

# Mitochondria as a Target of Intermittent Hypoxia

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**ABSTRACT:** The review is focused upon summarizing the current knowledge of the mechanisms of interval hypoxic training (IHT) impact on the mitochondria (Mt) structure and functions in comparison with the effects of acute hypoxia (AH). It has been revealed that AH causes mitochondrial swelling, vacuolization of organelles, disorganization and destruction of mitochondrial membranes. When exposed to IHT, the increase in the total number of mitochondria, the reduction of the number of structurally modified organelles, the appearance of energetically active Mt with vesicular cristae, and the micromitochondria (microMt) formation are observed. One of the key mechanisms of cell damage during hypoxia and reoxygenation is excessive production of reactive oxygen species (ROS) in the mitochondria which oxidize proteins, lipids and DNA. On the other hand, low level of ROS production is protective and serves as a trigger for adaptive responses. IHT leads to reprogramming of the mitochondria metabolism, ensuring adequate production of ATP. The activation of potassium transport in the mitochondrial matrix under IHT is a protective mechanism against  $Ca^{2+}$  overload caused by acute hypoxia. The intensity of neuronal mitochondrial energy production in the brain stem is directly related to the regulation of neurotransmitter metabolism, including glutamate and GABA, which are involved in the mechanisms of respiratory rhythmogenesis formation. All adaptive reactions to hypoxia are regulated by HIF-factors (HIF-1, HIF-2, HIF-3). Each of HIF-subunits plays a certain role depending on the mode of hypoxia-induced stress. These peculiarities can be important when choosing a mode of IHT to prevent and treat various diseases. New data on the organ specificity of HIF operation provide potential pharmacological regulation of HIFs as a new therapeutic approach for treatment of diseases.

**KEY WORDS:** mitochondrial dysfunction, intermittent hypoxia, morphology of mitochondria, free radical processes, glutamatergic system, hypoxia-inducible factor

## I. INTRODUCTION

Recent discoveries have demonstrated the key role of mitochondria in physiology and pathology of humans and animals, putting these sub-cellular structures, organized in a complicated way, in the center of biomedical research worldwide and initiating the development of new areas, such as mitochondrial physiology,<sup>1</sup> mitochondrial pharmacology,<sup>2</sup> mitochondrial medicine,<sup>3,4</sup> and mitochondrial genetics.<sup>5</sup> Mitochondria are the “gate-keepers” of life and death in the cell, they regulate energy metabolism, cell signaling and differentiation, redox balance, and ion homeostasis.<sup>6</sup>

During evolution, aerobic organisms have developed extremely sophisticated and harmonious cell system to respond to changes in extracellular oxygen concentration,

so far as molecular oxygen acts as a terminal electron acceptor in the mitochondrial respiratory chain for energy production in the process of oxidative phosphorylation. The respiratory chain of the mitochondria responds to changes in the extracellular oxygen content, initiating a cascade of intracellular functional and metabolic reactions that form the total response to these changes.<sup>7,8</sup> Inadequate oxygen supply results in dysfunction of mitochondrial system, which in turn serves as a leading basic molecular mechanism of cell response to the lack of oxygen. Mitochondrial dysfunction during hypoxia leads to accumulation of intermediates of lipid and carbohydrate metabolism in the cytoplasm, calcium accumulation, release of cytochrome *c* from the mitochondria, and development of cell apoptosis. Under the conditions of hypoxia, mitochondrial respiratory chain is the main intracellular source of reactive oxygen species (ROS) generation, excessive formation of which may disturb metabolic processes, the structure of proteins, and mitochondrial genome.<sup>9,10</sup> Thus, ROS act as major mediators of cell damage during hypoxia. Mitochondrial dysfunction induced by oxygen deprivation of the tissues is a mandatory component of most pathological processes in the body.

