



Effects of exercise on cations/anions in blood serum of English Thoroughbred horses

[Efeito do exercício sobre cátions/ânions em soro sanguíneo de equinos da raça Puro-Sangue-Inglês]

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ABSTRACT

English Thoroughbred horses, are widespread in Mexico and due to the lack of data on their exercise physiology, it is important to conduct exercise tests in order to obtain information the effects of exercise on more essential cations/anions in blood serum, as these horses are submitted to constant efforts. The study was carried out with 150 blood samples of English Thoroughbred horses clinically healthy. The blood sample collection was performed during three periods: 1) rest, 2) 30min after exercise (speed race of 12km/h for 30min with no rest) and 3) 60min after exercise. Mean values were calculated for cations (sodium and potassium) and anions (chloride and bicarbonate). The resulting data set was analyzed using Gaussian distribution and descriptive statistics. Confidence intervals of 95% were established. The linear relationships between ions were quantified, and an analysis of variance was performed to compare the mean values between groups. The concentrations of the described analytes are consistent with values reported by international literature. The comparison between groups, revealed that during exercise, sodium ion did not show changes 30min after exercise and increase 60min after. Potassium ion showed increase 30min after exercise and decrease 60min after. Chloride ion showed a decrease 30min after exercise, to recover gradually 60min after. Meanwhile, bicarbonate ion showed increase 30min after exercise, decreasing slightly in the final stage. Negative correlation between bicarbonate ion and chloride ion were determined. It was concluded that exercise tests are useful for the determination of acid-base balance and osmotic balance, and their main role is to evaluate the athletic ability of horses. Considering that chloride ion excretion and metabolic adjustments of potassium ion and bicarbonate ion are superior to water loss, compared to the normal osmolarity of blood serum. The results found can be used to structure an adequate replacement program of electrolytes lost in sweat.

Keywords: english thoroughbred horses, blood chemistry, metabolic profile, osmotic balance

RESUMO

Equinos da raça Puro-Sangue-Inglês são difundidos no México e, devido à falta de dados sobre sua fisiologia do exercício, é importante fazer testes de exercício para obter informações sobre os efeitos do exercício em cátions/ânions mais essenciais no soro do sangue, pois esses equinos são submetidos a esforços constantes. O estudo foi realizado com 150 amostras de sangue de equinos Puro-Sangue-Inglês, clinicamente saudáveis. A coleta de sangue foi realizada em três períodos: 1) descanso, 2) 30min após o exercício (corrida de velocidade de 12km/h por 30min, sem descanso) e 3) 60min após o exercício. Os valores médios foram calculados para cátions (sódio e potássio) e ânions (cloreto e bicarbonato). O conjunto de dados resultante foi analisado utilizando-se distribuição gaussiana e estatística descritiva. Intervalos de confiança de 95% foram estabelecidos. As relações lineares entre os íons foram quantificadas, e uma análise de variância foi realizada para se compararem os valores médios entre grupos. As concentrações dos analitos descritos são consistentes com os valores relatados na literatura internacional. A comparação entre os grupos revelou que, durante o exercício, o sódio íon não mostrou alterações 30min após o exercício e aumentou 60min após. O potássio íon mostrou aumento 30min após o exercício e diminuiu 60min após. O cloreto íon mostrou uma diminuição 30min após o exercício, para recuperar gradualmente 60min depois. O bicarbonato íon mostrou aumento 30min após o exercício, diminuindo ligeiramente no estágio final. Correlação negativa entre bicarbonato íon e cloreto íon

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foi encontrada. Concluiu-se que os testes de exercício são úteis para a determinação do equilíbrio ácido-base e do equilíbrio osmótico, e seu principal papel é avaliar a capacidade atlética dos equinos. Considerando-se que a excreção de cloro íon e ajustes metabólicos de potássio íon e bicarbonato íon são superiores à perda de água, comparada à osmolaridade normal do soro sanguíneo, os resultados encontrados podem ser usados para estruturar um programa adequado de reposição de eletrólitos perdidos no suor.

