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Criteria of diagnosing cardiomyopathies of different genesis in sport horses

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Sport horses suffering physical overexertion syndrome, anemic syndrome, and electrolyte imbalance may experience development of cardiomyopathy with various pathogenetic mechanisms. The objectives of our study were to determine the causes of pathogenesis, substantiate its individual links, and elaborate the methods of diagnosing cardiomyopathies of different genesis in sport horses (anemic syndrome, physical overexertion syndrome, electrolyte imbalance). The horses suffering cardiomyopathy were observed to have increased frequency of heart murmurs, arrhythmias, and valvular regurgitation. In the underperforming horses with metabolic disorders, the anemic syndrome manifested in a stable number of erythrocytes, content of hemoglobin, and hematocrit, whereas in the high-performing animals these parameters were significantly increased. Increase in the concentration of total bilirubin and iron in blood of the horses with anemic syndrome indicated anemia of hemolythic origin. In athletic horses, excessive physical exertion results in the development of the syndrome that manifests in dehydration (increase in the content of total protein), cholestasis (increase in the concentration of total bilirubin; tendency toward increase in the activities of alkaline phosphatese and gamma-glutamyl transpeptidase), cytolysis (increases in the activities of aspartate aminotransferase, total creatine kinase, cardiac isoenzyme of creatine kinase, lactate dehydrogenase, lactate dehydrogenase-1), and uremic syndrome (increase in the urea concentration and creatinine). A specific marker of cardiomyopathy in equine athletes was found to be heightened activity of cardiac isoenzyme of creatine kinase and concentration of lactate in blood. In horses subject to excessive exertion, cardiomyopathy develops as a result of electrolyte imbalance due to the loss of electrolytes with sweat (decline of sodium and potassium concentrations in blood serum). This is evidenced by dehydration (increase in the content of the total protein and hematocrit). Based on the conducted studies, we have elaborated the methods of diagnosing cardiomyopathies of different genesis in sport horses.

Keywords: heart murmurs; arrhythmias; markers; electrocardiography; echocardiography.

Introduction

As of now, people use horses in sport (Campbell, 2011), agriculture, recreation, and tourism (Ricketts, 2004). Equestrianism is an impressive sport, and interest to it is growing, including studying its effects on human health, opportunities of treating diseases of the cardiovascular and nervous systems, and rehabilitation of the ill (Montalcini et al., 2012). Horseback riding competitions have been gaining popularity, and therefore the numbers of sport equestrian clubs, schools, and private owners of horses are growing (Barandych, 2005).

Cardiology allows clinicians to diagnose heart diseases and determine the work capacity of horses (Yeates, 2009; Campbell, 2013). In horses, cardiac diseases rank third after gastrointestinal diseases and chronic obstructive pulmonary diseases. This is why it is necessary to take into account the effect of cardiac dysfunction on the health and work capacity of horses (Buhl et al., 2013), and to determine the severity of disorders of hemodynamics and accompanying diseases (Stevens et al., 2009), factors that are decisive for the course and prognosis (Andrijchuk et al., 2012). Most heart diseases in horses are asymptomatic and well-tolerated (Buhl & Ersbøll, 2006) because of the significant adaptive and compensatory properties of the cardiovascular system (Physick-Sheard & McGurrin, 2010; Sribhen et al., 2013). Cardiac diseases manifest at late stages (Buhl et al., 2013; Vazzana et al., 2014), when symptoms become clinically significant, with notable arrhythmias, loss of consciousness, and collapse. Therefore, timely diagnosis is crucial for the safety and wellbeing of people and horses (Nagy et al., 2012; Reef et al., 2014).

Horseback riding is characterized by high requirements to animals, significantly complicated contest conditions, changes in the methods of training, and increase in the intensity of exertions. Pursuit of high results leads to intensification of horse training, which enhances physical parameters, but also increases the likelihood of excessive physical exertion (Buergelt, 2003). Due to horses' insufficient fitness, they may experience pathological hypertrophy, myocardal hypoxia, and disorders in depolarization processes (Hinchcliff et al., 2002).

The work capacity of horses depends on the amount of oxygen (O_2) used by the organism during exertion, the ability to increase blood volume, and the efficiency of O_2 transport to the tissues (Gondim et al., 2009). Horses have several mechanisms of regulation of heightened O_2 consumption during exertion. Due to hemoconcentration in the spleen, the amount of circulating blood during exertion increases 65%, and a rise in heart contraction frequency and stroke volume can lead to a sixfold increase in the cardiac output (Hinchcliff et al., 2002).

Over the recent years, great achievements have been made in horse cardiology thanks to the studies in a number of institutions in Europe, North America, and Japan (Barbesgaard et al., 2010). The international veterinary community recognized the importance of diagnostics of heart diseases and arrhythmias in horses (Reef et al., 2014) and outlined the current recommendations for designing protocol for studying the cardiovascular system, since preservation of health and wellbeing of horses is key in races over long distances (Bennet & Parkin, 2018).

In horses, heart diseases manifest clinically under the conditions of expansion and remodeling of the myocardium (Clegg, 2012), when impairment in the blood supply of the cardiac muscle (ischemia) is accompanied by imbalance between the influx of O_2 and the cardiomyocytes' need of it. The myocardium is sensitive to tissue detoxication, which leads to coronary dysregulation, local inflammation, and peroxidation, cellular acidosis, ionic imbalance, and reduced synthesis of adenosine triphosphate acid, which cause qualitative changes in the membrane of cardiomyocytes and the development of dystrophy (Luethy et al., 2017). In human medicine, the reasons for myocardial dystrophy are classified into three groups. The first group includes

conditions when the influx of oxygen, oxidation substrates, and vitamins decreases. Such a myocardial dystrophy occurs in cases of starvation, hypovitaminosis, enteritis, liver failure, anemia, and is a result of myocardium hypoxia (hypoxic myocardial dystrophy). The second group of myocardial dystrophies comprises diseases that impair the process of cellular respiration, oxidative phosphorylation, and transmembrane cation exchange, and which reduce the formation of energy and effectiveness of its utilization. Those patterns of myocardial dystrophy are observed during electrolyte imbalance. The third group of myocardial dystrophies occurs in cases of elevated energy expenditures as a result of excessive load on the heart (dystrophy due to hyperfunction) (Dimopoulos et al., 2009).

During physical exertion, blood flow redistributes, and therefore a portion of blood is shunted to the muscles, leaving the tissues in the state of ischemia. Additionally, catecholamines – during oxidation of which active oxygen species form – are released into blood in greater amounts (Gunina & Nosach, 2012). Free radicals that form during dysoxia cause qualitative changes in the properties of membranes of cardiomyocytes and impair their functions.

Therefore, myocardial dystrophies develop due to discrepancy between the energy expenditures in the myocardium's functioning structures and their energy replenishment (Fielding et al., 2011). According to hypoxia theory, the pathological process is caused by oxygen insufficiency, which emerges in a hypertrophied myocardium, whereas the theory of neurodystrophy attributes it to the hypoxic effect of catecholamines (Dimopoulos et al., 2009).

Hypoxic myocardial dystrophy due to anemic syndrome. The health condition of horses subjected to exertion is monitored to exclude animals experiencing a metabolic crisis (Hinchcliff et al., 2004; Nagy et al., 2012). Exertion is accompanied by energy expenditures and development of hypoxia, and oxygenation of the tissues depends on the structural-functional condition of erythrocytes (Burak, 2008; Schöffel et al., 2008).