On the other hand, it is known that adaptation to interval hypoxic stimulation causes positive changes in the mitochondrial apparatus of the cells. The latter manifests itself as restructurization of the tissue energy supply due to more economical use of oxygen, stabilization of mitochondrial membranes, etc.<sup>7,10,11</sup> The mechanisms of adaptation to intermittent hypoxia enable the body not only survive in conditions of acute shortage of oxygen, but also increase body resistance to emotional stress, intense exercise, etc. Intermittent hypoxic training (IHT) implements its anti-hypoxic effect by stimulating its own endogenous defense mechanisms at all levels - from genes to the whole organ or tissue. For example, we know ROS as intracellular messengers that regulate a variety of cellular processes via an activation of antioxidant enzymes transcription factors, regulatory and protective proteins.<sup>12,13</sup> It is ROS that act as trigger of a cascade of intracellular redox signaling with a subsequent activation of redox-sensitive transcription factors and genes regulating the synthesis and migration of regulatory components in the cell.<sup>14</sup> In the last decade, new ways of mitochondrial signaling have been explored, including the release of metabolites, mitochondrial dynamics and mobility, interaction with organelles such as the endoplasmic reticulum.<sup>23</sup> It has been established that mitochondria-dependent signaling has divergent physiological and pathophysiological implications.

Until recently, the studies on intermittent hypoxia in Western Europe and North America were mainly focused on the negative effects of chronic intermittent hypoxia associated with sleep apnea syndrome. For example, Nanduri et al.,<sup>16</sup> Prabhakar and Semenza<sup>17</sup> believe that intermittent hypoxia almost always causes multiorgan pathology, biochemical violations, and changes in gene expression patterns. However, over the last decade, the gap in views between East and West is gradually decreasing, and common understanding in this area is becoming clearer.<sup>18,19</sup>

The question arises, what is the key mechanism that determines adaptive or maladaptive nature of different paradigms of intermittent hypoxia, which molecular

pathways mediate pathological or physiological response to hypoxia. Currently, there is no exact evidence concerning the mechanisms of switching adaptive to maladaptive response to hypoxic stimulus. Several attempts have been made to explore this question.<sup>7,20,21</sup> The most significant contribution to this point Prabhakar and Semenza<sup>17</sup> made, having described the transcriptional regulation of gene expression that is mediated by hypoxia-inducible factors 1 and 2 (HIF-1 and HIF-2). The basic idea of the authors is that HIF-signaling is not a linear process, but a complex network of perhaps hundreds of input stimuli and thousands of potential output stimuli, each of which represents different target genes. The discovery of HIF family allowed us to take a fresh look at the molecular basis of adaptive and maladaptive responses at the cellular and systemic level under the influence of continuous and intermittent hypoxia. However, until now the authors cannot answer the practical question, what dose and mode of hypoxic stimulation is the most advantageous for animals and humans.

In this review, we will get a view of the action of intermittent hypoxia on mitochondrial structure and function in different tissues, as well as the possibility of intermittent hypoxia to prevent mitochondrial dysfunction in different hypoxic states, focusing on recent works made in the Department for the Study of Hypoxic States, O.O. Bogomolets Institute of Physiology, National Academy of Sciences of Ukraine.

## **II. MORPHOLOGICAL CHANGES IN MITOCHONDRIA UNDER DIFFERENT TYPES OF HYPOXIA**

It should be noted that the effect of intermittent hypoxia on the mitochondrial ultrastructure is not very explored. In the literature, there are only a few reports, which state that intermittent hypoxia (stay at the altitude of 5000 m, 6 hours a day for 28 days) prevents the development of disorders in ultrastructure of the myocardial mitochondria and deletion of mtDNA in cardiomyocytes that occur during ischemia-reperfusion.<sup>22</sup>

In a series of experimental works on rats performed by Rozova<sup>23,24</sup>, it has been revealed that acute hypoxic hypoxia (breathing with gas mixture containing 7% oxygen in nitrogen for 30 min) caused swelling of the mitochondria of different degrees in lung tissues and the myocardium, partial or complete vacuolization of organelles, violation of cristae regularity, swelling of inter-cristae gaps, disruption and destruction of mitochondrial membranes (more often internal, sometimes external). In some mitochondria the formation of small optically dense structures was observed, surrounded by a membrane sheath (micromitochondria).

The most severe mitochondrial dysfunction is observed in the lung tissue under respiratory hypoxia that develops in experimental pneumonia: melting of cristae, full vacuolization, and violations of mitochondrial membrane integrity (both internal and external). The total number of lysosomes increases, especially the secondary ones, tightly adjacent to the mitochondria, which indicates strengthening of degenerative processes in them, as well as intense and less economical functioning of the mitochondria.

