

ADAPTATION TO INTERVAL HYPOXIA- HYPEROXIA IMPROVES EXERCISE TOLERANCE IN PROFESSIONAL ATHLETES: EXPERIMENTAL SUBSTANTIATION AND APPLIED APPROBATION

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Abstract

A theoretical substantiation and experimental testing of combined adaptation to *changing oxygen levels* in the enhancement of tolerance to physical loads and pilot study on the protective effects of a novel strategy for adaptation to interval hypoxia-hyperoxia aimed at eliminating the overtraining syndrome in professional athletes, have been carried out.

Methods On the experimental step, adaptation of male Wistar rats was performed in 2 modes: 1) hypoxia-normoxia (H/N): 2) a new model - hypoxia-hyperoxia (H/H), 1h daily for 15 days. Acute physical load (APL) consisted in swimming (21°C) to exhaustion. Intensity of free radical oxidation was estimated by the rate of accumulation of lipid peroxidation products in the course of induction in the Fe²⁺+ ascorbate system in vitro. Activities of antioxidant enzymes were measured by spectrophotometria, levels of inducible HSP72, HSP32 and constitutive HSC73 were measured by Western blot analysis with monoclonal antibodies.

On the pilot study 15 male and female middle-distance runners with overtraining syndrome were exposed to interval hypoxia-hyperoxia training (IHHT) sessions. Working capacity (PWC170), hypoxic tolerance, haematological parameters and heart rate variability (HRV) analysis were determined before and 3 days after IHHT sessions.

Results In experiments combination of adaptation to physical exercise with adaptation to hypoxia-hyperoxia improves tolerance under conditions of

APL: short-term adaptation to physical exercise compensates for stress, but not for the hypoxic component of APL, while physical training combined with adaptation to hypoxia-hyperoxia fully compensates for both components.

In 15 young professional athletes with overtraining syndrome combination of IHHT with low-intensity exercise restores the autonomic balance and physical capacity (significant elevation of PWC170 index, maximal oxygen consumption VO_2max , and VO_2max/kg).

Conclusion Adaptation to interval hypoxia-hyperoxia provides optimization of the hypoxic and stress components in exercise tolerance systemic response which is revealed in experimental studies and supported by the data of young athletes with overtraining syndrome rehabilitation.

Keywords: Adaptation to hypoxia, hypoxic-hyperoxic training, overtraining syndrome

Introduction

Nonmedicamentous methods for increasing general resistance of the organism have gained considerable recent attention of practitioners in the field because of the advent of novel technologies for rehabilitation in clinical practice and sports medicine (Trukhanov 2004; Ignatenko 2008; Manzhugetova et al. 2008). These include various types of adaptation to stress, physical and hypoxic exposures, ambient temperature, etc. A search for attractive adaptation strategies for fast generation of adaptive responses and minimization of related adverse effects is currently underway. The most recent trend is towards development of effective low-intensity stress and hypoxic training patterns able to provide adequate adaptive responses at cellular, tissue and organismic levels (Meersom et al. 1992; Ignatenko 2008).

In the past 20 years, keen attention of investigators was focused on interval normobaric hypoxic training (INHT) as a new method of adaptation to periodic hypoxia through enhancing the resistance of the human organism to damaging factors (Strelkov and Chizhov 2001, Chizhov and Potievskaya 2002). This method combines adaptation to hypoxia itself with reoxygenation, i.e., repeated inhalation of air with normal oxygen content. Under periods of normoxia, oxygen content in inhaled air is sufficient for an individual who has just been exposed to hypoxia (Arkhipenko et al. 1997). Reoxygenation initiates synthesis of reactive oxygen species (ROS), which may either exert damaging effects on the organism or launch a cascade of redox-signaling processes, which, in its turn, initiates generation of adaptive responses increasing the resistance of the organism to damaging factors (Arkhipenko et al. 1997; Das 2001).

Apart from the well-known role of excess ROS in the pathogenesis of diseases, their generation and the free radical reactions initiated by them are natural physiological processes occurring continuously in all living organisms. The most important physiological functions of ROS include (i) oxidation and utilization of damaged molecules (Zolotarjova et al. 1994; Sazontova 2008); (ii) synthesis of messenger molecules - e.g., eicosanoids during free radical oxidation of phospholipid polyunsaturated fatty acids (Hemler et al. 1979; Roberts et al. 1981), and (iii) participation in redox signaling pathways and intracellular transfer of external signals to cell nuclei terminated by protein synthesis (Semenza 1999; Chandel and Schumacker 2000). In the absence of specific receptors, initiation of redox signaling pathways by ROS triggers cell responses to hypoxia, oxidizing and reducing agents, etc. Mediators whose effects are controlled by specific receptors (e.g., hormone receptors) also possess an ability to stimulate nonspecific redox signaling processes, which constitute the basis for cross-protective effects where adaptation to one damaging factor increases the resistance to other factors. In our study, adaptation to changing oxygen levels was used to increase the efficiency of adaptation to physical exercise.