Palavras-chave: equinos Puro-Sangue-Inglês, bioquímica sanguínea, perfil metabólico, equilíbrio osmótico

INTRODUCTION

The sport of racing is the most demanding of equine athletic disciplines, with horses required to complete distances of up to 160km/d (Randle and Waran, 2017). In these activities, animals with highly developed metabolic responses are required (Doherty *et al.*, 2017). Because the evaporation of sweat is the major mechanism for the removal of excess heat produced during exercise, there is a substantial loss of body water and electrolytes, especially cations: sodium (Na^+) and potassium (K^+) and anions chloride (Cl^-) and bicarbonate (HCO_3^-) (Potts *et al.*, 2015; Randle and Waran, 2017). Additionally, dehydration and electrolyte imbalances increase the risk for metabolic disease, including synchronous diaphragmatic flutter (Nagy *et al.*, 2017), and rhabdomyolysis (Wilberger *et al.*, 2015).

Cations and anions losses during speed race, reflect a balance among sweat loss, plus water, influence of exercise intensity and duration, and index of the horse's adaptation to speed and endurance (Sanin *et al.*, 2015). These ions are electrically charged particles, and not just inert accumulations of salt suspended in an aqueous medium (Martins *et al.*, 2014). They are indispensable biochemical analytes in the acid-base balance of blood, the water balance of body, maintaining osmotic pressure, movement of electrical impulses and muscle contraction and relaxation (Soetan *et al.*, 2010; Terker *et al.*, 2015; Kataoka, 2017). For this reason, the present study determined the effect of physical exercise on the serum concentration of Na^+ , K^+ , Cl^- and HCO_3^- ions in English Thoroughbred horses for its application in sports medicine.

MATERIALS AND METHODS

All animals in this study were kept following the guidelines of the (Olfert *et al.*, 1993).

Evaluations were carried in the equestrian club Sayavedra in the Mexico City (latitude 19 25'42" N, longitude 99 07'39" O and altitude 2240m). The mean temperature during the exercise and relative humidity were 22°C, and 41%, respectively.

Fifty English Thoroughbred horses clinically healthy, (25 stallions and 25 mares), ranging from 2 to 3 years of age and weight 400 \pm 50kg, were included in the study. All the horses had been trained regularly for at least one year. Blood samples were collected, between 8:00 and 11:00 a.m., by puncture of the jugular vein during three periods: 1) rest, 2) 30min after exercise (speed race of 12km/h for 30min with no rest) and 3) 60min after exercise, using 8.5mL vacuum tubes with clot activator and serum separator gel (BD Vacutainer 367988; Becton-Dickinson Co., Franklin Lakes, United States). The serum was separated by centrifuging directly at the equestrian club at 1 500 x g for 10min using a portable centrifuge (Porta-Spin C828; UNICO., Dayton, United States). Subsequently, the serum samples were separated using 1.5mL tubes with lid (Tubes Safe-Lock 3810X; Eppendorf., Madrid, Spain) and transported at 4°C in a portable cooler (Thermoelectric Cooler Car/Home M5644-710; Coleman Company., Kansas, United States) to the clinical laboratory of the Universidad Autónoma Metropolitana campus Xochimilco, where they were frozen at -20°C (Biomedical Freezer MDF-U5412H-PE; PHC Europe B.V., Amsterdam, Netherlands) until analysis.

The concentration of Na^+ , K^+ , Cl^- and HCO_3^- ions was determined with an UV/Vis spectrophotometer (Biochemistry Analyzer ES-218; KONTROLab., Guidonia, Italy). Table 1 describes measured biochemical analytes, the analytical method employed to obtain each parameter, and the corresponding commercial reagents used.

Table 1. Biochemical analytes, units, analytical methods, and corresponding commercial reagents

	Unit	Method	Reagent
Cations			
Sodium (Na ⁺)	mM	Enzymatic ^a	1001385 ¹
Potassium (K ⁺)	mM	Enzymatic ^b	1001395 ¹
Anions			
Chlorine (Cl ⁻)	mM	Colorimetric. Mercuric Thiocyanate	1001360 ¹
Bicarbonate (HCO ₃ ⁻)	mM	Enzymatic by CO ₂ total and gas dissolved	99852 ²

^a Galactosidase; ^b Phosphoenolpyruvate-Lactate Dehydrogenase; ¹ Spinreact., Girona, Spain; ² Biolabo Laboratory., Grandcamp-Maisy, France.

