The pharmacokinetics and pharmacodynamics of intravenous magnesium sulfate in horses

Dissertation

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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2019

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Abstract

Intravenous administration of magnesium sulfate ($MgSO_4$) is used to calm horses in equestrian competitions, which is contrary to the rules, and there are no current means to regulate its administration. Initial pilot data from magnesium sulfate ($MgSO_4$) administrations identified increases in plasma ionized magnesium (Mg^{2+}) concentrations and changes in the plasma ionized calcium (Ca^{2+}) to Mg^{2+} concentration ratio. Three experiments were designed to evaluate these changes and to investigate the pharmacokinetics and pharmacodynamics of $MgSO_4$ administration with a goal to identify regulatory methods for its control in horses. The first study investigated the effect the processing, handling, and storage procedures, of the United States Equestrian Federations Equine Drugs and Medications Program, have on Mg^{2+} and related electrolytes in a sample of 50 normal horses. It was concluded that these procedures could be used to establish regulatory thresholds through the use of electrolyte biomarkers for the administration of $MgSO_4$.

The second study detailed the pharmacokinetics of $MgSO_4$ administration and included an investigation of the potential mechanism by which $MgSO_4$ exerts its calming effect. This study examined pharmacodynamic related changes in cardiovascular and fractional excretion variables, as well as, changes in the endocrine profile and electrolyte levels in cerebrospinal fluid as a result of a 60 mg/kg intravenous administration of

$MgSO_4$. This study did not include a control group as it was not possible to maintain restraint on these intensively instrumented horses for 6 hours without the aid of $MgSO_4$. It was concluded that the intravenous administration of $MgSO_4$ decreased mean arterial blood pressures, increased left ventricular function, increased the fractional excretion of electrolytes, altered the endocrine profile for calcitonin and parathyroid hormone, but did not result in changes to concentrations of Mg^{2+} in the cerebrospinal fluid. Additionally, this work confirmed the changes in plasma Mg^{2+} concentrations and the Ca^{2+} to Mg^{2+} ratio as seen in the pilot data.

In the third study, which was tightly controlled, a novel radiotelemetric approach was used to further elucidate the pharmacodynamics of intravenous $MgSO_4$ administration without the need to for restraint of the horses. This technology was used to document changes in blood pressure, electrocardiograms, and locomotion in response to horses administered 60 mg/kg of $MgSO_4$. We were able to demonstrate a decrease in blood pressure, changes in electrocardiogram conduction intervals, and a decrease in locomotion, all of which could be related to the calming effects of intravenous $MgSO_4$ in horses.

Conclusions drawn from these three studies include the identification of regulatory biomarkers capable of determining intravenous $MgSO_4$, a decrease in blood pressure as the likely mechanism for calming, a decrease in activity as a result of administration, and the identification of potential endocrine biomarkers for regulatory control in the form of calcitonin and parathyroid hormone. Additional work is necessary

and ongoing to further evaluate and develop thresholds for calcitonin and parathyroid hormone.

Dedication

This work is dedicated to my wife Erin and children, Nicholas and Elizabeth, my parents, Richard and Carol Schumacher, for all of their unquestionable love, support and understanding.

Acknowledgments

Throughout this process I have been blessed with so many great individuals willing to share their time, talent and experience. I would like to first thank my advisor, Dr. Alicia Bertone for her support, guidance and enthusiasm, and for never giving up on me. I would also like to thank the members of my committee for their advice and support, Drs. Ramiro Toribio, Jeffrey Lakritz, Phillip Lerche and Teresa Burns. I truly appreciate all of the following who answered questions, collected data, motivated and were supportive through it all, and generally put up with me.

Dr. Brian Scansen
Dr. Jonathon Yardley
Dr. Robert Hamlin
Dr. William Muir
Dr. Kasia Dembek
Dr. Allison Kilbourne
Dr. Nathalie Reisbig
Dr. Hayam Hussein
Dr. Ahmed Kamr
Dr. Mari Kaido
Dr. Jake Swink
Dr. Katy Timko

Judith Dutson
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Foreman, J. H., B. E. Bergstrom, K. S. Golden, J. J. Roark, D. S. Coren, C. R. Foreman, and S. A. Schumacher. "Dose Titration of the Clinical Efficacy of Intravenously Administered Flunixin Meglumine in a Reversible Model of Equine Foot Lameness." [In eng]. Equine Vet J Suppl, no. 43 (Dec 2012): 17-20.

