Comparison of serum parathyroid hormone and ionized calcium and magnesium concentrations and fractional urinary clearance of calcium and phosphorus in healthy horses and horses with enterocolitis

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Objective—To evaluate calcium balance and parathyroid gland function in healthy horses and horses with enterocolitis and compare results of an immunoradiometric assay (IRMA) with those of an immunoluminometric assay (ICMA) for determination of serum intact parathyroid hormone (PTH) concentrations in horses.

Animals—64 horses with enterocolitis and 62 healthy horses.

Procedures—Blood and urine samples were collected for determination of serum total calcium, ionized calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)), phosphorus, BUN, total protein, creatinine, albumin, and PTH concentrations, venous blood gases, and fractional urinary clearance of calcium (FCa) and phosphorus (FP). Serum concentrations of PTH were measured in 40 horses by use of both the IRMA and ICMA.

Results—Most (48/64; 75%) horses with enterocolitis had decreased serum total calcium, Ca\(^{2+}\), and Mg\(^{2+}\) concentrations and increased phosphorus concentrations, compared with healthy horses. Serum PTH concentration was increased in most (36/51; 70.6%) horses with hypocalcemia. In addition, FCa was significantly decreased and FP significantly increased in horses with enterocolitis, compared with healthy horses. Results of ICMA were in agreement with results of IRMA.

Conclusions and Clinical Relevance—Enterocolitis in horses is often associated with hypocalcemia; 79.7% of affected horses had ionized hypocalcemia. Because FCa was low, it is unlikely that renal calcium loss was the cause of hypocalcemia. Serum PTH concentrations varied in horses with enterocolitis and concomitant hypocalcemia. However, we believe low PTH concentration in some hypocalcemic horses may be the result of impaired parathyroid gland function. (Am J Vet Res 2001;62:938–947)

The primary function of the parathyroid gland is to prevent hypocalcemia by rapid secretion of parathyroid hormone (PTH) when serum ionized calcium (Ca\(^{2+}\)) concentrations decrease. In several species, PTH induces an acute increase in blood calcium concentration by enhancing renal reabsorption and bone release of calcium and a chronic increase by facilitating intestinal calcium absorption. For reasons not yet understood, serum Ca\(^{2+}\) concentrations decrease in humans and other animals with certain pathologic conditions (eg, endotoxemia, sepsis, severe burns), which often results in an increase in serum PTH concentration and subsequent return of serum Ca\(^{2+}\) concentration to within the physiologic range.

Clinical observation has revealed that many horses with severe enterocolitis are hypocalcemic. Hypocalcemia may result in neuromuscular, cardiovascular, and gastrointestinal tract dysfunction. Muscle weakness, tetany, synchronous diaphragmatic flutter, cardiac arrhythmias, ileus, and convulsions have been observed in hypocalcemic horses. Severe hypocalcemia can result in death. Supplementation with calcium added to replacement fluids appears to benefit horses with enterocolitis and hypocalcemia. Although the contribution of calcium to improvement in gastrointestinal tract and cardiac function in such horses has not been defined, we believe that restoration of calcium homeostasis is an important aspect of managing horses with enterocolitis.

We hypothesized that horses with severe enterocolitis and hypocalcemia would also have high serum PTH concentrations. High PTH concentrations would result in increased renal resorption of calcium, decreased fractional urinary clearance of calcium (FCa), and increased fractional urinary clearance of phosphorus (FP).

The role of ionized magnesium (Mg\(^{2+}\)) has received less attention than that of Ca\(^{2+}\) in critically ill patients. In humans, serum Mg\(^{2+}\) concentration can influence Ca\(^{2+}\) concentration, and Mg\(^{2+}\) deficiency can result in hypocalcemia attributable to impaired PTH secretion as well as altered renal and skeletal responsiveness to PTH. Treatment with magnesium restores these deficiencies. 1,25-Dihydroxyvitamin D metabolism is more sensitive to Mg\(^{2+}\) depletion than either PTH secretion or PTH-mediated osteoclastic bone resorption. The effects of Mg\(^{2+}\) on mineral metabolism may be more important in chronically ill patients than has been appreciated. Few data are available regarding serum Mg\(^{2+}\) concentrations in healthy or ill horses.
There are multiple radioimmunoassays for use in measuring PTH concentrations. The assays recognize either the N-terminal, C-terminal, or midregion fragment of PTH or the full-length (intact) hormone. Results of radioimmunoassays for the C-terminal and midregion fragments do not reflect biological activity of the hormone; only assays for the N-terminal region or intact hormone measure biologically active PTH.

The introduction of 2-site immunometric assays to measure intact PTH has resulted in an important advance, because these assays provide greater reliability and precision than earlier assays. Two-site immunometric assays for PTH use 2 antibodies, one to the N-terminal region and the other to the C-terminal region. Intact PTH is captured between these 2 antibodies. The immunoradiometric assay (IRMA) for PTH has been shown to be useful in dogs, cats, cows, and horses. In horses, measurement of serum PTH concentration has been used to aid in the diagnosis of iatrogenic hypocalcemia of foals, primary hyperparathyroidism, nutritional secondary hyperparathyroidism, pseudohyperparathyroidism, vitamin D toxicosis, and renal disease. Evaluation of serum PTH concentration has also been used to investigate the relationship between serum Ca\(^2+\) and PTH concentrations in healthy horses.

