Exercise-induced Hypoxia and Structural and Metabolic Adaptation of Skeletal Muscle

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Abstract

We hypothesised that elevated oxygen concentration during exercise on rats exhibits smaller muscle structural and metabolic adaptations of skeletal muscles than rats under the same training protocol in normoxia. Changes in m. tibialis anterior after 6-week training by swimming under hyperoxic condition (SO) were compared with those trained in normoxia (SN) and sedentary control rats (C). Capillary-to-fibre ratio was increased in SO and SN group as compared to C. The cross-sectional area was significantly increased in fast glycolytic (FTb) fibres of SO and SN but not in FTa fibres. Myoglobin (MG) concentration in SN was 21% higher in the fast oxidative (FTa) fibres, as well the succinate dehydrogenase (SDH), cytochrome c oxidase (cyt.c) and α-glycerophosphate dehydrogenase (α-GPDH) activity were 18, 56 and 45% higher respectively, than in C group ($p < 0.05$). Cyt.C and α-GPDH activity in FTb fibres in SN were 69 and 40% higher respectively than in C animals ($p < 0.05$). No significant changes were found either in SDH or α-GPDH activities or MG content in SO animals compared to the C. In conclusion, hypoxia arising in muscles during training stimulates those changes that promote better substrate and oxygen supply to muscles and better oxygen transport within fibres. Apparently, initiation of capillary growth is determined by factors other than hypoxia.

Key words: hyperoxia, skeletal muscle fibres, oxidative potential, capillary supply.

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Long duration aerobic training leads to a higher aerobic capacity and performance [25]. This phenomenon is accompanied by the muscle capillary growth, increase of fibre cross sectional area (CSA), myoglobin content and oxidative potential [10, 24, 26]. It is well known that endurance training induce much earlier changes of these parameters [5, 20], or their greater expression under hypoxic conditions. These changes are generally believed to improve oxygen supply and oxygen utilization in muscle fibres. These data make many authors suppose that work-induced muscle adaptations might be partly induced by the local-induced hypoxia in the working muscles [5, 9, 13, 29]. This hypothesis can be tested by reducing exercise-hypoxia in the working muscles, i.e. by providing training under conditions of hyperoxia, when the PO_2 in arterial and venous blood is higher than under normoxic conditions [16]. We hypothesized that skeletal muscles of rats supplemented with oxygen during exercise sessions exhibits smaller structural and metabolic adaptations than muscles of animals under the same training protocol in normoxia.

Animals

23 three-month-old Wistar rats capable to swim were selected from 45 animals. Nonswimming rats were eliminated from the study. The rats were then randomly subdivided into 3 groups. Animals of the first group were trained by swimming in normoxia (SN group) ($n = 9$; body weight 296 ± 9 g). Group 2 was trained by swimming according to the same protocol as group SN, but in the atmospheric with 65% O_2 (SO group, $n = 7$; body weight 323 ± 9 g). Group 3 remained intact and served as a control (C, $n = 7$; body weight 359 ± 13 g).

All groups were allowed food and water *ad libitum* and fed according to the standards for rodents. During the experiment, all rats were kept in the vivarium at an air temperature of 22°C and a day length of 12 h. All the experiments described below were approved by the Review Committee.

Training Design and Protocol

Before the experiment SN and SO rats were subjected to physical performance (swimming till exhaustion with a load of 5% of body weight). In the preliminary test SN and SO rats demonstrated the swimming time 25 ± 3 (n = 5) and 27 ± 1 (n = 4) min respectively. The water temperature was 37°C. Then SN and SO rats were subjected to training. During the initial period (1 week) the time of swimming with 5% b.w. load attached to the tail was increased from 10 minutes in the first day to the 60 minutes by the end of the week. The duration of a daily swimming session was 60 minutes, 5 day a week for the following six weeks. During the training session in hyperoxia, O_2 was continuously pumped through the swimming chamber and it's content was measured by means of oxyanalyser at the opposite end of the chamber. O_2 contend was maintained at the 65% level. The air was continuously agitated in the chamber (fig. 1). It was established that this hyperoxia regime induces neither vasoconstriction, nor a noticeable increase in free-radical formation, nor any other negative processes in the organism [15].

Tissue Processing

24 h after the experiment had been completed, the rats were euthanized by an overdose of nembutal (200 mg/kg). Under anesthesia the m. tibialis anterior was removed and immediately frozen in liquid nitrogen and stored there until morphological and histochemical analyses were performed. The frozen samples were transferred to the cryostat and then the transverse 10 μm thick sections were made at -20°C and mounted on the slides.

Staining

The staining procedure was carried out at room temperature. Muscle fibres were typed with myofibrillar adenosinethriphosphatase (ATP- ase) stains [7] (pH of preincubation solution 4,6). The fibres were classified into three major types (I, IIa, and IIb). But the number of I type muscle fibre was not enough for statistical analysis. The capillaries were performed by staining for myofibrillar ATP-ase but after preincubation at pH 4,0 [27]. We used Lojda's modifications [18] of specific staining for enzymes, including succinate dehydrogenase (SDH), NADH-tetrazolium reductase (NADH-tr), α-glycerophosphate dehydrogenase (α-GPDH) (by the tetrazolium Nachlas technique) and cytochrome c oxidase (cyt.c) (by the azocombination technique). Myoglobin (MG) was detected immunohistochemically by means of a Sigma Chemicals kit. All compared sections were treated simultaneously.

