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Effects of Different Modes of Interval Hypoxic Training on Morphological Characteristics and Antioxidant Status of Heart and Lung Tissues

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The effects of various modes of interval hypoxic training differing by the intensity and duration of hypoxic exposure on the morphology and antioxidant status of the heart and lung tissues were studied. Interval hypoxic training mode with more severe, but shorter hypoxic component led to the prooxidant/antioxidant imbalance in the myocardial and lung tissues, which was paralleled by significant disorders in their morphology and function. Moderate hypoxic exposure of different duration promoted the maintenance of optimum antioxidant homeostasis and development of compensatory adaptive changes in tissue structure.

Key Words: *interval hypoxic training; myocardium; lungs; morphology and function; antioxidant status*

Interval normobaric hypoxic training (IHT) is used with good results in the training of athletes and in the treatment of many diseases [3]. Favorable and destructive effects of hypoxic exposure on tissues are known [2]. This fact necessitates differentiated approach to the choice of the intensity and duration of hypoxic periods during IHT. It is known that short periods of hypoxia/reoxygenation are associated with induction of antioxidant response, in which first-line antioxidant defense enzymes (SOD and catalase) and the glutathione system play an important role [4,6]. Changes in reduced (GSH) to oxidized glutathione (GSSG) ratio reflect the level of nonspecific resistance to any stress exposure, including hypoxia [11]. Biochemical reactions under these conditions are paralleled by changes in energy metabolism reflected by tissue morphology and functions, specifically, by the status of biological barriers, cell mitochondrial system, capillary endothelium, *etc.* [9].

Virtually no comparative morphofunctional analysis of IHT modes differing by the intensity and duration of hypoxic exposure, which can validate the efficiency of its use, can be found in available literature.

Here we studied the effects of IHT modes differing by the intensity and duration of hypoxic exposure, on morphological characteristics and antioxidant status of the heart and lung tissues.

MATERIALS AND METHODS

The study was carried out on adult male Wistar rats (200±20 g). The animals were divided into 4 groups, 10 per group: 1) control (normoxia); 2) animals breathing gas mixture with 7% O_2 in nitrogen during 5 min with 15-min normoxic intervals (protocol I); 3) animals breathing gas mixture with 12%

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O_2 in nitrogen during 5 min with 15-min normoxic intervals (protocol II); 4) animals breathing gas mixture with 12% O_2 in nitrogen during 15 min with 15-min normoxic intervals (protocol III). The total duration of exposure in all trained groups was 65 min, duration of IHT course 15 days.

The choice of gas mixture composition is explained by the fact that mixture containing 12% O_2 is a critical mixture; exposure to this mixture initiates significant changes in metabolic processes, and adaptive reactions in tissues can be very effective; exposure to 7% O_2 shows the range of adaptive potential of the body at the systemic and tissue levels.

The animals were decapitated 24 h after the end of IHT course. Lung and heart tissue preparations for electron microscopy were prepared routinely with double fixation in glutaraldehyde and OsO_4, dehydrated in ascending alcohols, and embedded in epon [1]. Ultrathin sections (40-60 nm) were fixed in uranyl acetate and lead citrate and examined under a JEM 100-CX electron microscope. Morphometric studies were carried out after Weibel.

For biochemical studies, the heart and lungs were rapidly removed, washed in saline, and frozen in liquid nitrogen. Activities of SOD and catalase were measured by spectrophotometry [10]. The content of TBA-active products (TBA-AP) was evaluated as described previously [10]. Tissue levels of GSH and GSSG were measured as described previously [7], protein concentration was measured by the method Lowry.

The results were processed using Student's *t* test, the differences were considered significant at $p<0.05$.

RESULTS

Hypoxic training using gas mixture with 7% O_2 caused intensification of LPO processes, shown by an increase in TBA-AP content by 22% ($p<0.05$) in the myocardium and by 33% ($p<0.05$) in the lungs in comparison with the control. This was paralleled by an increase in GSSG content and reduction of GSH content in the heart and lung tissue. The shift of the GSH/GSSG balance towards disulfide accumulation indicated retained activity of oxidative processes and decreased reduction potential of glutathione under conditions of this training mode. Accumulation of GSSG under conditions of oxidative stress can lead to imbalance of the antioxidant system, because GSSG, as a toxic compound, easily forms mixed disulfides with thiol-containing enzymes impairing their activity [11]. IHT according to protocol I led to reduction of SOD activity and increase in catalase activity in the heart and lung tissues (Table 1), which is important, because catalase plays the key role in cell protection from active O_2 metabolites in oxidative stress [6].

