0.46±0.08	0.36±0.09	0.49±0.07
20: 4 n-6	14.36±1.07	17.32±3.56	16.21±1.78
22: 4 n-6	0.37±0.06	0.63±0.04**	0.41±0.10
18: 3 n-3	0.27±0.06	0.17±0.05*	0.14±0.03**
20: 5 n-3	0.27±0.06	0.13±0.04**	0.18±0.10**
22: 5 n-3	1.75±0.26	1.74±0.51	2.15±0.38
22: 6 n-3	9.45±1.38	12.26±4.24	17.02±3.07**
Σ SFA[×]	34.11±3.12	42.04±6.33*	24.16±2.00**
Σ MUFA	11.35±0.97	7.03±1.94*	19.41±1.99**
Σ PUFA	51.64±4.11	48.89±8.22	52.50±4.13
Σ n-6	40.16±3.63	34.57±4.25*	33.04±1.36*
Σ n-3	11.98±1.53	14.32±4.64	19.45±3.25**
n-6/n-3	3.39±0.42	2.64±0.74	1.75±0.34**
PUFA/SFA	1.53±0.24	1.21±0.33*	2.03±0.30*
20: 4/22: 6	1.53±0.16	1.42±0.26	0.96±0.12*

*Data are means ± S.D.; #SFA – saturated fatty acids, MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. Significantly different from the controls; *p<0.05, **p<0.001.*

Heart PL of rats fed the diet supplemented with margarine exhibited a decrease in the SFA content (both 16:0 and 18:0) and an increase in the MUFA content (18:1). PUFA content showed no difference so that the PUFA/SFA ratio was higher when compared to the control group. These changes are not in accordance with the generally accepted fact that feeding fats with a different composition of fatty acids does not appear to alter the ratio of PUFA/SFA, i.e. that biomembranes display a considerable degree of homeostasis with respect to this parameter. The PUFA n-6 content was lower, whereas PUFA n-3 content was higher indicating a qualitative difference. The 18:2 n-6 content significantly decreased and 20:4 was not significantly altered. Reduced levels of 18:2 n-6 are replaced by PUFA n-3, i.e. by 22:6 n-3 which was significantly increased. 20:5 n-3 is present in relatively small amounts in heart phospholipids. It appears that 20:5 n-3 is either poorly incorporated into heart phospholipids or that the amount available is readily desaturated and further elongated to form 22:6 n-3. It is now clear that 20:4 n-6 is the precursor of prostaglandins and leukotrienes that have potent modulatory functions in many organ systems 22:6 n-3 is a competitive inhibitor of their biosynthesis (McMurchie 1988). The significant change in the phospholipid 20:4/22:6 ratio in rats of the M group could have prolonged effects upon heart prostanoid metabolism and thus upon heart function. However, little is known about the role played by these fatty acids in the structure of biomembranes. Furthermore, the definitive function of 22:6 n-3 in the heart is still unknown.

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