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Effect of Physical Activity on Changes in Mitochondrial Pool of Muscle Fibers

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Abstract: *The purpose of this research was to study the restructuring of adaptive features of skeletal muscles (gastrocnemius and soleus) under conditions of prolonged physical activity on an ultramicroscopic level. Studies were carried out on laboratory animals (rats) of two groups - the control (intact) and the experimental group (exposed to a 30-minute swim test for 5 weeks). The muscle material was studied using electron microscopy. We found that during the process of adaptive restructuring of skeletal muscles, morphological transformations occur at different levels of structural organisation. Changes in muscle tissue during prolonged physical load include increasing deposits of myoglobin with additional focal changes in glycogen content and an increase in mitochondria. A significant increase in the volumetric density of mitochondria was noted in the symplasts of the gastrocnemius and soleus muscles of rats in the experimental group. Additionally, the average cut size of mitochondria increased compared to intact animals. This was due to the appearance of large organelles against the background of a decrease (almost three times) in the number of small mitochondria. The consequence of such transformation can be metabolic reorganisation in myocytes, and changes in the plastic properties of energy generators and contractile structures. The established processes can become a basis for understanding the adaptive restructuring of skeletal muscles of athletes under the influence of long-term physical activity.*

Keywords: *muscles, ultrastructural adaptive changes, mitochondria, physical activity*

INTRODUCTION

High physical loads, characteristic of modern sports, present increased demands on all organs and systems of the organism, including skeletal muscles. The study of changes occurring in muscles under the influence of different motor modes at macroscopic, microscopic and ultramicroscopic levels is of great theoretical and practical importance.

Sports practice shows that purposeful training increases strength and other functional properties of muscles. But there are also such phenomena when at maximum loads and insufficient rest time muscle strength begins to decrease and the athlete cannot repeat the high results shown before (Raeder et al., 2016; Weiss, 1991). It is important to know what changes occur in the muscles and what the athlete's motor regime should be in the future.

The adaptive mechanisms of response to physical activity of slow and fast contractile fibers are different and are of interest for more detailed study. Slowly contracting fibres are characterised by a small contraction speed, a large number of mitochondria, high activity of oxidative enzymes, wide vascularisation, and high glycogen accumulation potential. Rapidly contracting fibres break down ATP much faster, are well susceptible to endurance training, and their activity is associated with the use of anaerobic energy sources (Platonov, 2013). The higher plasticity of rapidly contracting fibres contributes to faster and more powerful contractions (Caiozzo, 2002; Fox et al., 1993; Shoepe et al., 2003). The gastrocnemius and soleus muscles of laboratory rats, which differ in the ratio of fast and slow contractile fibres, were chosen as models for further study.

The present article aims to study adaptive changes in the skeletal muscles of laboratory rats after exercise at the ultramicroscopic level.

MATERIAL AND METHODS

The studies were carried out on 20 white sexually mature rats of the Fischer line with an initial weight of 200-220 g. The animals were equally divided into control (intact rats) and experimental groups. In the experimental group, physi-

cal exercise was applied as daily swimming for 30 minutes with additional weight (10% of body weight) for 5 weeks. The maintenance and use of laboratory animals met the methods and norms of bioethics (Gnadt & Leland, 2001).

We studied both slow muscle fibres (predominant in the soleus muscle) and fast contracting fibres (predominant in the gastrocnemius muscle). Muscle material from both groups was studied using a PEM-125K transmission electron microscope.

After decapitation under ether anaesthesia, muscle pieces were fixed with 2.5% glutaraldehyde solution on phosphate buffer with additional fixation in 1% osmium tetraoxide solution (Madigan et al., 2021). Dehydration was carried out in alcohols of increasing concentration and acetone, poured into the epon-araldite mixture according to the generally accepted technique (Karupu, 1984).

The quantitative material was processed using descriptive statistics methods. The mean (M) and standard deviation (SD) of such indicators as the volumetric and quantitative density of mitochondria in symplasts of gastrocnemius and soleus muscles were evaluated. Statistical reliability in the difference between the results of the control (C) and experimental (E) groups was determined using the non-parametric Wilcoxon T-test (Wiedermann & von Eye, 2013). Differences between groups were considered statistically significant at $p<0.05$. All quantitative calculations were performed using the statistical environment R (https://www.r-project.org).