Changes in the antioxidant system in response to more severe hypoxic exposure were paralleled by significant changes in tissue ultrastructure. Changes in the lungs involved mainly the surfactant system and the cell mitochondrial system. They manifested in inhibited synthesis (devastation of lamellar bodies in type II pneumocytes) and secretion (no free surfactant on alveolar surface) of surfactant, mosaic vacuolation and swelling of mitochondria, dyscomplectation and destruction of cristae in 25-30% organelles (Fig. 1, *a*). Hypoxic edema of the lungs was mild, the thickness of the blood-

TABLE 1. Effects of Various IHT Modes on the Prooxidant/Antioxidant Balance in Heart and Lung Tissues ($M\pm m$; $n=10$)

Parameter		Normoxia	IHT		
			protocol I	protocol II	protocol III
TBA-AP, nmol/mg protein	heart	8.55±0.56	10.45±0.31*	9.08±0.69$^+$	9.68±0.32*
	lungs	15.08±1.07	20.06±1.02*	17.1±1.01$^+$	17.98±0.32*$^+$
SOD, arb. units/mg protein	heart	3.35±0.25	2.83±0.12*	4.22±0.35*$^+$	3.95±0.40*$^+$
	lungs	2.42±0.28	1.92±0.30*	2.16±0.34	2.36±0.26$^+$
Catalase, $\times10^2$ μmol H_2O_2/min/mg protein	heart	22.16±2.50	29.21±2.71*	21.68±3.16$^+$	23.42±3.20$^+$
	lungs	41.00±3.90	49.14±2.34*	47.98±1.60*	47.01±1.09*
GSH, μmol/mg tissue	heart	1.14±0.07	0.99±0.06*	1.17±0.07$^+$	1.15±0.08$^+$
	lungs	1.62±0.02	1.40±0.02*	1.88±0.05*$^+$	1.84±0.04*$^+$
GSSG, μmol/mg tissue	heart	0.056±0.002	0.067±0.002*	0.065±0.002*	0.066±0.002*
	lungs	0.067±0.003	0.088±0.003*	0.072±0.003$^+$	0.074±0.004$^+$

Note. Here and in Table 2: $p<0.05$ compared to: *control, $^+$IHT protocol I.

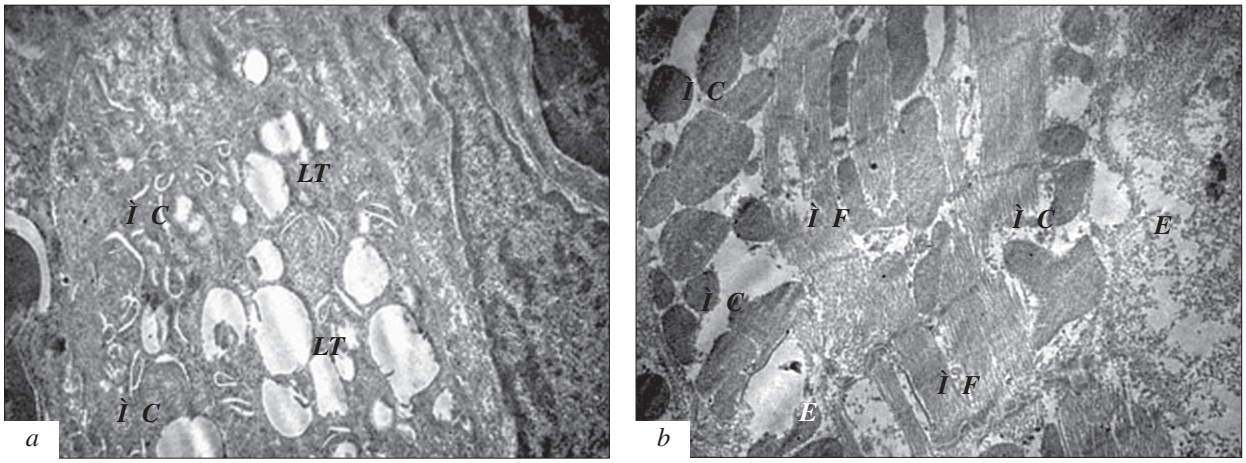

Fig. 1. Effects of IHT (protocol I) on lung (*a*) and heart (*b*) tissue ultrastructure. Here and in Fig. 2: MC: mitochondria; LB: lamellar bodies; E: edema; MF: myofibrils. ×9600.

air barrier exhibited a trend to an increase (Table 2), while intra-alveolar edema and destruction of individual layers of the blood-air barrier were observed in just solitary alveoles.

Electron microscopy of the myocardium showed vast areas impregnated with fluid and proteins, which indicated increased permeability of cytoplasmic membranes. This was associated with swelling, disorganization, and destruction of the myofibrils, pronounced edema of the blood-tissue barrier (Table 2) with marginal localization, cardiomyocyte destruction without appreciable disorders in the capillary endothelial ultrastructure.

Partial vacuolation was rarely seen in the mitochondria, but in the majority of cases the matrix was condensed, which can be regarded as an indirect evidence of inhibited glycolysis in this mode of hypoxic exposure (Fig. 1, *b*) [6]. These data are in line with the opinion that more intense and longer hypoxia stimulates free radical processes and causes morphofunctional changes in various tissues [5].

