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ROS GENERATION AND LIPID PEROXIDATION IN PROFESSIONAL SPORTSMEN (EXTREME SPORTS CONDITION)

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Abstract

Introduction: Too intensive physical exercise may lower NO bioactivity as a result of excessive production of reactive oxygen species (ROS), such as superoxide anion radical ($O_2^{\bullet -}$). This can lead to the intense peroxidation of cell membrane lipids in erythrocytes.

Aim of the study: The aim of the study was to evaluate the influence of dosed maximal exercise on the plasma nitric oxide content and the level of malonyl dialdehyde in red blood cells in professional sportsmen and in individuals of normal physical activity.

Materials and Methods: 41 rugby players and 21 men of normal physical activity (control group) were the subject of the study. NO and MDA concentrations were determined in three time points: before an effort, 1 minute after dosed maximal exercise and after 30-minute restitution period. Nitric oxide (NO) concentration was evaluated with Griess's indirect method and malonyl dialdehyde (MDA) concentration was measured with Placer's method.

Results: Mean NO concentration increased in the exercise phase and decreased after 30-minute restitution in all three studied groups. The highest MDA level was observed in the first minute of exercise in all studied groups.

Conclusions: Results of determination of plasma NO concentration does not confirm reduction of its bioactivity. Plasma NO level rose 1 minute after maximal exercise in all three groups of studied subjects. In trained professional sportsmen, adapted to the physical effort, this kind of load enables protection of cell structures against peroxidation process by the antioxidant barrier, which is reflected by the lower concentration of malonyl dialdehyde (MDA) in the red blood cells in comparison to the control group.

Key words: *nitric oxide, malonyl dialdehyde, dosed maximal physical exercise*

Introduction

Nitric oxide (NO) probably plays a regulatory role in the respiration process in the mitochondrial phase during physical exercise. To intensive physical exercise may lower bioactivity of NO because of the excessive production of reactive oxygen species (ROS). It is supposed that endothelial dysfunction and decreased activity of nitric oxide may result from excessive superoxide anion radical production ($O_2^{\bullet -}$), which is a consequence of lowered activity of ROS removing endogenous enzymes such as: superoxide dismutase (CuZn-SOD), or glutathion peroxidase (GSH-Px). This leads to the accumulation of superoxide anion radical molecules and intensified NO inactivation. A final product of this reaction is peroxynitrite ($ONOO^-$) molecule (1).

Furthermore, shortage of the substrates necessary in NO formation such as: arginines and insufficient saturation with tetrahydrobiopterine, which plays a cofactor role, not only causes NO production, but also simultaneous $O_2^{\bullet -}$ synthesis. Nitric oxide may react with $O_2^{\bullet -}$ forming peroxynitrite. This reaction is of great biological significance and leads to $O_2^{\bullet -}$ and NO inactivation. However, newly formed $ONOO^-$ which is not a free radical itself, may be the source of highly toxic oxygen forms. Peroxynitrite in the tissue fluid gets protonated to the peroxynitrous acid (HOONO). Molecules of this acid may disintegrate to hydroxyl radical ($OH^{\bullet}$) and nitrogen dioxide radicals ($NO_2^{\bullet}$) or to nitronium cation (NO_2^+) and hydroxyl anion (OH^-). These three products: $OH^{\bullet}$, $NO_2^{\bullet}$ and NO_2^+ are among the most aggressive and hazardous agents in biological systems. Excessively produced $ONOO^-$ causes modification of proteins, including enzymatic proteins, mostly these with iron-sulphur centers, transition metal ions and heme groups (2).

Physical effort increases oxygen consumption and is one of many factors influencing oxygen metabolism of particular cells in the human body, including erythrocytes. This leads to the production of a great number of ROS characterized by strong chemical reactivity, which, to a great extend, affect survival, growth and development of the cells. Strenuous exercise may intensify peroxidation of the erythrocyte cell mem-

brane, damage its cytoskeleton and finally decrease its capacity of reversible change of the shape (3). Malonyl dialdehyde (MDA) is the most frequently investigated peroxidation marker.

The aim of this study was to evaluate the influence of dosed maximal exercise on the plasma nitric oxide content and the level of malonyl dialdehyde in the red blood cells in the professional sportsmen and in individuals of normal physical activity. Influence of this kind of physical load on the sportsmen's body, depending on the experience in the training and adaptation to the physical effort was compared.

