

The Effects Live High, Train Low/High at Natural Altitude on Blood Variables and Endurance Performance

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Abstract

Purpose: The present study was done to specify the impacts of a protocol called “living high, training low and high (LHTLH)” on serum ET-1 and EPO levels as well as the 3000-m performance of endurance runners in Iran’s national team. **Method:** eight male runners (aged 24.4 ± 3.1 , height of 180.5 ± 4.2 , weight of 66.7 ± 3.4 and BMI of $20.1 \pm 5.0 \text{ kg/m}^2$) who cooperated voluntarily with regard to middle distance and marathon running (R2M system training) at high and low altitudes. All case studies did 3000-m test and blood samples were taken at a time period of 24-hours before and after the test. The intended tasks consisted of continuous, interval, aerobic and resistance exercises. The runners were to take part in 16 training sessions each week. It should be noted that the training lasted for 11 weeks according to (LHTLH) protocol. Variance with repeated measures ($P \leq 0.05$) was utilized as the method to analyze the collected data. **Results:** It was shown that 3000-m time underwent significant decrease with regard to the time of impact both before and after the training. However, there were not significant changes in EPO ($P \geq 0.05$). On the contrary the amount of ET-1 demonstrated significant increase ($P \leq 0.05$). **Conclusions:** The results indicated that 3000-m time at lower altitudes will decrease on account of certain training programs. The reason is not directly related to the level of EPO. It is through hematological and metabolic changes as well as increase of ET-1 levels that performance under hypoxic and normoxic states happens to improve in the real sense.

Keywords: Endothelin-1; Endurance Runners; EPO; Hypoxia; Normoxia

INTRODUCTION

Throughout the world, athletes draw from a protocol called “living high, training low” (LHTL) in order to improve the level of their performance. According to Wilber et al. (2007), athletes have increase their performance through training at high altitudes. There are a number of training protocols apart from living and training at high altitudes, one of which is training at low altitudes. Being termed “living high, training low and high” (LHTLH), this protocol has been a crucial to accomplish that goal (Levine, 1991). Conducted studies in this area have shown that LHTL as a technique leaves physiological impacts on the performance of athletes (Stray-Gundersen et al., 2001), ventilatory adaptations (Townsend et al., 2002) and hematological responses and the higher capacity of blood to carry oxygen (Saunders et al., 2009).

Nowadays, altitude/hypoxic training is considered to be a helpful and practical strategy which is used by athletes in order to escalate the quality of their performance especially in a series of competitions at sea level (Brocherie et al., 2015; Sinex and Chapman, 2015). By using LHTL protocol, the running performnace of professionally trained runners at sea level will improve for two reasons: adapting to hypoxia that brings about an increase in erythropoiesis and VO₂ max and maintaining the velocity of training at sea level which is in close affinity with the increase of running velocity and VO₂ max (Garvican et al., 2011). Saunders et al. (2004) and Sinex and Chapman (2015) conducted some research and accordingly concluded that LHTL has been influential on the performnace of elite endurance runners (e.g. 3000-meter runners) because the capacity of oxygen to transfer increases. Exposure to high altitudes paves the ground for the decrease of aerobic exercise capacity; in other words, the content of arterial oxygen decreases and the maximal cardiac output encounters limitation (Fulco et al., 2000).

A number of reasons including combined effects of decreased blood volume, hypocapnia, increased viscosity of blood, autonomic changes in nervous system, reduction of myocardial function, decrease of hemoglobin concentration as well as endothelin-1 have all been presented to account for the maximal cardiac output at altitudes (Wagner, 2000). The results of recent laboratorial studies demonstrated an increase of average acute erythropoietin (EPO) at a moderate altitude

of 150% in relation to the baseline of sea level. Furthermore, a 4-week stay at that altitude brought about an increase of 1.5-2.0 ml/kg in the average red cell mass (Stray-Gundersen et al., 2001; Levine, 2002). Based on the aforementioned literature, LHTL is under the influence of some factors such as increased erythropoiesis, improvement of exercise economy, rising accumulation of glycogen, consumption of fatty acids, capacity of muscle buffer, oxygenation of skeletal muscle and, last but not least, cardiovascular function (Park and Nam, 2017).

