ORIGINAL RESEARCH PAPER

Physiology

THE STRUCTURAL AND FUNCTIONAL RELATIONSHIP BETWEEN CHANGES IN BLOOD MICROCIRCULATION AND THE ULTRASTRUCTURE OF CAPILLARIES IN MUSCLES IN RESPONSE TO PHYSICAL TRAINING

KEY WORDS: microcirculation, dosed physical training, laser Doppler flowmetry, capillarization of muscle tissue, mitochondria.

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ABSTRACT

The relationship between changes in blood microcirculation (BM) and the ultrastructure of muscles capillaries in response to the dosed physical training (DPT) in young people of varying degrees of fitness and animals under experimental conditions was studied. It was found that lack of fitness and/or initially low microcirculation index (BMI) are accompanied by a decrease in the effectiveness of blood flow regulation in response to DPT, while initially high values of BMI in sportsman indicate the possibility of compensatory increase in blood flow efficiency in the microcirculation system at DPT due to predominance of active regulatory mechanisms. In the experiment with DPT were revealed the ultrastructural manifestations of muscle adaptation, including initial signs of primary angiogenesis, the activation of mitochondrial morphogenesis. The formation of such compensatory and adaptive changes aimed at providing adequate energy metabolism and preventing the development of secondary tissue hypoxia. Along with this, in the muscular tissue (especially with DPT in untrained animals) there was a site of hypertrophy of muscles, and sometimes an increase in the percentage of structurally damaged organelles. Such multidirectional changes may indicate the individual peculiarities in the formation of adaptive mechanisms in the organism in response to the DPT and explain the variability of changes in the microcirculation system.

INTRODUCTION

Human adaptation to stressful muscle activity and finding the ways to improve physical capacity and aerobic productivity remains an actual problem of physiology. Question about the features of the cardiovascular system functioning and, in particular blood microcirculation (BM) under the influence of physical training is one of the most relevant [1-3]. By increasing the load on the organism, accompanied by a significant increase in oxygen consumption, there arise the hypoxic states, which in the literature are isolated in a separate type of hypoxia - load hypoxia (LH) [4]. The latter limits the physical capacity, and one of the main characteristics of LH is limited by diffusion of oxygen in tissues, particularly in muscle tissue during its transport from the vascular microcirculation to mitochondria. To reduce the influence of this factor on the development of tissue hypoxia, the necessary (and often sufficient) condition may be the growth of tissue capillarization [5]. The activity of the cardiovascular system of practically healthy, not engaged in sports, people differs from those who regularly engage in sports, by a number of characteristic features. These differences are due to the processes of long-term adaptation of the circulatory system to systematic muscular stresses. However, the question of the BM changing under the influence of physical activity requires more detailed study due to lack of knowledge, multi-directional developing reactions, as well as due to lack of attention to the problem of structure-function relationships in the microcirculation system in terms of its efficiency and capabilities of active and passive modulation of blood flow fluctuations [6,7].

The aim of this study was to investigate the relationships between changes of blood microcirculation and the ultrastructure of capillaries in muscle in response to exercise in young people of varying degrees of fitness and in animals under the experimental conditions.

MATERIALS AND METHODS

Experimental studies were carried out on adult male rats (Wistar) weighing 220-250 g (experimental group, n = 10). The dosed physical training (DPT) was created by swimming of the animals (30 minutes, once daily, or once a day within 3 weeks) in heated to 30-320 ° C water at the height of water column of 80 cm. The consumption rate of O2 was 70-75% of the maximum, which corresponded with a conditions created during the training of students and sportsmen [8,9]. The control group included 10 intact animals.

At the end of the experiment, the animals were decapitated under a weak etheric anesthesia. Work on all stages of the experiment was performed in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Purposes (Strasbourg, 1986) and the principles of the Helsinki Declaration (2000).

In morphological and morphometric studies, samples of calf muscle were used. The preparation of the tissue samples for electron microscopic investigations was performed by the usual method [10], using reagents of Sigma (USA) and Fluka (Switzerland) firms. Viewing of ultrathin sections (40-60 nm thick) was carried out using an electron microscope JEM 100CX (Japan). Morphometric assessment of mitochondria was carried out using the computer program Image Tool Version 3 (USA) on 130-150 fields in each series of studies. The total number of functioning capillaries (FC) was determined according to the methodology, proposed by H. Hoppeler et al. [11] on the screen of an electron microscope with a small increase.

To identify the characteristics of BM, men at the age of 21 were examined: 15 students and 15 sportsmen (freestyle wrestling, level of candidates for master of sports). The dosed physical training (DPT) was performed on a veloergometer and was selected individually - the O2 consumption rate was 70-75% of the maximum [12].

