Effects of Intravenous Magnesium Sulfate on Calcium-Regulating Hormones, Plasma and Urinary Electrolytes in Healthy Horses

Short Title: Hypermagnesemia Alters Calcium-Regulating Hormones and Electrolytes in Horses

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Abstract

Intravenous magnesium sulfate (MgSO_4) is used in equine practice to treat hypomagnesemia, dysrhythmias, neurological disorders, and calcium dysregulation. MgSO_4 is also used as a calming agent in equestrian events. Hypercalcemia affects calcium-regulating hormones, as well as plasma and urinary electrolytes; however, the effect of hypermagnesemia on these variables is unknown. The goal of this study was to investigate the effect of hypermagnesemia on blood parathyroid hormone (PTH), calcitonin (CT), ionized calcium (Ca^{2+}), ionized magnesium (Mg^{2+}), sodium (Na^+), potassium (K^+), chloride (Cl^-) and their urinary fractional excretion (F) after intravenous administration of MgSO_4 in healthy horses. Twelve healthy female horses of 4-18 years of age and 432-600 kg of body weight received a single intravenous dose of MgSO_4 (60 mg/kg) over 5 minutes, and blood and urine samples were collected at different time points over 360 minutes. Plasma Mg^{2+} concentrations increased 3.7-fold over baseline values at 5 minutes and remained elevated for 120 minutes ($P < 0.05$), Ca^{2+} concentrations decreased from 30-60 minutes ($P < 0.05$), but Na^+ , K^+ and Cl^- concentrations did not change. Serum PTH concentrations dropped initially to rebound and remain elevated from 30 to 60 minutes, while CT concentrations increased at 5 minutes to return to baseline by 10 minutes ($P < 0.05$). The FMg, FCa, FNa, FK, and FCl increased, while urine osmolality decreased from 30-60 minutes compared baseline ($P < 0.05$). Experimental hypermagnesemia alters calcium-regulating hormones (PTH, CT), reduces plasma Ca^{2+} concentrations, and increases the urinary excretion of Mg^{2+} , Ca^{2+} , K^+ , Na^+ and Cl^- in healthy horses. This information has clinical implications on the short-term effects of hypermagnesemia on calcium-regulating hormones as well as plasma and urine electrolytes.

Introduction

Intravenous magnesium sulfate (MgSO_4) is used in equine medicine to treat dysrhythmias, neurological disorders, hypomagnesemia in critical illness, and refractory hypocalcemia [1]. More recently, intravenous MgSO_4 has been used in equine events as a calming and performance-enhancing agent, with the ultimate goal of giving a competitive edge to horses that receive it [2, 3].

Magnesium (Mg) is an essential macroelement involved in a multitude of physiological processes, including enzymatic activation, intermediary metabolism of carbohydrates, fats, and proteins, nucleic acid synthesis, cell membrane ion transport, neuromuscular excitability, cell proliferation, and calcium homeostasis [1]. The secretion of PTH and activity of the PTH receptor are modulated by Mg. Thus, Mg depletion could impair calcium regulation [4].

Similar to total calcium (Ca), in which ionized calcium (Ca^{2+}) represents the active fraction, ionized Mg (Mg^{2+}) is the active fraction of total Mg. Considering that a multitude of cellular processes are highly dependent on Mg, intracellular Mg concentrations are tightly regulated [1]. However, extracellular Mg^{2+} is not under tight hormonal homeostatic control as is Ca^{2+} , and its plasma concentrations depend on gastrointestinal absorption, renal excretion, and bone exchange [1, 5]. A number of hormonal and non-hormonal factors influence extracellular Mg^{2+} concentrations [6].

Parathyroid hormone (PTH) and calcitonin (CT) are important Ca-regulating hormones [7, 8]. Hypocalcemia stimulates and hypercalcemia suppresses PTH secretion, while the opposite occurs with CT release [9]. In addition to its effects on Ca^{2+} homeostasis, PTH stimulates renal reabsorption of Mg^{2+} and its release from bone [10]. Calcitonin (CT) is secreted by the thyroid

gland C cells in response to hypercalcemia to restore normocalcemia by blocking osteoclast-mediated bone resorption and reducing renal calcium reabsorption. There is evidence in other species that hypermagnesemia also stimulates CT secretion [11, 12].

