

The influence of 9-day trekking in the Alps on the level of oxidative stress parameters and blood parameters in native lowlanders

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Abstract

Background. The stimuli acting on a person in a high mountain environment (such as hypobaric hypoxia with subsequent reoxygenation, physical exercise) can significantly increase oxidative stress, stimulate erythropoiesis, lead to changes in the blood count and participate in the development of altitude sickness.

Objective. The aim was to investigate changes in haematological parameters, indicators of oxidative stress (malondialdehyde – MDA) and antioxidant defences: catalase (CAT), superoxide dismutase (SOD), and total antioxidant status (TAS) in the plasma of young, healthy people after a 9-day expedition in the Alps.

Materials and method. A total of 5 patients (4 men and 1 woman), members of the Wrocław Mountaineering Club, aged 24–26 years. Collection of blood samples was carried out immediately before departure and 3 days after the end of exposure to high-altitude conditions. During the expedition, the subjects were exposed to heights: 2,050–4,165 m.a.s.l., and exercise associated with climbing.

Results. Trekking in the Alps neither caused significant changes in the parameters of red blood cells nor increased the level of oxidative stress parameters in plasma. CAT activity increased, the ratio of SOD / CAT decreased. There was also a decrease in the total number of leukocytes, mainly monocytes and basophils.

Conclusions. 9-day exposure to high-altitude conditions is not a substantial burden for the organism of young, physically active people. The increase in antioxidant capacity is sufficient to stop oxidative processes, which are severe in these conditions, and to prevent the occurrence of significant oxidative stress. Discontinuation of exposure to allergens and dust pollution clears the airways, which is indicated by the reduction in the number of monocytes and basophils.

Key words

oxidative stress, blood count, high-altitude environment

INTRODUCTION

A stay in high-altitude conditions leads to the development of hypobaric hypoxia. This phenomenon activates adaptation mechanisms which allow continued function in hypoxia. The most important is intense erythropoiesis, which leads to an increase in the number of erythrocytes (RBC), haemoglobin concentration (HGB) and haematocrit (HCT) levels [1]. As a result of hypoxia, there is not just adaptation, there are also unfavourable phenomena, such as increased production of reactive oxygen species (ROS). The largest generation of ROS occurs during reoxygenation – after cessation of exposure to altitude hypoxia [2].

In addition to hypoxia associated with climbing, in high altitudes, the human body is exposed to other factors, increasing oxidative stress. These factors include: heavy exercise, dehydration, inadequate supply of antioxidants in diet, low ambient temperature, wind, and more intense UV radiation than in the lowlands. [3, 4]. Free oxygen radicals may accelerate swelling of cells in the development of high-altitude cerebral oedema (HACE). There are reports indicating the important role of oxidative stress in the development of altitude sickness [2].

Monitoring of indicators of oxidative reactions and antioxidant defence during staying in the high mountains can contribute to a better understanding of the mechanisms responsible for the development of pathological changes associated with exposure to the high-altitude environment.

Objective. The aim was to investigate changes in haematological parameters, indicators of oxidative stress (malondialdehyde, MDA) and antioxidant defences (catalase, CAT, superoxide dismutase, SOD and total antioxidant status, TAS) in plasma of young, healthy people after a 9-day expedition in the Alps.

MATERIALS AND METHODS

A trekking trip to the Italian Alps was organized for this study. The purpose of the visit was to climb the Matterhorn mountain (4,478 m above sea level). Apart from the effort directly related to the mountain operations, the participants did undertake any additional exercise. The stay in the mountains lasted nine days, during which time the participants lived at different heights above sea level (Fig. 1). Most of the time, especially at night, they spent at an altitude of 2,800 m above sea level.

A total of 5 people (4 men and 1 woman), members of the Wrocław Mountaineering Club aged 24–26 years, and all volunteers declared in good health.

