

Serum concentrations of calcium, phosphorus, magnesium and calciotropic hormones in donkeys

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Objective—To provide reference values for serum biochemical variables that are used for evaluation of mineral metabolism in donkeys and compare values with those in horses.

Animals—18 donkeys and 18 horses.

Procedures—Total calcium (tCa), total magnesium (tMg), and inorganic phosphorus (P) concentrations were measured in serum samples via spectrophotometry. Ionized calcium (iCa) and magnesium (iMg) concentrations were quantified with selective electrodes. By use of a micropartition system, tCa and tMg were fractionated to separate protein-bound (pCa, pMg) and ultrafiltrable fractions. Complexed calcium (cCa) and magnesium (cMg) concentrations were calculated by subtracting ionized fractions from ultrafiltrable fractions. Parathyroid hormone (PTH) and calcitriol (CTR) concentrations were measured via radioimmunoassay.

Results—Serum tCa concentration in donkeys (3.37 ± 0.21 mmol/L) was composed of pCa (1.59 ± 0.21 mmol/L [$47.0 \pm 4.2\%$]), iCa (1.69 ± 0.04 mmol/L [$50.4 \pm 3.0\%$]), and cCa (0.09 ± 0.08 mmol/L [$2.6 \pm 2.9\%$]). Serum tMg concentration (1.00 ± 0.08 mmol/L) was fractionated in pMg (0.23 ± 0.08 mmol/L [$23.4 \pm 8.1\%$]), iMg (0.59 ± 0.04 mmol/L [$58.8 \pm 5.1\%$]), and cMg (0.18 ± 0.08 mmol/L [$17.8 \pm 7.2\%$]). Serum concentrations of P (1.14 ± 0.30 mmol/L), PTH (20.4 ± 21.2 pg/mL), and CTR (13.4 ± 5.9 pg/mL) were determined.

Conclusions and Clinical Relevance—Serum variables of mineral metabolism in donkeys were within reference ranges for horses. However, when compared with horses, donkeys had higher iCa, cMg, and CTR and lower pMg and PTH concentrations. (*Am J Vet Res* 2006;67:1333–1336)

Diseases affecting mineral metabolism encompass a wide range of skeletal and soft tissue disorders related to alterations in the homeostasis of calcium (Ca) and phosphorus (P). In equids, these disorders may be broadly grouped as nutritional secondary hyperparathyroidism,¹ chronic renal disease,² primary hyperparathyroidism,³ pseudohyperparathyroidism,⁴

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ABBREVIATIONS

| | |
|-----|-------------------------------------|
| tMg | Total magnesium |
| iCa | Ionized calcium |
| iMg | Ionized magnesium |
| pCa | Protein-bound calcium |
| pMg | Protein-bound magnesium |
| cCa | Calcium complexed with weak acids |
| cMg | Magnesium complexed with weak acids |
| PTH | Parathyroid hormone |
| CTR | Calcitriol |
| tCa | Total calcium |
| uCa | Ultrafiltrable calcium |
| uMg | Ultrafiltrable magnesium |
| TP | Total protein |

and vitamin D toxicosis.⁵ Alterations in serum Ca and magnesium (Mg) are also common in horses with gastrointestinal diseases (colic and diarrhea).^{6,7} In addition, mineral metabolism is known to be affected by exercise of various intensities (show jumping,⁸ 3-day event,⁹ and endurance races¹⁰) in horses.

Mineral metabolism is routinely evaluated by measurement of the serum concentrations of Ca, P, and Mg. Total Ca and tMg are present in serum in 3 forms: iCa and iMg; pCa and pMg; and cCa and cMg, such as citrate, phosphate, and bicarbonate.¹¹⁻¹² The use of micropartitioning systems based on the filtration method allows separation of the Ca and Mg fractions. Micropartitioning systems contain a membrane that is highly retentive for serum proteins, through which serum is filtered. After centrifugation, the protein-bound fractions are retained and the ultrafiltrable fractions (ionized and complexed) pass through the membrane.^{13,14} Measurement of the ionized fractions by use of selective electrodes permits distinguishing between the ionized and complexed minerals in the ultrafiltrate.^{12,15}

