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EFFECT OF EXERCISE ON THE CONTENT AND COMPOSITION OF PHOSPHOLIPID-FATTY ACIDS IN RAT SKELETAL MUSCLES

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Abstract. Phospholipid-fatty acid composition of the plasma membrane determines many of its properties. There are no data on effect of exercise on the fatty acid content and composition of skeletal muscle phospholipid moieties and it was the aim of the present study to investigate this question. The experiments were carried out on male Wistar rats, 280-310 grams of body weight fed ad libitum a commercial pellet diet for rodents. The rats were divided into three groups: 1-control (sedentary), 2 –exercising 30 min, and 3 – exercising till exhaustion. The treadmill was set at +10^{o} incline and was moving with a speed of 1200m/h. The determinations were carried out on the following muscles: the white (WG) and red (RG) section of the gastrocnemius and the soleus (S). The muscles are composed of fast-twitch glycolytic, fast-twitch oxidative-glycolytic and slow-twitch-oxidative fibres. The phospholipid-fatty acids were identified and quantified by means of gas-liquid chromatography. The total content of phospholipid-fatty acids was reduced after each bout of exercise in WG and and remained stable in the other muscles. 30 min exercise reduced the content of stearic and arachidonic acids in WG, and Palmitoleic acid in RG. Exercise till exhaustion reduced the content of palmitic acid in WG, increased the content of myrystic and palmitoleic acid in S and reduced the content of oleic acid in the same muscle. All these changes, although statistically significant, were small. The ratio of the total content of saturated fatty acids to the total content of unsaturated fatty acids was stable both after 30 min and exhaustive exercise. It is concluded that acute exercise produces only minor changes in the content of phospholipid-fatty acids in skeletal muscles. It assures stability of the plasma membrane function.

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Key words: Phospholipid-fatty acids - Skeletal muscle – Exercise

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Introduction

Much attention has been paid to utilization of long chain fatty acids by working skeletal muscles. Oxidation of the acids has been repeatedly shown to increase during prolonged exercise. The process takes place predominantly in muscles composed of high oxidative fibres. The acids come mostly from the albumin-bound fraction of free fatty acids in the plasma. Relatively small amount of the acids is liberated from plasma triacylglycerols. Also triacylglycerols present in the high-oxidative myocytes are used during exercise [9,12,19]. A very rich intracellular sink of fatty acids are phospholipids. However, effect of exercise on the muscle phospholipid metabolism is only poorly recognized. Earlier studies showed that prolonged exercise did not affect the total content of phospholipids in working muscles of humans, dogs and rats [2,7,8,13,17]. This would indicate that acute exercise does not affect metabolism of this pool of lipids. A more recent in situ study, however, showed that a short-term contractile activity increases several-fold the incorporation of the plasma-borne 14C-palmitic acid into phospholipid moieties in each muscle type [10]. The latter would, in turn, suggest that exercise could change composition of the phospholipid-fatty acids. The composition of fatty acids in phospholipid moieties plays an important role in creation such features of the membrane like its fluidity, permeability and sensitivity to insulin [3,6,11,15]. There are no data, so far, on effect of acute exercise on the content and composition of phospholipid-fatty acids in skeletal muscles and it was the aim of the present study to examine this question.

Materials and Methods

The experiments were carried out on male Wistar rats, 280-310 g of body weight. They were fed ad libitum a commercial pellet diet for rodents and had free access to tap water. 12:12 h light/dark cycle was maintained in the animal quarters. The experimental protocol was approved by the Ethical Committee on the Animal Studies in the Medical University of Białystok. The rats were divided into three groups: 1-control (sedentary), 2-running 30 min, and 3-running until exhaustion. Rats were made run on a treadmill set at $+10^0$ incline and moving with a speed of 1200 m/h. Exhaustion was regarded as a point when rats refused of further running and placed on the table did not escape. The average running time until exhaustion was 4 h and 20 min. Rats of 2nd and 3rd group were accustomed to the treadmill by forcing them to run 10 min daily, five days preceding the final experiment. The animals were anaesthetized with thiopental (60 mg/100 g) administered

