УДК 636.1:591.111

OXIDATIVE STRESS BIOMARKERS AND ERYTHROCYTE HEMOLYSIS IN TRAINED UKRAINIAN WARMBLOOD HORSES UNDER THE INFLUENCE OF EXERCISE

Anastasiia Andriichuk[1], Halyna Tkachenko[2], Natalia Kurhaluk[2], Iryna Tkachova[1]

[1]Institute of Animal Breeding of National Academy of Agricultural Sciences, Kharkiv, Ukraine, anastasia.pohlyad@gmail.com
[2]Department of Animal Physiology, Department of Zoology, Institute of Biology and Environment Protection, Pomeranian University, Słupsk, Poland, tkachenko@apsl.edu.pl, kurhaluk@apsl.edu.pl, biology.apsl@gmail.com

The purpose of the present study was to investigate the effect of regular exercises on oxidative stress biomarkers, antioxidant enzymes activity, as well as resistance of erythrocytes to hemolytic agent in well-trained Ukrainian warmblood horses. Twenty three Ukrainian warmblood horses (9 females and 14 males), involved in jumping, eventing and dressage were used. Oxidative stress biomarkers (2-thiobarbituric acid reactive substrates level (TBARS), the carbonyl derivatives content of protein oxidative modification), antioxidant defenses (superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), level of ceruloplasmin (CP), total antioxidant capacity), and osmotic resistance of erythrocytes were assessed. Our results suggest that exercises cause different consequences in oxidative stress biomarkers in the blood, plasma, and erythrocytes of horses. Exercise lead to decrease TBARS level in erythrocytes while in blood and plasma does not. This difference in TBARS level before and after exercise, most likely is a consequence of differing levels of oxidative stress occurring in the tissues and erythrocytes. Decrease of carbonyl derivatives in erythrocytes' suspension is result of exercise-induced adaptation. A correlation between the oxidative stress biomarkers and antioxidant defenses in the horses after exercise was observed, which may indicate a protective response of GR, GPx and CP activities against exercise-induced oxidative stress. Statistically significant differences in the percentage of haemolysed erythrocytes between before and after exercise also were observed.

Keywords: HORSES, UKRAINIAN WARMBLOOD BREED, TRAINING, OXIDATIVE STRESS, ANTIOXIDANT DEFENSES, ERYTHROCYTE RESISTANCE

ЗМІНИ МАРКЕРІВ ОКСИДАТИВНОГО СТРЕСУ ТА ОСМОТИЧНОЇ РЕЗИСТЕНТНОСТІ ЕРИТРОЦИТІВ У СПОРТИВНИХ КОНЕЙ УКРАЇНСЬКОЇ ВЕРХОВОЇ ПОРОДИ В ДИНАМІЦІ ТРЕНІНГУ

А. В. Андрійчук[1], Г. М. Ткаченко[2], Н. М. Кургалюк[2], І. В. Ткачова[1]

[1]Інститут тваринництва НААН, 62404, Харківська обл, Харківський р-н, смт. Кулиничі, вул. 7-ї Гвардійської армії, 3, anastasia.pohlyad@gmail.com
[2]Department of Animal Physiology, Department of Zoology, Institute of Biology and Environmental Protection, Pomeranian University, Arciszewski Str., 22b, 76-200 Slupsk, Poland, tkachenko@apsl.edu.pl, kurhaluk@apsl.edu.pl, biology.apsl@gmail.com

Дослідження особливостей перебігу метаболічних змін в організмі коней, призначених для використання в класичних видах кінного спорту (конкур, виїздка, триборство) є важливою передумовою в аналізі їх адаптаційних можливостей та рівня тренованості. Мета даних досліджень полягала у визначенні вмісту маркерів оксидативного стресу (ТБК-активних продуктів, похідних оксидаційної модифікації

протеїнів), активності ензимів антиоксидантного захисту (супероксиддисмутази, каталази, глутатіонредуктази, глутатіонпероксидази), вміст церулоплазміну, як компонента системи антиоксидантного захисту та осмотичної резистентності еритроцитів у спортивних коней української верхової породи в динаміці фізичних навантажень (в стані спокою та після тренування). Дослідні коні (9 кобил та 14 жеребців), перебували у систематичному спортивному тренінгу та активно використовувалися в класичних видах кінного спорту. Після фізичних навантажень, в еритроцитах нами виявлено істотне зниження рівня маркерів ліпопероксидації та неістотне зменшення вмісту продуктів окиснювальної модифікації протеїнів, що свідчить про розвиток ефективних адаптаційних реакцій в організмі коней. В динаміці тренування спостерігалось також істотне підвищення активності супероксиддисмутази та глутатіонредуктази. Кореляційний аналіз залежності між маркерами оксидативного стресу та складовими системи антиоксидатного захисту виявив важливу роль ензимів глутатіонової ланки та церулоплазміну, які попереджують розвиток вільнорадикальних реакцій під час фізичних навантажень у спортивних коней. Осмотична резистентність еритроцитів спортивних коней після тренування істотно знижувалася. Фізичні навантаження спричинюють підвищення відсотку гемолізованих еритроцитів та зниження їх осмотичної резистентності у спортивних коней. Рівень маркерів оксидативного стресу,активність ензимів антиоксидантного захисту та резистентність еритроцитів можуть бути інформативними показниками для оцінки рівня тренованості коней спортивного напрямку роботоздатності.

Ключові слова: КОНІ, УКРАЇНСЬКА ВЕРХОВА ПОРОДА, ТРЕНІНГ, ОКСИДАТИВНИЙ СТРЕС, АНТИОКСИДАНТНИЙ ЗАХИСТ, ОСМОТИЧНА РЕЗИСТЕНТНІСТЬ ЕРИТРОЦИТІВ

ИЗМЕНЕНИЯ МАРКЕРОВ ОКИСЛИТЕЛЬНОГО СТРЕССА И ОСМОТИЧЕСКОЙ РЕЗИСТЕНТНОСТИ ЭРИТРОЦИТОВ У СПОРТИВНЫХ ЛОШАДЕЙ УКРАИНСКОЙ ВЕРХОВОЙ ПОРОДЫ В ДИНАМИКЕ ТРЕНИНГА

А. В. Андрийчук[1], Г. М.Ткаченко[2], Н. Н. Кургалюк[2], И. В. Ткачова[1]

[1]Институт животноводства Национальной Академии Аграрных Наук Украины, г. Харьков 62404, Харьковская обл., Харьковский р-н, пгт. Кулиничи, ул. 7-ой Гвардейской армии, 3, anastasia.pohlyad@gmail.com

[2]Department of Animal Physiology, Department of Zoology, Institute of Biology and Environmental Protection, Pomeranian University, Arciszewski Str., 22b, 76-200 Slupsk, Poland, tkachenko@apsl.edu.pl, kurhaluk@apsl.edu.pl, biology.apsl@gmail.com

Исследование особенностей метаболических изменений в организме лошадей, предназначенных для использования в классических видах конного спорта (конкур, выездка, троеборье) является важной предпосылкой в анализе их адаптационных возможностей и уровня тренированности. Цель данных исследований заключалась в определении содержания маркеров оксилительного стресса (ТБК-активных продуктов, производных окислительной модификации белков), активности ферментов антиоксидантной защиты (супероксиддисмутазы, каталазы, глутатионредуктазы, глутатионпероксидазы), содержание церулоплазмина, как компонента системы антиоксидантной защиты и осмотической резистентности эритроцитов у спортивных лошадей украинской верховой породы в динамике физических нагрузок (в состоянии покоя и после тренировки). Исследуемые лошади (9 кобыл и 14 жеребцов), пребывали в систематическом спортивном тренинге и активно использовались в классических видах конного спорта. После физических нагрузок, в

