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RESTING PLASMA CONCENTRATIONS OF REDUCED GLUTATHIONE AND THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS), AND ON THE ANTIOXIDANT ENZYME ACTIVITIES IN BLOOD

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Abstract. The aim of the study was to determine the resting activities of antioxidant enzymes (SOD, CAT, GPX) and the concentration of reduced glutathione (GSH) in blood, as well as of the lipid peroxidation products (TBARS) in plasma of subjects with similar maximal oxygen uptake but differing in oxygen uptake and utilisation at the anaerobic threshold (AnT). Twenty physical education students, characterised by a high (HT group) or low (LT group) oxygen uptake at AnT, participated in the study. The reduced glutathione (GSH) concentration in whole blood was significantly higher in the HT than in LT group. Resting SOD and CAT activities in erythrocytes and GPX in blood were similar in all subjects but HT and LT groups differed significantly in the SOD/GPX and CAT/GPX activity ratios. Resting plasma TBARS concentrations significantly correlated with oxygen uptake and oxygen utilisation (%VO_2max) at the anaerobic threshold only in the HT group (r=0.65 and 0.83, respectively). It was suggested that a higher endurance fitness, expressed by the oxygen uptake and utilisation at the anaerobic threshold, resulted in an increased production of lipid peroxides. Simultaneously, a higher resting blood GSH reflects a better antioxidant protection of erythrocytes. Although a higher oxygen uptake and utilisation at the anaerobic threshold did not increase the activities of antioxidant enzymes in erythrocytes, it affected activity ratios. These changes might represent the initial stage of the erythrocyte adaptation to the oxidative stress induced by an increased oxygen uptake.

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Key words: Lipid peroxides – Reduced glutathione – Antioxidant enzymes – Anaerobic threshold

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Introduction

Physical exercise is known to increase the generation of free oxygen species and of hydrogen peroxide. This may bring about an irreversible damage of cells and their organelles due to a high reactivity of those substances. The presence of the enzymatic antioxidant systems (superoxide dismutase – SOD, catalase – CAT and glutathione peroxidase – GPX), as well as of the low-molecular antioxidants (i.e. reduced glutathione, GSH), protect cells from harmful effects of the reactive oxygen species [16,27].

Physical training, especially of endurance type, elevates the maximal oxygen uptake and oxygen utilisation at the anaerobic threshold [11]. However, the antioxidant enzymes activities in tissues and organs of endurance athletes was reported to be elevated [5]. Recently it has been shown that similar effects were induced by high-intensity training, resulting in a higher generation of free oxygen species on one hand [21] and an increase of the muscle antioxidant capacity on the other [8]. Lipid peroxidation decreases, reducing the generation of lipid peroxides as measured by the concentration of malondialdehyde (MDA) or of thiobarbituric acid reacting substances (TBARS), and may reflect the adaptation to oxidative stress induced by physical training [30].

The reports on the effect of exercise and physical training on antioxidant enzyme activities and on lipid peroxide generation, conducted in humans and animals, are often contradictory and depend on the examined tissue or organ or on the type and duration of the exercise/training [1,13,18,31,32].

Erythrocytes are particularly susceptible to oxidative stress and even undergo an irreversible damage [5]. Physical training was shown to alter the antioxidant status of erythrocytes and to increase of the resting activities of antioxidant enzymes [22] and of GSH concentration in blood [10], resulting in a better protection of erythrocytes from the oxidative stress.

It has been demonstrated that oxygen uptake at the anaerobic threshold is a better index of aerobic capacity than is the maximal oxygen consumption [11]. Nevertheless, no relationships were found between oxygen utilisation at AnT and the resting activities of erythrocyte antioxidant enzymes, reduced glutathione concentration in blood and the concentration of lipid peroxides in plasma.

The aim of this study was to determine the resting activities of antioxidant enzymes (SOD, CAT, GPX) and the concentration of reduced glutathione (GSH) in blood, as well as of the lipid peroxidation products (TBARS) in plasma of subjects with similar maximal oxygen uptake but differing in oxygen uptake and utilisation at the anaerobic threshold (AnT).

Material and Methods

Twenty male physical education students volunteered to participate in the study. Their physical characteristics are given in Table 1. The experimental protocol was approved by the local Ethics Commission. All students were subjected to physical exercises lasting 8 h per week, after having been medically examined. None of them trained sport professionally however, 8 of them jogged recreationally for 3.5 h per week. All subjects were alike with respect to maximal oxygen uptake (VO_2max) but differed in the oxygen uptake at anaerobic threshold. They were classified into two groups [7]: of high (over 60%VO_2max; HT) or low (below 60%VO_2max; LT) threshold oxygen uptake. Relative body fat content was determined from three skinfolds using a Harpenden calliper [9].