intraperitoneally. Samples of the soleus, red and white gastrocnemius were taken, cleaned of any visible non-muscle tissue and frozen in liquid nitrogen. The muscles are composed mostly of the slow-twitch oxidative, fast-twitch oxidative-glycolytic and fast-twitch glycolytic fibres, respectively [1,16]. The muscle samples were pulverized in an aluminum mortar with the stainless steel pestle pre-cooled in liquid nitrogen. Lipids were extracted in chloroform methanol [5] as described by [18]. The chloroform/methanol mixture contained 0.01% butylated hydroxytoluene (Sigma) as an antioxidant. Heptadecanoic acid (17:0; Sigma) was added to correct for losses during the extraction and assay procedures. Phospholipids were separated from other lipids by means of thin-layer chromatography using developing solvent composed of heptane/isopropyl ether/acetic acid (60:40:3 vol/vol/vol) [4]. The band containing phospholipids was scraped off the plate into tubes containing methylpentadecanoic acid (15:0; Sigma) as an internal standard. Phospholipid-fatty acids were methylated with 14% boron trifluoride in methanol, 30 min at 100^0C [14]. The resulting methyl esters were extracted with pentane. Next, pentane was evaporated, the residue was dissolved in hexane and analysed by means of gas-liquid chromatography using Hewlett-Packard 5890 Series II Gas Chromatograph, capillary column Hp-INNOWax, 30 m long and FID detector. The starting temperature was 160^0C and the temperature was then increased at a rate of 5^0C up to 230^0C. The following fatty acids were identified: myrystic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:1), oleic (18:1), linoleic (18:2) and arachidonic (20:4).

The results obtained were evaluated statistically using the Student t-test for unpaired data. The data are presented as means±standard deviation. N=10 for each group.

Results

The major saturated acid was palmitic acid and the major unsaturated acid was oleic acid in each muscle type, both at rest and after either exercise (Tables 1-3)

White gastrocnemius (Table 1). 30 min exercise reduced the content of stearic and arachidonic acid. Also the total content of phospholipid-fatty acids decreased. After exercise till exhaustion, the total content of the phospholipid-fatty acids was reduced comparing to the respective resting value. Only the content of phospholid containing palmitic acid residue was significantly reduced in this group. Red gastrocnemius (Table 2). Neither 30 min nor exercise till exhaustion affected the total content of phospholipid-fatty acids. After 30 min the level of palmitoleic acid was significantly reduced whereas after exercise till exhaustion the level of all acids remained stable.

Table 1
Effect of exercise on the content and composition of phospholipid – fatty acids in the white gastrocnemius

Acid	Control	Exercise	
		30 min	Till exhaustion
14:0	0.05±0.02	0.05±0.02	0.05±0.01
	(0.33±0.14)	(039±0.16)	(0.37±0.09)
16:0	4.25±0.26	3.99±0.26	3.97±0.24[a]
	(31.0±0.89)	(31.43±1.01)	(31.15±0.64)
16:1	0.22±0.07	0.23±0.05	0.23±0.04
	(1.62±0.47)	(1.79±0.38)	(1.77±0.30)
18:0	1.79±0.15	1.63±0.12[a]	1.67±0.13
	(13.02±0.62)	(12.85±0.51)	(13.05±0.69)
18:1	1.29±0.14	1.28±0.11	1.24±0.16
	(9.41±0.86)	(10.09±0.81)	(9.70±0.90)
18:2	3.93±0.48	3.69±0.44	3.51±0.47
	(28.57±1.97)	(29.01±2.43)	(27.46±3.09)
20:4	2.20±0.14	1.84±0.25[c]	2.11±0.38
	(16.04±0.87)	(14.46±1.57)	(16.49±2.76)
Total	13.73±0.97	12.71±0.82[a]	12.77±0.85[a]

Values (μmol x g^{-1}) are means±standard deviation; n=10; numbers in brackets – percent of the total.
a-$p<0.05$; b-$p<<0.02$; c-$p<0.01$
Acids: 14:0-myrystic; 16:0-palmitic; 16:1-palmitoleic; 18:0 stearic; 18:1-oleic; 18:2-linoleic; 20:4-arachidonic

Soleus (Table 3). 30 min run had no effect on the content the phospholipid-fatty acids. Exercise till exhaustion resulted in elevation in the content of myrystic and palmitoleic acid and reduction in the content of linoleic acid but the total content of fatty acids remained stable.