Initiation of redox signaling pathways is accompanied by activation of transcription factors (e.g., NF- κ B (Sazontova et al. 1995; Sazontova and Arkhipenko 2004), AP-1 (Whelan and Hightower 1985), HIF-1 α , HIF-3 α (Semenza 1999; Zhukova and Sazontova 2005), which, in turn, stimulate the induction of a vast variety of protective molecules, e.g., antioxidant enzymes, HSP, Fe-regulating proteins, repair enzymes, peroxiredoxins, etc. (Sazontova et al. 1987; Graven et al. 1993; Maulik et al. 1999; Peng et al. 2000; Ryter and Tyrrell 2000), as a result of which body cells become saturated with protective substances. It is noteworthy that endogenously formed protective systems are far more efficient than exogenously formed ones (Hu et al. 1989). Continuously limited generation of ROS is a mechanism whereby the organism increases its resistance to stress (Sazontova et al. 2007), physical training (Powers et al. 1994), cold adaptation (Spasich et al. 2001), adaptogens (Sanz et al. 1994; Singh et al. 1994), diets enriched with oxidation substrates (e.g., n-3 PUFA) (Hu et al. 1989; Sazontova et al. 1995), interval hypobaric hypoxia (Arkhipenko et al. 1997), etc. Thus, periodic exposures to damaging effects of environmental factors stimulate periodically limited generation of ROS and enhanced synthesis of protective proteins (Sazontova and Arkhipenko 2004).

In the past three decades, INHT was widely employed for improving athletic achievements (Ignatenko 2008; Strelkov and Chizhov 2001; Bonetti and Hopkins 2009). A number of questions then arise: (i) what mechanisms, both systemic and molecular, are responsible for physical endurance increase of athletes and their enhanced tolerance to APL? (ii) what is the «value» of

such training? (iii) can the duration of the training period be reduced? (iv) how can the beneficial effect of adaptation be enhanced without related adverse side effects?

Our previous studies showed that more fast attainment of protective effects demands more drastic hypoxia and a significantly enhanced ROS signal, which can both initiate augmented synthesis of protective proteins, but do not lower the rate of ROS-mediated processes [30]. In our study, periods of normoxia were replaced by repeated episodes of moderate hyperoxia, which made it possible to enhance the ROS signal without any effect on the hypoxic component. The novel type of adaptation to hypoxia and moderate hyperoxia (H/H) (RF Patent No 2289432) Arkhipenko et al. 2006) competes favorably with hypoxia-normoxia (H/N) in the ability to produce faster effect on the resistance of membrane structures (Sazontova and Arkhipenko 2004). Its high efficiency in affording effective protection of cell membranes from ROS-induced injuries *in vitro* (Sazontova and Arkhipenko 2004; Boikova et al. 2006) and beneficial therapeutic effects of normobaric interval hypoxia with dosed oxygenation in the treatment of patients with lung diseases are documented (Strelkov and Chizhov 2001; Stepanov et al. 2005).

The design of this study was built up to meet the challenges of the aforesaid exploratory task. Stipulating that adaptation to combined effects of several environmental factors (hypoxia, cold, immobilization, physical exercise, etc.) has more pronounced effect than adaptation to each individual factor and that exhaustive physical exercise, similar to competitive one, possesses both hypoxic and stress components, we hypothesized that combined adaptation to physical exercise and interval hypoxia-hyperoxia (H/H) would be more efficient in respect of the ultimate goal of adaptation, viz., improvement of physical tolerance without depleting inner resources of the organism. Studies in this area are very scarce, while those concerning adaptation to hypoxia-hyperoxia and its beneficial effect on the organism are an entirely virgin field. This circumstance and our keen intention to find a clue to this problem gave an impetus to the present study.

The main goal was to study of feasibility of realization of protective effects of adaptation to hypoxia and hyperoxia in preventing stress- and hypoxia-related injuries and increasing tolerance to physical exercise.

Methods

Our experimental and applied investigations were carried out in two steps. In the first step, we compared the efficiency of different types of adaptation to changing oxygen levels in preventing disturbances induced by exhaustive APL and enhanced physical performance. The second step included a pilot study of various effects of combined adaptation to interval hypoxia-hyperoxia and regular physical training aimed at eliminating the

overtraining syndrome and enhancing physical capacity and exercise tolerance in professional athletes.

Step 1

Experimental design. The experiments were performed on 45 male Wistar rats (200–230 g) kept under standard vivarium conditions. The efficiency of exercise training used separately or in combination with adaptation to changing oxygen levels (H/N and H/H) in improving tolerance, normalizing free radical processes and HSP levels was investigated.

Acute physical load (APL) consisted in swimming (21°C) to exhaustion with additional weight (5% of body mass). The total duration of swimming sessions and the active swimming phase were recorded. Tissue samples were collected 2 h after APL.

