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Vascular Tissue Lipid Metabolism of *Rana hexadactyla* (Lesson) during Short Term and Prolonged Muscular Exercises

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The effect of short term (SME) and prolonged (PME) locatized muscular exercises on some aspects of lipid metabolism of vascular tissue was studied. Both Short term, excertised (SME) and prolonged exercised (PME) induced lipolysis and depleted the total lipid content of heart, dorsal aorta and plasma, the extent of depletion being more in PME. The phospholipid content of the tissues depleted during SME while the same was elevated during PME. The cholesterol content of heart, dorsal aorta and plasma was drastically depleted in PME animal tissues.

Key words: Muscular exercises, Heart, Dorsal aorta, Cholesterol, Phospholipids

Introduction

Physical exercise increases oxygen-demand of the myocardium (Harri & Voltola 1975) and elevates the heart rate, regional blood flow and systolic pressure (Kaufman & Sugimoto 1977, Oritsland et al. 1977) and these parameters attain normal level during the training programme (Link et al. 1972, Hickson et al. 1978). Whole animal training programmes lower the lipid level of the blood and prevent the atherosclerotic vascular lesions (Prior & Ziegler 1965, Paul 1973, Jones et al. 1979). The training programme influenced serum lipids (Rochelle 1961, Froberg 1969) heart lipid metabolism (Krul et al. 1967) and hepatic lipolysis (Narasimha Murthy et al. 1981). The reports on the effect of exercise on serum cholesterol and triglycerides are confusing and often contradictory (Taylor 1959, Goode et al. 1966, Mann et al. 1969). Studies on the impact of localized muscular exercises on the cardiac metabolism are scanty. The present study is aimed to elucidate the impact of localized muscular in vivo exercises on the lipid metabolism of plasma and vascular tissues.

Material and Methods

Male frogs of species *Rana hexadactyla* (Lesson) 30 $\pm$ 2 g were employed in the present investigation. Two platinum electrodes were placed 1 cm apart from each other

directly on the skin of right gastrocnemius muscles of intact animals. These muscles were stimulated with electronic stimulator (INCO/CSIO Research Stimulator—Ambala) as described by Reddanna et al. (1978) with a series of impulses (biphasic) of 5V at a frequency of 2 C/sec for 30 min per day for one day in one batch of experimental animals (short-term muscular exercised—SME) and for 10 successive days in another batch (Prolonged muscular exercised—PME). The duration of each impulse was 100 m sec.

The heart and dorsal aorta were isolated from freshly pithed control and experimental animals and placed in amphibian Ringer to recover from the shock effects. The heart and dorsal aorta were incised longitudinally and washed with amphibian Ringer to remove the traces of blood and taken for biochemical assays. Heparinised blood was centrifuged at 3000 rpm and plasma was separated.

The total lipids (Folch et al. 1957), lipase activity (Huggins & Lapides 1955), free fatty acids, cholesterol, triglycerides (Natelson 1965), phospholipids (Bieri & Prival 1965) and glycerol (Burton 1957) were estimated in heart, dorsal aorta and plasma.

Results and Discussion

SME animals had a decrease in cardiac total lipid content (table 1) suggesting the possible onset of lipolysis. Lipase activity was considerably elevated suggesting the degradation of lipid components in response to the induced muscular exercise. Triglyceride content was slightly elevated while phospholipids had a considerable drop suggesting the possibility of preferential utilization of phospholipids towards the energy release. Free fatty acid content was depleted indicating their involvement in the oxidative metabolism. Since the muscular exercises result into depleted total carbohydrate level of cardiac tissue (Reddanna & Govindappa 1978) and free fatty acids were metabolised during carbohydrate deficiency, it was likely that free fatty acids might have been utilized for energy demands leading to their depletion. Glycerol content showed non-significant change while total cholesterol content had considerable depletion suggesting the importance of short-term muscular exercises in decreasing the cholesterol content of the cardiac tissue.