Table 2
Effect of exercise on the content and composition of phospholipid – fatty acids in the red gastrocnemius

Acid	Control	Exercises	
		30 min	till exhaustion
14:0	0.08±0.02 (0.38±0.08)	0.08±0.03 (0.40±0.16)	0.07±0.02 (0.32±0.08)
16:0	4.72±0.28 (23.15±3.04)	4.43±0.38 (22.83±0.69)	4.42±0.33 (21.87±0.78)
16:1	0.36±0.07 (1.73±0.28)	0.27±0.05[b] (1.42±0.27)	0.31±0.04 (1.54±0.13)
18:0	3.56±0.67 (17.11±1.66)	3.48±0.42 (17.91±0.63)	3.73±0.41 (18.38±0.87)
18:1	2.19±0.37 (10.57±0.82)	2.09±0.25 (10.74±0.85)	2.28±0.24 (11.25±0.57)
18:2	6.95±1.04 (33.57±1.65)	6.61±0.59 (34.12±1.81)	6.90±0.42 (34.19±1.73)
20:4	2.78±0.31 (13.49±0.85)	2.45±0.40 (12.59±1.01)	2.53±0.41 (12.44±1.23)
Total	20.62±2.47	19.42±1.83	20.24±1.62

Legend as in Table 1

Discussion

The total content of phospholipid-fatty acids was stable after exercise in the two muscles with high oxidative capacity (the red section of the gastrocnemius and the soleus) and it is agreement with the previous data [7]. The total content of the acids in the glycolytic muscle (white section of the gastrocnemius) is reduced in the present study, which is in odd with the above quoted data. The reason of this discrepancy remains unknown. However, the reduction in the content of phospholipids is very small (7.4% after 30 min exercise and 7% at exhaustion) and most likely it has no physiological significance. Moreover, the ratio: total content of saturated-fatty acid to the total content of unsaturated fatty acids was not influenced by exercise in either muscle.

Table 3
Effect of exercise on the content and composition of phospholipid – fatty acids in the soleus

Acid	Control	Exercise	
		30 min	till exhaustion
14:0	0.05±0.02	0.06±0.04	0.08±0.04[a]
	(0.27±0.09)	(0.34±0.20)	(0.45±0.22)
16:0	3.47±0.19	3.44±0.28	3.53±0.38
	(17.67±0.90)	(18.40±1.17)	(18.7±1.54)
16:1	0.25±0.06	0.22±0.06	0.30±0.05[b]
	(1.28±0.30)	(1.20±0.30)	(1.56±0.22)
18:0	3.95±0.27	3.78±0.53	3.80±0.37
	(20.08±0.76)	(20.09±1.16)	(20.12±1.06)
18:1	2.27±0.21	2.40±1.03	2.27±0.18
	(11.52±0.90)	(12.44±3.43)	(12.03±0.52)
18:2	6.77±0.56	6.24±0.66	6.24±0.27[b]
	(34.36±1.08)	(33.28±1.73)	(33.15±2.11)
20:4	2.29±0.31	2.69±0.43	2.65±0.39
	(14.83±1.13)	(14.25±1.16)	(13.98±1.38)
Total	19`.68±1.22	18.84±2.54	18.86±1.19

Legend as in Table 1

As already mentioned, short-term contractile activity markedly increases incorporation of the blood-borne labelled palmitic acid into both glycerolipid and sphingomyelin moieties in each muscle type [10]. The results obtained in the present study clearly indicate that both 30 min exercise and exercise till exhaustion produced only rather small changes in the content of particular fatty acids in the phospholipid fraction. There is no reason to ascribe this discrepancy solely to different experimental conditions. The examined fraction of phospholipids consists of different glycerophospholipids and a sphingolipid-sphingomyelin. Glycerophospholipids contain two fatty acid residues: one is esterified at the sn1 position and it is occupied by a saturated fatty acid and another is one is esterified at the sn2 position and it is occupied by unsaturated fatty acids. The sn1 bond is hydrolysed by phospholipase A_1 and the sn2 bond by phospholipase A_2. The process of esterification is catalysed by the enzyme glycerol-3-phosphate

acyltransferase. Sphingomyelin contains only one long-chain fatty acid. Hydrolysis of this sphingolipid by the enzyme sphingomyelinase yields ceramide and phosphocholine. This reaction is reversible. The present data, along with these where labelled fatty acids were used [10] would rather suggest that though the turnover of the phospholipid-fatty acid residues undoubtedly increases during exercise it does not lead to more pronounced change in the composition of the residues. In other words, fatty acids released from phospholipids during exercise are replaced mostly by fatty acids of the same structure. It allows keeping the composition of the phospholipid-fatty acid in each muscle during exercise almost constant. This, in turn, assures stability of these functions of the plasma membrane, which depend on the composition of fatty acid residues in the phospholipid moieties.

In summary, we have found that both short-term and prolonged exercise of moderate intensity produces only minor changes in the content and composition of fatty acid residues esterified in phospholipid moieties in different skeletal muscle types.

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