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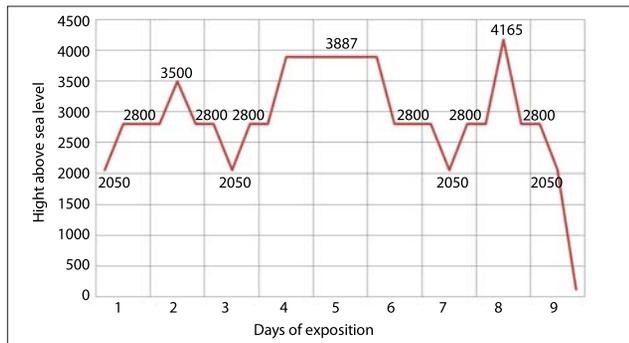


Figure 1. Changes in meters above sea level during the 9-day stay in the Alps

Before leaving, all volunteers were examined (blood, urine, ECG, chest X-ray), informed about the conditions of the experiment and familiarized with its successive stages. Each of them gave written consent to participate in the study.

Collecting venous blood samples before and after departure. Collecting venous blood samples was made in the Central Analytical Laboratory of the Independent Public Clinical Hospital No. 1 in Wrocław. Blood was collected immediately before departure and after 3 days exposure to the high-altitude environment. In the day prior to blood sampling, the subjects did not consume alcohol and did not practice sports. Blood was collected from the antecubital vein at the fasted state at 07:30 – 08:00 a.m., approximately 20 ml to each of 2 sample tubes. Some donations were used to determine the parameters of blood count with differential. The remaining plasma samples were collected, frozen at -80C, and kept in these conditions until biochemical measurements.

Determination of the cellular components of blood was conducted on a haematology analyzer BC-2800 Mindray's. Blood smears were evaluated independently.

The activity of catalase (CAT) was determined by the set of reagents OxiSelect Catalase Activity Assay Kit, (No. Cat. STA-341) produced by the Cell Biolabs company. The activity of superoxide dismutase (SOD), total antioxidant status (TAS) and malondialdehyde concentration was performed using reagents from kits Cayman Chemical Company: Superoxide Dismutase Assay Kit (No. Cat. 706,002), Antioxidant Assay Kit (No. Cat. 709,001) and TBARS Assay kit (No. Cat. 10,009,055).

All assays were performed strictly according to the instructions included by the manufacturer.

Statistical analysis. The results were presented as mean and standard deviation (Mean \pm SD). The occurrence of statistically significant differences between variables were tested using the Wilcoxon test for dependent variables. The level of significance was $p < 0.05$. Statistical calculations were made with use of the statistical program STATISTICA 10.0.

RESULTS

Results of complete blood count with differential before and after the trip to the Alps. Average values of the white blood cells parameters in the expedition (mean \pm SD) before and after exposure to high-altitude conditions are shown in Table 1. After exposure to high-altitude conditions, there was a statistically significant decrease in the number of white blood cells (about 10%) in comparison to the value before the trip. The decrease in the number of WBC was associated with decreased content of basophils (BASO) and monocytes (MONO). During the stay in the Alps, percentage and absolute value BASO decreased by 35% and 50%, the absolute value of MONO was decreased by 17%, compared to the value before the expedition, $p < 0.05$.

Table 2 shows other selected indicators of blood counts, such as haematocrit (HCT), the number of red blood cells (RBC), haemoglobin (HGB), mean corpuscular volume index (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and platelet count (PLT). The respondents had an increase in the average values of haemoglobin, haematocrit, and number of erythrocytes and platelets after exposure to high-altitude conditions. However, these changes were not statistically significant, and all the red blood cell parameters were within normal limits. The stay in the Alps did not cause significant changes in such red blood cell parameters as MCV, MCH, MCHC.

Changes in activity of antioxidant enzymes and the content of lipid peroxidation products. Table 3 shows the average activity of the antioxidant enzymes (CAT, SOD), SOD/CAT, TAS levels and plasma MDA concentration before and after the trip in. Results are expressed as mean \pm SD. In the activity of catalase and SOD / CAT values a statistically significant change was observed. CAT activity in plasma was higher than 50% after exposure to high-altitude environment, compared to the enzyme activity before departure. However, the ratio of

Table 1. Parameters of white blood cell counts with differential before and after exposure