The main role in the development of metabolic dysfunction in horses is played by exhausting physical exertion (Vollaard et al., 2005), in particular long-distance races, three-day races (Verdegaal et al., 2023). The mechanisms of physical exhaustion include loss of fluid and electrolytes, disturbed pH balance, and loss of glycogen in the muscular tissue, which more often develops in non-trained animals (Gomez et al., 2020).

Myocardial dystrophy due to hyperfunction. Physical overexertion causes hypertrophy of the cardiac muscle, thereby increasing its need of blood and oxygen (Treiber et al., 2006). Because of energy expenditures during exertion (Migio et al., 2021), horses experience intensive use of adenosine triphosphate and development of a stable need for energy. The commonest causes of exclusion of horses from sport competitions are metabolic crisis and cardiovascular dysfunction. Exhaustive physical exertion manifests in dehydration and oxidative and thermal stress (Gunina & Nosach, 2012), and therefore the health of horses is monitored so as to detect animals with metabolic crisis before competitions (Burak, 2008).

Myocardial dystrophy associated with electrolyte imbalance. Electrolytes play a key role in the regulation and balance of fluids and pH, and support of osmotic pressure and nervous-muscular activity. Electrolyte metabolism is subject to a number of factors, and working horses lose those electrolytes with sweat (Coenen, 2013). During exertion, perspiration causes dehydration and loss of the main elements, mostly sodium and chlorine, and in lower amounts potassium, magnesium, and calcium (Shcherbatyy et al., 2017). The loss of fluid in horses during exertion causes a deficiency of ions in the myocardium and therefore its dysfunction.

A specific reaction in horses during exertion is changes in erythropoiesis parameters, which characterize the degree of saturation of erythrocytes with hemoglobin. Therefore, erythropoiesis parameters are measured to determine the degree of fitness and suitability of horses for exertion (Jesty, 2012).

Over recent decade, research on the biochemical aspects of assessment of heart activity made considereable progress, in particular regarding the contents of proteins, hormones, and activity of enzymes that are considered biochemical markers of myocardial damage (Cullen et al., 2013). Correlation between the heart biomarkers and prognosis of the heart failure was determined in animals (Van Der Vekens et al., 2012).

Myocardial diseases in human medicine are diagnosed by evaluating the activities of the following enzymes: creatine kinase, lactate dehydrogenase, and aspartate aminotransferase. At the same time, myocardial infarction is diagnosed based on the activities of the isoenzymes creatine kinase-MB and lactate dehydrogenase-1 (Buergelt, 2003; Adamu et al., 2012).

In horses, the activities of creatine kinase, lactate dehydrogenase, and aspartate aminotransferase are considered markers of damaged skeletal muscles (Sribhen et al., 2013). Since all these enzymes are present in the cardiac and skeletal muscles, differential diagnostics involves the identification of their isoenzymes (creatine kinase-MB and lactate dehydrogenase-1) (Adamu et al., 2012).

An informative test for assessing the degree of horses' fitness is concentration of lactic acid (lactate) in blood, which in healthy animals does not exceed 4 mmol/L. Hyperlactatemia is a marker of insufficient oxygenation of the tissues, which allows detecting horses with metabolic crisis (de Miranda et al., 2009).

The horses' working capacity is assessed according to hematocrit and the concentration of total protein in blood (dehydration assessment), and also by studying the content of electrolytes in blood (sodium, chlorine) prior to and after exertion (Franklin et al., 2012).

The objectives of this study were to determine the causes, substantiate certain links in the pathogenesis, and elaborate the methods of diagnosing cardiomyopathies of different genesis in sport horses (anemic syndrome, physical overexertion syndrome, and electrolyte imbalance).

Materials and methods

During the scientific studies, we adhered to the established norms of laboratory practice GLP (1981) and the provisions of the General Ethical Principles of Experiments on Animals, adopted by the First National Congress of Bioethics (Kyiv, 2001). The experiments were performed according to international standards, in particular the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), Rules of Conducting Work with the Use of Experimental Animals, according to the Order of Ministry of Healthcare No. 281 On Measures of Improving the Organization of Work Involving Experimental Animals as of November 1, 2000, and also according to the Law of Ukraine On Protection of Animals from Abuse.

The material for the study was sport horses of Ukrainian Riding, Hanoverian, and Westphalian breeds, used in classical equestrian sports (show jumping, dressage, and eventing). The daily training adhered to the scheme for using the animals in sport. The regular average-intensity training of the horses lasted for one hour: walking 5 min; posting 10 min; walking 5 min; sitting trot 10 min; walking 10 min; gallop with transition to walking 10 min; walking 10 min.

The mares were not pregnant or lactating and were clinically healthy at the moment of the study. All the horses underwent deworming and vaccination, and were kept in the same maintenance conditions. Depending on the cause and mechanism of development, the sport horses were diagnosed three groups of cardiomyopathies: anemic syndrome, overexertion, and electrolyte imbalance.

In the sport horses with cardiomyopathies due to anemic syndrome, physical overexertion, and electrolyte imbalance, we conducted a complex of clinical and additional studies (morphological and biochemical blood parameters, electrocardiography, and echocardiography). We took into account the peculiarities of the course of the disease (physical activity, incidence of arrythmia).

The predictors of heart failure in horses were determined according to the pulse rate, color of mucous membranes, and capillary refill time. Also, we evaluated dehydration according to the skin elasticity. The heart rate was estimated prior to exertion, immediately after exertion, and 10 and 30 min after exertion. Horses whose heart rate exceeded 42 bpm at rest were excluded (Maksymovych, 2017). We carried out clinical studies and analysis of morphological blood pa-

rameters. The equine athlets with cardiomyopathy associated with anemic syndrome were divided into two groups: the first included the animals (n = 50) that demonstrated high performance over a distance of 1,600 m (2 min 02 sec – 2 min 20 sec) and the second (n = 50) comprised horses that showed low results, travelling this distance in 2 min 30 sec – 2 min 50 sec.

In order to assess the effect of exertion on the myocardium, we distinguished a group of horses (n = 50) with cardiomyopathy due to physical overexertion. According to the results of blood assays, we distinguished the group of horses (n = 50) with the development of cardiomyopathy due to electrolyte imbalance.

In the horses, we performed a general blood test and analyzed the biochemical parameters that characterize the functional condition of the organs (liver, kidneys, heart), content of electrolytes, and dehydration parameters (total protein in blood, hematocrit). The blood samples from the horses were collected from the jugular vein prior to morning feeding. The samples were drawn using Ø 16×40 mm injection needles into test tubes (2.0 mL; Sarstedt, Germany) with anticoagulant (EDTA-K) and test tubes (10 mL; Vacutest, Italy) without anticoagulant. The samples were transported in a thermal container and analyzed within six hours of collection. In blood, we determined the number of red blood cells (RBC), hemoglobin (Hb), hematocrit (PCV/Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), and plateletcrit (PCT). The general blood analysis was conducted on a Mythic 18 automated hematological analyzer (Orphee S.A., Switzerland) using reagents manufactured by PZ Cormay S.A. (Poland).

