Effect of altitude training on the peroxidation and antioxidant enzymes in sportsmen

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ABSTRACT

WOZNIAK, A., G. DREWA, G. CHESY, A. RAKOWSKI, M. ROZWODOWSKA, and D. OLSZEWSKA. Effect of altitude training on the peroxidation and antioxidant enzymes in sportsmen. *Med. Sci. Sports Exerc.*, Vol. 33, No. 7, 2001, pp. 1109–1113. **Purpose:** The aim of this work was an evaluation of the influence of physical exercise in high-altitude conditions (about 2000 m above sea level) on thiobarbituric acid reactive substances (TBARS) and superoxide dismutase (SOD) and catalase (CAT) activities in 10 kayakers and 10 rowers. **Methods**: During their training, the sportsmen performed different kinds of static and dynamic efforts. The blood samples were taken from the cubital vein on the control day at low altitude and at high altitude, and on the 4th, 10th, and 18th days of the training camp before and after exercise. The TBARS and lactic acid concentrations in blood plasma and SOD and CAT activities in erythrocytes were measured. **Results:** A statistically significant increase of SOD and CAT activities in erythrocytes after exercise on the 4th, 10th, and 18th days of training was found. The TBARS concentration in erythrocytes decreased in a statistically significant way after the end of the 10th day of exercise ($P < 0.01$), and on 18th day it more than doubled ($P < 0.001$) when preexercise values were compared with postexercise values of each day. A statistically significant increase of TBARS concentration in blood plasma was observed only after the end of exercise on the 10th day—using the same comparison as above. A statistically significant increased lactic acid concentration in blood plasma was noticed both on 4th or 18th days of training. **Conclusions:** The results obtained show the increasing generation of oxygen-derived free radicals and the compensatory intensification of SOD and CAT activities after training in altitude (high mountain) conditions. **Key Words:** SUPEROXIDE DISMUTASE, CATALASE, THIOBARBITURIC ACID REACTIVE SUBSTANCES, KAYAKERS, ROWERS

High-altitude conditions lead to changes in organism function. The cause of these changes is lower oxygen concentration in breathing air, low temperature, and long-lasting influence of ultraviolet radiation. The deeper respiratory movements, increase of heart rate, elevation of the number of circulating erythrocytes and concentration of hemoglobin, and increase of blood cells and whole blood volume are an effect of high-altitude condition adaptation (8,11).

Some studies have shown increasing generation of oxygen-derived free radicals during exercise (9,10). In hypoxic conditions, these processes seem to be more intensified. The main sources of reactive oxygen species during exercise in high-altitude conditions are reaction induced by reperfusion after hypoxia. First of all, conversion of xanthine dehydrogenase to oxidase is reached, and in the reaction catalyzed by this enzyme superoxide, anionradical and hydrogen peroxide are generated (16). Production of reactive oxygen species also takes place during reperfusion when arachidonic acid metabolism is intensified (14) and during enlarged monoelectronic respiratory chain mitochodrium leaking (15). The increase of oxygen-derived free radicals generation is also observed during migration and activation of granulocytes to hypoxic tissues (23). In addition, hypoxia leads to a freeing of transition metals (Fe, Mg, Cu) to catalyze hydroxyl radical (10). The consequence of the action of this reactive oxygen species is lipid peroxidation, whose the final products are substances reacting with thiobarbituric acid (TBARS)—mainly malondialdehyde (MDA). Antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), are responsible for the elimination of or decomposition of oxygen-derived free radicals into less reactive forms within the cell (10).

The aim of this work was an evaluation of the influence of high-altitude training on TBARS concentration in blood plasma and erythrocytes and on the activities of some antioxidant enzymes in red blood cells in kayakers and rowers. Additionally, correlation between chosen biochemical parameters was also analyzed.

METHODS

Subjects. The study was performed on 20 sportsmen (10 rowers and 10 kayakers) who took part in a sport camp in Bulgaria in high-mountain conditions—about 2000 m above sea level. Characteristics of the basic physical parameters of the studied group are shown in Table 1.

Sportsmen trained two times per day, in the morning and the afternoon for 2 h (running, ski running, power-house, football). During the first 3 d, sportsmen trained very intensively, as

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TABLE 1. Mean value (±SD) of basic characteristics of sportsmen.

Age (yr)	23.8 ± 2.9
Body height (cm)	183.4 ± 5.6
Body mass (kg)	81.4 ± 4.7
Training experience (yr)	9.7 ± 2.8
$\dot{V}O_{2max}$ (mL·min^{-1}·kg^{-1})	64.2 ± 4.9

at sea level. On the fourth day, intensity of training was diminished according to adapting to decreased efficiencies of sportsmen, but during the following days, intensity of exercise gradually increased. Training started on Monday, and Sunday was the resting day. On Saturday, only one exercise unit was reached. The training protocol is presented in Table 2.

