Influence of skilled athletes’ altitude training on erythropoiesis and angiogenesis processes

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Summary
The influence of altitude training exercises on the content of the HIF-1α factor induced by hypoxia, vascular endothelial growth factor (VEGF), and erythropoietin (EPO) in blood serum of athletes specialized in cyclic sports is studied in the article. The system approaches adaptation under the conditions of mountains, the analysis of compensatory resources, and an increase in the level of resistance of organism under the action of stressors at various levels (hypoxia, high temperature and humidity, violation of biological rhythms, etc.) as well as the search for new criteria of estimation and prediction of physical workability – the ones of the actual questions concerning estimation of efficiency and increase in the individualization of athletes training process. The HIF-1α factor controls a number of genes involved in various cellular functions, including the glycolysis, proliferation, and differentiation of cells. The HIF-1α-dependent activation of the expression of the gene of VEGF, which is the principal angiogenic factor responsible for the growth of new blood vessels, is critically crucial for skeletal muscles. The synthesis of EPO, which is a key regulator of erythropoiesis, is also connected with enhanced expression of HIF-1α.

The purpose of the work was the construction of an algorithm for the markers use of individual skilled athletes’ adaptation to altitude trainings.

Methods. Studies were executed with participation of 12 skilled athletes representing a cyclic sport before and after trainings in medium-altitude mountains (17 days). Levels of HIF-1α, VEGF, EPO, cortisol, and testosterone in blood serum were studied by the immunoenzymatic method with the use of reagents collections from the firm “VectorBest” (Russia). Measurements of concentrations of the indicated factors were executed on photometer “Sunrise” (Tecan, Austria). Indicators of hematologic and biochemical homeostases were studied, respectively, with the help of automatic hematologic analyzer “ERMA PCE-210” (ERMA Inc., Japan) and semiautomatic biochemical analyzer “HUMALYZER 3000” (Human Ltd., Germany).

Results. Obtained data show an increase in the amount of erythrocytes and the concentration of haemoglobin after trainings in medium-altitude mountains. We also found a noticeable decrease in the mean volume of erythrocytes and an increase in the mean concentration of haemoglobin in one erythrocyte, which indirectly indicates the improvement of the oxygen-transporting function of blood. The anabolic index shows the tendency of decreasing. It was also revealed that the trainings under the conditions of hypoxia lead to the increase of VEGF in athletes relative to the input level (p<0.05). The concentrations of HIF-1α and EPO in blood serum have increasing tendency. With regard for the remarkable individual variations in data, athletes were divided into two groups by the input level (before the beginning of trainings) of HIF-1α. The first group included athletes, whose level of HIF-1α was less than 1.0 ng⋅ml⁻¹ (from 0.51 ng⋅ml⁻¹ to 0.81 ng⋅ml⁻¹). Athletes with the level of HIF-1α higher than 1.0 ng⋅ml⁻¹ were referred to the second group. Before the beginning of trainings in medium-altitude mountains, the concentrations of HIF-1α and VEGF in and the anabolic indices of athletes from the first and second groups (p<0.05) were essentially different. After the trainings, athletes with initial concentrations of HIF-1α less than 1.0 ng⋅ml⁻¹ manifested a higher level of VEGF and a larger increase in the concentration of EPO relative to the athletes, whose initial level of HIF-1α was larger than 1.0 ng⋅ml⁻¹.

Conclusion. The determination of such modern markers allows one to estimate the functional state of athlete’s organism, to find the individual peculiarities of its reaction to the hypoxia in mountains, and to evaluate the necessary terms of trainings under the conditions of medium-altitude mountains at the use of a similar simulation technology of athletes’ physical workability.

Keywords: altitude training, adaptation, HIF-1α, angiogenesis, erythropoiesis.
**Introduction**

The conditions of hypoxia are actively used by representatives of various sports to increase the workability and to approach the athletic ability peak before the basic competitions. For these purposes, natural conditions in mountains and special equipment used under urban conditions (pressure chambers, climatic chambers, hypoxicators) are applied (Wilber, 2007). However, the duration of the acclimatization of athletes to mountainous conditions is of a special interest.