эритроцитах нами выявлено существенное снижение уровня маркеров липопероксидации и несущественное уменьшение содержания продуктов окислительной модификации белков, что свидетельствует о развитии эффективных адаптационных реакций в организме лошадей. В динамике тренинга наблюдалось также существенное повышение активности супероксиддисмутазы и глутатионредуктазы. Корреляционный анализ зависимости между маркерами окислительного стресса и составляющими системы антиоксидатной защиты определил важную роль ферментов глутатионового звена и церулоплазмина, которые предупреждают развитие свободнорадикальных реакций у спортивных лошадей во время физических нагрузок. Осмотическая резистентность эритроцитов спортивных лошадей после тренировки существенно снижалась. Физические нагрузки вызывают повышение процента гемолизированных эритроцитов и снижение их осмотической резистентности. Уровень маркеров окислительного стресса, активность ферментов антиоксидантной защитыи резистентность эритроцитов могут быть информативными маркерами для оценки уровня тренированности лошадей спортивного направления.

Ключевые слова: ЛОШАДИ, УКРАИНСКАЯ ВЕРХОВАЯ ПОРОДА, ТРЕНИНГ, ОКИСЛИТЕЛЬНЫЙ СТРЕСС, АНТИОКСИДАНТНАЯ ЗАЩИТА, ОСМОТИЧЕСКАЯ РЕЗИСТЕНТНОСТЬ ЭРИТРОЦИТОВ

Training is an effort promoting many metabolic and hormonal changes that also may affect the normal function of the organism. The pattern of changes in the physiological system depends on intensity, duration and frequency of exercises but may also vary among breeds and genders [1–5]. A number of studies have reported that exhaustive endurance training induces accelerated generation of reactive oxygen species (ROS). ROS result in lipid peroxidation (LPO), which induces adverse effects on the health status and performance of horses [6, 7]. After intense exercise in horses oxidative stress can occur and lead to cellular and muscular damage [8, 9]. The elevated metabolic rate associated with physical exercises can increase mitochondrial O_2 consumption in muscle tissue and, consequently, mitochondrial ROS generation. There are numerous reports that provide reasonable support to the notion that exercise increases the ROS production and that mitochondria are important sources of these oxidants [10]. Other sources of oxidative stress during physical exercise are inflammatory responses mediated by neutrophils [11], the interaction of metmyoglobin and methemoglobin with lipid peroxides [12], and the activity of xanthine oxidase [13], possibly within an ischemia-reperfusion model [14]. Oxygen- and nitrogen-derived free radicals are generated during cellular metabolism and mitochondrial energy production and are involved in oxidative damage to cell components, regulation of signal transduction and gene expression, and activation of receptors and nuclear transcription factors [15].

Conversely, cells can tolerate mild oxidative stress and often respond by raising their levels of antioxidant defenses. Intense or moderate exercise in horses may increase ROS production exceeding the capacity of antioxidant defences [7–9, 16]. However, severe oxidative stress and free radicals produce serious disturbances in cellular structure and metabolism [17]. The cell conserves highly specific (e.g., superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase) and less specific (e.g., vitamins C and E, glutathione) antioxidant mechanisms to counteract the effects of free radicals and/or oxidants [10].

Numerous studies have shown that exercise-induced oxidant/antioxidant changes in trained horses vary with regard to exercise type (race, standardized treadmill exercise, standardized race track exercise, endurance) and the markers assessed in blood, although it is generally agreed that exercise does induce significant alterations of the circulatory oxidant-antioxidant balance [7]. However, some controversy exists in terms of poorly

reproducible and even contradictory results that suggest that experimental design, the horses' fitness, the analytical approach and environmental factors strongly influence the study results [7].

Despite the increased ROS production during exercise, growing evidence derived from several studies strongly indicates that habitual, moderate physical activity reduces the incidence of oxidative stress-based diseases and retard the aging process [18]. This apparent paradox can be explained taking into account that ROS produced during repeated exercise bouts could serve as mild stimulating stressors able to adaptation to exercises [10]. Regular physical exercise has well-documented health benefits and can prolong mean life span in animals. Adaptive mechanisms seem to decrease oxidative stress and they encompass increased antioxidant defenses, reduced basal production of oxidants, and reduction of radical leak during oxidative phosphorylation [19]. Moderate exercise significantly decreases the age-associated development of oxidative stress in mice, increases life span, prevents decay of mitochondrial function, and even improves behavioral performance [20].

Thus, the aim of this study was to investigate the influence of regular exercises on oxidative stress biomarkers, antioxidant enzymes activity, as well as resistance of erythrocytes to hemolytic agent in well-trained Ukrainian warmblood horses before and after exercises

Materials and methods

Horses and training. A total of twenty three Ukrainian Warmblood sport horses in regular training were used in this study. Nine well trained mares (aged 7.11±2.2; body weight 501.38±40 kg; mean withers height 167.66±4.8 cm; mean girth measurement 190.77±5.1 cm; mean length measurement 163.66 ±6.3 cm; mean body conditional score 3.5) and fourteen stallions (aged 7.21±1.5; body weight 501.10±42.8 kg; mean withers height 166.86±4.57 cm; mean girth measurement 190.5±6.6 cm; mean length measurement 164±4.3 cm; mean body conditional score 3.5) involved in regular training. All horses had the same diet. These diets were composed of grass hay (6 kg), oats (6 kg) and 2 kg wheat bran, distributed in three times per day. The diet (hay and oat) that maintain the recommendation for exercising horses (5.9 % digestible protein on average, calcium=0.20 %, phosphorus=0.15 %, vitamin A=12.5 UI). Salt and water were available *ad libitum*.

Fitness training and general animal care were carried out by professional staff not associated with the research team. All horses were subjected to the same type of management: in stables with individual boxes, natural indoor temperature (17 °C–19 °C), and the same feeding schedule. The horses were involved in jumping (n=8), eventing (n=10) and dressage (n=5). All horses had been in regular training for several years. All horses were thoroughly examined clinically and screened for hematological, biochemical and vital parameters, which were within reference ranges. All the horses were dewormed and vaccinated at similar time and had the same vaccine status with no signs of clinical disease and with the females non-pregnant.

Since all horses were used in various kinds of equestrian sport, it was proposed common to all horses exercises average intensity: walk — 5 min (average speed 70 m/min); trot — 10 min (average speed 120 m/min); walk — 10 min; trot — 10 min; walk — 5 min; gallop — 10 min (average speed 300 m/min); walk — 10 min. Duration of training was 1 hours. Animal care and experimental procedure were in accordance with the Guide for the Care and Use of Laboratory Animals. Blood samples were collected from the jugular vein of each horse into ethylenediaminetetraacetic acid (EDTA) tubes at two points: baseline at rest (in the morning 90 minutes after feeding) and immediately after exercises.

Blood samples. Blood samples were obtained by jugular venipuncture, in the morning, 90 minutes after feeding while horses were in stalls, and immediately after

training session when horses came back to stalls from the horse walker. Samples were aspirated into 10 ml syringe and immediately transferred into sterile EDTA tubes. Samples were kept in a chilled cooler before transport to the laboratory. The plasma separation was performed immediately after blood collection.. For isolation of erythrocytes blood samples were centrifuged at 3,000 g for 10 min. The erythrocytes were washed three times with five volumes of saline solution and centrifuged at 3,000 for 10 min. After centrifugation, plasma samples were transferred to other tubes, frozen at -20 °C and stored until analysis.