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Fields of Study

Major Field: Comparative and Veterinary Medicine

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Chapter 1. Introduction

Human nature is evident in every aspect of life we endure, whether it be in business, education, or competition. To win is usually the endgame regardless of what the pursuit is. It is no different in sports with horses. The term 'hopped-up' comes from horse racing in the 1800's when it was used to describe the attempt to make a horse more competitive by increasing the amount of hops a horse was given in preparation for a race. It was in 1968 that Dancer's Image was stripped of his first place finish in the Kentucky Derby following the detection of phenylbutazone in his post-race blood sample. While the policy regarding medications were very permissive at that time, it was still prohibited to have this drug in the horse at race time. This case was the impetus for many other groups governing equestrian sport to enact equine drugs and medications rules. While the rules have changed over the years to reflect an ever changing environment of abuse and the increasing sensitivities for detection, it appears many of the oldest substances are still the most commonly abused. The goals of the competition typically dictates what the most likely substances are for abuse; whether the horse is supposed to race varied distances, perform a particular set of difficult movements, or to jump incredibly intricate fences. The potential for abuse varies greatly between anabolic steroids, non-steroidal anti-inflammatory drugs (NSAIDs), analgesics, and substances intended to calm horses.

In 1917, the American Horse Shows Association (AHSA) was formed to govern competition in the show ring. Its intention was to maintain clean competition and fair play in the show ring. In 1969, following the case with Dancer's Image, the AHSA began a surveillance program to detect drug use in show horses by collecting urine and saliva samples. All samples that first year were negative, but the die were cast and the AHSA Equine Drugs and Medications Program was launched in 1970. Through the years, challenges to the enforcement of the Equine Drugs and Medications rules have varied, but the one constant has been the desire for horses to be 'calm' during competition. The AHSA has changed names and is now the United States Equestrian Federation (USEF), but its purpose remains the same. The USEF is currently the National Governing Body (NGB) of equestrian sport in the United States.

Regardless of the breed, discipline or pursuit, a calm and responsive horse has always been the goal of competitors. This is especially important in competitions which are subjectively evaluated to determine the winner. Substances used for calming horses involve a wide variety of therapeutic and non-therapeutic drugs, medications, and 'herbal supplements'. When conducting drug testing, the detection of drugs and medications has gotten easier due to the advancement of analytical methods and instrumentation. Early in the enforcement of equine drugs and medications rules, the mere presence of a drug was grounds for a positive finding, but as techniques and technology have advanced, it has become necessary to establish levels at which drugs are considered 'negative' in a sample. Using administration studies, the pharmacokinetics of specific drugs with the potential for abuse have become routine and more simpler…while remaining expensive.

The basic premise is that performance altering or enhancing drugs are not endogenously produced, so the detection is not confusing, the issue is more about determining a limit, below which, is considered not to have the potential to effect the performance of a horse. However, there are exceptions. Some endogenously produced hormones are of benefit to the humans and horses, such as, testosterone and growth hormone. These substances exert a normalizing effect when produced endogenously in the normal course of homeostasis of the mammalian organism, but when exogenously administered the same substances, in large concentrations, serves to maximize or exceed the body's normal response. Methods have been developed to identify exogenously administered, endogenous performance enhancing substances (i.e. testosterone, erythropoietin, human growth hormone). These methods include the detection of recombinant technologies for erythropoietin (rEPO), for example, and the development of biological profiles and biomarkers to detect excessive responses to these administrations. One such profile developed uses the ratio of testosterone (T) to epitestosterone (E) to detect an exogenous administration. A great deal of work has focused on the appropriate ratio for screening and on identifying additional biomarkers definitive for the administration of testosterone. This work is a little more challenging than just detecting a drug. It is necessary to identify what 'normal' is and to identify additional biomarkers that can be utilized to clearly identify the nefarious use of endogenous substances.

