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ORIGINAL PAPERS

SELECTED ELECTROLYTIC, HAEMATOLOGICAL AND ENZYMATIC PARAMETERS IN HORSES DURING ENDURANCE RACES

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Abstract

Long-distance endurance races are a big challenge for a horse's body. The prolonged exercise and dehydration associated with it, in adverse circumstances, may dysregulate many metabolic processes. The aim of this study was to assess changes in electrolyte balance in horses during endurance races, and to explain the potential need for electrolyte supplementation. The experiment was performed on fifty-six horses aged 9-15 years. Haematological and biochemical parameters of horse blood were tested before, during and after the completion of a 120 km endurance race. Electrolyte status (Na^+, K^+, Cl^-) was assessed by analysing test results in horses that completed the race without any clinical symptoms of metabolic disorders and received no additional mineral supplements before the race. The following haematological parameters were determined: red blood cell and white blood cell counts, haemoglobin levels, haematocrit and red blood cell indices (MCV, MCH, MCHC). Enzymatic tests involved the serum levels of lactate dehydrogenase and creatine kinase. A significant increase was found in the levels of haemoglobin, lactate dehydrogenase and creatine kinase activity, but the values of these parameters were within the normal physiological ranges. Other parameters (WBC, RBC, Ht, MCV, MCH, MCHC, Na^+, K^+, Cl^-) changed slightly, but were also within the ranges characteristic of sporthorses. The results suggest that horses well prepared for effort do not require additional supplementation of minerals during endurance races under conditions of the Polish climate.

Keywords: horse, exercise, effort, enzymes, haematology, biochemical parameters, dehydration, endurance rides.

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INTRODUCTION

Success in sports, regardless of animal species, always requires the enhancement of metabolic processes to optimally use physical capabilities. Technical endurance training allows for the development of mechanisms helping to improve the performance of the whole body. Body fluids, i.e. blood with its all constituents, and extracellular and intracellular fluids with substances dissolved in them, play an extremely important role in the development and maintenance of adaptation processes during strenuous exercise (Munoz 2010, Nagy et al. 2012). These fluids are also involved in gas exchange, delivery of energetic and structural substances, elimination of metabolites, and in thermoregulation by the dissipation of excess heat from active tissues and its distribution in tissues of lower temperature. All these functions require close communication between the blood, and extra- and intracellular fluids (Bergero et al. 2005, Adamu et al. 2010).

High-intensity exercise, associated with enhanced metabolic processes in myocytes, stimulates ion exchange between the interior of the cells and their environment necessary for muscle work, but also reflects the regulatory activity of a living organism to maintain homeostasis despite the increased production of metabolites. The local circulation of fluids between watery spaces in muscles is also enhanced by differences in hydrostatic pressure resulting from changes in their volume due to work of myocytes. The electrolyte balance is additionally affected by an increased count of red blood cells released from the spleen and stimulated by catecholamines and the activation of sympathetic nerves during physical exercise in sporthorses, and also in animals during transportation (Niedźwiedź et al. 2012). This leads to increased blood viscosity and flow resistance, which, because of changes in the hydrostatic pressure gradient, provokes changes in the volume and ionic composition of the water spaces (Bergero et al. 2005, Piccione 2007), and these in turn can induce muscle damage (Wael, Sabry 2010).

Thermoregulation during exercise is another process that should be considered when analysing electrolyte balance in sporthorses. In horses, the excess heat generated during strenuous exercise is eliminated from the body through evaporation in the respiratory system, and mainly by the evaporation of sweat from the skin surface. This process results in the loss of water and also electrolytes and proteins, changing the volume and composition of fluids in intra- and extracellular spaces (Waller et al. 2007, Munoz et al. 2010). The complexity of these mechanisms responsible for the electrolyte balance in horses during strenuous effort makes it difficult, if not impossible, to develop a universal strategy aimed at minimising the negative effects of high-intensity exercise on animal health. Studies on the supplementation of water and electrolytes in horses, both before and during races, failed to provide conclusive results (Bergero et al. 2005, Waller et al. 2007, Fielding et al. 2011, 2012). Some authors (Burk, Williams 2008) suggest that right fe-

eding management can improve water and electrolyte balance in horses organism during exercise.

The objective of this study was to assess changes in electrolyte balance in horses during endurance races, and to explain the potential need for electrolyte supplementation.

MATERIAL AND METHODS

The study was conducted during 120 km endurance races in three different locations, in similar terrain conditions (on flat terrain - max. 30 m difference in elevation). Races took place in the summer months in similar weather conditions: air temperature 16-21°C, humidity 55-82%.