Table 1
The physical characteristics of subjects (means ± SD)

Variable	Group LT[A] (n=10)	Group HT[A] (n=10)
Age (years)	20.7±1.6	22.2±2.1
Body mass (kg)	71.4±7.8	77.5±10.1
Body height (cm)	176.9±5.9	185.2±7.4
Fat content (%)	11.0±3.3	10.0±2.1
LBM (kg)	63.5±6.9	69.6±8.2
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	54.0±4.1	53.3±2.7
VO_2 at AnT ($ml \cdot kg^{-1} \cdot min^{-1}$)	25.9±2.6*	36.9±3.9
%VO_2max at AnT	48.2±5.6*	69.2±5.4

[A]The LT and HT group – the oxygen uptake at the anaerobic threshold, lower and higher than 60% VO_2max, respectively;
*Significant differences vs. HT group ($p<0.001$)

All participants performed a graded cycle ergometer exercise (ER 900, Jaeger, Germany) for determining maximal oxygen uptake and anaerobic threshold. The initial workload was equal to 100 W and increased by 50 W every 3 minute [33]. Ergo-Oxyscreen analyser (Erich Jaeger, GmbH, Germany), calibrated with gases of known O_2 and CO_2 concentration, was used to determine the expired air

composition during the last minute of each subsequent load. The plateau value of oxygen uptake, i.e. no further increase with increasing power output, was considered maximal. Blood was sampled from fingertips at the end of each bout of exercise for determining lactate concentration. The anaerobic threshold (AnT) was defined as oxygen uptake corresponding to blood lactate equal to 4 $mmol \cdot l^{-1}$ [34]. The sessions took place in the morning (9^{oo}-12^{oo} a.m.), 2 h after a light breakfast (about 200 kcal).

Blood samples from the antecubital vein were collected into heparinized test-tubes 7 days after having completed the graded exercise, in the morning, in the preprandial state, in sitting position.

The activities of superoxide dismutase (SOD; EC 1.15.1.1) and catalase (CAT; EC 1.11.1.6) were determined in the hemolysates of erythrocytes, washed three times in cold saline (0.9%). SOD activity was measured colorimetrically according to Misra *et al.* [23], one unit of activity being defined as that corresponding to 50% inhibition of autooxidation of adrenaline at 25ºC and pH=10.2. CAT activity was assayed spectrophotometrically according to Beers *et al.* [2] at 240 nm (absorption decrease caused by hydrogen peroxide decomposition in the presence of the enzyme). The activity of glutathione peroxidase (GPX; EC 1.11.1.9) in whole blood hemolysates was determined spectrophotometrically at 37ºC using Ransel commercial kits (Randox, Great Britain) with cumene peroxide as substrate.

The concentration of reduced glutathione in blood (GSH) was assayed colorimetrically with Ellman's reagent [5,5'dithiobis-(2-nitrobenzoic acid)] (DTNB) according to Beutler [3].

The activities of enzymes and GSH concentration were expressed per g or mg of haemoglobin (Hb). Haemoglobin concentration in hemolysates was determined by the standard cyanmethemoglobin method with Drabkin's reagent.

Lipid peroxidation was evaluated indirectly in plasma, stabilised with BHT (butylated hydroxytoluene) to prevent polyunsaturated fatty acid oxidation, by determining the concentration of thiobarbituric acid reacting substances (TBARS) in plasma and using double wavelength measurements (535 and 520 nm). Reaction product was extracted with n-butanol [4,28].

The results were presented as means ± SD. Student's t-test for independent variables and Pearson's correlation coefficients were used in data analysis. The level of $p \leq 0.05$ was considered significant.

Results

As follows from data presented in Table 2, resting plasma TBARS concentration was slightly (by about 10%) higher in HT than in LT group (3.06±0.67 and 2.77±0.38 $\mu mol \cdot l^{-1}$, respectively). In better aerobically fit subjects (HT group), blood GSH concentration was significantly higher than in the LT group (2.83±0.54 and 2.36±0.35 $\mu g \cdot mg^{-1}$ Hb, respectively).

