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Responses of the metabolism in tired syndrome in endurance horses

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Abstract

Results from a study of biochemical blood parameters in performance horses are presented in this article. After strenuous activity, horses are more likely to have symptoms of the metabolic syndrome, which includes dehydration, cytolysis syndrome, uremic syndrome, and electro- lyte imbalance. In order to detect latent illness in horses, cardiac output must be measured before, during, and after exercise. Understanding how the metabolic processes in a horse's body change in response to different levels of physical exertion is crucial. Metabolic and cardiovascular dysfunction are the main reasons why horses are not allowed to participate in competitions. The research set out to learn how exercise affects the biochemical blood parameters of performance horses. Research subjects were horses, which are used in the equestrian disciplines. All of the horses had their blood analyzed for its overall composition and biochemical characteristics, which provide insight into the health of the horse's vital organs like the heart, liver, and kidneys. Increased weariness, dyspnea, tachycardia, and less often arrhythmias are the most commonly reported effects of physical stress on horses. After strenuous exercise, performance horses may experience a variety of physiological changes, including dehydration (hyperproteinemia), uremic syndrome (increased concentration of urea and creatinine), cytolysis syndrome (increased activity of AST and ALT), and electrolyte imbalance (decreased serum sodium and potassium). In horses, exercise-induced hyper- lactatemia alters the permeability of cardiomyocytes and the activity of blood-exit enzymes, which may play an important role in the etiology of myocardial dystrophy. Pre-exercise metabolic syndrome in horses may be detected with the use of installed tests.

Key words: horses, metabolic syndrome, myocardial dystrophy, hepatic and renal tests, blood lactate, electrolyteimbalance, exhausted syndrome.

Introduction

The combination of the cardiovascular system diseases and metabolic disorders in the humane medicine has been known since the 1940s. In the 1980s, this combination was determined by the term of the metabolic syndrome, which was included in the group of metabolic risk factors thatoccur simultaneously in one patient. Nowadays the term "metabolic syndrome" is common in the medical literature, but there are minor devergence about diagnostic criteria (Poryadin and Oskolok, 2011).

The physical activity of horses requires energy for a long time (Treiber et al., 2006). Under such conditions, a stable energy requirement is accompanied by metabolic disorders, and that causes dysfunction of the cardiorespiratory, endo- crine and neuromuscular systems (Flaminio and Rush, 1998; Schott et al., 2006).

The most common reason for excluding horses from sport competitions is colic (Fielding et al., 2009), whileother researchers have found that the main cause is metabolic disorders and disfunction cardiovascular system (Trigo et al., 2010).

Active muscular activity promotes hypertrophy of the heart muscle. However, with the maximum muscular load, the increased use of adenosine triphosphate occurs, and the rate of supply with substrates and oxygen is insufficient. The reason for the development of myocardial dystrophy is the inconsistency between the energy expenditure in the functioning structures of the myocardium and their restora- tion, caused by significant increase in energy costs. Accord- ing to the hypoxic theory, the pathological theory is caused by oxygen deficiency, and the neurodegenerative process connects myocardial dystrophy with the hypoxic effect of excess catecholamines (Kushakovsky, 2000).

However, due to physical activity, the electrolyte imbal- ance develops, as a result of which the processes of cellular respiration, oxidative phosphorylation and transmembrane exchange of cations are disturbed, which leads to a decrease in the formation of energy in the myocardium and the effec- tiveness of its use by functioning structures of the heart muscle (Tarmonova and Shutov, 2007). Due to energy defi- ciency the myocardial dystrophy develops, which is caused by metabolic disorders, which leads to the dystrophy and dysfunction of the heart muscle, accompanied by a disorder of electrical and metabolic processes in it (Dimopoulos et al., 2009). The development of electrophysiological and functional dysfunction of the myocardium is caused by cellular acidosis, local inflammation and peroxidation, vio- lation of ionic equilibrium, decrease in the synthesis of adenosine triphosphate (Chernaya et al., 2010).

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Evaluation of the horse's heart functional state must be done during and after activity, when the latent course of the disease manifests itself clinically. It is important to know theways of the metabolic processes flow that occur in a horse body during physical activity of different intensity (Bergero et al., 2005).