Blood samples were taken from the cubital vein on control day at low altitude and at high altitude, and before and after training on the 4th, 10th, and 18th days. All sportsmen gave their consent for taking blood, were informed about the aim of the studies, and gave their written consent. The studies obtained the agreement of The Local Ethical Committee at The Ludwik Rydygier Medical University in Bydgoszcz. The results obtained for rowers and kayakers were similar; therefore, both groups of sportsmen were combined into one group.

Procedures and measurements. The freshly withdrawn blood was centrifuged with 3.2% sodium citrate at 4°C. After removal of the blood plasma, erythrocytes were washed three times with triple-volume phosphate-buffered saline (PBS) and centrifuged after each washing. The concentration of hemoglobin was assayed by the standard colorimetric method, using Drabkin's reagent and expressed as g·dL^{-1}.

TBARS concentration in blood hemolysates that were obtained from erythrocytes suspensions and in blood plasma were assayed according to Buege and Aust's (2) method with later modifications (6). This procedure is based on formation of color complex of lipid peroxidation products reacting with thiobarbituric acid at 100°C and at acidic pH. Spectroscopically, the maximum absorption of the complex is at 532 nm. Because the predominant compound reacting with thiobarbituric acid is MDA, the TBARS levels in erythrocytes were expressed as nmol MDA·g Hb^{-1} and in plasma as nmol MDA·mL^{-1}.

The estimation of superoxide dismutase activity in hemolysates, which was obtained from erythrocyte suspensions, was measured according to Misra and Fridovich's (19) method. This method is based on inhibition of adrenaline auto-oxidation to adrenochrome by the enzymes in an alkaline solution. The activity unit was defined as the amount of the enzyme that inhibits the reaction by 50% during the maximal increase of absorption by 0.025 per minute and expressed as U·g Hb^{-1}.

The estimation of catalase activity in hemolysates, which were obtained from erythrocyte suspensions, was measured according to Beer and Seizer's (1) method. This method is based on decreasing hydrogen peroxide absorption measured at 240 nm, which is the result of the presence of catalase. The molar absorption coefficient, which is well known for H_2O_2, makes possible the estimation of the quantity of decomposed compound within a time period. This fact allowed the direct measurement of catalase activity, which was expressed as IU·g Hb^{-1}.

Additionally, on the 4th and 18th days of training, the concentration of L-lactic acid (L-lactate) in blood plasma was measured using commercial kits from Boehringer Mannheim. Absorption was measured at 340 nm at 25°C. L-lactic acid concentration was expressed as mmol·L^{-1}.

The results obtained were analyzed statistically by Student's *t*-test for paired data. The correlation coefficients between chosen biochemical parameters for evaluation of their relation has also been analyzed. Hypothesis about the statistical significance of correlation coefficients has also been verified.

RESULTS

It has been shown that there are statistically significant higher values of parameters assayed before exercise in high altitude as compared with values measured on the control day at low altitude. Statistically nonsignificant differences have been noticed only in the case of SOD activity assayed

TABLE 2. Training protocol.

	Kind of Training	Intensity of Training
4th Day and 10th day		
Morning	On foot excursion from 2200 m over sea level to 2800 m over sea level and back	Pulse rate assayed by sporttester 110–130
Afternoon	Continuous swimming in pool, distance 1000–1200 m.	Pulse rate about 130
18th Day		
Morning	Lifting maximal power lying on back on bench; catching up maximal weight lying ahead on bench; power training executed by repetition method on 6 stations	Maximal load, anaerobic effort not lactic acidosis, pulse rate 130–150
		Submaximal load
Afternoon	Ski running (endurance training)	Pulse rate 130–140 lasting 25 min, 150–160 lasting 20 min, 160–170 lasting 15 min, 180–190 lasting 10 min
Other day of training	Swimming	Pulse rate 140–170
	Team games	Pulse rate 130–170
	Ski excursion (110–160 min)	Pulse rate 120–160
	Continuous ski running	Pulse rate 130–180
	Kayak ergometer	Pulse rate 130–180
	Running (continuous)	Pulse rate 130–150
	Power endurance training	Pulse rate 150–170
	Maximal power training	Pulse rate 130–150

TABLE 3. Effect of training on the concentration of thiobarbituric acid reactive substances (TBARS) and on activities of superoxide dismutase (SOD) and catalase (CAT) of kayakers and rowers.