Biomarker analysis. 2-Thiobarbituric acid (TBA), oxidized and reduced glutathione (GSSG and GSH), NADPH, and 5,5-dithiobis-2-nitrobenzoic acid (DTNB), ethylenediaminetetraacetic acid (EDTA), thrichloroacetic acid (TCA), quercetin, tetramethylenediamine (TEMED), hydrogen peroxide, ammonium molybdate, sodium aside, t-butylhydroperoxide, Tween 80, urea acid, 2,4-dinitrophenyl hydrazine (DNFH) were obtained from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade. All enzymatic assays were carried out at 25±0.5 °C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany). The enzymatic reactions were started by adding the blood, erythrocytes suspension or plasma samples. The specific assay conditions are presented subsequently. Each sample was analyzed in duplicate.

Thiobarbituric acid reactive substrates (TBARS) assay. The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malondialdehyde (MDA) concentration [21]. This method is based on the reaction of the degradation of lipid peroxidation product, MDA, with TBA under conditions of high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. Briefly, 0.1 mL of sample (blood, plasma, and erythrocytes' suspension) was added to 2 mL of distilled water, 1 mL of TCA and 1 mL of TBA. The mixture was heated in a boiling water bath for 10 minutes. After cooling, the mixture was centrifuged at 3,000 g for 10 minutes. The μmol of MDA per l L was calculated by using $1.56 \cdot 10^5$ mM^{-1} cm^{-1} as extinction coefficient.

The carbonyl derivatives content of protein oxidative modification (OMP) assay. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with DNFH as described by Levine et al. (1990) [22] and as modified by Dubinina et al. (1995) [23]. DNPH was used for determining carbonyl content in soluble and insoluble proteins. Briefly, 1 mL 0.1M DNPH (dissolved in 2M HCl) was added to 0.1 mL of the sample (plasma and erythrocytes' suspension) after denaturation of proteins by 20 % TCA. After addition of the DNPH solution (or 2M HCl to the blanks), the tubes were incubated for a period of 1 h at 37 ^oC. The tubes were spun in a centrifuge for 20 min at 3,000g. After centrifugation, the supernatant was decanted and 1 mL of ethanol-ethylacetate solution was added to each tube. Following mechanical disruption of the pellet the tubes were allowed to stand for 10 min and then spun again (20 min at 3,000 g). The supernatant was decanted and the pellet washed thrice with ethanol-ethylacetate. After the final wash, the protein was solubilized in 2.5 mL of 8M urea solution. To speed up the solubilization process, the samples were incubated in at 90 ^oC water bath for 10–15 min. The final solution was centrifuged to remove any insoluble material. The carbonyl content was calculated from the absorbance measurement at 370 nm and 430 nm, and an absorption coefficient 22,000 $M^{-1} \cdot cm^{-1}$. Carbonyl groups were determined spectrofotometrically from the difference in absorbance at 370 nm (aldehyde derivates, OMP_{370}) and 430 nm (ketonic derivates, OMP_{430}).

Superoxide dismutase activity assay. Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was assessed by its ability to dismutate superoxide produced during quercetin auto-oxidation in an alkaline

medium (pH10.0) by Kostiuk et al. (1990) method [24]. Briefly, 1.0 mL of C reagent was mixed with 0.1 mL of blood sample (1:1000). C reagent was made *ex tempore* (mixture of equal volumes of 0.1M K,Na-phosphate buffer, pH7.8 and 0.08M EDTA); pH of C reagent was adjusted to 10.0 by adding TEMED. Distilled water (0.1 mL) was added to blank vials instead of blood sample. The total volume of all samples was brought up to 2.4 mL using distilled water. The reaction was initiated by adding 0.1 mL of quercetin (1.4 μM dissolved in dimethyl sulphoxide). Absorbance at 406 nm was measured immediately and after 20 min addition of quercetin solution. Activity is expressed in units of SOD per mL of blood.

Catalase activity assay. Catalase (CAT, E.C. 1.11.1.6) activity was determined by measuring the decrease of H_2O_2 in the reaction mixture using a spectrophotometer at the wavelength of 410 nm by the method of Koroliuk et al. (1988) [25]. The reaction was initialized by adding 0.1 mL of plasma into the incubation medium (2 mL of 0.03 % H_2O_2 solution). The duration of this reaction was 10 min at room temperature. The reaction was terminated by rapid adding of 1.0 mL of 4 % ammonium molybdate dissolved in 12.5 mM H_2SO_4 solution and 1 mL of 125 mM H_2SO_4. Blank assay instead of plasma sample included 0.1 mL of distilled water. All samples were centrifuged at 3,000 g for 5 min. The absorbance of the obtained solution was measured at 410 nm and compared with that of the blank. One unit of catalase activity is defined as the amount of enzyme required for decomposition of 1 μmol H_2O_2 per min per L of blood.

Glutathione reductase activity assay. Glutathione reductase (GR, E.C. 1.6.4.2) activity in the blood was measured according to the method described by Glatzle et al. (1974) [26]. The enzyme assay mixture contained 2.4 mL of 67 mM sodium phosphate buffer (pH 6.6), 0.2 mL of 7.5 mM oxidized glutathione, and 0.1 mL of blood sample (dilution in water 1:20). The rate of NADPH oxidation was followed spectrophotometrically at 340 nm. A control without NADPH was used. The GR activity was expressed as nmol NADPH per min per mL of blood.

Glutathione peroxidase activity assay. Glutathione peroxidase (GPx, E.C. 1.11.1.9) activity in the blood was measured spectrophotometrically as described by Moin (1986) [27]. The assay mixture contained 0.8 mL of 0.1M Tris-HCl buffer with 6 mM EDTA and 12 mM sodium aside (pH 8.9), 0.1 mL of 4.8 mM GSH, 0.2 mL of blood sample (dilution in water 1:20), 1 mL of 20 mM t-butylhydroperoxide, and 0.1 mL of 0.01M DTNB. The rate of GSH reduction was followed spectrophotometrically at 412 nm. GPx activity is expressed as μmol GSH per min per mL of blood.

Ceruloplasmin level assay. The ceruloplasmin (CP, EC 1.16.3.1) level in the plasma was measured spectrophotometrically at 540 nm, as described by Ravin (1961) [28]. The assay mixture contained 0.1 mL of plasma, 0.4M sodium acetate buffer (pH 5.5), and 0.5 % *p*-phenylenediamine. The mixture was incubated at 37 ^oC for 60 min. Before cooling at 4 ^oC for 30 min, the mixture was added to 3 % sodium fluoride for inhibition. Ceruloplasmin was expressed in milligrams per liter of plasma.

Total antioxidant capacity assay. The TAC level was estimated spectrophotometrically at 532 nm following the method with Tween 80 oxidation to MDA, as described by Galaktionova et al. (1998) [29]. Briefly, 0.2 mL of sample (plasma, erythrocytes' suspension) was added to 2 mL of 1 % Tween 80. Blank assay instead of sample included 0.2 mL of distilled water. The mixture was heated at 37 ^oC for 48 hours. After cooling, 1 mL of 40 % TCA was added and the mixture was centrifuged at 3,000 g for 10 min. After centrifugation, 2 mL of supernatant and 2 mL of 0.25 % TBA reagent was mixed. The mixture was heated in boiling water bath at 95 ^oC for 15 minutes. The absorbance of the obtained solution was measured at 532 nm and was compared with that of the blank. TAC was expressed in %.

