Plasma and urine electrolyte and mineral concentrations in Thoroughbred horses with recurrent exertional rhabdomyolysis after consumption of diets varying in cation-anion balance

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Objective—To determine whether plasma, urine, and fecal electrolyte and mineral concentrations differ between clinically normal horses and Thoroughbreds with recurrent exertional rhabdomyolysis (RER) after consumption of diets varying in cation-anion balance.

Animals—5 Thoroughbred mares with RER and 6 clinically normal mixed-breed mares.

Procedure—Each of 3 isocaloric diets designated as low, medium, and high on the basis of dietary cation-anion balance (DCAB) values of 85, 190, and 380, respectively, were fed to horses for 14 days. During the last 72 hours, 3 horses with RER and 3 control horses had daily urine and fecal samples obtained by total 24-hour collection. Remaining horses had urine samples collected daily by single catheterization.

Results—For each diet, no differences existed between horses with RER and control horses in plasma pH, electrolyte concentrations, and creatine kinase activity or in urine pH and renal fractional excretion (FE) values. Plasma pH, strong ion difference, bicarbonate and total carbon dioxide concentrations, and base excess decreased and plasma chloride and ionized calcium concentrations increased with decreasing DCAB. Urine pH decreased with decreasing DCAB. The FE of chloride and phosphorus were greatest for horses fed the low diet. The FE values for all electrolytes exept magnesium did not differ between urine samples obtained by single catheterization and total 24-hour collection. Daily balance of calcium, phosphorus, sodium, chloride, and potassium did not differ significantly among horses fed the various diets.

Conclusions—In clinically normal horses and in horses with RER, the DCAB strongly affects plasma and urine pH and the FE of sodium, potassium, chloride, and phosphorus. (*Am J Vet Res* 2002;63:1053–1060)

Exertional rhabdomyolysis is a common cause of pain and muscle necrosis in horses, occurring with a frequency of approximately 5% in racing Thoroughbreds.[1] Clinical signs vary from subclinical increases in muscle enzyme activity to severe muscle cramping, sweating, and reluctance to move. An episode of exertional rhabdomyolysis can occur as an isolated event, but in some horses, clinical episodes can be chronic, resulting in performance limitation.[2] Exertional rhabdomyolysis is most commonly triggered by exercise, but several other precipitating factors have been identified, including high grain diets, stress, and anesthesia.[3] In addition, electrolyte imbalances have been implicated as a cause of recurrent episodes of rhabdomyolysis.[3,4]

Although clinical signs were once believed to be the result of lactate accumulation during exertion, many afflicted horses have no lactate accumulation and develop signs of rhabdomyolysis during submaximal exercise below the lactate threshold.[5,6] In addition, metabolic alkalosis, rather than acidosis, is a common derangement associated with exertional rhabdomyolysis.[7] Harris and Colles[4] suggested that electrolyte imbalances determined by creatinine clearance ratios of sodium, potassium, and phosphate might trigger rhabdomyolysis in Thoroughbreds with exertional rhabdomyolysis.[4] A group of affected Thoroughbreds has been reported to have significantly lower erythrocyte potassium concentrations, compared with age-matched controls, potentially indicating low intracellular potassium concentrations.[8]

Exertional rhabdomyolysis has recently been further categorized into several distinct disorders with differing causes and clinical presentations. In Quarter Horses and draft horses and related breeds, exertional rhabdomyolysis is commonly attributable to polysac-

Received Sept 4, 2001
Accepted Dec 17, 2001
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Supported by the Southern California Equine Foundation.
Presented at the 18th Annual Forum of the American College of Veterinary Medicine in Seattle, May 25–28, 2000.
The authors thank Dr. Michael Murphy and Tom Arendt for technical assistance.
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charide storage myopathy, a disease associated with the accumulation of abnormal polysaccharide within myocytes.[5] **Recurrent exertional rhabdomyolysis (RER)** is a disorder specific to a particular subset of Thoroughbreds that appears to be inherited in an autosomal dominant fashion.[9] Affected horses have intermittent episodes of exertional rhabdomyolysis and have an abnormally low threshold for muscle contracture in response to caffeine and halothane in vitro.[1,9] An increase in ionized calcium concentration in skeletal muscle fibers during acute rhabdomyolysis in horses with episodes of exertional rhabdomyolysis has been proposed.[10] Thus, recent research suggests that RER in Thoroughbreds may be related to an abnormality in intracellular calcium regulation in myocytes.[10,11] It is currently not known whether plasma ionized calcium concentration has any impact on the occurrence of abnormal muscle contraction in RER.

Serum electrolyte and ionized calcium concentrations can be experimentally manipulated by altering the **dietary cation-anion balance (DCAB)**.[12,13] The DCAB refers to the difference in the concentration of strong cations and strong anions in a diet. A high DCAB value corresponds to a high concentration of cations in the diet, most importantly sodium and potassium. Likewise, a diet with a low DCAB contains a higher proportion of strong anions, such as chloride and sulfur, in relation to cations, such that the DCAB may approach 0 or even a negative value.[14,15] A diet with a high DCAB can induce nutritional metabolic alkalosis, whereas a low DCAB induces metabolic acidosis.[12,16] The effect of DCAB on acid-base and electrolyte variables has been thoroughly investigated in dairy cattle, in which diets with low DCAB have been proven to significantly reduce the incidence of postparturient hypocalcemia via alterations in calcium metabolism.[17,18] The mechanisms by which a low DCAB affects calcium metabolism are not entirely clear. Cattle consuming diets with a low DCAB have high plasma 1,25 dihydroxyvitamin D concentrations, high plasma ionized calcium concentrations, and concurrent marked hypercalciuria. High plasma hydroxyproline concentrations were also observed, suggesting that the resorption of calcium from bone is a major component of the response.[19] The amount of readily available soluble calcium in the bone pool may as much as double in metabolic acidosis.[17]

High grain diets, a causative factor for exertional rhabdomyolysis, tend to have a low DCAB and are acidogenic because of a relative cation deficit when compared with anions.[20,21] One explanation for the predisposition of horses with RER to develop rhabdomyolysis while consuming a high grain diet may be associated with the effect of the diet on electrolyte, acid-base, and mineral balance, including ionized calcium.