		Before ascent	Min-max (before)	After ascent	Min-max after	Statistical significance
WBC $10^3/uL$	(n=5)	5.78 \pm 0.91	4.76–7.22	5.20 \pm 0.42	4.65–5.84	$p < 0.05$
NEUT %	(n=5)	49.70 \pm 6.11	47.10–59.30	51.94 \pm 4.87	45.20–57.50	NS
LYMPH %	(n=5)	36.04 \pm 5.55	29.50–44.30	34.46 \pm 4.85	28.40–41.30	NS
MONO %	(n=5)	9.94 \pm 1.39	8.80–11.60	9.32 \pm 0.64	8.60–10.30	NS
EO %	(n=5)	3.26 \pm 1.47	1.50–5.10	3.58 \pm 1.35	1.40–4.90	NS
BASO %	(n=5)	1.08 \pm 0.13	1.00–1.20	0.70 \pm 0.14	0.60–0.90	$p < 0.05$
NEUT $10^3/uL$	(n=5)	2.86 \pm 0.52	2.24–3.56	2.69 \pm 0.27	2.31–3.03	NS
LYMPH $10^3/uL$	(n=5)	2.08 \pm 0.43	1.57–2.59	1.79 \pm 0.29	1.32–2.11	NS
MONO $10^3/uL$	(n=5)	0.58 \pm 0.14	0.47–0.84	0.48 \pm 0.07	0.40–0.60	$p < 0.05$
EO $10^3/uL$	(n=5)	0.19 \pm 0.10	0.14–0.37	0.18 \pm 0.07	0.18–0.26	NS
BASO $10^3/uL$	(n=5)	0.06 \pm 0.01	0.05–0.08	0.03 \pm 0.00	0.03–0.05	$p < 0.05$

Table 2. Parameters of red blood cells, haematocrit and platelet counts before and after exposure

		Before ascent	Min-max (before)	After ascent	Min-max (after)	Statistical significance
HCT %	(n=5)	43.26±2.47	39.20–45.60	44.02±1.63	41.80–46.10	NS
RBC 10 ⁶ /uL	(n=5)	4.86±0.19	4.91–4.97	4.95±0.26	4.93–5.23	NS
HGB g/dL	(n=5)	14.74±0.77	13.50–15.50	15.08±0.64	14.40–15.90	NS
MCV pg	(n=5)	88.86±2.69	86.70–92.90	88.94±2.54	85.90–92.30	NS
MCH g/dL	(n=5)	30.30±0.87	29.60–31.60	30.46±1.01	29.60–31.80	NS
MCHC g/dL	(n=5)	34.08±0.25	33.70–34.40	34.24±0.41	33.80–34.70	NS
PLT 10 ³ /uL	(n=5)	233±33.79	176–266	258±34.95	225–304	NS

Table 3. Changes in the activity of antioxidant enzymes (CAT, SOD, TAS, SOD / CAT) and quantity of the substance reacting with thiobarbituric acid (TBARS)

		Before ascent	Min-max (before)	After ascent	Min-max (after)	Statistical significance
CAT U/ml	(n=5)	7.81±3.49	3.35–12.44	15.96±6.36	11.33–25.75	p<0.05
SOD U/ml	(n=5)	49.89±6.00	40.91–56.54	46.29±4.35	41.70–51.12	NS
SOD/CAT U/ml	(n=5)	7.97±4.91	5.97–16.09	3.22±1.11	1.94–4.51	p<0.05
TAS mmol/l	(n=5)	1.28±0.37	0.71–1.74	1.74±0.43	1.39–2.47	NS
TBARS μM	(n=5)	9.34±1.77	6.37–10.72	9.86±2.21	7.39–13.23	NS

SOD / CAT decreased (by approximately 60%) in comparison to the value before the stay in the mountains.

In the levels of indicators of lipid peroxidation (TBARS) and total antioxidant status (TAS) in participants' plasma before and after the expedition there were no statistically significant changes.

DISCUSSION

Parameters of red blood cells. Staying at high altitudes is a commonly known factor contributing to the increase in haemoglobin, number of red blood cells and haematocrit value [1, 5]. However, no significant changes were observed in the HGB, RBC and HCT after 9 days of trekking (altitude 2,050–4,165 m above sea level) in the participants of the presented study. The mean values of these parameters did increase, but the increase was not statistically significant. The results can be explained by the relatively mild conditions of the experiment – the respondents spent 6 nights at the height of 2,800 m above sea level, and 2 nights at an altitude of 3,887 m above sea level. In addition, the speed of climbing was moderate and adapted to the capabilities of all participants. Apparently, the hypoxic stimulus was too weak to cause significant stimulation of erythropoiesis. Similarly, Michael et al [6] did not observe a statistically significant increase in haemoglobin in 6 persons who spent 23 nights at a simulated altitude of 3,000 m above sea level in normobaric hypoxic conditions, without exposure to physical activity.

