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CHANGES IN PROOXIDATIVE – ANTIOXIDATIVE BALANCE IN ROWERS FOLLOWING ERGOMETRIC EXERCISE TEST OF MAXIMAL INTENSITY

Key words: intensive physical exercise, antioxidative enzymes, oxidative stress, rowers.

ABSTRACT

The study was carried out on 24 athletes, who performed a 2000 m rowing ergometer test at the maximum rate and power of rowing. Before the exercise, one minute after its termination, and after the subsequent 24 hours, blood samples were drawn from the antecubital vein. The following parameters were evaluated in the blood: antioxidative enzymes activity (SOD, GPx) and thiobarbituric acid reactive substances (TBARS). Furthermore, the concentration of serum creatine kinase (CK) and Total Antioxidant Capacity (TAC) were assessed. The study results revealed that the rowers' peak exercise resulted in a significant increase in SOD, TAC and CK activity and free radical injury measured with TBARS concentration. During a 24-hour recovery period, none of the parameters evaluated returned to the rest values, which indicates persisting disturbances of the antioxidative defense mechanisms. The results of the study showed that intensive physical exercise increased free radical injury, in spite of an adaptive response of the antioxidative defense system.

INTRODUCTION

A number of authors [2, 8, 33, 34] show that physical training is an important factor increasing the body's antioxidative potential that protects athletes against oxidative stress induced by a single physical exercise. Brites et al. [5], showed significantly higher levels of endo- and exogenous antioxidants in football players, as compared with non-training subjects. Favourable adaptive changes in the antioxidative barrier system, secondary to physical training, were also noted by other authors [3, 10, 19].

However, some other authors emphasize that this stable redox balance is maintained only during

a single, moderate-intensity, short-term exercise, wheareas maximal intensity exercise can lead to increased production of free radicals that enhance oxidative modification of lipids, proteins or nucleic acids [9, 21, 22, 30]. Increase in exercise intensity is also associated with gradual depletion of the antioxidative protection barrier. which is manifested as decreasing concentration of intracellular antioxidative enzymes and extracellular non-enzymatic antioxidants [2, 15, 25]. According to Kretzschmar and Klinger [14] and Venditti et al. [32] these changes can be a sensitive indicator of peroxidation processes in the body.

Taking the above into account, we designed a study whose purpose was to determine whether

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athletes at the highest sport level possess sufficient antioxidative defense parameters protecting them against the oxidative stress induced by physical exercise, similar in its nature to sports competition.

METHODS

Subjects

The sample consisted of twenty-four male members of the Polish National Rowing Team taking part in a training camp. The characteristics of the subjects are presented in Table 1. The study was granted approval of the Research Ethics Committee. All subjects were informed about the purpose of the study and gave their informed consent to participate.

Experimental design and procedures

Aerobic capacity was estimated using oxygen uptake (VO₂max) obtained during an ergometer test. Each subject had to cover a 2000 m distance in shortest time on rower ergometer (Concept II - USA). Before the main test each subject performed a five-minute individual warmup. During the test, minute ventilation (VE), oxygen uptake (VO₂) and carbon dioxide elimination (VCO₂) were continuously recorded with the use of Oxycon Mobile ergospirometer (VIASYS Healthcare GmbH. - Germany). Heart rate (HR) was recorded using a sport tester (Polar PE 3000, Finland). On the basis of obtained gasometric values oxygen uptake was estimated for each subject. VO2max was also expressed in relative values $(ml/kg^{-1}/min^{-1})$.

Blood sampling and analysis

Blood samples for redox parameters were taken from the antecubital vein, on calcium disodium versenate as the anticoagulant, before the exercise test (in the morning, after an overnight fast), 1 minute after the test completion, and following the 24-hour recovery period. Samples were centrifuged to separate red blood cells from plasma. Packed erythrocytes were washed three times with saline and lysed with ice-cold, redistilled water. Plasma and lysed erythrocytes were frozen immediately and stored at -28°C until use (up to one week). Additionally, capillary blood samples were taken by fingerprick before and after each exercise test to assess the lactate levels (LA).

The Total Antioxidant Capacity (TAC), following Miller et al. [20], used as the overall measure of plasma antioxidant capacity, was assessed with the aid of commercial kits (Randox-TAS, Cat No. NX 2332, UK).

The superoxide dismutase (SOD) activity was measured in washed erythrocytes after their lysis, by means of commercial kits (Randox-Ransod, Cat No. SD 125, UK). The superoxide dismutase activity was expressed in U/gHb.

The glutathione peroxidase (GPx) activity in the hemolysate samples was measured using commercial kits (Randox-Ransel, Cat No. RS 506, UK) according to the method of Paglia [23], and the glutathione peroxidase activity was expressed in U/gHb.

