ELECTRON-MICROSCOPIC INVESTIGATION OF THE EFFECTS OF EXERTION AT HIGH ALTITUDES ON SKELETAL MUSCLE FIBERS

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The problem of adaptation of the organism to high-altitude hypoxia and maintenance of physical working capacity under these conditions is extremely important at the present time. Adaptation to high altitudes in the mountains is known to increase resistance to other extremal and subextremal factors, including physical exertion. However, the levels of working capacity of the body after different lengths of stay under conditions of high-altitude hypoxia differ substantially [1-5, 7].

Many problems connected with adaptive changes in the muscular system on the transition to a new level of function still remain unsolved. The particular features of the fine submicroscopic structure of skeletal muscle under conditions of crossed adaptation to exercise and high-altitude hypoxia have not been studied at all.

The aim of the present investigation was to study ultrastructural changes and to undertake a corresponding morphometric analysis on skeletal muscle fibers during combined exposure to exercise and high-altitude hypoxia in the mountains.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats taken by helicopter to an altitude of 4000 m above sea level (Pamir Mountains, District of the Fortambek Glacier, base of the Laboratory of High-Altitude Medico-Biological Research, Academy of Sciences of the Tadjik SSR*), and kept in a vivarium on a standard diet. Starting with the second day in the mountains the animals were made to exercise by running on a horizontal treadmill: the first three training sessions each 30 min in duration at a speed of 11.2 m/min, in all subsequent sessions for 1 h at a speed of 13.7 m/min. The animals were decapitated 30 min after the end of exercise on the 1st, 3rd, 7th, 15th, 30th, and 46th days of the experiment (five animals at each time). Red and white fibers of the tibialis anterior muscle were investigated. Pieces of muscle measuring 1 × 3 mm were fixed in a mixture of solution of glutaraldehyde and paraform by a modified Karnovsky's method and postfixed in 1% OsO_4 solution. Thereafter the material was treated in the usual way. Muscles of animals kept at a low altitude in the plains (Dushanbe) on a standard diet served as the control.

The ultrastructure of the muscle fibers was studied in longitudinal and transverse sections. No fewer than 220 electron micrographs for each period of the experiment, obtained at random from transverse sections of muscle fibers under a primary magnification of 15,000 times were subjected to stereomorphometric analysis. The relative volume of the mitochondria, sarcoplasmic reticulum (SPR), and the number of glycogen granules, calculated per square micron, were determined.

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Fig. 1

Fig. 2

Fig. 1. White muscle fiber (single exercise session after animal had stayed 1 day at an altitude of 4000 m). Degenerative changes in individual mitochondria. Moderate widening of SPR. 30,000 ×.

Fig. 2. Red muscle fiber (single session of exercise after animal had stayed 1 day at an altitude of 4000 m). Disturbance of structure of sarcomeres of myofibrils and fragmentation of Z bands.

EXPERIMENTAL RESULTS

The experiments showed that the level of physical working capacity at high altitudes in the mountains is much lower than in the plains. For instance, the volume of muscular exertion accomplished by the animals, which were selected previously for ability to undergo physical training, at the second day of the stay in the mountains was less than that which untrained animals could achieve in the plains (running for 30 min at a speed of 11.2 m/min and for 1 h at a speed of 18.5 m/min respectively). Although by the end of the experiment with simultaneous exposure to exercise and high altitude the animals could do a greater volume of muscular work than initially, they still did not achieve the values characteristic of animals adapted to exercise under adequate conditions (running for 1 h at a speed of 13.7 m/min and for 2 h at a speed of 27.5 m/min respectively) [6]. These data also indicate a reduction in the training effect of exercise under crossed adaptation conditions.

Electron-microscopic investigation of the skeletal muscle fibers of animals exposed to two extremal factors, namely intensive muscular exertion and high-altitude hypoxia, revealed considerable abnormalities of their ultrastructural organization, relating to both their energy-producing and contractile, myofibrillary apparatus. In some muscle fibers, for instance, swelling of the mitochondria with a pale matrix and with partially or completely reduced cristae, sometimes containing small myelin-like structures, was observed (Fig. 1). Degeneratively changed mitochondria, autophagosomes, and numerous vacuoles were found most frequently in the widened perinuclear and subsarcolemmal spaces. Considerable widening of various components of the SPR also was observed. Changes in the contractile myofibrillary apparatus were focal in character and manifested as disintegration of myofilaments, breakdown of their complex organization, disturbances of the I disks and destruction of the Z bands, and in some cases also total loss of the structure of the sarcomeres (Fig. 2).