Recently, an automated solid-phase 2-site immunonephelometric assay (ICMA) for quantitative determination of intact PTH has become available. Results of this assay and those of the IRMA are in good agreement for human serum. However, the ICMA can detect lower serum PTH concentrations and is more sensitive and more reliable than the IRMA.

The purposes of the study reported here were to compare the ICMA and IRMA for measurement of intact PTH in horses and to evaluate calcium balance and parathyroid gland function in healthy horses and horses with enterocolitis by measuring serum total calcium and Ca\(^2+\), phosphorus, Mg\(^2+\), and intact PTH concentrations and FCa and FP.

Materials and Methods

Animals—This study was approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee. The affected group consisted of 64 (39 sexually intact females, 18 castrated males, 7 sexually intact males) horses with enterocolitis that were selected over a 1-year period from horses admitted to The Ohio State University Veterinary Teaching Hospital with a history of severe diarrhea of < 48 hours duration. Horses in this group were between 1 and 22 years of age (mean ± SD, 6.3 ± 5.1 years) and comprised several breeds (19 Quarter Horses, 11 Thoroughbreds, 11 Standardbreds, and 23 other breeds). Affected horses had received no calcium and no more than 5 L of fluid, administered IV or PO, prior to admission. Complete physical examinations, including cardiovascular, respiratory tract, and gastrointestinal tract assessment and assessment of rectal temperature, hydration status, and mentation, were performed immediately after admission. Clinical evidence of acute profuse watery diarrhea and dehydration were required for entry into the study.

The control group consisted of 62 (42 sexually intact females, 14 castrated males, 6 sexually intact males) healthy horses between 2 and 21 years of age (6.9 ± 3.6 years). Healthy horses were selected from the Ohio State University College of Veterinary Medicine teaching herd during a 1-year period and comprised several breeds (35 Standardbreds, 15 Thoroughbreds, and 12 other breeds). Horses in this group were fed a diet of grass and grass hay, had no history of illness for the 3 months preceding the study, and had received no treatments for the month preceding the study. Horses were determined to be healthy on the basis of results of complete physical examinations. In addition, results of CBC and biochemical analyses, including determination of serum total calcium and Ca\(^2+\) concentrations and plasma fibrinogen concentration, were within reference ranges.

Venous blood samples from 3 horses with chronic renal failure and hypercalcemia (one 10-year-old Thoroughbred mare, one 7-year-old Thoroughbred gelding, and one 10-year-old Quarter Horse gelding) were used to determine serum intact PTH concentrations for PTH assay validation.

Sample collection—Venous blood samples for determination of serum total calcium, Ca\(^2+\), total protein, albumin, creatinine, BUN, phosphorus, and Mg\(^2+\) concentrations were collected into tubes without additives. Venous blood samples for CBC were collected into tubes with EDTA and, for determination of plasma fibrinogen concentration, into tubes with sodium citrate. Venous blood samples were also collected under anaerobic conditions into heparinized syringes for determination of blood gases. Urine was collected by free catch or catheterization for urinalysis and urine biochemical analyses. Blood samples for serum biochemical analyses were collected at the same time as urine.

Hematologic and biochemical analyses—Complete blood counts were performed, using an automated system. Serum total calcium and protein, creatinine, BUN, albumin, and phosphorus concentrations were determined by use of colorimetric reactions in an automated analyzer. Plasma fibrinogen concentrations were determined, using a nephelometric analyzer. Venous blood gases were measured, using a blood gas analyzer, and pH was corrected for rectal temperature. Blood samples for Ca\(^2+\) and Mg\(^2+\) determinations were processed immediately, using Ca\(^2+\)- and Mg\(^2+\)-selective electrodes.

Fractional clearance of calcium and phosphorus—Urine was acidified with 6 N HCl to dissolve calcium salts. Urine calcium and creatinine concentrations were measured as described for serum. Fractional clearances of calcium and phosphorus were calculated, using serum and urine calcium, phosphorus, and creatinine concentrations, as described by Roussel et al.

Determination of intact PTH concentration—Venous blood, collected into tubes without additives, was centrifuged at 4 C immediately after clotting, and serum was stored at –20 C for batch analysis. Serum PTH concentrations were measured by use of 2-site IRMA for human intact PTH that was validated for use in horses and a 2-site ICMA for human intact PTH.

Ten serum samples for which PTH concentrations were measured by the Nichols Institute IRMA and the ICMA were selected and sent to an independent laboratory. The PTH concentration range for these samples, determined by use of the ICMA, was 3.5 to 77.0 pmol/L. The independent laboratory routinely measures PTH in equine serum by use of the DPC IRMA, and we wanted to compare their results to ours. Intact PTH concentrations were reported in pmol/L (pmol/L = pg/ml X 0.105).

