Effects of Ten-Day Aerobic Training on the Energy Potential and Blood Supply of Skeletal Muscle in Rats

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The activity of mitochondrial creatine kinase is found to increase in skeletal muscles of rats after ten days of running a treadmill. Over that short period, however, no significant increases occur either in the maximal rate of mitochondrial respiration or in the number of capillaries per skeletal muscle fiber and their density, nor are the early adaptive changes in muscle tissue accompanied by modifications in the resistive or metabolic compartments of the vascular bed, i.e. by improvements in the blood supply to muscle tissue.

Key Words: *mitochondrial respiration; creatine kinase; capillarization and hydraulic conductivity of skeletal muscle blood vessels*

The main features of the structural and metabolic adaptation occurring in skeletal muscle tissue during training for endurance are well known. After a month of such training, significant increases were recorded in the number of capillaries per muscle fiber, mitochondrial volume density, and the rate of synthesis of protein molecules [8]. Yet during the first 2 to 3 weeks of training no changes were detected in the metabolism of muscles or in the activity of oxidative and glycolytic enzymes, and it remains unclear what muscle resources are utilized to make possible the performance of increased amounts of work early in the course of adaptation. In 1991, Green *et al.* [7] found that although the muscles of persons subjected to a test load after 12 days of aerobic training expended substrates more economically than before, the economy was achieved without any change in the activity of enzymes involved in muscle energy metabolism. These authors pointed out that metabolic changes may not be accompanied by peripheral adaptation in the muscular enzymatic potential. If it is so, then the adaptation of skeletal muscle during the early period of aerobic training could result from an improved delivery of substrates and oxygen owing to a structural anatomical dilatation of resistive vessels [2,10].

This study on rats was designed to examine the sequence of changes that occur in response to aerobic exercise in the activity of enzymes of the respiratory chain of skeletal muscle mitochondria and in the structural component of hydraulic conductivity in the blood vessels of the lower limbs.

MATERIALS AND METHODS

Twenty male Wistar rats weighing 244±11 g were exercised on a treadmill at a rate of 30 m/min for 35 min per day, 5 days a week for 2 weeks; 18 rats of the same strain and weight served as

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controls. For 13 of the test and control rats, parameters of mitochondrial respiration were measured in saponin-skinned skeletal muscle fibers from the lateral gastrocnemius muscle (red zone). The number of capillaries per fiber and their density were determined in the skeletal muscle tissue. In 23 rats of both groups, the structural component of vascular resistance in the right hind limb was assessed by estimating the maximal hydraulic conductivity of its blood vessels in perfusion tests. In these tests the vascular bed was perfused with Tyrode's salt solution (t=37°C, pH 7.4) through a cannula inserted into the right common iliac artery; perfusion flow rates were stabilized by a peristaltic pump (LKB, Sweden). Vessels perfused with a salt solution are dilated to the maximum [6], and the perfusion pressure at the cannula's entrance is therefore directly proportional to the magnitude of the structural component of vascular resistance. According to the perfusion pressures at different stabilized perfusion rates, the state of this component was judged in rats from both groups.

The condition of the mitochondria and the efficacy with which mitochondrial creatine kinase (MCK) was functioning were evaluated by recording the respiration of saponin-skinned muscle fibers [12]. Free Ca and Mg concentrations were calculated on the basis of equations described by Fabiato [5] using values of dissociation constants [4].

The relaxing solution (medium A) in which the muscle fibers were skinned with saponin contained 5 mmol/liter MgATP and 15 mmol/liter phosphocreatine. Medium B, which was used to measure mitochondrial respiration and to wash the fibers free of saponin, contained, in addition to the ingredients of medium A, 5 mmol/liter glutamate, 2 mmol/liter malate, and 2 mg/ml bovine serum albumin devoid of free fatty acids. For measuring oxygen uptake, 7-8 bundles of saponin-skinned fibers were transferred to a thermostatically controlled oxygraphic cell equipped with a magnetic stirrer and filled with 3 ml of medium B with albumin (2 mg/ml). Oxygen uptake was determined at 22°C by oxymetry using Clark's electrode and an oxygraph (Yellow Spring Instruments, USA) with a Linear recorder [13]. After the measurements, the fibers were removed from the cell and dried for 24 h at 100°C to determine their dry weight. Oxygen solubility at that temperature was taken to be 460 ng-atoms/ml [13]. The respiratory rate was expressed in ng-atoms O/min per mg dry weight. All reagents used in this part of the study were from Sigma (USA).