In equine competition, one of the most abused substances is one of the most common divalent cations found in all mammals; magnesium. Typically administered in the form of magnesium sulfate ($MgSO_4$), it is also available as magnesium chloride

($MgCl_2$). While $MgSO_4$ has some therapeutic uses in humans and horses, the nefarious purpose for its administration to show and sport horses is to make them calm for competition. In racing, $MgSO_4$ has been used as a pre-race treatment to keep horses from becoming nervous and 'washing out' prior to the race. Racehorses are typically high strung and easily get excited and 'worked up' prior to the race, thereby overexerting themselves before the race has even run. In sport and show horses, the need for a compliant and responsive horse is essential when being subjectively judged. The use of $MgSO_4$ has been reported to be used by trainers as long as drug testing has been around. However, as previously stated, it is more difficult to identify magnesium use than a typical drug as it is an essential element found in the horse, and expected to be present in any sample collected.

The focus of this research was to identify normal levels of ionized magnesium found in the horse, to characterize the pharmacokinetics and pharmacodynamics of ionized magnesium following the administration of $MgSO_4$, and to determine if there is a biomarker, or collection of biomarkers, that provide a biological profile for regulatory purposes. The pharmacodynamics of $MgSO_4$ administration evaluated were changes in the ratios of important plasma electrolytes, changes in the fractional excretion of electrolytes, changes in direct cardiac indices of function evaluated by echocardiography, changes in electrocardiography, changes in electrolytes found in cerebrospinal fluid, alterations in endocrine profiles, and changes in behavior and activity.

It was important to utilize the typical collection methods and techniques used in the regulatory process. The administration of $MgSO_4$ is prohibited by the Federation

Equestre Internationale (FEI), which is the International Governing Body for Equestrian Sport and a member of the International Olympic Committee. While the administration is prohibited, the substance is not prohibited at this time due to the lack of a means to detect its use. The use of $MgSO_4$ is a national and international issue and rules have been promulgated to prohibit its use by disallowing injections of any kind prior to competition; however, this is very difficult to enforce. This research attempts to provide an understanding of the effects $MgSO_4$ has on the horse and to provide support for regulatory control.

Chapter 2. Ionized magnesium and calcium concentration and their ratio in equine plasma samples as determined by a regulatory laboratory compared to a clinical reference laboratory

Drug Test Anal (Sep 25 2018). doi: 10.1002/dta.2509.

Abstract

Magnesium sulfate ($MgSO_4$) is administered to calm competition horses. We evaluated the impact of regulatory requirements for the handling of blood samples on plasma ionized magnesium (Mg^{2+}), ionized calcium (Ca^{2+}), the Mg^{2+} to Ca^{2+} ratio, and pH. We hypothesized that Ca^{2+}, Mg^{2+} and Mg^{2+}/Ca^{2+} would be similar among storage and collection methods. Four blood samples were collected from each of 50 horses on the same day: Group 1- collection in a heparinized syringe and processed within hours in a clinical laboratory; Group 2- collection into a plasma separator tube (PST) centrifuged just prior to analysis, and plasma processed as in (1); Group 3- collection into a PST, refrigerated, shipped via overnight carrier to the United States Equestrian Federation (USEF) Equine Drug Testing and Research laboratory, centrifuged just prior to analysis, and plasma processed; and Group 4- as in (3), but stored frozen at -80˚C for 90 days, thawed, and plasma processed as in (3). Results for Mg^{2+}/Ca^{2+} are unit-less, adjusted Mg^{2+} for potential influence of plasma protein and Ca^{2+}, and was highly correlated with Mg^{2+} pH ($r=-.933$; $P<0.01$). Samples processed immediately in a clinical reference

laboratory had the greatest Mg^{2+}/Ca^{2+}. Both Mg^{2+}/Ca^{2+} and pH predictably decreased after freezing ($P<0.001$). These data suggest that the Mg^{2+}/Ca^{2+}mirrors alterations in Mg^{2+} regardless of storage and collection methods. This understanding can facilitate the development of a regulatory threshold for the control of the nefarious use of magnesium sulfate in competing horses, and an understanding of potential changes to Mg^{2+}/Ca^{2+} with storage of B samples.

