



DOI: 10.1515/rmlm-2016-0008

Biochemical and functional modifications in biathlon athletes at medium altitude training

Modificările biochimice și funcționale ale atleților biatloniști după antrenament la altitudine medie

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Abstract

Objective: The aim of our research was to identify physiological and biochemical changes induced by training at medium altitude.

Methods: Ten biathlon athletes underwent 28-day training camp at medium altitude in order to improve their aerobic effort, following the living high-base train high-interval train low (Hi-Hi-Lo) protocol. There were investigated three categories of functional and biochemical parameters, targeting the hematological changes (RBC, HCT, HGB), the oxidative (lipoperoxid, free malondialdehyde and total malondialdehyde) and antioxidative balance (the hydrogen donor capacity, ceruloplasmin and uric acid) and the capacity of effort (the maximum aerobic power, the cardiovascular economy in effort, the maximum O₂ consumption).

Results: All the biochemical and functional parameters evaluated showed significant increases between the pre-training testing and post-training testing (5.13 ± 0.11 vs. 6.50 ± 0.09 , $p < 0.0001$ for RBC; 44.80 ± 1.22 vs. 51.31 ± 2.31 , $p < 0.0001$ for HCT; 15.06 ± 0.33 vs. 17.14 ± 0.25 , $p < 0.0001$ for HGB; 1.32 ± 0.04 vs. 1.62 ± 0.01 , $p < 0.0001$ for LPx; 1.61 ± 0.01 vs. 1.73 ± 0.01 , $p < 0.0001$ for free MDA; 2.98 ± 0.08 vs. 3.37 ± 0.03 , $p < 0.0001$ for total MDA; 45.92 ± 0.13 vs. 57.98 ± 0.12 , $p < 0.0001$ for HD; 25.95 ± 0.13 vs. 31.04 ± 0.06 , $p < 0.0001$ for Crp; 3.47 ± 0.03 vs. 7.69 ± 0.02 , $p < 0.0001$ for UA; 63.91 ± 1.00 vs. 81.53 ± 1.97 , $p < 0.0001$ for MAP; 33.13 ± 0.57 vs. 57.41 ± 0.63 , $p < 0.0001$ for C_{VEE}; 4190 ± 50.45 vs. 5945 ± 46.48 , $p < 0.0001$ for VO_{2max}).

Conclusions: Aerobic effort capacity of biathlon athletes has increased in the post-training period, using Hi-Hi-Lo protocol.

Keywords: acclimatization, medium altitude, hematological and functional explorations, biathlon, Hi-Hi-Lo protocol.

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Rezumat

Obiectiv: Scopul cercetării noastre a fost de a identifica modificările fiziologice și biochimice induse de antrenamentul la altitudine medie.

Material și metodă: Zece sportivi de biatlon au participat la antrenament timp de 28 zile, la altitudine medie, în scopul de a îmbunătăți efortul aerob, urmând protocolul (Hi-Hi-Lo). Au au fost investigați trei categorii de parametri funcționali și biochimici care vizează modificările hematologice (RBC, HCT, HGB), balanța oxidativă (lipoperoxid, malondialdehida liberă și malondialdehida totală) și antioxidantă (capacitatea de donator de hidrogen, ceruloplasmina și acidul uric) și capacitatea de efort (puterea maximă aerobă, economia cardiovasculară în efort, consumul maxim de O₂).