The ICMA is a solid-phase 2-site assay. The solid phase, a polystyrene bead in the test unit, is coated with an affinity.
ty-purified goat polyclonal antibody against amino acids 44 to 84 of human PTH. Serum samples and alkaline phosphatase-conjugated affinity-purified goat polyclonal antibody against amino acids 1 to 34 of human PTH were incubated for 60 minutes at 37°C in the test unit, with intermittent agitation. Intact PTH in the sample was bound and formed an antibody sandwich complex. The assay system automatically handled sample and reagent additions, incubation and separation steps, and measurement of photon output by use of a temperature-controlled luminometer. Results were calculated from an observed signal, using a stored master curve. The working range was 0.1 to 263 pmoL/L (1 to 2,500 pg/ml), and the sample volume was 50 μL.

The PTH IRMA (like the ICMA) is an immunometric assay based on 2 antibodies, in which a solid phase (a polystyrene bead or a polystyrene tube) is coated with a high affinity-purified goat polyclonal antibody against the midregion and C-terminal region of PTH. The second antibody binds the N-terminal region of PTH, and this antibody is labeled with 125I. Only intact PTH is able to form the antibody sandwich complex necessary for detection. This assay has been previously validated for determination of intact PTH concentration in equine serum. Sensitivity of the ICMA was defined as the smallest single value that could be distinguished from 0 at the 95% confidence limit, using equine serum. For the ICMA and IRMA, the manufacturers report a calculated sensitivity of 0.1 pmoL of intact PTH/L for human serum. We determined a sensitivity of 0.12 pmoL of intact PTH/L for equine serum by use of the ICMA.

The intra-assay coefficient of variation for the ICMA was determined by measuring 5 aliquots each of equine serum with low (2.5 pmoL/L), medium (19.0 pmoL/L), and high (180.0 pmoL/L) PTH concentrations. The interassay coefficient of variation for the ICMA was determined by comparison of results of 5 replicated measures of equine serum with low (8.1 pmoL/L), medium (20.5 pmoL/L), and high (170.0 pmoL/L) PTH concentrations. Equine serum samples with low (6.2 pmoL/L), medium (26.8 pmoL/L), and high (180.0 pmoL/L) PTH concentrations were assayed undiluted and diluted 1:2, 1:4, and 1:8, and results were evaluated for dilutional parallelism by comparing measured (observed) values with expected (calculated) values. Two additional dilutions were performed (1:16 and 1:32) for the sample with high PTH concentration. Expected values were calculated by multiplying measured values by the dilution factor. Correlations between observed PTH and calculated PTH concentrations were determined. Precision of the IRMA was not evaluated, because this assay has been validated for use in horses.

Comparison of results between the IRMA and ICMA—Serum PTH concentrations were determined by use of both the ICMA and IRMA for 32 horses with enterocolitis, 3 horses with chronic renal failure and hypercalcemia, and 5 clinically normal horses. Results were compared between tests.

Statistical analyses—Initial analysis consisted of descriptive statistics for all variables. Data were compared between affected and healthy horses by use of an unpaired t-test or the Mann-Whitney rank test. All analyses were performed with the assistance of commercially available software.

On the basis of serum Ca²⁺ concentration, horses with enterocolitis were assigned to a normocalcemic or hypocalcemic (serum Ca²⁺ concentration < 0.6 mg/dL) group. The serum Ca²⁺ concentration cutoff used to define hypocalcemia was derived from results of a previous report of Ca²⁺ concentrations in healthy horses and data from healthy horses in the present study. Calcium concentrations less than the minimum limit defined by the 95% confidence interval were considered below normal. The hypocalcemic group was further divided into 3 subgroups on the basis of serum PTH concentration. Affected horses with serum PTH concentrations within reference range were considered nonresponders, whereas horses with midrange PTH concentrations were considered midresponders, and horses with high PTH concentrations were considered high responders. Results were compared among these 3 subgroups by use of a Kruskal-Wallis 1-way ANOVA on ranks, with pairwise multiple comparisons performed by use of the Dunn method.

For affected horses, data regarding serum PTH concentrations failed the Kolmogorov-Smirnov test for normality. Therefore, Spearman rank tests for correlation were used to calculate correlation (ρ) between serum PTH concentration in affected horses and total calcium, Ca²⁺, Mg²⁺, and phosphorus concentrations and FCa and FP. A Pearson product moment test was used to calculate correlation (r) between PTH concentration and total calcium, Ca²⁺, Mg²⁺, and phosphorus concentrations and FCa and FP in healthy horses.

Because PTH concentrations in horses with enterocolitis were not normally distributed, and the test for equal variance between serum PTH concentration in healthy horses and horses with enterocolitis failed, a Mann-Whitney rank sum test was used to compare PTH concentrations in healthy and affected horses. Fractional urinary clearance of calcium and FP were compared between these 2 groups by use of a t-test. For all tests, P < 0.05 was considered significant.

Bland-Altman bias plots, in which differences between results of ICMA and IRMA for each sample were plotted against the mean of these 2 values, were used to determine agreement between the 2 PTH assays. Bias was defined as the mean difference between values obtained for each sample by the 2 different methods, and error or variability was defined as the SD of these differences.