The osmotic resistance of erythrocytes assay. The osmotic resistance

of erythrocytes was measured spectrophotometrically at the wavelength of 540 nm as described by Mariańska et al. (2003) [30]. The method is based on the determination of differences between osmotic resistance of erythrocytes to a mixture containing different concentration of sodium chloride (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 %). Absorbance of mixture contained erythrocytes and distilled water was determined as 100 % (standard). The degree of hemolysis in every test tube (%) was calculated in respect to the absorbance of standard. Hemolysis of erythrocytes (%) in every test tube with different sodium chloride concentration was expressed as curve [30].

Statistical analysis. Results are expressed as mean ± S.E.M. All variables were tested for normal distribution using the Kolmogorov-Smirnov test ($p>0.05$). In order to find significant differences (significance level, $p<0.05$) between states at the rest and after training, Wilcoxon signed-rank test was applied to the data [31]. All statistical analyses were performed using STATISTICA 8.0 software (StatSoft, Poland). In addition, the relationships between oxidative stress biomarkers level and enzymes activities of all individuals were evaluated using Spearman's correlation analysis [31].

Results and discussion

Lipid peroxidation is a complex phenomenon involving the generation of many products. However, the content of MDA, one of most important end-products of lipid peroxidation, in the tissues is usually accepted as an index of lipid peroxidation intensity [6].

The lipid peroxide level induced by the training session was measured through analysis of the TBARS level and shown in figure 1. TBARS level in the erythrocyte suspension of horses showed a significantly decrease by 14.3 % ($p<0.05$) immediately after exercise as compared the before exercises. TBARS level in the blood of well-trained horses after exercise was not-significantly decreased by 12.2 % ($p>0.05$). There were no significant differences in plasma TBARS level between before and after exercises (fig. 1).

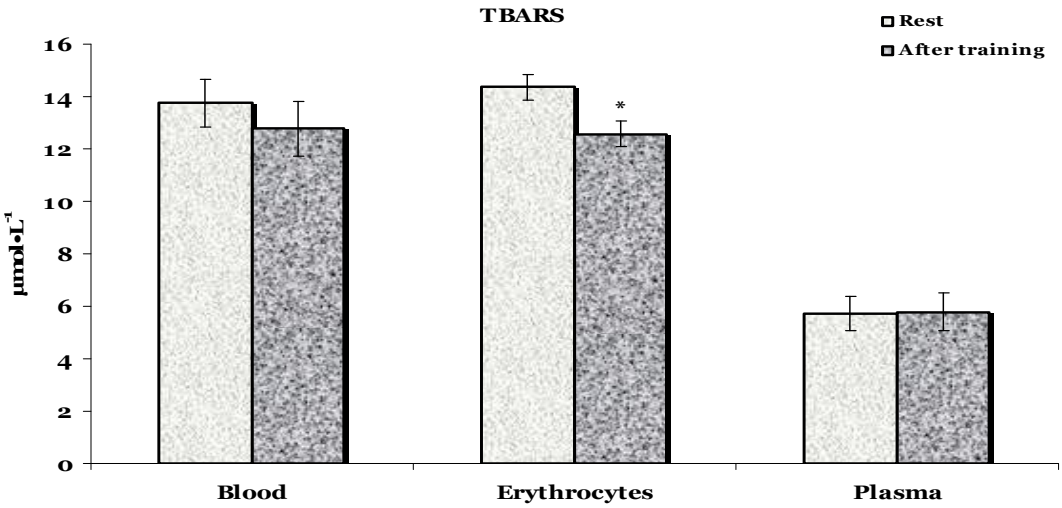

Fig. 1. The level of lipid peroxidation determined by quantifying the concentration of 2-thiobarbituric acid reactive substrates (TBARS) (µmol MDA·L^{-1}) in the blood, erythrocytes and plasma of horses of Ukrainian warmblood breed at the rest and after training session. Values expressed as mean ± S.E.M.
Note: * — the significant change was shown as $p<0.05$ when compared between values at the rest and after training (Wilcoxon signed-rank test)

ROS induced oxidation of arginine, lysine, threonine, or proline amino acid residues generates reactive carbonyl derivatives (RCD), which can be readily

measured by reaction with 2,4-dinitrophenyl hydrazine [32]. Protein carbonyls derivates are used very often as a marker of oxidative modification of proteins [18, 20, 32]. The increase in exercise mediated accumulation of oxidative protein damage in plasma could be due to the increased ROS production and/or the poor proteolytic breakdown of oxidatively modified proteins. Despite this, in general, oxidative modifications of proteins activate the proteolitic system.

The effects of training session on protein carbonyl contents in the erythrocyte suspension and plasma samples from horses are shown in figure 2. There were no statistically significant changes in the derivates of protein destruction level in the erythrocytes and plasma of trained horses during exercise. At the same time, after exercise, horses had non-significantly lower aldehyde (by 42 %, $p>0.05$) and ketonic derivates level (by 27 %, $p>0.05$) (fig. 2).

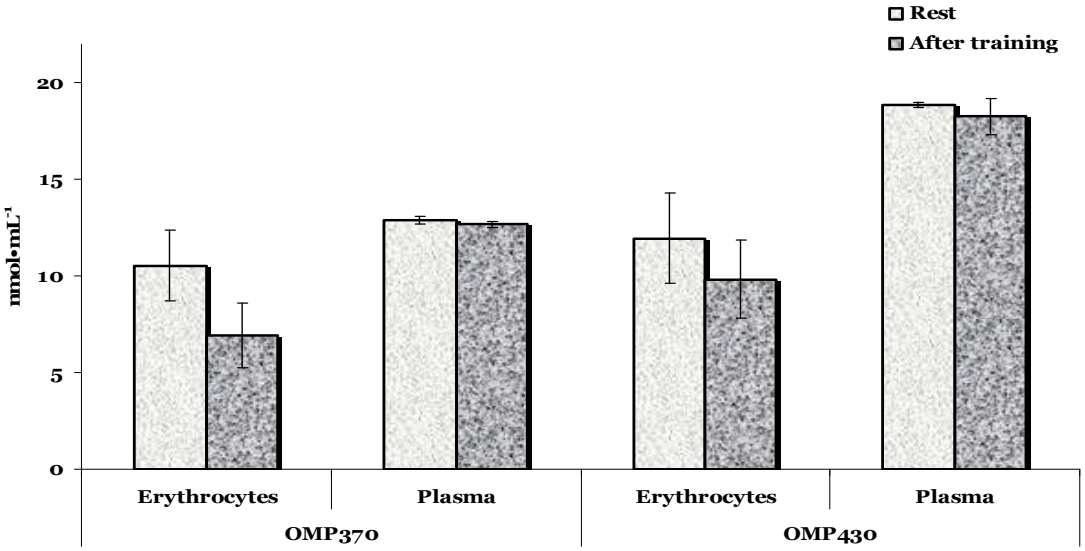

Fig. 2. Oxidatively modified proteins (OMP) content, measured by quantity of carbonyl oxidation (nmol·mL^{-1}) in the erythrocytes and plasma of horses of Ukrainian warmblood breed at the rest and after training session. Carbonyl groups were determined spectrofotometrically from the difference in absorbance at 370 nm (aldehyde derivates, OMP_{370}) and 430 nm (ketonic derivates, OMP_{430}). Values expressed as mean ± S.E.M

Antioxidant defenses in the blood of well-trained horses before and after exercise are shown in table 1. There were no statistically significant changes in the activities of antioxidant defenses instead SOD and GR activity after exercise (Table 1). SOD activity was higher by 34.4 % ($p<0.05$) in blood of horses after exercise compared to before exercise. After exercise, GR activity was higher by 60.4 % ($p<0.05$) compared to the resting period.

