ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/256539596

Tissue Oxygenation and Mitochondrial Respiration under Different Modes of Intermittent Hypoxia

Article *in* High Altitude Medicine & Biology · September 2013

DOI: 10.1089/ham.2013.1012 · Source: PubMed

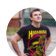
CITATIONS
13

READS
214

5 authors, including:

T. V. Serebrovskaya
Bogomolets Institute of Physiology NASU
104 PUBLICATIONS **991** CITATIONS

SEE PROFILE



Bronislav L. Gavenauskas
Bogomolets Institute of Physiology NASU
13 PUBLICATIONS **66** CITATIONS

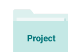
SEE PROFILE

Iryna Mikitivna Mankovska
Bogomolets Institute of Physiology NASU
31 PUBLICATIONS **130** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Effects of intermittent hypoxic trainings in patients with Prediabetes View project



Role of HIF-3alpha in cardiomyocytes responce on damage at anoxia/reoxygenation modeling . View project

All content following this page was uploaded by T. V. Serebrovskaya on 10 December 2014.

The user has requested enhancement of the downloaded file.

HIGH ALTITUDE
MEDICINE & BIOLOGY

High Altitude Medicine & Biology: http://mc.manuscriptcentral.com/highaltitudemedicine

TISSUE OXYGENATION AND MITOCHONDRIAL RESPIRATION UNDER DIFFERENT MODES OF INTERMITTENT HYPOXIA

Journal:	*High Altitude Medicine & Biology*
Manuscript ID:	HAM-2013-1012.R2
Manuscript Type:	Regular Scientific Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Serebrovskaya, Tatiana; Bogomoletz Institute of Physiology, Hypoxic States Nosar, Valentyna; Bogomoletz Institute of Physiology, Studying of Hypoxia States Bratus, Larissa; Bogomoletz Institute of Physiology, Hypoxic States Gavenauskas, Bronislav; Bogomoletz Institute of Physiology, Hypoxic States Mankovska, Iryna; Bogomoletz Institute of Physiology, Hypoxic States
Keyword:	Intermittent hypoxic training, Oxygen delivery, Adaptation, Normobaric hypoxia

SCHOLARONE™
Manuscripts

For Peer Review, Not for Distribution

For Peer Review ONLY/Not for Distribution

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

TISSUE OXYGENATION AND MITOCHONDRIAL RESPIRATION UNDER DIFFERENT MODES OF INTERMITTENT HYPOXIA

Tatiana V. Serebrovskaya, Valentyna I. Nosar, Larissa V. Bratus, Bronislav L. Gavenauskas, Iryna M. Mankovska

Bogomoletz Institute of Physiology, Kiev, Ukraine

Corresponding author:

Tatiana V. Serebrovskaya
Professor of Physiology, Doctor Biol. Sci.
Principal Researcher of the
Bogomoletz Institute of Physiology
4 Bogomoletz St.
Kiev 01024, UKRAINE
Tel: (38-044) 256-2557 (o); 411-6590 (h)
Fax: (38-044) 256-2000
e-mail: sereb@biph.kiev.ua (o)
tsereb@voliacable.com (h)

All other authors have the same mailing address and contact information, except for e-mail addresses:

Valentyna I. Nosar - nosar&biph.kiev.ua

Larissa V. Bratus - nosar&biph.kiev.ua

Bronislav L. Gavenauskas - b.gavenauskas@gmail.com

Iryna M. Mankovska – mankovsk@biph.kiev.ua

Running title: Intermittent Hypoxia Modes

Abstract

We compared the results of 5 modes of intermittent hypoxia training (IHT) on gastrocnemius muscle PO_2 and heart and liver mitochondrial respiration in rats. Minutes of hypoxia, $\%O_2$ and recovery minutes on air in each mode were: 1) 5, 12%, 5; 2) 15, 12%, 15; 3) 5, 12% 15; 4) 5, 7% 5; 5) 5, 7%, 15. Mode 1 proved best in that PmO_2 dropped minimally at the end of every hypoxic bout and recovered quickly after each bout. One, 2 and 3 week IHT in mode 1 each increased tissue PO_2 in both normoxic and 30 min severe hypoxic (7% O_2) tests. Adaptation to IHT in Mode 1 caused the substrate dependent reorganization of liver and heart mitochondrial energy metabolism favoring NADH-dependent oxidation and improving the efficiency of oxidative phosphorylation. Mitochondrial adaptation occurred after 14 days of IHT in liver tissue but after 21 days in myocardium, and was preserved during the three months following IHT termination. When using Mode 2, positive changes were also registered, but were less pronounced. Other IHT modes provoked negative effects on PmO_2 level both during hypoxic periods and reoxygenation. In conclusion, the most effective IHT regimen is 5 min 12% O_2 with 5 min breaks, five cycles per day during two or three weeks depending on the task of IHT.

Key words*: intermittent hypoxia, rat tissue oxygenation, mitochondrial respiration*

For Peer Review ONLY/Not for Distribution

For Peer Review ONLY/Not for Distribution

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

Widespread use of the intermittent hypoxic training/treatment (IHT) methods in sports, military and medical practice during recent decades has provoked discussion about the most beneficial regimens of hypoxic training, both normobaric and hypobaric. Traditional normobaric treatment protocols elaborated mostly by scientists from Eastern Europe comprise alternating periods of breathing with hypoxic gas mixtures followed by a subsequent oxygenation periods (breathing with ambient air). Many types of protocol with different numbers of hypoxia episodes, severity, and total exposure duration have been used by investigators and these combinations may have resulted in various physiological responses. Principals of IHT application for cell cultures, animal experiments (mice, dogs, cats, rabbits, pigs, horses and even insects) have been elaborated. A variety of technical implementations for treatment of humans have been tested and used in recent decades, including hypobaric chambers, normobaric reduced oxygen rooms, and mask-system hypoxicators producing hypoxic air in various ways [see reviews Serebrovskaya et al., 2003; Zieliński, 2005; Mateika & Sandhu, 2011; Lopata & Serebrovskaya, 2012] .

On the other hand, the intermittent hypoxia research in Western Europe and North America was primarily focused on the detrimental effects of chronic intermittent hypoxia associated with sleep-disorder breathing. For instance, Prabhakar & Semenza [2012] consider that intermittent hypoxia almost always represents a pathological stimulus that evokes maladaptive responses. However, during the past decade, such a gap of division between East and West is progressively shrinking, and mutual understanding on what "intermittent hypoxia" means, becomes clearer [Serebrovskaya & Xie, 2009; Semenza, 2012].

The questions that arise are, what are the key mechanisms determining the adaptive versus maladaptive nature of different paradigms of intermittent hypoxia, and, what molecular pathways are mediating the observed pathological or physiological response? It is appropriate to mention here the ancient wisdom well expressed by Paracelsus in the XYIth century: "Sola dosis facit venenum" (only the dose makes the poison).

Until now there is no exact evidence about the precise mechanism for switching adaptive or maladaptive responses to hypoxic impact. Several attempts were undertaken to analyze this question [Lukyanova, 2005; Serebrovskaya et al, 2008; Lukyanova et al, 2009, 2012; Yin et al., 2012]. The most significant contribution to this question was made by Prabhakar & Semenza [2012], who described transcriptional regulation of gene expression mediated by hypoxia-inducible factors 1 and 2 (HIF-1 and HIF-2). These factors constitute important components of the genetic makeup in the body, influencing hypoxic sensing by regulating intracellular redox state via transcriptional regulation of pro-and anti-oxidant enzymes. The

For Peer Review ONLY/Not for Distribution

large number of known feedback loops underscores the critical importance of precisely regulating O_2 delivery and utilization. The Authors basic message is that HIF signaling is not a linear pathway, but a complex web with perhaps hundreds of input stimuli and thousands of potential output responses, with each representing a different target gene. The discovery of the HIF family has led to novel insights into the molecular basis of adaptive and maladaptive cellular and systemic responses to continuous and intermittent hypoxia.