The precision and reliability of the techniques was controlled using lyophilized control serum (SPINTROL NORMAL 1002100; Spinreact., Girona, Spain). Hemolysis of serum was recorded on a qualitative scale of 0 (none) to 3 (dark). Samples showing hemolysis scores of 2 and above constituted less than 2% of all samples, and did not introduce a significant bias in any of the tested models after statistical analysis; thus, the influence of serum hemolysis was ignored.

Data were analyzed using Gaussian distribution, and percentiles: P₁₀-P₉₀, P₂₅-P₇₅ were determined by SPSS Univariate Procedure (SPSS..., 2013). The comparison between groups: 1) rest, 2) 30min after exercise and 3) 60min after exercise, was assessed by Analysis of Variance. A multiple comparison test of Tukey was performed when the effect of group was found to be significant (P<0.05). The linear relationships between biochemical analytes were identified by the use a Pearson Correlation Coefficient matrix. A diagnosis for outlier values was performed using robust multivariate outlier detection. This macro calculates the robust Mahalanobis distance for each observation. The following model was tested:

$$d_m(x, \bar{x}) = \sqrt{(x - \bar{x}) \sum_x^{-1} (x - \bar{x})}$$

where:

$d_m(x, \bar{x})$ = robust Mahalanobis distance;
 x = vector of the observation;
 \bar{x} = vector average of the observations; and
 \sum_x^{-1} = variance-covariance matrix of the observations.

A diagnosis for models main assumptions was performed. Linear functional form was visually checked by a normal plot. Shapiro-Wilk test was used to check normality. The Levene test was employed to check equality of variance. The Durbin-Watson test was employed to check for error uncorrelation.

RESULTS

The descriptive statistics for Na⁺, K⁺, Cl⁻ and HCO₃⁻ ions, determined from 150 blood serum of English Thoroughbred horses, and its respective international reference values are shown in Table 2.

Table 2. Mean (\bar{x}), standard deviation (SD), reference value, confidence interval (CI), and percentiles: P₁₀-P₉₀ and P₂₅-P₇₅ for different biochemical analytes (n= 150 blood serum of English Thoroughbred horses)

	$\bar{x} \pm SD$	Reference ^a	CI ^b	P ₁₀ -P ₉₀	P ₂₅ -P ₇₅
Cations					
Sodium (mM)	137.57±1.59	139±3.5	137.31-137.82	135.48-140.18	136.48-138.44
Potassium (mM)	3.91±0.25	3.51±0.57	3.87-3.95	3.61-4.25	3.78-4.06
Anions					
Chlorine (mM)	95.37±3.52	104±2.6	94.80-95.93	90.86-100.45	93.13-98.92
Bicarbonate (mM)	24.13±2.29	25±3.0	23.76-24.50	20.99-27.14	21.97-26.76

^a(Kaneko *et al.*, 2008) ^bconfidence interval of 95%.

In general, the values for all biochemical analytes are consistent with the reference values reported internationally. Table 3 describes the comparison between groups. The Na^+ ion not showed significant changes 30min after exercise, and increase 60min after. The K^+ ion showed

increase 30min after exercise and decrease 60min after. The Cl^- ion showed a decrease 30min after exercise, to recover gradually 60min after. And the HCO_3^- ion showed increase 30min after exercise, decreasing slightly in the final stage.

Table 3. Comparison of different biochemical analytes in English Thoroughbred horses undergoing exercise, ($n= 50$ horses/group)

	Rest	30min after exercise ¹	60min after exercise ¹
Cations			
Sodium (mM)	137.40±1.19 ^{a,b}	137.20±1.81 ^a	138.10±1.60 ^b
Potassium (mM)	3.71±0.13 ^a	4.10±0.27 ^b	3.93±0.13 ^c
Anions			
Chlorine (mM)	99.68±1.24 ^a	91.92±1.62 ^b	94.52±1.26 ^c
Bicarbonate (mM)	21.48±0.67 ^a	26.88±0.57 ^b	24.03±0.53 ^c

¹speed race of 12km/h for 30min with no rest; significant differences were obtained between groups indicated with different letters; ($P<0.05$); all data are presented means ± SD.

DISCUSSION

The Na^+ ion was the most stable electrolyte, because it did not show significant changes in blood serum concentration, 30min after exercise (Figure 1). Mansley *et al.* (2017) reported that fine-tuning of Na^+ ion content occurs within the aldosterone-sensitive-distal-nephron (ASDN),

whose activity increases during the exercise. They pointed out also that the aldosterone can promote Na^+ ion retention during exercise to offset changes in extracellular fluid volume, via the epithelial-sodium-channel (ENaC), in the kidney and the sweat glands. These assumptions could explain the stability of Na^+ ion in the blood serum.