In addition to measurement of the mineral ions (Ca, P, and Mg), the 2 main calciotropic hormones PTH and CTR are usually quantified in serum for evaluation of mineral metabolism. Parathyroid hormone is a polypeptide secreted by the parathyroid glands that promotes Ca and P release from bone and increases Ca reabsorption and P excretion in the kidneys. Thus, the combined action of PTH is to increase serum Ca and to decrease serum P. In addition to its direct effects on bone and the kidneys, PTH also has an indirect effect on the kidneys, promoting the synthesis of CTR.¹⁶ Calcitriol is the active form of vitamin D (1,25-dihydroxyvitamin D), which is synthesized in the kidneys from its precursor 25-hydroxyvitamin D. Calcitriol exerts hypercalcemic and hyperphosphatemic actions

by promoting release of Ca and P from bone and increasing intestinal absorption of Ca and P.¹⁷

Reference values for tCa, P, and tMg are available in horses, and the complete fractioning of tCa and tMg in horse serum has been described recently.¹⁸ An immunoradiometric method has been validated for measurement of PTH in horses, and the range of serum PTH concentrations in clinically normal horses has been reported.¹⁹ Likewise, serum CTR concentrations have been studied in horses.²⁰ Although disorders in mineral metabolism are also important in donkeys, there is an almost complete lack of information on serum variables of mineral metabolism in that species. Donkeys are becoming popular as pets, and there is an increasing demand for medical care for them. Therefore, it is important to know specific reference values for variables that allow a detailed study of mineral metabolism, instead of extrapolating reference ranges from horses. To our knowledge, only reference values for tCa, tMg, and P have been reported in donkeys,²¹ and there are no published data on either Ca and Mg fractions or calciotropic hormones.

The purpose of the study reported here was to provide reference values of serum biochemical variables used for evaluation of mineral metabolism, including the calciotropic hormones PTH and CTR, and determine results of complete fractioning of tCa and tMg in donkeys.

Materials and Methods

Animals—Blood samples were collected from 18 healthy adult (mean \pm SD age, 8.2 ± 5.5 years,) donkeys (15 jennets, 3 jacks). Because there are few reference data for calciotropic hormones in equids, a control group of 18 adult (mean age, 9.4 ± 3.8 years) horses (14 mares, 4 stallions) was also studied. These horses were housed at the same premises and had the same management as that of the donkeys. Animals were considered healthy on the basis of normal results of a clinical examination and values within the species' reference ranges for CBC and blood biochemical variables. Donkeys and 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 sampling. The experimental protocols were reviewed and approved by the Ethics Committee for Animal Research of the University of Cordoba.

Sample handling and measurement—Blood samples were obtained anaerobically from a jugular vein and transferred to 10-mL vacuum tubes for serum separation.^a Samples were centrifuged at $1,000 \times g$ for 5 minutes to separate serum. The serum was anaerobically transferred from the collection tube to a syringe. Serum iCa, iMg, and pH were measured immediately by use of selective electrodes.^b Total Ca and tMg were then measured by use of spectrophotometry.^{c,d}

Fractioning of serum tCa and tMg was performed on the fresh serum samples by use of a micropartitioning system as described in horses.¹⁸ Briefly, a serum aliquot (2 mL) was gently placed into a conical, disposable ultrafiltration membrane.^e The serum was centrifuged at $4,000 \times g$ for 15 minutes at 4°C to separate the protein-bound fractions, which were retained by the membrane, from the uCa and uMg, which were filtered. The ultrafiltrable fractions were quantified by use of spectrophotometry.^{c,d} From these measurements, cCa, cMg, pCa, and pMg fractions were calculated as follows:

$$\begin{aligned}cCa &= uCa - iCa, \\cMg &= uMg - iMg, \\pCa &= tCa - uCa, \\pMg &= tMg - uMg\end{aligned}$$

Serum was then frozen (-20°C) and stored until analysis for further biochemical measurements. Total proteins were quantified via spectrophotometry in serum (by use of the biuret technique)^f and in the ultrafiltrate.^g Inorganic P was measured via spectrophotometry,^h PTH was quantified by use of an immunoradiometric assay that has been validated for quantification of equine PTH,ⁱ and CTR was measured by use of a radioimmunoassay.^j

Statistical analysis—Results of the Ca and Mg fractioning were expressed in absolute values and as a percentage of tCa and tMg. A Pearson test was used to study correlation between the different Ca and Mg fractions. Comparison between donkey and horse values was made by use of unpaired *t* tests. All values are expressed as mean \pm SD. For all statistical comparisons a value of $P < 0.05$ was considered significant.

