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^oC) 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 Fe^{2+}+ ascorbate system in vitro. Activities of antioxidant enzymes were measured by spectrophotometria, levels of inducible HSP72, HSP32 and constituitive HSC73 were measured by Western blot analysis with monoclonal antibodies.

On the pilot study 15 male and female middle-distance runners with overtraining syndrome wereexposed 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 componentsinexercise 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 considerablerecent attention of practitioners in the field because of the advent of novel technologies for rehabilitation in clinical practice andsports medicine (Trukhanov 2004; Ignatenko 2008; Manzhugetova et al. 2008). These include various types ofadaptation to stress, physical and hypoxic exposures, ambient temperature, etc. A search for attractive adaptationstrategies 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 trainingpatterns 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 hypoxictraining (INHT) as a new method of adaptation to periodic hypoxia through enhancing the resistance of thehuman organism to damaging factors (Strelkov and Chizhov 2001, Chizhov and Potievskaya 2002). This methodcombines adaptation to hypoxia itself with reoxygenation, i.e., repeated inhalation of air with normal oxygencontent. Under periods of normoxia, oxygen content in inhaled air is sufficient for an individual who has justbeen 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-signalingprocesses, which, in its turn, initiates generation of adaptive responses increasing the resistance of the organismto 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 thefree radical reactions initiated by them are natural physiological processes occurring continuously in all livingorganisms. The most important physiological functions of ROS include (i) oxidation and utilization of damagedmolecules (Zolotarjova et al. 1994; Sazontova 2008); (ii) synthesis of messenger molecules - e.g., eicosanoidsduring 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 cellnuclei terminated by protein synthesis (Semenza 1999; Chandel and Schumacker 2000). In the absence ofspecific receptors, initiation of redox signaling pathways by ROS triggers cell responses to hypoxia, oxidizingand 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-protectiveeffects 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-kB(Sazontova et al. 1995; Sazontova and Arkhipenko 2004), AP-1 (Whelan and Hightower 1985), HIF-1a, HIF-3a(Semenza 1999; Zhukova and Sazontova 2005), which, in turn, stimulate the induction of a vast variety ofprotective molecules, e.g., antioxidant enzymes, HSP, Fe-regulating proteins, repair enzymes, peroxiredoxins,etc. (Sazontova et al. 1987; Graven at al. 1993; Maulik et al. 1999; Peng et al. 2000; Ryter and Tyrrell 2000), asa result of which body cells become saturated with protective substances. It is noteworthy that endogenouslyformed protective systems are far more efficient than exogenously formed ones (Hu et al. 1989). Continuouslylimited generation of ROS is a mechanism whereby the organism increases its resistance to stress (Sazontova etal. 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 etal. 1995), interval hypobaric hypoxia (Arkhipenko et al. 1997), etc. Thus, periodic exposures to damaging effectsof environmental factors stimulate periodically limited generation of ROS and enhanced synthesis of protectiveproteins (Sazontova and Arkhipenko 2004).

In the past three decades, INHT was widely employed for improving athletic achievements (Ignatenko2008; Strelkov and Chizhov 2001; Bonetti and Hopkins 2009). A number of questions then arise: (i) whatmechanisms, both systemic and molecular, are responsible for physical endurance increase of athletes and theirenhanced tolerance to APL? (ii) what is the «value» of

such training? (iii) can the duration of the training periodbe 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 drastichypoxia and a significantly enhanced ROS signal, which can both initiate augmented synthesis of protectiveproteins, but do not lower the rate of ROS-mediated processes [30]. In our study, periods of normoxia werereplaced by repeated episodes of moderate hyperoxia, which made it possible to enhance the ROS signal withoutany 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 theability to produce faster effect on the resistance of membrane structures (Sazontova and Arkhipenko 2004). Itshigh 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 intervalhypoxia with dosed oxygenation in the treatment of patients with lung diseases are documented (Strelkov andChizhov 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 effect**s** of several environmental factors (hypoxia, cold, immobilization,physical exercise, etc.) has more pronounced effect than adaptation to each individual factor and that exhaustivephysical exercise, similar to competitive one, possesses both hypoxic and stress components, we hypothesizedthat combined adaptation to physical exercise and interval hypoxia-hyperoxia (H/H) would be more efficient inrespect of the ultimate goal of adaptation, viz., improvement of physical tolerance without depleting innerresources of the organism. Studies in this area are very scarce, while those concerning adaptation to hypoxiahyperoxiaand its beneficial effect on the organism are an entirely virgin field. This circumstance and our keenintention 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 hypoxiaand 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, wecompared the efficiency of different types of adaptation to changing oxygen levels in preventing disturbancesinduced by exhaustive APL and enhanced physical performance. The second step included a pilot study ofvarious effects of combined adaptation to interval hypoxia–hyperoxia and regular physical training aimed ateliminating the