To obtain blood serum, the test tubes were centrifuged at 3,000 rpm for 10 min. In blood serum, we determined the contents of total protein, albumin, total bilirubin, glucose, urea, creatinine, total calcium, inorganic phosphorus, magnesium, iron, activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, total creatine kinase and its cardiac isoenzyme (creatine kinase-MB), and total lactate dehydrogenase and lactate dehydrogenase-1 (hydroxybutyrate dehydrogenase). The study was carried out with a Mindray BS-120 automated biochemical analyzer (China), using reagents manufactured by PZ Cormay S.A. (Poland). The contents of the electrolytes sodium and potassium in blood serum were determined with a BioChem SA semiautomatic biochemical analyzer (USA) using reagents manufactured by High Technology Inc., Production RD Walpole (USA). The concentration of lactate was determined using the non-enzymatic method in whole blood (Cottin et al., 2006). The degree of dehydration of the horses was determined according to clinical tests (BPM, color of mucous membranes, capillary refill time, skin elasticity), and also hematocrit (microcentrifugation according to Shklyar), and the content of total protein in blood serum, using the Mindray BS-120 automatic biochemical analyzer (China) and reagents manufactured by PZ Cormay S.A. (Poland).

The obtained results were mathematically processed using the Biostatistica software and Statistica 7.0. The difference between two values was considered significant at the levels of $P < 0.05$, $P < 0.01$, and $P < 0.001$.

Results

The studies of the horses that were subjected to maximum effort during races over long distances revealed that that 23.5% of 54.0% of the animals excluded from competitions had metabolic disorders.

The capillary refill time in the clinically healthy horses was 2 sec, whereas due to heart failure it increased to three and more seconds (4%). Against the background of heart failure, the mucous membranes were red with cyanotic hue (6%).

The functional condition of the horses' cardiovascular system was determined by measuring the heart rate before exertion and during recovery. Prior to exertion, the heart rate in the sport horses ranged 28–42 bpm (34.0 ± 0.68). After exertion, it increased to 46.7 ± 1.19 bpm (36–69), and in 10% of the horses remained above 64 bpm (65–69). Ten minutes after the test, the heart rate accounted for 38.0 ± 0.85 bpm (31–60), and 30 min after the test it measured 35.9 ± 0.65 bpm (30–55). In 6% (>50 bpm) and 4% (>47 bpm) of the horses, it did not normalize after 10 and 30 min, respectively.

In 2% of the horses, the skin fold remained raised for 3 and more seconds after exertion (compared with 1-2 seconds in the healthy horses), indicating dehydration.

In the underperforming horses with anemic syndrome, tachycardia was observed in 10% (52.6 ± 1.36; 47–70 bpm), fatigue in 8%, and shortness of breath and heart murmur in 6% (72% of the animals; 60% in the high-performing horses). Arrhythmias were diagnosed in 70% of the underperforming horses (62% of the high-performing animals). Valvular regurgitation was found in 46% of the underperforming animals (42% of the high-performing horses).

Before exertion, the number of erythrocytes, content of hemoglobin, and hematocrit in the sport horses of both groups ranged within the range of physiological limits and did not differ statistically (Table 1). After exertion, the number of erythrocytes in the high-performing horses increased: by 10.5% in the Ukrainian Riding ($P < 0.01$), by 15.8%in Hanoverian ($P < 0.001$), and 14.1% in Westphalian ($P < 0.01$) horses. In the second-group, underperforming horses, the number of erythrocytes displayed an upward tendency: 2.6% and 1.4% in the Hanoverian and Westphalian horses, compared with the period prior to exertion, and in the Ukrainian Riding horses this parameter did not change (Table 1).

After exertion, the content of hemoglobin in blood of the first-group horses increased: by 10.1% ($P < 0.05$) in the Ukrainian Riding, by 16.7 ($P < 0.01$) in Hanoverian, and by 18.0% ($P < 0.001$) in Westphalian horses, while in the second group this parameter only tended to increase – by 2.5%, 1.9%, and 3.2%, respectively (Table 1).

After exertion, in the first-group horses, hematocrit increased in the Ukrainian Riding (12.0%; $P < 0.01$), Hanoverian (14.9%; $P < 0.01$), and Westphalian (12.8%; $P < 0.001$) horses, whereas in the second group this parameter only tended to increase (3.9%, 1.8%, and 2.2%, respectively; Table 1).

Therefore, the increases in the number of erythrocytes, content of hemoglobin, and hematocrit after exertion in the first-group horses reflects the release of erythrocytes from the spleen during the tissues' heightened need of oxygen. The low parameters of erythropoiesis in the second-group horses after exertion are obviously a result of the development of anemic syndrome, as evidenced by the results of analysis of total bilirubin and iron in blood serum.

Under the conditions of anemic syndrome (the second experimental group), the concentration of total bilirubin in blood serum of the Ukrainian Riding horses was 63.5% higher, compared with the value prior to exertion ($P < 0.01$). This parameter was also higher than that of the clinically healthy horses (34.0%; $P < 0.05$). The total bilirubin content in the Hanoverian and Westphalian horses was 39.1% ($P < 0.01$) and 59.8% ($P < 0.001$) higher that prior to exertion, respectively, as well as higher compared with the clinically healthy animals (34.4%; $P < 0.01$ and 30.8%; $P < 0.01$, respectively).

The concentration of iron in blood serum of the sport horses prior to and after exertion was also elevated (Table 2). The values of hematocrit, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in the sport horses of both groups before and after exertion did not vary between the groups. Only after exertion, in the Westphalian horses, we determined higher indices of MCH and MCHC of the first ($P < 0.05$) and MCHC ($P < 0.05$) of the second group, compared with the parameters before exertion. This was obviously associated with a heightened saturation of erythrocytes with hemoglobin for provision of oxygenation in the tissues.

Before exertion, the number of platelets in the sport horses did not differ between the groups. After exertion, their number in the first-group horses increased, in particular by 26.0% ($P < 0.05$) in the Westphalian horses, while in the Ukrainian Riding and Hanoverian horses it tended to increase (18.6% and 19.9%). In the second-group horses, the number of platelets had an upward tendency in all three breeds, compared with the period prior to exertion: by 10.0% in the Ukrainian Riding, by 13.0% in Hanoverian, and by 5.3% in Westphalian horses. Certainly, the higher number of platelets in the first-group horses resulted from physical exertion.

Table 1
Parameters of erythropoiesis in the sport horses (x ± SD)

Breeds	Groups of horses	First group (n = 50)			Second group (n = 50)		
		erythrocytes, 10^{12}/L	hemoglobin, g/L	hematocrit, %	erythrocytes, 10^{12}/L	hemoglobin, g/L	hematocrit, %
Ukrainian Riding Horse (n = 20)	prior to exertion	7.62 ± 0.17^{ab}	126.4 ± 4.1^{a}	33.2 ± 0.8^{b}	7.74 ± 0.19^{b}	125.7 ± 3.6^{a}	33.1 ± 0.8^{a}
	after exertion	8.42 ± 0.19^{c}	139.2 ± 3.4^{b}	37.2 ± 1.0^{c}	7.71 ± 0.20^{b}	128.8 ± 3.3^{a}	34.4 ± 0.9^{b}
Hanoverian (n = 15)	prior to exertion	7.57 ± 0.17^{ab}	124.0 ± 3.2^{a}	32.1 ± 0.9^{a}	7.59 ± 0.24^{b}	125.0 ± 4.1^{a}	32.4 ± 0.7^{a}
	after exertion	8.77 ± 0.22^{c}	144.7 ± 4.8^{c}	36.9 ± 1.0^{b}	7.77 ± 0.25^{b}	127.4 ± 3.9^{a}	33.0 ± 1.0^{a}
Westphalian (n = 15)	prior to exertion	7.14 ± 0.16^{a}	122.0 ± 2.8^{a}	32.8 ± 0.8^{a}	7.16 ± 0.22^{a}	124.0 ± 3.3^{a}	32.3 ± 0.9^{a}
	after exertion	8.11 ± 0.21^{b}	144.0 ± 2.2^{c}	37.5 ± 0.8^{c}	7.19 ± 0.25^{a}	128.0 ± 2.2^{a}	33.0 ± 0.8^{a}

Note: different letters in a row indicate that data samplings are significantly (P < 0.05) different one from another according to the Tukey test.