There are a small number of reports about changes in the parameters of erythropoiesis, such as mean corpuscular volume (MCV), average weight of haemoglobin in red blood cells (MCH), and mean erythrocyte haemoglobin concentration (MCHC) at high altitudes. Esteva et al. [7] conducted a study in rats. They showed that exposure to intermittent hypoxia at an altitude corresponding to the height of 5,000 m above sea level during 4 weeks (4 hours a day / 5 days a week) resulted in a statistically significant increase of MCV and MCH in these animals. Participants in the presented study were highly exposed to much less

burdensome conditions. Measurements of red blood cell parameters, such as MCV, MCH and MCHC, after exposure to conditions of high-altitude did not show statistically significant differences. This is an advantageous phenomenon. The increase in the value of MCV, MCH and MCHC after exposure to a high-altitude environment indicates an increased number of abnormal erythrocytes. This may be caused by oxidative stress, which disturbs erythropoiesis, and erythrocyte membrane damage by ROS.

The obtained results indicate a moderate load and low stimulation of erythropoiesis. The results indirectly suggest an effective antioxidant defence and adequate levels of substances essential for producing red blood cells (iron, folic acid, vitamin B12) in the participants of the expedition.

Leukocytes – basophils, monocytes. The decrease in the total number of white blood cells (WBC), mainly monocytes and basophils, in the blood of participants of the expedition was observed after returning from the Alps.

Monocytes are directed against fungi, bacteria and large molecules, which are part of the particulate matter. The presence of contaminants increased the production and activity of monocytes in all participants in the presented study who live in a big city (over 600,000 inhabitants), and it may be assumed that most of the time during the year they are exposed to various forms of pollution transport, industrial and other (particulate matter, allergens).

Discontinuation of exposure to conditions of the urban environment and staying in a clean environment contributed to the respiratory tract clean attendees. No particulate matter in the mountains and the low level of antigens could lead to a decline in the number of monocytes due to lack of adequate incentives to their synthesis. Reducing the number of monocytes (within the physiological norm), can be regarded as a positive result for the stay in an unspoiled mountain environment.

The number of basophils after returning from a the expedition decreased by approximately 50%, in comparison to the value before the departure (the value was within within normal limits). Decrease in the number of basophils, as

monocytes can be associated with the discontinuation of exposure to the urban environment.

Other authors suggest the efficacy of the therapy in an alpine climate in the treatment of respiratory diseases and allergic reactions [8, 9].

Karagiannidis et al. [8] showed a beneficial effect of a 3-week long stay in the Alps for treatment of the local airway inflammation. Additionally, a reduction in the systemic activation of monocytes and T cells and an increase in the number of Treg cells was observed in the patients [8]. Also, Straub et al [9] reported improvement in respiratory function in asthmatic children after therapy altitude in a clinic located in the Swiss Alps. The beneficial effects of the mountain environment on the immune system was also revealed in increased levels of interleukin-10 (IL-10) in all patients [8, 9].

The positive impact on the treatment of allergic diseases and respiratory diseases is explained by the small amount of allergens in a mountain environment, high purity of the air and continuous winds. Increased synthesis of IL-10 during their stay in the mountains can also be associated with increased exposure to UV radiation [10].

Indicators of oxidative stress and antioxidant defense.

In the present study, no statistical significant changes in concentration of malondialdehyde (MDA) levels after exposure to hypoxia at altitude were detected, although most reports indicate the severity of oxidative reactions and increased levels of lipid peroxidation levels in people exposed to high-altitude environment [11, 12, 13, 14]. These differences can be explained by the fact that in most of the experiments are carried out in conditions which were more demanding than in the presented study (height, heavier exercise, exposure and other weather conditions).