Significant heterogeneity in the changes in mitochondria is also observed in the myocardium under acute hypoxia. Pronounced mitochondrial fission on organelles occurs in different energy-dependent states, which may indicate the presence of urgent adaptive changes in the heart muscle to maintain adequate energy supply in the changed conditions. Moreover, direct mitochondrial fission is frequently observed, which is the characteristic of acute hypoxic influence. Calcium precipitates are also formed on the inner membrane of the mitochondria.

Unlike acute hypoxia, changes in the morphological and functional state of the tissues exposed to IHT have mostly compensatory nature.<sup>25</sup> Regarding the heart tissue, this conclusion is based on the fact that in less severe forms of hypoxia-induced edema, the total number of mitochondria increases, while the number of structurally altered organelles is reduced twice, and energetically active mitochondria with vesicular cristae appear. In the lung and the heart mitochondria with altered ultrastructure the micromitochondria are formed with a diameter of 10 – 15 nm. This process occurs only in case of hypoxic hypoxia.

These results can be viewed from the perspective that IHT significantly improves the control of mitochondrial quality which is regulated by balance between the biogenesis and autophagic destruction of the mitochondria. Otherwise, this quality control is achieved by the establishment of fine balance between the elimination of damaged and dysfunctional mitochondria through autophagy (mitophagy) and the generation of new and “healthy” mitochondria through the processes of biogenesis, fusion and fission.<sup>26</sup> In general, the identification of molecular and cellular mechanisms of mitochondrial biogenesis and the auto / mitophagy now is the most important topic of mitochondriology.

According to these studies one can conclude that acute hypoxic hypoxia causes organ-specific destructive changes in mitochondrial ultrastructure of pulmonary and cardiac tissues. The most vulnerable is the lung tissue and the most resistant to different types of hypoxia is the myocardium. The most efficient IHT mode for positive ultrastructural changes is the inhalation of hypoxic gas mixture with 12% O<sub>2</sub>, 5-min exposures with 15-min normoxic breaks).

### **III. THE ROLE OF FREE RADICAL PROCESSES IN THE FORMATION OF MITOCHONDRIAL DYSFUNCTION AND IN ADAPTING TO IHT**

As discussed earlier, reactive oxygen species (ROS) play a key role in important cellregulatory mechanisms acting not only as damaging agents but also as physiological messengers that trigger different signaling cascades. They are original, sensitive to the redox status link of redox signaling that ensures signal transmission from the site of ROS generation to cell nucleus.<sup>27</sup> Lack of oxygen impairs energy metabolism and stimulates free radical oxidation. The activation of these processes increases energy deficit due to damaging the mitochondrial and lysosomal membranes and forming “vicious circle” which finally can cause irreversible damage and cell death.

On the other hand, in the mechanisms of protection from the negative ROS effects the antioxidant reactions are leading and the most powerful since they not only prevent the development of free radical reactions, accumulation of superoxide anions and peroxides, but also maintain a high activity of redox processes, ensure the elimination of final oxygen metabolites with further involving them in energy metabolism, and promote the activity of synthetic processes.<sup>28</sup>

The degree of oxidative processes impairment in the mitochondria (the intensity of lipid peroxidation (LPO) and oxidative modification of proteins) under acute hypoxic hypoxia have being actively studied in the Department of Hypoxic States of Bogomolets Institute of Physiology.<sup>25,29</sup> According to these investigations, the exposure of rats to acute hypoxia (gas mixture of 7% O<sub>2</sub>) results in LPO intensifying, an increase in oxidized (GSSG) and a decrease in reduced (GSH) glutathione content, an increase in oxidation-modified proteins. A high negative correlation was found between the oxidative changes in the mitochondria and indices of oxidative phosphorylation as well as between oxidative processes, antioxidant glutathione enzymes, and the level of Mn-SOD protein and mRNA expression<sup>30</sup> This suggests a key role of ROS in both the formation of mitochondrial dysfunction in acute hypoxia and in regulation of response of antioxidant systems to oxidative stress.