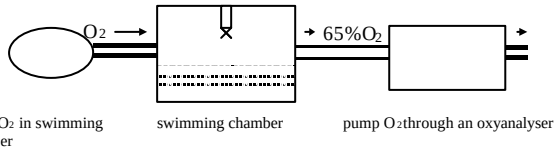

Figure 1. Scheme of the experimental facilities.

Image analysis

The CSA of no less than 100 fibres of each type were measured by means of the Leitz image analyser. Enzyme activities in different muscle fibres and MG concentration were evaluated in the central part of the fibres by point cytophotometrical method on Reichert microscope- photometer at wave length of 570 nm on the activity plateau (after the reaction had been terminated and the section fixed in 4% para-formaldehyde in phosphate buffer) and expressed in units of optical density. This method had been earlier verified against biochemical techniques [19] and serves as an equivalent of kinetic *in situ* cytophotometry [6]. Cytophotometrical assays for all enzymes were performed on fibres identified as fast oxidative (FTa) and fast glycolytic (FTb) fibre type by staining for myofibrillar ATPase. The quantity of slow twitch fibres was not sufficient for statistical analysis. Capillary and fibre counts to estimate capillary per fibre ratio values were calculated for 150-200 pictures per every slide with 7x40 magnification. The total number of capillaries was divided by the total number of fibres to determine the capillary-to-fibre ratio (cap/fib.). The capillary density (CD) was determined by calculating the area, in mm^2 (cap/mm^2), occupied by the designated skeletal muscle fibres.

Statistical Analyses

All values are reported as means ± SE. All statistical analyses were performed using the MS Excel software package. Comparison between means was done by unpaired t-test. The significance level of $P < 0.05$ of confidence was accepted as the critical point for determining differences between means.

Results

The results of the structural analyses are depicted in table 1. The cross-sectional area of the FTb fibres in SN and SO groups were 24 and 26% respectively higher ($p < 0.05$), than in the control rats. These were no significant differences between FTa fibres in experimental and control animals. There were no substantial differences in the cap/fib between SN and SO groups and this amount were 23 and 20% higher respectively, than in the control rats ($p < 0.05$). At the same time the CD increased significantly only in the animals trained in normoxia. But these differences were apparently due to the slight changes in CSAs and a little higher capillary per fibre ratio in this group than the ones produced by swimming in hyperoxia.

Table 1. Morphological characteristics of m. tibialis anterior (M ± m).

Parameter	Swimming	Swimming + O_2	Control
CSA of FTa	2339 ± 191	2444 ± 114	2196 ± 104
fibres (μm^2) FTb	3963 ± 100*	4005 ± 133*	3172 ± 96
cap/fib	1.96 ± 0.03*	1.90 ±0.05*	1.59 ± .01
cap/mm^2	752 ± 76*	637 ± 73	512 ± 31

*-Significant differences from the control ($P < 0.05$). CSA -the cross-sectional area, cap/fib- capillary-to-fibre ratio, capillary density - cap/mm^2

Meanwhile MG concentration in FTa fibres in the animals trained only in normoxia was 21% higher than in controlled ones. The SDH, cyt.C and α-GPDH enzyme activities in these fibres was correspondingly 18, 56 and 45% higher, than in the control group ($p < 0.05$) (table 2). The enzyme activities in FTb fibres in the group trained in normoxia, was also 69 and 40% higher for cyt.C and α-GPDH respectively than in the control animals ($p < 0.05$). SDH activity remained unchanged in trained animals as compared to control. By contrast, only cyt.c activity in FTb fibres in the hyperoxia swimming rats was 61% higher in comparison with the control. No significant changes were found in either SDH or α-GPDH activities or MG content in these animals compared to the control (tab. 1). NADH-tr activity did not reveal any significant difference among SN, SO and control groups.

Discussion

It was previously demonstrated that, when different gas mixtures containing 21-100% oxygen were inhaled during submaximal aerobic exercise, the oxygen content in arterial and venous blood flowing from the working muscles increased [16]. This was accompanied by a higher oxygen uptake by muscles, and the time of work without fatigue increased [23].