Living at an altitude of 2500 meters for a long period of time provides a hypoxic stimulus which, in its own turn, is powerful enough to enable acclimatization with regard to hematology in a significant way among most athletes; very interestingly, EPO and red cell mass react to a given altitude and, as a consequence, engender considerable individual variability (Gunga et al., 1994; Stray-Gundersen et al., 2001; Ge et al., 2002; Siebenmann et al., 2012). As a matter of fact, in those cases that the response of erythropoiesis is not present, it would be tough to show that altitude as a factor can improve endurance performance (Siebenmann et al., 2012).

Vascular endothelium is able to generate endothelin-1 (ET-1) through living at altitudes. Vasoactive substances like endothelin-1 and nitric oxide are released; as a result of this process, endothelial cells turn out to be influential on maintaining the vascular tone (Mather et al., 2002; Hubloue et al., 2003; Thijssen et al., 2007). It has been shown earlier that there is interaction between hypoxia and endothelin-1. Increase of hypoxia paves the ground for synthesis of endothelin-1 as well as the contraction of vascular smooth muscle; however, in a similar vein, nitric oxide decreases (Ni et al., 1998; Favret et al., 2006; Pham et al., 2010).

The pressure of pulmonary artery at the time of hypoxia depends on the balance between vasoconstrictors and dilators. It should be noted that pharmacological blockage of the receptors of endothelin-1 ends in decreasing hypoxia-caused escalations of pulmonary artery pressure (Pham et al., 2010). Therefore, it can be inferred that endothelin-1 plays a key role in alterations of pulmonary artery pressure (Calderón-Garcidueñas et al., 2007; Hiramoto et al., 2007). The vascular tone is highly contingent upon the balance between vasodilating and vasoconstricting factors like endothelin-1 and nitric oxide. In addition, it affects the response of the vascular system to exercise and hypoxia.

The latter can help increase HIF-1 which is highly related to factors in angiogenesis and erythropoiesis. As a result of these processes, there will be an increase of vessel formation, hem synthesis and red blood cell production (A O'Hagan et al., 2009). Consequently, the blood flow will be optimized the oxygen supplied to the tissues will be limited with regard to the oxygen supply. It should be highlighted that the mechanisms involved in hematological and non-hematological adaptations to LH-TL and, by extension, the improvement of athletic performance are not clearly determined (Lundby and Robach, 2016).

To address the proposed issue, the current research explores long-term exercises under hypoxic and normoxic states and thereby sheds light the probable impacts of changes in endothelin-1 and EOP blood serum levels on the performance of runners.

METHOD

Subjects

In order to achieve the goals of the present study, 8 male runners of track and cross-country (i.e. three 1500-meter, two 3000 and 10000-meter as well as three cross-country runners) volunteered to cooperate with Iran Association of Athletics Federations. The best performance of the runners in the preparatory season was the requirement to be accepted for the study. All participants signed the informed consent to the protocol which was prepared by the Institutional Review Board of the University of Tehran and national Olympic committee of Iran. The volunteer subjects attended the Asian Championship of the last three years. In addition, they were busy with national training camp for Asian Championships in Bahrain 2019 when the research was conducted.

Table 1: Participants' Characteristics

Variables	Age (y)	height (cm)	weight (kg)	BMI(kg/m ²)
	24.4±3	180.5±4	66.7±3	20.5±1

Initial measurement

One week before starting the test, a questionnaire related to medical health was utilized to ensure that the participants were healthy in terms of cardiovascular conditions, infectious diseases, diabetes, allergies, smoking and taking any kinds of medication or supplement. At the end of the season, the participants did cross-training (which included active rest with swimming, mountaineering, ping pong and so forth) in a 45-day course known as detraining period. Then, venous blood samples of 5 mL were taken from the antecubital vein. It should be mentioned that the sample were taken in the morning and the participant were required to be in a sitting position. Following that, the taken samples were centrifuged for 10 minutes at 3000 RPM. Once the serum were separated, the sample were kept under a temperature of -80 degrees centigrade.