An exposure to IHT evokes various changes in prooxidant-antioxidant status of the mitochondria in different tissues. In experiments, IHT was shown to evoke a significant increase in antioxidant enzymes activity (superoxide dismutase, catalase, glutathione reductase) in the myocardium, while the enhancement of such activity in the brain and the liver was less pronounced.<sup>31,32</sup> In patients with coronary heart disease and children with bronchial asthma, an exposure to IHT caused a decrease in lipid peroxidation and the increase in superoxide dismutase, catalase and glutathione reductase activity.<sup>33-35</sup>

An introduction of hyperoxic component to the training mode made it possible to achieve more rapid positive results.<sup>30</sup> Interval hypoxic / hyperoxic training (IHHT) make it possible to raise the level of ROS signal without increasing adverse effects of prooxidants.<sup>32</sup>

It has been reported<sup>36</sup> that IHHT increases the efficiency of oxidative phosphorylation, decreases the hyperactivation of MnSOD and intensifies its protein expression in the rat myocardial mitochondria following acute hypoxic test.

Summarizing all above, we can conclude that the use of IHT and especially IHHT to prevent oxidative disorders in the mitochondria is effective procedure that substantially increases the resistance of the cellular structures to acute hypoxia. The use of such training greatly inhibits the development of mitochondrial dysfunction under severe hypoxia, lowers oxidative damage of mitochondria, and increases the capacity of endogenous antioxidant system.

#### IV. MITOCHONDRIAL RESPIRATORY FUNCTION UNDER MITOCHONDRIAL DYSFUNCTION AND ADAPTING TO IHT

It has been proved that dysfunction of mitochondrial enzymes, especially mitochondrial enzyme complex I, is the basis of any form of hypoxia and the molecular mechanism that determines energy disorders in terms of limiting oxygen delivery to cells.<sup>7</sup> Restoration of electron transport function of the respiratory chain in hypoxia is an important task of anti-hypoxic protection which ensures creating anti-hypoxic means of ergotropic action.

It is known that enzymes for ATP synthesis and ion transport systems as well as electron carriers of the respiratory chain are localized on the inner mitochondrial membrane and directly involve in energy production. More recently, mitochondrial involvement in the regulation of cell calcium homeostasis and maintaining of physiologically required content of cytosolic calcium has been reported.<sup>37,38</sup> Under physiological conditions, the opening of recently discovered mitochondrial ATP-sensitive potassium channels occurs at a sharp decrease in intracellular ATP concentration, for example, in ischemia.<sup>39</sup> It has been proved that potassium ion transport, activated by potassium ATP channel opening, is a powerful modulator of basic mitochondrial functions: oxygen consumption,<sup>39,40</sup> generation of proton gradient and transmembrane potential,<sup>41</sup> synthesis<sup>42</sup> and hydrolysis of ATP,<sup>43</sup> in other words, all the main characteristics of the energy state of mitochondria.

Moreover, it was shown that an activation of adenosine triphosphate-sensitive potassium channels ( $K_{ATP}$ -channels) is a protective mechanism against mitochondrial overload by calcium ions, which is one of the causes of mitochondrial dysfunction.<sup>44</sup> A series of works made at Bogomolets Institute of Physiology deals with exploring the role of mitochondrial ATP-dependent potassium channels in the regulation of functional characteristics of mitochondrial respiratory chain in pathological conditions involving mitochondrial dysfunction.

During the development of experimental mitochondrial dysfunction caused by prolonged immobilization stress, rotenone injection or acute hypoxic exposure, the rate of mitochondrial respiration decreases in different tissues (liver, brain, periodontal). It is more significantly manifested under  $\alpha$ -ketoglutarate (NAD-dependent substrate of respiratory chain) oxidation, in other words, there is a tendency to limit the role of the NAD-dependent substrates in total oxidation.<sup>45-47</sup> This confirms the Lukyanova's idea<sup>7</sup> that just a decrease in the functioning of mitochondria on the substrate, not on the terminal part of the respiratory chain triggers the violation of oxygen utilization.

In the study of IHT effects on mitochondrial respiration using different oxidation substrates,<sup>11,45-47</sup> it was shown that changes in the functioning of liver mitochondria are associated with increased Chance respiratory control and ADP / O coefficient, increased mitochondrial respiration rate in active metabolic state under respiration and phosphorylation uncoupling, and decreased activity of succinate dehydrogenase. Positive changes are observed within 2 months after the last session of IHT. These data are consistent with the recent studies<sup>48-50</sup> indicating that intermittent hypobaric

hypoxia significantly increases the stability of mitochondrial energy metabolism of the myocardium against ischemia-reperfusion injury, anoxia-reoxygenation or intense exercise.

