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Alterations in Serum Parathyroid Hormone and Electrolyte Concentrations and Urinary Excretion of Electrolytes in Horses with Induced Endotoxemia

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Hypocalcemia and hypomagnesemia are common in horses with sepsis and endotoxemia. We hypothesize that endotoxemia triggers a systemic inflammatory response that results in hypocalcemia and hypomagnesemia. The goal of this study was to determine the effect of endotoxin (lipopolysaccharide [LPS]) administration to healthy horses on serum parathyroid hormone (PTH), ionized calcium (Ca^{2+}) and total calcium (tCa), ionized magnesium (Mg^{2+}) and total magnesium (tMg), phosphate (Pi), potassium (K^+), sodium (Na^+), chloride (Cl^-), and insulin concentrations, and on the urinary excretion of these electrolytes. Twelve mares were infused with *Escherichia coli* LPS (30 ng/kg/h IV) for 1 hour. Six mares were infused with saline (controls). In LPS-infused horses, heart rate increased significantly from (mean ± SD) 40.0 ± 1.3 to 70.0 ± 9.0 beats/min, respiratory rate from 12.7 ± 1.0 to 21.1±3.0 breaths/min, body temperature from 37.4 ± 0.3 to 38.9 ± 0.6°C, and tumor necrosis factor-α concentrations from 6.6 ± 3.5 to 507 ± 260 pg/mL ($P < .05$). White blood cell count decreased significantly from 7,570 ± 600 to 1,960 ± 560 cells/μL. Serum concentrations of Ca^{2+} decreased from 6.5 ± 0.3 to 6.0 ± 0.3 mg/dL, of Mg^{2+} from 0.53 ± 0.06 to 0.43 ± 0.04 mM, of tMg from 0.78 ± 0.05 to 0.62 ± 0.08 mM, of K^+ from 4.3 ± 0.4 to 3.0 ± 0.5 mEq/L, and of Pi from 3.4 ± 0.5 to 1.7 ± 0.5 mg/dL (all $P < .05$). PTH increased significantly from 1.3 ± 0.4 to 6.0 ± 5.2 pM; however, in some horses (n = 2), PTH did not increase despite hypocalcemia. Insulin increased significantly from 9.4 ± 3.6 to 50.5 ± 9.6 μIU/mL (n = 3). Urinary fractional excretion of Ca^{2+} decreased significantly from 4.7 ± 1.4 to 1.7 ± 1.2%, of Mg^{2+} from 36.6 ± 6.5 to 11.7 ± 7.3%, and of K^+ from 37.9 ± 11.3 to 17.7 ± 6.2%. Fractional excretion of Pi increased from 0.02 ± 0.02 to 0.14 ± 0.07% and of Na^+ from 0.26 ± 0.13% to 1.2 ± 0.5%. No changes were found in serum tCa, Na^+, and Cl^- concentrations. In conclusion, endotoxemia in horses resulted in electrolyte abnormalities that included hypocalcemia, hypomagnesemia, hypokalemia, hypophosphatemia, and increased serum PTH and insulin concentrations.

Key words: Equine; Hypocalcemia; Hypokalemia; Hypomagnesemia; Hypophosphatemia; Insulin; Lipopolysaccharide.

Electrolyte imbalances are common in critically ill humans and animals, and hypocalcemia constitutes a frequent finding in humans and animals with sepsis.[1–4] In septic horses and foals, low serum total calcium (tCa) and ionized calcium (Ca^{2+}) concentrations are common clinicopathologic abnormalities, especially in animals with gastrointestinal disease.[2–4] The causes of hypocalcemia in septic patients are multifactorial and not completely understood.[5] During hypocalcemia, the normal response of the parathyroid gland is an increase in parathyroid hormone (PTH) secretion; however, in septic patients, both increased and decreased PTH secretion have been reported.[4,5] Parathyroid gland dysfunction (decreased PTH secretion) has been proposed as one of the causes of hypocalcemia during sepsis in horses.[4] Increased PTH concentrations have been associated with the severity of illness in septic human patients. [1,5]

Hypomagnesemia is now recognized as one of the most frequent electrolyte disturbances found in human and veterinary critical care units,[3,4,6,7] and it has been associated with decreased survival in critically ill human and equine patients.[3,6] Like hypocalcemia, decreased total magnesium (tMg) and ionized magnesium (Mg^{2+}) concentrations are common findings in horses with gastrointestinal disease.[3,4,7]

Horses with gastrointestinal disease may have detectable concentrations of endotoxin in plasma,[8] and endotoxin administration to healthy horses results in increased blood concentrations of tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6.[9–12] Both, IL-1 and IL-6 have been documented to decrease PTH secretion in various species, including the horse.[13–15] Furthermore, increased plasma concentrations of TNF-α and IL-6 have been associated with hypocalcemia, hypomagnesemia, and decreased urinary excretion of Ca^{2+} in critically ill human patients.[5] We hypothesized that increased endotoxin concentrations may be the trigger that activates a series of mechanisms that result in hypocalcemia and hypomagnesemia in endotoxemic horses. Endotoxemia may also result in other electrolyte abnormalities as a consequence of a systemic inflammatory response and due to changes in serum PTH and insulin concentrations. The regulation of these electrolytes may be associated with calcium and magnesium homeostasis.

The objectives of this study were to determine the effect of administration of *Escherichia coli* endotoxin to healthy horses on serum PTH, Ca^{2+}, tCa, Mg^{2+}, tMg, phosphate (Pi), sodium (Na^+), potassium (K^+), chloride (Cl^-), and insulin concentrations, and on the urinary fractional excretion of these electrolytes.