The choice of such a program of DPT makes it possible to establish functional rearrangements in the organism of trained and untrained persons from the BM system [8,9]. The BM was evaluated using laser Doppler flowmetry (LDF) by LAKK-01 (Lazma, Russia) on the ventral surface of the distal phalanx of the 4th hand finger. Analysis of LDF-grams was performed in accordance with the Instruction for the apparatus [13]. Statistical processing was performed by applied program package "Microsoft Ecxel" using Student's t-test. The results were presented in the form mean ± SEM, since the data obtained fit into the normal distribution of the quantities. Differences between mean values were considered significant at $p < 0.05$. [14].

RESULTS AND DISCUSSION

Depending on the blood microcirculation index (BMI - the average perfusion value per unit volume of tissue per unit time, perfusion units – p.u.) students and athletes were divided into 2 subgroups: I subgroup - BMI was from 0.5 up to 12 p.u.; II subgroup - BMI was from 12 up to 25 p.u. At rest, the BMI in sportsmen's of both groups was higher than that in the students at 44% and 26%, respectively ($p < 0.001$), which indicates a more vigorous flow of red blood cells per unit volume of muscle tissue, i.e., on improving the supply of oxygen to tissues in sportsmen's.

After fulfilling the DPT BMI at the students of the I subgroup increased by 83%, whereas in the II subgroup it decreased by 14% (Fig. 1).

Figure 1 – here

In sportsmen's I subgroup BMI after DPT increased by 34%, whereas in the II subgroup it decreased by 33% (Fig. 1). The difference in the responses on the DPT of I and II subgroups of students and sportsmen's can indicate the inclusion of different adaptive mechanisms of the influence of the vegetative nervous system on BM: in the first case - sympathetic, in the second - parasympathetic.

The value of the mean square deviation (MSD - temporal variability of microcirculation) was lower for both students and sportsmen's of the I subgroup than in the second subgroup (Fig. 1); apparently, in the latter at the resting state, the mechanisms of modulation of tissue blood flow are more actively involved. After the DPT value of the MSD decreased in both groups of students (28% and 51%, respectively), which may indicate a decrease in the modulation capabilities of tissue blood flow. In sportsmen's after DPT, the opposite picture was observed: in the I subgroup there was a tendency to an increase of MSD ($0.1 < p < 0.2$), in II – there was an increase of MSD of 2.7 times (Fig. 1). Such changes may indicate a greater adaptive lability of tissue blood flow in DPT, aimed at optimizing of blood perfusion in muscle tissue.

The analysis of the variation coefficient (Kv), which gives information about the contribution of the vasomotor component to the modulation of tissue blood flow, showed the presence of a multidirectional response to DPT in trained and untrained individuals: at students of I and sportsmen's of II subgroups there were no significant changes in Kv; in the students of the I subgroup Kv decreased by 71%, and in the sportsmen's of the II subgroup this ratio increased by 4.1 times (Fig. 2). Consequently, in students with a low BMI level, DPT reduces the effectiveness of this protective mechanism in response to the disturbing factor, while in sportsmen's with a high BMI the contribution of the vasomotor component to the blood flow modulation sharply increases.

Figure 2 – here

Integrative characteristic of the ratio of active (due to myogenic and neurogenic activity of precapillary vasomotors and the vascular tone proper) and passive (fluctuations of blood flow synchronized with cardio- and respiratory rhythms) mechanisms of blood flow modulation demonstrates the flaksmotsy index (FI). In the students of the I subgroup, at rest, its value was 1.74 ± 0.046, and in II - 1.47 ± 0.001 (15.6% less), which indicates the predominance of the vascular tone over passive modulations in individuals with lower BMI values. After the influence of DPT in the students of the I subgroup, the FI decreased by 14%, and did not change in the II subgroup (Fig. 2). At the same time in sportsmen's of the I subgroup after the DPT, the FI did not change significantly, while in the II subgroup it increased 2.1-fold.

Such changes may indicate a low efficiency of blood flow regulation in the microcirculation system in students of the II and sportsmen's of the I subgroups and point to increasing of blood flow regulation efficiency, due to active modulation mechanisms. The obtained results show that the cardiovascular system of students and sportsmen's react differently on the DPT. This feature can be due to the structural features of the capillary network, formed under the influence of long-term sports training.

In the experiment with prolonged DPT were revealed ultrastructural signs of muscle adaptation: there was virtually no empty or stuck together capillaries; the number of functioning capillaries per unit area of tissue increased in experimental group of rats relative to untrained animals (Table 1), signs that were available primary angiogenesis as compensatory response to DPT. Formation of compensatory mechanisms in the BM, aimed at improving the metabolic processes associated with the supply muscle by oxygen under conditions of DPT. In the endothelial cells of capillaries, activation of pinocytosis was found, which is considered which is considered to be a reflection of the intensification of metabolic processes in response to DPT [15].