Adaptation to changing oxygen levels was performed under normobaric conditions using an air mixture with high and low oxygen content. The measuring device was designed at the Laboratory of Adaptive Medicine (Head – Prof. Yu.V. Arkhipenko) in collaboration with the Scientific-Production Complex «Metax» and was similar to that used for membrane separation of gases. In experiments with adaptation to interval H/N, 5-min episodes of 10% O₂ were interspersed with 3-min periods of normoxia (64-min sessions daily, for 15 days).

During H/H, 5-min 10% O₂ sessions were alternated with 3-min episodes of moderate hyperoxia (30% O₂) (64 min daily, for 15 days). The duration of H/H was increased from 20 min on days 1–3 to 64 min on the subsequent days.

Combined adaptation to changing oxygen levels and physical exercise included eight swimming sessions (8Sw) in a low-intensity regime (24°C) (30 min daily, for 8 days). The duration of swimming sessions was gradually increased from 10 min. The measurements were performed in the control group and 2 h after adaptation of rats to H/N or H/H, beginning with day 8.

Intensity of free radical oxidation was estimated by the rate of accumulation of lipid peroxidation (LPO) products in the course of LPO induction in the Fe²⁺ + ascorbate system *in vitro*. Concentrations of malondialdehyde and its derivatives were determined from their absorption maxima in a TBA test.

Activities of antioxidant enzymes were measured on a Cintra 10e spectrophotometer in the linear region of the spectrum: by H₂O₂ absorption at 240 nm (catalase), by deviation of the rate of superoxide anion radical formation in the xanthine-xanthine oxidase system at 560 nm (superoxide dismutase) and by the rate of formazan synthesis from NBT.

Prior to assay, hemoglobin was removed by extraction with a chloroform-ethanol mixture.

Levels of HSP proteins (inducible proteins HSP72 and HSP32 and constitutive protein HSC73) were measured in the cytoplasmic fraction of the heart using a Bio-Rad 3System. Primary monoclonal antibodies (Stressgen, Canada) and secondary peroxidase-labeled antibodies (SantaCruz, USA) were transferred to a PVDF membrane using a SemiDry system. Proteins were detected with the help of ECL reagents (Amersham) and the radiographic film Kodak. Samples isolated from thermally treated H35 cells (30 min, 41.5°C) were used as positive controls.

Step 2

To evaluate the efficiency of the novel version of interval normobaric adaptation to hypoxia, viz., interval hypoxic-hyperoxic training (IHHT), as a valuable tool for potentiating physical tolerance, we carried out a pilot study on 15 young professional track-and-field athletes. The sample included 7 males and 8 females aged 18–20, with 7–9 years sports experience) rated as Candidates or Masters of Sports who volunteered to participate in this study. All tested individuals had proven manifestations of overtraining syndrome (low fitness level, decreased endurance, etc.). To improve functional status, all examinees were suggested to undergo IHHT (fourteen 45-min sessions, 3 times a week) in the form of single exposures combined with standard low-intensity sport training sessions. IHHT was performed 1.5–2-h after sport training. Gas mixtures (10–35% O₂) were generated using a prototype version of the REOXY unit («AI Mediq», Luxembourg).

Prior to training, athlete's individual sensitivity to hypoxia was checked in a 10-min hypoxic test (HT), which included inhalation of a gas mixture (10% O₂) through a facial mask upon continuous (once-a-minute) monitoring of heart rate (HR) and arterial oxygen saturation (SaO₂). IHHT was implemented in an interval regime and included 5–7 min inhalations of a hypoxic gas mixture (HGM) (11% O₂) through a mask followed by 2–3 min inhalation of a hyperoxic (30% O₂) mixture. After each inhalation session, gas mixtures were automatically renewed according to specially constructed biofeedback algorithms (Arkhipenko et al. 2006). Each inhalation session included 6–8 alternating cycles.

Prior to IHHT and on days 3–4 thereafter, all tested individuals underwent comprehensive examination. The latter was performed before noon and included blood determinations (red and white blood cell count – RBC and WBC, reticulocyte count, haemoglobin concentration, haematocrite) and evaluation of the autonomic status by assessing heart rate variability (HRV) parameters with an ANS-Spektr device (LLC «Neurosoft», Ivanovo, 2002) and determining temporal and frequency

characteristics of HRV according to known standards (Mikhailov2000). Measurements of temporal characteristics of HRV included estimation of HR (bpm), standard deviation of RR intervals (SDNN, ms) and coefficient of variation (CV, %). The stress index (SI) was expressed in relative units (Mikhailov 2000). Spectral analysis of HRV included determination of total spectrum power (TP) of HRV and individual components (high frequency (HF), low frequency (LF) and very low frequency (VLF) expressed on a per cent basis. The sympatho-parasympathetic index (LF/HF) was calculated as described previously (Mikhailov 2000).

