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EXERCISE-INDUCED BIOCHEMICAL ALTERATIONS IN THE BLOOD OF HORSES INVOLVED IN RECREATIONAL HORSEBACK RIDING IN POMERANIAN REGION (NORTHERN POLAND)

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Abstract

The aim of our study was to determine changes in some biochemical indices (alanine (ALT) and aspartate aminotransferase (AST), lactate dehydrogenase (LDH)) as well as lactate and pyruvate level in the blood of horses exercised in recreational horseback riding from Pomeranian regions during a training session. Measurement of values of liver biomarkers (AST, ALT) and muscle damage indicator (LDH), followed by a variety of training programs, can help to better understand the acute and chronic effects of resistance training. Thirteen healthy adult horses from central Pomeranian region in Poland (village Strzelinko, N54°30'48.0" E16°57'44.9"), aged 9.5±2.4 years old, were used in this study. All horses participated in recreational horseback riding. Training started at 10.00 AM, lasted 1 hour and consisted from a ride of cross country by walking (5 min), trotting (15 min), walking (10 min), trotting (10 min), walking (5 min), galloping (5 min), and walking (10 min). Blood was drawn from jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8.30 and 10.00 AM), and immediately after exercise session (between 11.00 AM and 2.00 PM). Blood was stored in tubes with K_3-EDTA and sodium citrate (3.8%) and held on ice until centrifugation at 3,000 rpm for 15 minutes. The plasma was removed. Plasma was used for the determination of aminotransferases and lactate dehydrogenase activity; whole blood was used for determination of lactate and pyruvate level. The regular training lead to adaptive processes which provoke changes in biochemical indices. In our research, non-significant alterations of AST and LDH activities in horses involved in recreational horseback riding were observed. This may indicate a normal course of aerobic-anaerobic glycolysis in horses involved in recreational horseback riding during a training session. Moreover, ALT activity was decreased by 20.6% ($p = 0.000$) during a training session. Increased blood lactate level in horses involved in recreational horseback riding during training session could be explained by increasing lactate formation *via* the reduction of pyruvate catalyzed by LDH as a result of anaerobic energy supply. Based on these results, it is concluded that the endurance exercises lead to specific metabolic changes accompanied by a redistribution of energy supply for improving resistance to exercises and athletic performance of horses. Therefore, the present data can be useful to assess

the status of athletes and the degree of their training adaptability providing an opportunity to modify the training schedule to achieve the desired performance.

Key words: recreational horseback riding, Pomeranian region, horses, blood, aminotransferases, lactate dehydrogenase, lactate, pyruvate

INTRODUCTION

Poland has a long tradition of horsemanship stemming. Horses are still used extensively in agriculture and various sports including, in winter, skijoring. There are many riding clubs around the country as well as country estates that still have stables (Wójcik and Skrzypczak 2014). Some geographic areas are more closely associated with the use of horses than others, i.e. Pomeranian region in Poland. Pomerania is one of the most dynamically developing regions in Central and Eastern Europe. A range of actions taken for the last years makes the region become a more advanced and attractive place each day (Investment Areas in Poland 2013). In general, any horse can be a trail riding horse. Some breeds, however, are more common on the trails than others. These breeds usually have a gentle disposition, tend not to spook easily, are fairly sure-footed and are smooth enough that they are comfortable to ride for extended periods (Pagoulatos et al. 2008). For riding, often use horses of local breeds, which a bred directly in recreational areas. For example, Hucul horses which widespread in the Carpathians – in Poland and Ukraine.

The recreational horseback riding is used to improve physical and mental health of people with various conditions, including individuals suffering from dementia and Alzheimers, as well as children with attention deficit and hyperactivity disorder (ADHD) and improvements in behavioral and social interaction in individuals affected by autism spectrum disorder (ASD) (Ogrinc et al. 2018). It has also been found that riding improves physical strength and posture, and commanding such a large animal that responds to one's cues also improves self-esteem (Fine 2010). Although recreational riding is a non-competitive activity, physical fitness is necessary for horses, especially if these horses use for the long or difficult riding (Kimball 2005). Moreover, recreational horseback riding helps develop a health and promotes to improve physical fitness, as a tourists horse-riders and horses too (Ogrinc et al. 2018). Proper conditioning and feeding help horses meet the physical demands of recreational riding, but training and mental readiness also play important roles in preparing a horse for the ride (Kimball 2005).

With continued growth in the number of horses used for therapeutic riding, it is imperative to consider horse stress levels to ensure both the health and welfare of animals used (Johnson et al. 2017). Our prior studies have assessed the seasonal-induced variations of exercise impact on hematological indices and oxidative stress biomarkers in plasma and erythrocytes in horses involved in recreational horseback riding (Tkachenko et al. 2016). The results of our study showed statistically significant alterations of oxidative stress biomarkers in erythrocytes after exercise test in autumn. Due to their fundamental role in the transport of oxygen, erythrocytes provide a unique opportunity to study oxidative defense systems at the cellular level. The results observed in our study suggest that reactive oxygen species (ROS) production may contribute to exercise-induced damage to the erythrocyte membrane.