Table 1

Antioxidant enzymes activities in the blood of horses of Ukrainian warmblood breed in the rest and after training

Antioxidant defense parameters	At the rest	After training session
SOD, U·mL^{-1}	11.80±0.52	15.86±1.21*
CAT, μmol·min^{-1}·L^{-1}	2.77±0.29	3.06±0.40
GR, nmol·min^{-1}·mL^{-1}	0.96±0.12	1.54±0.23*
GPx, μmol·min^{-1}·mL^{-1}	0.92±0.16	1.37±0.28
CP, mg·L^{-1}	30.19±4.42	35.41±5.86

Note: * — the significant change was shown as $p<0.05$ when compared between values at the rest and after training (Wilcoxon signed-rank test)

The total antioxidant capacity (TAC) in erythrocyte suspension and plasma is shown in figure 3. No significant variations in TAC of horses during exercise were found.

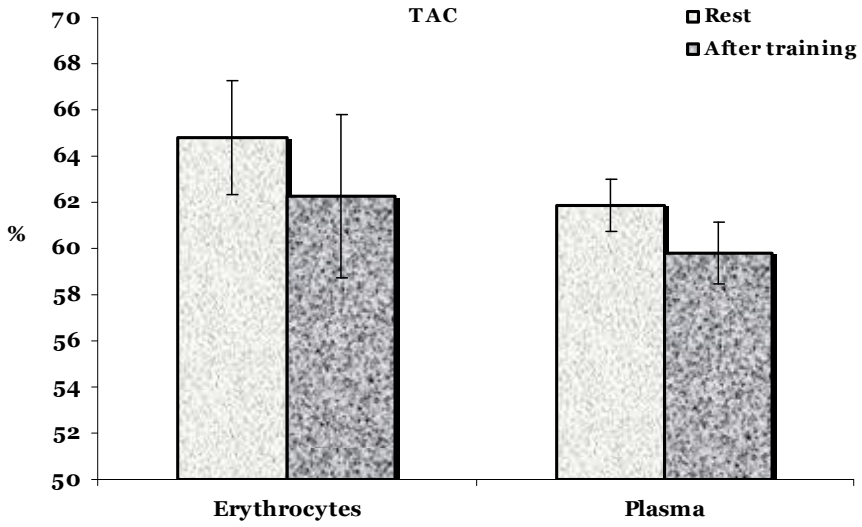

Fig. 3. Total antioxidative capacity (%) in the erythrocytes and plasma of horses of Ukrainian warmblood breed at the rest and after training session. Data are means ± S.E.M.

Correlative analysis of oxidative stress biomarkers and antioxidant parameters in the blood of horses at the rest and after exercise are presented in Table 2. There was a negative correlation between erythrocytes' TBARS level and CP content (r=-0.518, p=0.040), as well as plasma TAC and GPx activity (r=-656, p=0.006) in the blood of horses before exercise (tabl. 2). At the same time, erythrocytes' TBARS level correlated negatively with aldehyde derivates of protein oxidation in plasma (r=-0.761, p=0.001). Blood TBARS level correlated positively with aldehyde derivates of protein oxidation in erythrocytes' suspension (r=0.602, p=0.014). Significant correlation was noted between GR activity and TAC (r=-0.534, p=0.033) as well as plasma TBARS level (r=-0.523, p=0.038).

Table 2

Correlative analysis of oxidative stress biomarkers in the blood of horses of Ukrainian warmblood breed at the rest and after training session

Relation	Correlative coefficients, r	Significant difference level, p
	At the rest	
TBARS(blood)–OMP_{370}(erythrocytes)	0.576	0.020
TBARS(erythrocytes)–CP	-0.518	0.040
OMP_{370}(plasma)–CP	0.543	0.030
TAC(plasma)–GPx	-0.656	0.006
	After training session	
TBARS(blood)–OMP_{370}(erythrocytes)	0.602	0.014
TBARS(erythrocytes)–OMP_{370}(plasma)	-0.761	0.001
TBARS(plasma)–GR	-0.523	0.038
TAC(plasma)–CP	-0.705	0.002
TAC(erythrocytes)–GR	-0.534	0.033

Erythrocytes are the best indicators of increased generation of ROS and lipid peroxidation. Therefore, the next goal of our study was the measurement of erythrocytes' resistance to hemolytic reagent (solutions with different concentration of sodium chloride) in the blood of horses before and after exercise (fig. 4). Osmotic resistance of

erythrocytes (fig. 4) was significantly lower after exercise compared to before exercise (incubation with 0.1–0.7 % NaCl). Exercise caused increasing the percentage of hemolyzed erythrocytes and reducing their resistance.

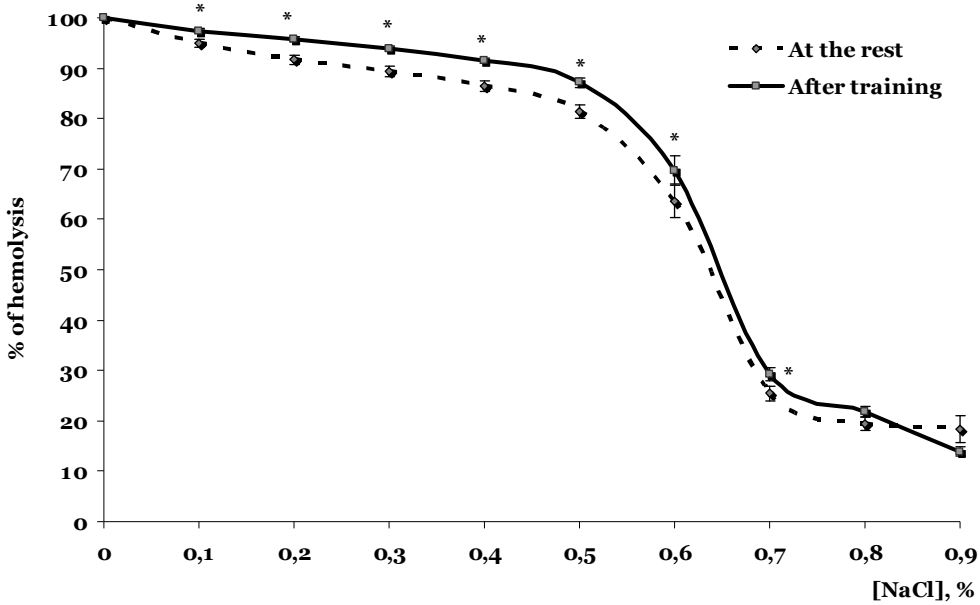

Fig. 4. Osmotic resistance of erythrocytes (% of hemolysed erythrocytes) in the blood of horses of Ukrainian warmblood breeds at the rest and after training session. Data are means ± S.E.M.
Note: * — the significant change was shown as $p<0.05$ when compared between values at the rest and after training (Wilcoxon signed-rank test)

Accumulating evidence suggests an association between oxidative stress and strenuous physical exercise [6, 7, 15] and the beneficial effects of chronic exercise training in physical condition [33, 34]. In this study we have investigated the direct link between oxidative stress biomarkers, antioxidant defenses and the beneficial role of moderate exercise training in well-trained horses. In this study we have investigated the direct link between oxidative stress biomarkers, antioxidant defenses and the beneficial role of moderate exercise training in well-trained horses. Our results suggest that exercises cause different consequences in oxidative stress biomarkers in the blood, plasma, and erythrocytes of horses. Exercise lead to decrease TBARS level in erythrocytes while in blood and plasma does not. This difference in TBARS level before and after exercise, most likely is a consequence of differing levels of oxidative stress occurring in the tissues and erythrocytes.