The purpose of the study presented here was to determine the effect of DCAB on plasma electrolyte and mineral concentrations and on urinary and fecal excretion of electrolytes and minerals in clinically normal horses and horses affected with RER. A second objective was to compare the **fractional excretion (FE)** results in urine samples obtained by 24-hour volumetric urine collection with those obtained by single daily catheterization. This was done to determine whether single urine samples provide an adequate reflection of the daily FE of electrolytes, because there has been conflicting data previously reported on this point.[22,23]

Materials and Methods

Five Thoroughbred mares with RER ranging in age from 2 to 15 years (mean, 8 years) and 6 clinically normal mixed-breed control mares ranging in age from 4 to 10 years (mean, 6 years) were used. Horses with RER were selected on the basis of a history of episodes of rhabdomyolysis and high serum creatine kinase activity following exercise. The diagnosis of RER had been previously confirmed in these horses via an abnormally low threshold for intercostal muscle contracture in the presence of caffeine.[1] Caffeine contracture thresholds were normal in intercostal muscle biopsy specimens from the 5 control horses.[1] Examination of middle gluteal muscle biopsy specimens performed in a previous study[5] did not identify abnormal polysaccharide storage in any of the horses.

Three diets with varying DCAB were designed by use of the equation:

$$\text{DCAB (mEq/kg/dry matter)} = (Na^+ + K^+) - (Cl^- + S^{2-})$$

A commercial pelleted concentrate composed of corn, wheat middlings, oats, and soybean material formed the basis of all diets (**Appendix 1**) and was fed twice daily in conjunction with grass hay at a 45:55 concentrate-to-roughage ratio by weight at approximately 2.2% of body weight. All diets were fed to achieve 28.8 Mcal in digestible energy, which surpassed the daily energy requirements of sedentary horses by 75%. Dietary energy content was formulated to match that used in a previous study[24] on horses with RER, which revealed that significant increases in postexercise creatine kinase activity occurred only when horses were consuming a high calorie ration rich in carbohydrate that surpassed maintenance requirements for sedentary horses by 70%.

A diet with a low DCAB was achieved through the incorporation of additional chloride (Cl^-) and sulfur (S^{2-}) into the concentrate pellet in the form of an organic soy product.[b] The medium diet consisted of the foundation pellet and was not supplemented with strong ions. A diet with a high DCAB was created by the addition sodium bicarbonate to the pellet formulation at 4.2% dry matter. The formulation of the diets was intended to meet the daily minimum electrolyte and mineral requirements of horses as according to 1989 National Research Council requirements for maintenance.[25] After formulation, the total DCAB values as measured by a commercial laboratory[c] were 85, 190, and 380 for low, medium, and high diets, respectively (**Appendix 2**). Final ration analysis revealed that the sodium (Na^+) concentration for the medium diet was lower than that requested in the original formulation. Horses were introduced to the pellets by gradually increasing the amount of the foundation pellet for the medium diet over a 5-day period. Each diet was fed for 14 days, and all horses received the same diet concurrently. The medium diet was fed between the high and low diets to avoid sudden extreme changes in DCAB.

Prior to feeding the 11 horses on the last 3 mornings on each diet, blood samples from the jugular vein were collected into sodium heparin tubes for determination of mineral and electrolyte concentrations. Concurrent urine samples were obtained by either single daily catheterization or total 24-hour urine collection from all horses for the determination of urine FE of electrolytes. Blood samples from the jugular vein were collected into lithium heparin blood gas syringes for measurement of plasma pH, bicarbonate (HCO_3^-), and ionized calcium (Ca^{2+}) concentrations. Creatine kinase activity was determined on a single venous blood sample on the final day of each diet.

To determine whether single daily urine samples could provide accurate FE values, urine samples were obtained by catheterization from 5 horses prior to the morning feeding, and total 24-hour urine collection was performed on the remaining 6 horses (3 control horses, 3 horses with RER) by use of self-retaining rubber urine collection harnesses. Horses that had urine samples obtained by 24-hour volumetric urine collection were observed continuously in tie stalls. Urine volume was measured every 6 hours, and an aliquot of urine was retained after vigorous stirring. Urine pH was determined for each aliquot. Six hourly urine aliquots were pooled for each 24-hour period, and each 24-hour pooled sample was submitted for analysis of mineral and electrolyte content. The FE value of each electrolyte was calculated by use of the formula:

$$FE\%(X) = \left(\frac{[Cr]plasma}{[Cr]urine} \times \frac{[X]urine}{[X]plasma}\right) \times 100$$

where Cr and X are creatinine and electrolyte or mineral concentrations, respectively, in urine or plasma.

Plasma **strong ion difference (SID)** was calculated by use of the equation:

$$SID = ([Na^+] + [K^+]) - [Cl^-].$$

Plasma anion gap was calculated using the equation:

$$\text{Anion gap} = ([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-]).$$

To determine fecal mineral balance, fecal weights were recorded every 6 hours for horses that were undergoing total 24-hour urine collection. Fecal samples were pooled from each 6-hour period. Fecal dry matter content was determined for each 24-hour period. Subsequently, the 3 daily samples from each horse were mixed to provide a single sample from each horse for 72-hour fecal mineral determination.