overtraining syndrome and enchancing physical capacity and exercise tolerance in professionalathletes.

Step 1

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

Acute physical load (APL) consisted in swimming (21^oC) to exhaustion with additional weight (5% ofbody mass). The total duration of swimming sessions and the active swimming phase were recorded. Tissuesamples were collected 2 h after APL.

Adaptation to changing oxygen levels was performed under normobaric conditions using an air mixturewith 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 wassimilar to that used for membrane separation of gases. In experiments with adaptation to interval H/N, 5-minepisodes of 10% O2 were interspersed with 3-min periods of normoxia (64-min sessions daily, for 15 days).

During H/H, 5-min 10% O2sessions were alternated with 3-min episodes of moderate hyperoxia (30% O2) (64min daily, for 15 days). The duration of H/H was increased from 20 min on days 1–3 to 64 min on thesubsequent days.

Combined adaptation to changing oxygen levels and physical exercise included eight swimmingsessions (8Sw) in a low-intensity regime (24^oC) (30 min daily, for 8 days). The duration of swimming sessionswas gradually increased from 10 min. The measurements were performed in the control group and 2 h afteradaptation 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 Fe^{2+} + ascorbate system *in vitro*. Concentrations of malonicdialdehyde 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 regionof the spectrum: by H2O2 absorption at 240 nm (catalase), by deviation of the rate of superoxide anion radicalformation in the xanthine-xanthine oxidase system at 560 nm (superoxide dismutase) and by the rate of formazansynthesis 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 constituitive protein HSC73) weremeasured 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 PVDFmembrane using a SemiDry system. Proteins were detected with the help of ECL reagents (Amersham) and theradiographic film Kodak. Samples isolated from thermally treated H35 cells (30 min, 41.5^{o}C) were used aspositive 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 outa pilot study on 15 young professional track-and-field athletes. The sample included 7 males and 8 females aged18–20, with 7–9 years sports experience) rated as Candidates or Masters of Sports who volunteered to participatein 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-intensitysport training sessions. IHHT was performed 1.5–2-h after sport training. Gas mixtures (10–35% O2) weregenerated 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 intervalregime and included 5–7 min inhalations of a hypoxic gas mixture (HGM) (11% O2) through a mask followed by2–3 min inhalation of a hyperoxic (30% O2) mixture. After each inhalation session, gas mixtures wereautomatically renewed according to specially constructed biofeedback algorithms (Arkhipenko et al. 2006). Eachinhalation 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 – RBCand WBC, reticulocyte count, haemoglobin concentration, haematocrite) and evaluation of the autonomic statusby 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 deviationof RR intervals (SDNN, ms) and coefficient of variation (CV, %). The stress index (SI) was expressed in relativeunits (Mikhailov 2000). Spectral analysis of HRV included determination of total spectrum power (TP) of HRVand individual components (high frequency (HF), low frequency (LF) and very low frequency (VLF) expressedon 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 2hafter 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 oxygenconsumption (VO2max and Vo2max/BW), and parameters of performance efficiency (inotropic (IRI) andchronotropic 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 sportsqualification but without overtraining syndrome. Their training was performed in a standard regime and at thesame performance level but without IHHT sessions.The data obtained were presented as M±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 placeboeffect, 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-normoxiawas 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 tochanging 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.

