Fractionation of calcium and magnesium in equine serum

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Objective—To establish reference values for protein-bound, ionized, and weak-acid complexed fractions of calcium and magnesium in equine serum and determine stability of ionized calcium (iCa) and ionized magnesium (iMg) in serum samples kept under various storage conditions.

Animals—28 clinically normal horses.

Procedure—Total calcium (tCa) and magnesium (tMg) in equine serum were fractionated by use of a micropartition system that allows separation of protein-bound calcium (pCa) and magnesium (pMg) and ultrafiltrable calcium (µCa) and magnesium (µMg) fractions. Serum concentrations of iCa and iMg were measured in the ultrafiltrate by use of selective electrodes. Serum concentration of complexed calcium (cCa) or magnesium (cMg) was calculated by subtracting iCa or iMg from µCa or µMg, respectively.

Results—Mean ± SE serum tCa concentration was 3.26 ± 0.06 mmol/L. Calcium fractions were as follows: pCa, 1.55 ± 0.03 mmol/L (47.4 ± 0.9%); iCa, 1.58 ± 0.03 mmol/L (48.5 ± 0.7%); and cCa, 0.13 ± 0.02 mmol/L (4.1 ± 0.9%). Serum tMg concentration was 0.99 ± 0.04 mmol/L. Magnesium fractions were as follows: pMg, 0.33 ± 0.04 mmol/L (33.3 ± 4.2%); iMg, 0.57 ± 0.02 mmol/L (57.6 ± 1.7%); and cMg, 0.09 ± 0.02 mmol/L (9.1 ± 1.9%). Refrigeration (4°C) did not affect iCa values, whereas iMg declined by 8% after 120 hours. Neither iCa nor iMg was affected by freezing (−20°C).

Conclusions and Clinical Relevance—In equine serum, iMg is less stable than iCa; thus, when serum samples are not going to be analyzed promptly, freezing may be preferable to refrigeration for storage. (Am J Vet Res 2006;67:463–466)

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Conclusions and Clinical Relevance—In equine serum, iMg is less stable than iCa; thus, when serum samples are not going to be analyzed promptly, freezing may be preferable to refrigeration for storage.
Materials and Methods

Animals—Blood samples were collected from 28 healthy horses (19 mares, 4 stallions, and 5 geldings). Horses were considered healthy on the basis of normal findings on physical examinations, CBCs, and blood biochemical analyses results within reference range. In addition to routine blood biochemical analysis, mineral metabolism was studied in all horses by measuring serum calcium, phosphorus, magnesium, PTH, and calcitriol concentrations. Horses were maintained in paddocks and were fed the same diet (hay, oats, and a vitamin-mineral supplement) for at least 2 months before blood sample collection. Experimental protocols were reviewed and approved by the Ethics Committee for Animal Research of the University of Cordoba (Spain).

Blood sample collection and measurements—Blood samples were obtained anaerobically from the jugular vein and transferred to 10-mL vacuum tubes for serum separation. Samples were spun at 1,000 × g for 5 minutes to separate serum. The serum was anaerobically transferred from the collection tube to a syringe. Serum iCa and iMg concentrations and pH were measured immediately by use of selective electrodes. The tCa and tMg were then measured by spectrophotometry.1

Fractionation of serum tCa and tMg was performed on the fresh samples of equine serum by use of a micropartition system as previously described for other species.13-18 Briefly, a serum aliquot (2 mL) was gently placed into a disposable ultrafiltration membrane with a conical shape. The serum was subsequently spun at 4,000 × g for 15 minutes at 4°C to separate the protein-bound fractions, which were retained by the membrane, from the free Ca and Mg, which were filtered. Ultrafiltrable fractions were quantified by spectrophotometry.4 From these measurements, serum concentrations of complexed (cCa and cMg) and protein-bound (pCa and pMg) fractions were calculated as follows: cCa = µCa – iCa; cMg = µMg – iMg; pCa = tCa – µCa; and pMg = tMg – µMg, respectively. Additional biochemical measurements included total protein concentrations that were quantified by spectrophotometry in the serum (by use of the biuret technique)3 and ultrafiltrate.6 Inorganic phosphorus was measured by spectrophotometry,7 PTH was quantified by use of an immuno radiometric assay that has been validated for quantification of equine PTH,10 and calcitriol was measured by use of a radioimmunoassay.3

To study the effect of storage time and conditions on iCa and iMg measurements, iCa and iMg were measured in 14 serum samples at various times as follows: just after collection; after the samples had been refrigerated at 4°C for 120 hours; and after the samples had been frozen at −20°C for 15 and 30 days. These serum samples were obtained from the collection tubes as already described and were maintained in anaerobiosis during the storage time.

Statistical analysis—Mean ± SE values were calculated for each calcium and magnesium fraction. Results were expressed in absolute values and as a percentage of tCa and tMg. A Pearson correlation test was used to study the correlation between different calcium and magnesium fractions. Changes in iCa and iMg during storage were studied by paired t tests. For all statistical comparisons, values of P < 0.05 were considered significant.