Analysis of plasma Na^+, Cl^-, potassium (K^+), Ca^{2+}, magnesium (Mg^{2+}), phosphorus (P), and creatinine concentrations and creatine kinase activity was performed by use of an automated chemistry analyzer.[d] Blood gas samples were stored on ice and analyzed within 30 minutes by use of a blood gas analyzer.

Urine pH was determined from litmus strips. The 24-hour aliquots of urine were refrigerated at 4 C and analyzed within 36 hours of collection. Urine concentrations of Ca^{2+}, P, Mg^{2+}, Na^+, and K^+ were determined via emission spectrometry. Urine aliquots were digested in concentrated nitric acid at a ratio of 1:2 to dissolve urine crystals prior to spectrometry, and the results were compared with a standard curve for each element. Urine Cl^- concentration was measured in solution on an automated chemistry analyzer.[c] Urine creatinine concentration was determined by the same equipment by use of the Jaffe rate method.[26]

Fecal dry matter was determined by heating samples to 135 C for 2 hours. Samples were heated to ashes in a muffle furnace at 500 C for 4 hours. Three milliliters of 6N hydrochloric acid was added to the ash residue and evaporated to dryness. Contents of Ca^{2+}, P, Mg^{2+}, Na^+, and K^+ were determined via ash inductively coupled plasma radial spectrometry following extraction with a solution of nitric and hydrochloric acids. Fecal S^{2-} percentage was determined following combustion of the samples in an oxygen-rich atmosphere at 1,350 C, which produced sulfur oxide. Sulfur oxide concentration was measured by infrared detection and reported as percent S^{2-} following conversion. Fecal Cl^- content was determined by potentiometric titration with silver nitrate by use of a titration unit with a silver electrode.[e]

Statistical analysis—Data from 3 of the horses undergoing total 24-hour urine collection while consuming the low diet (1 horse with RER and 2 control horses) were excluded from analysis on the basis of inadequate consumption of the diet. The remaining data, including data from the 3 horses that successfully consumed the low diet and data from all horses consuming the medium and high diets, were analyzed by use of a 1-way ANOVA, controlling for repeated measures, in a mixed model approach using a software program [27] Fractional excretion values for urine samples obtained by single catheterization and total 24-hour collection were pooled to compare data from horses with RER to control horses, as no significant differences in FE were found on the basis of the method of collection. Results are expressed mean values (± SE). Significance was set at $P < 0.05$.

Results

Plasma electrolytes and minerals—Plasma SID was significantly higher in horses with RER versus control horses consuming the medium diet (**Table 1**). No other significant differences in plasma electrolyte and mineral concentrations or acid-base status were observed between horses with RER and control horses

Table 1—Mean (± SEM) plasma electrolyte and mineral concentrations and acid base status of horses with recurrent exertional rhabdomyolysis (RER) and control horses consuming 3 diets that varied (ie, low, medium, high) in dietary cation-anion balance (DCAB)

	Control horses			Horses with RER		
Variable	**Low DCAB N = 4 (n = 9)**	**Medium DCAB N = 6 (n = 17)**	**High DCAB N = 6 (n = 15)**	**Low DCAB N = 4 (n = 10)**	**Medium DCAB N = 5 (n = 15)**	**High DCAB N = 5 (n = 13)**
Sodium (mmol/L)	136 ± 0.48[b]	137 ± 0.34[a]	138 ± 0.46[a]	137 ± 0.55[a]	138 ± 0.48[a]	138 ± 0.55[a]
Potassium (mmol/L)	4.0 ± 0.14[a]	4.3 ± 0.14[a]	4.0 ± 0.20[a]	4.20 ± 0.17[a]	4.90 ± 0.26[b]	3.70 ± 0.22[a]
Chloride (mmol/L)	109 ± 1.13[b]	104 ± 0.37[a]	105 ± 0.30[a]	110 ± 0.60[b]	103 ± 0.47[a]	103 ± 0.62[a]
Magnesium (mg/dl)	1.90 ± 0.09[a]	2.0 ± 0.04[a]	1.90 ± 0.03[a]	1.80 ± 0.07[b]	2.0 ± 0.02[a]	1.90 ± 0.04[a]
Phosphorus (mg/dl)	3.30 ± 0.20[a]	3.20 ± 0.12[a]	3.80 ± 0.16[b]	3.80 ± 0.26[a]	3.50 ± 0.28[a]	3.60 ± 0.32[a]
Calcium (mg/dl)	11.90 ± 0.12[a]	12.0 ± 0.04[a]	12.10 ± 0.10[a]	12.0 ± 0.14[ab]	12.20 ± 0.07[a]	11.80 ± 0.15[b]
Total CO_2 (mmol/L)	25.0 ± 1.37[a]	31.90 ± 0.42[b]	33.90 ± 0.46[c]	24.60 ± 0.57[a]	33.10 ± 0.42[b]	35.20 ± 0.67[c]
Base excess (mmol/L)	–1.47 ± 1.49[a]	4.78 ± 0.42[b]	6.88 ± 0.43[c]	–1.90 ± 0.65[a]	5.70 ± 0.29[b]	7.50 ± 0.54[c]
Strong ion difference (mmol/L)	31.0 ± 1.44[b]	37.80 ± 0.36[a]*	37.20 ± 0.58[a]	31.70 ± 0.63[b]	39.70 ± 0.35*[a]	38.60 ± 0.42[a]
Anion gap (mmol/L)	7.30 ± 0.35[a]	7.15 ± 0.41[a]	4.92 ± 0.54[b]	8.43 ± 0.47[a]	8.05 ± 0.42[a]	4.81 ± 0.60[b]
Creatine kinase (U/L)	159 ± 6.5[a]	150 ± 8.8[a]	170 ± 3.5[a]	187 ± 23.5[a]	159 ± 22[a]	172 ± 19[a]

*Significant difference ($P < 0.05$) between control horses and horses with RER.