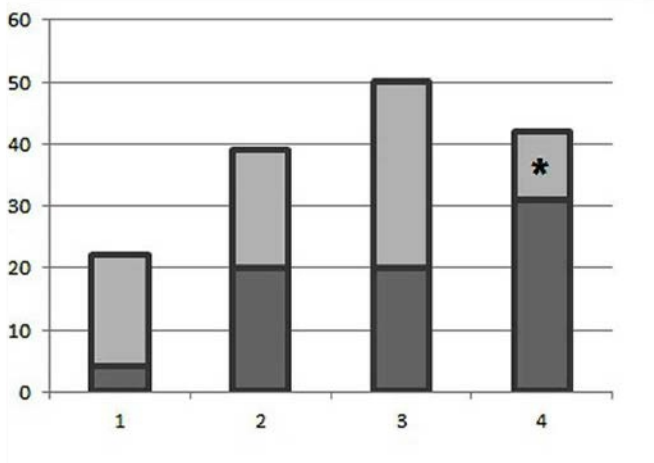


Figure 1. Effects of adaptation to hypoxia-normoxia (H/N) and hypoxia-hyperoxia (H/H) combined with training to physical exercise on total duration of swimming and active swimming phase (more intense colour). 1 - APL – acute physical load (forced swimming of control rats to exhaustion); 2 - 8Sw+APL – 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 incomparison 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 withadaptation to H/N and H/H. However,

only a combination of physical exercise with adaptation to H/H increase**s** the duration of the active phase and enhance**s** physical endurance.

Rates of free radical oxidation and activities of antioxidant enzymes were compared during APL andafter 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 changingoxygen 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).

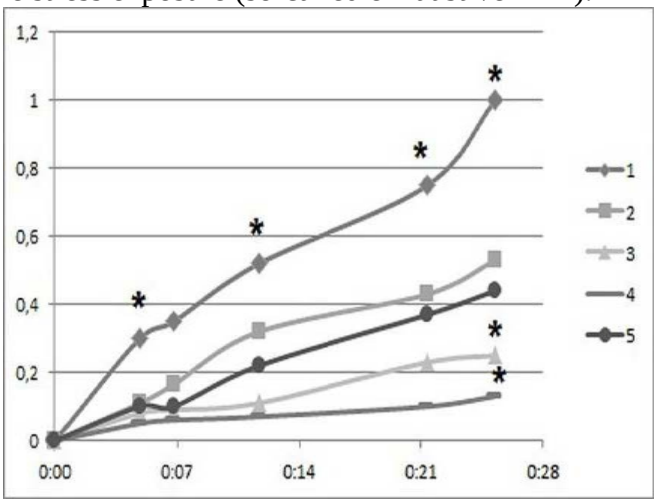


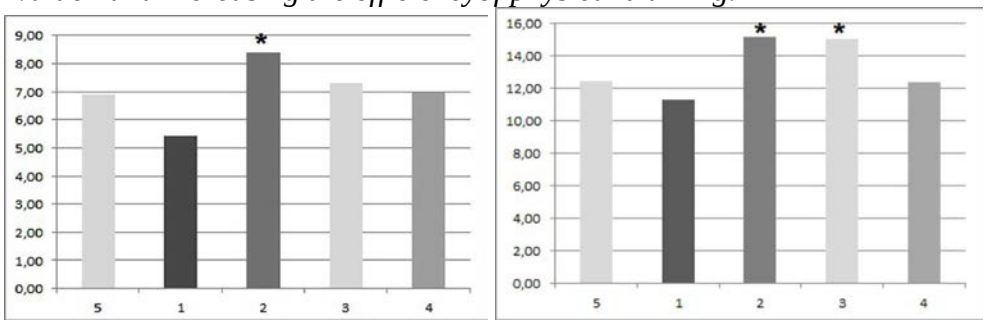
Figure 2. Effects of combined adaptation to H/N and H/H and physical exercise on the level of TBA- active products during induction of free radical oxidation in liver tissues in vitro. C – control. Designations as in Legends to Fig. 1; 5 – control.* - significance of differences ($P \leq 0.05$) from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

Compensation of free radical processes as a result of 8-day exercise training can also be due tosignificant (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 physicalexercise 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 efficiencyof physical training.*



A B

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 proteinsincluding three representatives of the HSP family, viz., the inducible proteins HSP72 and HSP32 and the constituitive 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 inductionof 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 constituitive 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 ourlaboratory 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.