While these fundamental molecular studies on cell culture models provided important insights into mechanisms of HIF activation by hypoxia, they can not answer as yet, the practical question of what dose and regimen of hypoxic impact could be mostly beneficial for animals and humans. In practice, hypoxic regimens that are used for the study of hypoxic adaptations vary broadly from 3-12 short hypoxic sessions (2-10 min) with 2-20 min normoxic breaks during 7-30 days to hypoxic influences lasting from 1 - 12 hours during 2-90 days [Nattie et al., 1978; McGuire & Bradford, 2001; Fagan, 2001; Lin et al., 2002; Neckár et al, 2002; Zong et al., 2004; Zhu et al, 2004; Vavilova et al., 2005; Joyeux-Eaure et al., 2005; Naryzhnaia et al., 2009; Manukhina et al., 2011; Rozova et al., 2012, and many others].

This wide spectrum of protocols for IHT is represented now in literature showing both beneficial and detrimental effects. In addition, simply the mode of hypoxic influence (depth, duration, and intermittence) appeared to be critical for the determination of healing or harmful result. Therefore, special purposeful investigations are needed to elucidate basic mechanisms of different IHT effects depending on the modality of hypoxic stimuli and elaborate most effective and safe regimen for the introduction in human practice.

The two most informative parameters of hypoxic influence on an organism are tissue oxygenation and tissue respiration. At the tissue level, hypoxia activates the transcription of multiple genes encoding angiogenic growth factors and cytokines. The result is an increase in the capillary density and tissue perfusion [Mankovska et al., 2005; Panisello et al., 2008; Xu WQ et al., 2011; Faiss et al., 2013], that increases in tissue oxygenation. At the cellular level, hypoxia leads to reprogramming of mitochondrial metabolism that ensures adequate ATP generation and prevents adverse consequences of excess mitochondrial ROS generation. These metabolic adaptations are due to HIF-1-mediated transcriptional regulation of glycolytic enzymes, mitochondrial electron transport chain components, and other metabolic enzymes [Prabhakar & Semenza, 2012]. Many studies confirm the close relations between physical performance and mitochondrial respiration during adaptation to intermittent hypoxia [Zoll et al., 2002; Mankovska et al., 2005; Saxena et al., 2012, and many others].

Based on the above summary of current knowledge, the aim of this study was to compare the effects of the five most widespread modes of hypoxic training on rat muscle pO_2 and

elucidate how the most beneficial modes influence mitochondrial respiration. We tested two hypoxic loads (breathing with 12% O_2 and 7% O_2) under different durations of hypoxic and normoxic periods in various combinations of 5 minute and 15 minutes.

MATERIAL AND METHODS

Animals

All procedures followed the criteria, technical standards and rights applied to animal research. All trials were followed in accordance with the statements of the European Union regarding handling of experimental animals. This investigation conforms to the law and local ethical committee guidelines for animal research. Experiments were conducted on adult male Wistar rats (weight range 200 to 220 gram). The animals were housed (4 per cage) in a room with 12:12-h light-dark cycle at 22oC and were provided with standard rat chow with water ad libitum.

Experimental design

Two series of experiments were provided. In Series I, muscle oxygen partial pressure (PmO_2) was recorded in *m. gastrocnemius* at the end of each of 6 hypoxic and end of each recovery period in each of the 5 modes during one day study. Then, the IHT in Mode 1 was administrated to animals for 3 weeks, and PmO_2 was recorded in both normoxic and 30 min severe hypoxic (7% O_2) tests after 7, 14 and 21 days of IHT as well as at 2 and 3 months post-training.

In Series II the investigation of liver and myocardium mitochondrial respiration was completed after a course of the two most beneficial IHT modes. In total, 91 animals were used in the study.

Series I: Measurements of Muscle PO2

Each of the 5 modes was studied in a separate group of animals consisting of 7 rats. Each rat was exposed 6 times to the chosen mode during one test day (see table 1). Animals of Gr. I-1 were furthermore subjected to three-week IHT in Mode 1, and PmO_2 was recorded

For Peer Review ONLY/Not for Distribution

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

before and at end of the severe hypoxia test (7% O_2 for 30 min) on days 7, 14, 21 and 2 and 3 months after finishing IHT.

Protocol

All manipulations were made under light etherization. Animals were fixed in supine position. Rats breathed through a two-valve respiratory mask which could feed gas mixtures directly to the nostrils, thus minimizing the dead space. A mask covered the animals' eyes protecting them from outside irritants. Investigations included PmO_2 continuous measurement during air breathing, inhalation of hypoxic gas mixtures according to prescribed modes or during a single AH test.

To determine PmO_2, the sterile open vitrified needle-shaped 0.2 mm diameter platinum electrode (chemical purity 99, 99%) was introduced through a skin puncture lengthwise approximatwly 10-15 mm along the inside of the muscle fibers [Berezovskii, 1975]. Polarographic measurements began after 5-10 minutes when the relatively stable PmO_2 values were attained. The oxygen electrode was calibrated before and after measurements in each animal.

Series II: Study of mitochondrial respiration.

Animal groups

Eight groups of rats, each group with 7 animals, participated in this series. Among them, group II-1 was the control group, exposed to a sham IHT, and five groups (Gr.II: 2-6) were exposed to IHT courses of different durations, Mode 1:

Gr.II-1 – control group;

Gr.II-2 – IHT during 7 days;

Gr.II-3 – IHT during 14 days;

Gr.II-4 – IHT during 21 days;

Gr.II-5 – 2 month residence in vivarium after three-week IHT;

For Peer Review ONLY/Not for Distribution

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Gr.II-6 – 3 month residence in vivarium after three-week IHT.

Gr.II-7 had sham treatment with IHT, Mode 2 (control group), and Gr.II-8 underwent to three-week IHT in Mode 2.

IHT Protocol

Rats underwent the IHT sessions in two gaseous chambers: hypoxic (ventilated by hypoxic gaseous mixture with 12 % O_2) and normoxic (ventilated by ambient air). Animals were placed inside a chamber in a metal cage which could be quickly moved from the air chamber and placed into the hypoxic chamber (there and back) every five or fifteen minutes depending on the IHT mode. This procedure was repeated 5 times daily during 7, 14 or 21 days depending on group number. The level of O_2 fraction in the hypoxic chamber was monitored by a gas analyzer.

Mitochondrial respiration protocol

Next day after the last hypoxic exposure, rats were anesthetized with 1–2% isoflurane, decapitated, and their livers and hearts were extracted for further analysis of mitochondrial respiration. Mitochondria were isolated by differential centrifugation according to Kondrashova et al. [2001]. Briefly, rat hearts and livers were quickly excised, washed in ice-cold buffer, weighed, and homogenized in a glass Potter-Elvehejm homogenizer with a motor-drive Teflon pestle. The homogenization medium contained for heart: 120 mM KCl, 10 mM HEPES, 10 mM EDTA, and 0.5% bovine serum albumin; pH 7.2; for liver: 120 mM KCl, 2 mM K_2CO_3, 10 mM Tris HCl, 1 mM EGTA; pH 7.2. Homogenate was centrifugated during 7 min at 700 G and 4^0C, then supernatant was centrifugated during 15 min at 11000 G and 4^0C. Mitochondrial suspension (3-4 mg protein/ml) was slurred in a medium without EDTA and kept on ice at 4^0C.