[a,b,c]Differing superscript letters indicate significant differences attributable to diet among control horses or among horses with RER.

N = Number of horses. n = Number of samples.

consuming any diet. No significant difference in resting plasma creatine kinase activity was observed between horses with RER and control horses for any of the diets.

Varying the DCAB had a significant effect on plasma electrolyte and acid-base variables (Table 1). Significant findings included an increased plasma Cl^- concentration in control horses and horses with RER consuming the low diet, compared with the medium and high diets. Plasma HCO_3^- and total carbon dioxide (CO_2) concentrations and plasma base excess differed across diets in both control and RER horses and were significantly less for horses consuming the low diet and significantly greater for horses consuming the high diet (**Fig 1**). Plasma ionized Ca^{2+} concentrations were significantly different across diets for the control horses and were increased on the low diet and decreased on the high diet (**Fig 2**). In horses with RER, plasma ionized Ca^{2+} was also significantly lower on the high diet, compared with the other diets, and plasma total Ca^{2+} was significantly lower on the high diet, compared with the medium diet as well. Plasma SID and plasma pH (**Fig 3**) were significantly lower in all horses consuming the low diet, compared with medium and high diets.

Urine pH and FE of electrolytes—Significant differences were not found in urine pH or the FE of electrolytes between horses with RER and control horses on all diets (**Table 2**). Urine pH was distinctly acidic when horses were consuming the low diet and was most alkaline when horses were consuming the high diet. Urinary FE of electrolytes and minerals was also affected by changes in DCAB. The FE of Na^+ in control horses and horses with RER was highest when horses were consuming the high diet. The FE of Na^+ for horses on the medium diet was significantly lower than the other diets. The FE of K^+ increased with increasing DCAB in horses with RER. Urinary FE of Cl^- decreased with increasing DCAB in horses with RER and control horses. In control horses, the FE of Cl^- was significantly higher on the low diet, compared with the medium and high diets. In horses with RER, the FE of Cl^- was also greatest in horses consuming the low diet and was significantly lower when horses were consuming the high diet, compared with the low and medium diets. The FE of P was significantly greater in all horses consuming the low diet. The FE of Ca^{2+} and Mg^{2+} was significantly higher in horses with RER on the medium diet.

The FE results for horses that had urine samples obtained by 24-hour volumetric collections were compared with those of horses that had urine samples obtained by single daily catheterization (**Table 3**). No significant differences were detected in FE of Na^+, K^+, Cl^-, Ca^{2+}, or P regardless of the method of urine collection or diet consumed. While consuming the high diet, the FE of Mg^{2+} was significantly higher for urine samples obtained by 24-hour volumetric collections, compared with urine samples obtained by single daily catheterization.

Fecal electrolytes and minerals—Fecal electrolyte and mineral concentrations were not different between horses with RER and control horses. Fecal electrolyte and mineral concentrations varied with DCAB (**Table 4**). Fecal Na^+ and Cl^- content was significantly increased in horses consuming the high diet. Fecal K^+

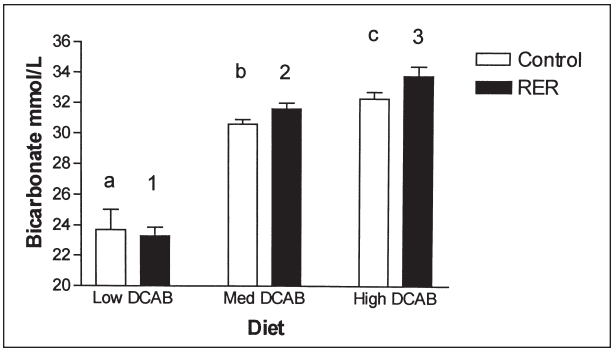

Figure 1—Mean (± SEM) plasma bicarbonate concentration in 5 horses with recurrent exertional rhabdomyolysis (RER) and 6 control horses consuming 3 diets that varied (ie, low, medium [med], high) in dietary cation-anion balance (DCAB). For low DCAB, data from 2 control horses and 1 horse with RER were not used because of inadequate consumption. Significant differences ($P < 0.05$) were not found between horses with RER and control horses. [a–c]Differing superscript letters indicate significant differences attributable to diet in control horses. [1–3]Differing superscript numbers indicate significant differences attributable to diet in horses with RER.

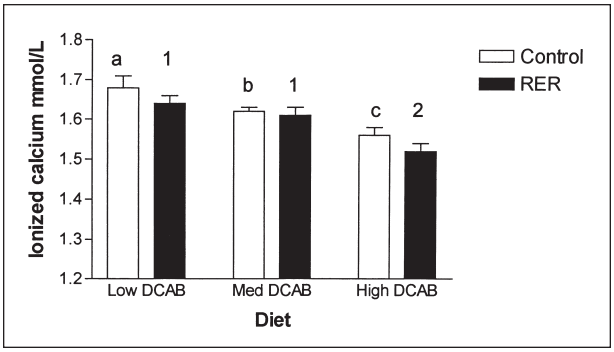

Figure 2—Mean (± SEM) plasma ionized calcium concentration in 5 horses with RER and 6 control horses consuming 3 diets that varied in DCAB. For low DCAB, data from 2 control horses and 1 horse with RER were not used because of inadequate consumption. Significant differences ($P < 0.05$) were not found between horses with RER and control horses. *See* Figure 1 for key.