Fifty-six horses (24 mares, 2 stallions and 30 geldings) aged 9-15 years, 32 Arabian and 24 Thoroughbred, were included in the study. The animals qualified for the study were clinically healthy, admitted to take part in races by professional regulatory veterinarians, had no symptoms of disease or dehydration at physical examination on the day of the competition, and completed the race with no pathological symptoms. For two months before the competition and during the race horses had been fed with standard fodder, watered with water from the mains or natural reservoirs or waterways, and were not given any supplements potentially affecting electrolyte balance.

The analysis of selected clinical parameters of animals and blood sampling for laboratory tests was carried out just before the race start, and within 3 minutes after completing distances of 60, 90 and 120 km. Horses with symptoms indicating a pathological condition were withdrawn from further stages of the race and received necessary veterinary treatment. Blood samples for tests were taken from the jugular vein into plastic test tubes prefilled with di-potassium EDTA (haematological analysis), and with a coagulation activator to obtain serum for biochemical tests. Blood for haematological tests was refrigerated at 8°C until used for analysis. Blood samples with the coagulation activator were kept at room temperature (16-21°C) for 25-45 min to allow clot formation, and then centrifuged for 15 min at 3000 rpm. The obtained serum was transferred into clean test tubes and stored at 8°C until transported to a laboratory.

The following haematological parameters were measured: white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin concentration (HGB), haematocrit value (Ht) and parameters of red blood cells, i.e. mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), using an ADVIA 2120i haematology analyser from Siemens. Serum samples were analysed for concentrations of sodium (Na), potassium (K), and chloride (Cl) by using an ion-selective method on a Rapidlab 348 blood gas analyser from Siemens. The

activity of lactate dehydrogenase (LDH) and creatine kinase (CK) was measured by using the kinetic method on an ACCENT-200 Cormay biochemical analyser. Reagents were supplied by Cormay.

Data from measurements were statistically analysed with the t-Student test.

RESULTS AND DISCUSSION

The values of haematological and biochemical parameters measured in blood samples taken just before race start, and within 3 minutes after completing distances of 60, 90 and 120 km are presented in Table 1.

Table 1

Mean values of haematological and biochemical parameters in endurance horses measured before race start, and after the completion of 60, 90 and 120 km distances (n = 56, mean ± s)

WBC (10^9 l^{-1})	9.32* ± 1.67	11.08 ± 2.04	11.31 ± 2.87	12.01 ± 2.06
RBC (10^{12} l^{-1})	8.56 ± 2.93	8.64 ± 3.12	8.97 ± 3.58	9.03 ± 2.71
HGB (g l^{-1})	132.12* ± 14.87	138.39 ± 17.02	139.58 ± 13.46	140.79 ± 16.99
Ht (%)	38.06 ± 6.09	40.27 ± 5.26	41.45 ± 7.89	41.63 ± 6.04
MCV (fl)	43.71 ± 7.58	44.16 ± 6.75	45.53 ± 7.12	45.42 ± 8.09
MCH (pg)	15.434 ± 2.34	16.017 ± 2.61	15.561 ± 3.29	15.591 ± 3.72
MCHC (g l^{-1})	347.136 ± 24.38	343.655 ± 26.109	336.743 ± 22.824	338.193 ± 26.018
Na^+ (mmol l^{-1})	142.45 ± 3.26	148.12 ± 4.23	149.02 ± 4.37	144.79 ± 3.95
K^+ (mmol l^{-1})	3.97 ± 0.96	3.72 ± 1.02	3.43 ± 1.28	3.58 ± 1.84
Cl^- (mmol l^{-1})	102.31 ± 6.54	104.01 ± 5.48	106.26 ± 6.81	105.87 ± 5.83
LDH (U l^{-1})	289 ± 42.08	352 ± 57.33	370 ± 61.12	439* ± 68.34
CK (U l^{-1})	237.9 ± 84.75	294.5 ± 98.18	387.9 ± 79.94	478.2* ± 64.13
Distance	0	60 km	90 km	120 km

* difference statistically significant at $p \leq 0.01$

There were no statistically significant differences between the sex, age and breed of the tested horse populations in measured parameters.