Materials and methods

41 rugby players from Budowlani Sports Club in Lodz were the subjects of this study. The group was divided into two categories of 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 sports was 5.17± 1.01 years for juniors and 10.25±2.36 years for seniors. Juniors' body mass index (BMI) was 26.24±1.5 kg/m^2, seniors' BMI was 29.69±2.18 kg/m^2 and BMI of untrained men was 25.06±2.18 kg/m^2.

Exercise tests were performed on a cycloergometer produced by Monark. Maximum effort was reached using a 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' refusal. Venous blood from a basilica 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. Nitric oxide content was determined in plasma, and erythrocyte mass was rinsed 3 times with 0.9% NaCl solution (+4°C) keeping the same conditions of centrifugation. After the supernatant was removed, rinsed, erythrocytes were hemolyzed by adding redistillated water ana partes equales and freezed at the temperature of – 18°C. The hemolysate, after being defrosted was used in the subsequent studies. Plasma nitric oxide (NO) concentration was evaluated with Griess indirect method, which determines a relationship between concentrations of nitrites and nitrates.

Malonyl dialdehyde (MDA) level in the red blood cells expressed by the concentration of compounds, reacting with thiobarbituric acid, was measured with Placer's method. (4). Hemoglobin (Hb) concentration in erythrocyte hemolysate, necessary to MDA level determination was evaluated with a standard colorimetric method with a Drabkin's reagent.

No contraindications to the exercise test were found in all the studied subjects.

Approval of Bioethics Committee of the Medical University in Lodz (nr RNN/36/05/KB) was obtained.

All individuals participating in the study were informed about the aim and the performance of the study and expressed their written consent.

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

Statistical analysis of the changes in nitric oxide content in the plasma and malonyl dildehyde level in the erythrocytes in the studied groups measured at the following time points: before exercise, 1 minute after the maximal exercise and after 30-minute restitution is shown in the table 1.

In both groups of the sportsmen, nitric oxide (NO) concentration increased in the exercise phase and the values were approximatte. After 30-minute restitution phase, nitric oxide concentration decreased in all three studied groups (fig1).

The highest level of malonyl dialdehyde (MDA) was observed after the first minute of the exercise in all the studied groups (fig 2). In all three periods: before exercise, exercise and restitution phases there were statistically significant differences between the con-

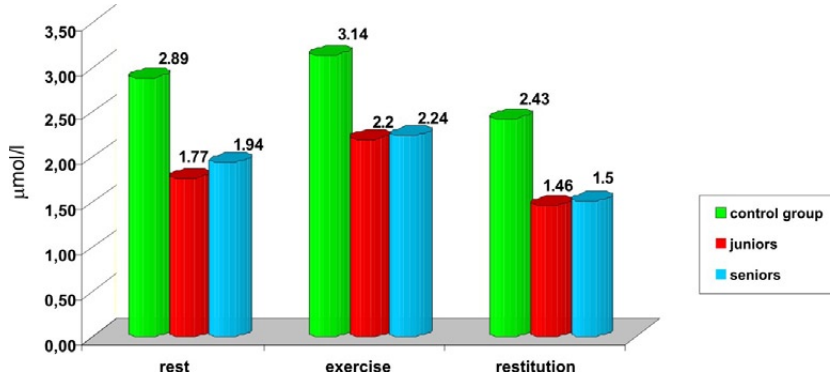

Fig.1. *Plasma nitric oxide concentration (NO) in sportsmen and in the control group submitted to the maximal exercise*

Table 1. *Statistical analysis of the nitric oxide (NO) level in the plasma and malonyl dialdehyde (MDA) content in the erythrocytes of the professional sportsmen and the control group before exercise, 1 minute after the maximal exercise and after restitution period (x±SD)*

