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THE EFFECT OF STRENGTH EXERCISE ON LIPID PEROXIDATION PRODUCTS CONCENTRATION AND GLUTATHIONE METABOLISM IN BLOOD OF THE BODY - BUILDERS

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Abstract. The study was carried out on twelve males subjected to strength exercise. Before exercise, immediately after exercise, after 30 min and 24 h rest, the following parameters were estimated in blood samples collected from the body builders: levels of lactate (LA), reduced (GSH) and total glutathione (GSH+GSSG), glutathione reductase (GR) and creatine kinase (CK) activity, and lipid peroxidation products (TBARS) concentration. The applied strength test at the maximal intensity (LA 9.83±3.08 mmol·l^{-1}) caused statistically significant increase of tested parameters after 24 h rest. The GSH/GSH+GSSG ratio decreased after 24 h ($P<0.05$). The positive correlation $r=0.821$ ($P<0.001$) between the creatine kinase activity (CK) and the lipid peroxidation products (TBARS) level was observed. It indicates there is relationship between the exercise – induced muscle damage and oxidative stress during the restitution.

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Key words: Exercise – Glutathione - Lipid peroxidation - Creatine kinase

Introduction

In 1982 Davis *et al.* [8], using electron resonance spectroscopy, proved reactive oxygen species (ROS) production in muscle tissue of rat subjected to intensive physical exercise. The ROS overproduction in the study of Davis *et al.* [8] contributed to decreased mitochondria respiratory capacity and sarcoplasmic reticulum integrity. Further studies showed that cellular peroxidative damage induced by the ROS is not restricted to skeletal muscles alone. Numerous authors analysing antioxidant activities and concentrations (superoxide dismutase SOD, catalase CAT, glutathione peroxidase GPx, glutathione reductase GR, α-tocopherol, ascorbic acid, glutathione etc.) and peroxidation products (malonyldialdehyde, lipid peroxides, conjugated dienes, hydrocarbons, carbonyl

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groups etc.) recognised the presence of oxidative stress in lungs, heart, liver and erythrocytes. Its intensity and moment of appearance depended on exercise duration and intensity as well as the kind of exercise (dynamic or static) [23,25,28].

According to numerous authors the strength exercise which producing eccentric and concentric muscle contractions induces typical series of reactions of reactive oxygen species generation [9,17,27]. The reason for it is extended hypoxia causing xanthine oxidase activity and the mass ROS production under reperfusion. Another reason are muscle contractions, which lead to muscle fibre damage and blood vessel micro-injuries followed by the ROS generation in granulocytes contributing to damage removal (phagocytosis). The third reason is the enhancement of aerobic metabolism and increase of the ROS production in mitochondria in the respiratory chain. The released ROS give rise to the range of cell damage and induce changes in antioxidant concentration in blood, including glutathione [17].

Glutathione (γ-glutamyl – cysteinyl – glycine) is frequently used as a marker of oxidative stress [4,14,26,31]. The decrease of reduced glutathione (GSH) or the elevation of the oxidised form (GSSG) in the subjects' blood was proportional to the ROS production in working muscles. The first study on changes of glutathione concentration in blood was carried out by Gohil *et al.* [15]. The authors recognised an enhancement of the glutathione oxidation (GSSG increase) and the GSH concentration decrease resulted from submaximal exercise. Evelo *et al.* [12] observed that training duration and running distance induce different response of the antioxidant glutathione system (GSH, S – glutathione transferase, glutathione reductase). However, Balakrishnan *et al.* [1] noticed decreased GSH concentration in blood of trained subjects compared with the untrained. Furthermore, the authors observed higher concentration of lipid peroxidation products (TBARS) in the subjects' blood. Our previous comparative study showed there was negative correlation (r=-0.703) between the resting concentration of total glutathione and TBARS concentration in blood. That suggests that the greater the ROS production and glutathione decrease during training the higher the intensity of the lipid peroxidation process [33].