Materials and Methods

Experimental Animals

Eighteen healthy mares (8 Standardbreds, 6 Thoroughbreds, 2 Appaloosas, and 2 Arabians), aged 3–14 years (8.2 ± 3.4 years), and weighing 460–570 kg, were selected from The Ohio State University College of Veterinary Medicine teaching herd. All horses were in good

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body condition, were fed the same diet of grass and grass hay (0.5% calcium and 0.25% phosphorus), and alfalfa hay (1.4% calcium and 0.25% phosphorus), had no history of illnesses, and had received no treatments for 1 month before sampling. To assure health, a complete physical examination was performed, and values for a CBC, serum chemistry profile; serum PTH, tCa, Ca^{2+}, tMg, Mg^{2+}, and Pi concentrations; plasma fibrinogen concentrations; blood gases; urine analysis; urine chemistry profile; and urinary excretion of electrolytes were within the reference ranges for all horses. Horses received no food or water during the first 6 hours of the study.

Horses were randomly divided into 2 groups. Horses in the 1st group (n = 12) were infused with *E coli* endotoxin (lipopolysaccharide ob; LPS). The 2nd group included control horses (n = 6) that were infused with 0.9% NaCl solution. The Ohio State University Institutional Laboratory Animal Care and Use Committee approved this study and animals were treated according to the National Institutes of Health *Institutional Animal Care and Use* guidelines.

Endotoxemic Horses. After baseline sampling, 12 healthy mares were infused with *E coli* serotype 0111:B4 LPS[a] at a rate of 30 ng/kg/h IV for 1 hour. The total amount of LPS to be administered was diluted in 250 mL of 0.9% NaCl. This dosage of LPS has been previously used in horses with predictable results and minimal complications.[11,12] To assess the systemic response to LPS administration, venous blood samples were collected to determine the white blood cell count (WBC) and TNF-α concentrations every 30 minutes for 360 minutes and at 12, 18, and 24 hours after the beginning of the experiment. To measure serum Ca^{2+}, tCa, Mg^{2+}, tMg, and PTH concentrations, blood samples were collected every 15 minutes for 120 minutes, then every 30 minutes until 360 minutes, and at 12, 18, and 24 hours. Serum concentrations of Pi, Na^+, K^+, Cl^-, total protein, albumin, and creatinine were determined every 30 minutes until 360 minutes, and at 12, 18, and 24 hours. To assess the acid-base status, heparinized samples for blood gas analysis were collected under anaerobic conditions at 0, 120, 180, 240, and 360 minutes. Because a pilot study in our laboratory indicated that some electrolyte abnormalities could be the result of increased insulin concentrations, serum insulin concentrations were measured in 3 horses from this group every 60 minutes for 360 minutes, and at 12 and 24 hours. To calculate the urinary fractional excretions of Ca^{2+} (FCa), Mg^{2+} (FMg), Pi (FP), Na^+ (FNa), K^+ (FK), and Cl^- (FCl), urinary catheters were placed in 6 mares of this group. Urine samples to determine the concentrations of creatinine and these electrolytes were collected every 30 minutes for 6 hours, and at 12 and 24 hours. To assure the health status of these horses after experimental endotoxemia, blood and urine samples were collected at 24 and 48 hours after the beginning of the LPS infusions for a CBC, serum chemistry profile, urine analysis, and urine profile.

Control Horses. Six mares were infused with an equivalent volume (250 mL) of 0.9% NaCl for 1 hour. Serum insulin concentrations were determined in 3 horses of this group. The urinary fractional excretion of electrolytes was determined in 4 mares of this group. Blood and urine samples were collected as in the horses with experimental endotoxemia.

Animal Monitoring

To monitor for adverse effects of endotoxemia, heart rate (HR), respiratory rate (RR), body temperature (BT), and electrocardiogram (lead II) were evaluated every 30 minutes during the experiments.

Sampling

An intravenous catheter was placed aseptically in each jugular vein. The catheter in the left jugular vein was used for the infusion of LPS or 0.9% NaCl and the catheter in the right vein was used for blood sample collection. Right catheter patency during rapid sample collection (0–120 minutes) was maintained by administration of 0.9% NaCl solution at a slow rate (0.5 mL/kg/h) in both groups of horses. Venous blood samples were collected in tubes with no additives, allowed to clot at 4°C for 1 hour, and centrifuged at 1,000 × *g* for 5 minutes at 4°C immediately after clotting. Serum samples for chemistry profile, tCa, tMg, Ca^{2+}, Mg^{2+}, and Pi concentrations were processed immediately, whereas samples to measure PTH and insulin concentrations were stored at −80°C until batch analysis. Venous blood samples for white cell counts were collected in tubes containing ethylenediaminetetraacetic acid. To determine TNF-α concentrations, blood samples were collected in heparinized tubes, immediately centrifuged at 1,000 × *g* for 5 minutes at 4°C, and the plasma samples were stored at −80°C until analysis. To determine venous blood gases, blood samples were collected in heparinized syringes under anaerobic conditions and processed immediately.

Urine sampling was achieved by placing indwelling 28F Foley catheters into the bladder of 6 mares in the endotoxemic group and 4 mares of the control group. The bladder was continuously emptied and urine samples were collected directly from the Foley catheters at the end of a 30-minute period. Before submitting samples for chemistry analysis, they were vigorously mixed and aliquoted. Urine samples to determine electrolyte concentrations were processed immediately after collection. The urinary fractional excretion of electrolytes was calculated as [(Ux/Sx)/(Ucr/Scr)] × 100, where U is urine, S is serum, x is electrolyte concentration, and cr is creatinine concentration. Therefore, the results were expressed as a fraction (%) of the urinary excretion of creatinine.