Evaluation of physical capacity was performed in a PWC170 test in the late morning (not earlier than 2h after breakfast). The following parameters were calculated: absolute and relative capacities (per kg of bodyweight, BW) by the Karpman method (Mikhailov 2005). (PWC170 and PWC170/BW), maximal oxygen consumption (VO_{2max} and Vo_{2max}/BW), and parameters of performance efficiency (inotropic (IRI) and chronotropic reserve (CRI) indexes and rate pressure product (RPP)) (Mikhailov 2005; Boreham et al. 1990)].

The IHHT data were compared to the results of dynamic testing of 19 athletes of the same sports qualification but without overtraining syndrome. Their training was performed in a standard regime and at the same performance level but without IHHT sessions. The data obtained were presented as $M \pm m$.

For some organizational, methodological and ethic reasons, we decided not to apply double blind design (athletes with overtraining syndrome undergoing simulated adaptation to hypoxia-hyperoxia) to exclude placebo effect, since the major objective of this pilot study was to check the efficiency and safety of the IHHT method. Comparative analysis of IHHT method with the results of overtrained athlete's adaptation to hypoxia-normoxia was not performed either. Limitations in the current research will be overcome in future studies.

Results

Step 1

Animal weight. Comparison of animal weights was performed before and after the experiment and did not establish any significant reduction in comparison with the control group, which prompted a conclusion that the training regime had only a moderate effect on the organism. However, there was significant difference between the H/N and H/H data. After APL, the weight of animals in the group where adaptation to physical exercise was combined with adaptation to H/H was significantly increased (19.4 g) and exceeded that in the H/N+8Sw+APL group. We hypothesized that combined

adaptation to H/H and physical exercise had no significant effect on animal weight, but enhanced protein synthesis.

Duration of swimming sessions. After adaptation to physical exercise combined with adaptation to changing oxygen levels or without it, the total duration of test swimming to exhaustion increased appreciably. In the (8Sw+APL) group, the total duration of swimming (APL) increased by 54%. Combined adaptation, i.e., physical exercise + adaptation to H/N (H/N+8Sw+APL) and H/H (H/H+8Sw+APL), increased this parameter (2.3- and 2-fold, respectively) in comparison with APL (Fig. 1). More impressive results were obtained when the duration of the active swimming phase was studied.

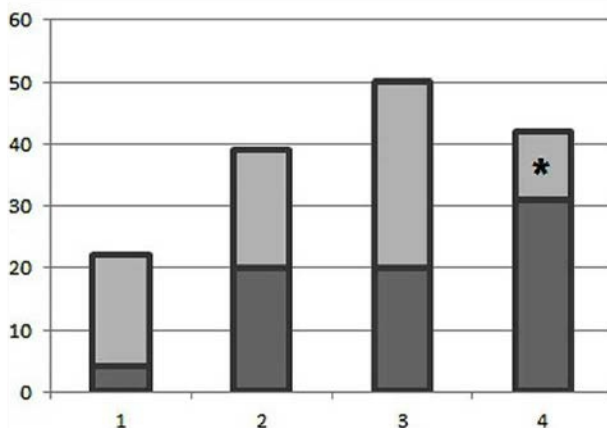


Figure 1. Effects of adaptation to hypoxia-normoxia (H/N) and hypoxia-hyperoxia (H/H) combined with training to physical exercise on total duration of swimming and active swimming phase (more intense colour). 1 - APL – acute physical load (forced swimming of control rats to exhaustion); 2 - 8Sw+APL – APL after adaptation to physical exercise (8 swimming sessions); 3 - H/N+8Sw+APL – APL after physical exercise + with adaptation to H/N; 4 - H/H+8Sw+APL – APL after physical exercise + adaptation to H/H. * - significance of differences - active phase ($P \leq 0.05$) from 8Sw+APL (Mann-Whitney U Test)

Physical exercise (8Sw+APL) significantly increased the duration of the active swimming phase in comparison with control rats (APL) (up to 52% of the total time of staying in water). On the whole, combined adaptation to H/N and physical exercise (H/N+8Sw+APL) prolonged the swimming period, but had no effect on the duration of the active swimming phase. Compared to 8Sw+APL, in the course of H/N the duration of the active swimming phase diminished from 52% to 40% of the total time of staying in water. The duration of the active phase increased by 1.5-fold only after addition of H/H (H/H+8Sw+APL) to the training protocol (8Sw+APL).

These findings suggest that APL can be prolonged through combination of physical exercise with adaptation to H/N and H/H. However,

only a combination of physical exercise with adaptation to H/H increases the duration of the active phase and enhances physical endurance.

Rates of free radical oxidation and activities of antioxidant enzymes were compared during APL and after adaptation to swimming (used alone or in combination with adaptation to changing oxygen levels). After APL, the intensity of inducible free radical processes in ROS-sensitive liver tissues increased more than twofold suggesting a drastic fall of the resistance of tissues to ROS-induced injuries. Adaptation to physical exercise used alone (8Sw+APL) or in combination with adaptation to changing oxygen levels (H/N+8Sw+APL and H/H+8Sw+APL) decreased the intensity of free radical reactions (Fig. 2). After direct adaptation to physical exercise (8Sw+APL), the rate of accumulation of LPO products did not differ from control, being significantly lower than in the APL group ($p < 0.05$). During adaptation to swimming combined with adaptation to changing oxygen levels, the decrease in the rate of free radical oxidation was even more apparent. It is significant that compensation of ROS-initiated processes was coupled with increased duration of the stress exposure (so-called exhaustive APL).