The aims of the present study were to determine the effect of maximal strength exercise on the reduced and total glutathione levels, glutathione reductase activity and lipid peroxidation products concentration in blood of the body-builders and to investigate whether there is a relationship between muscle damage and the estimated markers of oxidative stress.

Material and Methods

The research was carried out on 12 students of Physical Education Institute in Gorzów Wlkp., training regularly at a gym. The characteristics of the subjects are presented in Table 1.

Table 1
The characteristic of the body builders under study (mean ±SD).

Age (years)	Body mass (kg)	Height (cm)	Period of (special) training (years)
21.25±1.14	68.75±4.65	179.17±4.47	5.67±1.78

The body-builders performed a serial incremental exercise test consisting of 5-time repetition of track-athletics elements: knee bendings with the weight on the nape, weight lifting on a straight bench, death-pull. The subjects did from 3 up to 5 series, beginning with 60% of their maximal load (life record) increasing it individually.

The subjects were informed of the aim of the studies and gave their consent for taking blood samples. The studies obtained the agreement of the Local Bioethical Committee in Gorzów Wlkp.

Blood samples were collected from cubital vein before exercise, immediately after exercise, after 30 min and 24 h rest. The blood for lactate (LA) concentration measurement was taken from the ear lob before exercise, after each series and 30 min rest. Serum was obtained by centrifugation at 2500 x g for 10 min, then frozen and stored at –20^{o}C. Erythrocyte fraction was resuspended and washed threefold with cold isotonic saline solution. Washed erythrocytes were stored at –20^{o}C until analysis. All the samples were analysed within 7 days. In the whole blood the reduced glutathione (GSH) concentration by the method of Beutler *et al.* [2], total glutathione (GSH+GSSG) concentration by the method of Tietze [30] and lactate (LA) concentration using Dr Lange kit (Germany) were estimated. In the erythrocytes glutathione reductase (GR, EC 1.6.4.2) activity using Randox kit (England) and thiobarbituric acid – reactive substances (TBARS) concentration by the method of Buege and Aust [3] were measured. In the serum the creatine kinase activity (CK, EC 2.7.3.2) using Emapol kit (Poland) was estimated.

In the hemolysates the haemoglobin (Hb) concentration by Drabkin's standard method was assessed.

Statistical analysis was carried out using Statistica program. Data were tested by one – way ANOVA and Newman-Keuls post-hoc test. Between the tested parameters Pearson correlation coefficient was analysed. The accepted level of significance was $P<0.05$.

Results

Table 2
Changes in reduced glutathione (GSH), total glutathione (GSH+GSSG), lipid peroxidation products (TBARS) concentrations and glutathione reductase (GR), creatine kinase (CK) activity in blood of the body-builders (mean ±SD)

	P	E	R30	R24
GSH $\mu g \cdot ml^{-1}$ whole blood	247±26	247±43	242±43	284±34*
GSH+GSSG $\mu g \cdot ml^{-1}$ whole blood	419±59	475±84	457±80	639±236*
GR $U \cdot gHb^{-1}$ erythrocytes	12.8±2.41	12.14±2.17	15.01±3.01	17.14±2.62*
TBARS $\mu mol \cdot gHb^{-1}$ erythrocytes	3.28±0.45	3.48±1.64	2.85± 0.55	4.2±0.76*
CK $U \cdot l^{-1}$ serum	77.2±22.7	104.6±26.6	89.2±30.3	518.8±222*

*Indicates significant ($P<0.05$) differences from pre- exercise values
P- pre-exercise, E- exercise, R30- 30min rest, R24- 24 h rest