Laboratory Methods

CBCs were performed by an automated system.[b] For the specific measurements of urine calcium, magnesium, and phosphate, a 1/10 volume of 6 N HCl was added to the urine samples to dissolve crystals or to prevent crystal formation. Serum chemistry profiles, tCa, Pi, and urine calcium concentrations were measured by using an automated analyzer.[c] Plasma fibrinogen concentrations were determined by using a nephelometric analyzer.[d] Blood gases were measured with a blood gas analysis system.[e] For serum Ca^{2+} and Mg^{2+} concentrations, blood samples were collected under anaerobic conditions and processed immediately by using Ca^{2+}- or Mg^{2+}-selective electrodes.[f] Serum PTH concentrations were determined with an immunochemiluminometric assay[g] for human intact PTH previously validated for horses.[4] Serum insulin concentrations were determined with a human-specific insulin radioimmunoassay[h] previously used in horses.[16] Plasma TNF-α concentrations were measured by using a human-specific 2-site immunochemiluminometric assay[i] previously validated for horses.[11] Serum tMg and urine magnesium concentrations were determined by using a chemistry system.[j]

Statistical Analyses

Results are expressed as mean ± SD. Normality was determined by visual inspection of normal probability plots and by the Shapiro-Wilk goodness-of-fit test. Of the studied variables, serum PTH, serum Pi, plasma TNF-α, WBC, FCa, FMg, FP, FNa, FK, and FCl were not normally distributed. For correlation (*r*) between variables, the Pearson product moment test was used for normally distributed variables and the Spearman rank test was used for those that were not normally distributed.[17] Comparisons between 2 groups were made by using the *t*-test or the Mann-Whitney rank test depending on their normality. For variables that were normally distributed, one-way repeated measures analysis of variance (ANOVA) with multiple comparisons with the Dunnett's test were used to compare groups to baseline (time 0). When variables were not normally distributed, Friedman's one-way ANOVA for repeated measures was used, and multiple comparisons to time 0 were made with the Dunn's test. To assess the effects of treatment and time, two-way repeated-measures ANOVA was used and multiple comparisons to corresponding times were made with the Holm-Sidak method. Commercially available software was used for statistical analysis[k,l] and graph generation.[m] *P*-values < .05 were considered significant.

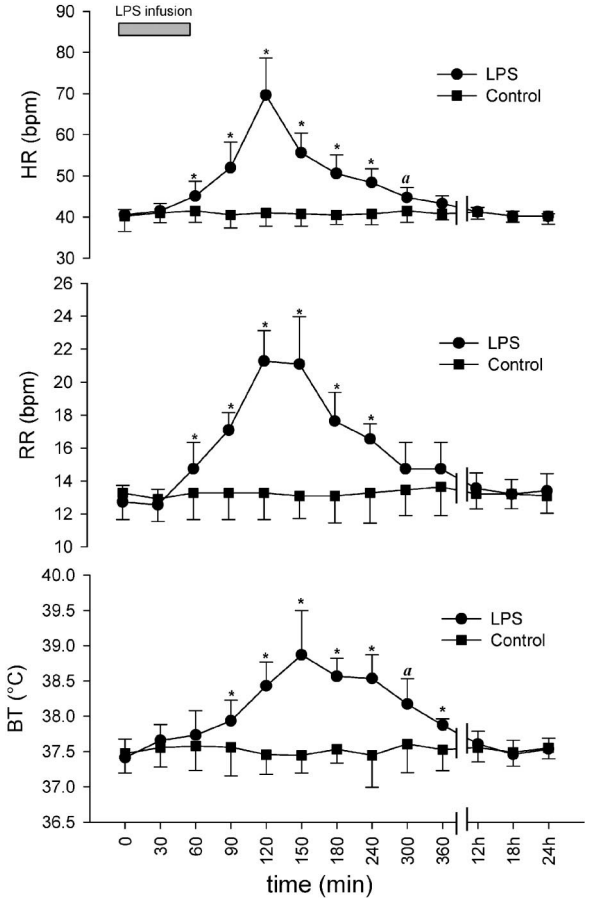

Fig 1. Effect of administration of *Escherichia coli* endotoxin (lipopolysaccharide [LPS], 30 ng/kg/h IV) to healthy horses (n = 12) on heart rate (HR), respiratory rate (RR), and body temperature (BT) (mean ± SD). Controls were 6 healthy horses infused with an equivalent volume of 0.9% NaCl. An asterisk indicates that the value is statistically different from baseline (time 0) and from the control value at the same time interval ($P < .05$). The letter *a* indicates that the value was statistically different from baseline ($P < .05$). Breaks in the *x*-axis represent the scale transition from minutes to hours.

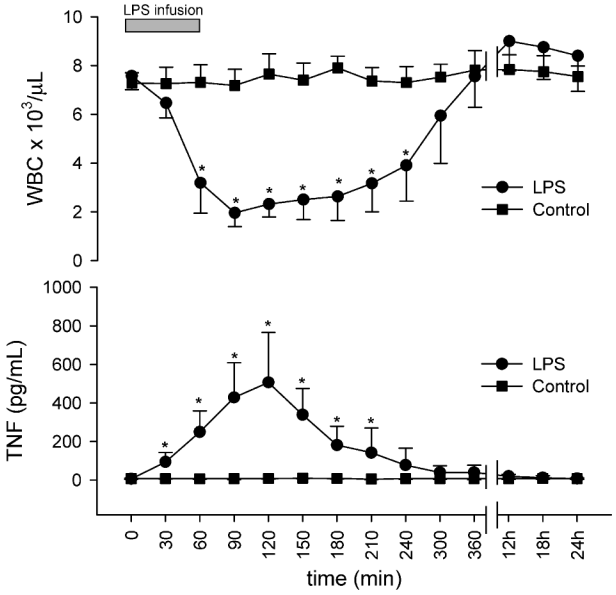

Fig 2. Effect of *Escherichia coli* endotoxin (lipopolysaccharide [LPS], 30 ng/kg/h IV) on white blood cell count (WBC) and plasma tumor necrosis factor-α (TNF-α) concentrations in 12 healthy horses (mean ± SD). Controls were 6 healthy horses infused with an equivalent volume of 0.9% NaCl. An asterisk indicates that the value is statistically different from baseline (time 0) and from the control value at the same time interval ($P < .05$). Breaks in the *x*-axis indicate the scale transition from minutes to hours.