Under such conditions, the main mediator of adaptive processes is HIF-1 (hypoxia inducible factor). In recent years, a high attention was paid to its investigation (Semenza, 2012; Лукьянова et al., 2011; Никулин, Родионова, 2011). HIF consists of α- and β-subunits. It is considered to be the leading transcriptive regulator of genes controlling the synthesis of erythropoietin (EPO), vascular endothelial growth factor (VEGF), glycolytic enzymes, ceruloplasmin, NO-synthase, and many other proteins ensuring the metabolic adaptation of organism under oxygen accessibility decrease (Mason, Johnson, 2007; Semenza et al., 1994; Semenza, 2004).

According to the literature data, trainings under the conditions of hypoxia in mountains are accompanied by increase in the levels of HIF-1α, myoglobin, and VEGF and by increase in the density of capillaries (Vogt et al., 2001). In this case, the HIF-1α-dependent activation of the VEGF gene expression, which is the principal angiogenic factor responsible for the growth of new blood vessels, is of critical importance for skeletal muscles (Breen et al., 2008). However, the studies of VEGF and HIF-1α in athletes under the conditions of altitude training are scanty and have mainly a starting character as compared with the initial levels (Schuler et al., 2005; Pialoux et al., 2009; Ogata et al., 2011).

EPO, which is a key regulator of erythropoiesis, is also a crucial indicator ensuring the saturation of organism by oxygen (Jelkmann, 2003). Under the normoxic conditions, physical loads with maximum and sub-maximum intensities have practically no effect on its content. However, the hypobaric hypoxia leads to increase in the EPO level already in 1-2 hours at altitudes of 1500-3000 m (Schuler et al., 2005). The direct influence of HIF-1 on the EPO level was demonstrated in many researches. However, the mechanisms that increase the number of red blood cells under the conditions of altitude trainings are not completely known (Chapman et al., 1998; Mairbäurl, 2013; Voss et al., 2014).

Thus, up to the date we have no clear prognostic criteria, which would be related to the adaptation of athletes to the altitude hypoxia (Ogata et al., 2011) and would allow one to consider individual peculiarities and to introduce some corrections for a more efficient running of the training process.

**Purpose of the work** was the construction of an algorithm for the markers use of individual skilled athletes’ adaptation to altitude trainings.

**Materials and methods**

In our studies, we involved athletes, who were representatives of a cyclic sport with aerobic provision of the muscular activity (n=12, male, the mean age was 25.3±6.9 years) on a special preparatory stage of the training period. The blood 7-ml sampling from ulnar vein for studies was executed in the morning on an empty stomach before the beginning and directly after the end of the trainings for 17 days (altitude above the sea level is 2050 m; the Belmeken base, Bulgaria). At the time of inspections, athletes were healthy and did not complain. The contents of HIF-1α, VEGF, EPO as well as cortisol and testosterone in serum were determined by the immonoenzymatic method with the use of the corresponding test-systems (“Abcam”, Great Britain; “Sigma”, USA; “Vectorbest”, Russia). The measurements of concentrations of the indicated factors were performed on a photometer “Sunrise” (Tecan, Austria). The anabolic index (AI) was calculated as the ratio testosterone/cortisol 100%. For the sake of comparison, we studied the contents of these indicators in blood serum of practically healthy men (n=10, the mean age was 25.4±3.5 years) not engaged in sports.