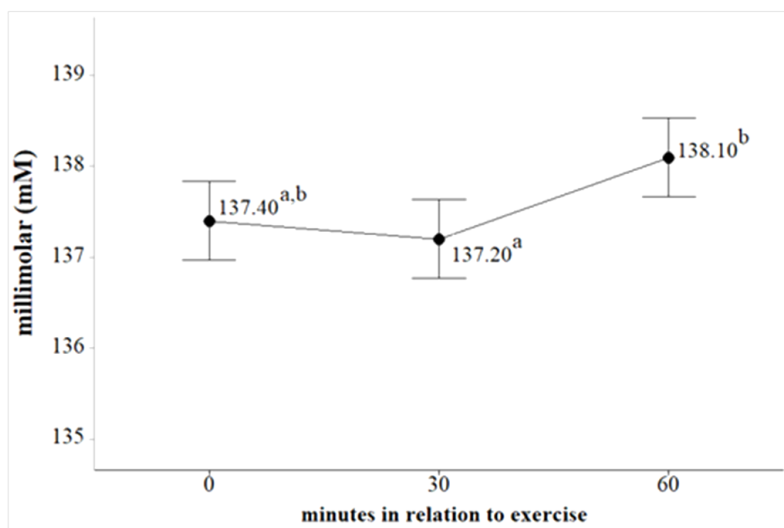


Figure 1. Comparison of sodium ion in English Thoroughbred horses undergoing exercise, ($n= 50$ horses/group). ^{a, b} significant differences were obtained between groups indicated with different letters ($P<0.05$); all data are presented means ± SD.

The aldosterone is a master regulator of renal Na^+ ion transport, but Hunter *et al.* (2014) reported that glucocorticoids are also influential, particularly cortisol elevated during exercise.

The hypothalamic-pituitary-adrenal (HPAA) axis can affect renal Na^+ ion homeostasis on multiple levels, systemically by increasing mineralocorticoid synthesis and locally by

actions on both the mineralocorticoid and glucocorticoid receptors, both of which are expressed in the kidney. Therefore, cortisol can stimulate renal transport processes conventionally attributed to the renin-angiotensin-aldosterone-system (RAAS), and its high concentration during exercise, could help maintain the stability of Na⁺ ion in the blood serum.

The K⁺ion is the most abundant intracellular cation, and its concentration in the extracellular space is low due to the action of the Na⁺/K⁺-

ATPase which pumps three Na⁺ion out of the cell in exchange for two K⁺ion (Rodan, 2016). Thus, 98% of total body K⁺ion is found in intracellular stores, chiefly in muscle (Terker *et al.*, 2015). Mora *et al.* (2015) reported that dehydrating exercise reduce muscle water (H₂O_{muscle}) seemly to restore plasma volume (PV). In response to the H₂O_{muscle} reductions the K⁺ion will exit the muscle cells and enter the bloodstream, increasing your concentration (Figure 2) (Rodan, 2016).

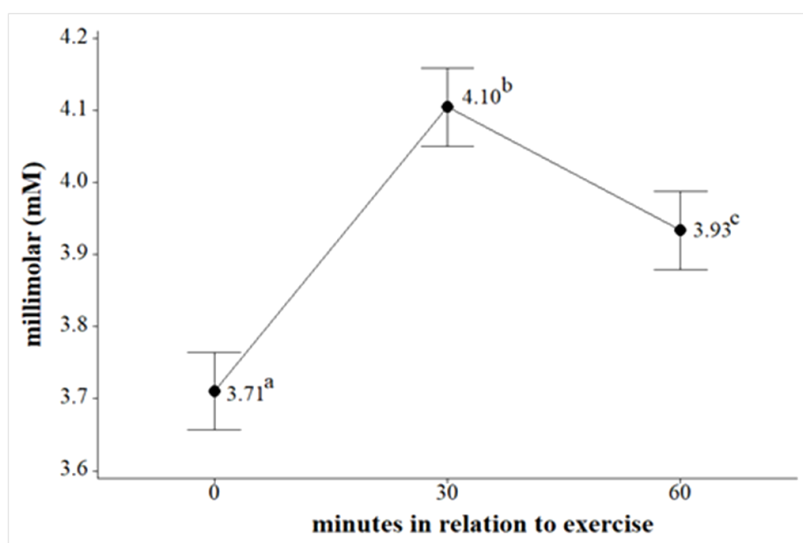


Figure 2. Comparison of potassium ion in English Thoroughbred horses undergoing exercise, ($n= 50$ horses/group). ^{a, b} significant differences were obtained between groups indicated with different letters ($P<0.05$); all data are presented means \pm SD.