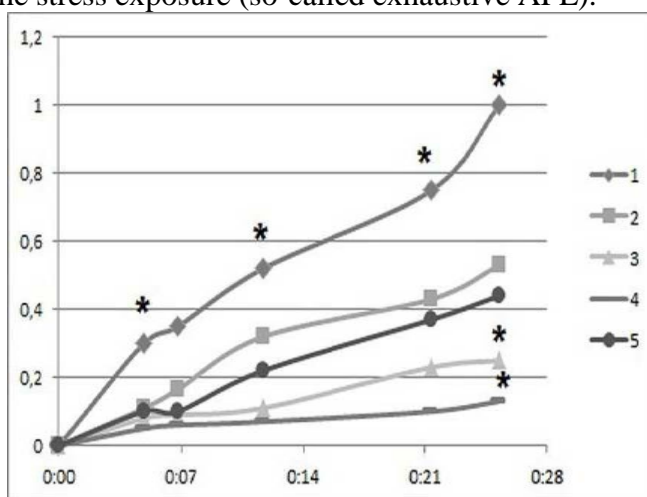


Figure 2. Effects of combined adaptation to H/N and H/H and physical exercise on the level of TBA- active products during induction of free radical oxidation in liver tissues in vitro. C – control. Designations as in Legends to Fig. 1; 5 – control. * - significance of differences ($P \leq 0.05$) from control (Mann-Whitney U Test)

Compensation of free radical processes as a result of 8-day exercise training can also be due to significant (in comparison with APL and control) enhancement of enzyme activity. The activity of SOD and catalase exceeded the control level by 24%. Similarly, the physiological ratio of pro- and antioxidant enzymes changed during APL and adaptation to physical exercise (8Sw+APL).

Combination of physical exercise with adaptation to H/N and, particularly, to H/H not only diminished the oxidation rate, but prevented activation of protective systems. After physical exercise combined with adaptation to H/N (H/N+8Sw+APE), this was manifested in lowered SOD levels, while after combined adaptation to H/H (H/H+8Sw+APL) neither SOD, nor catalase activity were significantly increased (Fig. 3).

In all probability, enhanced synthesis of antioxidant enzymes is a «value» of adaptation to physical exercise and improved tolerance. Combination of this adaptive procedure with adaptation to H/H provides a more efficient mechanism whereby the organism protects itself from ROS-induced injuries. It is significant that restoration of the balance between pro-oxidant and antioxidant enzymes significantly enhances the effect of physical training.

In other words, *combination of two different types of adaptation, viz., adaptation to changing oxygen levels and adaptation to physical exercise, prevents both the activity of protective systems during APL and their excessive activation during adaptation to physical exercise by reducing the «value» and increasing the efficiency of physical training.*

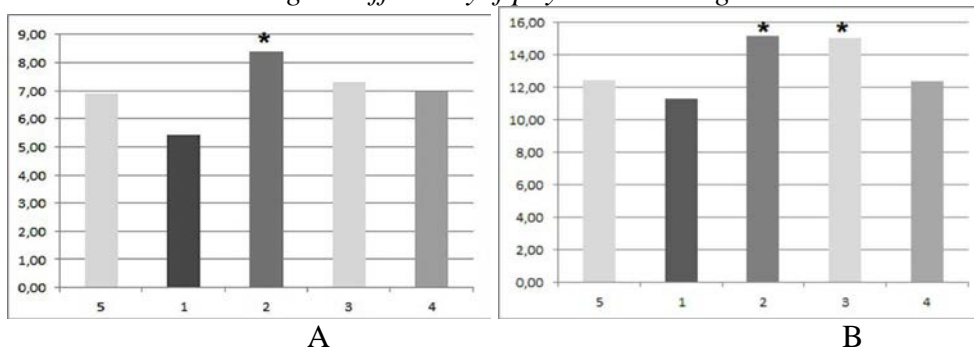


Figure 3. Effects of combined adaptation to H/N and H/H and physical exercise on: liver A – superoxide dismutase (SOD); B –catalase. Designations as in Legend to Fig. 1; 5 – control.*- significance of differences from control (Wilcoxon Matched Pairs Test, $p < 0.05$).

HSP (HSP72, HSP32, HSC73) levels. Similar regularities were established for other protective proteins including three representatives of the HSP family, viz., the inducible proteins HSP72 and HSP32 and the constitutive protein HSC73.