Table 2
Content of iron in blood serum of the sport horses before and after exertion (μmol/L)

Breed	Prior to exertion	After exertion
Ukrainian Riding	31.7 ± 2.12^{b}	32.0 ± 2.03^{b}
Hanoverian	29.9 ± 1.84^{a}	28.2 ± 1.24^{a}
Westphalian	29.4 ± 1.88^{a}	30.4 ± 1.79^{a}

Note: see Table 1.

Therefore, according to the results of the study, the sport horses with metabolic disorders experienced anemia of clearly hemolytic origin (increased concentrations of total bilirubin and iron in blood), accompanied by changes in the parameters of hematopoiesis, characteristic of the anemic syndrome.

In the horses subjected to physical overexertion, we registered heightened fatigue (36%), moderate shortage of breath (25.9 ± 0.82, 16–44 bpm), tachycardia (46.4 ± 1.20, 36–68 bpm, 64%), heart murmurs (70 %), arythmies (80%), and valvular regurgitation (44%).

Measuring the content of total protein allows determining the index of organism's dehydration, including loss or reduction of synthesis. After exertion, the content of total protein increased, obviously as a result of dehydration. This was especially notable in the Hanoverian (P < 0.01) and Westphalian (P < 0.05) horses (Table 2). In blood of the Hanoverian horses, we found an increase in albumins (P < 0.05; Table 2). After exertion, the concentration of total bilirubin increased in the Westphalian horses (P < 0.05), whereas in the other breeds we determined a tendency toward increase: by 22% in the Ukrainian Riding and 3.5% in Hanoverian horses (Table 2).

The increase in bilirubin was definitely a result of the development of cholestasis, which is associated with biliary dyskinesia. Cholestasis was evidenced by an upward tendency in the activities of alkaline phosphotase and gamma-glutamyl transpeptidase in blood of the horses (Table 3).

After exertion, the concentration of glucose in blood serum declined by 7.7% in the Westphalian horses (P < 0.05), and in the other breeds it had a tendency toward decrease: by 5.4% in the Ukrainian Riding and by 3.4% in Hanoverian horses (Table 2). The content of urea after exertion increased in the horses of all the breeds: by 17.2% (P < 0.05) in the Ukrainian Riding, 9.8 (P < 0.05) in Westphalian, and 5.4% in Hanoverian horses. At the same time, the content of creatinine increased in all the breeds, but was within the range of physiological fluctuations: by 22.2% (P < 0.01) in the Ukrainian Riding, by 13.9 (P < 0.05) in Hanoverian, and by 33.9% (P < 0.001; Table 3) in Westphalian horses. Therefore, when subjected to physical overexertion, horses can experience a disturbance of the glomerular filtration of the kidneys and development of uremic syndrome.

Table 3
Biochemical parameters of blood serum of the sport horses with cardiomyopathy associated with physical overexertion (x ± SD)

Breeds	Study period	n	Total protein, g/L	Albumines, g/L	Total bilirubin, μmol/L	Glucose, mmol/L	Urea, mmol/L	Creatinine, μmol/L
Ukrainian Riding	prior to exertion	20	61.3 ± 1.2^{a}	37.8 ± 0.8^{ab}	24.1 ± 2.1^{a}	5.53 ± 0.20^{b}	5.32 ± 0.23^{a}	132.8 ± 5.4^{ab}
	after exertion	20	63.8 ± 1.6^{ab}	38.0 ± 0.6^{ab}	29.4 ± 2.4^{c}	5.23 ± 0.19^{a}	6.23 ± 0.28^{b}	162.3 ± 5.9^{c}
Hanoverian	prior to exertion	15	60.9 ± 1.0^{a}	36.2 ± 0.8^{a}	28.1 ± 1.7^{bc}	5.48 ± 0.13^{a}	5.65 ± 0.16^{a}	127.7 ± 6.9^{a}
	after exertion	15	66.3 ± 1.3^{b}	39.4 ± 0.7^{b}	29.1 ± 1.8^{c}	5.30 ± 0.11^{a}	5.90 ± 0.29^{ab}	145.4 ± 4.1^{b}
Westphalian	prior to exertion	15	61.2 ± 1.6^{a}	38.7 ± 1.0^{ab}	26.6 ± 1.2^{b}	5.64 ± 0.09^{b}	5.21 ± 0.24^{a}	123.4 ± 3.9^{a}
	after exertion	15	67.1 ± 2.1^{b}	39.2 ± 0.4^{b}	32.5 ± 1.7^{d}	5.20 ± 0.11^{a}	5.53 ± 0.34^{a}	165.2 ± 5.1^{c}

Note: see Table 1.

As of now, to evaluate the functional condition of the myocardium, clinical and instrumental studies are conducted, and the diagnostics of cardiomyopathy is based on determining the activities of enzymes in blood serum.

In blood serum of the sport horses after exertion, we saw increases in the activity of aspartate aminotransferase: the Ukrainian Riding (P < 0.05), Hanoverian (P < 0.001), and Westphalian (P < 0.01) horses. The activity of alanine aminotransferase was increased only in the Westphalian horses (P < 0.01; Table 3).

Hyperenzymemia of aspartate aminotransferase is associated with disrupted permeability of the cell membrane of the skeletal muscules and development of cytolysis syndrome. The increased activity of the enzyme in blood of the horses after physical exertion could also have resulted from its elimination from cardiomyocytes. Therefore, the horses subjected to overexertion experienced increases in the concentration of total protein (dehydration) and the activity of aspartate aminotransferase (cytolysis syndrome). In addition, we determined upward tendencies in the concentrations of urea and creatinine (uremic syndrome), which can be useful for early diagnostics and prevention of physical overexertion syndrome (metabolic crisis). After exertion, the activity of total creatine kinase in blood serum increased by 17.3% (P < 0.05) in the Hanoverian horses. In the horses of the other breeds (Ukrainian Riding Horse and Westphalian), we found only an upward tendency (Table 4). According to the parameters of total creatine kinase, it is impossible to determine damage to cardiomyocytes. Therefore, the 'gold standard' for biochemical diagnosis of myocardial damage has recently been the isoenzyme of creatine kinase, particularly creatine kinase-MB.

Based on the results of the studies, after exertion, the activity of creatine kinase-MB in blood serum increased in all the breeds of horses: Ukrainian Riding Horse (P < 0.05), Hanoverian (P < 0.05), and increased the most in the Westphalian horses (P < 0.001; Table 5). Release of creatine kinase-MB from the cytolysis pool of cardiomyocytes in regularly trained horses indicates an irreversible damage to the myocardial cells, and hyperenzymemia of creatine kinase-MB can be an early diagnostic marker of their damaged state.