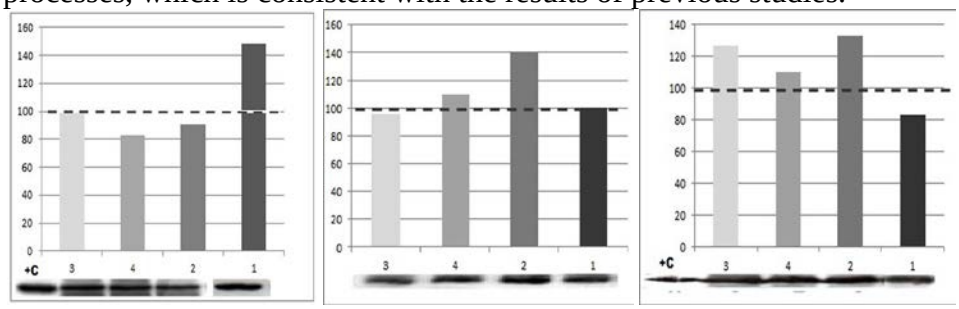


Figure 4. Effects of adaptation to H/N and H/H combined with adaptation to physical exercise on the level of the inducible stress protein HSP72 (A), the inducible protein HSP32 (B) and constituitive protein HSC73 (C) in the heart. +C – positive control (samples of H35 cells) after thermal treatment (30 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 VO_2max 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 (ΔRPP), CRI, IRI) (Table1) suggesting activation of the chronoinotropic 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 ± m)

NN	**Index**	Before IHHT	After IHHT
1.	VO_2max/BW, ml/кg	46,4±1,3 (53,4±1,8*)	50,3±1,4 (p=0,001) (54,7±1,6)
2.	PWC170, watt	170,8±11,8	191,9±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, $мс^2$	3118±456 (4503±512)	3890±337 (p=0,1) (4654±521)
9.	VLF, $мс^2$	1410±204 (1610±315)	1298±136 (1740±404)
10.	LF,$мс^2$	1300±566 (860±340)	801±209 (p=0,005) (828±420)
11.	HF, $мс^2$	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≤ 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 ± m)

NN	**Index**	Before IHHT	After IHHT
1.	SaO_2min, %	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 hypoxiaincreases the resistance of heart, liver and brain membranes to ROS. This phenomenon most likely underlies thecross effect of adaptation to IH under the conditions of physical load, based on the results of its application insports 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 hypoxianormoxia,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 ofmembrane 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 todemonstrate 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 traditionalmodes 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 functionalstatus, 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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References:

Andreeva LI, Goranchuk VV, Shustov EB, Boĭkova AA, Petrova VS, Rzhepetskaia MK (2001) Adaptation of humans to hyperthermia and changes in peripheral blood leucocytes. I.M. Sechenov Russian PhysiologicalJournal (in Rus.) 87 (9): 1208–1216.

Arkhipenko YuV, Sazontova TG, Tkatchouk EN, Meerson FZ (1997) Adaptation to continuous and intermittent hypoxia: role of the active oxygen-dependent system/ In: Sharma BK (Ed) Adaptation Biology and Medicine (Vol.1 Subcellular Basis) New Dehli, Narosa Publishing House: 251-259.

Arkhipenko YuV, Sazontova TG, Glazachev OS, Platonenko VI (2006) A method for enhancing nonspecific adaptive capacity of human beings based on the use of hypoxic-hyperoxic gas mixtures. RF Patent No 2289432 dtd 20.12.2006 (Rus).

Benjamin IJ, McMillan R (1998) Stress (Heat shock) Proteins. Molecular Shaperones in Cardiovascular Biology and Diseases. Circ Res 83: 117-132.

Bobyleva OV, Glazachev OS (2008) Some peculiarities of microcirculation in healthy individuals under acute hypoxia and after interval hypoxic training (in Rus). Human Physiology 34(6):92–99.

Boikova AA, Andreeva LI, Margulis BA (2006) A constituitive isoform of 70 kDa heat shock protein in human blood mononuclear cells as a marker of adaptation to normobaric hypoxic training (in Rus). I.M.Sechenov Russian Physiological Journal 92(7): 835–842.

Bonetti DL; Hopkins WG (2009) Sea-Level Exercise Performance Following Adaptation to Hypoxia: A Meta- Analysis. Sports Medicine 39 (2): 107-127.

Boreham CA, Paliczka VJ, Nichols AK (1990) A comparison of the PWC170 and 20-MST tests of aerobic fitness in adolescent schoolchildren. J Sports Med Phys Fitness 30(1):19-23.