Activation of HSP synthesis usually takes place in response to heat shock, oxidative stress (Sazontova TG, 2008), hypoxia (Sazontova TG, Arkhipenko YuV, 2009), etc. The inducible protein *HSP72* is actively and rapidly expressed under the influence of stress factors causing protein denaturation. *HSP32* represents an inducible isoform of heme oxygenase. Any change in its level should be interpreted in the paradigm of other ROS-induced proteins. *HSP32* induction, in particular, indicates simultaneous

presence of a ROS signal and a hypoxic component, while HSP72 elevation is more characteristic of ROS-induced stress than of isolated hypoxia (Andreeva et al. 2001). Correspondingly, simultaneous induction of HSP72 and HSP32 points to the presence of a ROS signal, while the lack of changes in HSP72 levels against the background of HSP32 activation is suggestive of hypoxia. In the absence of stress, the constitutive protein *HSC73* is continuously expressed under physiological conditions and plays the role of a molecular chaperone for newly synthesized proteins by participating in their transport or proteasome-induced degradation of irreversibly damaged proteins. Changes in HSC73 content are characteristic of hypoxia (Andreeva et al. 2001; Boikova et al. 2006).

A comprehensive approach to the analysis of changes in HSP levels recently developed in our laboratory is designed to evaluate the contribution of stress and hypoxic components of different factors. APL is associated with HSP72 induction, so its content increases in comparison with the control.

Adaptation to physical exercise (8Sw+APL) or its combination with adaptation to changing oxygen levels (H/N+8Sw+APL and H/H+8Sw+APL) compensates for stress as a result of which HSP72 content drops down to the control level (Fig. 4A). This suggests that APL plays the role of a stress factor, while adaptation to physical exercise combined with adaptation to H/N and H/H or without it prevents further activation of ROS-induced processes, which is consistent with the results of previous studies.

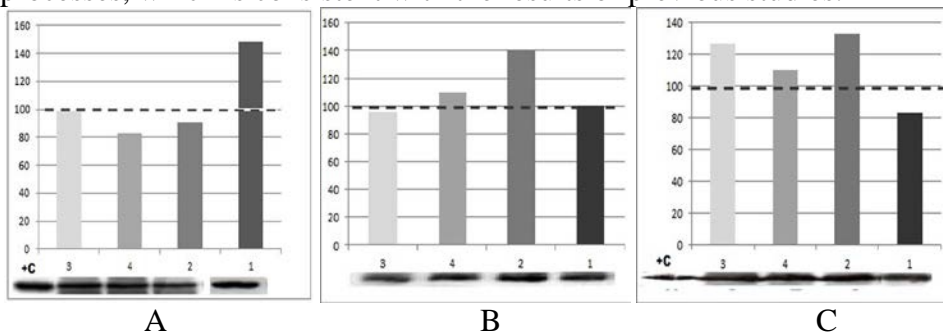


Figure 4. Effects of adaptation to H/N and H/H combined with adaptation to physical exercise on the level of the inducible stress protein HSP72 (A), the inducible protein HSP32 (B) and constitutive protein HSC73 (C) in the heart. +C – positive control (samples of H35 cells) after thermal treatment (30 min, 41.5°C). C – control group (100%), dotted line. Designations as in Legend to Fig. 1.

The situation is opposite for other representatives of the HSP family. After physical exercise

(8Sw+APL), the HSC73 level increased by 34% against control (cf. 43% for APL), and the HSP32 level increased against control and APL, suggesting predominance of the hypoxic component (Fig. 4, B and C).

Physical training combined with adaptation to changing oxygen levels attenuated this effect. When physical training was combined with adaptation to H/N (H/N+8Sw+APL), HSP32 level decreased, while in case of H/H (H/H+8Sw+APL) the adaptation effect was more pronounced and soon the activities of both proteins returned to normal values.

Short-term adaptation to physical exercise compensates for stress, but not for the hypoxic component of APL. Physical exercise combined with adaptation to H/N partly compensates for the hypoxic component, while adaptation to H/H provides complete normalization of both stress and hypoxic components of APL.

The totality of experimental data obtained in Step 1 testify to beneficial effects of adaptation to H/H on physical tolerance, normal balance of pro- and antioxidant enzymes and HSP and, in whole, demonstrate high efficiency of combined adaptation in training for high-intensity and competitive physical performance.

Step 2

After initial checkup, all test subjects in the IHHT group manifested low general physical tolerance and sport tests levels (low general health condition, fatigue (80%), anxiety (66.6%), signs of central sympathicotonic autonomic dysregulation, low TP levels, moderately elevated LF and VLF, decreased HF level, etc.) (Table 1).

Physical capacity was lowered in all tested individuals, while parameters reflecting the physiological „value“ of physical performance were significantly elevated in comparison with 19 athletes without overtraining syndrome whose training was performed in a standard mode and at identical levels of physical exercise.

After termination of IHHT combined with training in a low-intensity regime, the functional statuses and endurance of the tested subjects were significantly improved. The absolute and relative values of PWC170 and VO₂max were increased and maximally approximated those in the reference group, while the increment in other parameters in the sub-maximal test was decreased (for HR, BP, rate pressure product (Δ RPP), CRI, IRI) (Table1) suggesting activation of the chronotropic reserves of the myocardium and more economic systemic oxygen delivery during physical performance of the same degree of the sub-maximal test intensity.

