

ANTIOXIDANT DEFENSE SYSTEM DURING DOSED MAXIMAL EXERCISE IN PROFESSIONAL SPORTSMEN

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Abstract

Introduction Physical exercises and muscle work stimulate basic vital functions, which requires increase in the oxygen consumption and cell metabolism. Both these processes depend on the intensity and duration of the physical effort. Physical exercise is one of many factors influencing oxygen metabolism of the cells, including erythrocytes, leading to the production of substantial number of reactive oxygen species (ROS) (characterized by high chemical activity), which considerably affect survival, growth and development processes of the cells.

The aim of the work was to determine correlation between intensity of the physical exercise and enzymatic efficiency of the antioxidative defense: CuZn-SOD, CAT and GSH-Px in sportsmen, depending on the physical form and in individuals who do not practice sport professionally.

Material and methods: 41 rugby players from Budowlani Sports Club in Lodz and 21 men of normal physical activity (control group) were the subjects of the study. Exercise test was performed on the cycloergometer. Enzymes activities were determined in three time points: before the effort, 1 minute after the dosed maximal exercise and after 30-minute restitution period.

Results: Mean values of CuZn-SOD and CAT after the dosed maximal exercise were higher in the control group than in the professional rugby players (juniors and seniors) in all the studied time points, except lower activity of GSH-Px during the resting phase.

Conclusions: Increase in the activities of CuZn-SOD, GSH-Px, and CAT in the control group may suggest that the defense system of the antioxidative enzymes protects cells. Lowered activity of the studied enzymes in the first minute of physical exercise in the junior rugby players may be the evidence that long experience in professional sport may affect the oxidative stress.

Key words: antioxidant protection system, dosed maximal physical exercise

Introduction

During physical exercise oxygen consumption may increase 10 - or even 20-fold. Consequently, excessive number of reactive oxygen species (ROS), such as: superoxide anion radical ($O_2^{\cdot-}$), hydroperoxide (H_2O_2) and nitric oxide (NO) are produced. Overproduction of free radicals may be caused by the processes leading to the disarrangement of the respiratory chain, autoxidation of the respiratory proteins: hemoglobin and oxyhemoglobin, increased purine metabolism to uric acid, disrupted Ca^{2+} homeostasis or activation of the immunological system [1, 2].

A defense enzyme system, which neutralizes reactive oxygen species, controls production of ROS, limits or repairs damage induced by ROS, is consisted of superoxide dismutase (CuZn-SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). Superoxide dismutase catalyzes the dysmutation reaction of superoxide radical anion. The reaction product – hydrogen peroxide is then metabolized by the catalase. CAT is

an important enzyme protecting from reactive oxides. It removes H_2O_2 generated in various metabolic processes occurring in all cells of the body. In erythrocytes the catalase protects glutathione and hemoglobin from oxidation [3, 4].

Removing hydrogen and organic peroxides is a physiological role of the glutathione peroxidase. In erythrocytes this enzyme catalyzes oxidation of the reduced form of glutathione. The reaction involves hydrogen and organic peroxides, hydroxy derivatives of fatty acids, which are metabolized to water or stable peroxy acids [5]. Moreover, GSH-Px plays a substantial role in mitochondria, which lack the catalase capable of detoxification of H_2O_2 [6]. In addition, GPx is an essential component of the glutathione redox cycle of change.

The anti-oxidative response depends mainly on the type of tissue, which is related to different intensity of energetic changes, different localization and activation of the sources of reactive oxygen species [7]. Increased

ROS production may be particularly dangerous for skeletal muscles, since anti-oxidative enzyme activities and antioxidant concentrations are relatively low there (superoxide dismutase (CuZn-SOD) and catalase (CAT) activities are 40-fold lower, glutathione peroxidase (GSH-Px) activity is 7-fold lower in a gastrocnemius muscle than in the liver) [8].

Cell damage made by ROS may be one of important factor limiting working capacity of the body [9].

The aim of the work was to assess and compare the effects of dosed maximal physical exercise on the level of anti-oxidative status: superoxide dismutase, catalase and glutathione peroxidase activities, according to the age and adjustment to physical effort in professional sportsmen.

Material and methods

Forty one rugby players from Budowlani Sports Club in Lodz were the subjects of the study. No contraindications to the exercise test were found in all the studied subjects.

Approval of Bioethics Committee of the Medical University of Lodz was obtained.