There were no statistically significant alterations in the derivatives of protein destruction level in the erythrocytes of trained horses involved in recreational horseback riding before and after exercise. Only in the autumn season, aldehydic and ketonic derivatives of OMP were increased after exercise by 88% ($p < 0.05$) and 77% ($p < 0.05$), respectively (Pażontka-Lipiński et al. 2017b). There were no statistically significant alterations in the aldehydic derivatives of protein destruction level in the plasma of trained horses involved in recreational horseback ride before and after exercise in spring and summer seasons. In autumn season, aldehydic and ketonic derivatives of OMP were increased after exercise by 7.2% and 12.8% ($p < 0.05$), respectively. On the contrary, aldehydic and ketonic derivatives of oxidatively modified proteins were decreased after a training session in winter. Increasing the level of oxidative-modified proteins suggests the activation of oxidative stress during a training session in spring and autumn as adaptive changes in the horse's body due to changing ambient temperatures. Significant reductions in aldehyde derivatives of oxidatively modified proteins in plasma of horses after training in winter are the result of adaptation induced by constant ambient temperature and exercise. This is an important seasonal adaptive reaction to stabilize ambient temperature (Witaszek et al. 2017c). The increase in plasma lipid peroxidation level in horses after exercise could be attributed to oxidative damage in various organs mainly in muscle tissue, owing to free radicals being produced, as a consequence of endurance exercise. Based on our results, it is concluded that the endurance exercises lead to specific metabolic changes accompanied by a redistribution of energy supply for improving resistance to exercises and athletic performance of horses (Witaszek et al. 2017a,b). The significant increase of erythrocytes amount after exercise test both in the summer and autumn seasons was observed. Moreover, increased erythrocytes count by 18% ($p < 0.05$) in the autumn compared to the value in spring season after the exercise test was noted. Hemoglobin level was increased after exercise in the summer season (by 15%, $p < 0.05$). Our results also revealed the increased hematocrit level after exercise test in the summer, autumn and winter seasons. We assume that it was due to the release of erythrocytes into the circulation as a result of the increased splenic function (Andriichuk and Tkachenko 2015, Tkachenko et al. 2016).

The results also showed that training in spring and autumn might exert beneficial effects by enhancing the erythrocytes' resistance in horses involved in recreational horseback riding (Pażontka-Lipiński et al. 2017a). Training session led to decreased the maximum percentage of hemolyzed erythrocytes in horses by 16% (spring), 26.9% (summer), and 20.9% (autumn). In winter, the same value of the maximum percentage of hemolyzed erythrocytes in horses before and after the training session was observed. Percentage of hemolysis during the first 30 sec. as well as the maximum percentage of hemolyzed erythrocytes were significantly higher in the summer season both before and after training. The later time of maximum hemolysis was noted in the spring season before and after the training session (5.5 and 6.5 min, respectively). Both before and after a training session, an increase of time of hemolysis in horses in spring was observed. The least resistant erythrocytes to hydrochloric acid were in summer, more resistance – in autumn and winter, respectively. We can assume that seasonal alterations may occur due to the greater muscle activity of the horses, as well as higher environmental temperature, especially in the summer period (Pażontka-Lipiński et al. 2016, 2017a).

Blood consists of many components that play an essential role in supporting the increased metabolic rate during exercise by transporting oxygen, water, enzymes, electrolytes, nutrients, and hormones to working muscles. Physical effort influences many parameters in the horse blood and since training should lead to a more efficient energy metabolism, it seems advisable to determine biochemical parameters during training session and in horses from different environments (Hinchcliff and Geor 2008, Kirschvink et al. 2008, Fazio et al. 2011, Piccione et al. 2007, 2008a, b, 2009, 2010, Tkachenko et al. 2016). Therefore, the goal of our study was to determine changes in some biochemical indices (alanine (ALT) and aspartate aminotransferase (AST), lactate dehydrogenase (LDH)) as well as lactate and pyruvate level in the blood of horses exercised in recreational horseback riding from Pomeranian regions during a training session. Measurement of values of liver biomarkers (AST, ALT) and muscle damage indicator (LDH), followed by a variety of training programs, can help to better understand the acute and chronic effects of resistance training (Hinchcliff and Geor 2008).

MATERIALS AND METHODS

Horses. Thirteen healthy adult horses from central Pomeranian region in Poland (village Strzelinko, N54°30'48.0" E16°57'44.9", Fig. 1), aged 9.5±2.4 years, including 5 Hucul pony, 2 Thoroughbred horses, 2 Anglo-Arabian horses, and 4 horses of unknown breed, were used in this study.