Capillary supply and fibre CSA

After six weeks of physical exercise, an increase in the capillary per fibre ratio both in groups SO and SN compared to the control did not produce any significant differences. In rat muscles, the number of capillaries was earlier shown to increase after the animals had been trained by swimming for four weeks [1]. In literature there are two viewpoints on the origin of capillary growth during physical activity. Some authors suppose that the increase in the volume blood flow usually accompanying muscular work is particularly important for angiogenesis. The research team headed by O. Hudlicka believes that hyperemia affects the endothelium of the blood vessels through mechanical stimulation, which induces proliferation of the endothelial cells [4, 12, 30]. Others put forward the assumption that the capillary growth is stimulated by metabolic stimuli, such as local tissue hypoxia, which is characteristic of a working muscle [3, 13]. A similar increase in the number of capillaries found in rats trained at different oxygen concentrations might indicate that hypoxia is not the major stimulus for work-induced angiogenesis. However, the capillary density was significantly higher only in group SN, which trained under normoxic conditions, where the CSA of fibres were slightly smaller, while the capillary per fibre ratio was slightly higher than in the animals trained under hyperoxia condition. These non-significant changes being summarised result in a shorter diffusion path and improved transport for oxygen within muscle fibres in the group, which was trained in normoxia.

Rats swimming

In normoxia the MG concentration was raised in the oxydative-glycolytic muscle fibres (table 2). MG is known to be involved in facilitation of oxygen transport from a capillary to mitochondria, and its concentration is usually higher in muscles of trained animals [21]. Since the time of Krogh, it has been generally believed that the resistance to the mass O_2 flow is the highest within the muscle fibre, while the cross-sectional area is the major limiting factor in the diffusion rate of both O_2 and energy substrates to the mitochondria [17]. Therefore, an increase in capillary density and MG concentration in animals trained under condition of normoxia may be considered to be adaptive changes that facilitate oxygen diffusion within a fibre.

Table 2. Enzymatic activities in different types of muscle fibres of m. tibialis anterior (M ± m) (in units of optical density).

Parameter		Swimming	Swimming + O_2	Control
SDH	FTa	0.71 ± 0.02*	0.65 ± 0.03	0.60 ± 0.03
	FTb	0.44 ± 0.02	0,40 ± 0.03	0.44 ± 0.04
cyt.c	FTa	0.36 ± 0.04*	0.32 ± 0.04	0,23 ± 0.03
	FTb	0.22 ± 0.03*	0.21 ± 0.03*	0.13 ± 0.02
NADH-tr	FTa	0.27 ± 0.02	0.21 ± 0.03	0.22 ± 0.13
	FTb	0.15 ± 0.01	0.13 ± 0.02	0.13 ± 0.01
α-GPDH	FTa	0.58 ± 0.04*	0.52 ± 0.05	0.40 ± 0.03
	FTb	0.52 ± 0.03*	0.47 ± 0.05	0.37 ± 0.02
MG	FTa	0.17 ± 0.01*	0.13 ± 0.01	0.14 ± 0.01
	FTb	0.11 ± 0.01	0.09 ± 0.00	0.11 ± 0.01

*-Significant differences from the control ($P < 0.05$). SDH-succinate dehydrogenase, NADH-tr - NADH-tetrazolium reductase, α-GPDH -α-glycerophosphate dehydrogenase, cyt.c - cytochrome c oxidase, MG - myoglobin. Enzymatic activities are indicated in the units of optical density.

Enzyme activities

It has been hypothesized that the local work-induced hypoxia stimulates an increase in mitochondrial enzyme activity [8, 9, 29], and indeed, activities of SDH, cytochrome c oxidase, and α-GPDH were significantly increased in animals trained under normoxic conditions, but not in those trained under hyperoxia (table 2). α-GPDH is usually considered to be a marker of anaerobic glycolysis, whose stimulation results in a substantial increase in ATP production rate. Maintaining the level of ATP synthesis can compensate for the insufficient activity of the oxidative system in rats with exercise-induced hypoxia caused by training under normoxic conditions. In the same group, an increase in the activity of oxidative enzymes may result in a higher gradient of oxygen partial pressure between the capillaries and mitochondria and stimulate oxygen extraction [11, 14]. However, in group SO, trained under conditions of hyperoxia, there was no significant difference in enzyme activity from that in muscle fibres of the control rats. This suggests that decreasing the level of local exercise-induced hypoxia does not promote the growth of muscle-fibre oxidative potential. These data agree with the hypothesis that oxidative enzyme activities are stimulated by hypoxia [5, 9, 13, 29].

It's interesting that an increase in the activity of terminal mitochondrial enzymes of the cytochrome C oxidase complex was also observed in the FTb fibres of both trained rat groups. However, cytochrome c oxidase, which is an important enzyme in oxidative phosphorylation, is not a limiting factor for the mitochondrial respiratory chain properties of skeletal muscles [2, 22]. Therefore, changes in its activity do not necessarily indicate changes in muscle oxidative potential. For example, apart from true changes in the Vmax of cytochrome c oxidase [28], this enzyme exhibits not only quantitative, but also qualitative changes in response to training for endurance.

It is concluded that animals trained under conditions of hyperoxia exhibited neither increased capillary density and MG concentration, nor mitochondrial enzyme activities, as compared to the animals trained under normoxic conditions. This means that hypoxia arising in muscles in the course of training stimulates those changes that promote better substrate and oxygen supply to the muscles and better oxygen transport within muscle fibres. The capillary per fibre ratio in animals trained under normoxic and hyperoxic conditions increased to the same extent. Apparently, initiation of capillary growth is determined by factors other than hypoxia.

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