The result of insufficient provision of physiological needs of tissues and organs during physical activities is a metabolic crisis, which can be manifested by fatigue, dehydration, oxidative or thermal stress (Trigo et al., 2010; Niedźwiedź et al., 2017).

During physical activity the parameters of homeostasis change in horses, in particular their biochemical blood pro- file (electrolytes and acid-base balance) (Castejon et al.,2006).

A number of studies have been conducted in to investigate changes in hematological parameters in horses' blood during physical activity (Piccione et al., 2003; Golovacha et al., 2017). However, studies concerning horses excluded from the competition because of the metabolic disorders dueto physical exertion are not adequately covered (Fielding et al., 2009). Therefore, the *purpose* of our work was to inves- tigate changes in biochemical blood parameters of sport horses after physical activities.

1. Materials and methods

50 horses were included into our research, used in the classic equestrian sport of Ukrainian warmblood horses (n = 20), Hanoverian (n = 15) and Westphalian (n = 15) breeds horses. Among the experimental animals there were 25 mares, 9 stallions and 16 geldings. The average age of horses was 8.4 ± 0.71 (3.5–16.0), weight – 479.4 ± 8.54 kg
(350–605 kg).

The daily diet of horses included: meadow grass (6 kg), oats (6 kg), wheat bran (2 kg), three times per day. Salt and water were available without restrictions.

The horses were examined clinically: the internal body temperature was measured, the pulse and respiration rate were counted, the heart auscultation was performed, the color of the mucous membranes and the time of capillaries filling were determined.

For all horses, a general analysis and biochemical blood indices were analyzed, characterizing the functional state of the organs (heart, liver, kidneys). Mares were not enceinte. All horses were de-wormed and vaccinated, were in the same conditions of detention.

Studies were carried out immediately before physical ac-tivities and immediately afterwards. The duration of medi- um intensity of regular training was 1 hour: walk –5 minutes; rising trot – 10 minutes; walk – 5 minutes; sittingtrot – 10 min; walk – 10 minutes; gallop with transition to a walk – 10 min; walk – 10 min.Blood samples of horses were taken from the jugular vein using injection needles Ø 16 × 40 mm into tubes (10 ml; Vacutest, Italy) without anticoagulant. The analysis of blood samples was carried out in the laboratory of the Department of Internal Animal Diseases and Clinical Diagnosis of Stepan Gzhytskyi National University of VeterinaryMedicine and Biotechnologies Lviv.

To obtain blood serum, the tubes were centrifuged at 3000 rpm for 10 minutes. The concentration of total protein, albumin, total bilirubin, glucose, urea, creatinine, total cal- cium, inorganic phosphorus, magnesium, activity of aspar- tate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP) and gamma-glutamyl transpeptidase (GGT) in the serum were determined using an automatic biochemical analyzer Mindray BS-120 (China)with reagents PZ Cormay S.A. (Poland). The content ofpotassium and sodium in the horses' serum was determined on a semi-automatic biochemical analyzer BioChem SA (USA) using reagents High Technology Inc., Production RD Walpole (USA).Mathematical analysis of the obtained results was carriedout using software *Microsoft Office Excel* by means of con- ventional methods of variation statistics with estimation of the mean (M), its error (m), the probability by the Student's t-test.

2. Results and discussion

Most frequently increased fatigue, dyspnea, mild tachy- cardia (46.4 ± 1.20 beats per minute, 36–68 beats per mi- nute) were observed in horses during physical activity, less frequently arrhythmias (sinus arrhythmia, supraventricular extrasystole).

The determination of total protein, albumin and globulin content allows determine the status (index) of body hydra- tion, protein loss, or reduction of its synthesis (Rose and Hodgson, 1994). Studies of humans and dogs have shown that protein is not an important energy substrate during physical activity. For horses the role of protein during phys- ical activity is unknown, but it is assumed that carbohy- drates and fat oxidations predominate in energy supply (Pösö Reeta et al., 2004).