Training protocol

3000-meter time trial races on a 400-meter track were intended to assess the level performance of normoxia. Being held in Tehran, these time trial races started at 09:00 in the morning and lasted up to 10:00. The participants were required to endeavor to achieve the best possible time in the race. At the beginning, professional and competent pace setters were asked to set a quick and competitive pace in the 3000-meter race to be a benchmark for physiological performance in comparison with the tactical one. These pace setters who were athletes did not participate in the study. Being called “rabbit” as well, the pace setter ran the race pace for all time trials which were specified earlier. The time for each athlete was recorded to be near 0.1 second.

Then, it was the physician’s turn who took the blood samples after the 3000-meter race and transferred them to the laboratory immediately. The next step was general phase training based on R2M method. This method is considered to be an enhanced version of a protocol which has been developed earlier (Mohammadi Mirzaei and Mirdar, 2016; Roohollah and Shadmehr, 2016). This phase lasted for 4 weeks. Blood samples were taken from the participants and the 3000-meter running performance was conducted according to the specified rules at the end of week 4. Following that, the runners were taken to Zagros Mountain, Delfan City in Lorestan Province. The altitude was 2500 meters high which was regarded to be of relative hypoxia. It took

4 weeks to do the training course called R2M method under that hypoxic state. The training consisted of 160 kilometers in a week which was divided into 2-3 sessions during a day (at 06:00 a.m., 10:00 a.m. and 04:00 p.m.). As it is shown in Table 1, the training included endurance, interval, sprint, resistance, plyometric and isodynamic exercises. Both speed and volume were determined to be different.

Once the 3000-meter running performance was finished, the blood sample was taken from each runner at an altitude of 1200 meters at the end of week 4. The mentioned altitude is considered to be a normoxic state. Later, the runners went on with their training (R2M) at 1200-meter altitude. They were taken by bus to sea level and 3000-meter running performance was done. The blood sample was taken another time.

The runners were provided with equal accommodation, resting and exercises facilities. The trainings were based on two methods of “living high, training low” (LHTL) and “living high, training high” (LHTH). The results of 3000-meter running performance and information related to the samples are given in Table 2 and 3. Figure 1 contains the components of the training protocol under hypoxic and normoxic states.

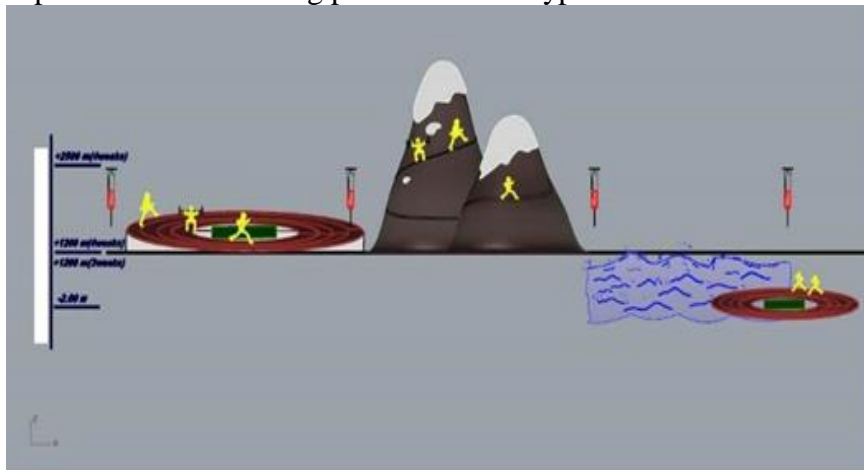


Fig 1: Components of Training Protocol under Hypoxic and Normoxic States

Blood serums assessment

In order to assess the alterations of EPO and ET-1 by drawing from enzyme-linked immune sorbent assay (Zellbio GmbH ELISA kit was made by UIM Company in Germany), the serum sample was removed from -80 degrees centigrade and 40 mL of EPO and ET-1 solutions were added to 40 mL of its content. Each sample (50 mL) was kept according to standard method; i.e. a temperature of 37 degrees centigrade and a time period of 60 minutes. Then, 300 microL buffer solution was utilized to wash the plates. They were washed five times. Then, A and B chromogen solutions were added and they were kept for 10 minutes under a temperature of 37 degrees centigrade. Finally, STOP solution was used in each plate.