The group was divided into two age categories: juniors (J) and seniors (S). There were 21 players aged 18.5 ± 0.43 in the group of juniors and 20 players aged 23.9 ± 1.65 in the group of seniors. A control group (K) consisted of 21 men aged 28.1 ± 1.44 who did normal physical exercise.

Mean experience in professional sport was 5.17 ± 1.01 years for juniors and 10.25 ± 2.36 for seniors. Juniors' body mass index (BMI) was 26.24 ± 1.5 $\text{kg} \cdot \text{m}^{-2}$. Mean seniors' BMI was 29.69 ± 2.18 $\text{kg} \cdot \text{m}^{-2}$ and 28.1 ± 1.44 $\text{kg} \cdot \text{m}^{-2}$ for control group.

Exercise tests were performed on a cycloergometer produced by Monark. Maximum effort was reached using load of increasing intensity, starting from 2.0 W/kg of a body mass and increasing it by 50 W each 2 minutes until the participants refused to continue the test. Venous blood from cubital vein was collected to the test tubes with lithium heparin. Material was collected three times: before the exercise test – a resting phase (S), 1 minute after the effort – an effort phase (W), after 30-minute restitution. Blood was centrifuged at 3000 rpm for 10 minutes. Erythrocyte mass was rinsed 3 times with 0.9% NaCl solution ($+4^{\circ}\text{C}$) keeping the same conditions of centrifugation. After the supernatant was removed, rinsed erythrocytes were hemolyzed by adding redistilled water *in partes equales* and then frozen at the temperature of -18°C . The hemolysate, after being defrosted was used in the subsequent studies.

Superoxide dismutase (CuZn-SOD) activity was measured with Misry and Fridovich's method [10]. Catalase (CAT) activity in the erythrocytes was determined with Beers and Sizer's method [11]. Glu-

tathione peroxidase (GSH-Px) activity was measured with Sedlak and Lindsay's method [12] modified by Little and O'Brien [13]. Hemoglobin (Hb) concentration in erythrocyte hemolysate, necessary to enzymes activities determination was evaluated with a standard colorimetric method with a Drabkin's reagent.

Statistics

The basic statistical parameters, mean, standard deviation, median, minimum, maximum, and asymmetry coefficient were calculated in the study. U Mann-Whitney test, nonparametric equivalent to Student's t-test for unpaired variables, was used. Statistical analysis was made with Statistica 6.0 software.

Results

In the control group mean values of the studied parameters: CuZn-SOD and CAT activities after the dosed maximal exercise measured in all time points were higher than in both groups of professional sportsmen.

In both junior and senior rugby players CuZn-SOD activity did not change substantially in relation to the load phase. However, before the effort activity of this enzyme was higher in juniors ($1.662 \pm 0.428 \times 10^3$ U/gHb) than in seniors ($1.486 \pm 0.43 \times 10^3$ U/gHb) (Fig. 1). Statistically significant difference ($P < 0.001$) was observed in the groups of seniors and juniors comparing to the control group in the resting phase, during the effort and in the restitution phase. CuZn-SOD activity was not statistically different in the juniors and seniors group.

In the group of senior sportsmen catalase (CAT) activity increased and reached maximum value (6.23 ± 2.36 BU/gHb) in the restitution phase. In junior rugby players mean CAT activity lowered in the first minute of the effort (5.80 ± 2.37 BU/gHb) in comparison to the resting phase (6.51 ± 2.91 BU/gHb). During the restitution phase of the enzyme activity of the value, as in phase before effort (6.55 ± 2.22 BU/gHb) (Fig. 2). During the effort and restitution phases statistically significant difference ($P < 0.001$) was observed in the control group in comparison to the groups of seniors and juniors. In all the studied phases CAT activity was not statistically different in the juniors and seniors.

In both studied groups of sportsmen glutathione peroxidase (GSH-Px) activity lowered during the effort and subsequently decreased more in the restitution phase. The opposite situation – increase in the GSH-Px activity was observed in the subjects who did not practice sport professionally (Fig. 3). In the effort and restitution phases statistically significant difference occurred in the control group in comparison to both groups of senior and junior rugby players.