RESULTS

The results of mitochondrial analysis in the soleus and gastrocnemius muscles for the control and experimental groups are summarised in Table 1.

Table 1. *Mitochondrial indices in symplasts of gastrocnemius and soleus muscles of rats of the control (C) and experimental (E) groups. M – mean, SD – standard deviation, E/C – a ratio of the experimental/control groups, p-value – the statistical significance of the Wilcoxon T-test.*

Indicator	Muscle	Control (C)		Experimental (E)		Changes (E/C ratio)	p-value
		M	Sd	M	SD		
Volumetric density,%	gastrocnemius	3.37	0.57	27.37	7.17	8.12	<0.05
	soleus	3.72	0.79	13.29	4.12	3.57	<0.05
Quantitative density, $10^{-2}/\mu m^3$	gastrocnemius	22.2	5.18	93.19	16.03	4.20	<0.05
	soleus	27.29	0.59	26.01	6.15	0.95	>0.05
Cutting area, $10^{-2}\,\mu m^2$	gastrocnemius	15.93	0.67	43.68	1.88	2.74	<0.05
	soleus	16.03	1.06	38.12	3.03	2.38	<0.05

Physical load in the experimental group caused a significant increase in the number of mitochondria. Not only the number but also the average mitochondrial slice area increased compared to control animals. This index was more than twice as large in rats subjected to exercise (Table 1). This was observed for both gastrocnemius and soleus muscles. The changes are due to the appearance of large-sized organelles against the background of a decrease in the number of small mitochondria.

The quantitative density of mitochondria in gastrocnemius muscle also increased more than 4 times. Similar changes in this parameter were not observed in the soleus muscle. Despite this, the volumetric density increased significantly in both muscle types. These changes indicate different structural adaptive rearrangements of fast and slow-contracting fibres under physical load.

In addition to quantitative changes in mitochondrial parameters, morphological changes associated with exercise were also observed. Mitochondria are located under the sarcolemma and between sarcomeres but differ in shape and size. The presence of a large number of cristae in mitochondria is also indicative of mitochondria functioning in a strained mode. The accumulation of calcium granules in mitochondria was also noted. Hypertrophy and hyperplasia of mitochondria in symplasts of the gastrocnemius muscle after exercise is accompanied by a significant decrease in the number of glycogen granules compared to the control group. Such changes do not exclude the decompensation of the energy supply of gastrocnemius muscle fibres in some rats under the condition of prolonged exercise.

At the microscopic level, the nuclei in the symplasts of animal gastrocnemius muscles had a well-structured caryolema, which forms deep invaginations. This increases the surface area. Euchromatin uniformly fills the

caryolema. Two adjacent nuclei are found in some places. All this indicates active transcriptional processes during physical activity.

Morphometric analysis of mitochondria of the soleus muscle showed that physical exercise also leads to an increase in their size. The average area of these organelles is more than twice as large as in control animals (Table 1). Mitochondria in the subsarcolemmal zone are larger than organelles located between the fibrils. The increase resulted from the decrease in the number of small mitochondria due to the appearance of larger organelles.

The sarcomeres of the flounder muscles in different animals have a typical ratio of A- and I-zones, Z-lines without compaction. The most characteristic changes in the sarcomeres of calf muscles are localised divergences of myofibrils. Changes in the fine structure of the muscle tissue in the experimental group are characterised by increased deposition of myoglobin, especially at the level of myofibrils, focal changes in glycogen content and an increase in mitochondria.

DISCUSSION

Prolonged physical exertion leads to the fact that muscle hyperfunction is fixed by the corresponding structural rearrangement (Grigoriev & Egorov, 1992; Krivoshchekov & Divert, 2001). In the process of adaptive reactions, morphological transformations occur at various levels of the structural organisation of skeletal muscles. The consequence of such transformations is metabolic rearrangement in myocytes, and, under certain conditions, changes in the properties of their energy-generating and contractile structures. An increase in the number of muscle fibres is not an obligatory characteristic of muscle hypertrophy, although it often accompanies it (Macdougall, 2003).