During physical overexertion, the sport horses experienced damage particularly to the myocardial cells, because the activity of CK-MB in blood increased by 26.1% in the Ukrainian Riding, 22.0% in Hanoverian, and 44.3% in Westphalian horses, which is 1.26, 1.22, and 1.44 times higher, respectively, compared with the values prior to exertion.

At the same time, the activity of total creatine kinase increased by 1.19 times in the Ukrainian Riding and by 1.17 times in Hanoverian

and Westphalian horses; whereas the activity of aspartate aminotransferase increased by 1.17, 1.14, and 1.16 times, respectively. During the development of rhabdomyolysis syndrome, the activities of creatine kinase and aspartate aminotransferase in the horses increased by 10–900 and 5–100 times, respectively.

Table 4
Activity of enzymes in blood serum of the horses with cardiomyopathy associated with physical overexertion (U/L, x ± SD)

Breeds of horses	Period of study	n	Aspartate aminotransferase	Alanine aminotransferase	Alkaline phosphatase	Gamma-glutamyl transpeptidase
Ukrainian Riding	prior to exertion	20	257.2 ± 10.1^{a}	6.41 ± 0.54^{b}	112.2 ± 8.4^{ab}	13.5 ± 0.9^{c}
	after exertion	20	300.0 ± 16.0^{b}	7.29 ± 1.05^{c}	116.5 ± 9.7^{ab}	14.2 ± 1.3^{d}
Hanoverian	prior to exertion	15	257.2 ± 7.0^{a}	5.53 ± 0.34^{a}	111.3 ± 5.6^{ab}	11.0 ± 0.7^{a}
	after exertion	15	292.9 ± 8.1^{b}	6.53 ± 0.46^{b}	120.3 ± 6.6^{b}	12.2 ± 0.9^{b}
Westphalian	prior to exertion	15	259.7 ± 11.3^{a}	5.20 ± 0.36^{a}	106.9 ± 5.0^{a}	12.8 ± 0.8^{b}
	after exertion	15	302.5 ± 6.9^{b}	8.71 ± 0.95^{d}	109.2 ± 7.6^{a}	13.8 ± 1.2^{c}

Note: see Table 1.

Table 5
Activity of cardiospecific enzymes in blood serum of the horses with cardiomyopathy associated with physical overexertion (U/L, x ± SD)

Breeds of horses	Period of study	n	creatine kinase	creatine kinase-MB	Lactate dehydrogenase	LDH-1
Ukrainian Riding	prior to exertion	20	160.7 ± 13.1^{a}	208.1 ± 9.7^{a}	502.7 ± 16.6^{b}	225.0 ± 9.9^{a}
	after exertion	20	190.8 ± 18.9^{b}	262.5 ± 21.3^{b}	555.1 ± 30.5^{c}	253.0 ± 17.9^{b}
Hanoverian	prior to exertion	15	189.5 ± 12.2^{b}	246.5 ± 15.3^{b}	551.3 ± 18.9^{c}	244.3 ± 10.0^{b}
	after exertion	15	222.3 ± 10.4^{c}	300.7 ± 19.5^{c}	614.7 ± 24.3^{d}	266.2 ± 11.9^{b}
Westphalian	prior to exertion	15	167.8 ± 10.2^{a}	203.1 ± 7.9^{a}	433.2 ± 19.6^{a}	205.8 ± 14.8^{a}
	after exertion	15	197.0 ± 13.5^{b}	293.1 ± 19.7^{c}	544.9 ± 34.4^{c}	251.6 ± 15.9^{b}

Note: see Table 1.

Another enzyme indicating the development of cardiomyopathy is total lactate dehydrogenase and its isoenzyme lactate dehydrogenase-1. The activity of total lactate dehydrogenase in the Ukrainian Riding and Hanoverian horses prior to exertion was the same. Then, in the Ukrainian Riding horses, it increased 10.4%, whereas in the Hanoverian horses it increased 11.5% ($P < 0.05$). The lowest values of the enzyme prior to exertion were observed in the Westphalian horses. However, after exertion, the value increased 25.8% ($P < 0.05$; Table 4).

The conducted studies of the activity of lactate dehydrogenase-1 revealed its 22.3% ($P < 0.05$) rise in blood of the Westphalian horses after exertion. At the same time, in the Ukrainian Riding and Hanoverian horses, only an upward tendency was observed (12.4 and 9.0%, respectively; Table 4).

Thus, the cardiomyopathy marker in Westphalian horses is hyperenzymemia of creatine kinase-MB, total lactate dehydrogenase, and its isoenzyme lactate dehydrogenase-1. In Hanoverian horses, the marker is hyperenzymemia of total creatine kinase, creatine kinase-MB, and total lactate dehydrogenase. In Ukrainian Riding horses, the marker is hyperenzymemia of creatine kinase-MB. The most informative parameter for diagnostics of cardiomyopathy in the sport horses after physical overexertion was the activity of cardic isoenzyme of creatine kinase (creatine kinase-MB).

A sensitive test to the metabolic changes in horses is lactate in blood. According to the results of the conducted studies, the content of lactate after exertion increased in the Ukrainian Riding ($P < 0.001$), Hanoverian ($P < 0.05$), and Westphalian ($P < 0.05$) horses (Table 6).

One of the possible determinants of changes in the permeability of cardiomyocytes is reduced synthesis of adenosine triphosphate, necessary for the support of membrane's integrity. Physical exertion results in anaerobic glycosis in blood, leading to accumulation of lactic acid. Hyperlactatemia, observed in the horses subjected to exertion, plays a key role in the pathogenesis of cardiomyopathy, because it increases the permeability of the muscle cells and release of enzymes into blood. Creatine kinase and lactic acid are positively associated, because the effect of increased lactate concentration on the permeability of cellular membranes has been demonstrated.

Table 6
Parameters of lactate in blood of the horses with cardiomyopathy associated with physical overexertion (mmol/L, x ± SD)

Breeds of horses	N	Prior to exertion	After exertion
Ukrainian Riding	20	4.93 ± 0.26^{b}	6.49 ± 0.18^{c}
Hanoverian	15	3.42 ± 0.20^{a}	4.33 ± 0.29^{b}
Westphalian	15	3.47 ± 0.15^{a}	4.29 ± 0.24^{b}

Note: see Table 1.

According to the study results, we may state that the diagnostic criteria of syndrome of physical overexertion in horses are increase in the content of total protein (dehydration), increases in the activity of aspartate aminotransferase, creatine kinase, creatine kinase-MB, lactate dehydrogenase, and lactate dehydrogenase-1 (cytolysis syndrome), and hyperlactatemia, indicating the development of cardiomyopathy, and also upward tendencies in the concentrations of urea and creatinine (uremic syndrome).

As a result of electrolyte imbalance, heart murmurs were observed in 66%, arrythmia in 78%, and valvular regurgitation in 38% of the animals.

The content of total calcium in blood of the sport horses did not differ significantly before and after exertion (Table 7). At the same time, in the Hanoverian and Westphalian horses, we observed a downward tendency after exertion (by 2.7% and 5.3%).

The measurement of inorganic phosphorus in the sport horses after exertion revealed a downward tendency: by 7.3% in the Ukrainian Riding, 12.2 in Hanoverian, and 6.8% in Westphalian horses (Table 7). The downward tendency in the content of inorganic phosphorus in blood of the horses after exertion was a result of using phosphate for providing the working muscles with energy.