Introduction

Magnesium (Mg) is the fourth-most abundant cation in mammals and essential to over 600 required enzymatic reactions including energy metabolism, protein synthesis, neuronal and cardiac excitation, skeletal muscle contraction, and vasomotor tone[1]. The assessment of Mg levels is difficult because of its vast residual storage in bone and muscle, distribution throughout all tissues of the body, and its close relationship with Calcium (Ca). Serum Ca is hormonally regulated by 1,25 dihydroxyvitamin D, calcitonin and parathyroid hormone via kidney and gastrointestinal absorption and secretion feedback loops, yet no hormone primarily regulates Mg balance[2,3]. Mg does interact with Ca by upregulating calcitonin and vitamin D concentrations. Active Mg transport does take place in the distal convoluted tubule of the kidney[2] and serves as the essential enzymatic co-factor for converting vitamin D into its active form thus turning on Ca absorption and bone formation[4]. All the enzymes metabolizing vitamin D require Mg as a necessary co-factor[4,5].

Serum or plasma total Mg and Ca include three forms: ionized (Ca^{2+} and Mg^{2+}); bound to proteins (pCa and pMg); and complexed with weak acids (cCa and cMg)[6]. The ionized

forms of Mg and Ca are thought to be the only physiologically active forms of Mg and Ca as they interact with Ca^{2+}/Mg^{2+}-sensing receptors and other cationic receptors[6]. Because Mg is important in the regulation of a number of physiological processes, there is an essential need to maintain both intracellular and extracellular concentrations of Mg within preset limits[7]. While some studies have demonstrated that Ca^{2+} may be a more potent stimulator of Ca^{2+}/Mg^{2+}-sensing receptors, there is evidence that mouse distal convoluted tubule cells possess a Ca^{2+}/Mg^{2+} sensing mechanism that is equally sensitive to extracellular Ca^{2+} and Mg^{2+} concentrations normally found in plasma[8]. The importance of the cation sensing receptors in the kidneys is central to the homeostatic maintenance of extracellular Ca^{2+} and Mg^{2+} concentrations and is an active area of investigation for understanding the regulatory loop of Mg in the body[2]. Characterization of Mg imbalance is often identified from the perspective of the integrated relationship between Mg and Ca, and described by the clinical signs displayed including muscle cramping in athletes with relative hypomagnesemia and neurologic calming with hypermagnesemia[2,4].

Accurate measurement of the normal ranges for Mg^{2+} and Ca^{2+} concentrations and their ratio (Mg^{2+}/Ca^{2+}) is important for determining alterations in their values on physiologic processes. Magnesium sulfate ($MgSO_4$) is an abused substance in equine competition and is administered illegally as a performance enhancing calming agent[9,10]. Administration of $MgSO_4$ results in an increase in Mg^{2+} and can be quantitated in order to determine the Mg plasma concentrations that produce this effect. Accurate measurement of normal plasma concentrations for both Ca^{2+} and $Mg^{2+}$is an important

first step to establishing reliable threshold concentrations for horses in competition. Since Ca^{2+} and Mg^{2+} are in flux with pCa and pMg and cCa and cMg, both Ca and Mg values may be affected by the collection methods, duration of collection to testing, and storage methods6[6]. Laboratory methods for processing and storage have been established in Standard Operating Procedures (SOP) for individual clinical reference and regulatory laboratories.

We evaluated the impact of collection method, sample processing (shipment and analysis) and long term storage on plasma Mg^{2+}, Ca^{2+}, pH and the Mg^{2+}/Ca^{2+} ratio. Our hypothesis was that there would be no significant difference in Mg^{2+}, Ca^{2+} and Mg^{2+}/Ca^{2+} in samples immediately processed in a clinical reference laboratory and samples collected, processed and stored as per a regulatory laboratory.