The indicators of hematologic and biochemical homeostasis were studied, respectively, with the help of automatic hematologic analyser “Erma PCE-210” (Erma Inc., Japan) and a semiautomatic biochemical analyzer “Humalyzer 3000” (Human Ltd., Germany) with the use of authentic standard diagnostic and control materials. For the statistical processing of data, we used the package of applied programs – “Statistica”. The obtained data were analyzed to check the correspondence to the Shapiro-Wilk normal distribution. The significance of variations of the indicators was determined with the help of the Fisher criterion (Яң, Сең 2011).
Results and discussion

On the first stage of studies, we determined the standard hematologic and biochemical parameters and the anabolic index reflecting the degree of organism adaptation to physical loads (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Indicators under study</th>
<th>Inspection stages of training</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes, 10¹²/l⁺</td>
<td>4.8±0.2</td>
<td>5.2±0.3*</td>
</tr>
<tr>
<td>Haemoglobin, g/l⁺</td>
<td>142</td>
<td>154</td>
</tr>
<tr>
<td>Haematocrit, %</td>
<td>39.1±2.6</td>
<td>43.2±2.2</td>
</tr>
<tr>
<td>Mean corpuscular volume, fl</td>
<td>80.2±1.7</td>
<td>76.8±1.5*</td>
</tr>
<tr>
<td>Mean corpuscular haemoglobin, pg</td>
<td>26.0±1.42</td>
<td>29.7±0.83*</td>
</tr>
<tr>
<td>Mean corpuscular haemoglobin concentration, g/dl</td>
<td>34.9±1.1</td>
<td>36.8±3.5</td>
</tr>
<tr>
<td>Anisocytosis, %</td>
<td>14.4±0.42</td>
<td>15.1±0.21</td>
</tr>
<tr>
<td>Testosterone, nmol/l⁺</td>
<td>21.43±2.77</td>
<td>26.1±6.58</td>
</tr>
<tr>
<td>Cortisol, nmol/l⁺</td>
<td>568.5</td>
<td>874.91</td>
</tr>
<tr>
<td>Anabolic index, %</td>
<td>4.41±1.26</td>
<td>3.63±1.49</td>
</tr>
</tbody>
</table>

Note: * – significantly relative to the data of athletes before the beginning of altitude trainings (p<0.05).

After the altitude trainings, we observed increase in the number of erythrocytes and a considerable growth in the concentration of haemoglobin in athletes, which agrees with the literature data (Sawka et al., 2000). In view of the remarkable individual variations of the content of haemoglobin, we failed to establish the significant differences in this indicator. However, the level of haemoglobin exceeded 160 g/l⁺ in 25% of athletes after the end of trainings, which was not observed in none of the athletes before the beginning of trainings. We also found a significant decrease in the mean volume of erythrocytes, which was compensated by an increase in the absolute concentration of haemoglobin in erythrocyte (p<0.05) and moderately caused the improvement of the oxygen-transporting function of blood (Robach et al., 2012).

The majority of indicators of biochemical homeostasis do not leave the limits of reference values (Table 2).

However, we observe in the growth of the urea level in blood serum in 10 athletes out of 12, which is a sign of low rate of recovery processes (Никулин, Родионова, 2011). As for the indicators reflecting a functional state of organs of the hepatopancreatoduodenal zone, the appearance of conjugated bilirubin connected with glucuronic acid in 4 athletes can testify the active haemolysis of erythrocytes, because bilirubin is a final product of the metabolism of haemoglobin in blood, and can be explained by the disappearance of young erythrocytes (by physical and phenotypic criteria) from the circulation after the descent from mountains, which was found in studies of the processes of acclimatization in experienced mountaineers (Risso et al., 2007). It is established that the activity of α-amylase, which characterizes a functional state of pancreas, was on the upper boundary in 7 athletes or considerably exceeded the reference value. This is also considered as a marker of over fatigue (Никулин, Родионова, 2011), especially, in the complex with the essential increment in the content of creatinine and with variations in the concentration of potassium (Гунина et al., 2013).

The level of glucose increases moderately, which indicates the adequate reaction of the indicators of the carbohydrate metabolism in athletes’ organisms to the imposed loads. The indicators of activities of AsAT, AIAT, and γ-GT increased slightly or were invariable and crossed the upper boundaries of the norm in none of the cases, which also testified to the normal support of the energy and nitrogen
metabolisms, to the absence of damages of hepatic tissue, and to the invariable functioning of bile-excreting channels (Никулин, Родионова, 2011). The other biochemical indicators of athletes after trainings under the conditions of altitude training did not significantly vary and did not leave the limits of reference values.