Rezultate: Toți parametrii biochimici și funcționali evaluați au prezentat creșteri semnificative între testarea pre-antrenament și testarea post-antrenament (5.13 ± 0.11 vs. 6.50 ± 0.09 , $p < 0.0001$ for RBC; 44.80 ± 1.22 vs. 51.31 ± 2.31 , $p < 0.0001$ for HCT; 15.06 ± 0.33 vs. 17.14 ± 0.25 , $p < 0.0001$ for HGB; 1.32 ± 0.04 vs. 1.62 ± 0.01 , $p < 0.0001$ for LPx; 1.61 ± 0.01 vs. 1.73 ± 0.01 , $p < 0.0001$ for free MDA; 2.98 ± 0.08 vs. 3.37 ± 0.03 , $p < 0.0001$ for total MDA; 45.92 ± 0.13 vs. 57.98 ± 0.12 , $p < 0.0001$ for HD; 25.95 ± 0.13 vs. 31.04 ± 0.06 , $p < 0.0001$ for Crp; 3.47 ± 0.03 vs. 7.69 ± 0.02 , $p < 0.0001$ for UA; 63.91 ± 1.00 vs. 81.53 ± 1.97 , $p < 0.0001$ for MAP; 33.13 ± 0.57 vs. 57.41 ± 0.63 , $p < 0.0001$ for CVEE; 4190 ± 50.45 vs. 5945 ± 46.48 , $p < 0.0001$ for VO₂max).

Concluzii: Capacitatea de efort aerob a sportivilor de biatlon a crescut în perioada post-antrenament, folosind protocolul Hi-Hi-Lo.

Cuvinte cheie: aclimatizare, altitudine medie, explorări hematologice și funcționale, biatlon, protocolul Hi-Hi-Lo.

Received: 11th October 2015; Accepted: 24th August 2016; Published: 30th August 2016.

Introduction

Body acclimatization to alpine climate with medium altitude induces physiological and biochemical changes in the body's systems, among which the circulatory and respiratory systems are essential. At 1000 m altitude the human body gets into hyperventilation to ensure the optimum O₂ need for body acclimatization; the increase in altitude implies a proportional increase in the intensity of hyperventilation.

The human body can compensate for the lack of O₂ up to an altitude of 5000 m, where the oxygen partial pressure (pO₂) reaches 25-35 mmHg, values that represent the minimum limit for survival [1-3].

The body has mechanisms to prevent tissue hypoxia by changing blood parameters, thus, at high altitude the hemoglobin concentration (HGB) and the red blood cell count (RBC) increase, reaching values of 8,000,000/mm³ at 500 m altitude, resulting in the doubling of normal values.

After a period of 2-4 weeks training and exposure at altitude, the athletes' bodies adapt by increasing the RBC rate formation and by the rapid mobilization of the blood reserves; in case they descend to a lower altitude where the pO₂ is normal, both the oxygen transportation and the oxygen consumption capacity increase, determining the improvement of the effort capacity on short or long term. These procedures have been adopted by biathletes and cross-country skiers [4].

These adaptations are modulated by many factors, including the degree of hypoxia related to altitude, time of exposure, effort intensity and individual conditions. It has been established that exposure to high altitude is an environmental stressor that elicits a response that contributes to many adjustments and adaptations that influence the effort capacity and the endurance performance. These adaptations include the increase in HGB concentration, ventilation, capillary density and tissue myoglobin concentration [5].

The high altitude environment may be divided into three zones according to their altitude above the sea level [6]:

1. medium altitude - 1,500-2,500 m;
2. higher altitude - 2,500-5,300 m;
3. extreme altitude - over 5,300 m.

The saturation with oxygen (SaO₂) of the arterial blood at medium altitude exceeds 90% and tissue oxygenation is not constrained [7]. Studies on adaptation to altitude are relatively numerous, but they do not fully highlight the correlation between the adaptive changes of the human body and the improvement of the physical performance. Most studies particularly analyze the hematological changes in relation with the effort or the motor capacity.

In setting the research hypothesis we started from the assumption that the athlete's body suffers a series of functional and biochemical changes in the case of exposure and training at medium altitude, changes that will determine an improvement of aerobic effort capacity.

The aim of our study is the assessment at medium altitude of three categories of parameters: the adaptive changes of the blood parameters, the oxidative and antioxidative balance parameters and the effort capacity parameters.