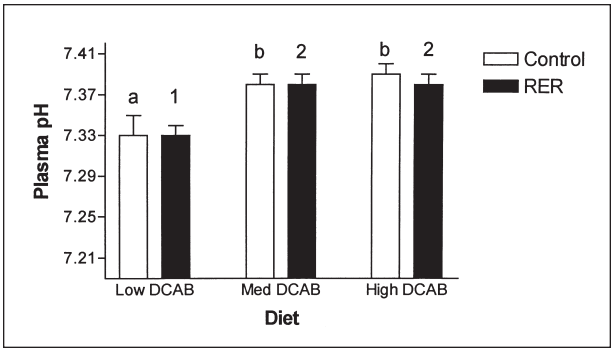

Figure 3—Mean (± SEM) plasma pH in 5 horses with RER and 6 control horses consuming 3 diets that varied in DCAB. For low DCAB, data from 2 control horses and 1 horse with RER were not used because of inadequate consumption. Significant differences ($P < 0.05$) were not found between horses with RER and control horses. *See* Figure 1 for key.

Table 2—Mean (± SEM) percent fractional excretion (FE) of electrolytes and minerals and pH of urine samples collected from horses with RER and control horses consuming 3 diets that varied in DCAB

	Control horses			Horses with RER		
Variable	Low DCAB N = 4 (n = 8)	Medium DCAB N = 6 (n = 16)	High DCAB N = 6 (n = 15)	Low DCAB N = 4 (n = 10)	Medium DCAB N = 5 (n = 14)	High DCAB N = 5 (n = 13)
FE sodium (%)	0.25 ± 0.02[a]	0.06 ± 0.02[b]	0.38 ± 0.07[a]	0.34 ± 0.07[a]	0.04 ± 0.01[b]	0.57 ± 0.12[a]
FE potassium (%)	33.20 ± 5.59[a]	30.90 ± 3.10[a]	39.70 ± 4.49[a]	23.81 ± 3.60[a]	38.90 ± 2.79[b]	48.30 ± 5.0[c]
FE chloride (%)	1.50 ± 0.22[a]	0.62 ± 0.11[b]	0.63 ± 0.12[b]	1.05 ± 0.13[a]	0.91 ± 0.12[a]	0.54 ± 0.09[b]
FE magnesium (%)	14.90 ± 1.68[a]	19.30 ± 1.42[a]	16.50 ± 1.57[a]	15.0 ± 1.30[a]	21.30 ± 1.52[b]	16.60 ± 2.10[a]
FE phosphorus (%)	8.26 ± 3.90[a]	2.13 ± 0.83[b]	1.99 ± 0.56[b]	4.74 ± 1.08[a]	0.23 ± 0.05[b]	1.24 ± 0.47[b]
FE calcium (%)	9.80 ± 2.28[a]	9.90 ± 1.56[a]	8.60 ± 0.87[a]	12.30 ± 2.10[ab]	14.80 ± 1.66[a]	7.70 ± 1.23[b]
Urine pH	5.25 ± 0.08[a]	8.0 ± 0.09[b]	8.61 ± 0.12[c]	5.31 ± 0.07[a]	7.79 ± 0.09[b]	8.70 ± 0.05[c]

Data are combined from urine samples obtained by single catheterization and by total 24-hour collection.
See Table 1 for remainder of key.

Table 3—Mean (± SEM) percent FE of electrolytes and minerals of urine samples collected by single catheterization or total 24-hour urine collection from horses with RER and control horses consuming 3 diets that varied in DCAB

	Single catheterization			Total 24-hour collection		
FE	Low DCAB N = 5	Medium DCAB N = 5	High DCAB N = 5	Low DCAB N = 3	Medium DCAB N = 6	High DCAB N = 6
Sodium (%)	0.33 ± 0.07[a]	0.05 ± 0.01[b]	0.40 ± 0.07[a]	0.27 ± 0.05[ab]	0.05 ± 0.01[a]	0.51 ± 0.10[b]
Potassium (%)	32.55 ± 4.50[a]	38.04 ± 4.50[a]	45.60 ± 7.37[a]	22.30 ± 4.30[a]	32.4 ± 2.10[b]	42.60 ± 3.50[c]
Chloride (%)	1.48 ± 0.17[a]	0.82 ± 0.19[b]	0.70 ± 0.20[b]	0.96 ± 0.15[a]	0.70 ± 0.06[a]	0.53 ± 0.04[b]
Magnesium (%)	15.90 ± 1.34[ab]	17.80 ± 1.80[a]	11.10 ± 2.20*[b]	13.70 ± 1.50[a]	21.80 ± 1.10[b]	19.60 ± 1.0*[b]
Phosphorus (%)	6.98 ± 3.27[a]	2.02 ± 1.10[a]	2.35 ± 0.80[a]	5.46 ± 0.90[a]	0.73 ± 0.25[b]	1.25 ± 0.35[b]
Calcium (%)	10.74 ± 2.10[a]	9.75 ± 2.30[ab]	4.87 ± 1.48[b]	11.80 ± 2.35[a]	13.80 ± 1.20[b]	10.03 ± 1.40[a]

*Significant difference ($P < 0.05$) between urine samples collected by single catheterization and total 24-hour urine collection.
[a,b,c]Differing superscript letters indicate significant differences attributable to diet among urine samples collected by single catheterization or among urine samples obtained by total 24-hour urine collection.
See Table 1 for remainder of key.