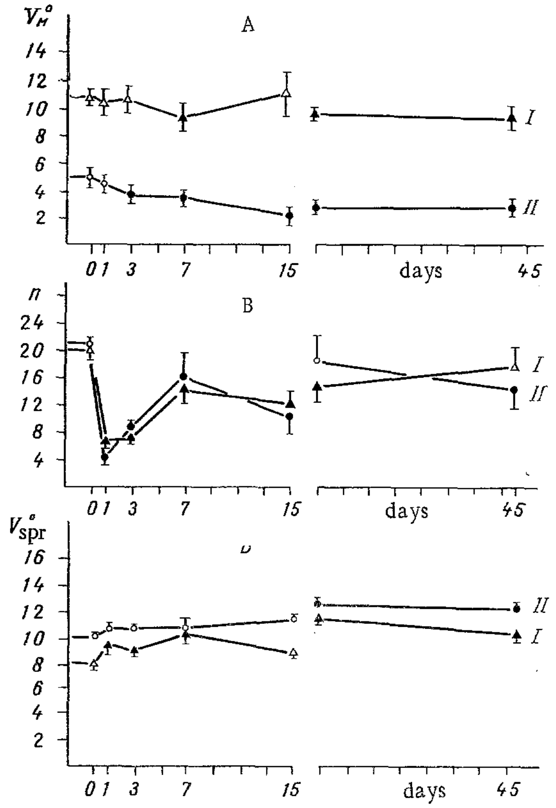

Fig. 3. Time course of changes in quantitative parameters of ultrastructures of muscle fibers during crossed adaptation to exercise and high altitude hypoxia. A) Relative volume of mitochondria in red (I) and white (II) muscle fibers; B) number of glycogen granules in red (I) and white (II) muscle fibers expressed per area of 1 μ^2; C) relative volume of SPR in red (I) and white (II) muscle fibers. Filled symbols indicate that differences compared with control are significant ($P < 0.05$).

These changes, incidentally, were more marked in the red muscle fibers and were observed more often during the first 7 days of the experiment. Later the severity of the degenerative changes in the muscle fibers gradually diminished and the number of fibers with a disturbed structure was reduced. The results of these observations suggest that under the influence of intensive physical exertion and high altitude factors, structures whose function is based on oxidative metabolism were most vulnerable. Under conditions of oxygen deficiency oxidation and oxidative phosphorylation processes in the mitochondria are inhibited and, as a result, there is a compensatory increase in the intensity of their function [3, 4]. Combined exposure to hypoxia and intensive physical exertion enhances this effect even more. "Running down" work of this kind may ultimately lead to exhaustion of the mitochondrial apparatus.

Morphometric analysis of the ultrastructural changes confirmed these suggestions. For instance, during the very first days of the experiment, a tendency was observed for the relative volume of the mitochondria in both types of muscle fibers to decrease; later this tendency grew stronger and led to a statistically significant decrease in this parameter (Fig. 3A).

By contrast with these results, those obtained during previous experiments to study the effect of graded training exercises on skeletal muscle ultrastructure of animals under adequate conditions [6] demonstrated a gradual increase in the relative volume of the mitochondria in red muscle fibers which, in the writer's opinion, play a more important role than the white fibers in the process of adaptation, if not the principal role. Perhaps the decrease in power of the mitochondrial apparatus is one of the causes of the decrease in the training effect of physical exertion under high altitude conditions 4000 m above sea leavel. Meanwhile the increase, small as it may be, in the volume of muscular work done suggests activation of certain adaptive mechanisms. The results of the present experiments suggest that intensive muscular work under conditions of oxygen deficiency is accompanied by activation of the glycolytic pathway of energy formation in skeletal muscles; this is evidently true, moreover, not only of white fibers, for which this metabolic pathway is adequate, but also, and this is particularly important, for red fibers.

Quantitative determination of glycogen, the energy substrate of glycolysis, in fact revealed a sharp fall in its concentration during the first 3 days of the experiment. However, after 7 days the reduction in the glycogen reserves was less marked, and toward the end of the experiment this parameter was close to the control level (Fig. 3B). This state of affairs, it is claimed, can be regarded as evidence of an increase in the initial level of the energy reserves through the more rapid synthesis of glycogen, including by glyconeogenesis.

This hypothesis also is confirmed by data on changes in the volume of the SPR in these experiments. The SPR, which is responsible for transport of Ca^{++} ions, is known to play an active part in contraction and relaxation of myofibrils. At the same time, however, the opinion is also held that it plays an active role in the glycolysis and glycogenolysis. Intensive exercise under hypoxic conditions in the high mountains was found to be accompanied by an increase in the relative volume of the SPR at all times of observation and in both types of muscle fibers (Fig. 3C), whereas training exercises under adequate conditions did not bring about any such effect on the SPR [6]. The possibility cannot be ruled out that these changes are connected with the greater degree of intensification of anaerobic metabolism than that associated with exercise under adequate conditions, and aimed at compensating disturbances in the aerobic pathway of energy formation.

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