Material and method

This research is an extension of a previous research performed between 15th of July and 11th of August 2014 on the same group of athletes, when the training took place at the sports center of Rasnov at an altitude of 676 m and only changes in the functional capacity were recorded.

Both studies were carried out by including the same training program and protocol, with the exception that the post-training testing conducted in 2014 was achieved after three weeks of physical training camp.

The present research was conducted on a group of 10 experienced male biathlon athletes, who underwent 28-day training camp at medium altitude in order to improve the aerobic effort. They followed the living high-base train high-interval train low (Hi-Hi-Lo) protocol.

The exposure to hypoxia and training was done between 13th of July and 9th of August 2015, in the sports center of *Cheile Gradistei - Fundata* at an altitude of over 1500 m and in *Postavarul Mountain* range at an altitude of over 1700 m.

The study was approved by the Ethics Committee of University of Medicine and Pharmacy Tîrgu Mureş. All the selected participants in this study were volunteers and gave their informed consent.

The athletes underwent the pre-training testing prior to the start of exposure to altitude and the post-training testing, 28 days after the exposure to altitude.

The study comprised three categories of biochemical and functional explorations: the exploration of the hematological parameters, the determination of the oxidative and antioxidative balance and the exploration of the capacity of effort.

The targeted hematological indicators were: red blood cells count (RBC $\times 10^6/\mu\text{l}$), the hematocrit (HCT %) and the hemoglobin concentration (HGB g/dl). The hematological determinations were made using the Celdyn 3700 Abbott analyzer.

For the determination of the oxidative and antioxidative balance we collected venous blood samples, pre-acclimatization, pre-training and post-acclimatization, post-training, and we targeted the following markers:

- For the oxidative balance: lipoperoxid (LPx), free malondialdehyde (MDA_{free}) and total malondialdehyde (MDA_{total}), all expressed in nmol/ml;
- For the antioxidative balance: the hydrogen donor capacity (HD%), ceruloplasmin (Crp mg/dl) and uric acid (UA mg/dl).

The exploration of the effort capacity was assessed at the County Medical Center MAI from Braşov, which is located at an altitude of 592 m, and consisted of an indirect determination of the aerobic effort capacity using the Astrand-Ryhming method. The Astrand-Ryhming test consisted of a submaximal effort carried out for 6 minutes, using a cycloergometer, with the rotation of 40-80/min and the intensity of 175 W/kg, maintained constant during the whole test procedure [8]. On the basis of the measured heart rate during the last 10 seconds of pedaling, the following indirect indicators of the effort capacity were calculated:

- The maximum aerobic power, expressed in ml/kg and calculated using the formula $MAP = VO_{2max}/kg$.
- The cardiovascular economy in effort, expressed in ml/min and calculated using the formula $CVEE = VO_{2max}/Maximum\ heart\ rate$.
- The maximum O_2 consumption (VO_{2max}) expressed in ml and calculated by the tailored formula for men [9] $VO_{2max} = (0.00212 \times workload + 0.299) / (0.769 \times HR_{ss} - 48.5) \times 100$.

The statistical analysis was performed using Graph Pad Prism 5 software. The following statistical indicators were calculated so as to

Table 1. The red blood cell parameters

Parameter*	Pre-training	Post-training	p value
RBC ($10^6/\mu l$)	5.13 ± 0.11	6.50 ± 0.09	< 0.0001
HCT (%)	44.80 ± 1.22	51.31 ± 2.31	< 0.0001
HGB (g/dl)	15.06 ± 0.33	17.14 ± 0.25	< 0.0001

*The parameters are expressed as Mean \pm SD.

highlight the qualitative differences between the variables: the mean, the standard deviation, the minimum and maximum values, the Student's t-test [10]. A p value < 0.05 was considered statistically significant.

Normality of the data was assessed using the Shapiro-Wilk test and the significance level was set at $\alpha=0.05$.

Results

The mean age of the study group was 24.11 ± 1.45 years and the mean weight 69.20 ± 1.26 kg.