Pfeifer et al. [11] observed an increase in lipid peroxidation products in the urine of people who were subjected to intense physical training at an altitude of 2,700 m above sea level for 14 winter days. The Everest III study conducted by Joan et al. [14] showed that increase in the oxidative stress is directly proportional to the height reached by volunteers. At an altitude of 6,000 m above sea level, an increase in the level of lipid peroxidation by 23% was observed, but at an altitude of 8,848 m the level indicators of oxidative stress increased by 79%.

The increase in the level of oxidative stress in addition to the height above sea level and additional incentives in the form of cold or heavy physical exertion is also caused by reduced exposure to atmospheric pressure. This is indicated by numerous studies carried out by various authors in animal models [7, 12, 13]. Exposure time significantly affects the level of lipid peroxidation products in rats subjected to intermittent hypobaric hypoxia corresponding to 4,000–6,700 m above sea level. In experiments in which the time of exposure was longer (4 weeks) no significant differences in the value of TBARS in comparison to the control group were detected [7]. However, in trials, the exposure time was 15 days [13], or 12 hours [12] statistically significant increases in the concentration of MDA was observed. It seems that the longer time of exposure allows mobilisation of adaptive mechanisms: an increase in haemoglobin, improvement in tissue oxygenation, and increased antioxidant defence. However, in a shorter period of time, the changes in the body may be insufficient to effectively oppose the effects of severe oxidative reactions.

Increased CAT activity was observed among the participants of the presented study, which indicates the occurrence of oxidative stress stimulus. No statistically significant changes in the concentration of MDA may be due to the time of blood sampling. Samples were collected 3 days after discontinuation of exposure to high-altitude conditions. This period of time may have led to a decrease in the concentration of lipid peroxidation products. In trained individuals, such as the participants in the current study, the rate of metabolism of MDA was higher, and its growth lower than in physically inactive people [15]. On the other hand, Vij et al [16] have shown that elevated levels of lipid peroxidation products may occur even 3 months after the end of exposure to a high-altitude environment. The most likely explanation for the observed lack of significant increase in lipid peroxidation products in the plasma is adequate activation of antioxidant defence.

The examined patients had a statistically significant increase in the activity of catalase (CAT) and a reduction in SOD / CAT, and no change in the activity of superoxide dismutase (SOD). The number of studies describing the changes in CAT and SOD activity under the influence of high-altitude environment is limited, the results obtained are not coherent, and the authors often have difficulty in interpreting the results.

Reports indicate that exposure to a significant height and excessive production of hydrogen peroxide inhibit the activity of SOD. In an experiment in which the rats were exposed to a simulated altitude of 5,500 m, there was increased production of hydrogen peroxide and a marked decline in mitochondrial superoxide dismutase activity [17]. Also, in rats exposed to intermittent hypobaric hypoxia (corresponding to 4,000 m above sea level) decreased activity of mitochondrial superoxide dismutase (SOD) in skeletal muscle was observed [12]. Sinha et al [18] observed people at an altitude of 4,560 m above sea level for 7 days. They were subjected to an exhaustive increase in physical exertion of catalase activity, and a decrease or no change in the superoxide dismutase activity. The increase in catalase activity is usual at high concentrations of hydrogen peroxide, for example, during exhausting physical activity in which there is an overproduction of H_2O_2 due to autoxidation of haemoglobin. An increase in enzyme activity in the erythrocytes, which were exposed to hydrogen peroxide, was observed [19]. People climbing in the mountains are exposed to increased physical activity and hypobaric hypoxia. This may lead to increased production of H_2O_2 in the reaction catalyzed by xanthine oxidase [20]. Overproduction of H_2O_2 , exceeding the possibility of its neutralization by glutathione peroxidase (GPx), leads to an increase in CAT activity [20].

Superoxide dismutase is very efficient enzyme which can break one million anion superoxide radicals per second [21]. No change in SOD under various stimuli may indicate that the activity is sufficient for the intensity of oxidative reactions in the body. A significant increase in superoxide dismutase is usually associated with exposure to a strong, long-term stressor [22]. With the rapid increase in the amount of hydrogen peroxide, a decrease in SOD activity is observed [17]. No significant increase in superoxide dismutase activity in participants in the presented study may indirectly reflect the proper preparation for the trip, and plan of the trek in gradually gaining height and the number of rest periods. However, increase in the activity of catalase in plasma and

lower rate of SOD / CAT than before the expedition indicates the stimulating effect of mountain environments, leading to oxidative capacity in the bodies of the participants.