Table 4—Mean (± SEM) total daily balance of electrolytes and minerals in horses with RER and control horses consuming 3 diets that vary in DCAB

Variable	Low DCAB N = 3	Medium DCAB N = 6	High DCAB N = 6
Calcium intake (mg/kg)	99 ± 3.6[a]	131 ± 3.8[b]	135 ± 4.1[b]
Urine calcium (mg/kg)	29 ± 8.7[a]	34 ± 4.6[a]	33 ± 5.0[a]
Fecal calcium (mg/kg)	51 ± 11[a]	129 ± 26[b]	114 ± 9[ab]
Calcium balance (mg/kg)	19 ± 20.5[a]	–33 ± 27.0[a]	–12 ± 13.1[a]
Phosphorus intake (mg/kg)	108 ± 4.0[a]	89 ± 2.6[b]	122 ± 3.8[c]
Urine phosphorus (mg/kg)	5 ± 0.9[a]	0.3 ± 0.2[b]	0.8 ± 0.4[b]
Fecal phosphorus (mg/kg)	86 ± 20.3[a]	108 ± 7.1[a]	149 ± 10.8[b]
Phosphorus balance (mg/kg)	18 ± 23[a]	–20 ± 8.5[a]	–28 ± 13.5[a]
Magnesium intake (mg/kg)	44 ± 1.6[a]	43 ± 1.2[a]	42 ± 1.3[a]
Urine magnesium (mg/kg)	5 ± 1.0[a]	9 ± 0.9[b]	8 ± 0.6[b]
Fecal magnesium (mg/kg)	27 ± 7.2[a]	43 ± 3.6[b]	37 ± 2.9[ab]
Magnesium balance (mg/kg)	12 ± 9.1[a]	–8 ± 4.0 [b]	–3 ± 4.0[ab]
Sodium intake (mg/kg)	31 ± 1.1[a]	18 ± 0.5[b]	107 ± 3.3[c]
Urine sodium (mg/kg)	19 ± 2.0[a]	3 ± 0.9[b]	36 ± 2.6[c]
Fecal sodium (mg/kg)	4 ± 1.45[a]	20 ± 7.4[a]	81 ± 9.4[b]
Sodium balance (mg/kg)	9 ± 2.5[a]	–5 ± 7.2[a]	–11 ± 11.3[a]
Potassium intake (mg/kg)	263 ± 6.9[a]	263 ± 7.6[a]	259 ± 8.0[a]
Urine potassium (mg/kg)	87 ± 9.9[a]	107 ± 5.8[a]	135 ± 9.0[b]
Fecal potassium (mg/kg)	70 ± 9.3[a]	97 ± 8.8[a]	76 ± 12.4[a]
Potassium balance (mg/kg)	107 ± 23.3[a]	59 ± 16.7[a]	47 ± 18.3[a]
Chloride intake (mg/kg)	143 ± 5.2[a]	78 ± 2.2[b]	78 ± 2.4[b]
Urine chloride (mg/kg)	87 ± 23.1[a]	51 ± 2.4[b]	42 ± 3.2[b]
Fecal chloride (mg/kg)	6 ± 1.45[a]	7 ± 1.1[ab]	11 ± 1.7[b]
Chloride balance (mg/kg)	52 ± 27.8[a]	20 ± 3.65[a]	25 ± 3.9[a]

[a,b,c]Differing superscript letters indicate significant differences attributable to diet.
See Table 1 for remainder of key.

content did not differ significantly among diets. Fecal P concentration was significantly increased when horses were consuming the high diet. Fecal Mg^{2+} and Ca^{2+} concentrations were significantly lower for horses on the low versus medium diet.

Total daily balance—The apparent daily balance (retention) of Na^+, Cl^-, K^+, P, or Ca^{2+} did not differ significantly between horses with RER and control horses or on the basis of varying DCAB (Table 4). A significantly greater daily balance (increased retention) of Mg^{2+} was found for horses on the low diet, compared with horses on the medium diet. A negative daily balance (decreased retention) of Ca^{2+}, P, Mg^{2+}, and Na^+ was observed when horses consumed the medium and high diets.

Discussion

Results of our study did not identify any significant difference in electrolyte and mineral metabolism between clinically normal horses and horses with RER. Imbalances in acid-base status, electrolytes, and minerals have previously been implicated as triggering factors for exertional rhabdomyolysis.[4,28] Sodium bicarbonate has been used as a treatment for exertional rhabdomyolysis and as a dietary supplement to prevent exertional rhabdomyolysis, purportedly by buffering lactic acid.[29-31] This appears controversial, however, because the most consistent metabolic abnormality with exertional rhabdomyolysis is a hypochloremic metabolic alkalosis, and lactic acidosis has not been identified in

horses developing exertional rhabdomyolysis during treadmill exercise.[7,32] For horses in our study, the addition of 4.2% sodium bicarbonate to achieve an increase in DCAB resulted in a significant increase in plasma HCO_3^- concentration, base excess, urine pH, and urinary FE of Na^+ and K^+ as well as a decrease in plasma ionized Ca^{2+} concentration, findings that are in agreement with those of previous studies.[12,33] These changes reflect alkalemia induced by the systemic generation of HCO_3^- following absorption of Na^+ from the gastrointestinal tract in exchange for hydrogen (H^+).[34]

Harris and Colles[4] described 4 horses with exertional rhabdomyolysis that had low FE values of Na^+ and showed clinical improvement by increasing dietary Na^+ and normalizing FE values to reference range. Increasing serum Na^+ concentration has been postulated to potentially increase plasma volume during exercise or improve neuromuscular function.[4,29,35] In our study, no differences in plasma electrolyte concentrations or FE values were observed in 5 horses with RER, compared with 6 clinically normal horses. Thus it appears unlikely that the clinical signs of RER are the result of a unique inherent electrolyte imbalance in horses with RER.