Results

Donkeys had a serum concentration of tCa of 3.37 ± 0.21 mmol/L; this value was not significantly different from tCa concentration in horses (3.29 ± 0.25 mmol/L). Values for tMg were also similar between donkeys (1.00 ± 0.08 mmol/L) and horses (0.98 ± 0.13 mmol/L). Serum P concentration, however, was significantly ($P = 0.002$) higher in donkeys (1.14 ± 0.30 mmol/L) than in horses (0.85 ± 0.25 mmol/L). In donkeys, lower serum PTH concentration (20.4 ± 21.2 pg/mL vs 46.6 ± 22.5 pg/mL; $P = 0.001$) and slightly higher serum CTR concentration (13.4 ± 5.9 pg/mL vs 8.6 ± 4.7 pg/mL; $P = 0.02$), compared with horses, were also found.

Results of fractioning of serum Ca in donkeys were determined. The tCa concentration (3.37 ± 0.21 mmol/L) was composed of 47% pCa (1.59 ± 0.21 mmol/L) and 53% uCa (1.78 ± 0.08 mmol/L). Serum pH and TP concentration were similar in donkeys (pH, 7.474 ± 0.027 ; TP, 66.0 ± 5.1 g/L) and horses (pH, 7.498 ± 0.047 ; TP, 64.3 ± 3.8 g/L). When standardized for serum TP concentration, the amount of Ca bound to proteins in donkeys was 0.024 ± 0.004 mmol Ca/g of protein. Ninety-five percent of uCa was iCa (1.69 ± 0.04 mmol/L), and 5% was cCa (0.09 ± 0.08 mmol/L). Excellent correlation between tCa and pCa ($r = 0.899$; $P < 0.001$) and weaker correlation between tCa and iCa ($r = 0.536$; $P = 0.02$) were found. Interestingly, uCa was much better correlated with cCa ($r = 0.939$; $P < 0.001$) than with iCa ($r = 0.300$; $P = 0.226$). When compared with the data of a previous study,¹⁸ no differences in Ca fractions were found between donkeys and horses, except for iCa, which was significantly higher in donkeys than in horses (1.69 ± 0.04 mmol/L vs 1.59 ± 0.13 mmol/L; $P = 0.01$).

Partitioning of serum tMg was determined in donkeys. The tMg concentration was fractioned in pMg (0.23 ± 0.08 mmol/L [$23.4 \pm 8.1\%$]), iMg (0.59 ± 0.04 mmol/L [$58.8 \pm 5.1\%$]), and cMg (0.18 ± 0.08 mmol/L [$17.8 \pm 7.2\%$]). On the basis of the TP concentration, binding of Mg to serum protein was 0.004 ± 0.001 mmol of Mg/g of protein. As with Ca, tMg was better

correlated with pMg ($r = 0.663$; $P = 0.01$) than with iMg ($r = 0.483$; $P = 0.04$). The ultrafiltrable fraction was better correlated with cMg ($r = 0.916$; $P < 0.001$) than with iMg ($r = 0.458$; $P = 0.08$). In comparison to the data of a previous study,¹⁸ donkeys had lower pMg (23% vs 33%; $P = 0.01$), higher uMg (77% vs 67%; $P = 0.01$), and higher cMg (18% vs 9%; $P = 0.01$) than horses.

Discussion

The study of serum variables related to mineral metabolism is of great importance in the diagnosis of several equine diseases and in studies of equine exercise physiology.¹⁻¹⁰ Although in recent years substantial advances have been made in the knowledge of equine Ca metabolism,¹⁸⁻²⁰ there is a lack of information on serum variables used for assessment of mineral metabolism in donkeys.

To our knowledge, only reference values for total serum concentrations of Ca, Mg, and P have been reported in donkeys.²¹ The data obtained in the present study for tCa, tMg, and P were in agreement with the values reported in the literature.²¹ No significant differences in serum tCa and tMg concentrations were found between horses and donkeys. Although results indicated that donkeys had higher P values than horses, the serum P concentration was within the reference range for horses. Thus, published reference values for tCa, P, and tMg in horses should also be valid for donkeys.