Table 1 indicates a significant difference between pre-training testing and post-training testing for the hematological parameter.

The results for the oxidative and antioxidative balance parameters are shown in **Table 2**.

Table 2. The oxidative and antioxidative balance parameters

Oxidative balance parameters*	Pre-training	Post-training	p value	Antioxidative balance parameters*	Pre-training	Post-training	p value
LPx (nmol/ml)	1.32 ± 0.04	1.62 ± 0.01	< 0.0001	HD (%)	45.92 ± 0.13	57.98 ± 0.12	< 0.0001
free MDA (nmol/ml)	1.61 ± 0.01	1.73 ± 0.01	< 0.0001	Crp (mg%)	25.95 ± 0.13	31.04 ± 0.06	< 0.0001
total MDA (nmol/ml)	2.98 ± 0.08	3.37 ± 0.03	< 0.0001	UA (mg/dL)	3.47 ± 0.03	7.69 ± 0.02	< 0.0001

*The parameters are expressed as Mean \pm SD.

Table 3. The parameters showing the aerobic effort capacity

Parameter*	Pre-training	Post-training	p value
MAP (ml/kg)	63.91 ± 1.00	81.53 ± 1.97	< 0.0001
CVEE (ml/min)	33.13 ± 0.57	57.41 ± 0.63	< 0.0001
VO ₂ max (ml)	4190 ± 50.45	5945 ± 46.48	< 0.0001

* The parameters are expressed as Mean ± SD.

The parameters showing the aerobic effort capacity are presented in **Table 3**.

Discussions

Acclimatization and training protocols like living high- training high protocol (Hi-Hi), living high-training low protocol (Hi-Lo), living low-training high (Lo-Hi), and intermittent hypoxic exposure (IHE) protocol provided natural and artificial methods that have been developed to improve aerobic and anaerobic capacity. Evidence in the literature suggests that the Hi-Lo or LH-TL model has advantages over SL training to improve performance, associated with an increase in VO₂max, hematological parameters, power output and economy [5].

Some authors [8, 11] consider that there is an “acclimatization memory” that is expressed through an accentuated and gradual attenuation of the adaptive changes to the training sessions at altitude. Thus, it was found that after a training period of 21-28 days, during the first 24-48 hours, the functional state presents no relevant changes, but after this short period, from the third day after exposure to altitude, the acclimatization phase installs, which induces a low yield for the next seven to ten days. After this acclimatization phase the positive phase follows, in which aerobic efficiency can increase by up to 15% and biochemical parameters reach very good values (HGB, RBC, VO₂max, alkaline reserve, glutathione, etc.). Sport yield is good in the range of 14 to 21 days after exposure to medium altitude, with a maximization peak between days 14 to 18.

Hematological parameters

Comparing the results of our study with the ones obtained by Martoma on alpine skiers, we found that the means were superior in the favor of the biathlon group [11, 12]. Thus, the mean differences among the tests between the biathlon group and alpine skiing group were: 0.16x10⁶/μl for RBC, 0.48 g/dl for HGB and 3.50% for HCT.

Studies have shown that the red cell mass slowly increases along with prolonged hypoxemia. HGB concentration increases, from the sea level value around 14-15 g/dl, to levels of over 18 g/dl and the HCT increases from 40-45% to more than 55%. Renal hypoxia, and norepinephrine, respectively, stimulates the production and the discharge of erythropoietin (EPO). EPO is a growth factor that stimulates the production of proeritroblasts in the bone marrow and determines the acceleration of the development of red blood cells from the progenitor cells [13].

Chapman et al. [14] proposed one modification in the LH-TL according to the new solution: athletes should stay and perform low-intensity training at an altitude of 2500 m for four weeks, while high-intensity interval training should be performed at 1250 m. This method was called Hi-Hi-Lo (live high-base train high-interval train low). In this respect, other studies found a significant increase in VO₂max accompanied by improvement of several hematological parameters (hemoglobin mass and hematocrit) that resulted in significant improvement of running time after the Hi-Hi-Lo protocol [4, 14, 15].