Fig. 1. Map of Strzelinko village (N54°30'48.0" E16°57'44.9") in the administrative district of Gmina Słupsk, within Słupsk County, Pomeranian Voivodeship, in northern Poland, where blood samples from horses were collected. It lies approximately 8 kilometers (5 mi) northwest of Słupsk and 111 km (69 mi) west of the regional capital Gdańsk

All horses participated in recreational horseback riding. Horses were housed in individual boxes, with feeding (hay and oat) provided twice a day, at 08.00 and 18.00 h, and water available *ad libitum*. All horses were thoroughly examined clinically and screened for hematological, biochemical and vital parameters, which were within reference ranges. The females were non-pregnant.

Exercise test. Training started at 10.00 AM, lasted 1 hour and consisted from a ride of cross country by the walking (5 min), the trotting (15 min), the walking (10 min), the trotting (10 min), the walking (5 min), the galloping (5 min), and the walking (10 min) (Fig. 2).

Fig. 2. Training session involved walking, trotting, and galloping. Photo by Paweł Pażontka-Lipiński and Marlena Witaszek

Blood samples. Blood was drawn from jugular veins of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8.30 and 10.00 AM), and immediately after exercise session (between 11.00 AM and 2.00 PM). Blood was stored in tubes with K_3-EDTA and sodium citrate (3.8%) and held on ice until centrifugation at 3,000 rpm for 15 minutes. The plasma was removed and defibrinated. Serum was used for the determination of aminotransferases and lactate dehydrogenase activity; while whole blood was used for the assessment of lactate and pyruvate level.

Assays of alanine aminotransferase (ALT, E.C. 2.6.1.2) and aspartate aminotransferase (AST, E.C. 2.6.1.1) activities. ALT and AST activity was analyzed spectrophotometrically by standard enzymatic method (Reitman and Frankel 1957). The substrates in the reaction were α-ketoglutaric acid (α-KG) plus L-aspartate for AST, and α-KG plus L-alanine for ALT. The products formed by enzyme action in the presence of plasma are glutamate and oxaloacetate for AST and glutamate and pyruvate for ALT at pH 7.4. Addition of 2,4-dinitrophenyl hydrazine resulted in the formation of the hydrazone complex with the ketoacids to form their respective hy-

drazone derivatives, which were measured colorimetrically at 530 nm. A red color was produced on the addition of 0.4M NaOH. The intensity of the color is related to the enzymatic activity. In the measurement of both AST and ALT, pyruvate is used as the standard for calibration graph composition. One unit/L of AST or ALT is defined as the liberation of 1 mmol of pyruvate per hour at 37°C incubation per L of plasma.

Assay of lactate dehydrogenase (LDH, E.C. 1.1.1.27) activity. The colorimetric method of Sevela and Tovarek (1959) was used for the determination of LDH activity. The pyruvate formed by LDH action in the presence of plasma in the presence of NAD^+. The reduction of NAD^+ is coupled with the reduction of L-lactate. Briefly, 0.1 mL of plasma was mixed with 0.3 mL of NAD^+ reagent (0.6 mg per sample), 0.8 mL of 0.03M tetrasodium pyrophosphate (pH 8.8), and 0.2 mL of 0.45M sodium lactate (pH 7.5) reagents. The samples were incubated at 37°C for 15 min. Addition of 2,4-dinitrophenyl hydrazine results in the formation of hydrazone complex with the ketoacids to form their respective hydrazone derivatives, which were measured colorimetrically at 530 nm. A red color is produced on the addition of 0.4M NaOH and is related to the enzymic activity. In the measurement of LDH activity, pyruvate is used as the standard for calibration graph composition. One unit/L of LDH is defined as the formation of 1 mmol of pyruvate per hour at 37°C incubation per liter of plasma.

Assays of lactate and pyruvate concentrations. Lactate and pyruvate concentrations were measured according to the procedure described by Herasimov and Plaksina (2000). One mL of total blood sample was added to 6 mL of distilled water and 1 mL of 10% metaphosphoric acid. The mixture was centrifuged at 800g for 5 min to separate the supernatant. One mL of 25% copper (II) sulfate and 500 mg calcium hydroxide were added to the supernatant and then mixed for 30 min. The mixture was centrifuged at 1,000g for 10 min. For lactate concentration assay the resulting supernatant was resuspended in 3 mL of *p*-dimethylamino benzaldehyde (0.5% in dimethyl sulfoxide), and 1 mL of 25% NaOH. The mixture was incubated at 37°C for 45 min, which was then centrifuged at 1,000g for 10 min. The absorbance was measured at 420 nm. Mixture with 0.5% *p*-dimethylamino benzaldehyde and 25% NaOH was used as a blank. For pyruvate concentration assay, the resulting supernatant was resuspended in 0.1 mL of 10% copper (II) sulfate, 4 mL of concentrated H_2SO_4, and 0.1 mL of 20% hydroquinone dissolved in alcohol, which was then heated in a water bath at 95°C for 15 min. The absorbance was measured at 430 nm. Calibration curves of lactate (0.1-5 mM) and pyruvate (0.1-5 mM) were used, and results were expressed in mmol lactate per L or mmol pyruvate per L.