Training quantification and standardization (R2M method)

The participants had training logs based on a daily basis. They contained information about the training load (in terms of miles during a day) and the number of “high intensity” workout sessions (i.e. whether it is interval training or tempo runs; the pace is specified by the athlete in accordance with lactate threshold and HR pace). Before conducting the research, a global training template was shared with the participant. It should be highlighted that the given template was utilized by Mohammadi Mirzaei and Mirdar (2016) and Roohollah and Shadmehr (2016) for an individual training plan. Considering the training impulse of various groups that live and train at different altitudes, the mentioned training template brought about successful outcomes. The athletes were required to do certain workouts (such as interval sessions, long runs, tempo efforts endurance, interval, sprint, resistance, plyometric and isodynamic trainings among others) at the same time during a specific day. As it is given in Table 1, the training atmosphere was clearly formulated for the participants.

Table 2: R2M Plan and Training System at Altitude (R2M stands for running at middle distance to marathon). Quantities of Training Intensity (Int), Maximal Aerobic Power (MAP), Maximal Heart Rate (MHR), First Ventilatory Threshold (VT1) and Second Ventilatory Threshold (VT2).

hypoxia							
normoxia							
Training load	Very high						
	high						
	medium			160 km		160 km	
	low	120 km	140 km		140 km		
	Very low						
Training intensity		Int≤VT1 MHR≤160	Int≤VT1 Int≤VT2 MHR 160-170	Int≤VT1 Int≤VT2 Int≤MAP1 MHR 170-180	Int≤VT1 Int≤VT2 Int≤MAP2 MHR 180-190	Int≤VT1 Int≤VT2 Int≤MAP2 MHR 180-190	
Training type		Speed endurance-Strength-endurance Tempo endurance-Running endurance	Speed endurance-Strength endurance Tempo endurance-Running endurance Power speed-speed-Strength Plyometric	Speed endurance-Strength endurance Tempo endurance-Running endurance Power speed-speed-Strength-Plyometric-Isodynamic	Speed endurance- Strength endurance- Tempo endurance- Running endurance- Power speed- speed- Strength- Plyometric- Isodynamic- Competition strategy	Speed endurance- Strength endurance- Tempo endurance- Running endurance- Power speed- speed- Strength- Plyometric- Isodynamic- Competition strategy	
Training duration at Altitude and normoxia (days)		28	9	9	10	21	
5km test and blood sample	Pre-test	Test-2			Test-3		Post-test

Data collection and Statistical analyses

SPSS Statistical Software (version 24) was utilized to analyze the collected data. The given data values are calculated based on means \pm SD. However there are some exceptions. This study make use of Shapiro-Wilk test and, accordingly, all dependent variables turned out to be distirubted in a normal way. Morevoer, for additional and complementary analyses, parametric statistic was utilized. In order to highlight the discrepancies that exist in the dependent measures at distinct times between normoxia and hypoxia at certain altitudes, two-way split plot repeated measures (ANOVA) with a priori tests that bear simple main effects were used. Additionally, Banferroni test as well as least significant difference post hoc test were intended for further analysis. The significance α -level was determined to be $P \leq 0.05$. Excel Software was used to draw the diagrams of this study.

RESULTS

All participants went through the whole protocol successfully. In the present study, the discrepancies in terms of physical features, performance in 3000-meter run, the protein level of EPO and protein level of ET-1 were not significant. However, the training load which was defined according to the training protocol was different with regard to normoxia, altitude and sea level.

Table 2 contains the results of ANOVA which were taken from the protein level of EPO serum, the protein level of ET-1 serum, the performance variables of 3000-meter run in the following four measurement stages: pre-examination (normoxia basic level), post-examination 1 (before hypoxia), post-examination 2 (after hypoxia and post-examination 3 (after sea level).