The Hi-Lo effect on the quantity of HGB and on the volume of RBC studied on resistance/ endurance athletes, elite athletes, mountain guides, who live at 2500 m, 18 hours a day and train at 1000 and 1800 m for 24 days, showed an increased HGB mass and red cell volume, which may help to increase the performance of endurance athletes [16].

The HGB decrease in subjects acclimatized on altitude to the pre-acclimatization values, does not affect $\text{VO}_{2\text{max}}$ during the effort in hypoxia. The acute HGB increase does not affect the maximum effort capacity or the $\text{VO}_{2\text{max}}$ during the effort in acute hypoxia [17].

Several studies show a relatively linear decrease in HGB and HCT starting as soon as the hypoxic stimulus is removed [13, 18-20].

For the athletes trained for endurance there is a decrease of the $\text{VO}_{2\text{max}}$ at the altitude of 1000, 2500, and 4500 m, respectively. This can be explained by the maintenance of a high arterial desaturation that is produced by the limited diffusion [21].

The results of another study have illustrated that it is possible for endurance athletes to expect a ~3% increase in HGB mass after only 14 days of LHTH training at 1800 m. This lowers the threshold altitude considered sufficient to increase the red blood cell production by ~200m. Given that the accelerated red blood production seems to depend on the total dose of sufficient 'altitude' our results highlight that LHTH is the most time efficient modality for athletes and coaches to consider, because it provides a 24-hour per day exposure to hypoxia [22]. A meta-analysis highlighted an additional ~2% increase in HGB mass in the third week [23].

The determinations of the oxidative and antioxidative balance

Studies on the oxidative and antioxidative balance changes in the case of medium altitude

training were simulated both on laboratory rats and on athletes in real conditions [11, 12].

The oxidative and antioxidative balance parameters recorded significant increases in conditions of altitude training in biathlon athletes compared to the studies performed on alpine skiers by Martoma [11, 12]. Analyzing the results obtained in our study with those obtained on the alpine skiers, the differences between the mean values, in terms of the oxidative balance parameters, were: 0.04 nmol/ml for LPx, 0.01 nmol/ml for MDA_{free}, 0.05 nmol/ml for MDA_{total}, and in terms of antioxidative balance parameters, the differences were: 1.06% for HD, 0.12 mg/dl for Crp and 0.11 mg/dl for UA. Although these differences are not very high, it should be noted that the biathlon group recorded higher values, both initially and finally, on all the oxidative balance parameters, but especially in the case of antioxidative balance parameters, thus contributing to a better adaptation to altitude, with direct effect on the motor capacity.

Hypoxia is a promoter of the oxidative enzymes expression in the mitochondria, determining the increase of the tissue ability to extract the O_2 from the blood. Acclimatization to high altitude does not only increase the release of the O_2 from the periphery but also the O_2 uptake by the tissues [11, 12]. The effect of the O_2 fractions inspired upon the oxygenation during the endurance athletes' training, at a moderate altitude (1800-1900 m) showed that supplementation in O_2 on Hi-Lo models determines a significant increase of the physical performance and a decrease in the oxidative stress indicators, reflected in the blood (lipid hydroperoxide and reduced glutathione) and urine (urinary malondialdehyde and 8-hidroxi-deoxi-guanine) [24].

The aerobic effort decreases the sensitivity of the tissue to insulin. This determines the decrease of the glucose transport in muscles, increasing the reconversion into free fatty acids and saving the muscle glycogen [25, 26].

Another study investigated if the Hi-Hi-Lo procedure performed in hypo-baric hypoxia conditions affects biathletes' aerobic capacity and effort energy cost in normoxia. Additionally, the erythropoietin and the selected hematological variables were evaluated, such as the potential factors influencing the aerobic capacity and the endurance performance. The main finding of the study was that the Hi-Hi-Lo 3-week protocol did not improve the aerobic capacity estimated by VO_2max in spite of the significantly higher values of the hematological parameters [4].