Burtscher M, Gatterer H, Szubski Ch, Pierantozzi E, Faulhaber M (2007) Effects of interval hypoxia on exercise tolerance: special focus on patients with CAD or COPD. Sleep Breath. 14 (3): 209-220.

Burtscher M, Gatterer H, Faulhaber M, Gerstgrasser W, Schenk K (2010) Effects of intermittent hypoxia on running economy. International J Sports Medicine 31 (9): 644-650.

Chandel NS, Schumacker PT (2000) Cellular oxygen sensing by mitochondria: old questions, new insight. J Appl Physiol 88: 1880–1889.

Chizhov AYa, Potievskaya VI (2002) Intermittent normobaric hypoxia in prevention and treatment of the hypertensive disease. Moscow, Russian Peoples‟ University Press (Rus) pp 1-187

Das DK (2001) Redox regulation of cardiomiocyte survival and death. Antiox Redox Signal 13 (1): 23-37.

Graven KK, Zimmerman LH, Dickson EW, Weinhouse GL, Farber HW (1993) Endothelial cell hypoxia associated proteins are cell and stress specific. J Cell Physiol 157 (3): 544-554.

Hamlin MJ, Hellemans J (2007) Effect of intermittent normobaric hypoxic exposure at rest on haematological, physiological, and performance parameters in multi-sport athletes. J Sports Sciences 25 (4): 431-441.

Hemler ME, Cook HW, Lands WE (1979) Prostaglandin biosynthesis can be triggered by lipid peroxides. Arch Biochem Biophys 193: 340–345.
Hu ML, Frankel EN, Leibowitz BE, Tappel AL (1989) Effect of dietary lipids and vitamin on in vitro lipid peroxidation in rat liver and kidney. J Nutr 119: 1574-1582.
Ignatenko GA (2008) Modern-day potentials of adaptive medicine. Clinical Medicine (Rus) 11(1): 56–57.
Manzhugetova M.R., Syzdykov M.S., Pak G.D., Dauletova S.A. (2008) Adaptive effects of hypoxic training on physical tolerance and central hemodynamics in flight personnel. In: Physiology of Adaptation: Proceedings of the First Russian Scientific and Practical Conference. (in Rus) Volgograd: 292–296.
Maulik N, Yoshida T, Das DK (1999) Regulation of cardiomyocyte apoptosis in ischemic reperfused mouse heart by glutathione peroxidase. Mol Cell Biochem 196: 13–21.
Meerson FZ, Malyshev YI, Zamotrinsky AV (1992) Differences in adaptive stabilisation of structures in response to stress and hypoxia relate with the accumulation of hsp70 isoforms. Mol Cell Biochem 111: 87–95.
Mikhailov VM (2000) Heart Rate Variability. Ivanovo (in Rus), pp 1-182.
Mikhailov VM (2005) ECG-controlled stress- tests: veloergometry, treadmill, step test and walking. Ivanovo (in Rus), pp. 1-282.
Peng J, Jones GL, Watson K (2000) Stress proteins as biomarkers of oxidative stress: effects of antioxidant supplements. Free Rad Biol Med 28 (11): 1598 – 1606.
Powers SK., Criswell D, Lawler J, Ji LL, Martin D, Herb RA, Dudley G (1994) Influence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. Am J Physiol 266 (2): 375-380.
Roberts AM, Messina EJ, Kaley G (1981) Prostacyclin (PGI2) mediates hypoxic relaxation of bovine coronary artery strips. Prostaglandins 21: 555–569.
Ryter SW, Tyrrell RM (2000) The heme synthesis and degradation pathway: role in oxidant sensitivity. Free Radic Biol Med. 8: 289-309.
Sanz MJ, Ferrndiz ML, Cejudo M, Terencio MC, Gil B, Bustos G, Ubeda A, Gunasegaran R, Alcaraz MJ (1994) Influence of a series of natural flavonoids on free radical generating systems and oxidative stress. Xenobiotica 24 (7): 689-699.
Sazontova TG, Arkhipenko YuV, Meerson FZ (1987) Increased activity of antioxidant enzymes in rat heart during adaptation to short-term stress. (in Rus). Biull Eksp Biol Med 103 (10): 411–413
Sazontova TG, Tkatchouk EN, Kolmykova SN, Ehrenbourg IV, Meerson FZ, Arkhipenko YuV (1994) Comparative analysis of peroxidation and