The ability of the parathyroid gland chief cells and thyroid gland C cells to detect extracellular Ca^{2+} concentrations depends on the calcium-sensing receptor (CaSR), a G-protein-coupled cell surface receptor [13, 14]. The CaSR is also expressed in the renal tubular cells where it regulates the reabsorption of Ca^{2+} , Mg^{2+} , Na^+ , K^+ , Cl^- , and water in various species, including the horse [4, 13, 15]. Excessive CaSR activation promotes diuresis and could lead to dehydration in patients with chronic hypercalcemia [4, 15]. Relevant to this study, the CaSR also detects other polyvalent cations, including Mg^{2+} , Sr^{2+} , Gd^{3+} , and neomycin [16]. Therefore, hypermagnesemia could potentially influence PTH and CT secretion, plasma Ca^{2+} concentrations, as well as the urinary excretion of electrolytes.

Hypercalcemia affects calcium-regulating hormones, as well as plasma and urinary electrolytes in horses [4]. In addition, hypercalcemia decreases Mg^{2+} concentrations in horses [4]. Therefore, based on the Ca^{2+} and Mg^{2+} interactions, as well as the ability of Mg^{2+} to activate CaSR, one can speculate that hypermagnesemia could affect a number of blood and urinary variables in horses, which could have clinical implications.

The goal of this study was to investigate the effect of experimentally induced hypermagnesemia with intravenous MgSO_4 on blood PTH, CT, Mg^{2+} , Ca^{2+} , Na^+ , K^+ , and Cl^- concentrations, as well as on their urinary excretion in healthy horses. We hypothesized that hypermagnesemia will reduce Ca^{2+} concentrations, initially suppress to later stimulate PTH secretion, with the opposite effects on CT concentrations. We also proposed that

hypermagnesemia, similar to hypercalcemia, will increase the urinary fractional excretion of Mg^{2+} , Ca^{2+} , Na^{+} , K^{+} and Cl^{-} in horses.

Materials and methods

Animals criteria and experimental design

Twelve healthy Standardbred mares with a median age of 8 years (4-18 years) and weighing 509 kg (432-600 kg) from The Ohio State University teaching herd were included in the study. Horses had no history of illness 6 months prior to the study, were under a routine health program, and were considered healthy based on physical examination, hematology, serum chemistry, and urine analysis. They were fed the same diet of grass and grass hay (0.5% calcium and 0.25% phosphorus), alfalfa hay (1.4% calcium and 0.25% phosphorus), and no concentrate feed.

MgSO₄ administration

All horses were administered a single intravenous dose of MgSO₄ (60 mg/kg) (Magnesium Sulfate 50% solution, Wedgewood Pharmacy, Swedesboro, NJ, USA) over 5 minutes to induce hypermagnesemia. This dose was chosen because it increases Mg^{2+} concentrations 3-fold over baseline values, which is expected to activate CaSR, and is also the dose anecdotally used for its calming effects at equestrian events [3]. This represented approximately 30 grams of MgSO₄ (60 ml) for a 500 kg horse [3].

Sampling

Polyurethane catheters (Mila International, Florence, KY, USA) were placed aseptically in the left and right jugular veins for MgSO₄ administration and blood sample collection and were removed after the last blood sample was collected. Blood samples were collected at 0 (baseline; before

MgSO₄ administration), 5, 10, 15, 30, 45, 60, 120, 180, 240, 300, and 360 minutes after MgSO₄ administration. A Foley catheter was placed aseptically into the bladder and urine samples collected at baseline (time 0; before MgSO₄ administration) and at 10, 30, 60, 120, 180, 240, 300, and 360 minutes after MgSO₄ administration.