Statistical analysis. Results are expressed as the 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 before and after exercise, the Wilcoxon signed-rank test was applied to the data (Zar 1999). Statistical significance between means in groups of horses in spring and summer both before and after exercise was evaluated by the Mann–Whitney *U* test (Zar 1999). All statistical analyses were performed using STATISTICA 8.0 software (StatSoft, Krakow, Poland).

RESULTS AND DISCUSSION

In our research, non-significant alterations of AST and LDH activities in horses involved in recreational horseback riding were observed. Moreover, ALT activity was decreased by 20.6% ($p = 0.000$) during a training session (Fig. 3). The regular training lead to adaptive processes which provoke changes in hematological and biochemical indices. The extent of changes depends on several factors: type of exercise, the intensity of work (strength, duration, and frequency) and individual variation. Physiological increases of ALT and AST have been shown to occur without any tissue destruction (Hinchcliff and Geor 2008).

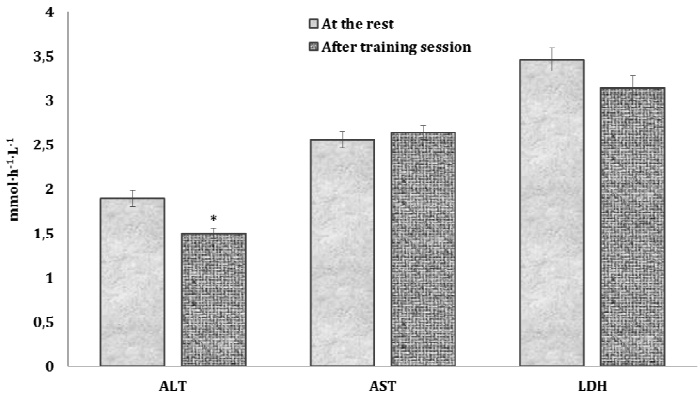

Fig. 3. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activity in the blood of horses involved in recreational horseback riding during a training session

Values expressed as mean ±S.E.M.

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

Aspartate aminotransferase is a cytoplasmic and mitochondrial enzyme that catalyzes the deamination of aspartate to form oxaloacetate, which can enter the Krebs cycle (Andrews et al. 1995). Increases in plasma aminotransferases activity may be due to hepatocyte damage, muscle damage, or *in vitro* hemolysis (Andrews et al. 1995). ALT is a pyridoxal-dependent enzyme that catalyzes the reversible transamination of L-alanine and α-ketoglutarate to form pyruvate and L-glutamate. The extent of changes in AST, ALT and LDH activities depends on the nature of the exercise (Hinchcliff and Geor 2008). Bashiri and co-workers (2010) found that resistance training for two months leads to a non-significant increase in serum ALT and AST levels in non-athlete students. Therefore, non-significant changes in LDH and AST activity suggests a special form of adaptation, and if exercise stress is followed by proper recovery, it will prevent more damage to the liver and muscles (Bashiri et al. 2010).

LDH catalyzes a redox reaction, the reversible conversion between pyruvate and L-lactate. L-lactate formation consumes reduced nicotinamide adenine dinucleotide ($NADH_2$) and generates oxidized nicotinamide adenine dinucleotide (NAD^+), whereas $NADH_2$ is produced during the oxidation of L-lactate to pyruvate. LDH converts py-

ruvate, the final product of glycolysis to lactate when oxygen is absent or in short supply, and it performs the reverse reaction during the Cori cycle in the liver (Andrews et al. 1995). In our study, a non-significant change in LDH activity in the serum of horses during a training session was noted. This may indicate a normal course of aerobic-anaerobic glycolysis in horses involved in recreational horseback riding during a training session. Based on these results, it is concluded that the endurance exercises lead to specific metabolic changes accompanied by a redistribution of energy supply for improving resistance to exercises and athletic performance of horses.