Mitochondrial respiratory function was measured in a water-jacketed chamber using a Clark O_2 electrode by the polarographic method of Chance and Williams [1956].

For Peer Review ONLY/Not for Distribution

For Peer Review ONLY/Not for Distribution
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Mitochondria were added to the thermostated and magnetically stirred respiration chamber containing a total volume of 1.0 ml of respiration medium. Medium composition: for heart - 30 mM tris-HCl, 125 mM KCl, 10 mM NaCl, 5 mM KH_2PO_4, 1.5 mM $MgCl_2$, and 3 mM EGTA; for liver - 120 mM KCl, 2 mM KH_2PO_4, 2 mM K_2CO_3, 10 mM TrisHCl, pH 7.2 at 25°C. Following oxidative substrates were used: 1 mM succinate in the presence of 2 µM of rotenone (S+R), or 3 mM glutamate + 2.5 mM of malate (G+M). ADP (phosphate acceptor) was administered in 0.2 mM concentration.

The following parameters were measured and calculated: V_4^s (oxygen consumption before ADP addition), V_3 (oxygen consumption stimulated by ADP), V_4^{ATP} (oxygen consumption after cessation of ADP phosphorylation), respiratory control ratio by Chance (V_3/V_4^{ATP}), and efficiency of phosphorylation (ADP/O, ADP-to-oxygen-ratio) [Estabrook, 1967]. Oxygen consumption was recorded as ng atoms oxygen per minute per mg mitochondrial protein, determined by the Lowry assay [Lowry et al., 1951].

Statistical analysis

Results were statistically treated with Student's t-test and ANOVA and presented as Mean±SD. Statistical differences were considered significant if the P value was <0.05.

Results

Fig 1 represents the results of PmO_2 measurements under one session of 5 different IHT modes. The initial values are taken as 100%. During Mode 1 (Gr. I-1, 12% O_2, 5 min + 20,9% O_2, 5 min), PmO_2 dropped by half by the end of every hypoxic period and recovered quickly after every hypoxic set, to the initial level or even exceeded it by 10-15%. After termination of the session, the value of PmO_2 quickly returned to its original level (Fig.1, Mode 1).

The increase of both hypoxic and normoxic exposures up to 15 min (Gr. I-2), showed

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

similar results as in Gr. I-1 during the first set but provoked a decrease in PmO_2 during following 2 to 5 normoxic periods by 20% and a delay in oxygenation restoration after the session finished (Fig.1, Mode 2).

The 5-min hypoxic load with 12% O_2 but with prolonged breaks of up to 15 min (Gr. I-3) did not lead to the full restoration of muscle oxygen supply after repeated sessions: PmO_2 during periods of reoxygenation was reduced by 20-40%, though during hypoxic exposures it dropped similar to Gr. I-1 (by 55-60%). The recovery period after the session was extended, and even at 15 min after training, PmO_2 did not reach the initial level (Fig.1, Mode 3).

More severe hypoxic exposure (7% O_2, 5 min) led to more severe drop in PmO_2 both during hypoxic and normoxic periods ((by 70-90% and 20-25%, respectively) (Gr. I-4). The lengthening of reoxygenation periods from 5 to 15 min (Gr. I-5) aggravated PmO_2 recovery compared with shorter periods (Fig.1, Modes 4 and 5).

Thus, the most advantageous mode, which provides the least drop in PmO_2 during hypoxic periods and rapid normalization of the skeletal muscle oxygen supply during reoxygenation, is Mode 1. Both the aggravation of hypoxic load and the prolongation of reoxygenation periods cause the disturbances in tissue oxygen supply. Among other modes, the Mode 2 seems to be considered as appropriate because of the smallest failures in oxygen supply during recovery periods.

Since fifth and sixth sets of hypoxia load did not differ significantly in all groups (Fig.1), we used in the sequel 5 hypoxic sets per day for IHT implementation.

To prove the beneficial effects of IHT with Mode 1, we further subjected animals of Gr.I to a 21-day IHT cycle using this mode. For more evidence, we provided PmO_2 measurements not only during air breathing but also during AH test (7% O_2, 30 min) (Fig.2). IHT increased normoxic PmO_2 from control on 7, 14 and 21 days of IHT and it remained up after 2 months. PmO_2 at the end of each acute severe hypoxia test (7% for 30 min) was increased from control by 46%, 63% and 89% after 7, 14, and 21 days of IHT, and was still

For Peer Review ONLY/Not for Distribution

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

elevated 42% 3 months later.

On this basis, we further investigated the liver and heart mitochondrial respiration during three-week course of IHT (Mode 1) and its consequences for 2 and 3 months. For comparison, we also tested IHT in Mode 2 during 21 days.

When using Mode 1, it was shown that adaptation to IHT caused the reorganization of mitochondria energy metabolism. Table 2 shows mitochondrial oxygen consumption data using FADH-(succinate) and NADH-dependent substrates (glutamate + malate). Seven days of IHT treatment (Gr.II-2) induced a decrease in liver mitochondrial O_2 consumption (V_3) at succinate oxidation and an increase in respiratory control ratio (V_3/V_4^{ATP}) indicating the enhancement of respiration and phosphorylation coupling. Meanwhile, the effectiveness of O_2 consumption (ADP/O ratio) did not change (Fig.3). This means that the significance of succinate oxidation pathway (mitochondrial enzyme complex II, MEC II), which is effective at acute hypoxia impacts, becomes insignificant under IHT treatment. Mitochondria switch the energy production to a more effective NADH-dependent pathway (mitochondrial enzyme complex I, MEC I). This is well illustrated by mitochondrial oxygen consumption under the MEC I substrates oxidation (Table 2): an increase in V_3/V_4^{ATP} and V_3 by 23% and 20%, respectively. ADP/O ratio increased by 13% (Fig.3). The defined IHT effects for liver mitochondria were also registered after 14- day IHT (Gr. II-3), diminished when using IHT during 21 days (Gr. II-4) and mostly preserved in 2 (Gr. II-5) and 3 (Gr. II-6) months after IHT finished (Table 2, Fig.3).

At the same time, the significant adaptive changes in the heart mitochondrial respiration developed later than in the liver, viz only after 3 weeks of training of training, showing a decrease in V_3 by 11%, an increase in V_3/V_4^{ATP} by 20% when using MEC II substrate, and an increase in V_3 and V_3/V_4^{ATP} by 16% and 26%, respectively, when using MEC I substrate (Gr. II-4). Beneficial effects continued during 2 and 3 months after IHT finishing.

For Peer Review ONLY/Not for Distribution

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

When using Mode 2 (Table 3), the positive changes in mitochondrial respiration were also registered, but they were less pronounced. For example, after 21-day IHT the V_3/V_4^{ATP} for heart mitochondria increased by 18% (Gr.II-8) as opposed to an increase in this parameter by 26% under Mode 1 (Table 2). For liver mitochondria, the respiratory control ratio changed insignificantly.