While studying AI, we observe the tendency to its diminution. This can indicate that the active duty altitude trainings, which are characterized by a stress-high environment, deteriorate the ratio of catabolic and anabolic processes in organism, because the decrease of AI down to a level of less than 3% is, according to the literature data, one of the signs of overtraining (Таймазов, Афанасьева, 2011).

The numerous works on the determination of the level of HIF-1α were executed with the help of the corresponding test-systems with the use of lysates of various cells (leucocytes, cells of the tissues of lungs, kidneys, liver, and muscles) mostly in experiments. But we carried out the studies of the indicated factor directly in blood serum. Similar studies were performed by other researchers with the use of the same test-systems, as in our work, but in patients with various pathologies (Левина et al., 2009; Макешова et al., 2012; Liang et al., 2013) and in native inhabitants of high-altitude mountains (Guan et al., 2013) for the clarification of variations during various pathological or physiological processes.

Our studies have shown that, before the beginning of altitude training, the contents of the studied indicators in blood serum of athletes are characterized by remarkable individual variations and the concentrations of VEGF and HIF-1α in athletes are significantly higher (p<0.05) than in practically healthy (donors) persons (Table 3).

At the influence of altitude trainings, the contents of all studied indicators in athletes increase; the growth of VEGF (p<0.05) is significant. Concentration variations of studied indicators in athletes, who trained themselves in medium-altitude mountains, were manifested in different degrees and were the adaptive answer to a decrease in the content of oxygen in the environment. Simultaneously, they allow one to develop theoretical foundation for the use of such method in order to increase the physical workability through the activation of the processes of angiogenesis and erythropoiesis without the intensification of training loads and without the application of additional pharmacological means.

The increase in the concentration of VEGF testifies the improvement of blood circulation by means of an increase in the density of capillaries in organism tissues (Jia et al., 2013), including muscular fibres it can be considered as the adaptive mechanism at the long-term action of hypoxia. The enlargement of a vascular network creates additional reserves for the supply of oxygen and energy resources to organs, which is crucial for the kinds of sport with the mainly aerobic ensuring of muscular activity. At the same time, the increase in the capacity of vascular channel decreases its total resistance and leads to a deceleration of blood turnover in separate vessels against the background of simultaneous increase in the blood supply to working muscles (Schuler et al., 2005).

With regard for obtained large range of data variations at the estimation of levels of the indicated factors sensitive to oxygen deficit, athletes were divided into two groups by the concentration of HIF-1α in blood serum. Group I included athletes with the initial level of this factor to be more than 1.0 ng·ml⁻¹. In group II, the content of the factor varied from 0.51 to 0.81 ng·ml⁻¹. Before the beginning of trainings, the indicators HIF-1α, VEGF, and AI differed significantly in athletes of the first and second groups (p<0.05). No significant differences in the contents of haemoglobin were determined (Table 4).

After the altitude trainings, we determined a higher level of VEGF in athletes of group II as compared with the results in group I (p<0.05). No significant differences in the concentrations of haemoglobin were found. However, it is worth noting that, after the end of trainings, the content of this indicator in 3 out of 8 athletes of group II (37.5%) exceeded 160 g·l⁻¹, which was not observed in representatives of group I. In this case, AI of the athletes of group II after trainings was less than 3%, as distinct from the athletes of group I, which was considered by us as one of the signs of overtraining.

