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ALTITUDE TRAINING AT SEA LEVEL AND ITS IMPACT ON EPO AND VEGF*

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Abstract

Introduction: Intermittent hypoxic exposure (IHE) is a no-invasive, no-pharmacological method enhancing performance, using the adaptive changes to decreased oxygen content in air. Erythropoietin (EPO) and vascular epithelium growth factor (VEGF) are very important factors in endurance sports, enhancing oxygen transport capacity and delivery to the working muscles.

Objective: To evaluate, if altitude training using IHE method stimulates synthesis of both EPO and VEGF, which among many other factors are products of expression of hypoxia inducible factor.

Methods: Twelve runners (age 25 ± 1 yrs, BMI 19.8 ± 0.5 kg/m^2) performed IHE for one hour daily for 2 weeks. During IHE athletes breathed subsequently with hypoxic and room air according to the results of hypoxia tolerance test, total hypoxic time in one session was 35 to 40 minutes and target oxygen saturation in blood 80%. Blood samples for determination of EPO and VEGF concentrations were obtained in the morning in the fasting state before, in 5th and 14th day of IHE.

Results: Blood concentration of EPO increased significantly after 4 days of IHE (from 6.7 ± 1.2 ml U/ml to 11.7 ± 0.9 ml U/ml) and remained elevated till the end of IHE (9.8 ± 0.9 ml U/ml). Also VEGF responded to IHE demonstrating the significant increase from 22.5 ± 6.0 pg/ml to 366.0 ± 49.2 pg/ml.

Conclusions: Altitude training at sea level using IHE method was well tolerated by all athletes and showed positive impact on molecular level on some important factors involved in exercise metabolism. Thus, IHE can be considered as a recommended option to be combined with classic altitude training.

Key words: *intermittent hypoxic exposure (IHE), erythropoietin (EPO), vascular epithelium growth factor (VEGF), altitude training, hypoxia inducible factor (HIF)*

Introduction

Scientific approach to altitude training is already half a century old. Still most athletes use a natural model of living and training at altitude, but many other strategies have developed – including living low-training high and living high-training low regimens [1-3]. Considering the logistics difficulties and weak points of those natural methods, the artificial manipulations of air composition were introduced and are nowadays commonly used by athletes [4,5]. They include either decrease of oxygen content (hypoxia) or the use of supplemental oxygen. Hypoxic training can be finalized in many ways, in a whole compartment (tent, room or house) or via the mask; with hypoxic air delivered from a container or supplied by a generator; at rest (hypoxic exposure) or during exercise (hypoxic training); in a constant or intermittent regimen. Intermittent hypoxic exposure (IHE) is a relatively safe, no-invasive, no-pharmacological method enhancing performance, using the adaptive changes to decreased oxygen content in air [6]. All those methods can be applied in any combination and have to be incorporated among the regular training sessions. Choosing the optimal regimen for each athlete and sports discipline is difficult but possible [7].

There are few reports on improved performance after IHE [8-10] or blood parameters [11,12], whereas in most cases no significant changes were found and authors do not recommend this method of altitude training [13,14].

Erythropoietin (EPO) and vascular epithelium growth factor (VEGF) are very important factors in endurance sports, enhancing oxygen transport capacity and delivery to the working muscles. Both of them cannot be administered to athletes as they are on the list of prohibited substances of the World Anti Doping Agency (WADA). Altitude training is supposed to induce expression of hypoxia inducible factor (HIF). Transcription of genes triggered by HIF influence many vital processes like erythropoiesis, angiogenesis, vascular tone, glucose metabolism, mitochondrial function, cell growth etc. [15]. Among many other factors HIF stimulates synthesis of both EPO and VEGF. Despite some controversies all means of altitude training, including IHE, are so far legal and accepted in sports society [16].

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Aim of the study

Present paper presents the methodology of the altitude training by the intermittent hypoxic exposure method developed in two years` practice. The aim of the study was to evaluate, if such altitude training stimulates synthesis of either EPO or VEGF in international level track and field athletes.

Methods

Participants

Twelve male runners of national track and field team participated in the study after signing the informed consent (age 25 ± 1 years, body mass 59 ± 2 kg, height 172 ± 2 cm and BMI 19.8±0.5 kg/m^2). The study protocol was accepted by the Ethical Committee of Warsaw Medical University (permission number KB/214/2010).