Status	SOD ($U \cdot g^{-1}Hb$)	CAT ($10^4 IU \cdot g^{-1}Hb$)	TBARS in Eryth. (nmol MDA·g^{-1} Hb)	TBARS in Plasma (nmol MDA·mL^{-1})	L-Lactate ($mmol \cdot L^{-1}$)
Control day (low altitude)	1200.12 ± 187.57	10.92 ± 2.16	18.01 ± 8.41	0.50 ± 0.17	2.10 ± 0.91
Control day (high altitude)	1291.18 ± 179.24	11.77 ± 2.41	23.14 ± 9.50	0.61 ± 0.19	2.43 ± 0.89
4th Day					
Before training	1159.22 ± 191.49[b]	16.01 ± 1.80[a,b]	52.67 ± 20.05[a,b]	1.26 ± 0.11[a,b]	3.26 ± 0.81[a,b]
After training	2428.74 ± 382.21***	21.95 ± 1.50***	59.17 ± 17.70	1.27 ± 0.15	11.20 ± 3.32***
10th Day					
Before training	1719.98 ± 206.98[a,b,c]	15.48 ± 3.19[a,b]	35.62 ± 16.91[a,b,c]	0.99 ± 0.13[a,b,c]	—
After training	2346.18 ± 180.82***	20.88 ± 2.40***	23.47 ± 4.15**	1.09 ± 0.21*	—
18th Day					
Before training	1879.90 ± 116.65[a,b,d,e]	9.98 ± 2.01[b,d,e]	33.78 ± 6.06[a,b,d]	1.22 ± 0.13[a,b]	3.02 ± 0.67[a,b]
After training	2598.10 ± 130.94***	17.40 ± 1.39***	74.07 ± 33.60***	1.21 ± 0.19	8.56 ± 2.93***

Values are means ± SD; statistical significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (before/after training).
[a] Significantly different from the respective value on control day (low altitude) and on control day (high altitude) and before training on 4th, 10th, and 18th day (all $P < 0.001$).
[b] Significantly different from the respective value on control day (high altitude) and on 4th, 10th and 18th day (SOD on 4th day at $P < 0.05$, on 10th and 18th day at $P < 0.001$; CAT on 4th and 10th day at $P < 0.001$, on 18th day at $P < 0.05$; TBARS in eryth. on 4th and 18th day at $P < 0.001$, on 10th at $P < 0.01$; TBARS in plasma all $P < 0.001$; L-lactate all $P < 0.05$).
[c] Significantly different from the respective value before training on 4th day and on 10th day (SOD at $P < 0.001$; TBARS in eryth. at $P < 0.01$; TBARS in plasma at $P < 0.001$).
[d] Significantly different from the respective value before training on 4th day and on 18th day (all $P < 0.001$).
[e] Significantly different from the respective value before training on 10th day and on 18th day (SOD at $P < 0.01$; CAT at $P < 0.001$).

on the control day versus the 4th day before training and between CAT activity on the control day and on the 18th day before exercise (Table 3).

Exercise in high-mountain conditions caused an increase of antioxidant enzyme activities, which were measured in erythrocytes. A statistically significant increase of superoxide dismutase activity and catalase was shown on the 4th, 10th, and 18th days of study (Table 3). On the 4th day, SOD activity doubled ($P < 0.001$) as compared with the value before the start of exercise; on the 10th day, it increased about 36% ($P < 0.001$) and on the 18th day about 38% ($P < 0.001$). Gradually increasing activity of SOD has been shown as compared with assayed before exercise. Activities of SOD before the start of exercise on the 4th, 10th, and 18th days were 1159.22, 1719.98, and 1879.9 $U \cdot g\ Hb^{-1}$, respectively. Differences between these days were statistically significant (between the 4th and 10th days and between the 4th and 18th days, the level of significance was $P < 0.001$ and between the 10th and 18th days $P < 0.01$).

The activity of CAT in erythrocytes increased on the 4th day of training by about 37% ($P < 0.001$), on the 10th day about 35% ($P < 0.001$), and on the 18th day increased about 75% ($P < 0.001$) as compared with the activity before the start of exercise. The activity of CAT gradually decreased during the training as compared with values assayed before exercise. On the 4th day, the activity of CAT was $16.01 \cdot 10^4$ $IU \cdot g\ Hb^{-1}$, on the 10th day $15.48 \cdot 10^4$ $IU \cdot g\ Hb^{-1}$, and on the 18th day $9.98 \cdot 10^4$ $IU \cdot g\ Hb^{-1}$. Statistically significant differences in activity of this enzyme were found between the 4th and 18th days ($P < 0.001$) and between the 10th and 18th days of training ($P < 0.001$).