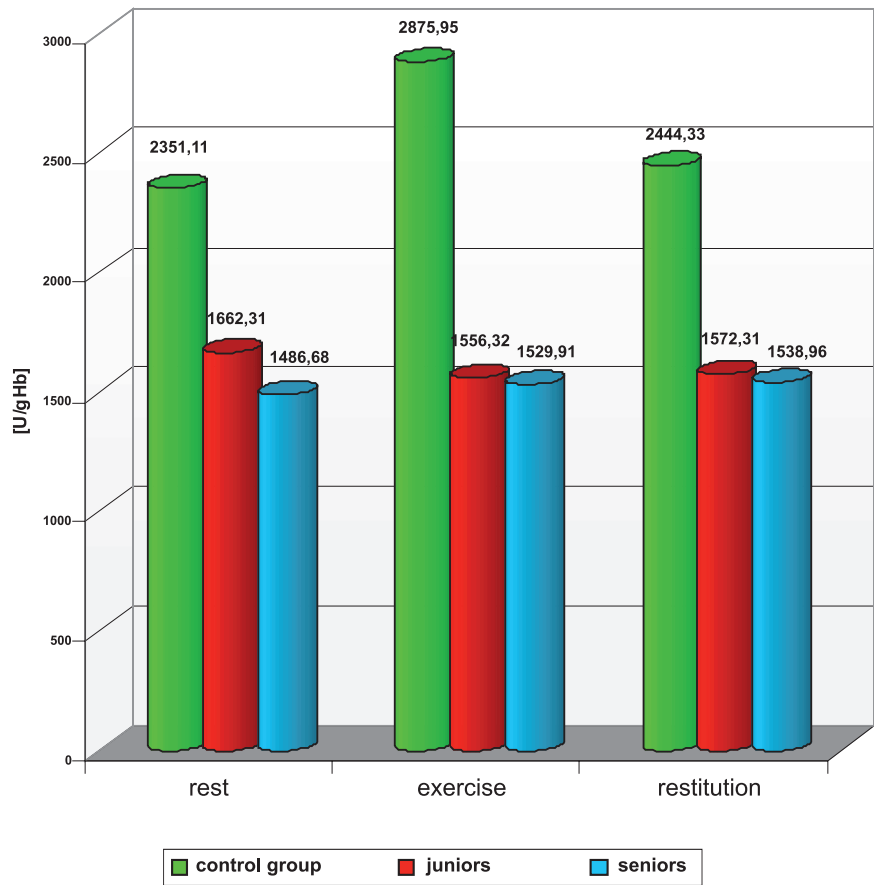


Fig. 1. Superoxide dismutase activity (CuZn-SOD) in the erythrocytes of sportsmen and the control group subjected to the dosed maximal exercise

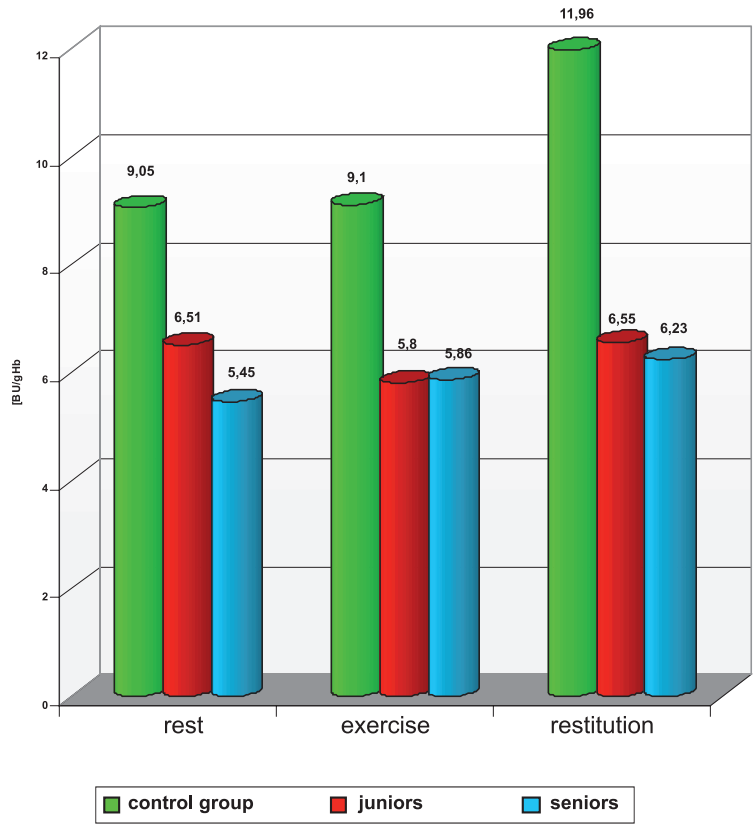


Fig. 2. Catalase (CAT) activity in the erythrocytes of sportsmen and the control group subjected to the dosed maximal exercise