Results

Baseline values for serum tCa, tMg, Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-}, K^{+}, Pi, and PTH concentrations were not statistically different between experimental groups. Infusion of *E coli* LPS resulted in a significant increase in HR, RR, BT, and TNF-α concentrations, and in a significant decrease in the WBC ($P < .01$; Figs 1, 2). The WBC decreased 90 minutes after the beginning of the infusion of LPS (Fig 2). In addition, endotoxemic horses became lethargic and had decreased gastrointestinal sounds, as reported by others.[12]

Serum Ca^{2+} and Mg^{2+} concentrations decreased during endotoxin infusion ($P < .001$; Fig 3). Serum Ca^{2+} concentrations were statistically below baseline at 120 and 150 minutes, and serum Mg^{2+} concentrations remained below baseline for 90–300 minutes. Furthermore, on a percentage basis, the effect of LPS infusion was more pronounced on serum Mg^{2+} concentrations than on serum Ca^{2+} concentrations; serum Ca^{2+} concentrations decreased by 8 ± 4.2% from baseline, whereas serum Mg^{2+} concentrations decreased by 19 ± 11%. Serum Ca^{2+} and Mg^{2+} concentrations did not decrease in 4 and 3 horses, respectively, in the LPS group. Changes in serum tCa concentrations were not statistically significant (Fig 3; $P = .10$). Serum tMg concentrations decreased at 150 minutes ($P = .002$; Fig 3). Plasma TNF-α concentrations were inversely correlated with serum Ca^{2+} ($r = -0.6$; $P < .001$) and Mg^{2+} ($r = -0.7$; $P < .001$) concentrations and the WBC ($r = -0.8$; $P < .001$).

No detectable differences were found in serum total protein and albumin concentrations in endotoxemic horses when compared to controls or time 0 at any time point (data not shown). No differences were found in plasma pH and bicarbonate concentrations in response to endotoxin administration.

Serum PTH concentrations increased in LPS-infused horses (90 minutes; Fig 3). This value was statistically higher than baseline ($P = .03$); however, serum PTH concentrations among horses were variable. Some horses (n = 3) responded to hypocalcemia with an increase in serum PTH concentrations, whereas other horses (n = 2) demonstrated no increase in PTH concentrations despite hypocalcemia (parathyroid gland dysfunction; Fig 4). Furthermore, 3 horses had an increase in serum PTH concentrations before serum Ca^{2+} concentrations decreased below the reference range (Fig 4). In 2 of the 4 horses with unchanged serum Ca^{2+} concentrations, no changes were found in serum PTH concentrations (Fig 4). In endotoxemic horses, serum PTH concentrations were negatively correlated with serum Ca^{2+} ($r = -0.35$; $P = .03$) and Mg^{2+} ($r = -0.4$; $P = .008$) concentrations.

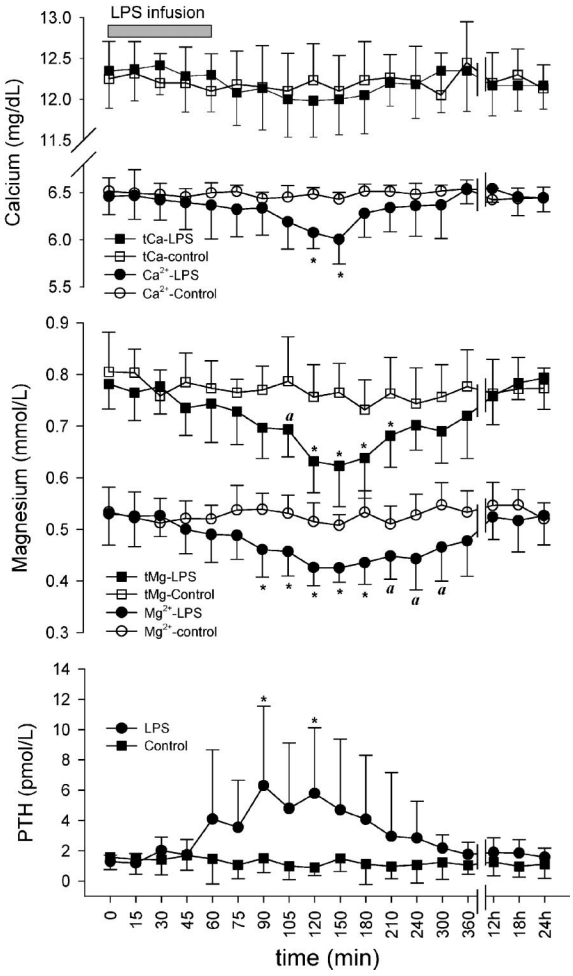

Fig 3. Effect of *Escherichia coli* endotoxin (lipopolysaccharide [LPS], 30 ng/kg/h IV) on serum total calcium (tCa), ionized calcium (Ca^{2+}), total magnesium (tMg), ionized magnesium (Mg^{2+}), and parathyroid hormone (PTH) concentrations in 12 healthy horses (mean ± SD). Controls were 6 healthy horses infused with 0.9% NaCl. The infusion of LPS resulted in a significant decrease in serum Ca^{2+}, Mg^{2+}, and tMg concentrations, and in a significant increase in serum PTH concentrations. An asterisk indicates that the value is statistically different from baseline (time 0) and from the control value at the same time interval ($P < .05$). The letter *a* indicates that the value was statistically different from baseline ($P < .05$). Breaks in the *x*-axis represent the scale transition from minutes to hours.