Serum total protein level horses of all breeds before physical activity was on the same level (table 1). After phys-ical activity, the protein level increased in the blood of sport horses from three research groups, obviously, that is the result of dehydration. It was especially evident among hors- es of the Hanoverian ($P < 0.01$) and Westphalian ($P < 0.05$) breeds, while the horses of the Ukrainian warmblood horses had only a tendency of increasing (table 1). Significant de- hydration observed in for horses that were excluded fromthe competition due to the metabolic crisis (Castejon et al., 2006; Francesca et al., 2007). After physical activity the content

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of albumin increased in blood of all research groups,

The concentration of total bilirubin in serum of horses after physical activity increased. In horses of the Ukrainian warmblood horses – on 22%, the Hanoverian – 3.5% com- pared to the parameters before physical activity, and on22.2% ($P < 0.05$) in the Westphalian breed (table 1). An increase in concentration of bilirubin in horses after physicalactivity may be the result of the development of cholestasis, which is obviously due to dyskinesia of the biliary tract during exercise (Golovacha et al., 2005), proved by the tendency to the increased level of AP and GGT in serum of all research groups (table 2).The gamma glutamine transferase (GGT) is consideredto be a marker of oxidative stress (Lee et al., 2004; Yang et al., 2007), which is a key in the development of metabolic imbalance (Lee and Jacobs, 2005; Fernando et al., 2009). The oxidative stress developed (Kinnunen et al., 2005; Demircan et al., 2009) in horses with a low training level during physical activity and an increased GGT serum levelis the result of insufficient training of horses (Noleto et al., 2016).

but a significant difference was established only in the ani-mals of the Hanoverian breed ($P < 0.05$; table 1).

Table 1

Biochemical parameters of blood serum sport horses before and after exercise

Breeds of horses	Groups	n =	Total protein, g/L	Albumins, g/L	Total bilirubin, µmol/L	Glucose, mmol/L	Urea, mmol/L	Creatinine, µmol/L
Ukrainian warmblood	Before exercise	20	61.3 ± 1.22	37.8 ± 0.78	24.1 ± 2.14	5.5 ± 0.22	5.3 ± 0.22	132.8 ± 5.42
	After exercise	20	63.8 ± 1.61	38.0 ± 0.56	29.4 ± 2.44	5.2 ± 0.21	6.2 ± 0.28*	162.3 ± 5.91**
Hanoverian	Before exercise	15	60.9 ± 0.95	36.2 ± 0.84	28.1 ± 1.76	5.5 ± 0.13	5.6 ± 0.16	127.7 ± 6.86
	After exercise	15	66.3 ± 1.34**	39.4 ± 0.68*	29.1 ± 1.81	5.3 ± 0.11	5.9 ± 0.28	145.4 ± 4.09*
Westphalian	Before exercise	15	61.2 ± 1.55	38.7 ± 0.96	26.6 ± 1.24	5.6 ± 0.15	5.1 ± 0.20	123.4 ± 3.88
		15	67.1 ± 2.07*	39.2 ± 0.44	32.5 ± 1.69*	5.2 ± 0.11*	5.6 ± 0.15*	165.2 ± 4.95***

Note: * – P < 0.05; ** – P < 0.01; *** – P < 0.001 (compared to exercises)

Table 2

Activity of enzymes of blood serum sport horses before and after exercise

Breeds of horses	Groups	n=	AST, U/L	ALT, U/L	AP, U/L	GGT, U/L
Ukrainian warmblood	Before exercise	20	257.2 ± 10.12	6.4 ± 0.54	112.2 ± 8.37	13.5 ± 0.90
	After exercise	20	300.0 ± 16.01*	7.3 ± 1.05	116.5 ± 9.69	14.2 ± 1.25
Hanoverian	Before exercise	15	257.2 ± 7.01	5.5 ± 0.34	111.3 ± 5.55	11.0 ± 0.73
	After exercise	15	292.9 ± 8.13***	6.5 ± 0.45	120.3 ± 6.60	12.2 ± 0.95
Westphalian	Before exercise	15	259.7 ± 11.27	5.2 ± 0.35	106.9 ± 5.01	12.8 ± 0.75
	After exercise	15	302.5 ± 6.89**	8.7 ± 0.95**	109.2 ± 7.60	13.8 ± 1.21

Note: * – P < 0.05; ** – P < 0.01; *** – P < 0.001 (compared to exercises)

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Serum glucose concentration tended to decrease in allexperimental groups of animals after physical activity (ta-ble 1). In horses of the Ukrainian warmblood horses, its blood concentration decreased on 5.4%, Hanoverian – by3.6%. In horses of the Westphalian breed the blood glucoseconcentration was reduced by 7.1% ($P < 0.05$) after physicalactivity, compared to the values before the activity (table 1).