Blood was collected into serum clot tubes and centrifuged at $2,000 \times g$ for 10 minutes at 4°C. Serum was aliquoted in smaller volumes and stored at -80°C until analysis. At each time point, blood was also collected into heparinized syringes for immediate electrolyte and creatinine measurements. Urine samples were processed immediately after collection to measure electrolyte and creatinine concentrations. The urinary fractional excretion of Mg²⁺ (FMg), Ca²⁺ (FCa), Na⁺ (FNa), K⁺ (FK), and Cl⁻ (FCl) was calculated as $[U_x/S_x]/[U_{cr}/S_{cr}] \times 100$, where U=urine, S=serum, x=each electrolyte, and cr=creatinine concentrations [4]. Results are expressed as a fraction (%) of the urinary excretion of creatinine [4].

Laboratory analyses

Blood samples for Mg²⁺, Ca²⁺, Na⁺, K⁺, Cl⁻, and creatinine concentrations were analyzed with a biochemistry analyzer (pHOx Ultra Analyzer, Nova Biomedical Corp., Waltham, MA, USA). Urine creatinine and electrolyte concentrations were measured using an automated chemistry system (Cobas C501, Roche Diagnostics, Indianapolis, IN, USA).

PTH and calcitonin measurements

Serum PTH concentrations were measured with a human-specific immunoradiometric assay (Scantibodies Laboratory, Santee, CA, USA) with a working range of 6.5- 2328 pg/mL, a sensitivity of 1 pg/mL, and previously validated for horses [17]. Serum CT concentrations were determined with a human-specific immunoradiometric assay (Scantibodies Laboratory, Santee, CA, USA) with a working range of 10-1000 pg/mL and a detection limit of 1 pg/mL. The CT assay

was validated for equine samples, with intra and inter-assay coefficients of variation <10%, and linear parallelism at 1:2, 1:4 and 1:8 dilutions ($R^2 = 0.96$).

Statistical analysis

Data were assessed for normality with Shapiro-Wilk statistic and were normally distributed. Therefore, results are expressed as means with standard errors (S.E.). Data were analyzed using repeated measures One-Way ANOVA with Tukey's test to determine significance between different time points (Prism 8.0, GraphPad Software, Inc., La Jolla, CA, USA). Figures were generated using graphics software (SigmaPlot 14, Systat Software, Inc., San Jose, CA, USA). Significance was set at $P < 0.05$.

Results

Plasma Mg^{2+} , Ca^{2+} , Na^+ , K^+ , and Cl^- concentrations and Mg^{2+}/Ca^{2+}

Plasma Mg^{2+} concentrations increased sharply by 5 minutes and remained significantly higher than baseline for 180 minutes (**Fig 1A and Table 1; $P < 0.05$**). At 5, 15 and 60 minutes, plasma Mg^{2+} concentrations were 3.7, 2.8, and 1.7-fold baseline values, respectively ($P < 0.05$). At 360 minutes, 4 horses still had Mg^{2+} concentrations that were 30% higher than their baseline values. Hypermagnesemia led to a sharp and short lasting increase in plasma Ca^{2+} concentrations to then cause a steady and statistically significant decrease in Ca^{2+} concentrations ($P < 0.05$), reaching its lowest values at 45 minutes (12.5% decrease from time 0) to subsequently return to baseline values at 120 minutes (**Fig 1A and Table 1**). Plasma Na^+ , K^+ , Cl^- , and creatinine concentrations and plasma osmolality did not change over time (**Table 1**). The Mg^{2+}/Ca^{2+} ratio was elevated by 5 minutes and remained above baseline values for 120 minutes (**Table 1; $P < 0.05$**).

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Fig 1. Plasma Mg^{2+} and Ca^{2+} **(A)** and serum PTH and CT **(B)** concentrations following IV administration of $MgSO_4$ (60 mg/kg) in healthy horses. Values expressed as mean with S.E. * indicates statistically different than time 0. Rectangle over X axis represents IV administration of $MgSO_4$ over 5 minutes. Mg^{2+} , plasma ionized magnesium; Ca^{2+} , plasma ionized calcium PTH, parathyroid hormone; CT, calcitonin

159 **Table 1.** Plasma Mg²⁺, Ca²⁺, Na⁺, K⁺ and Cl⁻, and serum PTH and CT concentrations, and Mg⁺²/Ca²⁺ ratio following IV administration
160 of MgSO₄ (60 mg/kg) in healthy horses. Values expressed as mean ± S.E.