Results

Physical examination results—Mean ± SD duration of enterocolitis before admission was 27.3 ± 15.4 hours for the 64 affected horses in our study. Physical examination on admission revealed that heart rate was significantly higher in horses with enterocolitis (71 ± 21 beats/min), compared with healthy horses (40 ± 1 beats/min). Respiratory rate, rectal temperature, and capillary refill time were also significantly higher in horses with enterocolitis (35 ± 17 breaths/min, 38.2 ± 0.9 °C, and 3 ± 1 seconds, respectively), compared with healthy horses (16 ± 3 breaths/min, 37.6 ± 0.4 °C, and < 2 seconds, respectively).

Serum analytes—Serum total calcium and Ca²⁺ concentrations were significantly less in horses with enterocolitis than in healthy horses (Table 1). Differences in the Ca²⁺-to-total calcium concentration ratio (Ca²⁺/Ca) were not detected between groups. Total calcium concentrations < 10.9 mg/dL were considered less than reference range, and 48 of 64 (75.0%) horses with enterocolitis had total hypocalcemia. In addition, Ca²⁺ concentrations < 6.0 mg/dL were considered less than reference range, and 51 of 64 (79.7%) horses with enterocolitis had ionized hypocalcemia. Serum phosphorus concentration was significantly higher in horses with enterocolitis than healthy horses,
Table 1—Results (mean ± SD) of serum biochemical analyses for healthy horses and horses with enterocolitis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy</th>
<th>All</th>
<th>Normocalcemic</th>
<th>Hypocalcemic (n = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 62)</td>
<td>(n = 64)</td>
<td>(n = 13)</td>
<td>(n = 15)</td>
</tr>
<tr>
<td>Total calcium (mg/dl)</td>
<td>11.9 ± 0.5†</td>
<td>10.08 ± 1.6</td>
<td>11.3 ± 0.9</td>
<td>9.7 ± 1.6</td>
</tr>
<tr>
<td>Ca²⁺/Ca</td>
<td>66.8 ± 0.3†</td>
<td>55.5 ± 0.9</td>
<td>64.4 ± 0.3</td>
<td>53.0 ± 0.4</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>66.8 ± 0.3†</td>
<td>55.5 ± 0.9</td>
<td>64.4 ± 0.3</td>
<td>53.0 ± 0.4</td>
</tr>
<tr>
<td>Mg²⁺/Ca</td>
<td>0.51 ± 0.17</td>
<td>0.40 ± 0.1</td>
<td>0.05 ± 0.1</td>
<td>0.045 ± 0.08</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>5.2 ± 1.0†</td>
<td>5.2 ± 2.4</td>
<td>4.6 ± 2.4</td>
<td>5.5 ± 2.8</td>
</tr>
<tr>
<td>Ca²⁺/Ca</td>
<td>14.1 ± 2.0†</td>
<td>40.8 ± 29.0</td>
<td>26.7 ± 16.2</td>
<td>122.2 ± 56.6</td>
</tr>
<tr>
<td>PTH (pmol/L)</td>
<td>1.3 ± 0.2†</td>
<td>3.6 ± 1.8</td>
<td>2.7 ± 1.6</td>
<td>3.1 ± 1.6</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.5 ± 0.1</td>
<td>0.8 ± 1.5</td>
<td>0.6 ± 1.3</td>
<td>5.8 ± 1.8</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>0.5 ± 0.0†</td>
<td>3.2 ± 0.7</td>
<td>2.7 ± 0.7</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>0.7 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.8 ± 0.6</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 ± 0.01</td>
<td>7.35 ± 0.08</td>
<td>7.36 ± 0.06</td>
<td>7.34 ± 0.11</td>
</tr>
<tr>
<td>Total CO₂ (mEq/L)</td>
<td>23.8 ± 4.9</td>
<td>24.2 ± 4.6</td>
<td>23.2 ± 6.2</td>
<td>25.0 ± 4.5</td>
</tr>
</tbody>
</table>

Table 2—Mean ± SD fractional urinary clearance of calcium (FCa) and phosphorus (FP) in healthy horses and horses with enterocolitis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy</th>
<th>All</th>
<th>Normocalcemic</th>
<th>Hypocalcemic (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 20)</td>
<td>(n = 20)</td>
<td>(n = 4)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>FCa (%)</td>
<td>3.4 ± 2.6†</td>
<td>0.8 ± 0.8</td>
<td>2.1 ± 1.8</td>
<td>0.5 ± 0.46</td>
</tr>
<tr>
<td>FP (%)</td>
<td>0.08 ± 0.2†</td>
<td>1.9 ± 2.6</td>
<td>1.8 ± 0.6</td>
<td>3.4 ± 3.6</td>
</tr>
</tbody>
</table>

*Horses with enterocolitis were assigned to normocalcemic and hypocalcemic groups on the basis of serum Ca²⁺ concentration (< 0.47 mmol/L). Horses in the hypocalcemic group were assigned to 3 groups on the basis of serum PTH concentrations (nonresponder, PTH concentration range of 0.5 to 3.0 pmol/L; midresponder, range of 3.1 to 12.0 pmol/L; high responder, range of 12.1 to 20 pmol/L). Significantly (P < 0.05) different from value for all horses with enterocolitis.