An important regulator of respiratory chain functioning and calcium-accumulating mitochondrial systems is transport of potassium ions. It has been reported that there is an inverse relationship between changes in calcium transport characteristics (initial rate of calcium accumulation,  $V_o$  and calcium capacity) and  $K^+$  content in the incubation medium: an increase in potassium concentration drastically inhibits calcium uptake by the mitochondria.<sup>51</sup> However, with increasing  $K^+$  concentration its input from the medium to the matrix increases. We can predict that the activation of potassium transport in the mitochondrial matrix under IHT provides a protective mechanism against  $Ca^{2+}$  overload. Changes in mitochondrial  $K^+$ (ATP)-channel activity are an important part of the mechanism of mitochondria regulation that are accompanied by a decrease in negative consequences of mitochondrial dysfunction under hypoxia.

## **V. EFFECTS OF DIFFERENT MODES OF INTERMITTENT HYPOXIA ON TISSUE OXYGENATION AND MITOCHONDRIAL RESPIRATION**

In recent decades, spreading of hypoxic training / treatment methods in clinic, sports and military practice led to a vivid discussion about the most effective modes of IHT. Traditional normobaric protocols include alternating of breathing hypoxic gas mixtures and periods of breathing ambient air or hyperoxic mixture (30%  $O_2$ ). To implement various kinds of hypoxic training, a lot of devices were developed, including hyperbaric chambers, rooms with low oxygen content in the air, individual hypoxicators that produce hypoxic air in a number of ways.<sup>52-54</sup> Hypoxic schemes used for investigation of IHT adaptation vary widely: from 3 – 12 brief hypoxic sessions for 2 – 10 min each with normoxic intervals of 2 – 20 min within 7 – 30 days to hypoxic effects lasting 1 – 12 hours for 2 – 90 days. To determine which type of treatment is more fruitful, various experiments on animals and people surveys have been made.<sup>55-62</sup>

The most informative indicators of hypoxic effects on the body is the degree of tissue oxygenation and tissue respiration. We compared the results of five modes of intermittent hypoxia training (IHT) on gastrocnemius muscle  $Po_2$  and heart and liver mitochondrial respiration in rats.<sup>47</sup> The most effective was the mode of breathing with 12%  $O_2$  gas mixture for 5 min, interrupted by 5-min intervals of air breathing, 5 – 6 times a day for 2 or 3 weeks, depending on the purpose of training / treatment. This mode results in the minimum reduction of muscle  $PO_2$  at the end of each hypoxic period and its quick recovery at breathing with the room air. Two-week course of IHT in this mode increased basal tissue oxygenation during normoxia and acute hypoxic test (breathing with 7%  $O_2$  gas mixture for 30 min). In addition, adaptation to IHT in 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 adaptation

occurred after 14 days of IHT in liver tissue, but after 21 days in myocardium, and was preserved during the 3 months following IHT termination. These pilot studies may be useful in the development of hypoxic training regimes for different categories of healthy people and patients with various diseases.

## **VI. FEATURES OF GLUTAMATERGIC SYSTEM FUNCTIONING IN THE BRAINSTEM RESPIRATORY STRUCTURES IN MITOCHONDRIAL DYSFUNCTION AND ITS CORRECTION BY IHT**

Changes in mitochondrial energy status of the brainstem neurons under mitochondrial dysfunction are of particular interest. There is a possibility that the intensity of mitochondrial energy production by the medulla oblongata neurons can be directly related to the regulation of the metabolism of certain neurotransmitters, namely glutamate and its biochemical derivative GABA, the major excitatory and inhibitory agents involved in the mechanisms of respiratory rhythmogenesis formation.<sup>63</sup> Glutamate and GABA metabolism depends on the production of macroergs.<sup>64</sup> A series of papers by Kolesnikova and coauthors<sup>45,65</sup> is devoted to this issue. The authors used a model based on the ability of toxic agent rotenone to cause blockage of mitochondrial respiratory chain complex I by binding to PPST-subunit of multipolipeptide complex of NADH-ubiquinone reductase. It has been shown that mitochondrial dysfunction of the brainstem neuronal structures is associated with lower peak and frequency of diaphragm electromyographic activity and energy metabolism of neurons, including a decrease in phosphorylation rate which eventually determines reduced brain sensitivity to hypoxic stimulation. An exposure of animals to moderate hypoxia for 30 minutes exerted the paradoxical stimulatory effect on respiration rate and coupling of oxidation and phosphorylation. Blockade of glutamate NMDA-receptors in the brainstem neurons helped raise the frequency of electromyographic activity, considered as a result of the abolition of the participation of these receptors and involvement of AMPA-receptor system in the mechanisms of central respiratory rhythm generator. The survey results indicate that the energy status of the brainstem neuronal mitochondria is probably one of the potential triggers of the respiratory activity formation in the respiratory rhythm generator for the central axis of the main neurotransmitters, namely "Glutamate-GABA". The use of IHHT contributed to optimization of the processes of oxidation and phosphorylation coupling and efficiency of the oxygen use in brain stem neurons mitochondria which eliminates metabolic dysregulation of main neurotransmitters that are involved in the formation of respiratory rhythmogenesis.