Variables	Time (minutes)											
	0	5	10	15	30	45	60	120	180	240	300	360
Mg²⁺ (mg/dL)	1.3±0.1	4.7±0.3*	3.7±0.1*	3.3±0.1*	2.7±0.1*	2.3±0.1*	2.2±0.1*	1.9±0.1*	1.7±0.1	1.6±0.1	1.6±0.1	1.6±0.1
Ca²⁺ (mg/dL)	5.9±0.1	6.0±0.1	5.9±0.1	5.9±0.1	5.6±0.1*	5.4±0.0*	5.6±0.1*	5.8±0.1	5.8±0.2	5.8±0.1	5.8±0.1	6.1±0.1
Mg²⁺/ Ca²⁺	0.2±0.0	0.8±0.1*	0.6±0.0*	0.6±0.0*	0.5±0.0*	0.4±0.0*	0.4±0.0*	0.4±0.0*	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0
Na⁺ (mmol/L)	137.5±0. 4	136.5±0. 5	137.0±0. 5	136.8±0. 5	137.1±0. 6	137.2±0. 4	136.6±0. 6	136.4±0. 7	137.0±0. 6	138.2±0. 5	137.2±0. 6	137.2±0. 4
K⁺ (mmol/L)	3.6±0.3	3.3±0.1	3.3±0.1	3.3±0.0	3.4±0.1	3.3±0.1	3.3±0.1	3.4±0.1	3.3±0.1	3.5±0.1	3.4±0.1	3.6±0.2
Cl⁻ (mmol/L)	104.0±0. 6	103.3±0. 6	102.9±0. 7	102.5±0. 6	102.7±0. 7	102.9±0. 7	103.0±0. 1	103.1±1. 2	103.5±0. 5	104.5±0. 3	104.3±0. 4	104.03±0. .8
PTH (pg/mL)	45.5±19. 3	10.1±1.4	5.0±1.1	11.0±2.7	113.9±37 .6*	109.8±40 .0*	92.8±30. 7*	62.6±15. 7	62.6±16. 2	64±18.8	80.3±17. 9	69.9±16. 8
CT (pg/mL)	13.4±9.9	116.3±32 .6*	22.0±7.5	9.7±2.3	5.1±1	4.2±0.1	4.8±1.7	3.2±0.8	4.0±0.8	3.0±0.7	4.0±1.2	2.7±0.7
Creatinine (mg/dL)	1.1±0.1	1.10±0.1	1.1±0.1	1.0±0.1	1.1±0.1	1.0±0.5	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.1	2.1±0.1
Osmolality (mOsm/kg)	275.9±1. 5	274.6±1. 7	275.1±1. 5	275.3±1. 3	276.0±1. 6	276.8±1. 8	275.2±1. 8	275.0±1. 2	275.6±1. 3	272.8±4. 5	275.2±1. 5	275.3±1. 8

161 Mg²⁺, ionized magnesium; Ca²⁺, ionized calcium; Na⁺, sodium; K⁺, potassium; Cl⁻, chloride; PTH, parathyroid hormone; CT,
162 calcitonin. *P<0.05.

PTH and CT concentrations

Serum PTH concentrations initially decreased to sharply increase at 30-60 minutes after MgSO_4 administration ($P < 0.05$), remaining elevated for the rest of the study although not statistically different than baseline (**Fig 1B and Table 1**). Serum CT concentrations increased rapidly at 5 minutes corresponding with the end of MgSO_4 administration and highest Mg^{2+} concentrations ($P < 0.05$), decreasing at 10 minutes and remaining at baseline values for the rest of the study (**Fig 1B and Table 1**).