Table 7
Content of mineral compounds in blood serum of the sport horses with cardiomyopathy associated with electrolyte imbalance (mmol/L, x ± SD)

Breeds of horses	Period of study	n	Total calcium	Inorganic phosphorus	Magnesium	Sodium	Potassium
Ukrainian Riding	prior to exertion	20	3.00 ± 0.06^{b}	0.823 ± 0.046^{b}	0.762 ± 0.018^{a}	140.3 ± 1.7^{b}	3.80 ± 0.13^{b}
	after exertion	20	2.97 ± 0.02^{b}	0.764 ± 0.030^{a}	0.759 ± 0.014^{a}	132.5 ± 2.8^{ab}	3.40 ± 0.11^{ab}
Hanoverian	prior to exertion	15	2.91 ± 0.07^{a}	0.981 ± 0.062^{b}	0.793 ± 0.018^{ab}	138.4 ± 2.7^{ab}	3.76 ± 0.11^{b}
	after exertion	15	2.83 ± 0.03^{a}	0.862 ± 0.054^{b}	0.804 ± 0.013^{b}	125.7 ± 3.2^{a}	3.44 ± 0.16^{ab}
Westphalian	prior to exertion	15	2.99 ± 0.07^{b}	0.880 ± 0.052^{b}	0.762 ± 0.017^{a}	141.9 ± 2.5^{b}	3.89 ± 0.11^{b}
	after exertion	15	2.83 ± 0.02^{a}	0.819 ± 0.040^{b}	0.790 ± 0.014^{ab}	123.0 ± 3.9^{a}	3.15 ± 0.18^{a}

Note: see Table 1.

The magnesium content in blood serum of the Ukrainian Riding, Hanoverian, and Westphalian horses before and after exertion underwent no changes (Table 6). Obviously, average-intensity exertion has no effect on exchange of this macroelement in blood of horses.

A significant role in the activity of the cardiovascular system and thermal regulation during physical exertion is played by fluid balance in the organism. The main role in water exchange is played by endocrine system through the balance of electrolytes, in particular sodium.

After exertion, the content of sodium in blood serum of the sport horses was decreased: by 5.6% in the Ukrainian Riding ($P < 0.05$), 9.2% in Hanoverian ($P < 0.01$), and 13.3% in Westphalian horses ($P < 0.001$; Table 7). Disturbance of the electrolyte balance was accompanied by a decrease in the potassium content in blood serum after exertion: by 10.5% in the Ukrainian Riding ($P < 0.05$) and 19% in Westphalian ($P < 0.01$) horses. In the Hanoverian horses, its content did not change significantly (8.5%; Table 7).

Obviously, the decreases in the contents of sodium and potassium in blood of the sport horses after average-intensity exertion resulted from the loss of electrolytes with sweat, which causes their imbalance. The loss of electrolytes with sweat and development of dehydration in the sport horses were evidenced by hyperenzymemia and the increase in hematocrit: Ukrainian Riding ($P < 0.01$–0.001), Hanoverian ($P < 0.001$–0.01), and Westphalian ($P < 0.01$–0.001) (Table 8). Perhaps, the most important factor in the development of cardiomyopathy in horses subjected to physical overexertion is the loss of electrolytes with sweat.

Table 8

Concentrations of total protein and hematocrit in the sport horses with electrolyte imbalance (x ± SD)

Breeds of horses	Period of study	n	Total protein, g/L	Hematocrit, %
Ukrainian Riding	prior to exertion	20	61.6 ± 1.1^{a}	32.7 ± 0.7^{a}
	after exertion	20	67.5 ± 1.5^{b}	38.4 ± 0.9^{b}
Hanoverian	prior to exertion	15	61.3 ± 0.8^{a}	32.2 ± 0.8^{a}
	after exertion	15	68.6 ± 1.5^{c}	37.9 ± 1.2^{b}
Westphalian	prior to exertion	15	62.9 ± 1.1^{ab}	31.2 ± 0.6^{a}
	after exertion	15	69.9 ± 1.7^{d}	38.1 ± 1.2^{b}

Note: see Table 1.

Therefore, in sport horses subjected to physical exertion, the loss of sodium and potassium with sweat causes dehydration (increases in the content of total protein and hematocrit), which leads to development of cardiomyopathies.

Discussion

Heart rate and rhythm are informative for assessment of metabolic status of horses subjected to physical exertion. Difference in clinical tests between horses with metabolic crisis and healthy horses is insignificant, although allows preventing the development of threatening symptoms. A prognostic parameter of cardiac insufficiency during physical exhaustion and metabolic crisis is tachycardia (Golovakha et al., 2017). An important marker of heart failure in horses is recovery of the heart rate after exertion to normal level.

It was determined that after physical overexertion, test with normalization of the heart rate allowed detecting horses with heart failure. In particular, right after exertion, tachycardia was registered in 10% of the horses; 10 min after exertion, this condition was found in 6%; and 30 min after exertion, 4% of the horses presented with this condition. The capillary refill time in the horses increased to 3 and more seconds (4%), the mucous membranes became red with cyanotic hue (6%), and the skin elasticity decreased, the fold persisting for 3 and more sec (2%).

According to the results of the clinical, laboratory, and instrumental studies, the horses were diagnosed with three groups of cardiomyopathies, in particular due to overexertion and anemic syndromes and electrolyte imbalance.

The horses subjected to exertions were observed to have increased cardiac output, heightened pulmonary arterial pressure, and optimized blood circulation due to erythrocytes, hemoglobin, hematocrit, and other erythrocyte parameters, which enhance the transport of oxygen to the tissues (Vazzana et al., 2014). Under such conditions, the circulatory system receives blood from the spleen, and thus the circulating volume of blood increases. In the spleen of horses, 30% of erythrocytes are deposited (6 to 12 L of blood) (Slivinska & Maksymovych, 2018). During anemic syndrome, 72% of the underperforming horses were diagnosed heart murmurs. At the same time, in the high-performing animals, heart murmurs were observed in 60%, arrhythmia in 70/62, and valvular regurgitation in 46/42% of the horses, respectively.

After exertion, blood of the high-performing horses contained significantly elevated concentrations of erythrocytes, hemoglobin, and hematocrit. Such hematopoietic adaptation allows for an increase in the oxygen volume in blood for the provision of muscular tissue, including myocardium cells with oxygen, increasing the aerobic capacity and decreasing the concentration of lactate in the muscules (Allsopp et al., 2024). At the same time, lower parameters of erythropoiesis in the second-group sport horses resulted from the development of anemic syndrome (Franklin et al., 2012). Our assumption is evidenced by the results of the studies of total bilirubin and iron in blood serum. Thus, total bilirubin increased in the Ukrainian Riding horses, compared with the value prior to exertion ($P < 0.01$) and the clinically healthy animals ($P < 0.05$); Hanoverian and Westphalian ($P < 0.01$; $P < 0.001$) and the clinically healthy horses ($P < 0.01$; $P < 0.01$). Physical overexertion has been observed to cause increase in total bilirubin concentration in blood in other studies (McLaren et al., 2019).

In sport medicine, there is a pathophysiological condition called sport anemia, characterized by low values of hemoglobin, hematocrit, and erythrocytes. Etiology of this condition is multifactorial, and one of the causes is increase in the blood volume, which occurs right after exertion or long training, as was determined in athletes. Other authors attribute 'sport anemia' to episodes of intravascular hemolysis. After maximum exertion, sport horses were also observed to have anemia of hemolytic origin (Burlikowska et al., 2015).

