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RESEARCH ARTICLE

The Distance Run is Not the Determining Factor on the Occurrence of Electrolytic Change for Equine Athletes Disqualified in Endurance Competitions

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A B S T R A C T

This study evaluated 18 equines, disqualified only by exhaustion in endurance championship, by having completed 30, 60 or 90 km distance. Immediately after disqualification by exhaustion, the animals received thorough clinical examination. Hematocrit, total plasma protein, anion gap, excess/deficit of base, osmolality, and pH were measured, as well as analysis of the concentration of ions hydrogen, bicarbonate, sodium, potassium, calcium and chloride. Moderate hypovolemia due to dehydration resulting from the exercise was observed. The exhaustion caused by physical exertion altered the thermoregulatory mechanism, lowering the osmolality which was accompanied by sodium depletion. The distance run was not the determining factor for the occurrence of exhaustion; this condition was associated with the intensity of training.

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INTRODUCTION

The equestrian endurance is characterized as a sports activity of varying intensity and prolonged effort (Dumont *et al*., 2011). This type of exercise promotes an intense sweating, resulting in loss of body fluids and electrolytes (Dumont *et al*., 2012). The volume of fluid lost through sweating can be very high and its inadequate replacement may result in dehydration that when associated with energy deficits may be responsible for the occurrence of exhaustion which can lead the animal to death (Lacerda-Neto *et al*., 2003; Silva *et al*., 2009)

Exhaustion is defined as a metabolic syndrome resulting from the deleterious effects caused by dehydration, electrolyte disturbances, hyperthermia and depletion of energy substrates (oxidative metabolism). Affected animals may exhibit depression, weakness, stumbling in walking, lameness, anorexia, glassy stare, expressionless, and dry and congested mucous membranes (Wickler *et al*., 2006).

Clinical and laboratory evidences of mild to moderate dehydration are present in horses finalists in endurance competitions (Dumont *et al*., 2012). However, a more intense dehydration may have negative effects on performance and on vital functions. Hypovolemia, for example, reduces the perfusion to skeletal muscle and other major organs, resulting in inefficient supply and use of energy substrates. Furthermore, in severe dehydration, heat dissipation is compromised, and if exercise is continued, the thermoregulatory mechanisms are overwhelmed with serious consequences for the health of the equine athlete. Another consequence of excessive sweating is electrolyte depletion, once equine sweat is isotonic or slightly hypertonic in relation to plasma containing high concentrations of Na^+, K^+ and Cl^- and moderate concentrations of Ca^{+2} and Mg^{+2} (Teixeira-Neto *et al*., 2004). Because of this, prolonged sweating causes significant deficits, promoting weakness, muscle cramps, acid-base imbalances, cardiac arrhythmias, decreased performance and eventually a condition of exhaustion (Munoz *et al*., 2010).

Acid-base and electrolyte disorders have been described in horses associated with prolonged and resistance exercises, however, many of these studies used exercise testing, laboratory evaluations on high speed treadmill, and dehydration induced processes. Therefore, the aim of this study was to evaluate the electrolyte parameters of equine athletes immediately after disqualification by metabolic disorders in endurance competitions.

MATERIALS AND METHODS

This study evaluated 18 equines, 14 males and four females, disqualified only by exhaustion of the Regional

Championship of the Equestrian Federation of Brasilia, in Brasilia/DF, Brazil, by having completed 30, 60 or 90 km distance. The exercise was of low intensity and long duration, with an average speed of 20 km/h. Animals disqualified by exhaustion were those affected by metabolic disorders with involvement of vital functions (Wickler *et al*., 2006). The study followed the standards of the Ethics Committee on Animal Use of the University of Brasilia protocol #88-2009.

Animals were evaluated at the time of disqualification, i.e., immediately after the official veterinary inspection (vetcheck), before any treatment. Complete clinical examination was conducted, with collection of venous blood samples for evaluation of hematocrit (Hct) and total plasma protein (TPP) through blood analysis (Abacus Junior Vet®, Diagon, LTDA, Belo Horizonte, Minas Gerais State, Brazil).

Venous blood was collected anaerobically, through jugular vein puncture, withdrawing 1.6ml using syringes for sampling blood for blood gas analysis (3ml, 0.7x25mm, 22G-BD Preset Eclipse®) as described by Dumont *et al*. (2012). Samples were identified and kept refrigerated for up to four hours until processing. The analysis was performed on an analyzer of blood gas, electrolytes, hemoglobin, hematocrit and O_2 saturation (OMNI C®-Roche Diagnostics), determining the concentration of ions hydrogen (H+), bicarbonate ($HCO^{-}{}_{3}$), sodium (Na+), potassium (K+), calcium (Ca+2) and chloride (CL-), besides the values of anion gap (AG), excess/deficit of base (EB), osmolality (Osm), and negative logarithm of the activity of hydrogen ions (pH). AG was calculated according to Fettig *et al*. (2012): $AG=([Na^+]+[K^+])-([Cl^-]+[HCO_3^-])$.