Table 3: ANOVA Results of EPO and ET-1 Protein Levels and 3000-Meter Performance (Four Measurement Stages)

	Source	Sum of square	df	Mean square	f	sig
ET-1 (pg/ml)	Time	81.37	3	27.12	9.50	0.01
	Standard Error	343.10	21	16.33		
EPO (mIU/ml)	Time	64643.53	3	59594.83	3.01	0.12
	Standard Error	149944.9	21	7140.2		
Performance 3000 m	Time	20156320.09	3	6717873.36	247.58	0.01
	Standard Error	569873.1	21	27136.8		

According to the results, the change in protein level of EPO serum during the training protocol was not meaningfully significant ($p \geq 0.05$). The results (Figure 2) showed that there are meaningfully significant changes in the protein level of ET-1 serum before and after the training period ($p \leq 0.05$). In addition, the results of post hoc Banferroni test which are given in Table 3 demonstrated that the changes in the measurement stages were significant.

Table 4: Post-Hoc Test Results of ET-1 Protein Level and 3000-Meter Performance

sources		The P after sea level than basic normoxia(Post-test 3 and pre-test)	The P after sea level than before hypoxia(post-test3 and post-test1)	The P after sea level than after hypoxia(post-test3 and post-test2)	The P after hypoxia than basic normoxic level(post-test2 and pre-test)	The P after hypoxia than before hypoxia(post-test2 and post-test1)	The P before hypoxia than basic normoxic level(post-test 1and pre-test)
3000 M	Intergroup interaction	0	0	0	0	0.01	0
	Standard Error	105.41	95.18	37.93	100.33	87.33	37.34
ET-1	Intergroup interaction	0.92	1.00	1.00	0.05	1.00	1.00
	Standard Error	1.33	1.42	1.99	85.33	0.70	2.64

The results clearly showed that the performance in 3000-meter run turned out to be better in a significant way before and after the training period ($p \leq 0.05$). Tracing the four measurement stages of the current study, the results of post hoc Banferroni test shed light on the meaningfully significant changes toward improvement (Table 3). The discrepancies were calculated to be as follows respectively: between pre-examination and post-examination 44s (4.86%), post-examination-2 and pre-examination 27s (2.84%), post-examination-1 and pre-examination 15s (1.6%), post-examination-2 and post-examination-1

12s (1.35%), post-examination-3 and post-examination-1 29s (3.25%), post-examination-3 and post-examination-2 17s (1.93%). Lastly, as it is given in Figure 3, the difference between hypoxic and normoxic states during all four measurement stages was significant.

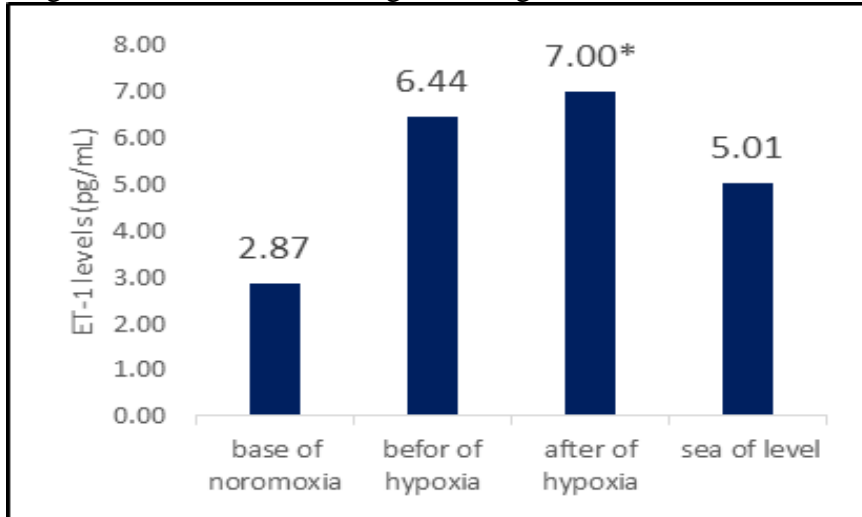


Fig 2: Mean ET-1 Difference (Hypoxic and Normoxic States)
*significant Mean performance time