The concentrations of basic haematological parameters measured directly before the race start were within the normal ranges characteristic for healthy adult horses. The mean WBC count at race start was 9.32 x 10^9 l^{-1}, and gradually increased with the race distance to a mean of 12.01 x 10^9 l^{-1} after 120 km, which was above the normal physiological limit. Lower fluctuations and increase were found for the RBC count (mean 8.56 x 10^{12} l^{-1} at start and 9.03 x 10^{12} l^{-1} after race completion). A slight increase was also observed for the haemoglobin (HGB) concentration in the blood of the analysed horses. The mean HGB concentration was 132.1 g l^{-1} directly before the start, increased to 138.4 g l^{-1} after 60 km, and later stabilised at 139.6 g l^{-1} after 90 km, and at 140.8 g l^{-1} after 120 km. The increase in the haematocrit value (Ht) was most significant during the first 60 km (from 38.06% to 40.27%). Less pronounced changes in Ht were found after longer distances (41.45% after 90 km and 41.63% after 120 km). The mean corpuscular volume (MCV) increased slightly from the start (43.71 fl) to sampling 90 km (45.53 fl), and then slightly decreased after race completion (45.42 fl). The lowest mean corpuscular haemoglobin (MCH) was recorded before the start (15.43 pg); and the highest after 60 km (16.02 pg). After another 30 km, the MCH decreased to 15.56 pg and remained stable until the end of the race (15.59 pg). The mean MCHC decreased slightly between the start (347.1 g l^{-1}) and 90 km (336.7 g l^{-1}), and then increased after race completion to 338.2 g l^{-1}.

The concentrations of electrolytes measured in serum were in the normal range for racing horses. The mean sodium (Na^+) concentration was 142.4 mmol l^{-1} at the start, increased significantly to 149.0 mmol l^{-1} after 90 km, and decreased at the end of the race to 144.8 mmol l^{-1}. The mean potassium (K^+) concentration measured in serum was 3.97 mmol l^{-1} before the start, decreased to 3.43 mmol after 90 km, and increased to 3.58 after 120 km. The mean concentration of chlorides (Cl^-) increased significantly from 102.3 mmol l^{-1} before the start to 106.3 mmol l^{-1} after 90 km, and then decreased to 105.9 mmol l^{-1} after race completion.

The activity of lactate dehydrogenase (LDH) in the serum of the analysed endurance horses increased significantly from 289.0 U l^{-1} before the start to 439.0 U l^{-1} after the end of the race. The activity of creatine kinase (CK) also increased significantly from 237.9 U l^{-1} to 478.2 U l^{-1}.

Physical effort in endurance racehorses is associated with intensive muscle work. An increased metabolic rate results in the generation of a large amount of heat which has to be eliminated by the animal to maintain a constant body temperature optimal for health. Thermoregulation is achieved by using the excess energy to evaporate water from the skin surface and the respiratory system (Munoz et al. 2010). Sweat evaporation decreases the temperature of the skin and blood in subcutaneous vessels. Because of the composition of sweat, electrolytes, mainly sodium and chloride ions, as well

as proteins, are also lost from the organism of a horse which is sweating (Teixeira-Neto et al. 2012). In the respiratory system, hyperventilation associated with physical exercise mainly leads to water loss (Munoz et al. 2010). Intensive muscle work also causes changes in the acid-base status, which leads to changes in the level of electrolytes in water spaces. These changes, reflecting an animal's adaptation to workload and maintaining homeostasis, should not have pathological consequences for equine health. Negative changes in the horse's body can be prevented by suitable training, nutrition and the well-planned strategy for completing an endurance ride (Piccione et al. 2007).

Changes in the values of haematological parameters reflect the adaptation of an organism to prolonged physical exercise. In the examined horses, water loss associated with thermoregulation resulted in increased blood density, which was manifested by the increasing haematocrit, red blood cell count and white blood cell count. Changes in these parameters can also result from the release of reserve red blood cells, stored in the spleen, stimulated by increased demand for oxygen and in response to stress caused by taking part in the race (Piccione et al. 2007, Adamu et al. 2010). However, this process has no effect on changes in white blood cell counts, which Adamu et al. (2012) attributed to increased blood density due to dehydration during exercise, or response to infections and infectious agents or tissue damage. The observed increase in the WBC count, although still in the normal physiological range, may also be associated with the effect of endogeneous adrenalin and cortisol released into the blood stream in response to the stress associated with endurance rides (Larsson et al. 2013). The neurohormonal background of increase in haemoglobin concentration, RBC and WBC counts is also suggested by changes in the mean corpuscular haemoglobin, which decreased during our study, although it should increase in a case of dehydration and increased blood density. This mechanism was reported by Teixeira-Neto et al. (2012). Indirectly, the lack of drastic changes in electrolyte balance is also suggested by minor, normal fluctuations for healthy horses in the mean corpuscular haemoglobin and the mean corpuscular volume. Similar findings were reported by Larsson et al. (2013) and Teixeira-Neto et al. (2012) who did not link them with pathological changes in the horse's body during physical exercise.