The results were tested for homoscedasticity using the Kolmogorov-Smirnov test. The parametric parameters were assessed by the one-way ANOVA with post hoc Holm-Sidak test, and nonparametric data were subjected to Kruskal-Wallis with Dunn's post-test. Data were also tested using the Pearson correlation. For all analyses $P \leq 0.05$ was considered as statistically significant. Data were presented as mean±SE (GraphPad Prism 6.02 for Windows, Graph Pad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Data concerning to values of Hct, TPP, H^+, HCO^{-3}, Na^+,Ca^{+2}, K^+ and Cl^-, as well as values of AG, EB, Osm and pH are shown in Table 1.

Analysis of hematocrit and total plasma protein: The plasma protein concentration was higher than the reference value, the same was observed to the hematocrit value. These parameters, when taken together, suggest that the disqualified animals had severe degree of dehydration. Severe dehydration corroborates data from Muñoz *et al*. (2010), which stated that the disqualified animals undergo more substantial changes in the homeostasis than animals more apt to exercise, derived from the increased water loss and electrolyte change.

Acid-base status: The value of the H^+ ion (38.51±0.78 mmol/L) differed from that obtained from polo horses of high handicap (Ferraz *et al*., 2010) due to increased production of H^+ protons as a function of the effort, however, was similar to values obtained for equine finalists in endurance races (Viu *et al*. 2010; Dumont *et al*. 2012). Therefore, despite disqualification, the physical activity performed by these animals was not able to induce metabolic acidosis, being compatible with submaximal exercise.

The plasma concentration of HCO^{3-} was determined by PCO^{-2} and it is expected to compensate for the increased lactate, aiming its reduction (Ferraz *et al*., 2010). In a previous study with equine finalist of endurance (Dumont *et al*., 2012) there was an increase of PCO^{-2}, however, the increase in lactate annulled the elevation of HCO^{-3}. For disqualified animals, there was no change in this parameter (27.45±0.64 mmol/L), coinciding with that established as physiological (Wickler *et al*., 2006). Although the analysis was performed immediately after the disqualification of animals, this result has led us to understand that between the time of vet check, until the moment of evaluation, there might have been a respiratory compensation (Viu *et al*., 2010), by increasing the PO^{-2}, neutralizing the PCO^{-2} and in turn nullifying the changes resulting from the increased concentration of HCO^{-3}. This result showed that despite the disqualification by exhaustion, the acid-base balance, with regard to the PO^{-2} and PCO^{-2} was rapidly restored.

Regarding EB, this involves and quantifies other aspects of the acid-base balance (Viu *et al*., 2010). Once it would be possible to express what should be added (negative BE) or subtracted (BE positive) from bases for the body to maintain its optimum pH, that is, associating with variations of HCO^{-3} and pH, which would characterize the status of this balance (Silva *et al*., 2009). The EB behavior was similar to that of HCO^{-3} and of pH, with values below those found by Di Filippo *et al*. (2009) in healthy horses that completed courses of 60 km. Therefore, it can be considered a limiting factor and not very suitable to use as a single parameter for determining the occurrence of electrolyte imbalance in horses.

Anion gap: There was some similarity when comparing the values of AG found herein with those obtained in horses finalists of 90 km endurance races (Dumont *et al*., 2012). In horses finalists in 120 km races (Viu *et al*., 2010), a significant increase was detected in the AG during the race, when set a direct relationship with the distance traveled, emphasizing that animals at the end of the race had their parameters returned to baseline values. For those disqualified, we did not analyze the parameters during the race, but the AG found at the time of disqualification was slightly increased, at the upper limit of the reference value (Table 1). This suggests that during the race, the AG increased gradually and after the stop of animals the index decreased, with tendency to restore to the physiological level. Besides that, animals showed signs of dehydration at the time of disqualification, i.e., the increase in this variable would be associated with lactic acidosis due to anaerobic exercise with lactate accumulation, dehydration, organic acidosis, renal failure and hypovolemia, factors that could be related to slight compensatory acidosis (Viu *et al*., 2010).