Pyruvate is formed predominantly in the cytoplasm of cells from glucose *via* glycolysis and from alanine *via* ALT action, being conveyed to the mitochondrial matrix *via* unclear ways of transportation (Bricker et al. 2012). Inside the mitochondrial network, pyruvate may be acted upon by two enzymes, the pyruvate dehydrogenase (PDH) complex and pyruvate carboxylase, being forwarded respectively to the oxidative pathway to supply adenosine triphosphate (ATP) through the tricarboxylic acid (TCA) cycle and the oxidative phosphorylation reaction, or to the gluconeogenesis pathway that produces endogenous glucose (Adeva et al. 2013). Oxidation of pyruvate to carbon dioxide to generate energy requires the collaboration of the pyruvate dehydrogenase complex, the tricarboxylic acid cycle, and the mitochondrial respiratory chain to finally produce ATP in an oxygen-consuming reaction termed oxidative phosphorylation. Therefore, tissue hypoxia and congenital or acquired dysfunction of the pyruvate dehydrogenase complex, the TCA cycle, and the mitochondrial respiratory chain deteriorate mitochondrial ATP production. Under these circumstances, glycolysis becomes the most important source of energy for the cell, increasing L-lactate generation, as this anion is produced by LDH in the last step of the glycolytic pathway (Adeva et al. 2013). Increased blood lactate level in horses involved in recreational horseback riding during training session could be explained increasing lactate formation *via* the reduction of pyruvate catalyzed by LDH in a result of anaerobic energy supply (Fig. 4). There were non-significant changes in the blood pyruvate level between the resting period and after training (Fig. 4).

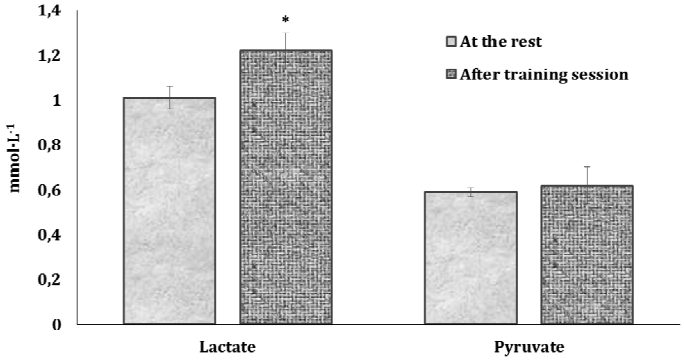

Fig. 4. Lactate and pyruvate level in the blood of horses involved in recreational horseback riding during a training session

Values expressed as mean ±S.E.M.

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

Blood and plasma lactate concentrations have been used in humans and horses to quantify the relative intensity of exercise (Rainger et al. 1995, Hodgson and McGowan 2014, Munsters et al. 2014). The blood and plasma lactate concentration is a result of the production of lactate in the muscle, the diffusion of lactate from muscles to the blood and the uptake of lactate into several tissues (Munsters et al. 2014). Accumulation of lactate level was dependent to activation of aminotransferases' reaction *via* ALT and AST activation. The correlative analysis confirmed our assumption. The increase of AST activity caused to increase ALT activity ($r = 0.563$, $p = 0.000$) and lactate level ($r = 0.351$, $p = 0.014$) in the blood of horses involved in recreational horseback riding before a training session (Fig. 5).

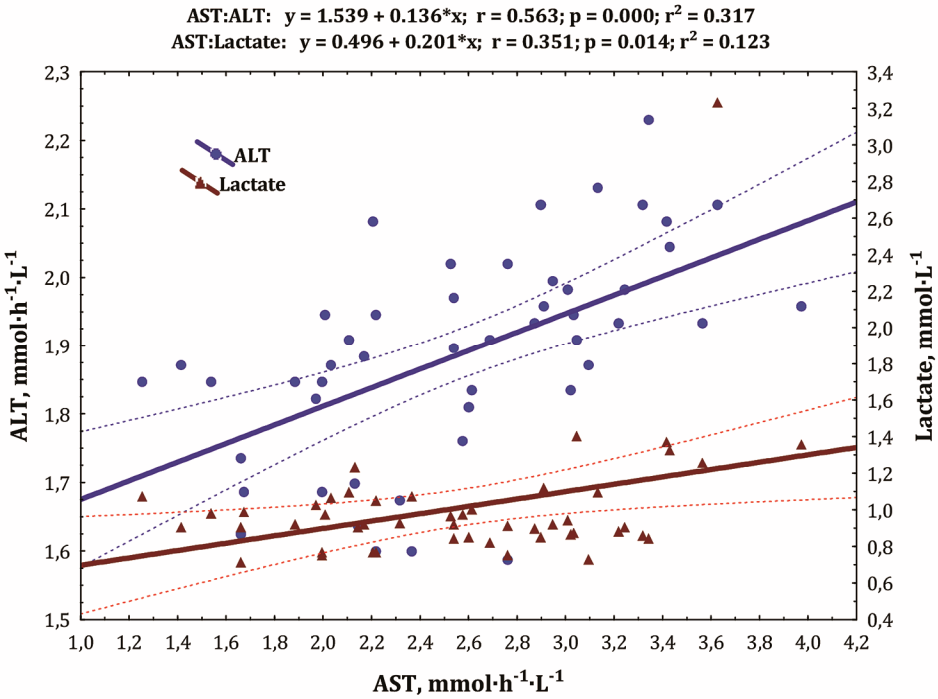

Fig. 5. Correlations between blood ALT, AST activity and lactate level in the blood of horses involved in recreational horseback riding before a training session