A slight increase in the mean corpuscular volume observed in subsequent tests may suggest electrolyte imbalance, associated with a drop in the osmotic pressure of serum and migration of free water into red blood cells. However, the increasing concentration of the major extracellular cation – sodium – contradicts this hypothesis. Moreover, the concentration of chlorides also increased in the studied horses. These fluctuations, not reaching abnormal levels, may indicate optimal preparation of the horses for the race and their proper performance (Teixeira-Neto et al. 2012), but could have also resulted from the supplementation of water or other fluids containing ap-

propriate electrolytes. Such a correlation was reported by Drobnik et al. (2012) in people drinking water from medicinal springs. As previously mentioned, a large amount of heat is generated during intensive muscle work, and this activates the evaporation of water from the surface of the respiratory system, and sweat, together with the electrolytes, evaporating from the skin surface. In unfit or overworked horses, the adaptability of the body is exhausted, which leads to dehydration, loss of electrolytes and an increase in blood density. This initiates a cascade of homeostatic disorders as described by Bergero et al. (2005) and by Scarpa et al. (2007), which may even lead to the animal's death. Increased blood density is associated with increased blood viscosity and reduced perfusion of capillary vessels, and thus impaired exchange of metabolites, gases and heat in tissues. Intensified sweating increases dehydration and loss of electrolytes, mainly sodium and chloride. This leads to an imbalance in the regulation of the electrolyte and acid-base status in the kidneys, and more severe pathological conditions. One such condition is metabolic acidosis caused by intensified anaerobic processes in the working muscles, leading to the increased synthesis of lactic acid. Intracellular buffering is an example mechanism maintaining the pH of a body at an acceptable level. This process relies on the exchange of potassium and hydrogen cations between the cytoplasm and extracellular space (Piccione et al. 2007). The concentration of H^+ decreases and the concentration of K^+ increases in the extracellular water space, resulting in the normalization of its pH. After normalization of acid-base disorders the exchange of cations occurs again and homeostasis is recovered, unless cell damage has taken place (Foreman et al. 2003, Larsson et al. 2013). In our study, no increase was found; contrary, a slight decrease in the concentration of K^+ in serum was detected, which indicates the efficient performance of adaptation mechanisms in the horses in terms of acid-base metabolism, mainly gas exchange in the lungs. Increase in the concentration of H^+ associated with acidosis stimulates the respiratory centre and leads to hyperventilation. This results, among other things, in the increased elimination of carbon dioxide (carbonic acid anhydride) and reduction of acidosis. The respiratory centre responds to disorders of the acid-base status soon after their onset (several minutes), so the mechanism is activated after 10-20 min of high-intensity exercise. Similar conclusions were reached by Fazio et al. (2014), who studied horses during simulated show jumping tests.

Prolonged acidosis, especially during strenuous muscular work, promotes changes in the configuration of contractile proteins and myocyte damage. Damage to cellular structures is reflected in an increased activity of enzymes, normally found inside cells, observed in serum or plasma. Creatine kinase and lactate dehydrogenase are characteristic enzymes of mammalian muscle tissue (Niedźwiedź et al. 2012, Żarczyńska et al. 2013). They are involved in carbohydrate and energy metabolism in the cytoplasm of myocytes, and after cell damage their activity in blood increase. Adamu et al. (2010, 2012, 2014) and Larsson et al. (2013) explained similar changes in the acti-

vity of enzymes by the increased permeability of cytoplasmatic cell membranes during intensive metabolic transformations. The activity of CK and LDH increased significantly in the serum of the horses submitted to our experiment, but were still in the normal ranges for sporthorses, which, according to the aforementioned authors, may be associated with the enhanced perfusion of capillary vessels in muscles during exercise, and does not indicate muscle damage.

CONCLUSIONS

Haematological and biochemical blood tests in horses carried out during long-distance endurance rides revealed that appropriate preparation of animals before the ride, well-planned performance strategy, and standard feeding and watering prevent electrolyte imbalance in endurance horses racing in Poland during the summer season. We found no reasons for using electrolyte supplementation in horses without pathological symptoms, or to rehydrate horses beyond their natural instinct for drinking.

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