Table 1: Hemato-biochemical parameters of horses disqualified by exhaustion after prolonged endurance exercise

Parameters	Units	Mean±SEM	Normal Range
Hct	%	52.34±1.35	32-42
PPT	(g/dL)	8.9±0.18	6.6-7.4
H^+	(mmol/L)	38.51±0.78	32-44
HCO^{3-}	(mmol/L)	27.45±0.64	27.1-31.9
AG	(mmol/L)	15.20±0.74	6-15
EB	(mEq/L)	2.40±0.65	-6±6
Osm	(mOsm/L)	265.86±1.9	268.60-279.12
Na^+	(mmol/L)	132.97±1.01	133-144
K^+	(mmol/L)	3.36±0.15	3.2-4.2
Ca^{+2}	(mmol/L)	1.29.3±0.6	1.54-1.69
Cl^-	(mmol/L)	93.36±1.03	94-104
pH	-	7.44±0.01	7.32-7.44

Hct-hematocrit, TPP-total plasma protein, H^+, hydrogen ion, HCO^{-3}-bicarbonate ion, AG-anion gap, EB- excess of base; Osm-osmolality, Na^+-sodium ion, Ca^{+2}- ionized calcium, K^+-potassium ion, Cl^--chloride ion, pH-potential of hydrogen.

Table 2: Hemato-biochemical parameters evaluated according to different distances traveled until the time of disqualification of horses in endurance race (Mean ± SEM)

Parameters	Units	30 km	60 km	90 km
Hct	%	44±4.2[a]	50±1.6[b]	55±1.41[b]
PPT	(g/dL)	8.7±0.3	8.8±0.3	9.0±0.2
H^+	(mmol/L)	39.45±3.45	37.43±1.35	38.98±1.02
HCO^{3-}	(mmol/L)	25.40±1.8	26.62±0.88	28.37±0.91
AG	(mmol/L)	18.60±0.6[a]	17.77±0.64[a]	13.61±0.86[b]
EB	(mEq/L)	0.6±2.3	2.08±1.01	2.96±0.93
Osm	(mOsm/L)	277±1.25	268±2.39	262±2.24
Na^+	(mmol/L)	139.2±0.46	133.3±0.94	130.9±1.2
K^+	(mmol/L)	3.5±0.07	3.22±0.24	3.48±0.23
Ca^{+2}	(mmol/L)	1.61±0.1[a]	1.37±0.08[a]	1.17±0.06[b]
Cl^-	(mmol/L)	98.85±0.45	93.23±1.79	92.35±1.32
pH	-	7.40±0.03	7.43±0.01	7.41±0.01

Hct-hematocrit, TPP-total plasma protein, H^+, hydrogen ion, HCO^{-3}-bicarbonate ion, AG-anion gap, EB- excess of base; Osm-osmolality, Na^+-sodium ion, Ca^{+2}- ionized calcium, K^+-potassium ion, Cl^--chloride ion, pH-potential of hydrogen. Values bearing different letter in a row differ significantly (P<0.05).

Relationship between Anion gap and Bicarbonate: The Pearson correlation test between the evaluated parameters allowed observing the existence of a strong negative linear correlation (r=-0.76) (Fig. 1) between the AG and the HCO^{-3}, corroborating studies of Viu *et al*. (2010), who suggested that animals were subjected to a complex electrolyte imbalance in which the hypochloremic metabolic alkalosis would be minimized by the presence of a mild metabolic acidosis.

Total concentration of sodium ion: The values of the sodium ion were lower than of animals finalists of 90 km races (Dumont *et al*., 2012), when they were slightly below the lower limit established as physiological for horses (Wickler *et al*.. 2006). Thus, it was possible to understand that blood concentrations of this ion would undergo subtle changes during maximal or submaximal exercises (Teixeira-Neto *et al*., 2004). The decrease of this ion can be related to losses through sweating, a fact noted by Di Filippo *et al*. (2009) up to an hour after the end of the endurance.

Total concentration of calcium ion: Total concentrations of calcium ions did not change during prolonged exercise (Schott *et al*., 2006). However, considering the ionized calcium (Ca^{+2}), the values found for disqualified equines were lower than those registered for equine athletes (Berlin and Aroch, 2009), and even for animals finalists of endurance races (Di Filippo *et al*., 2009; Dumont *et al*., 2012). This coincides especially with that found in horses with more severe electrolyte disturbances that run at high speed (Viu *et al*., 2010). The reduction in plasma concentrations of Ca^{+2} was caused by its loss through sweating and loss to the intracellular environment, when the concentration increased, in order to maintain muscle contraction (Wickler *et al*., 2006).

Total concentration of chloride ion: Values of chloride ion (Cl^-) were below the reference, about 4.2% lower than those found in horses finalists of endurance races (Dumont *et al*., 2012) and 7.65% lower than those established as baseline for endurance horses (Wickler *et al*., 2006). The decrease was also observed in horses finalists in 120 km-competitions (Robert *et al*., 2010; Viu *et al*., 2010). When associating the value found for the chloride ion with pH, it was possible to observe that the initial changes would induce hypochloremic metabolic alkalosis, if the animals continued the route, and showed complications in exhaustion syndrome.