### Table 3

<table>
<thead>
<tr>
<th>Indicators under study</th>
<th>Donors before the beginning</th>
<th>Donors after the end</th>
<th>Inspection stages of training</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIF-1α, ng·ml⁻¹</td>
<td>0.45±0.31</td>
<td>0.85±0.23*</td>
<td>1.09±0.27</td>
</tr>
<tr>
<td>VEGF, pg·ml⁻¹</td>
<td>38.8±10.6</td>
<td>90.0±3.46*</td>
<td>97.5±3.11**</td>
</tr>
<tr>
<td>EPO, mIU·ml⁻¹</td>
<td>19.20±2.3</td>
<td>22.0 (11.1; 49.85)</td>
<td>55.94 (19.04; 96.32)</td>
</tr>
</tbody>
</table>

*– p<0.05 relative to the data of donors; **– p<0.05 relative to the data of athletes after the end of trainings.
The values of VEGF and haemoglobin (Hb) in athletes after the trainings in medium-altitude mountains as compared with the content of HIF-1α.

<table>
<thead>
<tr>
<th>Indicators under study</th>
<th>Groups of athletes and inspection stages of training</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before the beginning</td>
<td>after the end</td>
</tr>
<tr>
<td>HIF-1α, ng⋅ml⁻¹</td>
<td>1.14±0.08</td>
<td>1.26±0.06*</td>
</tr>
<tr>
<td>VEGF, pg⋅ml⁻¹</td>
<td>88.0±3.46</td>
<td>95.75±2.5 #</td>
</tr>
<tr>
<td>Haemoglobin, g⋅l⁻¹</td>
<td>143.25 (129; 150)</td>
<td>150.25 (140; 156)</td>
</tr>
<tr>
<td>Anabolic index, %</td>
<td>4.37±1.17</td>
<td>3.86±2.11 #</td>
</tr>
</tbody>
</table>

Note: # – significantly relative to the data for the same group before the beginning after the end of trainings (p<0.05); * – significantly relative to the data for group I after the end of trainings (p<0.05); ** – significantly relative to the data for group I before the beginning of trainings (p<0.05).

The high concentrations of EPO after the trainings under the conditions of altitude training were determined in athletes of both groups: 58.57 mIU⋅ml⁻¹ (from 19.04 to 96.32 mIU⋅ml⁻¹) and 68.25 mIU⋅ml⁻¹ (from 36.22 to 90.50 mIU⋅ml⁻¹). However, the analysis of the increment in this indicator showed that it was larger in representatives of group II: in 4 out of 8 athletes, the increment of the level of EPO was higher than 60 mIU⋅ml⁻¹, what was not observed in group I. No associations with other indicators of hematologic and biochemical homeostasis in athletes of both groups were revealed.

It is quite difficult to explain the mechanisms mediating such dynamics of the indicators in both groups of athletes, which undoubtedly requires the additional studies. However, it is well known that the level of HIF-1α in organism is genetically determined (Ahmetov, 2010). In addition, the data exist on the different resistances to hypoxia of volunteers-donors, who are different primordially by the state of their parameters of red blood, functional activity of the immune system, and the expression of the reaction to hypoxic trainings (Serebrovskaya et al., 2012). Most probably, the skilled athletes have also the different stability to hypoxia in the environment and to physical loads under such conditions and, respectively, different adaptive mechanisms.

Conclusions

The usage of the proposed determination algorithm of complex currently applied indicators allows us to characterize the functional state of athlete’s organism, to determine the individual specific features of his reaction to the hypoxia in mountains, and, thus, to evaluate the necessary terms of trainings under the conditions of medium-altitude mountains at the use of a similar technology of stimulation of the physical athletes workability.