No statistically significant changes of thiobarbituric reactive substances in erythrocytes were shown after 4 d of training. On the 10th day of training, TBARS concentration decreased statistically significantly ($P < 0.01$), and on the 18th day it more than doubled ($P < 0.001$). The TBARS concentrations in erythrocytes were also gradually lower when values measured before exercise in each day of training were compared. This concentration decreased from 52.67 $nmol \cdot g\ Hb^{-1}$ on the 4th day to 35.62 $nmol \cdot g\ Hb^{-1}$ on the 10th day ($P < 0.01$) and to 33.78 $nmol \cdot g\ Hb^{-1}$ on the 18th day ($P < 0.001$) (as compared with the concentration on the 4th day).

A statistically significant increase of TBARS concentration in blood plasma was observed only on the 10th day of training. Concentration of TBARS on this day increased by about 10% ($P < 0.05$) as compared with the concentration before the start of exercise. The TBARS concentration in blood plasma before beginning exercise also decreased during these 18 d of sport camp. A statistically significant difference in TBARS concentration in blood plasma was noticed between the 4th and 10th days of training ($P < 0.01$) before exercise.

A statistically significant increase of lactic acid concentration in blood plasma was noticed after the end of exercise on both the 4th and 18th days of training. On the 4th day, lactic acid concentration increased about 244% ($P < 0.001$) and on 18th day about 183% ($P < 0.001$). Some statistically significant correlations were shown between measured parameters (Table 4).

DISCUSSION

Training in a moderate hypoxic condition (2000–3000 m above sea level) is more often used regarding the possibility of good formation of oxygenic skill of sportsmen (18). There are some studies showing that exercises in moderate hypoxic conditions are accompanied by higher oxidative stress than in similar training executed in normoxic conditions (24).

In this work, a statistically significant increase of activities of superoxide dismutase and catalase was shown in the erythrocytes of kayakers and rowers after exercise on the 4th, 10th, and 18th days of training. A higher generation of

TABLE 4. Statistically significant correlation coefficients between measured parameters.

	SOD/TBARS in Eryth.	SOD/TBARS in Plasma	CAT/TBARS in Eryth.	CAT/TBARS in Plasma	L-Lactate/ TBARS in Eryth.	L-Lactate/ TBARS in Plasma
4th Day after training	r = 0.550*					
18th Day after training	r = −0.437**	r = −0.442**	r = −0.396*	r = −0.470**	r = 0.497*	r = 0.465

* $P < 0.05$; ** $P < 0.01$.

superoxide anionradical results in increasing SOD activity, but increasing activity of CAT is a result of the formation of large quantities of hydrogen peroxide. Only these two reactive oxygen species (superoxide anionradical and hydrogen peroxide) as a substrates of reaction catalyzed by SOD and CAT may lead to the elevation of activities of these enzymes. SOD and CAT could not be synthesized *de novo* in erythrocytes because these cells do not posses a nucleus. For these reasons, the lack of inhibitors or the presence of activators probably causes the increase of activities of SOD and CAT but not their synthesis. A similar opinion is presented by Ohno et al. (21). At low altitude a statistically significant increase of SOD and CAT activities in the erythrocytes of wrestlers as an influence of testing exercise on cycloergometer with growing intensity has been shown by Hübner-Wozniak et al. (7). Also, Drewa et al. (3) noticed postexercise increasing activities of SOD and CAT in weightlifters who were tested during training. Some studies of antioxidant enzyme activities after training in high-altitude conditions have been performed, mainly in animals. Radak et al. (22) showed an increase of mitochondrial-SOD activity in the muscles of rats trained in the mountain conditions (4000 m above sea level). The authors did not observe changes of Cu-Zn-SOD, catalase, or glutathione peroxidase activity. However, Nakanishi et al. (20) noticed an increase and decrease of glutathione peroxidase and mitochondrial SOD and catalase in various tissues of rats after various time exposures to hypobaric hypoxia (5500 m over sea level) without any exercise.