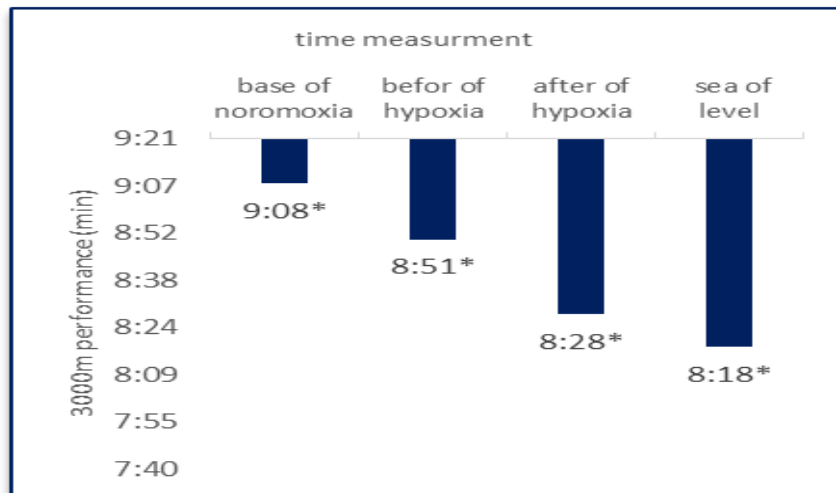


Fig 3: Mean 3000-Meter Performance Time Difference (Live High, Train Low/High).
*Significant Mean Performance Time Difference (p=0.05)

DISCUSSION

The current study was conducted to specify the impacts of a protocol called “living high, training high and low” (LHThL) on the protein levels of two serums, namely endothelin-1 and erythropoietin, as well as the performance of Iran National Team’s endurance runners in 3000-meter run. The main findings of this study showed that in case of using LHThL protocol, the endurance performance of athletes as well as ET-1 improve after 11 weeks of living under a normoxic state and altitude training. As a case in point, no matter after return or four weeks of training, the participant who accomplished 2500-meter (considered to be relatively hypoxic) returned to 1200-meter altitude and, then, opted for 1200-meter sea level four weeks later turned out to improve in a way that was meaningfully significant. As it is shown in Figure 3, the improvement of time trial performance was calculated to be mean 5-km (4.86%). Although such improvements were observed in the performance of the participants, EPO level did not change. Therefore, it seems unavoidable and vital to have erythropoiesis in accordance with altitude because in case of using a training protocol, it is not enough to bring about improvement only through one factor. The collected data evinced that R2M training model in a time period of 4 week at an altitude camp paves the ground for a living altitude as a target about 1200-2500 meters high (considered to be relatively hypoxic) which in its own turn ends in having a better sea level performance due to optimal acclimatization response.

The findings of Eckardt et al. (1989) highlighted the disproportionate relationship between EPO in reaction to LHThL protocol on the one hand and the stress level of hypoxia on the other. However, it must be mentioned that there is no overlapping with the research done earlier about hypobaric chamber exposure data and athlete cohort (Ge et al., 2002). Ge et al. found out that EPO reaction to a simulated altitude of 2500 meters (considered to be relatively hypoxic) was almost half in comparison with the amount before being exposed to the altitude. For Levine et al. (1991), the level of erythropoietin nearly doubled and the concentration of hemoglobin (Hb) showed increase. Levine’s case study run for 27 days in which a group of professional athletes who lived at an altitude of 2500 meters (considered to be relatively hypoxic) and did their workout at an

altitude of 1250 meters. In another study conducted by Dehnert et al. (2002), 15 male and 6 female triathletes spent two weeks at an altitude of 800 meters (comparatively low). It should be noted that 13 hours of the day was spent at an altitude of 1960 meters (regarded as moderate). According to the findings of this study that dealt with the relationship between hematological acclimatization and intensive training, EPO underwent significant increase by 30%. However, considering the LHTL protocol, the given increase was temporary. By the same token, Hb did not change so much among LHTL group while there was minor significant decrease for control sea-level group.

In case the aforementioned findings are applied to altitude exposure for a long period of time, it would seem sound to hypothesize that the athlete who lives at a higher altitude during the training time will have a significant response of EPO. Indeed, the given response can be describe as acute and chronic. It is important to note that once exposure to hypoxia is finished, EPO will decrease in comparison with normoxic baseline. Although it is hard to prove, it would be probable to argue that the time period dedicated to training at moderate and high altitudes by the participants has not be adequate to trigger a supplemental hypoxic stimulus in order to escalate the impact of the background altitude environment.