In our study, the highest level of oxidative stress biomarkers (TBARS, carbonyl derivates of oxidative modified proteins) was noted in erythrocytes (figs 1 and 2). Earlier study suggest that erythrocytes are susceptible to oxidative damage as a result of the high polyunsaturated fatty acid content of their membrane and the high cellular concentrations of oxygen and hemoglobin are potentially powerful promoter to oxidative processes [35]. The increase in cellular metabolism, especially during strenuous exercise, increases hemoglobin turnover and hemoglobin autoxidation that can result in an increased carbonyl content of the globin moiety. Erythrocytes may accumulate such molecular modifications and modifications of the membrane components because of their limited biosynthetic capacity and poor repair mechanisms whenever they are exposed to unusual oxidative stress [36].

In general, our results are in agreement with previous studies that have suggested a correlation of oxidative stress limiting with

exercise-induced adaptation. Exercise as a result of adaptive responses eventually lead to improvements in sports performance, but also can induce physiologic effects most likely by increase the antioxidant homeostasis and ability for adaption. Training-related oxidant stress, is associated with adaptations that improve the ability of the muscle cells to quench ROS [19]. It has been suggested that acute and irregular exercises have negative effects, whereas regular physical activity creates an advanced antioxidant system and decreases oxidative damage that trained individuals have a higher antioxidant capacity and lower levels of oxidative damage than sedentary individuals in resting conditions [37, 38]. Moreover, sedentary individuals have higher levels of oxidative damage caused by acute exhaustive exercise than athletes [39, 40]. No increase was observed in lipid peroxides after maximal bicycle exercise in either trained or untrained human subjects [41]. While the concentration of erythrocytes' TBARS increased in untrained but not trained rats after exhaustive exercise [42]. Endurance training of mice reduced the susceptibility of mouse skeletal muscle to lipid peroxidation *in vitro* [43]. In the present study the decrease in erythrocytes' TBARS level in horses after exercise could be attributed as adaptation to training which accompanied activation the antioxidant defenses and changes in antioxidant enzyme activity in erythrocytes (Table 1).

The exercise-induced adaptation involves the process of activation of the antioxidant system, interferes with the oxidative damage repair/eliminating systems, and influences redox-sensitive transcription, hence the gene expression and protein assembly. The stimulating effect during exercise is ROS formation, which evokes specific adaptation, such as increased antioxidant/oxidative damage-repairing enzyme activity, increased resistance to oxidative stress, and lower levels of oxidative damage [16, 34].

LPO has been established as a major pathogenetic mechanism of cellular injury in humans [17]. Although LPO is causally linked to structural and functional disturbance of biomembranes and has been implicated in numerous disorders and diseases, recent in vitro studies suggested a potential role of lipid peroxidation products as regulators and modulators of cellular signaling and gene expression [34]. Nagy et al. (1998) demonstrated that oxidized lipids can interact with receptors for peroxisomal proliferators, known activators of antioxidant enzymes, such as CAT and SOD [44]. Moreover, pretreatment of cells with various LPO products at sublethal level improves cellular tolerance against forthcoming pro-oxidant attacks [45].

Exercise training tends to increase the activity of the proteasome complex which involved the removal of oxidatively modified proteins is efficient in the muscle of old trained rats [18]. This is an important adaptive response because the induction of a repair mechanism – proteasome complex is regarded as a repair enzyme. This could be due with decrease in the accumulation of potentially harmful post-translationally modified proteins. Exercise can induce the activity of the proteasome complex, which is significantly involved in the degradation of oxidatively modified proteins [20]. Increased activity of proteasome could be an important factor that affects the rate of protein turnover and the remodeling of skeletal muscle after injury [20]. An increased rate of protein turnover with exercise training decreases the accumulation of oxidative damage, hence beneficially affecting the physiological function of proteins. The proteasome complex plays a critical role in this process [20]. Thus, in our study, decrease of carbonyl derivatives in erythrocytes' suspension is result of exercise-induced adaptation.

As the second part of this study, we investigated the effect of moderate exercise on antioxidant defenses of horses. Our interest in this model of equine training is based upon several recent reports that have suggested that moderate exercise increases antioxidant enzyme activity and attenuates oxidative stress [8, 9, 46]. The major antioxidant enzymes include SOD, GPx, GR, CAT [16].

SOD acts on superoxide radicals to form oxygen and the lesser reactive nonradical species, hydrogen peroxide, while GR can regenerate oxidized glutathione (GSSG, glutathione disulfide) to reduced glutathione (GSH). CAT is located mainly in peroxisomes and mitochondria and also removes H_2O_2. CAT requires iron as a cofactor, and similar to GPx and SOD, its activity is highest in highly oxidative muscle fibers [16].

The enhancement SOD activity in our study suggest that its activation probably is results of exercise-linked mitochondrial $O^{2-\bullet}$ production. As SOD provides the first line of defense against produced mitochondrial superoxide radicals, the increase in SOD activity shown in well-trained horses could reduce the exposure to superoxide and even to the hydroxyl radicals formed via the Haber–Weiss reaction [47]. It has been shown that training causes the increased SOD activity in the muscle and erythrocytes of students, swimmers, cyclists, and volleyball players [16]. A large number of studies have shown increases SOD activity following training, but a considerable proportion also show no change, with a small number also showing a decrease. In relation to acute exercise, erythrocyte SOD and GPx activities were not changed in pentathlon horses following show jumping [16]. Increase in different antioxidants activity, such as, GPx and SOD, has been observed in Standardbreds after a 12-week period of aerobic and anaerobic training, while the increase in oxygen consumption (measured as change of VO_2 max) was positively correlated with the increase of erythrocyte SOD activity [7].

GR is not considered as a primary detoxificant of ROS; however GR recycles oxidized glutathione to its reduced form and has a central role in the glutathione dependent antioxidant protection [8, 9]. Our results are consistent with the previous findings where endurance training resulted in increase in GR activity [8, 9, 46]. In addition, Tauler et al. (1999) suggested that ROS could preferentially activate GR because of the presence of more thiol groups in GR's structure [48]. Correlative analysis between GR activity and oxidative stress biomarkers (TBARS and protein derivates) suggest that increased GR activity plays a central role in the glutathione-dependent antioxidant protection in the blood of horses after exercise (Table 2).

Antioxidant activity of the serum of humans and animals attributed to the presence of transferrin and ceruloplasmin (CP) [49]. Ceruloplasmin is a copper-containing glucoprotein with multiple physiological functions, including ferroxidase and oxidase activity [50]. It has been suggested that CP might be the major antioxidant in plasma as a scavenger of oxygen radicals. CP being an acute phase reactant protein, its level rise immediately after cellular damage. It acts as an antioxidant through ferroxidase activity, and it also scavenges superoxide anion. Physiological factors like cancer, exercise, chronic inflammation, pregnancy increases CP level [50]. It can be hypothesized that non-significant increase of CP content in horses after exercise is a manifestation of the compensatory and adaptive reaction of the organism to physical activity (Table 1). Moreover, correlative analysis between CP content and oxidative stress biomarkers (TBARS and protein derivates) suggest that CP also plays a main role in antioxidant defenses in the blood of horses both before and after exercise. Antioxidant enzymes may be activated during strenuous exercise depending on the oxidative stress imposed on the specific tissues as well as the intrinsic antioxidant defense capacity. In our study, training session caused non-significant decrease of TAC (Fig. 3).