Complete fractioning of serum Ca and Mg in horses has been recently reported.¹⁸ Results of fractioning of serum Ca were similar between donkeys and horses; the proportion of pCa and uCa was almost identical. The relationship between pCa and uCa in donkeys was also within the range of reported values in humans^{11,13,15} and carnivores,²² although absolute values are different because of higher tCa in donkeys. By contrast, when compared with horses, donkeys have slightly higher iCa concentrations.¹⁸ The ionized fraction, which is able to interact with the Ca sensing receptor, is thought to be the only physiologically active Ca fraction.¹⁵ This theory is reinforced by results of studies^{7,23} that reveal that quantification of serum iCa is superior to measurements of the total electrolyte concentration for diagnosis of hypocalcemia in horses. Thus, although the iCa values of donkeys were within the reference range for horses, results indicated that donkeys had higher active Ca concentrations in serum than horses.

Although tMg values were almost identical in donkeys and horses, there were significant differences among the Mg fractions in both species. In contrast with Ca, the proportion of pMg and uMg was significantly different between donkeys and horses. In comparison to the data of a previous study,¹⁸ donkeys had lower pMg and higher uMg than horses. Comparison between protein-bound and ultrafiltrable fractions in different studies is complicated by the fact that filtration membranes may differ in permeability.¹³ However, differences between donkeys and horses in the present study were not influenced by this factor because the same membrane was used in both.¹⁸ Although comparison among studies is difficult because of differences in methodology, the proportion of pMg and uMg in donkey

serum was similar to that of human serum.¹² However, canine serum has pMg and uMg values closer to those of equine serum than to those of donkey serum.²⁴ It is also interesting that although uMg concentration was higher in donkeys than in horses, serum iMg concentration was almost identical in both species. Thus, the difference in uMg concentration was attributable to higher cMg concentration in donkeys (0.18 ± 0.08 mmol/L), compared with horses (0.09 ± 0.08 mmol/L). As with Ca, the active form of Mg is iMg; however, cMg may also have a biological role because it has been suggested that divalent cations loosely bound to weak acids may be able to interact with cationic receptors.²⁵ Measurement of the cMg fraction has clinical interest because cMg concentration has been reported to increase in certain diseases (eg, renal disorders).²⁶ Therefore, the differences in cMg concentration between donkeys and horses may be clinically relevant.

Total Ca and Mg concentrations in donkey serum were poor predictors of the ionized fractions, as indicated by the study of correlation. This is in agreement with a previous study¹⁸ in horses and supports the need for measurement of iCa and iMg concentrations. Likewise, the ultrafiltrable fractions were better correlated with the complexed than with the ionized fractions; therefore, ultrafiltration should not be used as an alternative to measurement of iCa and iMg concentrations by use of selective electrodes.

Measurement of serum PTH concentrations is an important diagnostic tool in the diagnosis of a variety of equine diseases.¹⁻⁷ Immunoradiometric assays have been validated for quantification of equine PTH, and the reference range of PTH concentration in equine serum has been reported.¹⁹ Results of the present study indicated that donkeys have serum PTH concentrations in the low portion of the reference range for horses. These low PTH concentrations are likely to be related to the high iCa concentrations that were also detected in donkey serum.

There is considerable controversy about serum CTR concentrations in horses. Horses have been reported to lack renal 1- α -hydroxylase activity and to have considerably lower serum CTR concentrations than other species (range, 8 to 20 pg/mL).²⁰ The present study revealed serum CTR concentrations within the equine reference range in both donkeys and horses. The higher CTR concentrations found in donkeys were consistent with the higher iCa and P values (CTR has hypercalcemic and hyperphosphatemic actions)¹⁷ and with the lower PTH concentration (CTR inhibits parathyroid gland function).²⁷

- a. Vacutainer, Becton-Dickinson, Plymouth, UK.
- b. Nova Stat Profile Critical Care Xpress, Nova Biomedical, Waltham, Mass.
- c. Calcium, Sigma Diagnostics, St Louis, Mo.
- d. Magnesium, Sigma Diagnostics, St Louis, Mo.
- e. Amicon Ultra-4, Millipore, Carrigtwohill, Ireland.
- f. BioSystems Reagents & Instruments, Barcelona, Spain.
- g. Bio-Rad protein assay, Bio-Rad Laboratories GmbH, Munchen, Germany.
- h. Phosphorus inorganic, Sigma Diagnostics, St Louis, Mo.
- i. Nichols Institute Diagnostics, San Juan Capistrano, Calif.
- j. Immunodiagnostic Systems, Bolton, UK.

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