Results

Horses of this study had a normal mineral metabolism on the basis of the following serum biochemical parameters: calcium, 3.26 ± 0.06 mmol/L; inorganic phosphorus, 0.84 ± 0.06 mmol/L; magnesium, 0.99 ± 0.04 mmol/L; PTH, 42.9 ± 4.1 pg/mL; and calcitriol, 8.9 ± 1.2 pg/mL. The serum tCa concentration (3.26 ± 0.06 mmol/L) was composed of 47% pCa (1.55 ± 0.03 mmol/L) and 53% µCa (1.71 ± 0.04 mmol/L; Table 1). When standardized for the serum total protein concentration (64.3 ± 0.9 g/L), the amount of calcium bound to proteins was 0.024 mmol Ca/g of protein. Greater than 90% of µCa was in the ionized form (iCa, 1.58 ± 0.03 mmol/L), and approximately 9% was complexed (cCa, 0.13 ± 0.02 mmol/L). A good correlation between tCa and µCa (r = 0.602; P = 0.006) was found. However, iCa was poorly correlated with tCa (r = 0.023; P = 0.929). Interestingly, µCa was better correlated with cCa (r = 0.951; P < 0.001) than with tCa (r = 0.264; P = 0.290).

Serum tMg concentration was 0.99 ± 0.04 mmol/L, and values for the magnesium fractions were as follows: iMg, 0.57 ± 0.02 mmol/L (58%); pMg, 0.33 ± 0.04 mmol/L (33%); and cMg, 0.09 ± 0.02 mmol/L (9%; Table 2). Based on the total protein concentration, the binding of magnesium to serum protein was 0.005 mmol of Mg/g of protein. As with calcium, tMg was well correlated with pMg (r = 0.823; P < 0.001) but not with iMg (r = −0.160; P = 0.527). Again, the ultrafiltrable fraction was better correlated with cMg (r = 0.959; P < 0.001) than with iMg (r = 0.470; P < 0.001).

Refrigeration of serum samples for up to 48 hours did not affect iCa values (Figure 1). However, samples that had been refrigerated for 120 hours had a small decline in iCa (from 1.38 ± 0.02 mmol/L to 1.53 ± 0.01 mmol/L; P = 0.04). The iMg was more affected by refrigeration, resulting in a time-dependent decrease in iMg that was significant after only 6 hours of storage. Although the decrease in magnesium values was sig-

<table>
<thead>
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<th>Variable</th>
<th>Absolute values (mmol/L)</th>
<th>% of tCa</th>
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<tr>
<td>tCa</td>
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<tr>
<td>µCa</td>
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<td>µMg</td>
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<td>48.5 ± 0.7</td>
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<td>cMg</td>
<td>0.13 ± 0.02</td>
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Figure 1—Serum iCa and iMg concentrations as a percentage of baseline values (iCa [mean ± SE], 1.58 ± 0.02 mmol/L; iMg, 0.58 ± 0.02 mmol/L) versus time in storage under refrigeration (4°C) or freezing (−20°C) conditions (n = 14 samples). *Significantly (P < 0.05) different from baseline value (0 hours).
Holley and Evans also analyzed some samples of serum. However, it should be taken into account that calcium seems to represent a smaller fraction in equine serum in samples of serum from 28 clinically normal horses. AJVR, Vol 67, No. 3, March 2006 465

In conclusion, our study reports the complete fractionation of serum calcium and magnesium in horses. In addition, our results indicate that serum magnesium is less stable than serum calcium, and therefore should be frozen.

Information available regarding serum magnesium concentration in horses is more limited than that of calcium. The tMg values were within the reference range for horses. As with calcium, pMg was slightly higher than previously reported values. In agreement with a prior study, our data indicate that horses have less pMg than tCa, not only in absolute values (0.005 vs 0.024 mmol/g of protein) but also in percentage (33.3 ± 4.2% vs 47.4 ± 0.9%). Compared with other species, humans have lower (19%) pMg than horses. However, the relationship between pMg and tMg is similar in horses (33% and 67%, respectively) and in dogs (37% and 63%, respectively).

Measurement of serum calcium and magnesium has important clinical applications in horses. Results of several studies indicate that for diagnostic purposes, measurement of the ionized fractions (iCa and iMg) is superior to quantification of serum tCa and tMg. In addition, as demonstrated by the lack of correlation between the total and ionized fractions, the total serum concentration is a poor estimator of the ionized value. Thus, it becomes clinically important to quantify iCa and iMg. However, most equine practitioners do not have ready access to ion-selective electrodes and may need to submit samples for analysis. Therefore, it is important to know the stability of iCa and iMg in samples of equine serum. Our results confirm previous findings indicating that iCa is fairly stable and that samples can be safely refrigerated without interfering with iCa values. To our knowledge, no previous data on iMg stability in equine serum are available. Information from other species indicates that iMg is stable in samples of human and canine serum. Our data reveal that although the changes are not prominent, iMg content significantly declines in refrigerated samples of equine serum. By contrast, no changes in either iCa or iMg values were found in samples frozen for up to 30 days. Thus, bearing in mind that in a clinical setting the storage conditions may be less perfect in comparison to our study (ie, complete anaerobiosis), we recommend that serum samples in which iMg cannot be promptly measured should be frozen.

In our study, samples were analyzed promptly, freezing is preferable to refrigeration for sample storage.
h. Phosphorus inorganic, Sigma Diagnostics, St Louis, Mo.

i. Nichols Institute Diagnostics, San Juan Capistrano, Calif.


References