Urinary electrolytes and osmolality

Urine Mg^{2+} concentrations increased steadily to reach statistical significance at 120 and 180 minutes compared to time 0 (**Table 2; $P < 0.05$**), to decrease to baseline values by 240 minutes. Urinary Ca^{2+} concentrations also increased by 180 minutes (**Table 2; $P < 0.05$**), to return to baseline at 240 minutes. Urine Na^+ concentrations increased at 60 minutes, while urine K^+ concentrations decreased at 30 minutes and returned to baseline at 360 minutes (**Table 2; $P < 0.05$**). Urinary Cl^- concentrations did not change overtime. Urinary osmolality decreased at 30 and 60 minutes to return to baseline thereafter (**Table 2; $P < 0.05$**).

180 **Table 2.** Urine Mg^{2+} , Ca^{2+} , Na^+ , K^+ , Cl^- , and creatinine concentrations and urine osmolality following IV administration of MgSO_4
181 (60 mg/kg) in healthy horses. Values expressed as mean and S.E.

Variables	Time (minutes)								
	0	10	30	60	120	180	240	300	360
Mg^{2+} (mg/dL)	49.6±11.8	109.9±27.7	143±36	111.8±20.5	138.9±20.6*	154.2±22.1*	125.3±20.3	98.0±17.4	96.7±8.5
Ca^{2+} (mg/dL)	59.7±28.2	67.2±26.5	51.4±8.1	71.9±45.4	109.5±84.8	119.5±94.7*	64.3±31.5	19.9±8.7	88.5±44.0
Na^+ (mEq/L)	34.2±9.7	64.9±13.8	74.3±17.2	115.3±37.1*	62.8±20.2	38.0±14.0	34.3±12.5	38.0±19.7	18.4±0.9
K^+ (mEq/L)	272.8±17.6	206.3±28.2	134.5±21.7*	172.8±31.5	221.4±24.8	263±26.3	266.2±26.4	226±34.7	267.9±24.7
Cl^- (mEq/L)	174.1±23.8	150.8±18.8	153.6±16.6	152.8±21.4	145.1±17.3	133.3±11.9	139.9±23.4	131.6±36.1	139±35.1
Creatinine (mg/dL)	316.1±53.6	204.5±60.2	97.7±27.8*	92.9±19.2*	183.7±39.6	287±54.78	336.5±65.2	412.9±117.1	463.6±132.9
Osmolality (mOsm/kg)	1249±125.9	856±143	624±75.8*	674.2±74.0*	845±116.2	1028±129.8	1053±138.8	1103±145.1	1292±148.6

182 Mg^{2+} , ionized magnesium; Ca^{2+} , ionized calcium; Na^+ , sodium; K^+ , potassium; Cl^- , chloride. *P < 0.05.

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Urinary fractional excretion of electrolytes

The FMg increased by 3-fold at 30-120 minutes after MgSO₄ administration to return to baseline values by 360 minutes (**Fig 2A; Table 3; P < 0.05**). The FCa, FNa, FK, and FCl increased statistically by 60 minutes to return to baseline thereafter (**Fig 2A/2B; Table 3; P < 0.05**).

Fig 2. FMg and FCa (**A**) and FNa, FK and FCl (**B**) (%) following IV administration of MgSO₄ (60 mg/kg) in healthy horses. Values expressed as mean with S.E. * indicates statistically different than time 0 (P < 0.05). Rectangle over X axis represents IV administration of MgSO₄ over 5 minutes. FMg, urinary fractional excretion of Mg²⁺; FCa, urinary fractional excretion of Ca²⁺; FNa, urinary fractional excretion of Na⁺; FK, urinary fractional excretion of K⁺; FCl, urinary fractional excretion of Cl⁻.

198 **Table 3.** Urinary fractional excretion of Mg^{2+} , Ca^{2+} , Na^+ , K^+ , and Cl^- following IV administration of MgSO_4 (60 mg/kg, IV) in healthy
 199 horses. Values expressed as mean $\pm$ S.E.