Discussion

In this study we compared for the first time the effects of five widespread modes of IHT on rat gastrocnemius muscle PO_2, and heart and liver mitochondrial respiration. We have proved that 5 hypoxic bouts consisting of 5 min breathing with 12% O_2 and 5 min air breathing is more beneficial than other studied modes, since such regimen led to the minimal drop of the muscle PO_2 at the end of every hypoxic bout and quick recovery after each bout. One, 2 and 3 week training with this mode raised basal tissue oxygenation during normoxia and provided higher PmO_2 level during a subsequent acute hypoxia test. Adaptation to IHT under this mode caused the substrate dependent reorganization of liver and heart mitochondrial energy metabolism favoring NADH-dependent oxidation and improving the efficiency of oxidative phosphorylation. Mitochondrial adaptations developed after 7 days of IHT in liver but after 21 days in myocardium and lasted for three months after IHT termination. When using the IHT mode with the same oxygen content but prolonged normoxic periods up to 15 min, positive changes were also registered, but were less pronounced. Other IHT modes provoked negative effects on PmO_2 levels both during hypoxic periods and reoxygenation.

To determine the optimal mode of IHT in sports and military activities, many authors use such key parameters as increased ventilation, arterial oxygen saturation, hemoglobin or hematocrit values, etc [Musa, 2007; Wilber et al., 2007; Katayama et al, 2009; Millet et al., 2010]. In our experiments on animals, we took the most informative integral parameter of muscle oxygen supply - PmO_2.

Animal investigations have also used a variety of exposure paradigms to study intermittent hypoxia. Foster et al. [2005] using two regimens - (1) 5 min of 12% O_2 / 5 min of air, 1 hr, 12 day, and (2) 30 min of 12% O_2, 12 days - showed that both short and long hypoxic durations had similar effects on the ventilatory and cardiovascular response to acute

For Peer Review ONLY/Not for Distribution

For Peer Review ONLY/Not for Distribution

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

progressive hypoxia. The similar results were obtained on humans [Ainslie et al., 2007] who compared short-term intermittent hypoxia (IH: 5-min hypoxia to 5-min normoxia, 90 min/day, 12 days) with continuous hypoxia (CH: ascending to 1,560 m altitude for 12 days) and showed that IH and mild CH can equally enhance the HVR which facilitates alterations in blood pressure and middle cerebral artery blood flow velocity.

But most authors consider that the shorter periods of hypoxic and normoxic exposures have more beneficial effects on an organism. Currently, there is practical necessity to decrease training time for achieving long-term adaptation, while maintaining its effectiveness. As Lukyanova [2005] suggested, short term hypoxia doesn't cause tissue damage and permits changes of mitochondrial enzymes to occur during normoxic intervals.

Peng & Prabhakar [2004] exposed rats to either short-duration intermittent hypoxia (SDIH, 15 s of 5% O_2 at 5 min intervals, 8 hr day^{-1}) or long-duration hypobaric intermittent hypoxia (LDIH, 4 h day^{-1}, 0.4 atm for 10 days) and showed that exposure to SDIH enhanced carotid body sensory response to hypoxia, but exposure to LDIH did not. Satriotomo et al [2012] believe that acute intermittent hypoxia (5-min episodes of 10.5% O_2 with 5-min normoxic intervals) initiates plasticity in respiratory motor control. Hu et al [2010] using rat model's hypoxic preconditioning, concluded that 15%-10% oxygen concentration range with short breaks could be regarded as a helpful effective area.

One of the key mechanisms of cell damage during hypoxia and reoxygenation is an excessive production of reactive oxygen and nitrogen species in mitochondria. These considerations may partly explain why the prolongation of reoxygenation periods in our studies from 5 to 15 minutes (Modes 2, 3 and 5) led to the deterioration of muscle oxygen supply. Probably, longer normoxia provokes greater free radicals production preventing normal oxygen delivery to tissues. On the other hand, there is evidence that by using 30% O_2 during recovery, adaptation to IHT could be achieved earlier and provide the upregulation of adaptive ROS signals compared to classical intermittent hypoxic training [Zhukova et al.,

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

2005; Gonchar & Mankovska, 2012]. Studies support the viewpoint that brief hypoxic generation of free radicals induces antioxidant enzyme protein synthesis that may be an important trigger for specific adaptations. Unfortunately, fundamental molecular studies on cell culture models [Mansfield et al., 2005; Kim et al, 2006; Chua et al., 2010] which provided important insights into mechanisms of HIF activation by hypoxia, can not explain exactly why the prolongation of reoxygenation periods during IHT treatment provoked less positive effects on PmO_2 level. We understand that a more reasonable explanation of this phenomenon needs further investigation.

Additionally, reactive oxygen species (ROS) also function as secondary messengers in a variety of physiological processes increasing antioxidant defense. It was shown that ROS participate in the signaling pathways involved in the activation of multiple transcription factors such as HIF-1, NF-κβ, c-fos, c-Jun under IHT [Sasaki et al., 2001; Huang et al., 2005; Prabhakar & Semenza, 2012]. In our experiments the extension of reoxygenating periods both after moderate (12 % O_2) and severe (7% O_2) hypoxia resulted in the deterioration of muscle oxygen supply. In addition, a decrease in oxygen content per se from 12% to 7% also led to worsening of tissue PO_2. As Wang et al [2007] suggest, severe but not moderate IH regimens decrease anti-oxidative capacity and increase lipid peroxidation leading to the suppression of vascular endothelial function, causing impairment of hemodynamic.

Our investigations also have shown that adaptation to IHT in Mode I caused reorganization of liver and myocardium mitochondrial energy metabolism favoring NADH-dependent oxidation. Since mitochondrial superoxide production is inversely related to MEC I activity, the activation of the latter by IHT could limit the oxidative stress development under acute hypoxia [Verkaart et al., 2005; Koopman et al., 2005]. So, increasing oxidation of NAD-generated substrates may preserve the mitochondrial oxygen consumption capacity during stress conditions. One can also assume that adaptation to IHT decreased the vulnerability of heart and liver mitochondria to ROS as demonstrated by elevated respiratory control ratio,

For Peer Review ONLY/Not for Distribution

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

ADP/O ration, and maximal rate of oxidative phosphorylation.

When using Mode 2, positive changes in mitochondrial respiration were also registered, but they were less pronounced.

Our findings are in agreement with the previous reports demonstrating the activation of mitochondrial respiration by IHT [Prabhakar & Kumar, 2004; Kurhaliuk et al., 2002; Kurhaluk et al., 2013]. As Lukyanova et al. [2009] suggest, the suppressed function of MEC I and the alternative activation of MEC II comprise a signaling regulatory mechanism which contributes to the formation of tissue-specific and general resistance of the body to different types of hypoxia.

Recent studies demonstrated the mechanisms by which intermittent hypoxia reversibly inactivates MEC I. The primordial function of HIF-1 appears to involve finding the optimal balance between oxidative and glycolytic metabolism for any given cell as a function of the local O_2 concentration [Prabhakar & Semenza, 2012]. Based on cell culture experiments with very short (30 sec) intermittent hypoxic exposures, Khan et al [2011] identified NADPH oxidases and mitochondrial electron transport chain at MEC I as major cellular ROS sources mediating systemic and cellular responses to intermittent hypoxia and demonstrated a functional cross-talk between NADPH oxidases and MEC I activity.

According to our results, we consider that the Mode 1 of IHT (5- min 12% O_2 with 5-min normoxic intervals) is more effective and less dangerous for the studied organs and tissues and may be recommended for sports as well as prophylaxis and treatment of cardiac and lung pathology. However, we must take into account that all these beneficial results were obtained on a rat model. In actual human practice [Musa, 2007], the regimen of IHT (the degree of hypoxia, exposure duration and number of sessions) could be related to the mission requirements, such as the operational target altitude, the risk of developing acute mountain sickness, or the anticipated physical activity levels.