Table 1 *The levels of total lipids, triglycerides, phospholipids lipase activity, glycerol, free fatty acid and cholesterol in heart of control (C), shor-term muscular exercised (SME) and prolonged muscular exercised (PME) animals. Values are mean of eight observations*

Component	C	SME	PME
Total lipids (mg/g wet wt)	62.74 ± 3.41	58.1 ± 2.73 — 7.39 $P<0.001$	46.56 ± 2.58 —25.79 $P<0.001$
Triglycerides (mg/g wet wt)	1.81 ± 0.08	1.91 ± 0.11 + 5.74 $P<0.05$	2.17 ± 0.14 +19.94 $P<0.001$
Phospholipids (mg/g wet wt)	17.13 ± 1.2	15.49 ± 0.92 — 9.57 $P<0.01$	18.68 ± 0.71 + 9.05 $P<0.01$
Lipase activity (μ mol P. nitrophenol formed/mg protein/hr)	0.22 ± 0.02	0.26 ± 0.001 +18.47 $P<0.001$	0.31 ± 0.01 +39.64 $P<0.01$
Glycerol (mg/g wet wt)	0.74 ± 0.04	0.72 ± 0.07 — 2.56 NS	0.54 ± 0.03 —26.91 $P<0.001$
Free fatty acids (mg/g wet wt)	18.46 ± 1.31	15.82 ± 0.93 —14.30 $P<00.001$	13.85 ± 0.73 —24.97 $P<00.001$
Total cholesterol (mg/g wet wt)	2.61 ±0.1	2.11 ±0.22 —19.12 $P<0.001$	1.24 ± 0.18 —52.51 $P<0.001$

Mean ± SD; + and — indicate percent increase and decrease over control, 'P' indicates the level of significance and NS non-significance

However, prolonged muscular exercise recorded more effective changes in lipid metabolism of cardiac tissue than that of SME. The total lipid content was highly depleted, which was due to higher lipase activity. There was a drastic decline in the levels of free fatty acids, glycerol and cholesterol of the tissue. The free fatty acids might have been utilized in oxidative metabolism. Since the level of citric acid cycle operation was enhanced in the cardiac tissue of PME animals (Reddanna & Govindappa 1978), it is likely that the depleted lipid content might have been diverted towards the oxidative activities of the tissue. The possibility of glycerol getting diverted towards the formation of carbohydrates (cf. Winkels et al. 1970), in the cardiac tissue of PME animals cannot be ruled out. This explains lesser depletion of total carbohydrates in the cardiac tissue of PME frogs in comparison to that of SME (Reddanna & Govindappa 1978). The elevated phospholipid content might be attributed to increased number, and size of mitochondria (Gollnick et al. 1971, Narasimha Murthy et al. 1981). The total cholesterol content of the cardiac tissue was significantly depleted and hence prolonged in vivo muscular stimulations seem to effectively decrease the cholesterol content of the heart.

The dorsal aorta of SME animals recorded non-significant change in total lipid content while PME showed significant drop (table 2), which can be explained on the basis of higher lipase activity in PME animal tissue. The glycerol content was elevated and triglyceride content was depleted due to elevated lipase activity. However, free fatty acid content showed non-significant change in SME animals while it was significantly decreased in PME tissues, suggesting that the dorsal aorta of PME animals was actively utilizing the free fatty acids for oxidative metabolism. The phospholipid content had significant depletion in SME animals while there was non-significant change in PME tissue indicating that the energy-rich lipid components are preserved in the tissue during PME. The total cholesterol content showed non-significant

Table 2 *The levels of total lipids, triglycerides, phospholipids, lipase activity, glycerol, free fatty acids and cholesterol in dorsal aorta of control (C), short-term muscular exercised (SME) and prolonged muscular exercised (PME) animals. Values are ... of eight observations*