Variables	Time (minutes)								
	0	10	30	60	120	180	240	300	360
FMg (%)	16.7 $\pm$ 4.3	27.2 $\pm$ 10.5	56.4 $\pm$ 6.3*	64.1 $\pm$ 13.5*	50.9 $\pm$ 7.1*	39.1 $\pm$ 5.6	30.6 $\pm$ 7.6	20.0 $\pm$ 5.8	25.7 $\pm$ 4.5
FCa (%)	4.9 $\pm$ 2.3	11.2 $\pm$ 4.4	10.8 $\pm$ 1.4	14.3 $\pm$ 7.1*	11.6 $\pm$ 6.4	9.1 $\pm$ 6.5	7.0 $\pm$ 3.6	0.7 $\pm$ 0.2	6.7 $\pm$ 3.8
FNa (%)	0.13 $\pm$ 0.1	0.7 $\pm$ 0.4	1.0 $\pm$ 0.4	1.2 $\pm$ 0.4*	0.8 $\pm$ 0.4	0.3 $\pm$ 0.3	0.1 $\pm$ 0.0	0.04 $\pm$ 0.0	0.1 $\pm$ 0.0
FK (%)	30.4 $\pm$ 3.8	43.6 $\pm$ 7.7	44.7 $\pm$ 5.1	57.4 $\pm$ 5.3*	48.0 $\pm$ 8.1	33.8 $\pm$ 5.3	27.5 $\pm$ 6.4	18.6 $\pm$ 4.2	27.1 $\pm$ 5.7
FCl (%)	0.8 $\pm$ 0.2	1.7 $\pm$ 0.7	2.1 $\pm$ 0.5	2.4 $\pm$ 0.8*	1.6 $\pm$ 0.6	0.9 $\pm$ 0.3	0.7 $\pm$ 0.3	0.4 $\pm$ 0.2	0.6 $\pm$ 0.2

200 FMg, urinary fractional excretion of magnesium; FCa, urinary fractional excretion of calcium; FNa, urinary fractional excretion of
 201 sodium; FK, urinary fractional excretion of potassium; FCl, urinary fractional excretion of chloride. *P < 0.05.

Discussion

In the present study, experimentally-induced hypermagnesemia in healthy horses led to a short lasting decrease in Ca^{2+} concentrations, with transient changes in PTH and CT concentrations, increasing the urinary fractional excretion of Mg^{2+} , Ca^{2+} , Na^+ , K^+ , and Cl^- , and decreasing urine osmolality.

A single intravenous dose of MgSO_4 at 60 mg/kg resulted in sustained hypermagnesemia for over 120 minutes. Similar results were observed in horses in which MgSO_4 was evaluated as a treatment for trigeminal headshaking syndrome and in human patients with preeclampsia [18, 19]. These findings also suggest that the physiological effects of a single dose of MgSO_4 in horses at the dose used in this study could last up to 6 hours. In addition, this information may be relevant to venues such as equestrian competitions, knowing that for at least 60 minutes after administration of this dose, serum $[\text{Mg}^{2+}]$ will be twice baseline values, potentially explaining the calming effects of hypermagnesemia and also carrying potential regulatory implications. An increase in Mg^{2+} concentrations coupled with a high $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio could be a potential biomarker for nefarious administration of MgSO_4 to competing horses [3, 20].

A decrease in Ca^{2+} concentrations after MgSO_4 infusion has been documented in human patients, horses and dogs [3, 19, 21-24] and could be attributed to excessive Mg^{2+} activation of CaSR in the renal tubules, increasing the urinary excretion of Ca and Mg, as reported in human patients [21, 24, 25]. We anticipated this response based on a previous study where experimental hypercalcemia decreased Mg^{2+} in healthy horses [4]. It is also possible that hypermagnesemia suppressed PTH secretion by activating CaSR [21], at least at early time points in the horses of this study, because subsequently there was a PTH rebound response that we attributed to the drop

in Ca^{2+} concentrations. One difference between this study and publications using MgSO_4 as a tocolytic agent was the rapid intravenous administration of MgSO_4 to these horses compared to how it is administered to pregnant women [26]. One study in healthy horses showed that hypercalcemia has a similar effect as hypermagnesemia, increasing the urinary excretion of Ca^{2+} , Mg^{2+} , Na^+ , K^+ , and Cl^- , increasing urine output, and decreasing urine osmolality [4].