For Peer Review ONLY/Not for Distribution

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

CONCLUSION

Our experimental data indicates that among 5 tested modes of IHT, optimal hypoxic dose for muscle oxygen supply is 5-min breathing with 12 % O_2 gas mixture and 5-min breaks (Mode 1), 5-6 times a day during two or three weeks. Under such mode, PmO_2 dropped minimally to the end of every hypoxic period and recovered quickly after every hypoxic set to initial level or even exceeded it. One, 2 or 3 week training with this mode raised basal tissue oxygenation during normoxia and provided higher PmO_2 level during acute hypoxia. Under other modes, PmO_2 did not reach normoxic level during every set, and the recovery period after one IHT session was delayed for 10-15 min.

Using Mode 1, it was shown that adaptation to IHT caused the substrate dependent reorganization of liver and heart mitochondrial energy metabolism favoring NADH-dependent oxidation and improving the efficiency of oxidative phosphorylation. Mitochondrial adaptation developed after 7 days of IHT in liver tissue, but after 21 days in myocardium and was preserved for three months after IHT termination. When using Mode 2 (15-min 12% O_2 with 15-min breaks), positive changes in mitochondrial respiration were also registered, but were less pronounced.

References

1. Ainslie P.N., Barach A., Cummings K.J., Murrell C., Hamlin M., and Hellemans J. (2007). Cardiorespiratory and cerebrovascular responses to acute poikilocapnic hypoxia following intermittent and continuous exposure to hypoxia in humans. J. Appl. Physiol. 102(5):1953-1961.
2. Berezovskii V.A. (1975). Tissue oxygen tension in animals and humans. Naukova Dumka, Kiev, Ukraine [In Russian].
3. Chance B. and Williams G.R. (1957). The respiratory chain and oxidative phosphorylation. Adv. Enzymol. Relat. Subj. Biochem. 17:65-134.
4. Chua Y.L., Dufour E., Dassa E.P., Rustin P., Jacobs H.T., Taylor C.T., and Hagen T. (2010). Stabilization of hypoxia-inducible factor-1ά protein in hypoxia occurs independently of mitochondrial reactive oxygen species production. J. Biol. Chem.

For Peer Review ONLY/Not for Distribution

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

For Peer Review ONLY/Not for Distribution

285: 31277–31284.

5. Estabrook R.W. (1967). Mitochondrial respiratory control and the polarographic measurements of ADPO:O ratios. Methods Emzymol.10: 41-47.
6. Fagan K.A. (2001). Physiological and genomic consequences of intermittent hypoxia. Selected contribution: Pulmonary hypertension in mice following intermittent hypoxia. J. Appl. Physiol. 90:2502–2507.
7. Faiss R., Léger B., Vesin J.M., Fournier P.E., Eggel Y., Dériaz O., and Millet G.P. (2013). Significant molecular and systemic adaptations after repeated sprint training in hypoxia. PLoS One.;8(2):e56522.
8. Foster G.E., McKenzie D.C., Milsom W.K., and Sheel A.W. (2005). Effects of two protocols of intermittent hypoxia on human ventilatory, cardiovascular and cerebral responses to hypoxia. J. Physiol. 567(Pt 2):689-699.
9. Gonchar O. and Mankovskaya I. (2009). Effect of moderate hypoxia/reoxygenation on mitochondrial adaptation to acute severe hypoxia. Acta Biol Hung. 60(2):185-94.
10. Gonchar O. and Mankovska I. (2012). Moderate hypoxia/hyperoxia attenuates acute hypoxia-induced oxidative damage and improves antioxidant defense in lung mitochondria. Acta Physiol. Hung. 99(4):436-446.
11. Hu D.Y., Li B., Dai R.J., Geng L.N., and Deng Y.L. (2010). Suitable oxygen concentration for rat model's hypoxic preconditioning. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 26(2):137-41.
12. Huang L., Lin W., and Weng X. (2005). Effects of gene regulation by reactive oxygen species during intermittent hypoxic training. In: Mitochondrial Physiology. The many Faces and Functions of Organelle. Ed.: E.Gnaiger, Schrocken: Vorarlberg, pp. 30-31.
13. Joyeux-Faure M., Stanke-Labesque F., Lefebvre B., Béguin P., Godin-Ribuot D., Ribuot C., Launois S.H., Bessard G., and Lévy P. (2005). Chronic intermittent hypoxia increases infarction in the isolated rat heart. J. Appl. Physiol. 98(5):1691-1696.
14. Katayama K., Ishida K., Iwasaki K.I., and Miyamura M. (2009). Effect of two durations of short-term intermittent hypoxia on ventilatory chemosensitivity in humans. Eur. J. Appl. Physiol. 105(5):815-821.
15. Khan S.A., Nanduri J., Yuan G., Kinsman B., Kumar G.K., Joseph J., Kalyanaraman B., and Prabhakar N.R. (2011). NADPH oxidase 2 mediates intermittent hypoxia-induced mitochondrial complex I inhibition: relevance to blood pressure changes in rats. Antioxid Redox Signal. 15;14(4):533-42.
16. Kim J.W., Tchernyshyov I., Semenza G.L., and Dang C.V. (2006). HIF-1-mediated

expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 3: 177–185.

17. Kondrashova M.N., Fedotcheva N.I., Saakyan I.R., Sirota T.V., Lyamzaev K.G., Kulikova M.V., and Temnov A.V. (2001). Preservation of native properties of mitochondria in rat liver homogenate. Mitochondrion. 1(3):249-267.
18. Koopman W.J., Verkaart S., Visch H.J., van der Westhuizen F.H., Murphy M.P., van den Heuvel L.W., Smeitink J.A., and Willems P.H. (2005). Inhibition of complex I of the electron transport chain causes O2-. -mediated mitochondrial outgrowth. Am. J. Physiol. Cell Physiol. 288(6):C1440-C1450.
19. Kurhaliuk N.M., Serebrovs'ka T.V., and Koliesnikova Ie.E. (2002). Role of cholino- and adrenoreceptors in the effects of intermittent and acute hypoxia on rat liver mitochondrial respiration. Ukr. Biokhim. Zh. 74(6):114-119 [In Ukrainian].
20. Kurhaluk N., Tkachenko H., Nosar V. (2013). The effects of intermittent hypoxia training on mitochondrial oxygen consumption in rats exposed to skeletal unloading. Annals of Clinical & Laboratory Science. 43(1):54-63.
21. Lin A.M., Chen C.F., and Ho L.T. (2002). Neuroprotective effect of intermittent hypoxia on iron-induced oxidative injury in rat brain. Exp. Neurol. 176(2):328-335.
22. Lopata V.A. and Serebrovskaya T.V. (2012). Hypoxicators: Review of the Operating Principles and Constructions. In: Lei Xi & Tatiana V. Serebrovskaya (Eds). Intermittent Hypoxia and Human Diseases. Springer, UK, Chapter 24, pp. 291-302.
23. Lowry O.H., Rosebrough N.J., Farr A.L., and Randall R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193(1):265-275.
24. Lukyanova L.D. (2005). Novel approaches to understanding of molecular mechanisms of adaptation. In: Hargens A., Takeda N., Singal P. K. (Eds). Adaptation Biology and Medicine. Vol.4: Current concepts. New Delhi: Narosa Publ.House; pp. 1-19.
25. Lukyanova L.D., Germanova E.L., Kopaladze R.A. (2009). Development of resistance of an organism under various conditions of hypoxic preconditioning: role of the hypoxic period and reoxygenation. Bull. Exp. Biol. Med. 147(4):400-404 [In Russian].
26. Lukyanova L.D., Kirova Yu. I., and Germanova E.L. (2012). Energotropic Effects of Intermittent Hypoxia: Role of Succinate-Dependent Signaling. In: Lei Xi & Tatiana V. Serebrovskaya (Eds). Intermittent Hypoxia and Human Diseases. Springer, UK, Chapter 20, pp. 239-252.
27. Mankovska I.M., Gavenauskas B.L., Hosar V.I., Nazarenko A.I., Rozova K.V., and Bratus L.V. (2005). Mechanisms of muscular tissue adaptation to intermittent hypoxia.