antioxidant enzyme activities in rats adapted to different regimes of normobaric hypoxia. Hypoxia Med J 2(4): 4-7.
Sazontova TG, Arkhipenko YuV, Meerson FZ (1995) Adaptation to intermittent hypoxia and PUFA n-3- enriched diet as cardioprotective tools increasing the resistance of the myocardial sarcoplasmic reticulum Ca-transport system to free radical oxidation (in Rus). Biull Eksp Biol Med 120 (7): 42–45.
Sazontova TG, Arkhipenko YuV (2004) The role of free radical proteins in adaptation to changing oxygen levels In: Lukyanova LD and Ushakov IB (Eds) Problems of Hypoxia: Molecular, Physiological and Clinical Aspects. Moscow, Istoki Press: 112–137.
Sazontova TG, Zhukova AG, Anchishkina NA, Arkhipenko YuV (2007) Transcription factor HIF-1a, urgentresponse proteins, resistance of membrane structures and their dynamics after acute hypoxia (in Rus). Vestnik RAMN 2: 17–25.
Sazontova TG (2008) Stress-induced changes in the function of the sarcoplasmic reticulum calcium transportsystem of the heart and its resistance to endogenous damaging factors. (in Rus) Biull Eksp Biol Med 108: 271–274.
Sazontova TG, Arkhipenko YuV (2009) Intermittent hypoxia in resistance of cardiac membrane structures: Role of reactive oxygen species and redox signaling. In: Xi L, Serebrovskaya TV (eds) Intermittent hypoxia: from molecular mechanisms to clinical applications. Nova Science Publishers, Inc, New York, pp147-187.
Semenza GL (1999) Perspectives on oxygen sensing. Cell 98: 281-284.
Singh B, Sharma SP, Goyal R (1994) Evaluation of Geriforte, an herbal geriatric tonic, on antioxidant defense system in Wistar rat. Ann NY Acad Sci 717: 170-173.
Spasic MB, Saicic ZS, Buzadzic B, Korac B, Blagojevic D, Petrovic VM (2001) Effect of long term exposure to cold on the antioxidant defense system in the rat. Free Rad Biol Med 15 (3): 291-299.
Stepanov VK, Dvornikov MV, Mayev EZ, Emelyanov BN, Kozyrev PV, Vinogradov NV, Kozyreva EP (2005) Normobaric interval hypoxic therapy and dosed oxygenation in pulmonology (in Rus). In: Intermittent Normobaric Hypoxic Therapy: Proceedings Internat. Acad. Probl. Hypoxia.Moscow, Bumazhnaya Galereya IV: 154–163.
Strelkov RB, Chizhov AYa (2001) Intermittent normobaric hypoxia in prophylaxic, treatment and rehabilitation. Ekaterinburg (in Rus) pp 17-19.
Tanskanen M, Atalay M, Uusitalo A (2010) Altered oxidative stress in overtrained athletes Journal of Sports Sciences 28(3): 309–317.
Trukhanov AI (2004) Modern Technologies in Rehabilitation Medicine.Moscow, Medica (in Rus) pp 1-244.

Vollaard NB, Cooper CE, Shearman JP (2006) Exercise-induced oxidative stress in overload training and tapering. Medicine and Science in Sports and Exercise, 38, 1335–1341.
Whelan SA, Hightower LE (1985) Differential induction of glucose regulated a heat shock proteins; effect of pH and sulfhhydryl-redusing agents on chiken embryo cells. J Cell Physiol 125 (2): 251-258
Wilber RL (2007) Application of Altitude/Hypoxic Training by Elite Athletes. Medicine & Science in Sports &Exercise 39(9): 1610-1624.
Zhukova AG, Sazontova TG (2005) Hypoxia inducible factor-1□ function and biological role. Hypoxia Med J 3-4: 34-41.
Zolotarjova N, Ho C, Mellgren RL, Askari A, Huang WH (1994) Different sensitivities of native and oxidized forms of Na+/K+-ATPase to intracellular proteinases. Biochim Biophys Acta 1192 (1): 125-131.