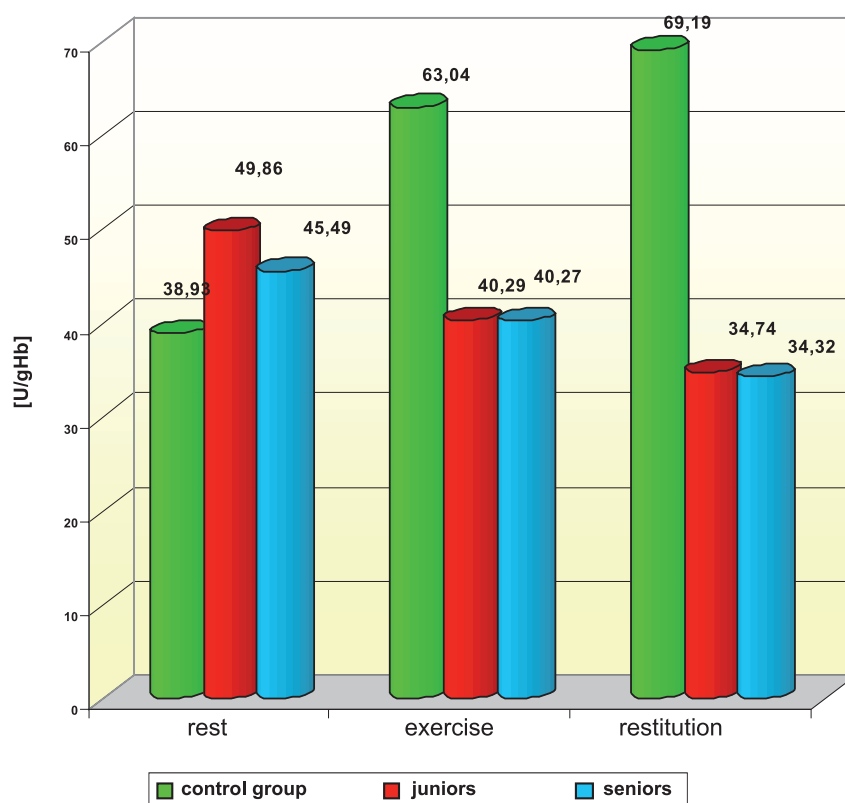


Fig. 3. Glutathione peroxidase (GSH-Px) activity in the erythrocytes of sportsmen and the control group subjected to the dosed maximal exercise

Discussion

In physiological conditions approximately 3% of oxyhemoglobin is converted to methemoglobin in the red cells [14]. However, increase in the oxygen consumption during intensive physical exercise leads to growing hemoglobin oxidation and intensified production of the superoxide radicals [15]. This is the only intraerythrocyte reaction in which reactive forms of oxygen are generated.

A review of the literature on the influence of the physical exercise on the prooxidative-antioxidative parameters emphasizes the role of the extra-erythrocyte sources of toxic oxygen species and reactive nitrogen species production [2, 16], which include: mitochondria, peroxisomes, respiratory chain [17, 18], vascular endothelium, purine metabolism to hypoxanthine and uric acid [19, 20], activation of fagocytes in the immunological system and respiratory burst [21-24]. Activation of the reactive oxygen species in the above-mentioned sources occurs during physical exercise, however the reports concerning results of the studies on the pro-oxidative parameters and anti-oxidative barrier characteristics are often conflicting.

In our research professional rugby players constituted the study group. Rugby is a team game requiring endurance effort which involves straining the whole body. During a rugby match aerobic and anaerobic potentials are consumed. Anaerobic process are involved in the sudden spurts and accelerations followed by the periods of the increased oxygen intake. During inten-

sive sustained muscle work and exercises of high risk of traumas micro-inflammatory processes stimulating immunological response and leading to the respiratory outburst may be the additional sources of ROS.

Activities of CuZn-SOD, CAT and GSH-Px (main enzymes involved in the protection against free radicals) reflect ROS concentration in the erythrocytes of the study subjects in the exercise test.

Considering both positive and negative effects of physical exercises it should be emphasized that specific kinds of trainings, intensity of the effort and individual reaction depending on the age, coexistent risk factors, physical efficiency are of great importance.