Determination of urea and creatinine blood concentration is used to assess glomerular filtration of the kidneys (Braun et al., 2003). For horses of the Hanoverian breed, the urea content tended to increase (by 5.4%), while in horses of the Ukrainian warmblood and Westphalian breeds it was in-creased significantly on 17.0% ($P < 0.05$) and 9.8% ($P < 0.05$; table 1).

The creatinine concentration in the blood of horses of three research groups increased after physical activity: hors-es of the Ukrainian warmblood horses on 22.2% ($P < 0.01$), Hanoverian – 13.9% ($P < 0.05$) and Westphalian breeds on 33.9% ($P < 0.001$; table 1).

Creatinine is a metabolite of creatin found in high con-centrations in tissues that have a high energy demand, in-cluding skeletal muscles (98% of the total body creatin pool) (Rose and Hodgson, 1994; Sewell and Harris, 1995).

Physical activity causes an increase in the level of free oxygen radicals (Snaders, 1995) that have a vasoconstrictive effect, reduces the rate of glomerular filtration by direct inactivation of cyclooxygenase in epithelial cells (Ohtra etal., 2004). Physical activity also leads to an increase in the content of endothelin, catecholamines, angiotensin II, cyto-kines (Snaders, 1995) that release mediators, which promote the development of kidney ischemia (Sewell and Harris, 1995) and the development of acute renal failure (Hisanaga et al, 1999). So, in terms of physical activity, glomerular filtration of the kidneys is disturbed in horses, and the ure-mic syndrome develops.

According to the results of our study sport horses have significantly increased serum activity of AST after physical activity: Ukrainian ($P < 0.05$), Hanoverian ($P < 0.001$) and Westphalian ($P < 0.01$; table 2) breeds. Serum level of ALT also tended to increase after physical activity in horses of the Ukrainian warmblood and Hanoverian breeds (table 2), whereas in Westphalian horses the increase was significant (7.8%; $P < 0.01$).

The increased activity of AST and ALT in serum of horses is directly related to a violation of the permeability ofthe muscle cell membranes, it can be registered in case of myopathy during physical activity. However, the enzymes activity in the horses' blood depends on the level of physicalpreparation, intensity and duration of the load (Wanderley etal., 2015).

It is possible that the increase in activity of AST in the blood serum of horses after physical activity can be the result of a violation of the permeability of the membranes ofcardiomyocytes due to damage to the myocardium (Singh et al., 2011).

Violation of the blood supply to the heart muscle leads to an imbalance between the supply of oxygen and the need for it in the myocardium and causes changes in the metabolism of cardiomyocytes. A limited amount of oxygen is distribut- ed between the oxidation of glucose and free fatty acids, andthe activity of both pathways of metabolism is reduced. In ischemia, glucose is cleaved mainly by anaerobic glycolysis, and the resulting pyruvate is not oxidizing decarboxylation, and is converted into lactate, which potentiates intracellular acidosis (Amosova, 2000; Lishnevskaja, 2008).

During exercise as a result of anaerobic glycolysis, mus-cle accumulates lactic acid, which causes a decrease in pH.

One of the possible determinants of permeability changes in cardiomyocyte cells is the decrease in the synthesis of aden- osine triphosphate (ATP), which is necessary to maintain theintegrity of the membranes. It is shown that exercise results in ischemia and cellular acidosis, peroxidation and ion bal- ance disturbances, which is the cause of the development of electrophysiological and functional myocardial dysfunction (Räsänen et al., 1996; Fernando et al., 2009).

According to the results of the research, it was found thatconcentration of lactate after exercise was likely to increase in horses Ukrainian warmblood ($P < 0.001$), Hanoverian($P < 0.05$) and Westphalian ($P < 0.05$) breeds horses (Fig. 1).