A potential explanation for the reported putative beneficial effects of sodium bicarbonate in horses with exertional rhabdomyolysis may be to modulate serum ionized Ca^{2+} concentration. Horses with RER have a lower threshold for muscle contracture in response to agents such as halothane and caffeine. These agents cause sarcoplasmic reticulum Ca^{2+} release by activation of the ryanodine receptor in the sarcoplasmic reticulum.[1] An alteration in serum ionized Ca^{2+} concentration could potentially alter intracellular ionized Ca^{2+} concentration in Thoroughbreds with RER. Although there is no precedent for a relationship between plasma ionized Ca^{2+} and IM Ca^{2+} concentrations in clinically normal horses, such a relationship could possibly exist in horses with RER.

A significant decrease in plasma ionized Ca^{2+} concentration was observed in horses consuming the high diet that was independent of changes in ionization induced by differences in plasma pH. For example, ionized Ca^{2+} concentrations for the high and medium diets were 1.52 ± 0.02 versus 1.61 ± 0.02 mmol/L, respectively, in horses with RER, despite virtually identical mean blood pH values. Kohn and Brooks[36] found a weak correlation between plasma pH and ionized Ca^{2+} concentration and concluded that several biochemical variables other than pH must play an important role in determining plasma ionized Ca^{2+} concentration in horses.[36] One explanation for the lower plasma ionized Ca^{2+} concentration in horses consuming the high diet may be that metabolic alkalosis reduces the ability of bone and kidney receptors to respond to parathyroid hormone, therefore decreasing serum Ca^{2+} concentration.[37] Metabolic alkalosis can decrease the net efflux of Ca^{2+} from bone reserves by inhibiting osteoclastic resorption of bone and increasing bone formation by osteoblasts.[38] This is of importance in dairy cattle where a high DCAB prepartum greatly increases susceptibility to postparturient hypocalcemia.[18,37]

Our study was not designed to address the effect of low plasma ionized Ca^{2+} concentration and high DCAB on the development of rhabdomyolysis. An exercise trial in horses consuming diets of varying DCAB would be necessary to investigate any potential benefit of such diets in preventing rhabdomyolysis.

A low DCAB in horses in our study resulted in metabolic acidosis and an increase in plasma ionized Ca^{2+} concentrations. The development of metabolic acidosis in response to a low DCAB has been demonstrated in cattle, horses, small ruminants, and swine.[12,21,39] In our study, the addition of Cl^- (0.77% of dry matter) to the diet created a DCAB of 85 and resulted in a moderate hyperchloremic metabolic acidosis. This was likely caused by an exchange of Cl^- for HCO_3^- in the gastrointestinal tract, resulting in a net loss of HCO_3^- and an increase in blood H^+ concentration.[12,40] In addition, a further increase in plasma Cl^- concentration may have occurred to compensate for decreased plasma HCO_3^- concentration secondary to metabolic acidosis. Stewart's approach suggests that the acidosis is a result of the decreased SID observed in horses fed a diet with a low DCAB.[41]

Metabolic acidosis appears to induce a greater uptake of parathyroid hormone by bone and may also decrease renal resorption of excreted Ca^{2+}.[42] The increased plasma ionized Ca^{2+} concentration observed in horses consuming the low diet has been previously reported in cattle consuming a diet with a low DCAB and may be the result of metabolic acidosis, which increases sensitivity of bone and kidney tissue receptors to parathyroid hormone.[37,43] Increased tissue sensitivity to parathyroid hormone was suggested in a previous study[37] by the higher concentrations of plasma Ca^{2+} and plasma 1,25 dihydroxyvitamin D in cattle consuming an anionic (low DCAB) diet, compared with a cationic (high DCAB) diet, despite similar blood parathyroid hormone concentrations in the 2 groups. Parathyroid hormone increases Ca^{2+} resorption from bone by stimulating osteoclastic activity and increases Ca^{2+} retention in the distal renal tubules. Furthermore, parathyroid hormone stimulates renal production of 1,25 dihydroxyvitamin D, which promotes Ca^{2+} absorption from the gastrointestinal tract via active transport.[17] The significantly lower fecal excretion of Ca^{2+} observed in horses on the low diet in our study may be a reflection of improved gastrointestinal Ca^{2+} resorption stimulated by high plasma concentrations of 1,25 dihydroxyvitamin D. Measurement of plasma parathyroid hormone activity and plasma 1,25 dihydroxyvitamin D concentrations was not performed in our study but would be necessary to confirm the suspected mechanisms behind the changes in Ca^{2+} metabolism we observed. Results of previous studies[37,44,45] in cattle and horses consuming diets with low DCAB reveal no significant increase in plasma parathyroid hormone concentration despite hypercalciuria and high plasma Ca^{2+} concentration, suggesting that the effects of a low DCAB are largely mediated by vitamin D.