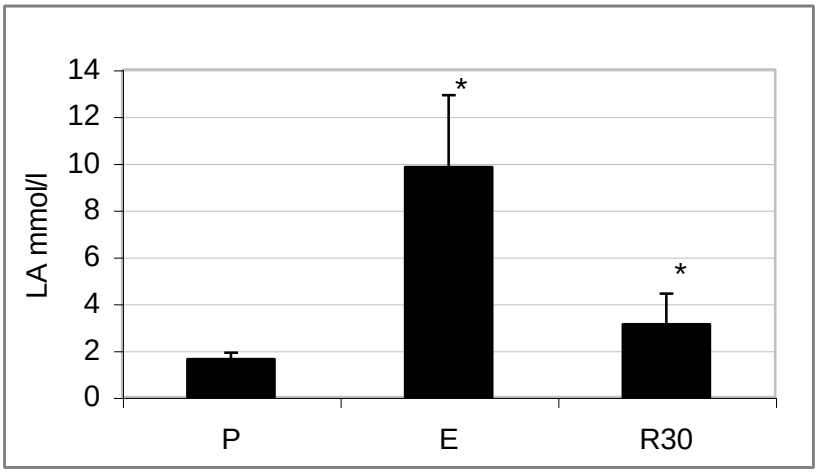
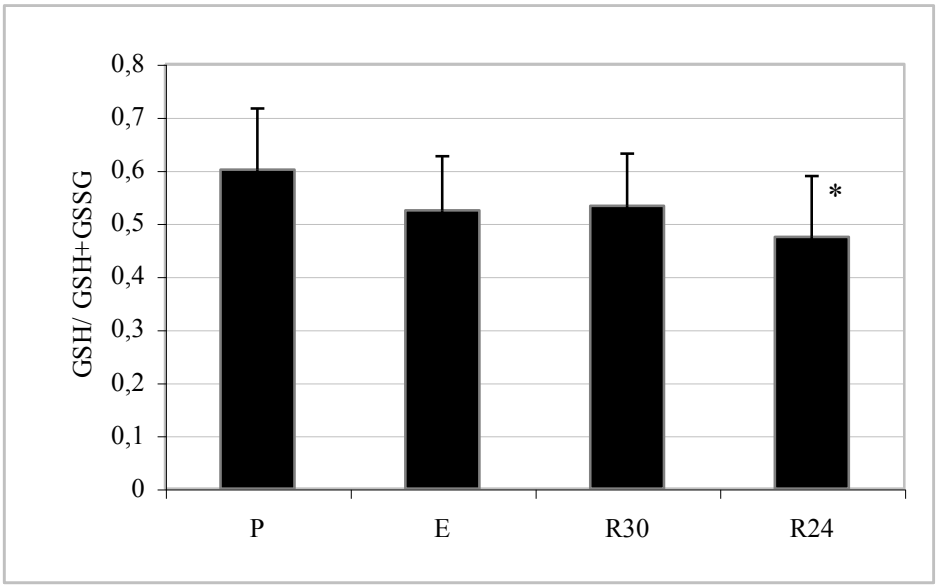


Fig. 1
Changes in blood lactate (LA) concentration in the body - builders

The data illustrated in tables 2-3 and Figs. 1-2 show that strength exercise at maximal intensity (LA 9.83±3.08 $mmol \cdot l^{-1}$) resulted in statistically significant post-exercise changes in the tested parameters. After 24 h rest an increase in the GSH and GSH+GSSG concentrations as well as a 34% increase in the GR activity were observed. However, the rise of the GSH+GSSG (+53%) was significantly higher than the GSH level (+15%). The GSH/ GSH+GSSG ratio decreased from the rest value 0.61±0.12 to 0.48±0.11 ($P<0.05$) after 24 h rest (Fig. 2). It was accompanied by the intensification of peroxidation process in erythrocytes. The TBARS concentration increase (+23%) after 24 h rest was observed. The serum CK activity increased six-fold after 24 h rest. Between the CK activity and the other analysed parameters the positive correlation was recognised (Table 3). The highest value of correlation coefficient was observed between the CK activity and the TBARS concentration $r=0.821$ ($P<0.001$).