For Peer Review ONLY/Not for Distribution

For Peer Review ONLY/Not for Distribution

Sport Medicine (Ukr). 1:3-11 [In Ukrainian].

28. Mansfield K.D., Guzy R.D., Pan Y., Young R.M., Cash T.P., Schumacker P.T., and Simon M.C. (2005). Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF-α activation. Cell Metab 1: 393–399.
29. Manukhina E.B., Jasti D., Vanin A.F., and Downey H.F. (2011). Intermittent hypoxia conditioning prevents endothelial dysfunction and improves nitric oxide storage in spontaneously hypertensive rats. Exp. Biol. Med. (Maywood). 236(7):867-873.
30. Mateika J.H., Sandhu K.S. (2011). Experimental protocols and preparations to study respiratory long term facilitation. Respir. Physiol. Neurobiol. 176(1-2):1-11.
31. McGuire M. and Bradford A. (2001). Chronic intermittent hypercapnic hypoxia increases pulmonary arterial pressure and hematocrit in rats. Eur. Respir. J. 18:279–285.
32. Millet G.P., Roels B., Schmitt L., Woorons X., and Richalet J.P. (2010). Combining hypoxic methods for peak performance. Sports Med. 40(1):1-25.
33. Muza S.R. (2007). Military applications of hypoxic training for high-altitude operations. Med. Sci. Sports. Exerc. 39(9):1625-1631.
34. Naryzhnaia N.V., Neckar J., Maslov L.N., Lishmanov Iu.B., Kolar F., and Lasukova T.V. (2009). The role of sarcolemmal and mitochondrial K(ATP)-channels in realization of the cardioprotection and antiarrhythmic effect of different regimens of hypobaric adaptation. Ross. Fiziol. Zh. Im. I.M. Sechenova..95(8):837-849. [In Russian].
35. Nattie E.E., Bartlett D. Jr., and Johnson K. (1978). Pulmonary hypertension and right ventricular hypertrophy caused by intermittent hypoxia and hypercapnia in the rat. Am. Rev. Respir. Dis. 118:653–658.
36. Neckár J., Papousek F., Nováková O., Ost'ádal B., and Kolár F. (2002). Cardioprotective effects of chronic hypoxia and ischaemic preconditioning are not additive. Basic Res. Cardiol. 97(2):161-167.
37. Panisello P., Torrella J.R., Esteva S., Pagés T., and Viscor G. (2008). Capillary supply, fibre types and fibre morphometry in rat tibialis anterior and diaphragm muscles after intermittent exposure to hypobaric hypoxia. Eur. J. Appl. Physiol. 103(2):203-13.
38. Peng Y.J. and Prabhakar N.R. (2004). Effect of two paradigms of chronic intermittent hypoxia on carotid body sensory activity. J. Appl. Physio. 96: 1236–1242.
39. Prabhakar N.R., Kumar G.K. (2004). Oxidative stress in the systemic and cellular responses to intermittent hypoxia. Biol. Chem. 385(3-4):217-221.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

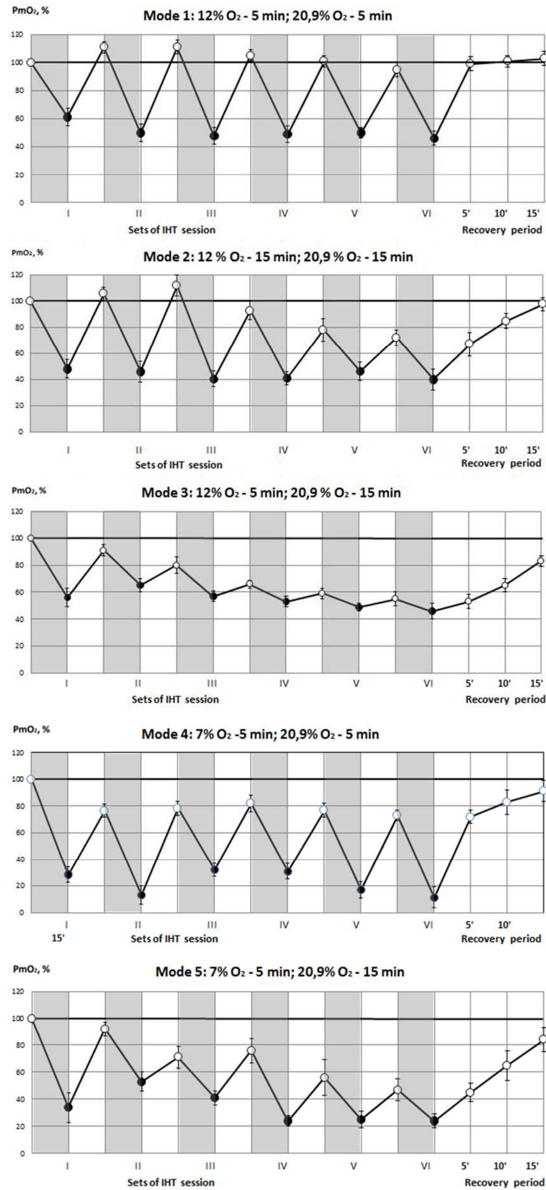
40. Prabhakar N.R., Peng Y.J., Yuan G., Kumar G.K. (2006). Reactive oxygen species facilitate oxygen sensing. Novartis Found Symp.; 272:95-99.
41. Prabhakar N.R., Semenza G.L. (2012). Adaptive and maladaptive cardiorespiratory responses to continuous and intermittent hypoxia mediated by hypoxia-inducible factors 1 and 2. Physiol. Rev. 92: 967–1003.
42. Rozova K., Gonchar O., and Mankovska I. (2012). Benefits and Risks of Different Regimen of Intermittent Hypoxic Training. In: Lei Xi & Tatiana V. Serebrovskaya (Eds). Intermittent Hypoxia and Human Diseases. Springer, UK, Chapter 22, pp. 273-280.
43. Sasaki H., Ray P.S., Zhu L., Otani H., Asahara T., and Maulik N. (2001). Hypoxia/reoxygenation promotes myocardial angiogenesis via an NF kappa B-dependent mechanism in a rat model of chronic myocardial infarction. J. Mol. Cell. Cardiol. 33(2):283-294.
44. Satriotomo I., Dale E.A., Dahlberg J.M., and Mitchell G.S. (2012). Repetitive acute intermittent hypoxia increases expression of proteins associated with plasticity in the phrenic motor nucleus. Exp. Neurol. 237(1):103-115.
45. Saxena S, Shukla D, and Bansal A. Augmentation of aerobic respiration and mitochondrial biogenesis in skeletal muscle by hypoxia preconditioning with cobalt chloride. Toxicol. Appl. Pharmacol. 2012 Nov 1;264(3):324-34.
46. Semenza G.L. (2012). Foreword. In: Lei Xi & Tatiana V. Serebrovskaya (Eds). Intermittent Hypoxia and Human Diseases. Springer, UK, p.V.
47. Serebrovskaya T.V., Swanson R.J., and Kolesnikova E.E. (2003). Intermittent hypoxia: mechanisms of action and some applications to bronchial asthma treatment. J. Physiol. Pharmacol. 54,Suppl 1:35-41.
48. Serebrovskaya T.V., Manukhina E.B., Smith M.L., Downey H.F., and Mallet R.T. (2008). Intermittent Hypoxia: Cause of or Therapy for Systemic Hypertension? Exp. Biol. Med. (Maywood), 233(6):627-50.
49. Serebrovskaya T.V. and Xi L. (2009). Historical Overview of Intermittent Hypoxia Research. In: Intermittent Hypoxia: From Molecular Mechanisms to Clinical Applications/. Editors: Lei Xi & Tatiana V. Serebrovskaya/ Nova Science Publishers, p. 17-33.
50. Vavilova H.L., Serebrovs'ka T.V., Rudyk O.V., Bielikova M.V., Koliesnikova Ie.E., Kukoba T.V., and Sahach VF. (2005). Effect of the hypoxia training on the sensitivity of phenylarsineoxide-induced mitochondrial permeability transition pore opening in the rat heart. Fiziol. Zh. 51(4):3-12 [In Ukrainian].