The level of total antioxidant status (TAS), which reflects the concentration of low molecular weight antioxidants, depends on the amount of food consumed. A diet rich in fruits and vegetables, moderate consumption of red wine and taking nutritional supplements promotes the growth of TAS [23]. Factors that reduce the value of TAS include dependence on cigarettes and alcohol. Reduction of the TAS could be the effect of the exercise, during which there is consumption of low molecular weight antioxidants [15]. An increase in TAS after exercise was observed, which is explained with higher concentrations of uric acid [24]. There is little research about the changes in the concentration of low molecular weight antioxidants at high altitudes. In one study in physically active people at an altitude of 4,560 m above sea level, a slight increase in the value of TAS was observed [18]. The authors explained this fact with the increased production of uric acid.

In the presented study, the 9-day exercise at high altitudes and a diet poorer in antioxidant components did not result in statistically significant changes in the level of TAS in the volunteers. Changes in the content of low molecular weight antioxidants were much slower than the changes in the activity of antioxidant enzymes [25]. This may result in minor fluctuations in the values of these parameters during a short observation.

CONCLUSION

The results of the presented study indicate the possibility that the 9-day trek in the Alps, at altitudes of 2,050–4,165 m above sea level does not cause significant changes in red blood cell parameters in young, healthy people. A reduced number of monocytes and basophils was observed, which seems to be most likely caused by the interruption of exposure to allergens and metropolitan air pollution. The exposure of 5 trained persons for high-altitude environmental conditions and moderate load effort in this study increases antioxidant defence activity (CAT activity increases and decreases the value of the coefficient of SOD / CAT), but does not cause a significant increase in indices of oxidative stress in plasma. However, the group examined in the study was small, and the conditions of the trekking were not very demanding in comparison to the other studies. Further studies with a larger sample size must be conducted to authenticate and confirm the obtained results.