In our previous study, we have determined the level of oxidative stress biomarkers, AST, ALT, and LDH activity, as well as lactate and pyruvate concentrations in sports horses involved in eventing before and after training (Andriichuk et al. 2013). All horses were in a regular systematic training and had the same diet. Significant increases in the 2-thiobarbituric acid reactive substrates (TBARS) level in the blood of horses after exercise was observed. There were no significant differences in erythrocyte TBARS level between the resting period and after exercises. Significant decreases by 8% ($p < 0.05$) in the aldehyde derivatives of protein oxidation in the plasma after training was noted. Exercise can induce the activity of the proteasome complex, which is significantly involved in the degradation of oxidatively modified

proteins. ALT and AST activity was lower by 4% and 15% ($p < 0.05$) in horses after training compared to the rest period. In post-exercise horses, lactate level was higher by 12% ($p < 0.05$) compared to the rest period. No statistically significant differences in pyruvate level between the rest period and after training were observed. A significant increase of lactate concentration after training indicates the level of contribution of anaerobic glycolysis to the total energy supply of muscle activity in eventing horses. The preventive effect of regular exercise leads to adaptation to prolonged exercises, which is accompanied by an increase of oxidative stress-induced adaptation and changes in redox homeostasis, increased antioxidant defenses, lower oxidative damage, and increased resistance to oxidative stress. Regularly performed exercise might induce an adaptive enhancement in skeletal muscle and erythrocytes of the defense mechanisms that protect them against oxidative stress. The level of oxidative stress markers and activity of aminotransferases, lactate dehydrogenase and concentration of lactate and pyruvate in the blood of sports horses can be sensitive and informative parameters for the assessment of equine athletic performance (Andriichuk et al. 2013).

In our previous study, we also have investigated the effect of an exercise test of moderate intensity on oxidative stress biomarkers, antioxidant enzymes activity, and osmotic resistance of erythrocytes in well-trained equine athletes (Andriichuk et al. 2016). Eighteen middle-aged horses of Ukrainian warmblood (8.3±1.6 years) and Holsteiner (7.4±1.9 years) breeds were used in this study. All horses have been in regular training for several years. The exercise test induced a significant increase in erythrocyte values, hemoglobin concentration, and hematocrit in horses of both breeds. Regular training induces activation of the antioxidant enzymes and thereby can reduce oxidative stress in athletic horses. The exercise test in horses of both breeds attenuates oxidative stress and accompanied with a significant decrease of lipid peroxidation and oxidatively modified proteins in erythrocytes after exercise. The findings of the present study demonstrated the elevated level of erythrocytes' catalase and glutathione reductase in Ukrainian warmblood horses, as well as the decreased level of superoxide dismutase and glutathione reductase in Holsteiner horses reporting changes in levels of exercise-induced oxidative stress biomarkers in horses of both breeds. Statistically significant differences in the percentage of hemolyzed erythrocytes between pre-exercise and post-exercise tests were observed and thereby signifying an oxidative stress-dependent impairment of erythrocyte stability. Our data suggest that oxidative stress and enzymatic antioxidant defense biomarkers can be used for the monitoring of fitness level, health benefits, and performance of equine athletes (Andriichuk et al. 2016).

The effects of gender differences on the blood oxidative stress biomarkers, antioxidant defenses, and resistance of erythrocytes to hemolytic agents of trained horses before and after exercise were evaluated in other our study (Andriichuk et al. 2014). The study was carried out on nine mares and 14 stallions of Ukrainian Warmblood well-trained horses, involved in jumping, eventing, and dressage. Trained stallions showed a decrease in lipid peroxidation and higher glutathione reductase activity, whereas mares presented a higher superoxide dismutase activity after exercise. The resistance of erythrocytes was similar in female and male. No statistically significant

differences were observed in the percentage of hemolyzed erythrocytes between after and before exercise. A correlation between the oxidative stress biomarkers and antioxidant defenses in the stallions after exercises were observed, which may indicate a protective response of superoxide dismutase and catalase against exercise-induced oxidative stress (Andriichuk et al. 2014). Stallions showed a significant increase in leucocytes and granulocytes amount, as well as erythrocytes, hemoglobin and hematocrit levels after the exercise test. Pre-exercise level of mean corpuscular hemoglobin concentration was higher in stallions. At the same time, mares showed a significant decrease in platelet volume after the exercise test. The physical effort in stallions leads to significant increase in aspartate aminotransferase activity. Trained mares and stallions showed a decrease in lipid peroxidation after exercise. Exercise also caused an increase in oxidatively modified protein of erythrocytes in stallions indicating by exercise-induced oxidative stress. The resistance of erythrocytes in 0.1N HCl was similar between females and males. No statistically significant differences in the percentage of hemolysed erythrocytes before and after exercise were observed (Andriichuk and Tkachenko 2017).