Statistically higher TBARS has been shown in blood plasma concentration in the all terms of studies in high altitude (except high altitude control) compared with values assayed at low altitude. Significantly higher TBARS concentration proves that the intensity of lipid peroxidation is probably not only a consequence of training but also of high-altitude conditions. Higher levels of conjugated dienes (CD) in serum—indicating early events of lipid peroxidation before the start of running of skiers at moderate altitude rather than at sea level—were shown by Vasankari et al. (24). In our study, a statistically significant increase of TBARS concentration in blood plasma after ending exercise on the 10th day of training was shown. The higher concentration of TBARS in blood plasma after training exercise was probably caused by intensification of lipid peroxidation of plasma low-density lipoprotein (LDL) and oxygen-mediated injury of muscle cell membranes. On the 10th day of training, a positive correlation between TBARS concentration in blood plasma and CAT activity was observed—this may suggest participation in lipid peroxidation of some reactive oxygen species other than hydrogen peroxide. On the 18th day of training, TBARS concentration in blood plasma had not changed—it was probably the result of a compensating increase of SOD and CAT activities. A confirmation of this hypothesis may be observed in this day's reverse correlation between TBARS concentration in blood plasma and SOD activity ($r = -0.442$; $P < 0.01$) and between TBARS concentration in blood plasma and CAT activity ($r = -0.470$; $P < 0.05$). Also, other authors have observed changes in lipid peroxidation products' concentration in blood plasma or serum after exercise in high-altitude and low-altitude conditions. Vasankari et al. (24) showed an increase of CD concentration in serum of skiers after a 20- to 30-km race in moderate-altitude conditions. Elevated MDA concentration in blood plasma of rats after exposure to simulated hypoxia at 7576 m above sea level has been observed by Kumar et al. (13); however, Radak et al. (22) noticed an increase of TBARS concentration in muscles of rats after 4 wk of exercise at an altitude of 4000 m above sea level. An increase of MDA in blood plasma in runners after a semimarathon run at low altitude was shown by Marzatico et al. (17) and by Hübner-Wozniak et al. (7) in wrestlers tested on a cycloergometer. Kretzschmar et al. (12) noticed a 40% decrease of TBARS concentration in long-distance runners tested under maximal load exercise.

Differences in research results also refer to TBARS concentration in erythrocytes. A statistically significant decreasing TBARS concentration was shown in our study in the erythrocytes of kayakers and rowers after training on the 10th day, as was a statistically significant increase after training on the 18th day. But all concentrations were statistically significantly higher as compared with the control day in low and high altitude. The differences that have been observed between the10th and 18th days may be linked not only with a different intensity of the erythrocytes cell membrane lipid peroxidation process but also with the rate of MDA, which is the predominant compound of substances reacting with thiobarbituric acid. MDA is metabolized in the liver and probably also in trained skeletal muscles of a human (9). One supposes that observed changes of TBARS concentration are the result of the rate of formation and decomposition of these compounds. The decreasing rate of decomposition of TBARS may be influenced, for example, by disturbances in the circulatory system, which is often in a hypoxic condition in high altitude. The decreasing of TBARS concentration in the erythrocytes on the 10th day of training may be a result of the statistically significant increasing of activities of SOD and CAT. But the increasing TBARS concentration on the 18th day of the study, in spite of elevated activities of SOD and CAT, may show an insufficient deactivation of generated superoxide anionradi-

cal and hydrogen peroxide or the taking part by other reactive oxygen species in the lipid peroxidation process in erythrocyte membranes. The acidosis of an organism that accompanies exercise may contribute to the transformation of superoxide anionradical to hydroperoxyl radical ($HO_2^{\cdot}$). The fact that acidosis may have an influence on the lipid peroxidation process is shown in our study by the positive correlation between lactic acid concentration and TBARS concentration in erythrocytes ($r = 0.497$; $P < 0.05$) and between lactic acid concentration and TBARS concentration in blood plasma ($r = 0.465$; $P < 0.05$) on the 18th day of training. The reverse correlation between TBARS concentration in red blood cells and CAT activity ($r = -0.396$; $P < 0.05$) was also observed on this day, which shows the role of $O_2^{\cdot-}$ and H_2O_2 in the lipid peroxidation process. Therefore, it is difficult to unequivocally explain the changes of TBARS concentration in erythrocytes. It may be supposed that it could be connected with changes in the physical form of kayakers and rowers and acclimatization to high-mountain conditions, which developed gradually during the time at high altitude. Publications of other authors about changes of TBARS concentration in erythrocytes are not uniform in their conclusions. Drewa et al. (4) observed statistically significant increases of TBARS concentration in the erythrocytes of weightlifters after training sessions at low altitude with an intensity of about 80–90% of maximal loading. Dudek et al. (5) noticed a statistically nonsignificant increase of MDA concentration in untrained men who were tested for 20 min on a cycloergometer with an intensity of about 75% $\dot{V}O_{2max}$.

On the basis of the present work's results, one can ascertain that physical training in high-mountain conditions is accompanied by increasing generation of oxygen-derived free radicals. But it is difficult to unequivocally explain what kind of mechanism leads to the observed changes.

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