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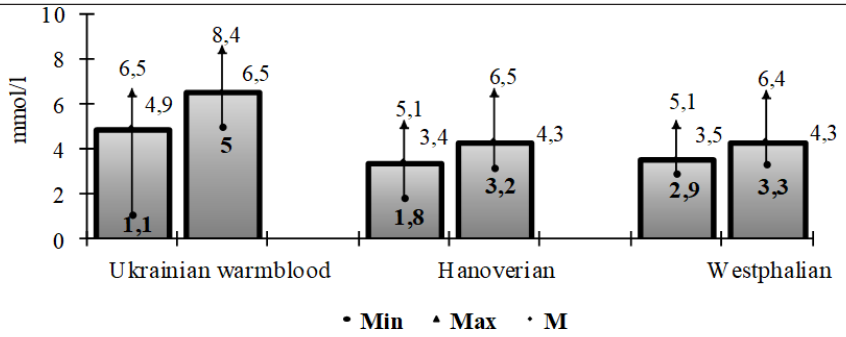

Fig. 1. The content of lactate in blood serum of sports horses before and after exercise

Increased lactate content is set in the blood of horses involved in eventing under after exercise (Andriichuk et al., 2014). One of the possible determinants of permeability changes in cardiomyocyte cells is the decrease in the synthesis of ATP, which is necessary to maintain the integrity of the membranes. During exercise due to anaerobic glycolysis in the blood accumulates lactic acid, which causes a decrease in pH (Fernando et al., 2009). Increasing the lactate that occurs in horses during exercise can play a key role in the pathogenesis of myocardial dystrophy in animals (Slivinska et al., 2018).

Our previous studies have established (Maksymovych, 2017) that the total calcium and inorganic phosphorus serum content in horses after a moderate-intensity activity tended to decrease. Physical activity of sport horses did not affect the exchange of magnesium in blood.

In sporting horses, after exercise the natrium blood serum content significantly decreases: in Ukrainian warmblood on 5.6% ($P < 0.05$), Hanoverian – 9.2% ($P < 0.01$) and Westphalian breeds on 13.3% ($P < 0.001$). The potassium serum content in the Hanoverian horses after the physical activity had the tendency to decrease (on 8.5%), whereas in the horses of the Ukrainian warmblood and Westphalian breeds the decrease was significant, respectively, on 10.5% ($P < 0.05$) and 19% ($P < 0.01$). Obviously that after physical activity, the reduction of sodium and potassium in serum of horses is due by the loss of electrolytes with sweat, which also causes the development of electrolyte disbalance (Maksymovych, 2017).

During activity in horses with metabolic imbalance, factors that are not related to feeding are more important. These include the loss of electrolytes with sweat, resulting in a violation of the neuromuscular transmission, and loss of water with sweat (dehydration) causes hemodynamic disturbances and impares supply of the oxygen and substrates, to the heart muscle (Lee and Jacobs, 2005). At the same time, the loss of water and electrolytes leads to the development of progressive metabolic alkalosis (Rose et al., 1979; McKeever, 2004; Piccione et al., 2008).

The results of our studies showed that an increase in the total protein content (dehydration), urea and creatinine (a violation of the functional state of the kidneys, which is characteristic of development uremic syndrome), a decrease in sodium and potassium levels (electrolyte imbalance), an increase in AST activity (cytolysis syndrome) can serve for early diagnosis of metabolic syndrome in horses during physical activity.

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3. Conclusions

1. Horse's metabolic syndrome is the result of insuffi- cient provision of organs and tissues with energy, accompa- nied by the development of dehydration, cytolysis, nephroticsyndrome, electrolyte imbalance.

2. Sport horses, have dehydration (hyperproteinemia), uremic syndrome (increased urea and creatinine concentra- tion), cytolysis syndrome (increased activity of AST and ALT) after physical activity.

3. The concentration of lactate increases in the blood af- ter exercise of sports horses of the Ukrainian warmblood, Hanoverian and Westphalian breeds.

4. The serum levels of sodium and potassium in horses decrease after physical activity due to the loss of electrolytes with sweat and the development of electrolyte imbalance.

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