Altitude training - Intermittent Hypoxic Exposure

Intermittent hypoxic exposure was performed using a four person hypoxicator delivered by Go2Altitude (Australia). Each IHE session lasts approximately one hour and consists of subsequent periods of breathing with hypoxic and room air. Preprogrammed personal station informs each IHE participant when to inhale hypoxic air from the mask and when to put the mask away and breath normally with room air. The length of those intervals is essential in IHE and is determined with hypoxia tolerance test performed before the first IHE session. The test is conducted by breathing the air of 11% of oxygen and measuring the time in which blood oxygen saturation drops to 85%. At this moment subject removes the mask and breaths with room air, time required for recovery to saturation of 95% is recorded. According to the results of hypoxia tolerance test the time of breathing hypoxic air was determined ranging from 3 to 8 minutes with rest intervals of breathing room air of 2 to 4 minutes. In most athletes whole IHE was completed using time intervals determined by this method, only a few subjects required slight readjustments which in all cases took place during the first two sessions of IHE and were caused by not fully relaxed breathing during the test. Number of intervals creating the single IHE session was set so that each subject in total breaths hypoxic air for 35 to 40 minutes, what means that the total hypoxic dose was the same in all subjects. In first three IHE sessions the air of 14% oxygen concentration was used (which usually corresponded to saturation of 85%) and in consecutive days it was gradually lowered to the value resulting in oxygen saturation in subjects` blood of 78-80%. The minimum oxygen concentration used was 11.5% what represents the altitude of approx. 4800 meters above sea level.

Due to noise emitted by the hypoxic air generator it was placed in a separate room and connected with a supplied 12 mm tubing to the main unit placed in the room where four deckchairs were available for athletes. Fully relaxed, supine body position is very important, as the hypoxic stimulus impact on cardio respiratory system should not be disturbed even by slight effort of postural muscles. For the same reason all participants of IHE should be peaceful and quiet, as enhanced ventilation induced by talking and laughing would influence the response of respiratory system. There also must be a sufficient, at least two hours interval between IHE and previous exercise or meal. Combining these conditions with two regular training sessions a day enables to perform IHE in the afternoon before the second exercise or in the evening before going to sleep. During the whole IHE altitude training each athlete had a one day of rest between day 5 and 7 according to the workload of physical training sessions.

Evaluation of EPO and VEGF

Blood samples for determination of EPO and VEGF concentrations were obtained from antecubital vein in the morning in the fasting state before, in 5th and 15th day of IHE.

Concentration of both EPO and VEGF were determined using the ELISA kits(R&D Systems, Catalog Number: DEP00 and R&D Systems, Catalog Number: DVE00).

Statistical analysis

Statistical analysis was performed with Statistica 6.0 software. After the normal distribution was revealed by analysis with Shapiro-Wilk`s test samples were compared with paired Student`s *t*-test. Level of significance was accepted at $P < 0.05$, data are presented as means ± SE.

Results

Blood concentration of EPO increased significantly after 4 days of IHE (from 6.7 ± 1.2 mlU/ml to 11.7 ± 0.9 ml U/ml, $P < 0.001$) and remained elevated till the end of IHE (9.8 ± 0.9ml U/ml, $P < 0.001$) (Fig. 1). Also VEGF levels responded to IHE demonstrating the

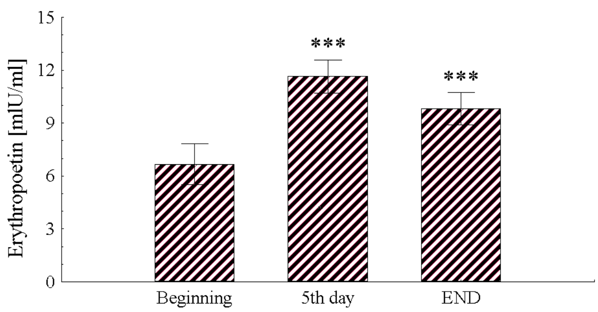

Fig. 1. *Blood erythropoietin concentration in the morning at the beginning, at 5th day and at the end of 2 weeks of intermittent hypoxic exposure; *** P < 0.001 compared with "Beginning"*

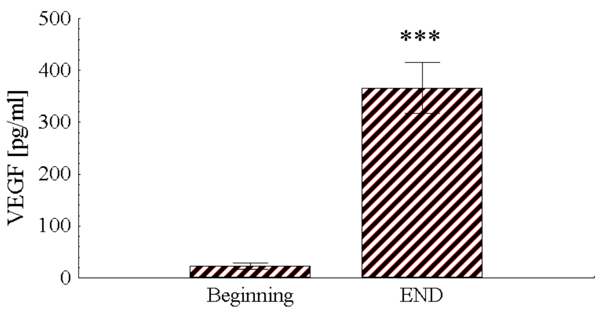

Fig. 2. *Blood vascular epithelium growth factor concentration in the morning at the beginning and at the end of 2 weeks of intermittent hypoxic exposure; *** P < 0.001*

significant increase from 22.5 ± 6.0 pg/ml to 366.0 ± 49.2 pg/ml, $P < 0.001$ (Fig. 2).