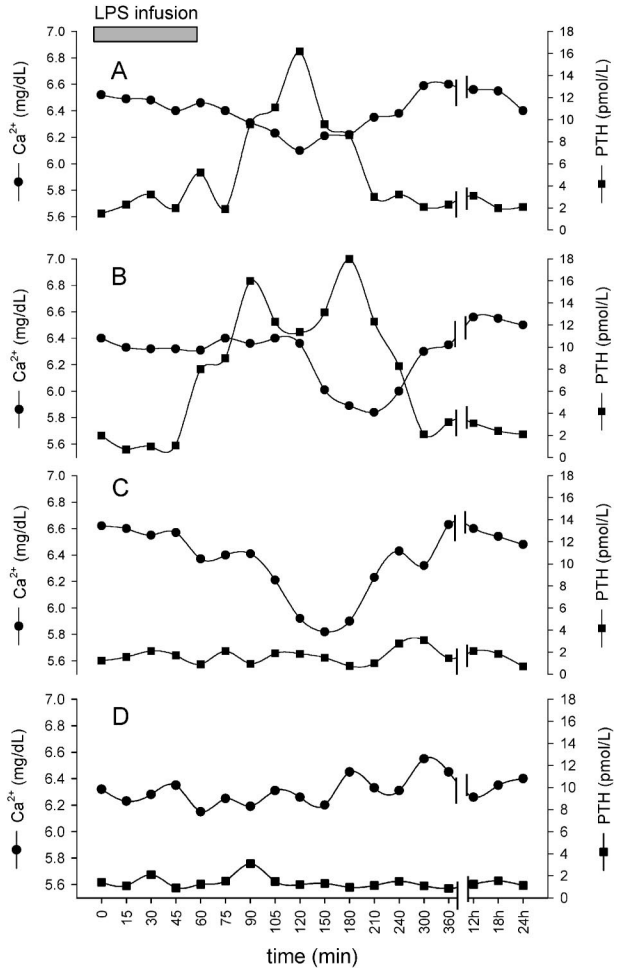

Fig 4. Examples of the effect of experimental endotoxemia on serum ionized calcium (Ca^{2+}) and parathyroid hormone (PTH) concentrations in horses. The infusion of *Escherichia coli* endotoxin (lipopolysaccharide [LPS]) for 1 hour resulted in variable responses in serum Ca^{2+} and PTH concentrations among horses. In the horse in panel A, an increase in serum PTH concentrations was associated with the decrease in serum Ca^{2+} concentrations. In the horse in panel B, an increase in serum PTH concentrations occurred before serum Ca^{2+} decreased below the reference range (premature parathyroid response), whereas the horse in panel C had no response of the parathyroid gland (PTH secretion) to hypocalcemia (parathyroid gland dysfunction). The horse in panel D had no changes in serum Ca^{2+} and PTH concentrations. Breaks in the *x*-axis represent the scale transition from minutes to hours.

Serum K^+ and Pi concentrations were lower in endotoxemic horses ($P < .01$; Fig 5). No significant changes were detected in serum Na^+ and Cl^- concentrations (data not shown). No detectable statistical relationship was found between serum PTH and Pi and K concentrations, likely because by the time Pi and K were below the reference range, serum PTH concentrations have returned to within the reference range. Serum insulin concentrations in endotoxemic horses (n = 3) increased from 9.4 ± 3.6 to 50.5 ± 9.6 μIU/mL (180 minutes; $P = .012$; data not shown). No changes were found in insulin concentrations in control horses.

Endotoxemia resulted in a decrease in FCa, FMg, and FK, and in an increase in the FP and FNa ($P < .05$; Figs 6, 7). FCa was correlated with FMg ($r = 0.62$; $P = .011$), FK ($r = 0.4$; $P = .024$), and FP ($r = -0.40$; $P = .05$). FMg was correlated with FK ($r = 0.5$; $P < .001$) and FP ($r = -0.7$; $P < .001$). No detectable association was found between FNa and other fractional excretions, except for FCl ($r = 0.62$; $P < .001$). No effect of endotoxin infusion was found on FCl ($P = .093$; Fig 7). Serum PTH concentrations and FP ($r = 0.64$; $P = .008$) and FNa ($r = 0.63$; $P < .001$) were correlated. No significant relationships were found among serum PTH and FCa, FMg, and FK. However, exclusion of 2 horses with no PTH response to endotoxemia from the analysis revealed an inverse and statistically significant relationship between serum PTH concentrations

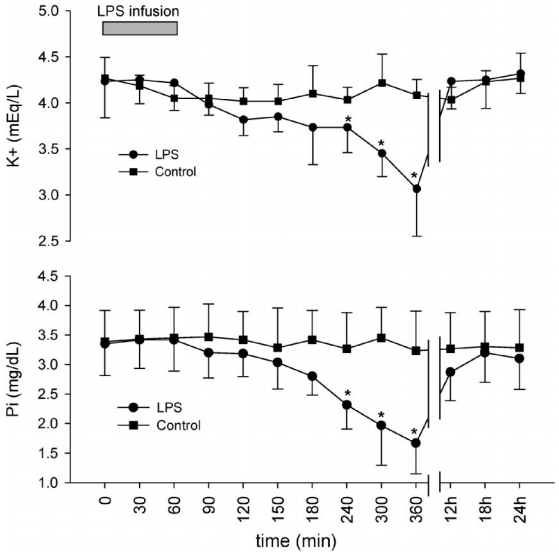

Fig 5. Effect of *Escherichia coli* endotoxin (lipopolysaccharide [LPS], 30 ng/kg/h IV) on serum potassium (K^+) and phosphate (Pi) concentrations in 12 healthy horses (mean ± SD). Controls were 6 healthy horses infused with 0.9% NaCl. An asterisk indicates that the value is statistically different from baseline (time 0) and from the control value at the same time interval ($P < .05$). No statistical changes in serum sodium (Na^+) and chloride (Cl^-) were found (not shown). Breaks in the *x*-axis represent the scale transition from minutes to hours.