Currently, there is an opinion that mitochondrial swelling is a sign of increased activity of oxidative enzymes, as well as a sign of decreased ATP content in cells and stimulation of glycolysis (Chinopoulos & Adam-Vizi, 2010; Jeong et al., 2004). The latter plays an important role in the energy supply of myocytes during intensive muscle work. These reversible changes in mitochondria are considered signs indicating an increase in metabolic processes in mitochondria and surrounding cytoplasm as a result of increased energy consumption during muscular work (Liesa & Shirihai, 2013; Schirrmacher, 2020).

The increase in the number of mitochondria in skeletal muscle cells during prolonged exercise is a known phenomenon and its molecular basis is being studied (Safdar et al., 2011). It is known that during exercise, the amount of PGC-1a protein in the cell increases and it moves from the cytoplasm to the nucleus. Recently, it was found that PGC-1a can penetrate not only into the nucleus but also into mitochondria, where it can participate in the activation of genes of the mitochondrial genome (Safdar et al., 2011).

When muscle cells are engaged in prolonged physical work, their energy requirements increase. The adaptive response is to increase the number of mitochondria and the number of enzymes providing ATP synthesis in the already existing mitochondria. Therefore, PGC-1a also moves into the mitochondria where it helps transcription factors to activate gene function. Thus, PGC-1a is part of the mechanism that helps muscles adapt to prolonged exercise (Little et al., 2010; Safdar et al., 2011).

Overall, mitochondrial changes during exercise are multidirectional and involve a variety of adaptive mechanisms:

- increase in the number of mitochondria – physical activity stimulates the process of mitochondrial biogenesis, which leads to an increase in the number of mitochondria in cells. This occurs by increasing the fission of existing mitochondria and the formation of new ones;
- increase in mitochondrial density – physical activity helps to increase the density of mitochondria in muscles. This means that muscle cells contain more mitochondria per unit volume, which promotes more efficient energy production;
- increased mitochondrial enzyme activity – exercise stimulates the activity of mitochondria-related enzymes involved in oxidative phosphorylation. This improves the mitochondria's ability to produce energy;
- increased mitochondrial adaptation – exercise promotes the activation of various signalling pathways that regulate genes associated with mitochondria. This leads to improved mitochondrial adaptation, increased aerobic metabolism and increased efficiency of energy metabolism in muscle;
- increase in mitochondrial efficiency – physical activity improves the efficiency of mitochondria in energy production. This is achieved by increasing the mitochondria's ability to oxidise fats and carbohydrates, which improves energy metabolism in the body.

The outlined changes at the mitochondrial level during exercise have positive effects on overall physical endurance, body adaptation to physical activity, general health and fitness.

Finally, we cannot fail to mention such general adaptation mechanisms as increased local blood flow in skeletal muscles. They were noted for both groups of the studied muscles. The reaction of the final blood flow of skeletal muscles at physical load is working hyperaemia, which creates conditions for blood inflow due to the opening of reserve capillaries. Anaerobic processes cannot ensure tissue functioning for a long period. The increase in the functional activity of the organ occurs with an obligatory increase in tissue metabolism. Acceleration of oxidative metabolism is impossible without increasing the delivery of blood and, together with it, oxygen to the working organs (Kalliokoski et al., 2005; Saltin et al., 1998).

CONCLUSION

We have investigated the features of adaptive changes in skeletal muscles (gastrocnemius and soleus muscles) of laboratory rats after prolonged physical activity. A complex structural rearrangement at different levels in the mitochondrial pool was found. The gastrocnemius and soleus muscles respond differently to prolonged physical activity, as evidenced by the quantitative density of mitochondria. Adaptive changes were observed in both muscle groups (gastrocnemius and soleus muscles). Mitochondrial volumetric density and mean slice area in the experimental group of rats subjected to exercise increased significantly. The general mechanisms consist of rearrangement at the level of myocytes, replacement of small mitochondria by larger ones, and enhancement of blood flow of skeletal muscles. All this contributes to the understanding of the deep mechanisms of adaptive reorganisation of skeletal muscles under the influence of physical exercise. The changes detected in the study are the basis for understanding the adaptive restructuring of skeletal muscles in athletes under the influence of prolonged exercise.

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