Table 2
Resting plasma TBARS and blood GSH concentrations in students with high (HT) or low (LT) oxygen uptake at the anaerobic threshold (means ± SD)

Group	TBARS ($\mu mol \cdot l^{-1}$)	GSH ($\mu g \cdot mg^{-1}$ Hb)
LT[A]	2.77±0.38	2.36±0.35
HT[A]	3.06±0.67	2.83±0.54*

[A] see Table 1; *Significant difference vs. LT group ($p<0.05$)

The antioxidant enzyme activities in erythrocytes (SOD, CAT) and in whole blood (GPX) were similar in both groups (Table 3). Nevertheless, both groups differed significantly with respect to the SOD/GPX ratio (58.2±13.9 and 74.3±18.3 for LT and HT groups, respectively) and the CAT/GPX ratio (2.83±0.55 and 3.71±1.16, respectively). In addition, plasma TBARS concentration was significantly correlated with oxygen uptake ($ml \cdot kg^{-1} \cdot min^{-1}$) and fractional oxygen utilisation ($\%VO_2max$) at the anaerobic threshold in HT group (r=0.69 and 0.83, respectively; Figs. 1 and 2).

Table 3
Resting activities of SOD and CAT (erythrocytes) and GPX (whole blood) in students with high (HT) or low (LT) oxygen uptake at the anaerobic threshold (means ± SD)

	Group LT[A]	Group HT[A]
SOD (U/g Hb)	1869±209	1979±248
CAT (U/g Hb)	92.0±13.6	98.9±19.7
GPX (U/g Hb)	33.2±5.9	27.9±7.2
SOD/GPX	58.2±13.9	74.3±18.3*
CAT/GPX	2.83±0.55	3.71±1.16*

[A] see Table 1; *Significant differences vs. LT group ($p<0.05$)

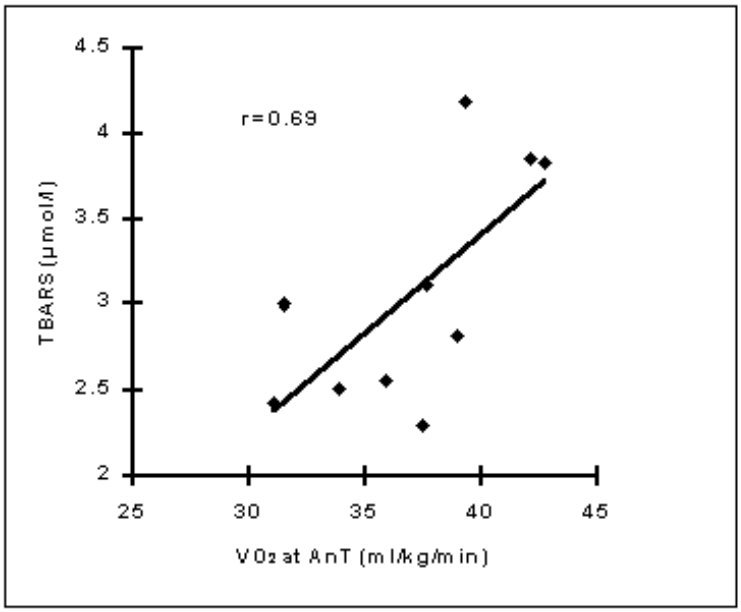

Fig. 1
The relationship between oxygen uptake at the anaerobic threshold and plasma TBARS concentrations in the HT group

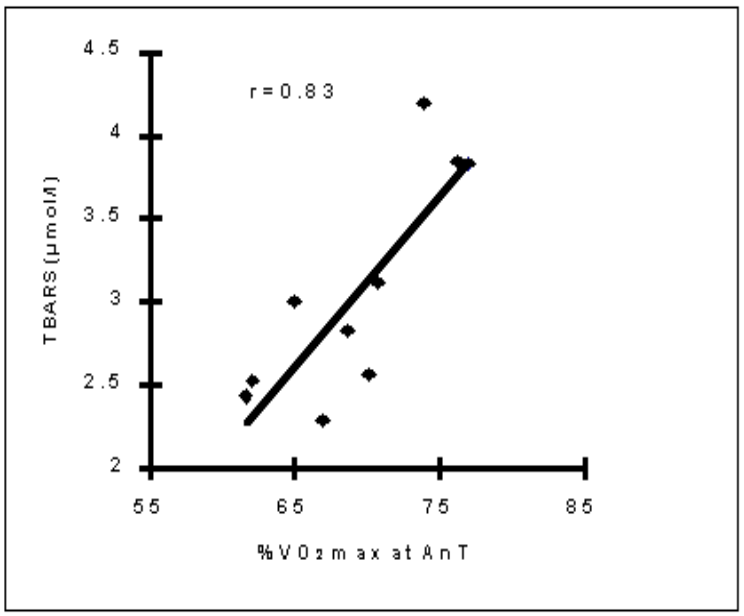

Fig. 2
The relationship between oxygen utilisation at the anaerobic threshold (%VO_2max) and plasma TBARS concentrations in the HT group

Discussion

Lipid peroxidation: The reports on the influence of increased oxygen uptake on lipid peroxidation product concentrations in plasma are ambiguous. Marzatico *et al.* [20] reported that the MDA concentration was higher in sprinters and marathon runners than in untrained subjects. In contrast, Miyazaki *et al.* [24] showed a lack of differences in resting plasma TBARS concentrations before and after 12-week training (running at 80% of the maximal heart rate, 60 min/day, 5 times a week). Similar data were presented by Robertson *et al.* [29], who found no differences in plasma TBARS concentrations between subjects who exercised running for 10 weeks (80–147 km/week) and the control ones.