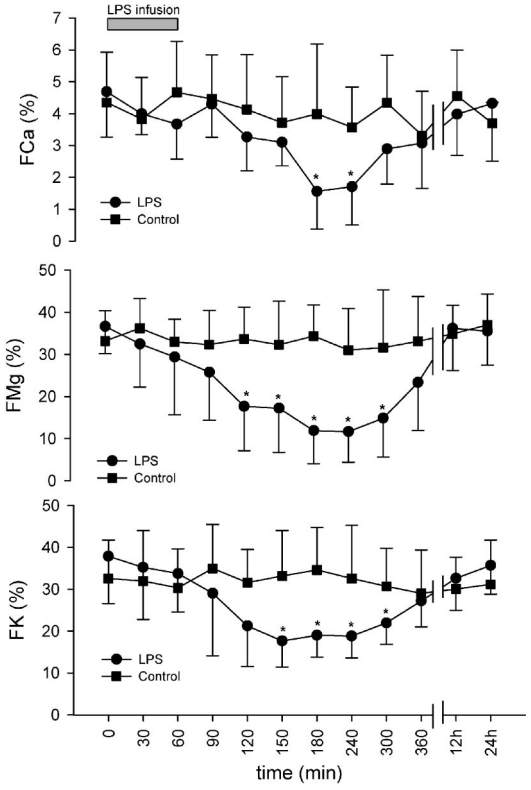

Fig 6. Effect of *Escherichia coli* endotoxin (lipopolysaccharide [LPS], 30 ng/kg/h IV) infusion in healthy horses (n = 6) on the urinary fractional excretion of calcium (FCa), magnesium (FMg), and potassium (FK) (mean ± SD). Controls were 4 horses infused with an equivalent volume of 0.9% NaCl. An asterisk indicates that the value is statistically different from baseline (time 0) and from the control value at the same time interval ($P < .05$). Breaks in the *x*-axis represent the scale transition from minutes to hours.

and FCa ($r = -0.26$; $P = .04$), FK ($r = -0.55$; $P = .037$), and FMg ($r = -0.25$; $P = .023$).

CBCs, serum chemistry profiles, urine profiles, and urine analyses were within the reference ranges 48 hours after the beginning of the experiment in all horses. In control horses, no statistically significant changes were found in any variable measured.

Discussion

In the present study, experimental endotoxemia in healthy horses resulted in electrolyte abnormalities, including hypocalcemia, hypomagnesemia, hypophosphatemia, and hypokalemia. The parathyroid gland response to endotoxemia was variable among horses; some horses responded to hypocalcemia with an increase in serum PTH concentrations whereas other horses did not. We speculate that variable secretion of PTH during endotoxemia in horses is the result of a systemic inflammatory response.

Hypocalcemia and hypomagnesemia are common findings in horses with severe gastrointestinal disease.[2–4] Horses with gastrointestinal disease may have detectable concentrations of endotoxin in plasma.[8] Horses are sensitive to the detrimental effects of LPS in that several hundred-fold lower dosages of LPS (ng/kg) are required to induce clinical signs of endotoxemia in horses when compared to dosages required in other species (μg/kg).[18–20] For reasons not as yet understood, hypocalcemia and hypomagnesemia are frequent findings in humans with sepsis and endotoxemia.[1,5,18,21] Sepsis and endotoxemia are thought to be the most common causes of hypocalcemia and hypomagnesemia in horses admitted to equine critical care units.[4] This study demonstrates a direct association between endotoxemia, hypocalcemia, and hypomagnesemia, and factors that regulate extracellular Ca^{2+} and Mg^{2+} concentrations, such as PTH and urinary excretion of Ca^{2+} and Mg^{2+} in horses.

Several mechanisms for the development of hypocalcemia during sepsis and endotoxemia have been proposed including renal loss of Ca^{2+},[21] sequestration of Ca^{2+} in the gastrointestinal lumen,[19] intracellular accumulation of Ca^{2+},[22] impairment in Ca^{2+} mobilization,[23] interstitial and tissue Ca^{2+} chelation or sequestration,[18,21] decreased Ca^{2+} release by the target tissue in response to PTH,[5,21] failure to synthesize 1,25-dihydroxyvitamin D_3,[5,21] increased glucocorticoids concentrations,[20] Mg^{2+} depletion,[24] and parathyroid gland dysfunction.[4,5] Renal loss of Ca^{2+} as a cause of hypocalcemia in septic horses seems unlikely. On the contrary, horses with enterocolitis and hypocalcemia have low FCa,[4] and results from this study confirm this, indicating that the equine kidney conserves Ca^{2+} during endotoxemia and hypocalcemia. Decreased urinary excretion of Ca^{2+} is documented in human patients with sepsis and hypocalcemia.[5] Impaired Ca^{2+} mobilization may result from impaired PTH secretion,[4,5] decreased bone resorption,[21] high concentrations of peptides of the calcitonin gene family,[21] and increased cortisol concentrations.[20] Decreased bone resorption does not seem to be the cause of hypocal-