For Peer Review ONLY/Not for Distribution

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

51. Verkaart S., Koopman W.J., Nijtmans L.G. et al. (2005). Mitochondrial superoxide production to complex I activity in human complex I deficiency. In: Mitochondrial Physiology. The many Faces and Functions of Organelle. Ed.: E.Gnaiger, Schrocken: Vorarlberg, p. 90.
52. Wang J.S., Chen L.Y., Fu L.L., Chen M.L., and Wong M.K. (2007). Effects of moderate and severe intermittent hypoxia on vascular endothelial function and haemodynamic control in sedentary men. Eur. J. Appl. Physiol. 100:127-135.
53. Wilber R.L. (2007). Application of Altitude/Hypoxic Training by Elite Athletes. Med. Sci. Sports. Exerc. 39(9):1610-1624.
54. Xu W.Q., Yu Z., Xie Y., Huang G.Q., Shu X.H., Zhu Y., Zhou Z.N., and Yang H.T. (2011). Therapeutic effect of intermittent hypobaric hypoxia on myocardial infarction in rats. Basic Res. Cardiol. 106(3):329-42
55. Yin X., Zheng Y., Liu Q., Cai J., and Cai L. (2012). Cardiac response to chronic intermittent hypoxia with a transition from adaptation to maladaptation: the role of hydrogen peroxide. Oxid Med Cell Longev. 2012:569520.
56. Zhu W.Z., Dong J.W., Ding H.L., Yang H.T., and Zhou Z.N. (2004). Postnatal development in intermittent hypoxia enhances resistance to myocardial ischemia/reperfusion in male rats. Eur. J. Appl. Physiol. 91(5-6):716-722.
57. Zhukova A.G., Sazontova T.G., and Arkhipenko Ju.V. (2005). Adaptation to intermittent hypoxia and hyperoxia: the increase of heart membranes resistance. In: Hypoxia, mechanisms, adaptation, correction. Proceedings of IY Russian conference. Academy of Medical Sciences, Moscow: Russian, pp. 44-45 [In Russian].
58. Zieliński J. (2005). Effects of intermittent hypoxia on pulmonary haemodynamics: animal models versus studies in humans. Eur. Respir. J. 25(1):173-180.
59. Zoll J., Sanchez H., N'Guessan B., Ribera F., Lampert E., Bigard X., Serrurier B., Fortin D., Geny B., Veksler V., Ventura-Clapier R., and Mettauer B. (2002). Physical activity changes the regulation of mitochondrial respiration in human skeletal muscle. J Physiol. 15;543(Pt 1):191-200.
60. Zong P.., Setty S., Sun W., Martinez R., Tune J.D., Ehrenburg I.V., Tkatchouk E.N., Mallet R.T., and Downey H.F. (2004). Intermittent hypoxic training protects canine myocardium from infarction. Exp. Biol.Med. (Maywood). 229(8):806-812.

For Peer Review ONLY/Not for Distribution

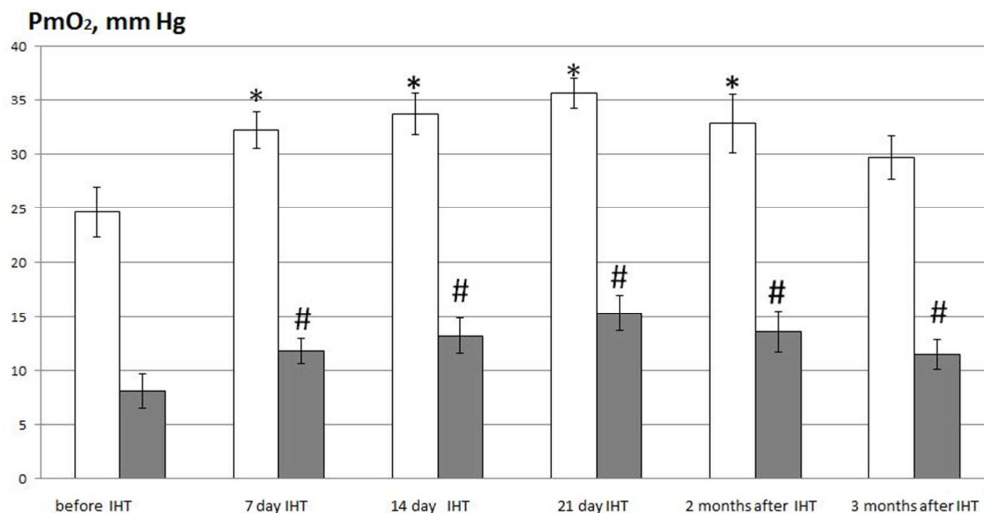


Oxygen tension (PmO2) in rat m. gastrocnemius under one session of 5 different IHT modes.
The initial values are taken as 100%. I-YI – sets of IHT. Gray panels – hypoxic perods; white panels – normoxic periods.

168x363mm (96 x 96 DPI)