Ca²⁺ = Ionized calcium. Ca²⁺/Ca = Ratio of ionized calcium to total calcium concentration. Mg²⁺ = Ionized magnesium. PTH = Parathyroid hormone.

whereas Mg²⁺ concentration was significantly less. Ionized magnesium concentrations < 0.47 mmol/L were considered less than reference range; 50 of 64 (78.0%) horses with enterocolitis had ionized hypomagnesemia.

Fractional urinary clearance of calcium and phosphorus—Urine and blood samples were collected simultaneously from 20 healthy horses and 20 horses with enterocolitis and hypocalcemia. Fractional urinary clearance of calcium was significantly less, and FP significantly greater, in horses with enterocolitis than in healthy horses (Table 2). Fractional urinary clearance of calcium in all horses with enterocolitis was less than the mean FCa of the control group (Fig 1).

Evaluation of the ICMA—The ICMA had intra-assay coefficients of variation of 5.6, 6.3, and 7.7% for serum samples with low (2.5 pmol/L), medium (19.0 pmol/L), and high (180 pmol/L) concentrations of PTH, respectively. The interassay coefficients of variation for serum samples with low (8.1 pmol/L), medium (20.5 pmol/L), and high (170 pmol/L) PTH concentrations were 6.4, 6.3, and 5.9%, respectively. Determination of PTH concentration, using serial dilutions of serum samples containing low (6.2 pmol/L), medium (26.8 pmol/L), and high (180 pmol/L) concentrations, revealed parallelism between measured (observed) and expected (calculated) values (r = 0.99).

Comparison of the IRMA and ICMA—When results were compared by use of the Mann-Whitney test, significant differences were not detected between assays (P = 0.80). Results of these 2 tests were correlated (r = 0.98). The 2 assays were also compared by use of difference or bias plots (ie, Bland-Altman plots). Analysis of results for all serum samples suggested that differences between methods were proportionally greater as PTH concentration increased (Fig 2). However, all but 3 data points were within 2 SD of the mean difference. The bias over the whole range of values was 1.9 pmol/L, and the error (variability) was 6.9 pmol/L. When the 5 serum samples with PTH concentrations > 50 pmol/L were excluded from analysis, the bias was 0.18 pmol/L, and error was 2.5 pmol/L, confirming our impression that there was proportional bias in these data. Results of the ICMA were usually greater than those of the IRMA for serum samples with high PTH concentrations. In addition, at high PTH concentrations, the difference in results between the 2 assays was greater than at lower concentrations. Our interpretation of these analyses was that, despite some proportional bias, agreement between results of ICMA and IRMA was good, and differences in values obtained by use of these 2 methods are not likely to be clinically important.
Serum intact PTH concentrations—In healthy horses (n = 62), serum PTH concentration as measured by use of the ICMA was 5.8 ± 5.7 pmol/L (median, 3.7 pmol/L; range 0.12 to 21.9 pmol/L). Horses with enterocolitis had a wide range of serum PTH concentrations (median, 29.1 pmol/L; range, 0.4 to 290 pmol/L). On the basis of serum Ca²⁺ concentration, 13 of these affected horses were further assigned to the normocalcemic group and 51 to the hypocalcemic group.

Affected horses with ionized hypocalcemia (n = 51) were then assigned to 3 subgroups on the basis of serum intact PTH concentration (Fig 3). Fifteen horses were classified as nonresponders; these horses had hypocalcemia and PTH concentrations within our reference range (0.6 to 21.9 pmol/L). Twenty-six were midresponders; horses in this group had hypocalcemia and moderately high serum PTH concentration (range, 23.3 to 121 pmol/L). Finally, 10 horses were considered high responders. These horses had high serum PTH concentrations (184 to 290 pmol/L). Significant differences in serum total calcium, Ca²⁺, phosphorus, creatinine, and BUN concentrations were not detected among these 3 subgroups (Table 1).

In control horses, serum Ca²⁺ and intact PTH concentrations were significantly (r = –0.46) correlated. These values were also significantly (ρ = –0.54) correlated in horses with enterocolitis. Serum Mg²⁺ concentration was significantly less in horses with enterocolitis than healthy horses.