The concentrations of the thiobarbituric acid reactive substances (TBARS) in the hemolysate samples were assessed as a measure of oxidative damage to red blood cells. TBARS concentrations were evaluated with the method described by Buege [6] involving the acidic breakdown of lipid peroxides into malonaldehyde molecules. The concentrations of TBARS (malondialdehyde equivalents) were expressed as µmol/gHb.

The creatine kinase activity was determined in plasma samples with a laboratory kit (Dr Lange, Cat No.LCN 282, Germany). The results were expressed as U/l.

The hemoglobin concentration was assessed using the cyanmethemoglobin method with the Drabkin's reagent and maximal absorbance at 540 nm. The results were expressed as g/100 ml.

The lactate levels in capillary blood were determined immediately after the collection of the samples using the diagnostic cuvette kit (Dr Lange, Cat No. LKM 140, Germany). The lactate concentration was expressed as mmol/l⁻¹.

Statistical analysis

Statistical analyses were performed with STATISTICA v. 6.0 software package. The normally distributed data (TAC, LA) were compared by oneway analysis of variance (ANOVA), with Tukey's (post hoc) test for multiple comparisons. These data were also analyzed with paired Student's t-tests. The data without normal distribution (SOD, GPx, TBARS, CK) were analysed with nonparametric tests. The Wilcoxon test was used to compare mean values within each group. All values were reported as mean \pm SD. Spearman or Pearson correlations coefficients were used to test the relationship among means. Statistical significance was set at $p \leq 0.05. \label{eq:planet}$

RESULTS

The results of the study are summarised in Tables 1-2 and presented graphically in Figures 1-5.

Table 1 contains the basic anthropometrical characteristics and training experience of the subjects. Physiological characteristics of the exercise and the results of lactic acid concentration measurements can be found in Table 2. Mean values of physiological parameters achieved during the 2000 m test (HR, minute pulmonary ventilation) indicate that the maximum exercise intensity was reached and the mean value of maximum oxygen uptake is a proof of the subjects' high physical efficiency. Post-exercise lactate concentration is indicative of significant contribution of anaerobic processes in coverage of the energetic cost of the exercise.

Fable 1. Basic characteristics of the studied groupsmeans \pm standard deviations)

Athletes (n=24)
20.0 ± 1.3
82.0 ± 8.9
190.0 ± 6.7
6.0 ± 1.7
14
10

Fable 2. Exercise characteristics (means \pm standard leviations)

Parameters	Athletes (n=24)
IR (ud./min)	191 ± 8.64
'ower (W)	402 ± 40.0
/'E (l/min ⁻¹)	190 ± 20.19
$/'O_2$ (l/min ⁻¹)	5.48 ± 56.07
$/O_2/kg (ml/kg^{-1}/min^{-1})$	67 ± 7.0
$A_{\text{max}} (\text{mmol/l}^{-1})$	15.5 ± 2.78
Time (s)	381 ± 19.80

Performance of the ergometric test resulted in an increase in subjects' SOD activity measured directly post-exercise and its further rise following a 24-hour recovery (Fig. 1). The GPx level also increased post-exercise; however, a significant difference was observed only in the recovery period (Fig. 2).



Figure 1. Changes of superoxide dismutase activity in the blood of rowers (n=24, data as shown as mean \pm SD; * – difference with respect to the rest period, † – difference with respect to exercise, p < 0.05)



Figure 2. Changes of glutathione peroxidase activity in the blood of rowers (n=24, data as shown as mean \pm SD; * – difference with respect to the rest period and with respect to exercise, p < 0.05)

As compared to the rest period values, the TBARS concentration and CK activity in the blood sampled one minute post-exercise increased almost twofold (by 97% and 81%, respectively). However 24 hours after the exercise a much lower increase in the discussed parameters was noted (Fig. 3, Fig. 4).

The performed physical exercise resulted also in an increase in the total serum antioxidant capacity post-exercise ($p \le 0.05$) that persisted without significant changes for 24 hours (Fig. 5).



Figure 3. Changes in concentration of lipids peroxidation products (n=24, data as shown as mean \pm SD; * – difference with respect to the rest period, † – difference with respect to exercise, p < 0.05)



Figure 4. Changes of creatinine kinase activity (n=24, data as shown as mean \pm SD; * – difference with respect to the rest period, p < 0.05)



Figure 5. Changes of total antioxidants level in the blood of rowers during the 2000m test (n=24, data as shown as mean \pm SD; * – difference with respect to the rest period, p < 0.05)

DISCUSSION

Rowing – as compared with other sports – involves exceptionally high muscle volume of the athlete, which results in a significant increase in energetic expense and its commensurate load of circulatory and respiratory systems [4, 29]. Based on the results of Messonnier et al. [18], Russell et al. [26], and Secher et al. [28] it is estimated that the energetic expense required to cover a regatta distance (2000 m) is covered in about 80% by aerobic metabolism, 12% of energy is released from anaerobic glycolysis and 8% from anaerobic phosphagenic processes.