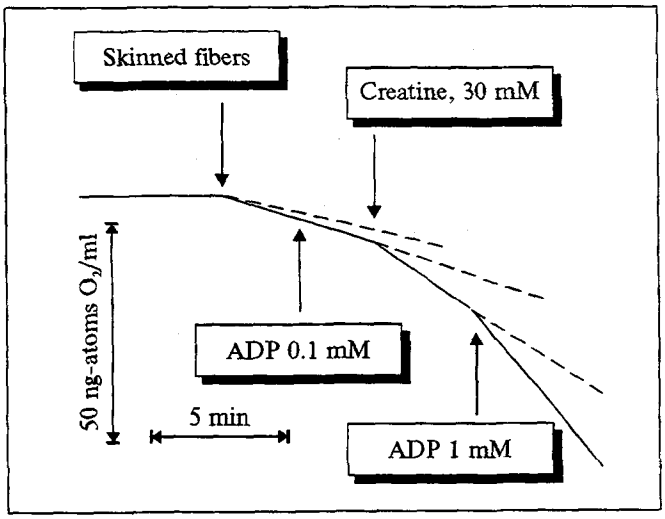

Fig. 1. Respiration oxygram for skinned fibers. The medium (3 ml) initially contained substrates for mitochondrial respiration. The arrows mark the times when skinned fibers, ADP, and creatine were added.

For histochemical tests, muscle tissue samples were frozen in liquid nitrogen and serial 10 μ cross sections were prepared in a cryostat at -20°C. Capillaries were detected by a histochemical test for alkaline phosphatase [1]. The number of capillaries per fiber (cap./fiber) and per mm² of cross section (cap./mm²) were counted. The reagents used for the histochemical part of the study were from Serva (USA).

The data were treated statistically using the paired and unpaired Student t tests.

RESULTS

After the 10-day treadmill runs, the number of capillaries per fiber and their density in the lateral gastrocnemius muscle of rats did not change significantly as compared to control animals (1.22±0.09 vs. 0.97±0.10 cap./fiber and 699±113 vs. 499±78 cap./mm² in the test and control rats, respectively). Nor did test rats differ significantly from the controls in the maximal hydraulic conductivity of perfused hind limb vessels (Table 1), indicating that

TABLE 1. Maximal Hydraulic Conductivity of Perfused Limb Vessels in Rats (X±x)

Group	Stabilized perfusion flow rate (ml×min/100 g) at			
	20 mm Hg	40 mm Hg	60 mm Hg	120 mm Hg
Control(n=10)	0.10±0.03	0.40±0.06	0.80±0.10	2.30±0.40
Test (n=13)	0.20±0.08	0.50±0.03	0.90±0.10	2.50±0.30

TABLE 2. Biochemical Parameters of Mitochondrial Respiration in the Gastrocnemius Muscle of Rats (ng-atoms O/min per mg tissue). The Values are Means±SEM

Group	V_0	V_{adp}	V_{cr}	V_{max}	V_{max}/V_0	%Cr
Control ($n=7$)	4.7±0.7	9.0±0.7	11.7±1.0	22.8±0.9	5.2±0.5	29.9±3.9
Test ($n=6$)	5.2±0.6	9.5±0.9	14.2±1.5	25.0±2.4	5.0±0.4	49.8±7.5*

Note. V_0: mitochondrial respiration in medium B; V_{adp}: mitochondrial respiration in medium B supplemented with 6 μl of 50 mmol/liter ADP; V_{cr}: mitochondrial respiration after addition of 9 mg creatine to the preceding medium; V_{max}: mitochondrial respiration after addition of 6 μl of 500 mmol/liter ADP to the preceding medium; V_{max}/V_0: respiratory control; %Cr: rate of creatine-stimulated respiration in muscle tissue samples under study, as calculated by the formula $V_{cr} - V_{adp}$(0.1 mmol/liter)/V_{adp}(0.01 mmol/liter). The asterisk indicates a significant difference from the control ($p<0.05$).

the structural component of vascular resistance remained unchanged.