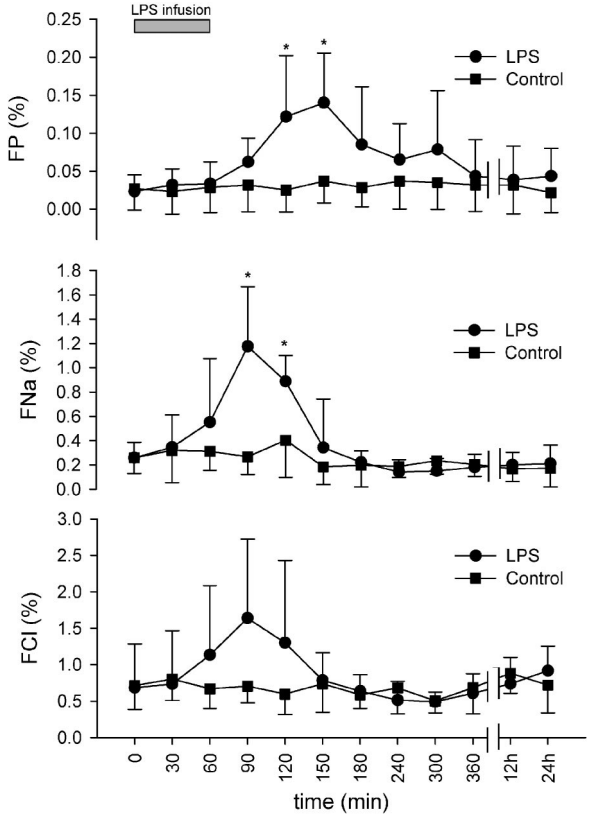

Fig 7. Effect of *Escherichia coli* endotoxin (lipopolysaccharide [LPS], 30 ng/kg/h IV) infusion in healthy horses (n = 6) on the fractional urinary excretion of phosphate (FP), sodium (FNa), and chloride (FCl) (mean ± SD). Controls were 4 horses infused with an equivalent volume of 0.9% NaCl. An asterisk indicates that the value is statistically different from baseline (time 0) and from the control value at the same time interval ($P < .05$). Breaks in the *x*-axis represent the scale transition from minutes to hours.

cemia during sepsis in humans[5] and the role of peptides of the calcitonin family in the development of hypocalcemia during sepsis remains controversial.[21] Based on work on other species, it is unlikely that vitamin D metabolites play an important role in the development of acute hypocalcemia during endotoxemia in horses. LPS infusion to healthy cows induced hypocalcemia, but failed to induce changes in serum concentrations of vitamin D metabolites.[20] Moreover, the role of vitamin D metabolites in the equine calcium metabolism is unclear.[25] Plasma cortisol concentrations increase during endotoxemia in horses,[26] and cortisol has calcitoninlike effects, decreasing serum Ca^{2+} concentrations[25]; however, these are long-term actions and do not explain the acute hypocalcemia seen in endotoxemic animals.

Intracellular accumulation of Ca^{2+} may contribute to hypocalcemia; both increased intracellular influx of Ca^{2+} from the extracellular compartment and decreased intracellular efflux of Ca^{2+} to the extracellular compartment have been documented during sepsis.[27]

We speculate that the most likely causes of hypocalcemia during sepsis are mobilization of Ca^{2+} to intracellular subcompartments and interstitial sequestration of Ca^{2+}, likely the result of a systemic inflammatory response. Tissue and interstitial sequestration of Ca^{2+} as causes of hypocalcemia have not been evaluated in horses; however, administration of LPS to healthy pigs resulted in ionized hypocalcemia and in increased Ca^{2+} accumulation in the liver and peritoneal fluid.[18] Because the interstitial fluid volume is much greater than the blood volume, interstitial accumulation of Ca^{2+} could result in hypocalcemia.[18]

Intracellular and interstitial movement of Mg^{2+} may play an important role in the development of hypomagnesemia, because a considerable decrease was found in serum Mg^{2+} and tMg concentrations, and in the urinary excretion of Mg^{2+} of endotoxemic horses. A major limitation in understanding the pathogenesis of hypomagnesemia during sepsis is that the intracellular and extracellular regulation of Mg^{2+} is poorly understood when compared to the regulation of other ions. Vasopressin, which may be increased early in sepsis and endotoxemia in many species, including the horse,[26] can induce a massive intracellular uptake of Mg^{2+}.[28] The role of vasopressin in the development of hypomagnesemia during sepsis remains to be determined. Insulin, which is increased in horses with experimental endotoxemia,[29] including horses of this study, increases the cellular uptake of extracellular magnesium.[30]

Both increased and decreased serum concentrations of PTH have been documented in critically ill horses.[4] In horses with severe enterocolitis admitted to The Ohio State University Equine Intensive Care Unit, 80% had ionized hypocalcemia and 70% had ionized hypomagnesemia.[4] Some horses with clinical evidence of sepsis and hypocalcemia had low serum PTH concentrations for their degree of hypocalcemia, indicating an inappropriate response of the parathyroid gland to low serum Ca^{2+} concentrations. Inflammatory mediators known to be increased in horses with endotoxemia, such as IL-1 and IL-6[9,10] decrease PTH secretion in several species, including the horse.[13–15] Furthermore, increased plasma concentrations of IL-6 and TNF-α have been associated with hypocalcemia in septic humans.[5] Hypomagnesemia may also result in parathyroid gland dysfunction and hypocalcemia.[24]

It is likely that the high serum PTH concentrations during sepsis, in both humans and horses, are the result of a systemic inflammatory response rather than a reflection of hypocalcemia.[1,4,5] The precise reason for the increase in serum PTH concentrations in this study when serum Ca^{2+} concentrations were within the reference range is unclear. One possibility is β-adrenergic stimulation of the parathyroid chief cells by catecholamines, which are increased during sepsis and are known the stimulate PTH synthesis and secretion.[31] Another possibility is increased concentrations of inflammatory mediators. One candidate is IL-8, which increases during endotoxemia,[32] and has been shown to stimulate PTH secretion by bovine parathyroid cells.[33] IL-1 and IL-6 decrease PTH secretion and mRNA expression in equine parathyroid chief cells,[15] making these 2 cytokines unlikely causes of increased PTH secretion, but likely causes of parathyroid gland dysfunction.

High serum PTH concentrations in critically ill humans have been associated with increased mortality,[1] suggesting that PTH may have other functions.[34,35] For example, leukocytes (granulocytes, lymphocytes, and monocytes) ex-

press PTH receptors and increased PTH concentrations alter their function.[34,35]

The effects of endotoxemia were more pronounced on serum Mg^{2+} than on serum Ca^{2+} concentrations. Serum Ca^{2+} concentrations decreased by 8% from baseline, whereas serum Mg^{2+} concentrations decreased by 19%. Moreover, the decrease in serum Mg^{2+} concentrations in these horses did not result from changes in plasma pH or in ionization of extracellular Mg^{2+} because serum tMg concentrations also decreased, and blood pH did not change, indicating that there was a net loss of Mg^{2+} from the intravascular compartment, perhaps to the interstitium or to the intracellular compartment. Clinical endotoxemia results in metabolic acidosis; however, we could not demonstrate a statistically significant decrease in blood pH and bicarbonate concentrations (data not shown).