Discussion

Altitude training at sea level using intermittent hypoxic exposure method was well tolerated by all athletes. Most of them reported increased sleep requirement, which is a normal response to increased training load as IHE should be considered as an additional training session during a day. Slight headaches occurred when saturation was dropped to much and too rapidly. Symptoms were reversible within minutes and subjects learned how to avoid them by taking single breaths of room air during hypoxia if saturation was dropping beyond expected limits. It is important to emphasize that no one complained of decreased performance after IHE, whereas approx. half of athletes reported improvements in perceived exertion. Several personal best times were also recorded, but there is no evidence allowing to assume, that the IHE was the key factor in those results. Comparison of performance factors and blood morphology was not considered, as subjects were training hard in small, different groups and it was not possible to create sufficient control to distinguish the effect of IHE from regular training.

Among the indices determined in blood the increased concentration of erythropoietin was found with peak secretion in 4th-5th day of IHE, what is consistent with old IHE literature originating from Russia [17]. Elevated EPO concentration was present to the end of 2-weeks of blood monitoring, being consistent with results of many other studies describing EPO release after various hypoxic stimuli [18-20]. Enormous magnitude of vascular epithelium growth factor production observed at the end of the study confirms the effectiveness of IHE in induction of molecular response to hypoxia [21,22]. As both EPO and VEGF are secreted in response to stimulation of hypoxia inducible factor, thus it can be assumed with high probability, that IHE resulted in increased expression of HIF [23]. In such case, enhanced production of many other molecules governed by HIF can be expected and most of them are in favor for exercise capacity, constituting the non-erythropoietic benefits of altitude training [24,25].

There are five main reasons for which IHE can be applied in athletes:

1. Pre-acclimatization before going for LH-TH real altitude training camp (duration up to 14 days).
2. Simulation of real LH-TH altitude training camp (3-4 weeks).
3. Maintenance of effects of previous altitude training (10-14 days).
4. Stimulation right before competition (3-4 days).
5. Preparation for competition at altitude (combined with exercise sessions in hypoxia – intermittent hypoxic training).

As with classic LH-TH altitude training, there are big inter-individual differences in response to IHE, but it is definitely easier to personalize this method. It is also extremely important not to overload the athlete, who has less rest due to IHE sessions. In such case there will be no benefits in performance or blood parameters, what probably had occurred in many studies involving hypoxic training.

We have observed that athletes involved in IHE have reported perceived positive effects mostly in two of mentioned above applications: pre-acclimatization preceding altitude camp and stimulation before competition. Even few days of IHE before going to the altitude camp enabled athletes to start regular training earlier than without it. Stimulation before competition with IHE was very helpful, also in case of several events repeated in weekly intervals. Such sensations are reasonable, considering the peak EPO secretion after just 3-4 days of IHE, resulting from enhanced HIF expression and having in mind other factors beneficial for exercise capacity induced by HIF, including VEGF. Hemoglobin content and maximal oxygen consumption should not be the only indices determining whether altitude training was successful or not, changes within muscle tissue could be very important and difficult to detect "in the field" [26]. For example in a recent, perfectly designed study [14], which concludes that IHE is useless for elite triathletes, lower lactate concentrations and maximal heart rate were observed, whereas oxygen consumption and running times were unaffected. From performance point of view there is nothing, but physiological cost of exercise is probably lower with all accompanying, obviously positive implications. Deeper knowledge of phenomena induced by hypoxic conditions will increase the probability of proper altitude training application in different conditions, therefore fulfilling the expectations of its users [27,28].

Conclusions

Altitude training at sea level using IHE method was well tolerated by all athletes and showed positive impact on molecular level on some important factors involved in exercise metabolism. Thus, IHE can be considered as a recommended option to be combined with classic altitude training.

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Declaration of interest

The authors report no conflict of interests.

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