A typical oxygraphic record of respiration obtained for the saponin-skinned fibers is shown in Fig. 1. It can be seen that the respiration was stimulated by ADP added in a concentration of 0.1 mmol/liter and that the addition of creatine in the presence of this submaximal ADP concentration caused a further increase in the rate of oxygen uptake by mitochondria. The latter increase was due to efficient functioning of the mitochondrial isoenzyme creatine kinase coupled with the oxidative phosphorylation system [9]. The highest respiration rate was observed following the addition of 1.0 mmol/liter ADP. That such a high ADP concentration was required for complete activation of respiration in the skinned fibers is indicative of the diffusional difficulties experienced by ADP [11].

The treadmill runs slightly increased the absolute value of the respiratory rate shown by skinned fibers when their respiration was stimulated by ADP at 1 mmol/liter. When ADP was added at 0.1 mmol/liter, the rate of ADP-dependent respiration (V_{adp}) remained virtually unchanged. However, the absolute values of the respiratory rate showed by fiber mitochondria increased in the presence of 30 mmol/liter creatine with a consequent significant increase in the activation of respiration by creatine, which reflects the functional efficiency of MCK and the degree of its coupling: the value of %Cr rose from 29.0±3.9 to 49.8±7.5% ($p<0.05$) (Table 2).

The adaptive changes preceding the growth of the oxidative potential exhibited by skeletal muscle in the course of aerobic training remain unknown. In 1991, Green *et al.* reported for the first time that glycogen and phosphocreatine were expended more economically in human subjects during a test load after 12 days of aerobic training on a bicycle ergometer [7], but these authors could not detect any appreciable changes in enzyme activity (MCK activity was not measured). They noted that the more economical glycogen and phosphocreatine consumption was not paralleled by adaptation of the metabolic potential of skeletal muscle.

In the present study, likewise, no significant changes in the maximal velocity of mitochondrial respiration were found to have occurred over a comparable period of aerobic training, although, as follows from Table 2, the respiration was significantly activated by creatine, indicating that the MCK was more active than before the training. It was shown in an earlier study that the isoenzymic composition of creatine kinase was modified in response to endurance training, which was manifested in a redistribution of the MM and MB isoenzymes and in a significant increase in the proportion of the mitochondrial fraction, whereas the total MCK activity was not changed [3]. The indicated changes, however, were detected after a more prolonged period of training. The mechanism by which the redistribution of MCK isoenzymes is regulated and the mitochondrial isoform increases significantly remains to be elucidated.

MCK is known to play a key role in intracellular energy transport through a shuttle transfer of phosphocreatine from the mitochondria and myofibrils for its subsequent utilization to produce ATP directly at the sites of its consumption [12]. It is also known that the creatine kinase systems including the mitochondrial isoenzyme, promote facilitated ADP diffusion in muscle cells by decreasing the K_m for ADP [11]. For cardiomyocytes, for example, a 10-fold reduction in K_m was shown to occur in the presence of creatine, i.e. when MCK was working [11]. More effective functioning of MCK in skeletal muscle can therefore facilitate energy transfer within the fiber. Identical changes could have been initiated by the metabolic shifts observed by Green *et al.* in a standard test after 10 training sessions [7].

Augmented activity of mitochondrial enzymes in muscles is frequently seen to be attended by improved delivery of oxygen and substrates owing to growth of the capillary bed or to anatomical dilatation of resistive vessels [10]; moreover, it should be noted that structural changes in the

resistive compartment of the vascular bed may occur without any increase in capillary numbers [2,10]. In the present study, MCK activity in the skeletal muscles of rats was found to have increased after 10 days of aerobic training, but the changes in the energy metabolism of these muscles during that period were not accompanied by structural modifications either in the resistive or the metabolic compartments of the vascular bed, i.e., by improvements in the blood supply to muscle tissue.

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Parameters of Lipid Peroxidation in Erythrocytes of Children Being Treated for Acquired Severe Aplastic Anemia

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It is shown that a peroxidase-catalase mechanism of antioxidant defense predominates in the erythrocytes of children with acquired aplastic anemia, and that the high level of lipid peroxidation (LPO) processes in such children dictates the need for continued antioxidant therapy during all phases of the disease. The antioxidant effect of glucocorticoids appears to be quite sufficient for eliminating the more severe effects of LPO.

Key Words: *lipid peroxidation; anemia*

Aplastic anemia is a collective term used to designate conditions of various origin in which injury to the bone marrow results in pancytopenia. The etiology of most forms of acquired aplastic anemia remains unknown, and for this reason they are referred to as idiopathic. In idiopathic aplastic anemia, which accounts for 60-70% of cases, the reduction in erythrocyte numbers in the peripheral blood is due to a defect in the hemopoietic sys-

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