Table 1. Dynamics of physical working capacity and autonomic balance parameters of young athletes after IHHT (n = 15, M \pm m)

NN	Index	Before IHHT	After IHHT
1.	VO ₂ max/BW, ml/kg	46,4 \pm 1,3 (53,4 \pm 1,8*)	50,3 \pm 1,4 (p=0,001) (54,7 \pm 1,6)
2.	PWC170, watt	170,8 \pm 11,8	191,9 \pm 71 (p=0,01)

		(204,2±13,8*)	(1278±93)
3.	PWC170/BW, watt/kg	2,63±0,12 (3,24±0,1*)	3,01±0,12(p=0,005) 3,31±0,11
4.	IRI, %	65,8±3,6 (50,8±4,1*)	54,8±5,4 (p=0,01) (49,6±3,8)
5.	CRI, %	50,0±5,3 (37,5±4,9)	38,0±5,9 (p=0,01) (36,8±5,0)
6.	RPP, cond.units	248±8,5 (208±8*)	213±11 (p=0,08) (199±11)
7.	ΔRPP, cond.units	167±8 (128±9)	132±12 (p=0,007) (127±9)
8.	TP, ms ²	3118±456 (4503±512)	3890±337 (p=0,1) (4654±521)
9.	VLF, ms ²	1410±204 (1610±315)	1298±136 (1740±404)
10.	LF,ms ²	1300±566 (860±340)	801±209 (p=0,005) (828±420)
11.	HF, ms ²	277±170 (1100±344)*	624±168 (p=0,005) (1167±501)
12.	LF/HF	8,01±5,51 (2,2±1,0)*	1,45±0,71 (p=0,007) (1,81±0,95)
13.	HR at rest, bpm	68,2±5,3 (62,4±3,8)	67,12±3,7 (60,4±4,6)

Designations: The figures in parenthesis designate reference group data, n = 19. * - significance of differences, $P \leq 0.05$ with respect to the reference group (Mann-Whitney U Test). The values of p in parenthesis designate significance of differences with respect to baseline values for the IHHT group.

After termination of IHHT sessions, all tested individuals manifested significantly improved tolerance to acute simulated hypoxia in a repeated hypoxic test. Arterial oxygen saturation diminished appreciably, while HR improved markedly after repeated HT (Table 2). The lack of significant hematologic shifts testifies to high physiological efficiency of exercise training, its low „value“ for the organism and lack of necessity to stimulate erythropoiesis in trained individuals (Hamlin and Hellemans 2007).

Table 2. Dynamics of hypoxic resistance and haematological shifts after IHHT ($M \pm m$)

NN	Index	Before IHHT	After IHHT
1.	SaO ₂ min, %	77,9±1,8 (83,7±2,1) *	84,2±1,5 (p=0,001) (85,7±3,0)
2.	HRmax, bpm	82,2±3,9 (79,7±3,1) *	76,6±3,0 (p=0,01) (77,7±2,3)
3.	ΔSaO ₂ , %	-19,3±2,1 (-15,7±3,1)	-12,2±1,5 (p=0,002) (-13,7±2,1)
4.	ΔHR, bpm	14,6±2,7 (9,7±1,8) *	9,1±2,2 (p=0,016) (10,0±2,4)

5.	Haemoglobin, g/L	138,3±2,6	140,7±2,7
6.	Haematocrite, %	40,5±0,7	41,6±0,7
7.	RBC, 10 ¹² /л	4,82±0,09	4,84±0,09
8.	Reticulocyte count, ‰	9,05±1,15	9,79±1,09

Designations: SaO₂min and HRmax – minimum saturation of blood by oxygen and maximum HR during HT, respectively; ΔSaO₂ and ΔHR – mean values of arterial oxygen desaturation and increment of HR in HT. The figures in parenthesis designate reference group data, n = 19, haematological parameters were not measured. Significance of differences as in Legends to Table 1.

These findings are consistent with the results of other investigators suggesting high efficiency of short-Legends to term (2–3 weeks) simulated interval hypoxia in improving aerobic and running performance (Wilber 2007; Manzhugedova et al., 2008; Bobyleva and Glazachev 2008, Burtcher at al. 2007, 2010). As regards the psychological and autonomic dynamics, subjective estimates of chronic fatigue diminished, while the general power of HRV showed a tendency to improve. LF decreased, while HF and LF/HF were at normal level. These findings point to significant activation of parasympathetic regulatory mechanisms and restoration of the regulatory sympatho-parasympathetic balance (Table 1). By and large, our data suggest that hypoxia-hyperoxia provides optimization of both hypoxic (high tolerance to hypoxia) and stress components (improved myocardial function during acute experimental exercise training) of systemic reactions during adaptation and rehabilitation of athletes with overtraining syndrome.