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TRENIRUOTĖS AUKŠTIKLALNĖSE POVEIKIS TALENTINGŲ SPORTININKŲ ERITROPOEZĖS IR ANGIogeneZĖS PROCESAMS

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SANTRAUKA

Straipsnyje nagrinėjamas treniruotės aukštkalnėse poveikis HIF-1α faktorui, kurį ciklinių sporto šakų sportininkų kraujo serume sukelia hipoksija, kraujagyslių endotelių augimo faktorius (VEGF) ir eritropoetinas (EPO). Organismo adaptacija treniruojantiesi kalnuotomis sąlygomis, kompensacinių mechanizmų analizė ir padidėjęs organizmo atsparumas įvairių lygiu ir lygių stresiščiams faktoriams (hipoksijos, audros temperatūros ir didelės drėgmenės, pažeidus biologinius ritmus ir pan.), naujų kriterijų paieška fizinio darbingumo nustatymui ir prognozavimui yra svarbiausi klausimai treniruotės aukštkalnėse. EPO, pagrindinio eritropoezės reguliatoriaus, sintezė taip pat yra susijusi su padidėjusiui HIF-1α pasireiškimu. Darbo tikslas – sukurti algoritmą, nustatantį individualius talentų sportininkų adaptacijos poveikį treniruotės aukštkalnėse. Tyrimo metu dalyvavo 12 talentinių ciklinių sporto šakų sportininkų, kuriems buvo atlikti tyrimai prieš ir po 17 dienų treniravimosi vidutinio aukščio kalnuose. HIF-1α, VEGF, EPO, kortizolio ir testosterono kraujo serume lygis buvo nustatomas imunoenziminiu metodu, naudojant „VectorBest“ (Rusija) firmos reagentus. Minėtų faktorių koncentracija buvo matuojama fotochromatojimu „Sunrise“ (Tecan, Austrija). Hematologinių ir biocheminių markerių koncentracija buvo matuojama fotochromatojimu „HUMALyzer 3000“ (Human Ltd., Vokietija).
Iš gautų rezultatų matyti, kad po treniruotės vidutinio aukščio kalnuose padidėja eritrocitų kiekis ir homoglobinio koncentracija, taip pat smarkiai sumažėja eritrocitų vidutinis kiekis ir padidėja vidutinė homoglobinio koncentracija viename eritrocite; tai netiesiogiai rodo pagerėjusią deguonies pernešimo kraujyje funkciją. Anabolinis indeksas buvo linkęs mažėti. Taip pat nustatyta, kad treniruotė hipoksijos sąlygomis lemnia sportininkų VEGF padidėjimą priklausomai nuo poveikio lygio (p < 0,05). HIF-1α ir EPO koncentracija kraują serume turėjo polinkį didėti. Atsižvelgiant į gana didelis individualių rezultatų įvairijas, sportininkai pagal HIF-1α lygi prieš treniruotės pradžią buvo padalyti į dvi grupes. Pirmajai grupėi priskirti sportininkai, kurių HIF-1α lygis buvo mažesnis nei 1,0 ng⋅ml⁻¹ (nuo 0,51 ng⋅ml⁻¹ iki 0,81 ng⋅ml⁻¹). Sportininkai, kurių HIF-1α buvo didesnis nei 1,0 ng⋅ml⁻¹, sudarė antrąją grupę. Prieš treniruotės vidutinio aukščio kalnuose pradžią pirmos ir antros grupės sportininkų HIF-1α ir VEGF koncentracija bei anaboliniai rodikliai reikšmingai skyrėsi (p < 0,05). Pasibaigus treniruotei aukštkalnėse sportininkai, kurių pradinė HIF-1α koncentracija buvo mažesnė nei 1,0 ng⋅ml⁻¹, pasižymėjo aukštesniu VEGF lygiu ir didesne EPO koncentracija, lyginant su tais, kurių pradinis HIF-1α lygis buvo aukštesnis nei 1,0 ng⋅ml⁻¹.

Tokų šiuolaikiškų rodiklių nustatymas leis įvertinti sportininko organizmo funkcinę būklę, atskleisti individualias jo reakcijos į hipoksiją kalnuose ir patybes, taip pat nustatyti būtiną treniravimosi vidutinio aukščio kalnuose laikotarpį, naudojant panašią sportininkų fizinio darbingumo ugdymo technologiją.

*Raktažodžiai*: treniravimas aukštkalnėse, adaptyva, HIF-1α, angiogenezė, eritropoezė.