*Indicates significant (P<0.05) differences from the pre- exercise value values
P- pre-exercise, E- exercise, R30- 30 min rest, R24- 24 h rest

Fig. 2
The reduced (GSH) and total (GSH+GSSG) glutathione ratio in blood of the body builders

Table 3
Relationship between creatine kinase activity (CK) and other parameters

	Coefficient of correlation	Significance
GSH	r=0.330	0.023*
GSH+GSSG	r=0.518	0.001*
GR	r=0.387	0.008*
TBARS	r=0.821	0.001*

*P<0.05

Discussion

Changes in the glutathione concentration in blood depend directly on the number of produced and released reactive oxygen species from skeletal muscles, vascular endothelial cells, neutrophils, macrophages, etc. Physical exercise may induce in a decrease in blood glutathione concentration as a result of a higher absorption of the GSH by tissues (mainly muscles) or an increase in glutathione due to enhanced glutathione synthesis.

The main location for the glutathione synthesis is liver, delivering about 80-90% of GSH into the circulation. Active transport of GSH from hepatocytes into blood in rats is 12.4±1.4 nmol/min/g tissue and exercise induces it to 26.4±1.2 nmol/min/g [19]. The glutathione transport is hormonally regulated (adrenaline, glucagon, vasopresin), which what was confirmed by Ji *et al.* [18] in the study on cyclists. The carbohydrates intake decreased the glucagon level and, at the same time, reduced the GSH efflux from liver during 120 min exercise at 70% VO_2max intensity on a cycle-ergometer.

Another source of glutathione in blood are skeletal muscles which release glutathione into blood despite low GSH especially slow - twitch muscles fibres. The level of GSH export from rat muscles amounts to 2 nmol/min/g of tissue [19]. Physical exercise diminishes the GSH release but increases its intake by muscles. It is confirmed by findings of Sen *et al.* [28] who observed an increase in the γ-glutamyl-transpeptidase activity (enzyme responsible for the glutathione transport through cell membrane) in limb muscles of dogs trained for 55 weeks.

Red blood cells are the third glutathione source synthesising the GSH *de novo*. An independent synthesis of the GSH indicates the contribution of erythrocytes to intra-molecular glutathione delivery. Dass *et al.* [7] supposed that the glutathione is "extracted" from erythrocytes by tissues of high GSH consumption (lungs, heart, intestine, brain and skeletal muscles) during intensive exercise. The detailed mechanism of the process is unclear.

The applied strength test caused no changes of the reduced glutathione (GSH) concentration in blood during exercise and after 30 min rest. A similar result was obtained by Camus *et al.* [4] investigating the influence of eccentric and concentric exercises on the GSH and GSSG concentrations in blood. The authors did not observe any changes in concentration of the analysed parameters during exercise and after 20 min rest.

However, in most the studies the decrease as large as 40% of GSH concentration was observed [10,11,14,15,31]. Our findings and those obtained by other authors vary possibly due to different tests used in the studies. Dynamic

exercise influence (e.g. running, ergometer cycling) was most frequently measured. Furthermore, the differences may be attributable to various measurement time. The glutathione concentration was determined immediately after exercise. In the present study the last measurement was conducted 24 h after exercise recognising increase in the GSH concentration. It was probably induced by increased demand for the antioxidant in tissues with the ROS generation and peroxidative injuries followed by an increase in glutathione synthesis in blood cells, hepatocytes and the GSH release from liver to blood after 24 h rest.