Robertson et al. (2010) contended that living high in tandem with training high and low is potential to bring about an increase of Hb mass. The group that they used for comparison consisted of athletes who followed living low and training high, not living high and training low. Apart from these observations, there is enough evidence to back up the argument that altitude training does not lead to an increase of EPO in a linear way which is needed to improve performance in accordance with LHTL protocol. It should be noted that this factor is not sufficient by itself.

From another perspective, EPO apart from its impacts on erythropoiesis (helping the cardiac and endothelial systems function more properly) is influential on performance (Cariou et al., 2008). However, it is a tough task to pinpoint such beneficial impacts (Lundby et al., 2007; Robertson et al., 2010). The vasoconstrictor reaction to a hypoxic state is highly dependent upon endothelial cells (Graser and Vanhoutte, 1991; Chan et al., 2011). To cite another relevant case, contraction through exposure to a hypoxic state by means of

endothelium increased in femoral and coronary arteries of dogs (De Mey and Vanhoutte, 1983; Graser and Vanhoutte, 1991; Pearson et al., 1996), pigs (Chan et al., 2011) and humans (Thorne et al., 2004). By the same token, a hypoxic state can leave long-term impacts on vasculature. Several studies have shown that viability and proliferation of vascular muscle cells which are smooth will increase after treatment through long-term hypoxia. Therefore, a link can be found between improvement and performance according to Kiefer et al. (2002).

In this study, male endurance runners followed an 11-week period of LHTHL protocol. The results showed that endothelin-1 underwent change in a significant way before and after the training period. The mean of ET-1 changes turned out to increase in post-examination-2 and pre-examination by 133%. Contrary to the findings of this study, Giles et al. (2012) showed that plasma ET-1 level under hypoxic and normoxic states did not increase after 30-minute training at ventilatory threshold. Very interestingly, normoxic trainings did not change plasma endothelin-1 level among healthy people (Lenz et al., 1998; Petidis et al., 2008).

The findings of the present study are in the same line with those of Maeda et al. (2009). They similarly concluded that endothelin-1 level will increase significantly after a 30-minute training at ventilatory threshold by 90%. In other words, the participants did the workout at 130% of the power which was measured at the ventilatory threshold and, in the meantime, the level of plasma endothelin-1 increased continuously. In addition, it was meaningfully greater than levels which were recorded at 90% of the ventilatory threshold. However it should be noted that the present study and the one conducted by Maeda et al. (1994) revealed some differences with regard to the reaction of plasma endothelin-1 to exercise, the reason of which was related to the training condition of the participants. The participants of the present study were endurance trained while in that of Maeda they were intercollegiate athletes. Therefore, it would be possible to infer that the reaction of plasma endothelin-1 to exercise among aerobically trained athletes is somehow less in comparison with non-aerobically trained ones. Matsakas and Mougios (2004) bolstered this argument in their study according to which the post-exercise reaction of endothelin-1 was shown to be less in aerobically trained individuals in comparison with non-aerobically trained individuals.

Based on the findings of this study, 11-week training period according to LHTHL protocol will improve endurance. For example, 3000-meter performance after LHTHL training camp turned out to be faster by 44 seconds in comparison with the same try on the first day. Although the findings of different studies are not unanimous, it would be helpful to mention them: according to Hellman (1999), the endurance performance improves significantly (3%); Rodriguez et al. (2000) revealed that power output increases in a significant way at non-aerobic threshold while VO₂max and cycling exercise time will not bear significant change. A number of studies did not report significant changes (Vallier et al., 1996) or improvement in normoxic VO₂max after LLTH in comparison with training at sea level (Geiser et al., 2001). The mentioned results were backed up by the findings of Roels et al (2005, 2007), according to which IHIT and IHT in a time period of 115 minutes in a week did not bring about a significant increase in aerobic performance or hematological variables in comparison with the increased caused by similar normoxic interval training.

CONCLUSIONS

An 11-week period of training based on LHTHL protocol led to improvement of race performance at sea level in a significant way. Similarly, ET-1 level appeared to increase for a group of trained distance runners. Those athletes who live under hypoxic and normoxic states did not show any significant change in EPO level after going through the training protocol. Therefore, according to the given data, in case an altitude training camp is intended, in order to expect improvement in hematology, an optimal living altitude must be taken into account.

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