## **VII. PARTICIPATION OF HYPOXIA INDUCIBLE FACTOR IN ADAPTATION TO IHT**

Reaction of cells to a lack of oxygen is of particular importance for understanding

pathological processes in the body. Several years ago it became known that the most important role in this process plays oxygen-sensitive protein complex with transcriptional activity, named hypoxia-inducible factor (HIF). Numerous reviews in recent years indicate that this problem attracts attention of physiologists, geneticists and clinicians. We will focus on some recent reports about the involvement of HIF in mitochondrial dysfunction and the impact of intermittent hypoxia on these processes.

HIF is a heterodimer transcriptional complex which consists of oxygen-dependent  $\alpha$ -subunits [HIF-1 $\alpha$ , HIF-2 $\alpha$ , or HIF-3 $\alpha$ ] and oxygen-independent  $\beta$ -subunit. HIF-2 $\alpha$  shows functional similarities to HIF-1 $\alpha$ , which has been investigated in detail, while the features of HIF-3 $\alpha$  expression and functional role in different tissues under hypoxic and normoxic conditions are poorly known. In addition, in the literature, there is mostly evidence for the activation of HIF proteins by hypoxia, but information regarding mRNA expression of different HIF subunits is very limited.

Recently it has been shown that HIF-1 plays a critical role in regulating the production of oxygen free radicals in the mitochondria through different mechanisms: direct – regulation of biosynthesis and autophagy of the mitochondria, the restructuring of expression pattern of cytochrome c oxidase subunits, and indirect – regulation of expression of pyruvate dehydrogenase kinase-1 (PDK-1), which phosphorylates and inactivates prolyl hydroxylase.<sup>17</sup> In the study of mitochondrial metabolism regulation via HIF-1, it has been shown that during hypoxia and, thus, enhanced ROS mitochondrial production the, expression of HIF-1 $\alpha$  and its target genes increases.<sup>20,66</sup>

So, on the one hand, HIF-1 causes the development of cellular adaptation to hypoxia through active reduction of oxygen consumption in the mitochondria via PDK-1, which in hypoxic conditions stimulates glycolytic processes in the cell and starts up the process of autophagy through BNIP-3.<sup>67,68</sup> On the other hand, HIF-1 affects the expression of miR-210 that can reduce the severity of apoptosis and regulate the expression of cytochrome oxidase subunit COX-4, related to ATP production, the rate of oxygen consumption, and ROS generation in the mitochondria. Obviously, the disruption of HIF system operation by different stress factors can cause the development of mitochondrial dysfunctions. However, the mechanisms of these effects remain to be elucidated.

In particular, it has been revealed that suppression of mitochondrial genes by doxorubicin affects energy-sensitive molecules such as ATP-activated protein kinase (AMPK), HIF-1, nuclear respiratory factor 1 (NRF-1), and proliferator-activated receptor  $\gamma$ -co-activator-1 $\alpha$  (PGC-1 $\alpha$ ).<sup>69,70</sup> Proteomic analysis allowed to show successive changes in proteins included in mitochondrial energy production and antioxidant defense.<sup>71</sup> This analysis also proved that expression of at least 9 proteins involved in mitochondrial energy metabolism very clearly increased during intermittent hypoxia.<sup>49</sup>

In the Department for the Study of Hypoxic States of the Bogomolets Institute of Physiology on the basis of a comprehensive study of the distribution of messenger RNA of all HIF subunits in various rat organs in normoxic and hypoxic conditions,

new data on changes in the levels of HIF-1 $\alpha$ , HIF-1 $\beta$ , HIF-2 $\alpha$ , and HIF-3 $\alpha$  mRNAs have been received.<sup>72,75</sup> Real time PCR made it possible to establish for the first time a significant increase in HIF-3 $\alpha$  mRNA expression in the heart, lungs and kidneys of rats exposed to IHT.