Numerous studies showed the effects of altitude on the physical performance of the athletes, caused by the decrease of O_2 availability under hypoxia. Anaerobic trials are not affected by the moderate altitude while aerobic trials are [3, 11, 12, 27, 28].

The determination of the aerobic effort capacity parameters

The aerobic capacity parameters showed significant increases in the post-training testing at medium altitude, compared with the initial testing, and contributing to the increase of the effort motor potential of the athletes.

The comparative analysis of our 2014 recorded results (the same group of biathletes) highlights statistically significant lower values than those obtained in the current research. Thus, the differences between the two researches for MAP were 1.40 ml/kg at the initial testing and 8.40 ml/kg at the final testing; for C_{VEE} were 1.40 ml/min at the initial testing and 8.90 ml/min at the final testing and for VO_2max of 72 ml at the initial testing and 200 ml at the final testing.

Comparing the alpine skiers' groups of Martoma et al. [11, 12] with our biathlon group, the mean of the recorded results of the biathletes is above the mean of the skiers group. The superior results of the biathlon group are influenced by the specificity of the practiced sport and the

athlete's selection and training are customized to endurance sports.

Studies on effort physiology prove that 1800-2400 m altitude area is the ideal place for performing sports training stages. The adaptation time increases according to the increase in altitude and the physiological changes are more intense, in order to allow acclimatization to high altitude. Adapting can have immediate effects, taking place in a few days or on a long-term, requiring weeks or even months [8, 11, 29-31]. A minimum period of three weeks at altitude will cause physiological changes in the body in order to adapt to a better use of the received O_2 . With the return to low altitude, these adjustments will help athletes to improve their performance capacity, because the skiers' performance is limited by the ability to use O_2 [11, 12].

Chapman et al. in 2014 observed a significant improvement of the sport performance in groups living at two medium altitudes (2085 and 2454 m) for four weeks, but not in groups living at 1780 and 2800 m. Moreover, EPO levels were significantly higher in all groups at 24 and 48 h, but returned to basic levels after 72 h in the 1780 m group. These results suggest that, the altitude between 2000 and 2500 m, during the LH-TL protocol, allows an optimal acclimatization response in sea level performance [32].

The positive changes observed in our participants led to the conclusion that Hi-Hi-Lo training method could effectively improve endurance in normoxia, since most of the biathlon competitions are performed at submaximal intensities.

The fact that the study was conducted with a convenience sample of a fairly small number of participants is a limitation of the present investigation.

Our future research will focus on the individual differences in terms of the altitude adaptation through the correlation of some other morpho-functional and motor parameters.

Athletes' adaptation to altitude will require more complex interdisciplinary research. Also, the relationship between the adaptation to altitude, the types of effort and the period of sports training, rehabilitation and rest or recreation will represent the important parameters of investigation for the understanding of the complex mechanisms of athletes' adaptation to different altitudes.

Conclusions

Our results confirm the hypothesis that the acclimatization to medium altitude and 28 days of training sessions determines complex biochemical and functional adaptive changes in the bodies of biathlon athletes. All the biochemical and functional evaluated parameters showed significant increases between the pre-training testing and post-training testing.

Abbreviations:

SD	- standard deviation
pO ₂	- oxygen partial pressure
MAP	- maximal aerobic power
CVEE	- cardiovascular economy in effort
VO ₂ max	- maximum O ₂ consumption
LPx	- lipoperoxide
MDA _{free}	- free malondialdehyde
MDA _{total}	- total malondialdehyde
HD	- hydrogen donor capacity
Crp	- ceruloplasmin
UA	- uric acid
RBC	- red blood cells
HCT	- hematocrit
HGB	- hemoglobin

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