Table 1 - here

There was a significant increase in the total number of subarcholemal and intramyofibrillar subpopulations of mitochondria (MC) (Table 1); therefore activation of MC morphogenesis took place. Such changes are considered as manifestations of the compensatory and adaptive changes formation, to ensure the adequate energy metabolism and prevention of secondary tissue hypoxia [11].

Along with the aforementioned, in the muscular tissue (especially with single DPT in untrained animals) there were the areas of muscle fibers hypertrophy, the increaseing in the number of vacuoles between them. The percentage of structurally damaged MC increased significantly: the main violations consisted in the presence of partially or completely vacuolized organelles (Table 1).

Such multi-directional changes may indicate the individual peculiarities of the formation of adaptive mechanisms in the organism in response to DPT and, to a certain extent, explain the multidirectional changes in the microcirculation system during exercise.

The conducted studies revealed that lack of training and/or initially low BMI are accompanied by a decrease in the effectiveness of blood flow regulation in response to DPT, while initially high values of BMI in sportsmen's indicate the possibility of compensatory increase of blood flow regulation efficiency in the microcirculation system under DPT due to the prevalence of active regulatory mechanisms.

CONCLUSION

It was shown that lack of fitness and/or initially low blood microcirculation index are accompanied by a decrease in the effectiveness of blood flow regulation in response to dosed physical training, while initially high values of blood microcirculation index in sportsman indicate the possibility of compensatory increase in blood flow efficiency in the microcirculation system at dosed physical training due to predominance of active regulatory mechanisms. In the experiment with dosed physical training were revealed the ultrastructural manifestations of muscle adaptation, including initial signs of primary angiogenesis, the activation of mitochondrial morphogenesis. Along with this, in the muscular tissue (especially with dosed physical training in untrained animals) there was a site of hypertrophy of muscles, and sometimes an increase in the percentage of structurally damaged organelles. Such multidirectional changes may indicate the individual peculiarities in the formation of adaptive mechanisms in the organism in response to the dosed physical training and explain the variability of changes in the microcirculation system.

Figure (1): Changes in blood microcirculation index (a) and mean square deviation (b) at students and sportsmen's before and after DPT. * - p <0.05

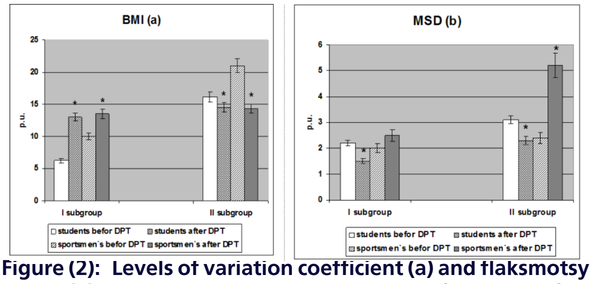

Figure (2): Levels of variation coefficient (a) and flaksmotsy index (b) in students and sportsmen's before and after dosed physical training. * - p <0.05

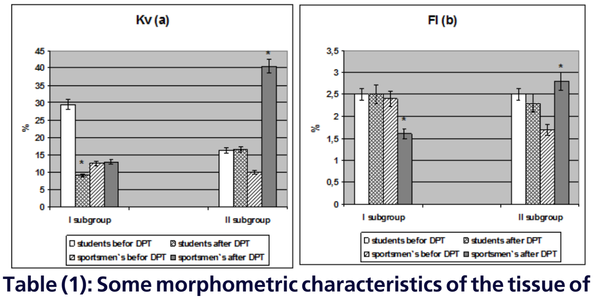

Table (1): Some morphometric characteristics of the tissue of the gastrocnemius muscle under the DPT (mean±SEM)

Indexes	Control group (n=10; a=135)	Experimental group (n=10; a=130)
Total number of mitochondria, units μm^{-2}: subsarkolemal mitochondria intramyofibrillar mitochondria	10,32,4 6,11,3	17,83,1* 10,11,6*
Number of structurally altered mitochondrial, %: subsarkolemal mitochondria intramyofibrillar mitochondria	3,8±0,7 0,9±0,2	13,2±1,4** 11,0±2,1**
Number of functioning capillaries, units μm^{-2}	10,81,4	19,52,0*

* - the mean difference in significant at the 0.05 level; ** - the mean difference in significant at the 0.01 level; a - the number of regions randomly chosen for calculationsa

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