It was determined that after intensive training, athlets experience deficiency of iron, which leads to anemia. According to McLaren et al. (2019), the presented pathogenetic mechanism is related to accumulation of iron in the liver, which, during training, limits the utilization of this element from the bone marrow and reduces its availability for hemapoiesis.

Another study revealed that the content of iron in blood of the sport horses was higher than the reference values (13.0–25.0 µmol/L). Such a mechanism is associated with intensification of erythropoiesis and is reflected in high values of erythrocytes, hemoglobin, and hematocrit. At the same time, a loss of iron in blood of the horses was observed, with the concentration ranging 12.0–18.3 µmol/L. In another study, high values of iron were determined in the healthy horses (29.3 µmol/L).

In the sport horses with anemic syndrome, the concentration of iron in blood serum before and after exertion was increased, measuring 31.7 ± 2.12 and 32.0 ± 2.03 µmol/L in the Ukrainian Riding, 29.9 ± 1.84 and 28.2 ± 1.24 in Hanoverian, and 29.4 ± 1.88 and 30.4 ± 1.79 µmol/L in Westphalian horses, respectively.

Our results are consistent with the literature data, where sport horses were observed to have increased content of iron in blood (Andriichuk et al., 2014). This has been attributed to exertion-induced hemoconcentration. However, other studies (Fielding et al., 2009) found an increase in iron concentration in athletes 30 minutes after exertion, indicating that this was unrelated to hemoconcentration.

Therefore, during physical overexertion, sport horses experience development of anemia of hemolytic genesis (concentrations of total

bilirubin and iron in blood increase) (Ussher & Lopaschuk, 2006). Such horses travelled a 1,600 m distance in 2 min 30 sec – 2 min 50 sec (low performance), whereas healthy high-performing horses travelled this distance in 2 min 0 sec – 2 min 20 sec.

Is also plausible that exertion is accompanied by the activation of the blood coagulation system (Holovakha et al., 2005). Change in the reactivity of platelets can affect hemostasis and play an important role in pathogenesis of exercise-induced pulmonary hemorrhage in horses (Hudson et al., 2008).

After exertion, the number of platelets increased in both the high-performing and underperforming horses, respectively: Ukrainian Riding (by 18.6%/10%), Hanoverian (19.9%/13.0%), and Westphalian horses ($P < 0.05$/5.3%).

Exertion in horses requires energy expenditures. A stable energy need can be accompanied by metabolic disorders and multiple organ failure (cardiorespiratory, endocrinous, and nervous-muscular systems) (Miglio et al., 2021).

Besides diseases with colic syndrome, a common reason for the exclusion of horses from sport competitions is metabolic crisis and cardiovascular dysfunction.

One of the determinants of changes in the permeability of cellular membranes, including cardiomyocytes, is lowered synthesis of adenosine triphosphate. During maximal exertion, the use of adenosine triphosphate is intensive, while the rates of provision of substrates and oxygen are insufficient. The main substrates for producing energy in cardiomyocytes are free fatty acids and glucose, from which – with participation of oxygen – adenosine triphosphate is formed. During hypoxia, glucose is broken down by anaerobic glycosis, with formation of pyruvate, which under the conditions of O_2 deficit transforms to lactate, which accumulates in the muscles. Increase in the concentration of the latter enhances the intracellular acydosis, overloads the cells with sodium and calcium, and damages cardiomycete membranes (Demircan et al., 2009).

Of the horses with cardiomyopathy associated with physical overexertion, heart murmurs were heard in 70%, arrhythmias were detected in 80%, and valvular regurgitation was found in 44%.

Metabolic dysfunction due to excessive physical overexertion was manifested in hyperenzymemia, especially in the Hanoverian ($P < 0.01$) and Westphalian ($P < 0.05$) horses. It should be noted that dehydration was observed in the horses that were excluded from competitions due to metabolic crisis.

In blood of the horses, we observed a rise in total bilirubin: Westphalian ($P < 0.05$), Ukrainian Riding (by 22%), Hanoverian (3.5%). These increases resulted from the development of cholestasis, associated with biliary dyskinesia (Ohtra et al., 2004), the presence of which was indicated by the upward tendency in the activities of alkaline phosphatase and gamma-glutamyl transpeptidase in blood.

Furthermore, gamma-glutamyl transpeptidase is considered a marker of oxidative stress (Maksymovych & Slivinska, 2017), which is key in the development of metabolic dysfunction (Böhm et al., 2011). The development of oxidative stress was observed in horses subjected to physical exertion. Also, heightened activity of gamma-glutamyl transpeptidase in blood is attributed to low level of fitness (Maksymovych et al., 2017).

Physical exertion causes increase in the level of free radicals of oxygen that exert a vessel-narrowing effect, thereby decreasing the rates of glomerular filtration through inactivation of cyclooxygenase in endothelial cells. Release of mediators – endothelin, catecholamines, angiotensin II, cytokines – also promotes the development of renal ischemia and acute kidney failure (Slivinska et al., 2018).

After exertion, the horses of all the breeds exhibited increases in the concentrations of urea (Ukrainian Riding and Westphalian ($P < 0.05$–0.05), and Hanoverian (by 5.4%) horses) and creatinine (Ukrainian Riding ($P < 0.01$), Hanoverian ($P < 0.05$), Westphalian ($P < 0.001$) horses). Therefore, horses subjected to physical overexertion experience malfunctioning glomerular filtration and development of uremic syndrome (Richard et al., 2009).

Diagnostics and prediction of cardiac diseases in people are carried out according to the contents of proteins, hormones, activities of enzymes, myoglobin, and cardiospecific troponins T and I in blood, which serve as biochemical markers of myocardial damage (Brown et al., 2007).

Myocardial diseases are diagnosed according to the activities of creatine kinase, lactate dehydrogenase, and their cardiac isoenzymes (creatine kinase-MB, lactate dehydrogenase-1), and also by analyzing the enzymatic profile of blood (combination of activities of several enzymes and their isoenzymes). Since cardiac pathology in horses can be clinically asymptomatic, study of heart markers is necessary (Buergelt, 2003).

It is unknown to what extent physical overexertion in horses is accompanied by myocardial damage and the release of cardiac markers into the bloodstream (Mythili & Malathi, 2015). It was determined that in blood of the sport horses subjected to physical overexertion, the activity of asparatate aminotransferase increased: Ukrainian Riding ($P < 0.05$), Hanoverian ($P < 0.001$), and Westphalian ($P < 0.01$) horses.

Creatine kinase is an enzyme that catalyzes a highly energetic compound creatine phosphate from adenosine triphosphate and creatine. This compound provides energy to the muscular fibers, and the enzyme's activity reflects the processes of catabolism in the unaltered tissue. In the horses subjected to overexertion, the activity of creatine kinase increased: by 17.3% in the Hanoverian($P < 0.05$), by 18.7 in Ukrainian Riding, and by 17.4% in Westphalian horses.

Creatine kinase is composed of subgroups M (muscular type) and B (brain type), which unite three isoforms: creatine kinase-MM (muscular fraction), creatine kinase-BB (brain, absent in blood serum), and creatine kinase-MB (cardiac fraction) (Zhuravlova & Kulikova, 2019).

It was determined that the activity of CK-MB in blood of the Ukrainian Riding ($P < 0.05$) and Hanoverian ($P < 0.05$) horses increased, with hyperenzymemia being most pronounced in the Westphalian horses ($P < 0.001$). The activity of creatine kinase-MB in blood increases within 3–4 hours after myocardial damage, reaching a diagnostically significant level after 4–6 hours, and remains elevated for 48–72 hours. At the same time, aspartate aminotransferase and lactate dehydrogenase peak after 24 h. Therefore, release of creatine kinase-MB from the cytosolic pool within cardiomyocytes in horses can indicate damage to myocardial cells.