	rest			exercise			restitution		
	control C	juniors J	Seniors S	control C	juniors J	seniors S	control C	juniors J	seniors S
MDA nmol/gHb	0.15 ±0.03	0.10 ±0.01	0.10 ±0.01	0.20 ±0.03	0.13 ±0.01	0.13 ±0.02	0.15 ±0.02	0.11 ±0.02	0.11 ±0.02
Statistical analysis	Levene's test = 5.67 p<0.01 Kruskal-Wallis test = 35.70 p<0.001 Mann-Whitney$Z^{C.J}$ test = 5.06 p<0.001; Mann-Whitney$Z^{C.J}$ test = 5.21 p<0.001 Mann-Whitney$Z^{C.J}$ test = 0.53 p>0.05			Levene's test =26.07 p<0.001 Kruskal-Wallis test = 30.85 p<0.001 Mann-Whitney$Z^{C.J}$ test = 4.32 p<0.001; Mann-Whitney$Z^{C.J}$ test = 4.45 p<0.001 Mann-Whitney$Z^{C.J}$ test = 0.52 p>0.05			Levene's test = 1.21 p>0.05 analysis of variance F test = 43.03 p<0.001 Dunkan's test Statistically significant differences: X_C: X_J p<0.001; X_C:X_S p<0.001; X_J: X_S p>0.05		
NO µmol/l	2.89 ±1.11	1.77 ±0.90	1.94 ±0.88	3.14 ±0.86	2.20 ±1.08	2.24 ±1.01	2.43 ±1.08	1.46 ±0.75	1.50 ±0.95
Statistical analysis	Levene's test = 0.83 p>0.05 analysis of variance F test = 7.98 p<0.001 Dunkan's test Statistically significant differences: X_C: X_J p<0.001; X_C:X_S p<0.001; X_J: X_S p>0.05			Levene's test = 0.90 p>0.05 analysis of variance F test = 6.00 p<0.01 Dunkan's test Statistically significant differences: X_C: X_J p<0.001; X_C:X_S p<0.001; X_J: X_S p>0.05			Levene's test = 1.17 p>0.05 analysis of variance F test = 7.14 p<0.01 Dunkan's test Statistically significant differences: X_C: X_J p<0.001; X_C:X_S p<0.001; X_J: X_S p>0.05		

MDA- malonyl dialdehyde, NO- nitric oxide

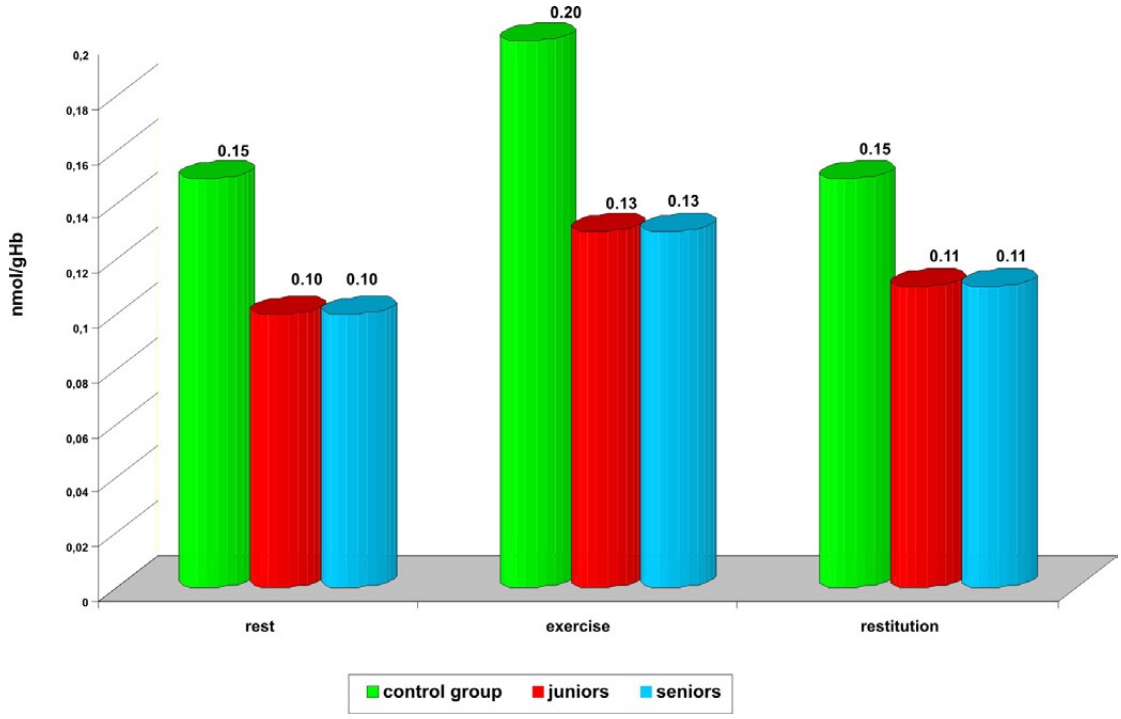

Fig.2. *Malonyl dialdehyde concentration (MDA) in the red blood cells in sportsmen and in the control group submitted to the maximum exercise*

trol group (K) and juniors (J) ($p<0.001$) and between control group (K) and seniors (S) ($p<0.001$). Malonyl dialdehyde concentration was not significantly different between juniors and seniors.