The increase in the total glutathione (GSH+GSSG) concentration in blood of the subjects was higher than in the GSH. That resulted in the decrease of the GSH/GSH+GSSG ratio and possibly related to a greater increase in concentration of the GSSG oxidised form. The elevated GSSG level in blood proves the glutathione contribution to reduction of reactive oxygen species (2GSH+ROS→GSSG) [17]. The process is necessary but harmful to a cell it proceeds rapidly. Too high the concentration of the GSSG inside endangers the proper functioning of enzymes. The glutathione disulfide and enzymatic proteins create the GSS-protein links or intramolecular disulfide bridges. It may induce temporary inhibition of the enzymes activity and oxidative stress enhancement [20]. For this reason, upon insufficient glutathione reductase responsible for glutathione reduction, the cells release the GSSG to extracellular environment. The GSSG concentration increase in the bioptic samples of the athletes' muscles after a marathon was observed by Corbbucci *et al.* [6]. Sahlin *et al.* [26] found an increase in total glutathione concentration in blood of the subjects as early as during incremental exercise to maximal intensity and after 10 min rest.

An other possible reason for such a great increase in total glutathione was its release from injured muscles. The positive correlation between CK activity (enzyme widely used as a marker of the exercise muscle injuries) and GSH+GSSG concentration in blood of the body builders was recognised.

The glutathione reductase (GR) activity in the body-builders' blood, similarly the total glutathione concentration, increased after 24 h rest following exercise.
The findings resembles results of the study by Ohno *et al.* [22] suggesting that the post-exercise GR activity increase may by explained by the pH decrease during exercise and changes in the GR and NADPH affinity (the hydrogen donor in the glutathion disulfide reduction) [24]. The pH decrease occurred in the subjects' blood an increase in the lactate concentration. However, due to intraerythrocytic mechanisms which maintain the pH constant, the GR increase in the subjects' erythrocyte is unlikely have been caused by the low pH in serum.

The enzyme activity may be regulated by another mechanisms directly related to the ROS production, similarly, when superoxide dismutase and catalase are concerned [16,29].

The ROS released from tissues may induce peroxidation in the erythrocyte membrane unless they are reduced by extracellular antioxidants. Erythrocytes are exposed to not only to external attacks since the ROS enter intracellular environment. The superoxide radical anion ($O_2^{-\cdot}$) permeates through the erythrocyte membrane due the content of an anion exchanger (band 3). It may also diffuses across membrane as a hydroperoxyl radical ($HO_2^{\cdot}$). The above process enabled by the pH decrease in blood during exercise (protonation: $O_2^{-\cdot} + H^+ \rightarrow HO_2^{\cdot}$) [32]. Red blood cells are well protected against oxidative stress by e.g. glutathione. Yet, the ROS tend to induce damage in erythrocytes. This has been proven conclusively in the studies by Duthie *et al.* [11], Drewa *et al.* [9], Miyazaki *et al.* [21].

The applied strength test in this study resulted in erythrocyte damage induced by the ROS reflected by increased lipid peroxidation products concentration (TBARS). The magnitude of the increase in lipid peroxidation was recognised as late as 24 h after exercise, similarly, the changes in the other estimated parameters.

Each cell damage gives rise to the ROS concentration in blood, which is followed by larger changes in glutathione metabolism and an increase in peroxidation products concentration [17]. Positive correlations were recognised between the creatine kinase activity (CK) and the other parameters nothing the highest between the CK and TBARS. That proves the exercise – induced muscle damage to be related to the ROS production. Such relationship was also found by Frankiewicz-Jóźko *et al.* [13] and Drewa *et al.* [9]. The running test until exhausting used by Frankiewicz-Jóźko *et al.* [13] caused the CK activity increase in serum and the peroxidation enhancement in rat muscle. Drewa *et al.* [9] observed a high positive correlation between the CK and TBARS in the weight-lifters' blood after 4 h training. Child *et al.* [5] also noticed the proportional increase in the malondialdehyde level and CK activity in blood of the long-distance runners performing laboratory test on a treadmill (20 km).

The obtained results of this study show that strength exercise induces changes in glutathione metabolism and elevates lipid peroxidation in the body-builders' blood, related to the ROS production at rest following exercise as well as the relationship between muscle damage and oxidative stress.

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