Total concentration of potassium ion: The reduction of potassium by the end of submaximal exercise may be enhanced by the intake and consequent mobilization of water into the intravascular compartment, promoting its dilution (Lacerda-Neto *et al*., 2003). Meantime, its reduction results from high losses through sweating and renal excretion, and thus sodium retention in order to promote the expansion of the extracellular fluid in response to dehydration (Schott II *et al*., 1997). In intense exercise, this ion was released by myocytes to plasma (Ferraz *et al*., 2010) and under submaximal exercise, its concentration increased in the initial phase and decreased at the end. This is a result of its return to the myocytes, sweating and renal excretion (Hess *et al*., 2005).

Relationship between osmolality with ions: The plasma osmolality followed the behavior of sodium, indicating a perfect positive linear correlation (r=1.0) (Fig. 2A). This suggests that this cation is the main regulator of this variable in the organism, thus coinciding with the findings of Silva *et al*. (2009) that verified its direct relationship with the sodium ion and confirmed the occurrence of its depletion through sweating.

Likewise the sodium, the ionized calcium and chloride also showed a strong positive linear correlation (r=0.78 and r=0.86, respectively) (Fig. 2B and 2C). Thus, it is believed that the drop in the plasma concentration of these ions and the reduction of osmolality were more pronounced in disqualified animals due to dehydration justified by the absence of thirst, as a function of the decrease in solute concentration, and osmolality (Teixeira-Neto *et al*., 2004). Otherwise, there was a negative and weak linear correlation (r=-0.39) between osmolality and potassium ion (Fig. 2D), which can be explained by the fact that this is the predominant intracellular ion. In addition, no changes were observed compared to baseline values.

Distance run versus training: According to the distance traveled until the time of disqualification, it was possible to verify that for all parameters (Table 2), when comparing

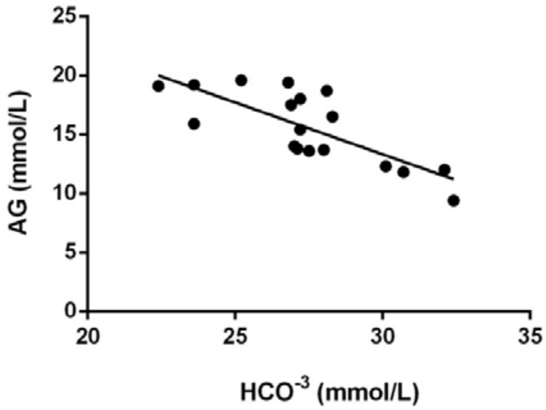

Fig. 1: Graphical representation of the Pearson correlation test, with a confidence interval of 95%, between the anion gap (AG) and bicarbonate (HCO^{-3}), r=-0.76.

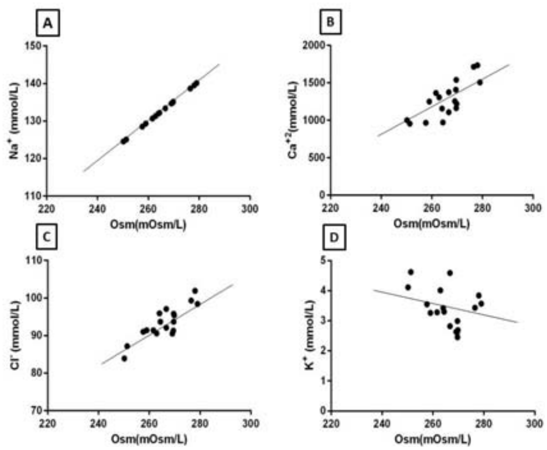

Fig. 2: Graphical representations of the Pearson correlation test, with a confidence interval of 95%, between the plasma osmolality (Osm) and (A) sodium (Na^+), (B) ionized calcium (Ca^{+2}), (C) ions chloride (Cl^-) and (D) potassium (K^+) in disqualified equines of endurance races.

different distances, there were no statistical differences. This result suggests that the determining factor in relation to equine exhaustion syndrome is not directly associated with the distance traveled, but with the intensity of training, i.e., the fitness of the animals to perform the effort.

Conclusion: A moderate hypovolemia due to dehydration induced by prolonged exercise in horses disqualified by exhaustion was observed. The effort has supplanted the thermoregulatory mechanism, reducing the osmolality, which was accompanied by sodium depletion. Also, the effort disabled animals to promote the expansion of plasma volume leading to increased hematocrit and total plasma protein concentration. The distance traveled was not the determining factor for the occurrence of exhaustion; this condition was associated with the intensity of training. There were no severe electrolyte imbalances; however, it is assumed that a continued effort could cause deleterious and irreversible disorders.

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