A positive correlation between serum PTH concentration and FP was detected in healthy horses (r = 0.52) and in horses with enterocolitis (ρ = 0.37). However, no association between PTH concentration and FCa was detected in horses with enterocolitis.
Discussion

The majority (31/64; 79.7%) of horses with enterocolitis in the present study had ionized hypocalcemia; however, parathyroid gland responses to hypocalcemia were variable. Possible causes of hypocalcemia in these horses included renal loss of calcium, sequestration of calcium in the lumen of the gastrointestinal tract as a result of loss or poor absorption associated with inflammation, impairment in calcium mobilization, tissue sequestration of calcium, and impairment of calcium release by the target tissue in response to PTH. Renal loss as a cause of hypocalcemia in horses in our study was unlikely; mean FCa was significantly less in affected horses, compared with healthy horses. Reduction in FCa is an appropriate homeostatic response to hypocalcemia. Low FCa has also been reported in association with hypocalcemia in other species. Low urinary calcium excretion may be explained by a slow rate of calcium filtration at the glomerulus or by efficient reabsorption of filtered calcium in the renal tubules. The mechanism of renal tubular cell reabsorption of calcium in humans and other animals with hypocalcemia has not been well described but may be PTH-mediated via an increase in tubule intracellular cAMP concentration. An association between high PTH and intracellular cAMP concentrations has been described in some humans with sepsis and hypocalcemia. Alternatively, renal reabsorption of calcium may be mediated by non-PTH dependent mechanisms. It has been shown that the cell membrane Ca2+-sensing receptor plays an important role in the control of renal calcium reabsorption in the thick ascending limb of the loop of Henle via phospholipase A2, adenylate cyclase, and the sodium-potassium-chloride cotransporter.

Impairment of calcium mobilization may be the result of impaired parathyroid gland secretion of PTH, poor osteoclast response to PTH, or high serum concentrations of calcitonin and procalcitonin. Secretion of PTH may have been impaired in the nonresponder group of affected horses in the present study. Serum PTH concentrations are low in some humans with sepsis and hypocalcemia or hypocalcemic and severe burns and in sheep with burns and hypocalcemia. Impaired PTH secretion in our nonresponder horses could have resulted from upregulation of the Ca2+-sensing receptor on parathyroid gland chief cells. Stimulation of the Ca2+-sensing receptor results in inhibition of cellular cAMP via inhibitory G-proteins and, consequently, in decreased PTH secretion. Inflammatory mediators that may increase in concentration in horses with enterocolitis (eg, interleukin-1, IL-6, and tumor necrosis factor-α) may induce increased expression of Ca2+-sensing receptors on chief cells. Endotoxin, another important mediator of enterocolitis in horses, has been shown to increase IL-1, IL-6, and TNF-α concentrations in horses. A diminished osteoclastic response to PTH could result in inadequate calcium mobilization from bone. High-responder horses in our study had high serum PTH concentrations but remained hypocalcemic.

Calcitonin concentrations in hypocalcemic horses have not been assessed. In other species, increased calcitonin concentration results in decreased PTH secretion and decreased calcium mobilization from bone. In humans, it appears that calcitonin and procalcitonin concentrations may increase in response to inflammation. Inflammatory mediators such as IL-1, IL-6, and TNF-α concentrations in horses have been associated with increased concentrations of calcitonin and procalcitonin. In addition, endotoxin, an important mediator in severe enterocolitis in horses, induces an increase in serum concentration and gene expression of procalcitonin in humans. Tissue sequestration as a cause of hypocalcemia has been studied in humans and other animals but was not evaluated in the present study. In humans with sepsis, intracellular calcium concentrations are high in hepatocytes, aortic smooth muscle cells, and blood cells, whereas in rats with sepsis, skeletal muscle calcium uptake is increased. Calcium accumulates in abdominal fluid and liver of pigs with endotoxemia. Carlstedt suggested that because interstitial fluid volume is much greater than blood volume, interstitial accumulation of calcium could result in hypocalcemia. Several mechanisms have been suggested for increased calcium entry into cells of septic patients, including IL-1-mediated increased influx across the cell membrane, depletion of intracellular stores of calcium that results in a retrograde signal and activates Ca2+ influx across the membrane, insulin resistance, and impaired Ca2+-dependent ATPase activity. It has been shown that during sepsis and endotoxemia in rats, there is an increase in cytosolic calcium that can cause cell toxicity and death by activation of proteases,
phospholipases, and other enzymes.\textsuperscript{63} We do not believe that influx of calcium from the extracellular to the intracellular compartment in our horses can explain the observed ionized hypocalcemia, because intracellular concentrations are low (100 nM), and an increase in intracellular Ca\(^{2+}\) concentrations sufficient to disrupt cellular functions would be unlikely to cause a major change in extracellular Ca\(^{2+}\) concentration.

In most species, the action of vitamin D to increase calcium absorption in the proximal portion of the small intestine is an important homeostatic response to hypocalcemia. Zaloga and Chernow\textsuperscript{6} reported that some humans with sepsis caused by infection with Gram-negative bacteria also have renal 1\(\alpha\)-hydroxylase deficiency, vitamin D deficiency, and acquired calcitriol resistance. We did not assess serum concentrations of vitamin D in our horses with enterocolitis and hypocalcemia. Plasma concentrations of calcidiol and calcitriol are reported to be much lower in horses than in people. The importance of vitamin D and its metabolites in the regulation of calcium metabolism in horses requires further study.