Parathyroid hormone promotes renal calcium reabsorption and bone resorption to maintain normocalcemia [27, 28]. The PTH receptor, via cAMP, mediates the renal actions of PTH by increasing the activity of the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ type 2 cotransporter (NKCC2), raising the electropositivity of the renal tubular lumen to promote paracellular cation reabsorption [29]. Activation of CaSR interferes with NKCC2, similar to furosemide, and inhibits vasopressin-mediated traffic of aquaporin-containing vesicles, explaining the diuretic actions of hypercalcemia in multiple species [15, 30, 31]. Under a similar premise and physiological studies showing that Mg^{2+} activates CaSR [13], hypermagnesemia could interfere with PTH secretion and renal reabsorption of electrolytes and water. In fact, CaSR has been described as a promiscuous receptor because it can sense multiple cations at different extracellular concentrations [13].

The initial PTH suppression could be attributed to excessive CaSR activation by Mg^{2+} . This effect was likely counteracted by the drop in Ca^{2+} concentrations, which likely triggered a rebound PTH response, reflecting the preference of CaSR for Ca^{2+} over Mg^{2+} [13]. The increase in serum PTH concentrations occurred in parallel with the decrease in Ca^{2+} concentrations, supporting the rapid response of this system to protect against hypocalcemia [32, 33]. After its rapid rise, CT concentrations returned to baseline values for the rest of the study, despite hypermagnesemia. This indicates that Mg^{2+} stimulated the secretion of CT stored in granules but was not sufficient to stimulate additional synthesis and secretion, in particular in the presence of

low to normal Ca^{2+} concentrations. It is likely that a mechanism similar to what occurred in the parathyroid gland chief cells, but with opposite endocrine secretion, was involved in thyroid gland C cell CT secretion. A similar CT response was observed in pigs after magnesium chloride infusion [34].

In addition to the increased FMg and FCa, the most feasible explanation for increased FNa, FK, and FCl were the effects of increased extracellular Mg^{2+} on transepithelial ion transport and water reabsorption, as previously discussed [35, 36].

While no effect of hypermagnesemia on plasma Na^+ , K^+ , and Cl^- concentrations was documented, its evident effect on urinary electrolyte excretion indicates that in the short term, other homeostatic systems could maintain their concentrations. Evaluating the effect of prolonged hypermagnesemia on calcium-regulating hormones and electrolyte balance could provide additional insight on equine Mg^{2+} physiology.

Conclusions

A single intravenous bolus administration of MgSO_4 to healthy horses causes prolonged hypermagnesemia, a transient decrease in Ca^{2+} concentrations, and rapid changes in calcium-regulating hormones, increasing the urinary excretion of electrolytes and decreasing urine osmolality. This information enhances our understanding of equine Mg and Ca biology and has clinical implications. For example, based on information from this study, it would be of interest to know whether prolonged hypermagnesemia from excessive Mg supplementation leads to electrolyte depletion, water wasting, and subsequent clinical consequences.

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289 **Conflict of interest:** Stephen Schumacher is employed by USEF

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404 **Fig 1.** Plasma Mg^{2+} and Ca^{2+} (A) and serum PTH and CT (B) concentrations following IV
 405 administration of $MgSO_4$ (60 mg/kg) in healthy horses. Values expressed as mean with S.E. *
 406 indicates statistically different than time 0. Rectangle over X axis represents IV administration of
 407 $MgSO_4$ over 5 minutes. Mg^{2+} , plasma ionized magnesium; Ca^{2+} , plasma ionized calcium PTH,
 408 parathyroid hormone; CT, calcitonin.

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Fig 2. FMg and FCa (**A**) and FNa, FK and FCl (**B**) (%) following IV administration of MgSO₄ (60 mg/kg) in healthy horses. Values expressed as mean with S.E. * indicates statistically different than time 0 (P < 0.05). Rectangle over X axis represents IV administration of MgSO₄ over 5 minutes. FMg, urinary fractional excretion of Mg²⁺; FCa, urinary fractional excretion of Ca²⁺; FNa, urinary fractional excretion of Na⁺; FK, urinary fractional excretion of K⁺; FCl, urinary fractional excretion of Cl⁻.

A

B

Figure 1AB

A

B

Figure 2AB