The amount of K⁺ion entering the bloodstream is dependent on the intensity and duration of the workload (Mora *et al.*, 2015). This is a transient effect, as K⁺ ion levels in the blood will peak and then begin to decline as it recovers in organism (Terker *et al.*, 2015), which can be attributed to losses in the sweat (Potts *et al.*, 2015).

The nature of equine sweat is alkaline, since their sweat glands are primarily secretory of Cl⁻ ion, sulphates and phosphates (Tomich *et al.*, 2018). Therefore, the equine sweat is hypertonic with respect to blood serum (Potts *et al.*, 2015). During exercise the sweating of horse is

accompanied by a significant loss of Cl⁻ ion (Figure 3), with a consequent reduction in blood serum (Demirtaş *et al.*, 2015).

Arias *et al.* (2014) quantified the losses of Cl⁻ ion in horse sweat, and when comparing their concentration: 110 ± 12.3 m-equiv/L in blood serum with 280.6 ± 18.5 m-equiv/L in sweat, reported that the loss of this electrolyte for every L of sweat is elevated. The amount of Cl⁻ ion entering the bloodstream is dependent on the intensity and duration of the workload and then begin to decline 60min after exercise as it recovers in organism (Demirtaş *et al.*, 2015).

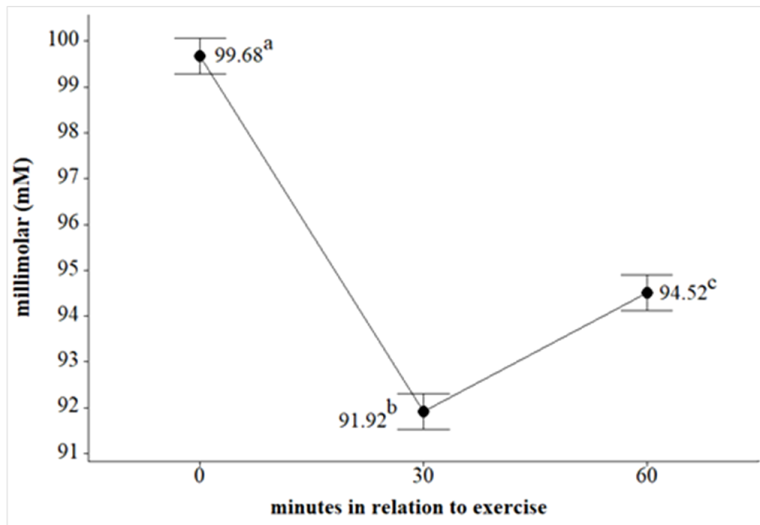


Figure 3. Comparison of chlorine ion in English Thoroughbred horses undergoing exercise, ($n= 50$ horses/group). ^{a, b} significant differences were obtained between groups indicated with different letters ($P<0.05$); all data are presented means \pm SD.

The loss of high amount of Cl^- ion causes deficit in blood serum (hypochloremia). Hypochloremia increases strong-ion-difference (**SID**) value. In these cases, Cl^- ion depletion also plays a crucial role in maintaining metabolic alkalosis (Hamilton *et al.*, 2017). The lower Cl^- ion

concentration in the tubular lumen also impairs activity of luminal ($\text{Cl}^-/\text{HCO}_3^-$) exchanger in type B intercalated cell and leads to reduced excretion of HCO_3^- ion. Therefore, the body presents high levels of HCO_3^- ion, 30min after exercise as anion compensation (Figure 4).