Discussion

Many authors of the studies (1,5-10) conclude that in healthy people, production of nitric oxide increases as a result of physical exercise.

In our study, nitric oxide concentration was measured with Griess's indirect method consisting in determination of a relationship between concentrations of nitrites and nitrates.

Plasma NO level increased 1 minute after maximal exercise in all the studied groups. The highest plasma NO concentration was observed in individuals who did not practice sport professionally. Therefore, in the circulatory system of professional sportsmen, trainings may cause different adaptive changes, affecting structure and function of blood vessels. Increase in the density of the capillary system decreases space between blood and the inside of the cells, which contributes to the higher rate of gas, metabolites and substrates exchange.

Results of this study are consistent with the reports of the aforementioned researchers, who described beneficial influence of the muscle work on the adaptation of the circulatory system to the load (submaximum or maximum) while taking exercise. The authors of the above-mentioned studies suggest that increase in the endothelium activity is caused by higher involvement of cutting forces on the vessel walls during the exercise, which is manifested by the augmentation of nitric oxide concentration. Mean blood flow velocity increases, enabling more effective substrates and metabolites exchange in a dense capillary system of a trained muscle.

Fukai T et al (11) described experiment on mice submitted to physical exercise, which had beneficial and protective influence on dilatation of the blood vessels. The mechanism was based on the increased expression of eNOS (endothelial nitric oxide synthase) together with extracellular superoxide dismutase (EC-SOD). This enzyme significantly reduces superoxide radical ($O_2^{\bullet -}$) reaction with the nitric oxide. Authors of this study prove that EC-SOD and physical exercise play a very important role in the protection against pathophysiological effects of $O_2^{\bullet -}$ on the vessel wall.

Silveira LR et al (12) showed that intense muscular contractions in rats led to the increased production of nitric oxide and hydrogen peroxide in the cells of skeletal muscles. Authors maintained, that large amounts of NO and H_2O_2 are probably produced inside a cell, then, as a result of permeability of the muscular cells membranes, released to the extracellular space, where they may be a part of an important mechanism of defense against peroxynitrite ($ONOO^-$) and hydroxyl radicals ($OH^{\bullet}$), produced intracellularly.

On one hand, physical exercise has beneficial effect on the expression of endothelial synthase (eNOS), increasing the local level of NO. On the other hand, it should be remembered that physical exercise always increases oxygen consumption and, consequently, augments production of the reactive oxygen and nitrogen species (13).

In the research work Patwell DM et al (14) described the influence of skeletal muscle contractions of the mouse on the release of free radicals to the extracellular space, dependently on the frequency of electrically stimulated muscle cell. Stimulation induced release of the superoxide radicals and nitric oxide, which contributed to the high level of the hydroxyl radical.

In our study, after 30-minute restitution phase, oxygen requirement decreases, vascular lumen narrows and well-trained organism does not need such long distribution of high level of NO, which is confirmed by the results.

Some researches, also proved that in studies on lipid peroxidation, most frequently used method of malonyl dialedehyde (product of this process) determination, based on the reaction with thiobarbituric acid (TBARS) was sensitive, but not specific.

Groussard C et al (15) reported 2,7-fold increase in the production of lipid radicals in the plasma, evaluated with electron- paramagnetic-resonance spectroscopy and simultaneous paradoxical decrease in the thiobarbituric reactive substances (TBARS) level in the plasma (-23.7%) after short supramaximal anaerobic exercise. The investigators assume, that changes in peroxidation-antioxidation balance depends on the exercise type and its intensity. MDA determination is not always an appropriate indicator of oxidative stress generated during physical exercise.

Many authors (16-22) evaluated of influence of physical exercise on parameters of oxidative stress and reported induction of changes in the cell membrane integrity by peroxidation products and consequent release of intracellular enzymes to the bloodstream.

In our study, maximal exercise, both in junior and senior groups, caused an increase in MDA content in the red blood cells. This may indicate changes in cellular membrane structure – MDA concentration remains the same after a restitution phase. However, in trained professional sportsmen adapted to the physical effort, this kind of load enables protection of cell structures against peroxidation process by the antioxidant barrier, which is confirmed by lower malonyl dialdehyde concentration in the erythrocytes in comparison to the control group.

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