Although hypoproteinemia and hypoalbuminemia were common findings in horses with enterocolitis, we did not detect a significant difference in Ca\(^{2+}\)/Ca between control and affected horses. This finding indicates that, overall, total calcium and Ca\(^{2+}\) concentrations changed proportionally in the same direction in affected horses. Although a decrease in serum total calcium concentration is expected in the face of hypoalbuminemia, we did not expect that Ca\(^{2+}\) concentration would decrease as a result of low serum albumin concentration. Low Ca\(^{2+}\) concentrations in affected horses with hypoalbuminemia were likely the result of impaired calcium homeostasis.

Serum Mg\(^{2+}\) concentration was significantly decreased in horses with enterocolitis and hypocalcemia, compared with healthy horses. Unlike Ca\(^{2+}\) concentration, for which complex regulatory mechanisms safeguard homeostasis, regulation of the Mg\(^{2+}\) concentration is dependent on gastrointestinal tract absorption and renal reabsorption with little endocrine control.\textsuperscript{41} The low serum Mg\(^{2+}\) concentrations that we observed in our affected horses may, therefore, have been the result of poor Mg\(^{2+}\) absorption from the lumen of inflamed bowel or diminished renal reabsorption of magnesium resulting in excessive urinary losses. Because we did not determine fractional urinary clearance of magnesium, we could not assess urinary magnesium losses.

The role of Mg\(^{2+}\) in critically ill patients has received less attention than that of Ca\(^{2+}\). It has been suggested that Mg\(^{2+}\) may serve as a Ca\(^{2+}\) antagonist and prevent Ca\(^{2+}\) entry into cells during sepsis or endotoxemia.\textsuperscript{42} Magnesium may also have a protective effect in endotoxemia, because magnesium deficiency predisposes humans with endotoxemia to a poor outcome.\textsuperscript{43}

A major complication in humans with moderate to severe magnesium depletion is hypocalcemia.\textsuperscript{44} Most humans with magnesium depletion and hypocalcemia have inappropriately low serum PTH concentrations with respect to degree of hypocalcemia, suggesting impairment in PTH secretion or synthesis. Administration of magnesium to these patients results in an increase in serum PTH concentration, whereas in clinically normal humans, magnesium administration results in a decrease in PTH concentration.\textsuperscript{45} Although horses with enterocolitis in the present study had significantly lower serum Mg\(^{2+}\) concentrations than healthy horses, serum Mg\(^{2+}\) concentrations also decreased in affected horses as PTH concentrations increased. The relevance of this observation is not known. However, some humans with Mg\(^{2+}\) deficiency and hypocalcemia have high serum PTH concentrations, suggesting end-organ resistance (renal or skeletal) to PTH.\textsuperscript{46} Both renal and skeletal PTH resistance have been detected in humans with hypomagnesemia.\textsuperscript{47} In addition, serum 1,25-dihydroxyvitamin D concentrations are low in humans with hypomagnesemia,\textsuperscript{48} suggesting that formation of vitamin D is sensitive to magnesium depletion. Experimentally induced magnesium depletion in humans resulted in renal resistance to PTH-induced 1,25-dihydroxyvitamin D synthesis.\textsuperscript{49} The role of magnesium and its interactions with calcium in horses with enterocolitis deserves further study.

The increase in FP in horses with enterocolitis and hypocalcemia was consistent with high PTH concentrations in these horses. However, the correlation between PTH concentration and FP was poor (\(\Delta = 0.37\)), indicating that factors other than PTH are likely important in regulating renal phosphorus clearance in horses with enterocolitis and hypocalcemia.

In 56% (36/64) of our horses with enterocolitis, serum Ca\(^{2+}\) concentrations were low and PTH concentrations high. However, a subgroup of these horses had low serum concentrations of PTH despite concomitant hypocalcemia. We speculate that this low PTH response may have been attributable to suppression of PTH secretion or synthesis by inflammatory mediators such as IL-1, IL-6, and TNF-\(\alpha\). The mechanisms by which cytokines may alter PTH release remain to be determined. Inflammatory cytokines in horses with hypocalcemia may facilitate over expression of the Ca\(^{2+}\)-sensing receptor on parathyroid cells and permit increased concentrations of intracellular calcium, thus indirectly decreasing PTH release.\textsuperscript{1} Additionally, it is known that the effects of Ca\(^{2+}\) and Mg\(^{2+}\) on PTH secretion are interdependent. It is possible that hypomagnesemia in some horses may have permitted intracellular accumulation of calcium and reduced cAMP production in the parathyroid gland, resulting in decreased PTH synthesis or release. Moreover, low serum Mg\(^{2+}\) concentrations may have resulted in renal and skeletal PTH resistance.