Discussion

Our study demonstrates remarkable potentials and obvious merits of combined intervalhypoxic-hyperoxic adaptation in improving physical working capacity, cardiovascular performance, autonomic and prooxidant-antioxidant balance.

In previous papers it was established that long-term adaptation to intermittent normobaric hypoxia increases the resistance of heart, liver and brain membranes to ROS. This phenomenon most likely underlies the cross effect of adaptation to IH under the conditions of physical load, based on the results of its application in sports medicine (Sazontova et al. 1987; Sazontova and Arkhipenko 2004). However, the procedure of adaptation to intermittent hypoxia/normoxia takes a lot of time for its development, whereas its short duration may be achieved with more severe hypoxia and enhancement of the ROS signal (Sazontova et al. 2007). In order to enhance the ROS signal without inducing hypoxic side effects, we hypothesized that normoxia as a constituent element of adaptation to hypoxia/normoxia should be replaced by moderate hyperoxia. Compared to hypoxia/normoxia, the novel type of

adaptation to hypoxia and moderate hyperoxia (Russian Federation Patent No.2289432) affords a faster increase in the resistance of membrane structures (Arkhipenko et al. 2006). The efficiency of the novel adaptation procedure in protecting membrane structures against *in vitro* ROS-induced injuries was previously demonstrated (Sazontova and Arkhipenko 2009); however, little is known about its effects at the whole body level. The first examples of the defensive potential of the novel adaptation procedure to variable oxygen level (hypoxia and hyperoxia) under conditions of sharply decreased (hypokinesia) or increased (acute exhaustive physical training) physical activity are described in our recent publication (Powers et al. 1994).

The mechanism of the damaging effect of exhaustive physical load on body cells is based on excessive activation of ROS-associated processes. The exhaustive physical load has both hypoxic and stress components.

Interestingly, simultaneous adaptation to several external factors (hypoxia+cold, hypoxia+immobilization, cold+physical training, etc.) often gives more impressive results than adaptation to each individual factor. It was also suggested that training to physical loads combined with adaptation to hypoxia and/or hyperoxia is much more effective with regard to the ultimate effect of physical training.

As it is shown in the experimental part of the study, short-term physical training increases the duration of swimming in acute physical load test. Its combination with adaptation to variable oxygen levels has no effect on this parameter, while adaptation to physical load combined with adaptation to hypoxia-hyperoxia increases the duration of the active swimming phase and, as a consequence, the efficiency of adaptation.

Adaptation to physical load and its combination with adaptation to IHHT increases the resistance of membrane structures to free radical oxidation at the expense of excessive activation of antioxidant defense enzymes in the course of physical training, which is partly compensated by adaptation to hypoxia/normoxia and is fully prevented by adaptation to hypoxia/hyperoxia. The combination of two forms of adaptation (*i.e.* direct adaptation to physical load and cross adaptation to variable oxygen levels) compensates for the markedly elevated content of HSP proteins in the course of physical training, which is especially well-pronounced during adaptation to hypoxia/hyperoxia. This novel technique is apparently less “physiologically demanding” and more beneficial for the organism.

The benefits and high efficiency of interval hypoxia-hyperoxia training is confirmed in pilot study to demonstrate improving the functional status, autonomic balance, physical endurance and aerobic performance in athletes with overtraining syndrome. Repeated and well individually dosed hypoxic-hyperoxic exposures combined with physical exercises seem to be capable to evoke beneficial adaptations in terms of neurohumoral,

antioxidant, respiratory and cardiovascular mechanisms, enhancing physical working capacity and exercise tolerance in overtrained athletes, which usually demonstrate weakened exercise tolerance and autonomic dysregulations partially induced by altered oxidative stress (Vollaard et al 2006, Tanskane 2010).

Undoubtly further studies are needed to prove the IHHT efficiency in comparison with traditional modes of passive interval hypoxia training. Yet, more research work has to be done to explain basic molecular mechanisms of IHHT and, in applied aspects, - to optimize the optimal individual dosing of Hypoxia and Hyperoxia training sessions depending individual subject's peculiarities.

Conclusion

Our study demonstrates remarkable potentialities and obvious merits of combined interval hypoxic-hyperoxic adaptation in improving physical working capacity, cardiovascular performance, autonomic and prooxidant-antioxidant balance. Combination of two different types of adaptation (to physical exercise and hypoxia-hyperoxia) potentiates effects of physical training, enhances exercise tolerance, normalizes the intensity of ROS-mediated reactions and activities of antioxidant enzymes and is physiologically more efficient than any other individually performed adaptation.

The benefits and high efficiency of interval hypoxia-hyperoxia training in improving the functional status, physical and aerobic performance in athletes are also quite promising. The totality of experimental data unequivocally demonstrate that interval normobaric hypoxia-hyperoxia holds considerable promise as a highly efficient and experimentally verified procedure and an attractive strategy for sports and rehabilitation medicine and might become a method of choice in large-scale training/rehabilitation programs for athletes.

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