In our study we also observed in erythrocytes subjected to the different concentration of sodium chloride had a higher level of haemolysis after exercise. (Fig. 4). After exercise, the osmotic resistance of erythrocytes was decreased; the difference was statistically significant at the presence of 0.1–0.7 % NaCl. There is increasing evidence to suggest that ROS production may contribute to exercise-induced damage to the erythrocyte membrane with consequent modification of membrane fluidity [36]. It

also has been suggested that modifications of cell membranes caused by lipid peroxidation following ROS overproduction may be one of the causes of exercise-induced myopathies and haemolysis in horses [35].

Conclusions

The results of present study showed an increase antioxidant enzyme activity (SOD and GR) and reducing of exercise-induced oxidative stress in the blood of horses after exercise. It has been observed a decrease of lipid peroxidation level in erythrocytes of well-trained horses after exercise, while it did not change both in the plasma and blood. This difference in TBARS level between before and after training most likely is a consequence of differing levels of oxidative stress occurring in the tissues and erythrocytes. Decrease of carbonyl derivatives in erythrocytes' suspension is result of exercise-induced adaptation. A correlation between the oxidative stress biomarkers and antioxidant defenses in the horses after exercise was observed, which may indicate a protective response of GR, GPx and CP activities against exercise-induced oxidative stress. Statistically significant differences in the percentage of haemolysed erythrocytes before and after training also were observed.

Prospects for further research. Studies of the course of metabolic processes related to the functioning of antioxidant defense and intensity of lipid peroxidation and oxidative modification of proteins in sport horses is important. Because it allows to study the adaptation processes to physical activities of various duration and intensity, to evaluate of horses fitness level and identify factors that limit their workability. Further research pro-and antioxidant balance equine athletes will allow improved the scientific substantiation of adaptation to physical activity and the develop of effective correctional training programs of sport horses.

Acknowledgments

This study was carried out during Scholarship Program of Anastasiia Andriichuk supported by The The Polish National Commission for UNESCO in the Department of Animal Physiology, Institute of Biology and Environmental Protection, Pomeranian University (Slupsk, Poland). We thank to The Polish National Commission for UNESCO for the support of our study.

1. Freestone J. F., Wolfsheimer K. J., Kamerling S. G., Church G., Hamra J., Bagwell C. Exercise induced hormonal and metabolic changes in Thoroughbred horses: effects of conditioning and acepromazine. *Equine Veterinary Journal*, 1991, 23 (3), pp. 219–223.

2. Alexander S., Irvine C. H. G. Stress in the race horse: coping vs. not coping. *Journal of Equine Veterinery Science,* 1998, 9 (3), pp. 77–81.

3. Marlin D. K., Nankervis K. 2002. *Equine Exercise Physiology.* Iowa State Press, Ames, Iowa, USA, 2002. 296 p.

4. Hinchcliff K. W., Kaneps A. J., Geor R. J *Equine Sports Medicine and Surgery: Basic and clinical sciences of the equine athlete*. Edinburgh: Saunders, 2004. 1364 p.

5. Escribano B. M., Castejón F. M., Santisteban R., Aqüera E. I., Tovar P., Vivo R., Rubio M. D. Gender differences in non-specific immune response to exercise in the lactate threshold: a study in equine athletes. *Research in Veterinary Science*, 2008, 85 (2), pp. 250–256.

6. Chiaradia E., Avellini L., Rueca F., Spaterna A., Porciello F., Antonioni M. T., Gaiti A. Physical exercise oxidative stress and muscle damage in race horses. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology*, 1998, 119 (4), pp. 833–836.

7. Kirschvink N., de Moffarts B., Lekeux B. The oxidant/antioxidant equilibrium in horses. *The Veterinary Journal*, 2008, 177 (2), pp. 178–191.

8. Kinnunen S., Hyyppä S., Lehmuskero A., Oksala N., Mäenpää P., Hänninen O., Atalay M. Oxygen radical absorbance capacity (ORAC) and exercise-induced oxidative stress in trotters. *European Journal of Applied Physiology*, 2005, 95 (5–6), pp. 550–556.

9. Kinnunen S., Atalay M., Hyppä S., Lehmuskero A., Hänninen O., Oksala N. Effects of prolonged exercise on oxidative stress and antioxidant defense in endurance horse. *Journal of Sport Science and Medicine*, 2005, 4, pp. 415–421.

10.Packer L., Cadenas L., Davies K. J. Free radicals and exercise: an introduction. *Free Radical Biology & Medicine*, 2008, 44 (2), pp. 123–125.

11.Jenkins R. R. Free radical chemistry. Relationship to exercise. *Sports Medicine*, 1988, 5 (3), pp.156–170.

12.Cooper C. E., Vollaard N. B., Choueiri T., Wilson M. T. Exercise, free radicals and oxidative stress. *Biochemical Society Transaction*, 2002, 30 (2), pp. 280–285.

13.Heunks L. M., Viña J., van Herwaarden C. L., Folgering H. T., Gimeno A., Dekhuijzen P.N. Xanthine oxidase is involved in exercise-induced oxidative stress in chronic obstructive pulmonary disease. *The American Journal of Physiology*, 1999, 277(6), pp. 1697–1704.

14.Koyama K., Kaya M., Ishigaki T., Tsujita J., Hori S., Seino T., Kasugai A. Role of xanthine oxidase in delayed lipid peroxidation in rat liver induced by acute exhausting exercise. *European Journal of Applied Physiology and Occupation Physiology*, 1999, 80 (1), pp. 28–33.

15.Lamprecht M., Greilberger J., Oettl K., Analytical aspects of oxidatively modified substances in sports and exercises. *Nutrition*, 2004, 20 (7–8), pp. 728–730.

16.Deaton C. M, Marlin D. J. Exercise-associated oxidative stress. *Clinical Techniques of Equine Practice*, 2003, 2 (3), pp. 278–291.

17.Halliwell B, Gutteridge J. *Free Radicals in Biology and Medicine.* Third ed., Oxford University Press, New York, 1999. 704 p.

18.Radák Z., Naito H., Kaneko T., Tahara S., Nakamotoo H., Takahashi H., Cardozo-Pelaez F., Goto S. Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle. *European Journal of Physiology*, 2002, 445 (2), pp. 273–278.

19.Leeuwenburgh C., Heinecke J. Oxidative stress and antioxidants in exercises. *Current Medicinal Chemistry*, 2001, 8 (7), pp. 829–838.

20.Radak Z., Young Chung H., Goto S., Systemic adaptation to oxidation challenge induced by regular exercise. *Free Radical Biology and Medicine*, 2008, 44 (2), pp. 153–159.

21.Kamishnikov V. S. *Spravochnik po kliniko-biohimicheskim isslidovanijam i laboratornoj diagnostike* [Reference book on clinic and biochemical researches and laboratory diagnostics]. Moscow, MedPress-Inform., 2004. 589 p (in Russian).

22.Levine R. L., Garland D., Oliver C. N., Amici A., Climent I., Lenz A. G., Ahn B. W., Shaltiel S., Stadtman E. R. Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymolology*, 1990, 186, pp. 464–478.