IHT by protocol II caused just a trend to an increase in the levels of LPO products in the heart and lung tissues, in contrast to protocol III, when these parameters surpassed the control level by 13 and 19%, respectively ($p<0.05$). Activity of SOD in the myocardium increased by 26% in response to IHT by protocol II and by 18% in response to IHT by protocol III ($p<0.05$), while catalase activity remained normal. Catalase activity in the lungs increased by 15-17% ($p<0.05$), while SOD activity remained at the level of control. Analysis of the glutathione pool of the studied tissues showed that hypoxic training according to protocols II and III led to an increase in GSH content in the lungs, while in the myocardium increased level of GSSG was retained in comparison with the control (Table 1). Better balance of the antioxidant system in the lung tissues can be explained by the ability of the alveolar cells to maintain the antioxidant balance under extreme conditions at the expense of the fluid covering the epithelium and containing numerous enzymatic and non-enzymatic antioxidants, including glutathione (100-fold higher content than in the plasma), primarily in reduced state (90%) [8,11].

TABLE 2. Mean Thickness of Blood-Air and Blood-Tissue Barriers in Different IHT modes (nm; *M*±*m*)

Experiment conditions		Blood-air barrier	Blood-tissue barrier
Control		163±8	221±14
IHT	protocol I	198±21	396±29*
	protocol II	186±19	285±21*+
	protocol III	194±28	272±17*+

Changes in the morphology and function of the studied tissues in response to IHT with a hypoxic component of moderate intensity were largely compensatory and adaptive. Mild hypoxic edema of the blood-tissue barrier (23-29% increase of the thickness; $p<0.05$; Table 2) in cardiac tissue was paralleled by an increase in the total number of mitochondria in comparison with the control (by 8-10/μ^{-2}, $p<0.05$) and 2-fold decreased number of structurally changed organelles in comparison with the level in IHT protocol I. Mosaic disorders of myocardial ultrastructure with cardiomyocyte edema, myofibrillar swelling and sometimes disorganization were seen (Fig. 2, *a*). These changes were characteristic of tissue reaction to hypoxic exposure [2].

Activation of the surfactant synthesis and secretion with restoration of the surfactant lining in the

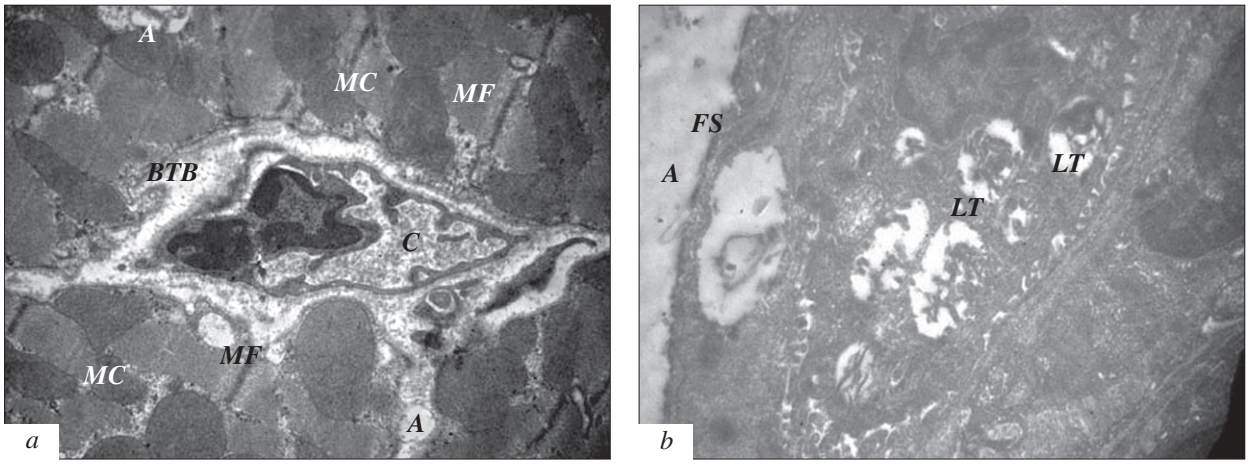

Fig. 2. Effects of IHT (protocol III) on heart (*a*) and lung (*b*) tissue ultrastructure. A: alveolar lumen; C: capillary lumen; BTB: blood-tissue barrier; FS: free surfactant. ×9600.

greater part of the alveoles was observed in lung tissue. The ultrastructure of the blood-air barrier and lung stroma corresponded to intact tissue in the majority of regions (Fig. 2, *b*). Partial vacuolation and swelling of the mitochondria and low (up to 10%) number of organelles with manifestations of crist dyscomplectation and destruction were observed.

The results indicate that the effects of IHT on the ultrastructure of the studied tissues are organ-specific.

Hence, hypoxic exposure in the studied IHT modes differing by duration and intensity leads to the development of different morphofunctional responses of tissues: from defense to destruction, depending on tissue sensitivity to active oxygen forms and the balance between the pro- and antioxidant systems ratio. Highly intensive repeating free radical signal in the IHT mode I acts as a destructive factor for antioxidant homeostasis and for tissue structure, while more or less compensated induction of active oxygen forms in IHT modes II and III stimulates the defense systems of the cell and development of the compensatory adaptive changes in tissue structure.

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