A second subgroup of affected horses with hypocalcemia had high serum PTH concentrations (high responders). The high PTH concentrations in these horses could represent a supraphysiologic response of the parathyroid gland to the combined effects of hypocalcemia and increased concentrations of inflammatory mediators. In humans and rats, hyperphosphatemia may directly stimulate PTH release.\textsuperscript{40,46} High PTH concentrations in the high-responder horses may have resulted in increased serum phosphate concentrations; mean phosphorus concentration in high-responder

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horses was 7.2 ± 2.5 mg/dl, whereas mean phosphorus concentrations in the mid- and nonresponders were 5.7 ± 2.8 and 5.5 ± 2.8 mg/dl, respectively. However, the difference in phosphorus concentrations among groups was not significant (P = 0.3). Serum concentrations of PTH in the high-responder group may have been high because target tissues in these horses were resistant to PTH; hypomagnesemia may induce PTH resistance. Alternatively, high-responder horses may have had low serum concentrations of 1,25-dihydroxyvitamin D. Low 1,25-dihydroxyvitamin D concentrations appear to be permissive for enhanced PTH secretion.60

Some (13/64; 20.3%) horses with enterocolitis had serum Ca\(^{2+}\) concentrations within reference range. We believe that in these horses, calcium homeostatic mechanisms were efficient enough to maintain extracellular Ca\(^{2+}\) concentration within the physiologic range. Another explanation may be that these horses were assessed early in the disease process. In addition, 3 affected horses with normocalcemia had serum PTH concentrations greater than the upper limit of our reference range. It is possible that this increase in serum PTH concentration was the direct result of inflammatory mediators or endotoxins acting on the parathyroid gland. Alternatively, efficient PTH release may have resulted in correction of hypocalcemia, but serum PTH concentration remained high. Nakamura et al7 found that endotoxin infusion in rats resulted in an increase in serum PTH concentration in some animals.

Methods for describing agreement between results of 2 tests, such as the ICMA and the IRMA, are controversial when neither test is considered the gold standard. We included assessments of correlation and bias between the 2 tests. The Spearman correlation coefficient was high (\(\rho = 0.98\)), indicating a good association in results between tests. The Bland-Altman plot for all data revealed that bias over the whole range of values was 1.9 pmol/L, indicating that the ICMA yielded PTH concentrations 1.9 pmol/L greater than the IRMA. However, inspection of the Bland-Altman plot for all data suggests that this assessment may have overestimated the bias for low PTH concentrations and underestimated the bias for high concentrations.61 For this reason, we examined a second Bland-Altman plot in which data were restricted to those obtained by analysis of serum samples that yielded PTH concentrations >50 pmol/L by use of the ICMA. In the second plot, the bias was reduced to 0.2 pmol/L. Agreement between results of the 2 tests was thus better at PTH concentrations <50 pmol/L, and inspection of the second plot indicates that only 2 values were outside 2 SD of the mean of the differences. Serum PTH concentrations >50 pmol/L are 2 times greater than the upper limit of our reference range for the ICMA. The proportionally higher values for ICMA, compared with IRMA PTH determinations, are unlikely to be clinically important, because values for PTH determined by use of both methods will be clearly above the reference ranges. Enhanced sensitivity, shorter incubation time, automation, and lack of radioactivity are important advantages of the ICMA over the IRMA.

Mean and reference range values for serum PTH concentrations in the healthy horses of this study, measured by use of the ICMA (mean ± SD, 5.8 ± 5.7 pmol/L; range, 0.12 to 21.9 pmol/L), were greater than those reported by Estepa et al,65 measured by use of the human intact PTH IRMA (mean ± SD, 3.3 ± 2.4 pmol/L; range, 0.8 to 8.7 pmol/L) and the rat PTH IRMA (mean ± SD, 4.6 ± 3.1 pmol/L; range 1.1 to 10.1 pmol/L). Breidenbach et al,66 who used the human intact PTH IRMA to measure plasma PTH concentrations in horses, reported a higher mean PTH value than ours (mean ± SD, 22.9 ± 19.0 pmol/L). Serum PTH concentrations for clinically normal horses have been determined by use of C-terminal assays; values were similar to or greater than those of the present study.1,7,20 The PTH response of healthy horses to acute hypocalcemia was evaluated by Roussel et al,67 using a C-terminal immunoassay. The reference range for PTH concentrations in that study was greater than values we determined for healthy horses in the present study. This is likely attributable to the prolonged serum half-life of the C-terminal portion of PTH.7 We do not recommend the use of the older C-terminal PTH assays, because they do not measure intact hormone. With the new 2-site immunometric assays for intact PTH, it is now possible to accurately determine concentrations of biologically active PTH in horses. We believe that the variation among results of previous studies is related to assay variation. Differences in the calcium and phosphorus content of diets fed to horses may also have contributed to these apparent differences.

Most (31/64; 79.7%) horses with enterocolitis in the present study also developed hypocalcemia. The expected response of the parathyroid gland to hypocalcemia is to increase secretion of PTH. Serum PTH concentrations in most (36/51; 70.6%) of the affected hypocalcemic horses was high. However, in others (15/51; 29.4%), serum PTH concentrations did not increase in proportion to the degree of hypocalcemia. Reasons for this variation in response to hypocalcemia among horses with enterocolitis are not clear. We found that renal calcium loss (ie, FCa) was significantly lower in horses with enterocolitis than in healthy horses. We believe that impaired calcium mobilization from bone, sequestration or loss of calcium from the gastrointestinal tract, or failure of the parathyroid gland to secrete adequate PTH are the most likely causes of hypocalcemia in horses with enterocolitis.
References