In order to explain the possible reaction of increasing just HIF-3 $\alpha$  mRNA expression, we made attempts to find sensitive to hypoxia elements (sequence HRE-A / GCGTG) in the promoters of such rat genes: ERO, VEGF, IGF-2 (which are already well aware that their expression is regulated by HIF) and HIF-3 $\alpha$ . Search results made it possible to assume that it is the expression of HIF-3 $\alpha$  gene can be controlled by other HIF  $\alpha$ -subunits. Thus, HIF-1 $\alpha$  and HIF-2 $\alpha$  proteins can stimulate the expression of messenger RNA of HIF-3 $\alpha$  gene under hypoxia.

Thus, increased HIF-3 $\alpha$  mRNA expression in acute hypoxia and IHT, to some extent, may be due to the presence in the promoter of this gene an element sensitive to hypoxia. This increase serves as a marker of HIF mRNA transcription factors, and of their target gene erythropoietin in response to even the mild hypoxia. In literature, there are a few details about what molecular mechanisms are involved in the induction of HIF-1 $\alpha$  expression in the intermittent hypoxia, including ROS generation through NADPH-oxidase, ROS-dependent calcium signaling pathway that includes an activation of phospholipase C- $\gamma$  and a group of kinases: protein C, mTOR, and S6-kinase. As a result, the increase in mTOR-dependent synthesis of HIF-1 $\alpha$  and the reduction of prolyl hydroxylase-dependent HIF-1 $\alpha$  degradation contribute to the accumulation of HIF-1 $\alpha$  during adaptation to intermittent hypoxia.<sup>76</sup>

Portnichenko et al.<sup>77,78</sup> investigated the changes in oxygen consumption, body temperature, HIF-1 $\alpha$  and HIF-3 $\alpha$  gene expression in the lungs of young and mature rats, adapted to the effects of chronic hypoxia at an altitude of approximately 2100 m, and the adaptation to intermittent hypoxia in the barochamber. Four phases of IHT-induced physiological changes were identified. The first phase, hypometabolic (1 – 3 sessions), is characterized by reduced oxygen consumption, reduced body temperature, as well as induction of HIF-1 $\alpha$  and HIF-3 $\alpha$ . In the second, transitional phase (sessions 3 – 4), rearrangement of metabolism and reduction of hypoxic reactivity occurs. The third phase, hypermetabolic (4 – 5 sessions), is characterized by increased energy metabolism and compensation of hypoxic disorders. The fourth phase (after the 5<sup>th</sup> session) is a state of metabolic adaptation with normalization of oxygen consumption and body temperature, HIF-1 $\alpha$  and HIF-3 $\alpha$  expression, mitochondrial respiration, NAD-dependent oxidation of carbohydrate and lipid substrates. An exposure to IHT resulted in transcriptional activation of HIF-1 $\alpha$  gene in the lungs and contributed to the rapid recovery of metabolism of 6-month-old rats. In the 12-month-old animals, forming of HIF-3 $\alpha$ -mediated protective mechanisms in the lungs and slow recovery of metabolic processes were observed.

The regularities of reaction of different HIF subunits to acute and intermittent hypoxia may be important when choosing IHT protocols to prevent and treat various diseases, as well as to train athletes. In addition, new data on the HIF functions in energy metabolism (due to organ specificity) clarify the possibility of pharmacological

regulation of HIF as a new therapeutic approach to the treatment of many diseases, including cancer, diabetes, fatty liver, etc.<sup>20</sup>

Thus, detection of molecular, genetically determined mechanisms of adaptation to intermittent hypoxia is a fundamental aspect of physiology and pathophysiology, which could serve as a potential new therapeutic approach to the treatment of human diseases associated with the effect of chronic hypoxia of different genesis. The studies have revealed patterns of mitochondrial dysfunction in various tissues of animals and humans and their relationship with disorders of systemic, tissue, cellular, and genetic mechanisms of oxygen delivery and utilization in the mitochondria. This provides the basis for further improvement of IHT methods considering the individual characteristics of each patient's body.

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