It is known that the exercise induces a variety of changes depending on its characteristics, duration, and intensity (Rivero et al. 2007). The muscular adaptations to training in horses occur on a continuum that is based on the exercise intensity and duration of training. Exercises for 15 to 25 min/day at velocities between v2.5 and v4 can improve in the short term (3 weeks) the muscular stamina in Thoroughbreds. However, the exercises of 5-15 min at v4 are necessary to enhance muscular features related to strength (hypertrophy) (Rivero et al. 2007). The present research has focused on exercise-induced modifications of biochemical parameters in response to training protocol. As observed previously, physiological changes in several blood clinical chemistry parameters occur in response to exercise training in Thoroughbreds and Standardbreds during 80 days of training (Fazio et al. 2011). Training, moreover, is associated with transient modifications in the concentrations of the commonly measured chemical constituents of the blood such as enzymes like aspartate aminotransferase, creatine kinase (CK) and γ-glutamyl transferase, as well as total proteins, urea, and creatinine concentrations. The activities of AST and CK enzymes change following muscle damage or muscular exercise (Balogh et al. 2001, Tyler-McGowan et al. 1999, Fazio et al. 2011). Fazio and co-workers (2011) findings showed a gradual and significant increase of AST and CK activities until the 60^{th} day of training and a decrease at the end of the training period (80th day) in Thoroughbreds. These increases could be explained in relation to V4 values (anaerobic threshold) that showed an average increase of 185 m/min between the second and the third test. In this period there was a higher response to glycolytic metabolism that caused permeability changes in muscle fiber membranes (Fazio et al. 2011).

The type of exercise, which can be considered quite usual for pentathlon horses, caused detectable biochemical and lipid peroxidative changes in plasma and erythrocytes. Balogh and co-workers (2001) have examined exercise-induced changes in some plasma and erythrocyte biochemical and antioxidant variables in pentathlon horses immediately after, and 24 hours after competing in two 1-minute runs of intense exercise over jumps. The peak intensity periods were preceded by a 20-minute

warm-up and separated by a 20-minute break. Significantly increased concentrations of total protein, lactate, and the ferric reducing ability of plasma, and increased activities of CK and LDH were observed immediately postexercise compared with pre-exercise samples ($p < 0.05$). All results returned to approximately initial values after 24 hours of rest (Balogh et al. 2001).

The distribution of lactate between red blood cells and plasma was examined by Rainger and co-workers (1995) at rest, during exercise and 30 min after exercise in six Standardbred horses. Lactate and water concentrations were measured in blood and plasma samples collected prior to exercise, during the last 15 s of each step of an incremental exercise test and at 5 min intervals during the first 30 min after exercise. The mean ratio of erythrocytes' lactate concentration to plasma lactate concentration prior to exercise was 1.02±0.34. Haemoconcentration during exercise was associated with more rapid accumulation of lactate in plasma than in erythrocytes. Mean whole blood lactate concentration was only 59% of plasma lactate concentration in samples collected during exercise. Blood lactate concentration was highly correlated with plasma lactate concentration during exercise ($r = 0.98$; $p < 0.001$), but individual plasma lactate concentration values differed from predicted blood lactate concentration values by up to 2.1 mmol l^{-1} when plasma lactate concentration exceeded 8 mmol l^{-1}. At each exercise speed and time after exercise, there was a large variation between horses in the ratio of blood lactate concentration/plasma lactate concentration. During exercise at 11 m s^{-1}, the ratio ranged from 0.46-0.73. The blood lactate concentration/plasma lactate concentration ratio was significantly correlated with increasing exercise intensity ($r = -0.68$, $p < 0.001$) and with hematocrit ($r = -0.69$, $p < 0.001$). The effects of plasma lactate concentration and hematocrit on the blood lactate concentration/plasma lactate concentration ratio during exercise and the post-exercise period varied greatly between horses. The ratio of lactate concentrations in water of erythrocytes and plasma varied greatly during and after exercise (0.61-1.22). The ratio also varied considerably between horses, with coefficients of variation ranging from 14-34%. Lactate concentrations in erythrocytes and plasma water vary greatly between horses during and after exercise (Rainger et al. 1995).

A possible reason to explain our results is that horses have a higher capacity for blood lactate removal. Blood lactate removal is determined by several factors, such as whole-body muscle mass, local blood flow and consequently muscle capillary content, active and inactive muscles oxidative capacity, other tissues transport and gluconeogenesis capacity and arterial lactate availability as well (Gollnick et al. 1986, Brooks 1991). It is pointed out that many conditions influence the rate and magnitude of the accumulation of lactate in blood and muscles. Included are diet, state of physical fitness, and the type and duration of the exercise (Gollnick et al. 1986). These are several corollaries between muscle and whole-body lactate metabolism: temporal dependence on lactate uptake and release, the effects of beta-adrenergic stimulation on lactate formation and release, the effect of prior endurance training on lactate metabolism, the effect of lactate on glucose uptake and utilization, and the role of low oxygen tension (hypoxia) in loosening the control of glycolysis. The formation, exchange, and utilization of lactate represent a central means by which the coordination of intermediary metabolism in diverse tissues and different cells within tissues can be accomplished (Brooks 1991).