In this study, a significant correlation was found in the HT group between oxygen uptake and its utilisation (%VO_2max) at the anaerobic threshold, and plasma TBARS concentration. Similar results were reported by Kostka *et al.* [17], who found a positive relationship between resting TBARS concentrations in plasma and VO_2max in 70-years old women. However, Child *et al.* [6] showed a lack of correlation between VO_2max and resting MDA concentrations, and Robertson *et al.* [29] demonstrated a negative correlation between VO_2max and plasma TBARS. Our previous results [14] also showed a positive, significant relationship between those parameters in the group of trained rowers but not in physical education students.

Reduced glutathione: The reduced glutathione (GSH) is one of the most important components of the antioxidant system in erythrocytes. Whether endurance training may induce increases in resting GSH concentrations in blood remains, however, unclear. It was demonstrated in this study that resting GSH concentrations were significantly higher in HT than in the LT group. Evelo *et al.* [10] reported that training, which elevated maximal oxygen uptake, brought about increases in resting blood GSH. Moreover, that increase was higher in trained rowers than in physical education students [19]. Similarly, Robertson *et al.* [29] reported that after a running training lasting 10 weeks (16-43 or 80-147 km/week), the VO_2max was increased and resting GSH concentrations in blood were higher than in the control group. On the other hand, Ohno *et al.* [25] found no increase in resting GSH concentrations following a 10-week running training (5 km/week, 6 times a week).

Antioxidant enzymes: The antioxidant enzyme activities were similar in both HT and LT groups. However, the relationships between activities of the examined enzymes were significantly different and related to oxygen uptake and utilisation at the anaerobic threshold. It was found that the SOD/GPX and CAT/GPX ratios were

significantly higher in HT than in LT group. Marzatico *et al.* [20] reported that those ratios were significantly higher in marathon runners and sprinters than in the control group, which could suggest that it might represent an adaptive response to an increased oxygen uptake. The results of this study suggest that a higher oxygen uptake at the anaerobic threshold increased the lipid peroxide generation and that the disturbance of the SOD-GPX balance, associated with the hydrogen peroxide accumulation, may be responsible for increasing the oxidative stress [17].

Data concerning the effects of an increased oxygen uptake on resting SOD, CAT and GPX activities in blood are contradictory, probably due to differences in training regimens and/or fitness of subjects. Robertson *et al.* [29] demonstrated that the GPX activity in erythrocytes was elevated as an effect of endurance training, while SOD and CAT activities remained unchanged. According to Hübner-Woźniak *et al.* [12], the resting GPX activity in erythrocytes was higher in highly fit endurance athletes (rowers or cyclists) than in the strength ones (e.g. wrestlers or weight-lifters). However, Miyazaki *et al.* [24] noticed increases in SOD and GPX activities in erythrocytes following a 12-week training, the CAT activity remaining unchanged. Also, Mena *et al.* [22] showed that the resting SOD, CAT and GPX activities in blood were significantly higher in professional cyclists than in control subjects or amateur cyclists. Marzatico *et al.* [20] have also demonstrated increases in resting SOD and CAT activities in erythrocytes in marathon runners and sprinters. However, CAT activity was elevated in marathon runners but was decreased in sprinters, compared with the control group. On the other hand, Ortenblad *et al.* [26] did not find increased activities of any antioxidant enzyme in blood after a strength-speed training (series of jumps) but SOD and GPX activities in the vastus lateralis muscle were elevated. The data presented by Ohno *et al.* [25] show that a 10-week running training resulted in a significant increase in the CAT activity, but SOD and GPX activities in the erythrocytes remained unchanged.

In conclusion, a higher endurance fitness, expressed as oxygen uptake and utilisation at the anaerobic threshold, induced an increased generation of lipid peroxides. Simultaneously, a higher resting GSH concentration in blood reflects a better antioxidant protection of erythrocytes. Although a higher oxygen uptake and utilisation at the anaerobic threshold did not induce adaptive increases in the activities of antioxidant enzymes in erythrocytes, it affected the enzyme activity ratios. It may be presumed that these changes represent the initial stage of adaptation of the erythrocytes to the oxidative stress induced by an increased oxygen uptake.

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