In the present study showed higher activity of investigated enzymes in the control group compared with the sportsmen. The body of persons engaged in regular physical activity with a maximum intensity is particularly vulnerable to oxidative stress, because the sport training is associated with a systematic, intermittent exposure of tissues to high concentrations of oxygen. As a result of the effort of physical changes occur not only in the adaptive processes related to providing energy for muscle work (glycolysis, respiratory chain, citric acid cycle, β -oxidation), but also changes in the antioxidant, protecting cells against the harmful effects of ROS. Magnitude of changes in this system depends on the intensity and duration of exercise.

SOD plays a key role in the removal of free radicals. In our study lower SOD activity was observed in the subjects with shorter experience in professional sport

after the dosed maximal exercise. Lower CuZn-SOD was reported during the effort and in the restitution phase. In the senior sportsmen positive, defense reaction of this enzyme was observed in the first minute of the exercise.

Our observations are consistent with the study results described in the literature [9, 25, 26]. Authors of the above-mentioned studies explain that decrease in the SOD activity may result from overproduction of hydrogen peroxide, which suppress activity of SOD. Moreover, imbalance between SOD and CAT may lead to the increase in the production of hydrogen peroxide, which when excessive may damage cells directly or indirectly through generation of very reactive hydroxyl radicals in the presence of iron ions [9]. CAT is an enzyme of double activity, which depends on the concentration of hydrogen peroxide in the environment. It has catalase and peroxidase activities. At high concentration of H_2O_2 it acts as protection against reactive oxides catalyzing reaction of hydrogen peroxide disproportionation and converting it to water and oxygen [3]. Hydrogen peroxide - a product of numerous metabolic processes occurring in the nervous tissue is co-responsible for aging and degeneration [27]. Catalase specificity to H_2O_2 increases at higher concentrations of the substrate $>10^{-6}$ M, which may be the effect of the respiratory outburst or at the over-expression of SOD, contributing to the release of great amount of hydrogen peroxide [3].

In our studies maximal exercise led to the increase in the catalase activity after the exercise in the erythrocytes of both junior and senior sportsmen and the control group. The enzyme activity was highest after 30-minute restitution. High activity of SOD suppresses CAT activity and conversely low activity of SOD induces high activity of CAT. Imbalance between CAT and SOD leads to overproduction of H_2O_2 . Peroxide anion radicals are likely to play an important role in the process of CAT activation. Increase in their concentration inside the erythrocytes results from the damage of CuZn-SOD by the hydrogen peroxides [9].

GSH-Px was the enzyme of the greatest range of activity, depending on the studied group. The enzyme activity lowered during the effort and in the restitution phase in both groups of rugby players. On the contrary, in the control group GSH-Px activity increased and reached maximum level in the resting phase

Results of the studies, described in the literature, concerning the influence of the muscle work on the GSH-Px activity in blood are not unambiguous. On one hand some researchers reported increase in the GSH-Px activity in the red blood cells [28]. On the other hand, other authors observed stable level [26] or decrease in the activity of this enzyme despite intensive physical exercises [29-31]. Discrepancy in

the obtained results seems to depend on the type and intensity of the effort.

Decrease in the CuZn-SOD and GSH-Px activities in the erythrocytes after the maximal physical exercise is an additional indicator of the induction of the oxidative stress. Reduction of the activities of the above-mentioned enzymes may result from the proteolysis stimulated by free radicals [32]. Furthermore, production of great amount of ROS may surpass defensive capacity of the anti-oxidative enzymes, which is likely to cause changes in the intra-cellular redox potential and/or modifications of the active centers of the enzymes, leading to suppression of the enzymatic activities of CuZn-SOD and GSH-Px in the erythrocytes. Moreover, effort the variability of antioxidant system depends on the intensity and type of the exercises, which affect the direction and magnitude of changes in the prooxidant-antioxidant balance.

Decrease in the GSH-Px activity observed in the sportsmen after the maximal physical effort suggests need for greater number of anti-oxidants rich in selenium. There is correlation between selenium content and glutathione peroxidase level: activity of this enzyme increases along with the linear rise in the selenium concentration. Low activity of the GSH-Px coexist with symptoms of selenium deficit [33].

Conclusions

Dosed maximum exercise:

1. observed direction of change in the control group may indicate that the antioxidant enzyme system of defense protects the body's cells against their harmful effects (increased activity of CuZn-SOD, GSH-Px, CAT);
2. decreased activity of enzymes studied in the 1st minute of exercise in a group of junior shows that length and degree of training of the players can affect the size of oxidative stress;
3. in sportsmen reduces the activity of GSH-Px, which may indicate the need for support supplements rich in selenium.

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