Lactate dehydrogenase is an enzyme that is localized in the organs and tissues as five isoenzymes: lactate dehydrogenase-1 and lactate dehydrogenase-2 in the cardiac muscle; lactate dehydrogenase-3 in the pancreas, lung tissue, and suprarenal glands; and lactate dehydrogenase-4 and lactate dehydrogenase-5 in the muscular tissue and liver (Butudom et al., 2004). The activity of total lactate dehydrogenase during overexertion increased: Ukrainian Riding (by 10.4%), Hanoverian ($P < 0.05$), and Westphalian ($P < 0.05$) horses.

Also, an increase was observed in the activity of lactate dehydrogenase-1 in blood of the horses: Westphalian ($P < 0.05$), and Ukrainian Riding (12.4%), and Hanoverian (9.0%).

Therefore, hyperenzymemia of aspartate aminotransferase, creatine kinase-MB, lactate dehydrogenase, lactate dehydrogenase-1 in blood of horses during physical overexertion is a result of elimination of the enzyme from cardiomyocytes as a result of their damage and development of cytolysis syndrome (Sribhen et al., 2007). A specific marker of cardiomyopathy in sport horses can be considered the cardiac isoenzyme creatine kinase-MB, whose activity grew in blood of all three breeds of horses. A less specific marker was lactate dehydrogenase-1.

Since the enzymes aspartate aminotransferase, creatine kinase, and lactate dehydrogenase are present in the heart and skeletal muscles, differential diagnostics of rhabdomyolysis syndrome and cardiomyopathy is performed by analyzing isoenzymes (Brown et al., 2007; Harris & Schott, 2013; Lesyk et al., 2022; Verveha et al., 2023; Dunaievska et al., 2024). In sport horses subjected to physical overexertion, myocardial cells are damaged. This was evidenced by the activity of creatine kinase-MB in blood, which increased by 26.1% in the Ukrainian Riding, by 22.0% in Hanoverian, and by 44.3% in Westphalian horses, or by 1.26, 1.22, and 1.44 times, respectively. In addition, the activity of creatine kinase increased by 1.19 times in the Ukrainian Riding and by 1.17 in Hanoverian and Westphalian horses; and the activity of aspartate aminotransferase increased by 1.17, 1.14,

and 1.16 times, respectively. During the development of rhabdomyolysis syndrome, the activities of creatine kinase and aspartate aminotransferase increased by 10–900 and 5–100 times, respectively (Zhuravlova & Kulikova, 2019).

An informative biomarker for evaluation of the health condition of horses is measuring lactate concentration in blood. During exertion, hyperlactatemia causes damages to muscles, including cardiomyocytes (Demircan et al., 2009), and plays a key role in the pathogenesis of cardiomyopathy (Crocomo et al., 2009). The lactate content during physical overexertion increased in blood of the sport horses of all the breeds ($P < 0.05–0.001$).

An important role in the regulation and balance of fluid, pH, and nervous-muscular activity is played by electrolytes. During exertion, perspiration promotes thermal regulation in horses, and at the same time is accompanied by dehydration and loss of sodium, chlorine, potassium, magnesium, and calcium. During electrolyte imbalance, impairments occur in the processes of cellular respiration, oxidative phosphorylation, and transmembrane exchange of cations, leading to reduced generation of energy in the myocardium (Noleto et al., 2016). Due to electrolyte imbalance, 66% of the horses presented with heart murmurs, 78% with arrhythmias, and 38% with valvular regurgitation.

Muscular cells contain higher concentration of phosphorus, which is necessary for providing them with energy. Magnesium is a universal regulator of biochemical and physiological processes in the organism, which enables metabolism of many enzymes and transmembrane transport of ions. Moreover, it plays an important role in the exchange of calcium, phosphorus, sodium, and potassium (Maksymovych, 2017). In the sport horses, prior to and after exertion, the content of total calcium did not differ significantly, although in the Hanoverian and Westphalian horses it displayed a downward tendency (by 2.7% and 5.3%, respectively). Also, after exertion, there was determined a downward tendency in inorganic phosphorus: 7.3% in the Ukrainian Riding, 12.2% in Hanoverian, and 6.8% in Westphalian horses. Obviously, the downward tendency in the concentration of inorganic phosphorus in the horses after exertion was a result of using phosphate through ATP-phosphorilation in order to provide the muscles with energy. At the same time, exertion had no effect on the magnesium exchange.

After exertion, the sport horses exhibited development of electrolyte imbalance, because the concentrations of sodium and potassium decreased in the Ukrainian Riding ($P < 0.01–0.05$), Hanoverian ($P < 0.01$), and Westphalian ($P < 0.001–0.01$) horses. This occurred as a result of its loss with sweat. The degree of dehydration is determined based on the concentrations of total protein and hematocrit in blood (Franklin et al., 2012). In horses participating in long-distance races, fluid loss through sweat can reach 25%, leading to electrolyte imbalance (decreases in the concentrations of sodium and potassium in blood), which is a cause of cardiomyopathies. The equine athletes subjected to overexertion experienced hyperenzymemia and increased hematocrit: the Ukrainian Riding ($P < 0.01–0.001$), Hanoverian ($P < 0.001–0.01$), and Westphalian ($P < 0.01–0.001$) horses, respectively. Electrolyte imbalance causes impairments in the nervous-muscular conductivity, whereas dehydration disturbs the hemodynamics and transport of oxygen and substrates to cardiomyocytes, thereby causing cardiomyopathies.

Conclusions

The sport horses were diagnosed with three groups of cardiomyopathies: due to physical overexertion and anemic syndromes and electrolyte imbalance, during which we found high-level heart murmors (70/72/66%), arrhythmias (80/70/78%), and valvular regurgitation (44/46/38%).

Anemic syndrome in the sport horses manifested in tachycardia (10%), fatigue (8%), shortness of breath (6%); and also stable parameters of the number of erythrocytes, hemoglobin content, and hematocrit. During asthmatic syndrome, the horses developed hemolytic anemia, accompanied by the increase in the concentration of total bilirubin: 63.5% in the Ukrainian Riding ($P < 0.01$), compared with the value prior to exertion ($P < 0.01$) and the clinically healthy animals (34.0%; $P < 0.05$); 39.1 ($P < 0.01$) and 34.4 ($P < 0.01$) in Hanoverian; and 59.8 ($P < 0.001$) and 30.8% ($P < 0.01$) in Westphalian horses, respectively.

The sport horses subjected to physical overexertion experienced metabolic syndrome, which manifested in fatigue (36%), tachycardia (64%), dehydration, and cytolysis syndrome (hyperenzymemia of aspartate aminotransferase).

Cardiospecific markers of damaged cardiac muscle and development of cardiomyopathies due to metabolic syndrome in the horses were the increase in the activity of creatine kinase-MB, increase in the concentration of lactate in blood, and less notable was hyperenzymemia of lactate dehydrogenase-1.

Physical overexertion in the bred sport horses was accompanied by electrolyte imbalance and development of cardiomyopathies (decrease in sodium concentration in blood serum) and dehydration (increase in concentrations of total protein and hematocrit in blood).

The authors claim no conflict of interest.

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