REFERENCES

1. Roach RC, Hackett PH. Frontiers of hypoxia research: acute mountain sickness. *J Exp Biol.* 2001; 204(18): 3161–3170.
2. Bailey DM, Davies B, Young IS, Hullin DA, Seddon PS. A potential role for free radical-mediated skeletal muscle soreness in the pathology of acute mountain sickness. *Aviat Space Environ Med.* 2001; 72(6): 513–521.
3. Askew EW. Nutrition and performance in hot, cold, and high altitude environments. In: Wolinsky I. (eds.), *Nutrition in Exercise and Sport*, third ed. CRC Press, Boca Raton, FL 1997, pp. 597–619.
4. Huey RB, Eguskita X. Limits to human performance: elevated risks on high mountains. *J Exp Biol.* 2001; 204(18): 3115–3119.
5. Guzek JW. *Patofizjologia człowieka w zarzysie*. Wydawnictwo Lekarskie PZWL, Warszawa 2008 (in Polish).
6. Ashenden MJ, Gore CJ, Dobson GP, Hahn AG. Hahn Live high, train low” does not change the total haemoglobin mass of male endurance athletes sleeping at a simulated altitude of 3000 m for 23 nights. *Eur J Appl Physiol.* 1999; 80(5): 479–484.
7. Esteva S, Pedret R, Fort N, Torrella JR, Pagès T, Viscor G. Oxidative Stress Status in rats after intermittent exposure to hypobaric hypoxia. *Wilderness & Environmental Medicine* 2010; 21(4): 325–331.
8. Karagiannidis C, Hensey G, Rueckert B, Mantel PY, Ichters B, Blaser K, et al. High-altitude climate therapy reduces local airway inflammation and modulates lymphocyte activation. *Scandinavian Journal of Immunology* 2006; 63(4): 304–310.
9. Straub DA, Ehmann R, Hall GL, Moeller A, Hamacher J, Frey U, et al. Correlation of nitrites in breath condensates and lung function in asthmatic children. *Pediatr Allergy Immunol.* 2004; 15(1): 20–25.
10. Van Loveren H, Boonstra A, Van Dijk M, Fluitman A, Savelkoul HF, Garssen J. UV exposure alters respiratory allergic responses in mice. *Photochem Photobiol.* 2000; 72(2): 253–259.
11. Pfeifer JM, Askew EW, Roberts DE, Wood SM, Benson JE, Johnson SC, et al. Effect of antioxidant supplementation on urine and blood markers of oxidative stress during extended moderate-altitude training. *Wilderness and Environmental Medicine* 1999; 10(2): 66–74.
12. Radak Z, Lee K, Choi W, Sunoo S, Kizaki T, Oh-ishi S, et al. Oxidative stress induced by intermittent exposure at a simulated altitude of 4000m decreases mitochondrial superoxide dismutase content in soleus muscle of rats. *Eur J Appl Physiol Occup Physiol.* 1994; 69(5): 392–395.
13. Vani R, Reddy CS, Devi S. Oxidative stress in erythrocytes: a study on the effect of antioxidant mixtures during intermittent exposures to high altitude. *Int J Biometeorol.* 2010; 54(5): 553–562.
14. Joanny P, Steinberg J, Robach P, Richalet JP, Gortan C, Gardette B, et al. Operation Everest III (Comex’97): the effect of simulated severe hypobaric hypoxia on lipid peroxidation and antioxidant defence systems in human blood at rest and after maximal exercise. *Resuscitation* 2001; 49(3): 307–314.
15. Woźniak B, Miła-Kierzenkowska C, Drewa G, Woźniak A, Kalinowski A, Drewa T, et al. Stres oksydacyjny po wysiłku fizycznym u wioślarzy różniących się wiekiem i stopniem wytrenowania. *Medycyna Sportowa* 2007; 23(4): 229–232 (in Polish).
16. Vij AG, Dutta R, Satija NK. Acclimatization to oxidative stress at high altitude. *High Alt Med Biol.* 2005; 6(4): 301–310.
17. Nakanishi K, Tajima F, Nakamura A, Yagura S, Ookawara T, Yamashita H, et al. Antioxidant system in hypobaric-hypoxia. *J Physiol.* 1995; 15(489–3): 869–876.
18. Sinha S, Ray US, Saha M, Singh SN, Tomar OS. Antioxidant and redox status after maximal aerobic exercise at high altitude in acclimatized lowlanders and native highlanders. *Eur J Appl Physiol.* 2009; 106(6): 807–814.
19. Cimen MY. Free radical metabolism in human erythrocytes. *Clin Chim Acta.* 2008; 390(1–2): 1–11.
20. Johnson TE, Henderson S, Murakami S, de Castro E, de Castro SH, Cypser J, et al. Longevity genes in the nematode *Caenorhabditis elegans* also mediate increased resistance to stress and prevent disease. *J Inher Metab Dis.* 2002; 25(3): 197–206.
21. Bartosz G. *Druga twarz tlenu. Wolne rodniki w przyrodzie*. Wydawnictwo Naukowe PWN, Warszawa 2008 (in Polish).
22. Sinha S, Ray US, Tomar OS, Singh SN. Different adaptation patterns of antioxidant system in natives and sojourners at high altitude. *Respiratory Physiology & Neurobiology* 2009; 167(3): 255–260.
23. Haddad M, Herbeth B, Vincent-Viry M, Lamont JV, Fitzgerald PS, Visvikis S, et al. Serum total antioxidant status, erythrocyte superoxide dismutase and whole-blood glutathione peroxidase activity in the Stanislas cohort: influencing factors and reference intervals. *Clin Chem Lab Med.* 2003; 41(2): 209–215.
24. Matuszkiewicz A. Białka i kwas moczowy jako potencjalne zmiażdżacze wolnych rodników w organizmie sportowca wyczynowego. *Medycyna Sportowa* 2000; 5(106): 31–39 (in Polish).
25. Pittaluga M, Parisi P, Sabatni S, Ceci R, Caporossi D, Valeria Catani M, et al. Cellular and biochemical parameters of exercise-induced oxidative stress: relationship with training levels. *Free Radic Res.* 2006; 40(6): 607–614.