CONCLUSION

The regular training lead to adaptive processes which provoke changes in biochemical indices. In our research, non-significant alterations of AST and LDH activities in horses involved in recreational horseback riding were observed. This may indicate a normal course of aerobic-anaerobic glycolysis in horses involved in recreational horseback riding during a training session. Moreover, ALT activity was decreased by 20.6% ($p = 0.000$) during a training session. Increased blood lactate level in horses involved in recreational horseback riding during training session could be explained by increasing lactate formation *via* the reduction of pyruvate catalyzed by LDH as a result of anaerobic energy supply. Based on these results, it is concluded that the endurance exercises lead to specific metabolic changes accompanied by a redistribution of energy supply for improving resistance to exercises and athletic performance of horses. Therefore, the present data can be useful to assess the status of athletes and the degree of their training adaptability providing an opportunity to modify the training schedule to achieve the desired performance.

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ZMIANY BIOCHEMICZNE WYWOŁANE WYSIŁKIEM FIZYCZNYM WE KRWI KONI BIORĄCYCH UDZIAŁ W REKREACYJNEJ JEŹDZIE KONNEJ W REGIONIE POMORSKIM (PÓŁNOCNA POLSKA)

Streszczenie

Celem niniejszej pracy było określenie zmian wybranych wskaźników biochemicznych (aminotransferaza alaninowa (ALT) i asparaginianowa (AST), dehydrogenaza mleczanowa (LDH)) oraz poziomu mleczanu i pirogronianu we krwi koni z regionu Pomorza biorących udział w rekreacyjnych jazdach konnych. Pomiar wartości biomarkerów wątrobowych (AST, ALT) oraz wskaźnika uszkodzenia mięśni (LDH), a następnie różnorodne programy treningowe, mogą być pomocne w lepszym zrozumieniu ostrych i przewlekłych skutków treningu odpornościowego. W badaniach wykorzystano trzynaście zdrowych dorosłych koni z centralnego Pomorza w Polsce (wieś Strzelinko; N54°30'48,0" E16°57'44,9"), w wieku 9,5±2,4 lat. Wszystkie konie brały udział w rekreacyjnej jeździe konnej. Ćwiczenia rozpoczynały się o godz. 10 rano, trwały 1 godzinę i obejmowały: chód (5 min), kłus (15 min), chód (10 min), kłus (10 min), chód (5 min), galop (5 min) i chód (10 min). Krew od zwierząt pobierano z żył szyjnych rano, 90 minut po karmieniu (między godziną 8.30 a 10.00), podczas gdy konie były w stajni, oraz natychmiast po treningu (między godziną 11.00 a 14.00). Krew przechowywano w probówkach z K_3-EDTA i cytrynianem sodu (3,8%) na lodzie do momentu odwirowania przy 3000 obrotów przez 15 minut. Osocze zostało zlane. Osocze stosowano do oznacze-

nia aminotransferaz i aktywności dehydrogenazy mleczanowej; do oznaczania poziomu mleczanu i pirogronianu zastosowano pełną krew. Regularne treningi prowadzą do procesów adaptacyjnych, które prowokują do zmian wskaźników biochemicznych. W naszych badaniach zaobserwowano nieistotne zmiany aktywności AST i LDH u koni uczestniczących w rekreacyjnej jeździe konnej. Może to wskazywać na normalny przebieg tlenowej/beztlenowej glikolizy u koni podczas treningu. Ponadto aktywność ALT została zmniejszona o 20,6% ($p = 0,000$) podczas sesji treningowej. Zwiększony poziom mleczanu we krwi u koni biorących udział w rekreacyjnej jeździe konnej można wytłumaczyć wzmożonym wytwarzaniem mleczanu poprzez redukcję pirogronianu katalizowanego przez LDH w efekcie beztlenowego dostarczania energii. Na podstawie naszych wyników można stwierdzić, że ćwiczenia wytrzymałościowe prowadzą do określonych zmian metabolicznych, którym towarzyszy redystrybucja zaopatrzenia w energię w celu poprawy odporności na ćwiczenia i wyniki sportowe koni. Dlatego też niniejsze dane mogą być przydatne w ocenie statusu koni sportowych i stopnia ich zdolności adaptacyjnych do treningów, dając możliwość modyfikacji harmonogramu treningu, by osiągnąć pożądaną wydajność.