Despite the fact that (according to literature data) the proportionate anaerobic processes contribution to coverage of the energetic cost of the standard rowing exercise is insignificant, the postexercise blood lactate concentration in the examined rowers was high and, on average, exceeded 15 mmol/l (Tab. 2). This was most probably due to the high volume of active muscles and relatively long exercise duration. Lactic acid concentration noted in the examined athletes correlated positively with TBARS concentration $(r = 0.631; p \le 0.05)$. This correlation confirms that high intensity of exercise is one of the principal factors shifting the prooxidative – antioxidative balance towards oxidation reactions. It is further confirmed by a 97% increase in the TBARS concentration in the blood taken one minute postexercise and its subsequent rise in the recovery period. Ilhan et al. [12] evaluated the influence of exercise of varying intensity on the TBARS level and showed that exercise characterised by mixed aerobic-anaerobic - work resulted in a higher level of injury in comparison to aerobic or anaerobic exercise. On the other hand, Marzatico et al. [17], in a study involving athletes performing an exercise test typical for their specialties (sprinters covered the distance 6 x 150 m and marathon runners – half of the marathon distance), noted a post-exercise increase in malondialdehyde (MDA) in the blood serum in both groups. However, during the recovery period a decrease in the examined parameter was observed in marathon runners, while in sprinters its further increase was noted. The quoted authors suggest that, contrary to long-term exercise, during anaerobic exercise NADH and NADPH production decrease, contributing in this way to the reduction of antioxidative enzymes activity and accumulation of ROS-generating

substrates. Enhancement of prooxidative changes following increased exercise intensity was also confirmed by other authors [1, 2, 9].

The increase in creatinine kinase activity noted in this study, both directly after exercise and during the recovery period, points to muscle fibers cell membrane injury which results in the loss of cell membrane integrity and, in consequence, release of enzymatic proteins (among others) into the bloodstream. Kanter et al. [13] point to the fact that the process of lipids peroxidation is one of the major factors contributing to muscle cell membrane damage. The positive correlation between CK activity and TBARS level (r = 0.313; $p \le 0.05$) and LA concentration (r = 0.467; p \leq 0.05), noted in this study, proves that high exercise intensity maintained by athletes for 381 ± 19.80 seconds (Tab. 2) significantly affects the level of postexercise myofibrillar membranes injury. Antioxidative enzymes provide protection for and supplement the non-enzymatic defense mechanisms. According to the literature data, subjects with high physical capacity (VO₂max <60.0 ml/kg/min) reveal a higher activity of these enzymes, and the rate of biosynthesis of these enzymes depends on intensity and duration of physical exercise [16, 17]. Endurance exercises have a higher stimulatory effect on the antioxidative enzymes activity, in comparison with anaerobic ones. It is confirmed by our study results. At rest, the activity of the studied enzymes was lower in rowers than in hurdle runners [24] and sprinters [17]. Intensive physical exercise, in the form of a 2000 m run on the rowing ergometer, contributed to an increase in the superoxide dismutase activity, catalysing the reaction of superoxide anion radical dismutation to hydrogen peroxide and oxygen, both in blood taken one minute post-exercise and after a 24-hour recovery period. Furthermore, the SOD activity showed a significant correlation with the TBARS $(r = 0.543; p \le 0.05)$ and LA concentration (r = 0.775; $p \le 0.05$). As far as GPx is concerned, we noted a significant increase in its activity only during the recovery period ($p \le 0.05$). It means that not only intense physical exercise with a marked anaerobic component but also the post-exercise recovery period generates free oxygen radicals, and that dismutation of superoxide anion radical to hydrogen peroxide by SOD is more active during the recovery than during the ergometer exercise.

The serum total antioxidant capacity is an element of the non-enzymatic component pro-

tecting the body against the effects of excessive ROS activity. In our opinion, the significant TAC enhancement noted in this study is an adaptive response to high exercise intensity and is indicative of its mobilisation. Schneider et al. [27] used exercise of varying intensity in their study and showed that an increase in the serum antioxidants level accompanied only exercise of highest intensities. The increase in the serum antioxidants level due to intensive physical exercise is probably associated with a shift of some of its components from the tissues into the serum. This is confirmed by the results of the study of Duthie et al. [7]. The quoted authors revealed an increase in vitamins A and C and uric acid concentrations, Aslan et al. [2] - increased level of uric acid and Hübner-Woźniak et al. [11] as well as Vasankari et al. [31] demonstrated an increase in α – tocopherol concentration. Thus activation of the serum antioxidative defense system, manifested with the enhanced TAC level, seems to be a response to the oxidative stress induced by physical exercise.

The results of the study show that intense physical exercise enhances free radical injury despite the adaptive response of the antioxidative defense system.

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