For Peer Review Not for Distribution

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60



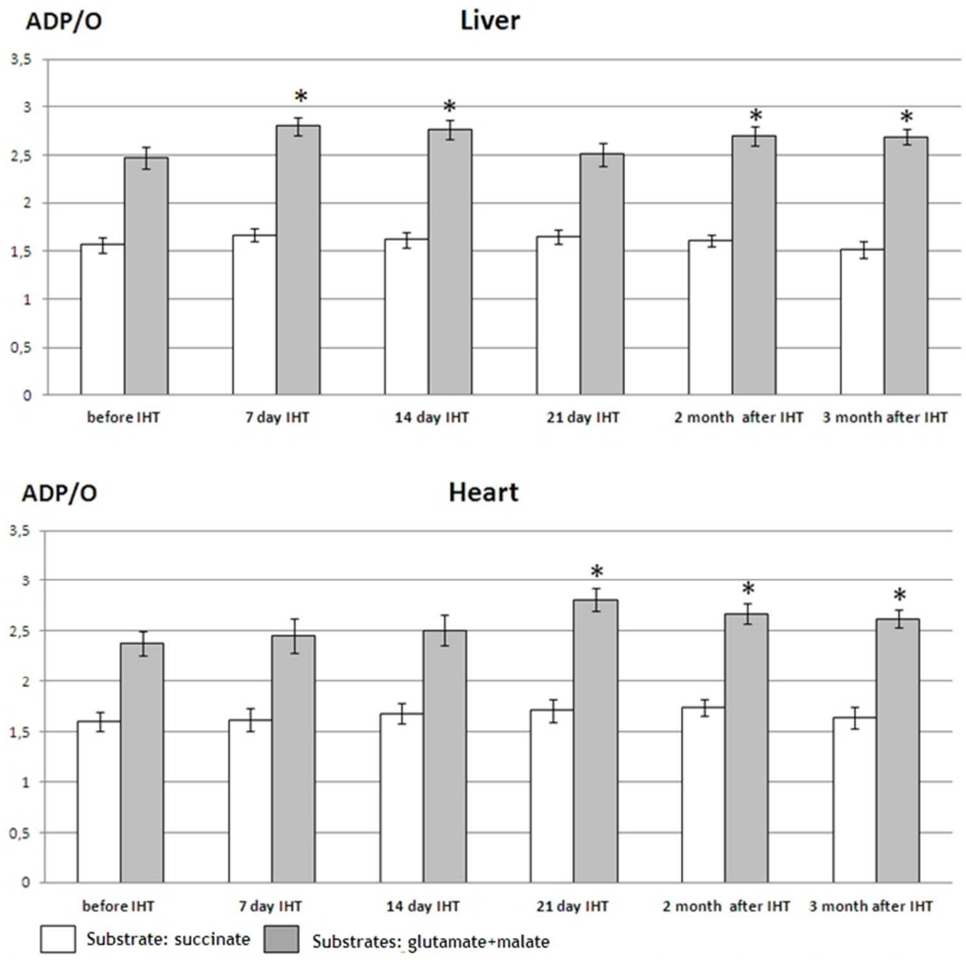
Oxygen tension (PmO2) in rat m. gastrocnemius under acute hypoxia test (breathing with 7% O2, 30 min). White columns – measurements during air breathing; gray columns – measurements at 30th min hypoxia. *, P<0.05, significantly different from initial normoxic value (before IHT); #, P<0.05, significantly different from 30th min hypoxia before IHT.

231x121mm (96 x 96 DPI)

For Review Only/Not for Distribution

For Peer Review, Not For Distribution

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60



205x202mm (96 x 96 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure legends

Figure 1. Oxygen tension (PmO_2) in rat *m. gastrocnemius* under one session of 5 different IHT modes.

The initial values are taken as 100%. I-YI – sets of IHT. Gray panels – hypoxic perods; white panels – normoxic periods.

Figure 2. Oxygen tension (PmO_2) in rat *m. gastrocnemius* during 1, 2 and 3 week IHT in Mode 1 under normoxia and acute hypoxia test (breathing with 7% O_2, 30 min).
White columns – measurements during air breathing; gray columns – measurements at 30th min hypoxia test.
*, $P<0.05$, significantly different from initial normoxic value (before IHT); $^{\#}$, $P<0.05$, significantly different from 30th min hypoxia before IHT.

Figure 3. Effectiveness of O_2 consumption by liver and heart mitochondria under IHT.
White columns characterize ADP/O ratio when using the MEC II substrate succinate (in the presence of rotenone) for oxidation; gray columns – ADP/O ratio when using the MEC I substrates (glutamate+malate) for oxidation.
*, $P<0.05$, significantly different from initial value (before IHT).

For Peer Review ONLY/Not for Distribution

For Peer Review ONLY/Not for Distribution

Table 1. Description of IHT modes

Mode	Group of animals	Hypoxic periods		Normoxic periods	
		O_2 content, %	Duration, min	O_2 content, %	Duration, min
1	Gr. I-1	12	5	20,9	5
2	Gr. I-2	12	15	20,9	15
3	Gr. I-3	12	5	20,9	15
4	Gr. I-4	7	5	20,9	5
5	Gr. I-5	7	5	20,9	15

For Peer Review ONLY/Not for Distribution

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

Table 2. Oxygen consumption of rat liver and heart mitochondria under IHT, Mode 1.

	Liver		**Heart**	
MEC II substrate: succinate (in the presence of rotenone)				
	V_3	V_3/V_4^{ATP}	V_3	V_3/V_4^{ATP}
Gr. II-1: Control (sham IHT)	51.41 ± 2.62	2.58 ± 0.09	54.11 ± 2.22	2.81 ± 0.14
Gr. II-2: 7 days of IHT	43.75 ± 2.0*	2.88 ± 0.08*	52.16 ± 2.76	2.88 ± 0.15
Gr. II-3: 14 days of IHT	45.12 ± 2.37*	2.76 ± 0.07*	50.72 ± 3.15	3.0 ± 0.17
Gr. II-4: 21 days of IHT	51.82 ± 2.84	2.70 ± 0.10	48.08 ± 1.84*	3.36 ± 0.11*
Gr. II-5: 2 months after IHT	48.33 ± 3.11	2.76 ± 0.07*	51.27 ± 2.70	3.16 ± 0.11*
Gr. II-6: 3 months after IHT	50.17 ± 2.83	2.77 ± 0.07*	53.88 ± 3.09	3.09 ± 0.10*
MEC I substrates: glutamate + malate				
Gr II-1: Control (sham IHT)	48.62 ± 2.90	3.12 ± 0.13	50.5 ± 2.20	3.05 ± 0.19
Gr. II-2: 7 days of IHT	58.40 ± 2.46*	3.84 ± 0.17*	53.80 ± 2.71	3.18 ± 0.22
Gr. II-3: 14 days of IHT	59.24 ± 2.38*	3.76 ± 0.14*	51.78 ± 2.54	3.26 ± 0.17
Gr. II-4: 21 days of IHT	53.85 ± 2.70	3.48±0.12*	58.44 ± 2.45*	3.84 ± 0.27*
Gr. II-5: 2 months after IHT	54.72 ± 3.13	3.42 ± 0.11*	55.16± 3.07	3.66 ± 0.17*
Gr. II-6: 3 months after IHT	51,18 ± 2.84	3.37 ± 0.10*	51.74 ± 2.94	3.45 ± 0.18*

Values are means ± SD.
V_3, oxygen consumption stimulated by ADP; V_4^{ATP}, oxygen consumption after cessation of ADP phosphorylation; V_3/V_4^{ATP}, respiratory control ratio.
(*) - statistical difference comparing to Gr II-1, $p < 0.05$.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

Table 3. Oxygen consumption of rat liver and heart mitochondria under IHT, Mode 2.

***MEC II* substrate: succinate (in the presence of rotenone)**

	Liver		Heart	
	V_3	V_3/V_4^{ATP}	V_3	V_3/V_4^{ATP}
Gr.II-7: Control (sham IHT)	58.24 ± 2.48	2.97 ± 0.12	65.54 ± 3.18	2.22 ± 0.12
Gr.II-8: 21 days of IHT	52.17 ± 3.05*	3.12 ± 0.10	79.86 ± 3.84*	2.43 ± 0.11

***MEC I* substrates: glutamate + malate**

	Liver V_3	Liver V_3/V_4^{ATP}	Heart V_3	Heart V_3/V_4^{ATP}
Gr.II-7: Control (sham IHT)	53.76 ± 2.36	3.48 ± 0.11	50.5 ± 2.2	3.24 ± 0.13
Gr.II-8: 21 days of IHT	58.87 ± 2.40*	3.61± 0.12	58.44 ± 2.45*	3.82 ± 0.12*

Values are means ± SD.
V_3, oxygen consumption stimulated by ADP; V_4^{ATP}, oxygen consumption after cessation of ADP phosphorylation;
V_3/V_4^{ATP}, respiratory control ratio.
(*) - statistical difference comparing to Gr.II-7, $p < 0.05$.

For Peer Review ONLY/Not for Distribution

View publication stats