A significant decrease occurred in serum Mg^{2+} during experimental endotoxemia. Moreover, evidence exists that hypomagnesemia predisposes horses to a worse outcome from endotoxemia,[3] and replacement therapy is warranted, especially because Mg^{2+} may have a protective effect in experimental animals.[36] Thus, magnesium replacement in endotoxemic and septic horses with hypomagnesemia should be considered.

The decrease in urinary excretion of Ca^{2+} and Mg^{2+} noted in the present study was likely the result of increased PTH concentrations, as well as the response of the renal Ca^{2+}-sensing receptor to hypocalcemia.

LPS administration resulted in hypophosphatemia and hyperphosphaturia, which could have been, in some horses, the result of increased concentrations of PTH, together with increased concentrations of inflammatory mediators such as IL-6 and TNF-α. Both PTH and TNF-α concentrations were increased in horses of this study. Increased concentrations of IL-6 and TNF-α in critically ill human patients have been associated with hypophosphatemia.[37] Likewise, LPS administration and increased TNF-α concentrations have been associated with hypophosphatemia in cows.[20] LPS administration to rats enhanced urinary phosphate excretion.[38] Increased concentrations of PTH have been documented in critically ill humans[1,5] and horses[4] and in endotoxemic rats.[19] In a clinical study in critically ill horses with evidence of endotoxemia, the urinary excretion of phosphate was increased.[4] In the same study, a number of horses had extremely high serum PTH concentrations, which could not be explained by hypocalcemia.[4] Other possibilities for the hyperphosphaturia in these animals are PTH-related peptide and atrial natriuretic peptide (ANP), which may be increased during sepsis and increase the urinary excretion of phosphate.[39,40] Another possibility is endotoxin-induced hyperinsulinemia, because increased insulin concentrations in humans induce hypophosphatemia,[41] and we showed that horses in this study were hyperinsulinemic.

Serum and urinary excretion of K^+ decreased, making urinary losses of K^+ an unlikely cause of hypokalemia. Evidence supports a role of sodium- and potassium-activated adenosine triphosphatase (Na^+/K^+ ATPase) in the development of hypokalemia during sepsis and endotoxemia. In healthy human volunteers with experimental endotoxemia, plasma K^+ concentrations decrease and an increase occurs in the activity of the Na^+/K^+ ATPase in skeletal muscle, resulting in skeletal muscle K^+ accumulation.[42] Adrenergic stimulation also increases the activity of the Na^+/K^+ ATPase,[42] contributing to hypokalemia. Another possibility for the hypokalemia is an increase in serum insulin concentrations, which are known to be increased during endotoxemia in several species, including the horse.[29,43] Insulin increases the activity of the Na^+/K^+ ATPase.[44] The decreased urinary excretion of K^+ could have been affected by the activity of PTH and the Ca^{2+} receptor on the thick ascending loop of Henle in response to hypocalcemia and hypomagnesemia.[45]

The increase in FNa could have been the result of renal tubular dysfunction or increased plasma concentrations of ANP, which are known to increase during sepsis and endotoxemia[40]; or due to increased PTH concentrations. PTH is a is natriuretic hormone.[46] Based on these mechanisms, it is important to mention that the increased FNa present in some critically ill horses may not necessarily indicate severe tubular damage.

We determined that hypocalcemia and hypomagnesemia may develop in horses as a direct effect of endotoxemia. We also found that the parathyroid gland response to endotoxemia was variable among horses; some horses responded to hypocalcemia with an increase in serum PTH concentrations, whereas other horses did not. Of interest, however, was that some horses had an increase in serum PTH concentrations before serum Ca^{2+} concentrations decreased (premature parathyroid gland response), indicating that factors other than serum Ca^{2+} concentrations may regulate the response of the parathyroid gland during sepsis. Moreover, it is likely that in horses, as in humans, PTH has immunomodulatory functions.[34,35] Based on the results of this study, it is clear that there are considerable individual variations in the response to endotoxemia with regard to Ca^{2+}, Mg^{2+}, and PTH concentrations. Therefore, we believe that other factors, in addition to parathyroid gland dysfunction, such as a systemic inflammatory response, are important in the development of hypocalcemia during sepsis and endotoxemia. However, it is less clear which factors may regulate extracellular Mg^{2+} during sepsis and endotoxemia.

Footnotes

[a] *E coli* serotype 0111:B4 LPS, Sigma-Aldrich Corporation, St Louis, MO
[b] Cell-Dyn 3500, Abbott Diagnostics, Santa Clara, CA
[c] Boehringer Mannheim/Hitachi 911 system, Boehringer Mannheim Corp, Indianapolis, IN
[d] ACL 200 Automated Coagulation Laboratory, Instrumentation Laboratory, Lexington, MA
[e] ABL 500, Radiometer Copenhagen, Radiometer Medical A/S, Copenhagen, Denmark
[f] Nova 8, Nova Biomedical, Waltham, MA
[g] Immulite PTH, Diagnostic Products Corporation, Los Angeles, CA
[h] Coat-A-Count Insulin, Diagnostic Products Corporation, Los Angeles, CA
[i] Immulyte TNF-α, Diagnostic Products Corporation, Los Angeles, CA
[j] Vitros DT60 II, Ortho-Clinical Diagnostics, Rochester, NY
[k] JMP 5.1, SAS Institute, Cary, NC

[l] SigmaStat 3.1, SPSS Inc, Chicago, IL
[m] SigmaPlot 8.0, SPSS Inc, Chicago, IL

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