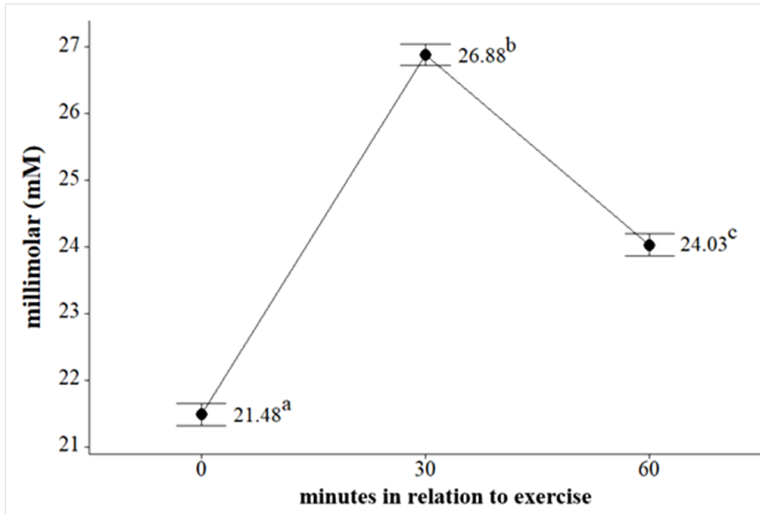


Figure 4. Comparison of bicarbonate ion in English Thoroughbred horses undergoing exercise, ($n= 50$ horses/group). ^{a, b} significant differences were obtained between groups indicated with different letters ($P<0.05$); all data are presented means \pm SD.

To maintain acid-base balance of blood, there are many different *buffer* systems in the body, but the key one for compensate most acid-base disorders is the HCO_3^- ion (Kataoka, 2017). The Cl^- ion is reabsorbed and excreted in inverse

proportion to HCO_3^- ion ($r= -0.87$; $P<0.05$), equation $\text{HCO}_3^- \text{ ion} = 78.86 - 0.5739 \text{ Cl}^- \text{ ion}$ (Figure 5). Conversely, hyperchloremia would lead to metabolic acidosis and renal elimination of HCO_3^- ion (Kataoka, 2017).

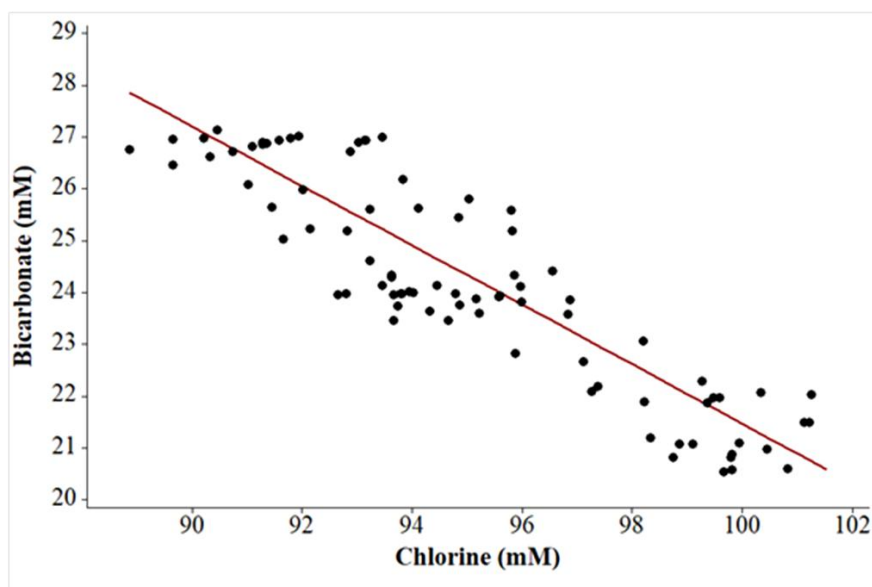


Figure 5. Relationship between bicarbonate and chlorine. Bicarbonate (●); predicted response (—).

CONCLUSIONS

The exercise tests are useful for the determination of acid-base balance and osmotic balance, and their main role is to evaluate the athletic ability of horses. The calculated confidence intervals could be used at herd level to detect alert situations when at least 5% of the sampled cows would fall outside of the calculated reference interval for a given parameter. Considering that chloride ion excretion and metabolic adjustments of potassium ion and bicarbonate ion are superior to water loss, compared to the normal osmolarity of blood serum. The results found can be used to structure an adequate replacement program of electrolytes lost in sweat.

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