23.Dubinina E. E., Burmistrov S. O., Khodov D. A., Porotov I. G. Okislitel'naja modifikacija belkov syvorotki krovi cheloveka. Metody ejo opredelenija [Oxidative modification of human serum proteins. A method of determining it]. *Voprosy medicinskoj himii — Questions of medical chemistry*, 1995, 41 (1), pp. 24–26 (in Russian).

24.Kostiuk V. A. Prostoj i chuvstvitel'nyj metod opredelenija superoksiddismutazy, osnovannyj na reakcii okislenija kvercetina [A simple and sensitive method of determination of superoxide dismutase activity based on the reaction of quercetin oxidation]. *Voprosy medicinskoj himii — Questions of medical chemistry*, 1990, 2, pp. 78–91 (in Russian).

25.Koroliuk M. A., Ivanova L. I., Majorova I. G., Tokarev V. E. Metod opredelenija aktivnosti katalazy [A method of determining catalase activity]. *Laboratornoe Delo — The Laboratory practice*, 1988, 1, pp. 16–19 (in Russian).

26.Glatzle D., Vuilleumier J. P., Weber F., Decker K. Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in human. *Experientia*, 1974, 30 (6), pp. 665–667.

27.Moin V. M. Prostoj i specificheskij metod opredelenija aktivnosti glutationperoksidazy v eritrocitah [A simple and specific method for determining glutathione peroxidase activity in erythrocytes]. *Laboratornoe Delo — The Laboratory practice*, 1986, 12, pp. 724–727 (in Russian).

28.Ravin H. A. An improved colorimetric enzymatic assay of ceruloplasmin. *The Journal of Labaratory and Clinical Medicine*, 1961, 58, pp. 161–168.

29.Galaktinova L. P., Molchanov A. V., El'chaninova S. A., Varshavskij B. Ja. Sostojanie perekisnogo okislenija bol'nyh s jazvennoj bolezn'ju zheludka i dvenadcatiperstnoj kishki [Lipid peroxidation in patients with gastric and duodenal peptic ulcers]. *Klinicheskaja laboratornaja diagnostika — Clinical Laboratory Diagnostics*, 1998, 6, pp. 10–14 (in Russian).

30.Mariańska B., Fabijańska-Mitek J., Windyga J. *Badania laboratoryjne w hematologii: podręcznik dla słuchaczy studiów medycznych* [Laboratory studies in hematology. Textbook for

Medical Students]. Warszawa : Wydawnictwo Lekarskie PZWL, 2003. 296 p. (In Polish).

31.Zar J. H. *Biostatistical Analysis*. Fourth ed. New Jersey: Prentice-Hall Inc., 4th ed., Englewood Cliffs, 1999. 594 p.

32.Radák Z., Sasvári M., Nyakas C., Taylor A. W., Ohno H., Nakamotoo H., Goto S. Regular training modulates the accumulation of reactive carbonyl derivates in mitochondrial and cytosolic fractions of rat skeletal muscle. *Archives of Biochemistry and Biophysics*, 2000, 383 (1), pp. 114–118.

33.Cunningham P., Geary M., Harper R., Pendleton A., Stover S. High intensity sprint training reduces lipid peroxidation in fast-twitch skeletal muscle. *Journal of Exercise Physiology online*, 2005, 8(6), pp.18–25.

34.Falone S., Mirabilio A., Pannelli A., Cacchio M., Di Baldassare A., Gallina S., Passerini A., Amicarelli F. Different impact of acute bout of exercise on redox- and oxidative damage related profiles between untrained subject and amateur runners. *Physiological Research*, 2010, 59 (6), pp. 953–961.

35.Clemens M., Waller H. Lipid peroxidation in erythrocytes. *Chemistry and Physics of Lipids*, 1987, 45 (2–4), pp. 251–268.

36.Cazzola R., Russo-Volpe S., Cervato G. Biochemical assessments of oxidative stress, erythrocyte membrane fluidity and antioxidant status in professional soccer players and sedentary controls. *European Journal of Clinical Investigation*, 2003, 33 (10), pp.924–930.

37.Ortenblad N., Madsen K., Djurhuus M. S. Antioxidant status and lipid peroxidation after short-term maximal exercise in trained and untrained humans. *American Journal of Physioljgy*, 1997, 272 (4), pp. 1258–1263.

38.Selamoglu S., Turgay F., Kayatekin B.M., Gönenc S., Yslegen C. Aerobic and anaerobic training effects on the antioxidant enzymes of the blood. *Acta Physiologica Hungarica*, 2000, 87 (3), pp. 267–273.

39.Metin G., Gümüstas M.K., Uslu E., Belce A., Kayserilioglu A. Effect of regular training on plasma thiols, malondialdehyde and carnitine concentrations in young soccer players. *The Chinese Journal of Physiology*, 2003, 46 (1), pp. 35–39.

40.Caimi G., Canino B., Amodeo G., Montana M., Lo Presti R. Lipid peroxidation and total antioxidant status in unprofessional athletes before and after a cardiopulmonary test. *Clinical Hemorheolgy and Microcirculation*, 2009, 43 (3), pp. 235–241.

41.Kretzschmar M., Müller D., Hübscher J., Marin E., Klinger W. Influence of aging, training and acute physical exercise on plasma glutathione and lipid peroxides in man. *International Journal of Sports Medicine*, 1991 12 (2), pp. 218–222.

42.Sentürk U. K., Gündüz F., Kuru O., Aktekin M.R., Kipmen D., Yalçin O., Bor-Küçükatay M., Yeşilkaja A., Başkurt O.K. Exercise-induced oxidative stress affects erythrocytes in sedentary rats but not exercise-trained rats. *Journal of Applied Physiology*, 2001, 91 (5), pp. 1999–2004.

43.Salminen A., Vihko V. Endurance training reduces the susceptibility of mouse skeletal muscle to lipid peroxidation in vitro. *Acta Physiologica Scandinavica*, 1983, 117 (1), pp. 109–113.

44.Nagy L., Tontonoz P., Alvarez J. G., Chen H., Evans R. M. Oxidized LDL regulates macrophage gene expression through ligand activation of PPAR gamma. *Cell*, 1998, 93 (2), pp. 229–240.

45.Niki E. Lipid peroxidation: physiological levels and dual biological effects. *Free Radical Biology & Medicine*, 2009, 47 (5), pp. 469–484.

46.Gondim F.J, Zoppi C.C, Silveira L, Pereira-da-Silva L, de Macedo D. Possible relationship between performance and oxidative stress in endurance horses. *Journal of Equine Veterinary Science,* 2009, 9 (4), pp. 206–212.

47.Halliwell B., Gutteridge J. M. C. The antioxidants of human extracellular fluids. *Archives of Biochemistry and Biophysics*, 1990, 280, pp. 1–8.

48.Tauler P., Gimeno I., Aguiló A., Guix M. P., Pons A. Regulation of erythrocyte antioxidant enzyme activities in athletes during competition and short-term recovery. *Pflügers Archive: European Journal of Physiology*, 1999, 438 (6), pp. 782–787.

49.Kim L. B. Age-related changes in ceruloplasmin content in W/SSM rats. *Bulletin of Experimental Biology and Medicine*, 2008, 146 (6), pp. 680–681.

50.Harma M. I., Harma M., Erel O. The FORT test: a novel oxidative stress marker or a well-known measure of ceruloplasmin oxidase activity? *Atherosclerosis*, 2006; 187 (2), pp. 441–442.