Component	C	SME	PME
Total lipids (mg/g wet wt)	53.33 ± 4.51	51.16 ± 3.23 — 4.07 NS	41.0 ± 2.1 −23.12 $P<$ 0.001
Triglycerides (mg/g wet wt)	1.3 ± 0.07	1.17 ± 0.09 −10.28 $P<$ 0.01	1.19 ± 0.1 − 8.46 $P<$ 0.00...
Phospholipids (mg/g wet wt)	5.93 ± 0.51	4.36 ± 0.42 −26.47 $P<$ 0.001	6.12 ± 0.31 + 3.27 NS
Lipase activity (μ mol P nitrophenol formed/mg protein/hr)	0.18 ± 0.01	0.2 ± 0.02 +10.67 $P<$ 0.05	0.22 ± 0 02 +24.72 $P<$ 0.001
Free fatty acids (mg/g wet wt)	3.46 ± 0.23	3.69 ± 0.43 + 6.65 NS	2.77 ± 0.25 −19.94 $P<$ 0.001
Glycerol (mg/g wet wt)	0.54 ± 0.04	0.59 ± 0.04 + 9.26 $P<$ 0.02	0.6 ± 0.03 +11.11 $P<$ 0.01
Total cholesterol (mg/g wet wt)	0.9 ± 0.19	0.94 ± 0.08 + 4.44 NS	0.54 ± 0.03 −40.44 $P<$ 0.001

Mean ± SD; + and — indicate percent increase and decrease over control, 'P' indicates the level of significance and 'NS' non-significance

Table 3 *The level of total lipids, triglycerides, phospholipids, lipase activity, free fatty acids, glycerol and total cholesterol in plasma of control (C), short-term muscular exercised (SME) and prolonged muscular exercised (PME) animals. Values are mean of eight observations*

Component	C	SME	PME
Total lipids (mg/100 ml)	250 ±12.3	243 ±10.3	192 ±11.2
		− 2.8	−23.2
		NS	$P<$ 0.001
Triglycerides (mg/100 ml)	15.19 ± 1.27	14 ± 0.96	9.62 ± 0.7
		− 7.85	−36.67
		$P<$ 0.05	$P<$ 0.001
Phospholipids (mg/100 ml)	75 ± 2.34	70 ± 2.76	87 ± 4.21
		− 6.66	+16.0
		$P<$ 0.001	$P<$ 0.001
Lipase activity (μ mol P. nitrophenol formed/mg protein/hr)	0.012 ± 0.001	0.011 ± 0.001	0.025 ± 0.02
		− 8.33	+108.33
		$P<$ 0.05	$P<$ 0.001
Free fatty acids (mg/100 ml)	110 ± 8.36	108 ±11.26	130 ±13.14
		− 1.82	+18.18
		NS	$P<$ 0.001
Glycerol (mg/100 ml)	18.71 ± 1.28	19.76 ± 1.17	22.04 ± 1.86
		+ 5.59	+17.77
		$P<$ 0.05	$P<$ 0.001
Total cholesterol (mg/100 ml)	37.63 ± 2.13	27.8 ± 1.84	12.15 ± 2.15
		−26.12	−67.71
		$P<$ 0.001	$P<$ 0.001

Mean ± SD; + and − indicate percent increase and decrease over control, 'P' indicates the level of significance and 'NS' nonsignificance.

change in SME animal tissue while it was significantly depleted in PME ones suggesting the utility of prolonged muscular exercises—in depleting cholesterol content of dorsal aorta in frogs.

The total lipid content of plasma recorded slight depletion in SME animals and significant drop in PME animals (table 3). This difference between the plasma of SME and PME animals, might be due to differential lipolytica ctivities in the plasma of these animals. The triglyceride content of the plasma was depleted in both SME and PME animal plasma, the extent of depletion being more in latter case. The phospholipid content was elevated in PME animals while the same was depleted in SME animals. The total cholesterol content was slightly depleted in SME animals and significantly depleted in PME animal plasma.

Hence in general it can be concluded that the programme of in vivo muscular exercises leads to effective depletion of lipid components associated with cardiovascular disorders. The work pertaining to the utility of this exercise programme in averting cardiovascular disorders of mammals is in progress.

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