Results of previous studies[12,21,43,46] in several species have consistently indicated that hypercalciuria develops in animals consuming diets with a low DCAB. The urinary FE of Ca^{2+} was not significantly impacted by

DCAB in our study. Hypercalciuria is suggested to be the result of an inhibitory effect of acidosis on tubular resorption of Ca^{2+}, resulting in an overall increase in urinary Ca^{2+} excretion despite increased renal sensitivity to parathyroid hormone.[47] This inhibitory effect is dependent on the dietary Ca:P ratio as well as the total dietary Ca^{2+} content.[48,49] The absence of hypercalciuria in horses consuming the low diet in our study may reflect the unexpectedly low Ca:P ratio (0.9:1) of that diet identified after the diet had been formulated. Studies[48,49] in other species have indicated that a low dietary Ca^{2+} content can induce a significant decrease in urinary Ca^{2+} excretion. The magnitude of the decrease in urine Ca^{2+} excretion is greatly accentuated by concurrent nutritional metabolic acidosis.[49] Increased intestinal absorption of dietary Ca^{2+} during metabolic acidosis is a significant contributing factor to the hypercalciuria observed in animals consuming an anionic diet but may not develop if the diet has a low Ca^{2+} content or low Ca:P ratio.[49,50] Previous studies[33,51] have reported either no change or a decrease in urinary P excretion in horses consuming a diet with a low DCAB. The significant phosphaturia observed in horses consuming the low diet in our study is likely to reflect increased sensitivity to parathyroid hormone, stimulating increases in blood ionized Ca^{2+} concentration and promoting P excretion. An increased FE of K^+ in horses on the high diet was likely the result of sequestration of K^+ in the renal filtrate.[33]

The increased FE of Cl^- observed in horses consuming the low diet has been reported previously and results from increased dietary Cl^- intake and maximal resorption of HCO_3^- in exchange for Cl^- in the renal filtrate to combat metabolic acidosis.[33,52] Chloride excretion results in sequestration of H^+ in the filtrate of the urine, accounting for the low urine pH observed in horses consuming a diet with a low DCAB.[12,20] In our study, the greater urinary excretion of Na^+ on the low diet, compared with the medium diet, might reflect the sequestration of a cation to accommodate increased Cl^- excretion. Furthermore, it may also reflect the inadvertently lower Na^+ content of the medium diet than had been requested in the original formulation. A similar pattern of Na^+ and Cl^- excretion in response to consumption of an anionic diet was reported by Baker et al[33] in sedentary horses and horses undergoing anaerobic exercise. As a means to compensate for systemic acidosis, the decreased FE of K^+ observed in horses on the low diet might reflect the preferential excretion of H^+ as a urinary cation to accompany Cl^-.[23]

In our study, no significant differences in urine FE values were observed between volumetric and single catheterization methods of urine collection for any electrolyte or mineral measured, apart from a greater FE of Mg^{2+} in 24-hour collection samples in horses consuming the high diet. The changes induced in the FE of Cl^-, K^+, Na^+, and P by varying DCAB were similar whether calculated from single urine samples or a composite 24-hour urine collection. When consuming the high diet, horses from which a total 24-hour urine sample was obtained had a significantly greater FE of Mg^{2+}, compared with horses that had a urine sample obtained by single catheterization. This may have resulted from substantial crystalluria induced by the more alkaline urine pH. Urine acidification was used prior to analysis of all samples to ensure dissolution of urine crystals. A more thorough recovery of Ca^{2+} and Mg^{2+} containing urine crystals may explain the high FE of these substances in urine samples obtained by total 24-hour collection.[23] Single sample urine collection has been reported as an unreliable method for the determination of Na^+, Cl^-, and Ca^{2+} concentrations.[22,23] However, using the methods used in our study, single sample urine collection appeared to accurately reflect the total 24-hour excretion of all electrolytes and minerals on a basal DCAB diet.

[a]KER 5X premix, Kentucky Equine Research, Versailles, Ky.
[b]West Central Soy, Ralston, Iowa.
[c]Dairy One, Ithaca, NY.
[d]Beckman CX4 analyzer, Beckman Instruments Inc, Brea, Calif.
[e]Fecal analyses performed by DHI Forage Testing Laboratory, Ithaca, NY.

Appendix 1

Percent composition of the 3 types of pellets as fed

	Pellet types		
Ingredient	**Low DCAB**	**Medium DCAB**	**High DCAB**
Ground corn (%)	34.9	37.1	39.9
Wheat middlings (%)	29.6	32.5	20.8
Oats (%)	15.0	15.0	15.0
Soybean meal (%)	None	4.0	6.9
Soybean hulls (%)	4.8	5.4	6.6
Sodium bicarbonate (%)	None	None	4.2
Molasses (%)	3.7	3.7	3.75
Dicalcium-phosphate (%)	0.75	None	2.0
Lignin sulfonate (%)	0.6	0.6	0.6
Premix[a] (%)*	0.5	0.5	0.5
Flavor (%)	0.05	0.05	0.05
Limestone (%)	None	1.1	None
Organic soy product[b] (%)	9.99	None	None
Total (%)	**100**	**100**	**100**

*A blend of essential vitamins and trace elements that was added to meet the National Research Council requirements.
DCAB = Dietary cation-anion balance.

Appendix 2

Digestible energy (DE), percent electrolyte and mineral composition, calcium-to-phosphorus ratio, and calculated DCAB value of the 3 rations (hay plus pellet) on a dry matter basis

	Ration type		
Variable	**Low DCAB**	**Medium DCAB**	**High DCAB**
DE (Mcal/kg)	28.8	28.8	28.8
Sodium (%)	0.17	0.09	0.56
Potassium (%)	1.41	1.39	1.35
Chloride (%)	0.77	0.41	0.41
Magnesium (%)	0.24	0.23	0.22
Phosphorus (%)	0.58	0.47	0.64
Calcium (%)	0.53	0.69	0.71
Calcium:phosphorus	0.9:1	1.5:1	1.1:1
Sulfur (%)	0.21	0.14	0.15
DCAB	85	190	380

See Appendix 1 for key.

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