Materials and Methods

Horses- A total of 50 healthy horses were included in the study. All horses were part of the university owned teaching and research herd and housed at pasture turnout at a university owned equine research farm. Horses were fed grass and water ad libitum. All horses were vaccinated and dewormed as per USDA standards at least 1 month prior to the study. The horses represented various breeds that included males (26; 24 castrates) and females (24 horses) ranging in age from 2-23 years. (Table 1) An approved IACUC protocol for blood draws and fulfillment of ARRIVE guidelines for the humane use of animals in research were approved and followed. (https://www.nc3rs.org.uk/arrive-guidelines) Included horses had not received any medications for at least one month prior to the blood draws.

Experimental Design- Blood samples (200 samples total) were collected from all horses on the same day and assigned to one of four methods of analysis: Group (Grp) 1- collection in a heparinized syringe and processed within 2-3 hrs in an clinical reference laboratory (standard); Grp 2 – collection into a plastic plasma separator tube [BD Vacutainer® PSTTM Gel and Lithium HeparinN 126 units], centrifuged just prior to analysis, and plasma processed within 2-3 hrs in the same clinical reference laboratory; Grp 3- collection into a plasma separator tube, shipped in a refrigerated state, centrifuged just prior to analysis, and plasma processed within 48 hrs in an regulatory laboratory; and Grp 4- collection into a plasma separator tube, shipped in a refrigerated state, centrifuged, and the plasma separator tube stored at -80˚C for 90 days, thawed and processed 90 days after collection in an regulatory laboratory.

Blood Collection- Blood was collected in one 3cc heparinized syringe (120 units of heparin) by a single venipuncture. Air was evacuated from the syringe and the syringe hermetically sealed. (Grp 1). A separate direct venipuncture was used to collect blood samples into each of three plasma separator tubes for Grps 2, 3, and 4. All samples were immediately placed on ice and transported to a university veterinary clinical pathology laboratory for processing or storage.

Processing and Analysis- The syringe containing the heparinized whole blood sample (Grp 1) was analyzed for Ca^{2+}, Mg^{2+}, and pH by a pHOx Ultra Analyzer by Nova Biomedical (Waltham, MA, USA) in the clinical reference laboratory. The analyzer was maintained and calibrated according to manufacturer's recommendation, which included daily calibration and multiple internal levels of QC using manufacturer provided Auto-

Cartridge QC. One plasma separator tube (Grp 2) was centrifuged for 8 min (3800g) in an Allegra X-30 (Beckman-Coulter, Brea, Ca), and subsequently plasma was aspirated and analyzed using the same equipment, in the same clinical reference laboratory. The remaining two plasma separator tubes (Grps 3,4) were refrigerated overnight and shipped the following day for overnight delivery in a refrigerated state to the regulatory laboratory (United States Equestrian Federation's Equine Drug Testing and Research Laboratory, Lexington, Kentucky). Upon arrival, one plasma separator tube (Grp 3) was centrifuged for 10 mins (1300g) in a ThermoFischer CL-30 (Thermo Fisher Scientific, Waltham, MA) and the plasma analyzed for Ca^{2+}, Mg^{2+} and pH by a pHOx Ultra Analyzer by Nova Biomedical (Waltham, MA, USA). The analyzer was maintained and calibrated according to manufacturer's recommendation, which included daily calibration and multiple internal levels of QC using manufacturer provided Auto-Cartridge QC. The remaining plasma separator tube (Grp 4) was centrifuged similarly and the plasma placed in storage at -80 ˚C within 48 hours of collection. This plasma separator tube was removed from storage on day 90, thawed and plasma analyzed using the equipment as for Grp 3.

Statistical Analysis

Raw data from all four groups were analyzed using commercially available software (SPSS®, IBM). Raw data were graphed and all variables were checked for normality using the Shapiro-Wilk test. Data not normally distributed was log-transformed to achieve the residual distribution in close agreement with a normal distribution. The coefficient of variation (standard deviation/mean x 100) was calculated for each group for

each variable. Data for all variables were compared among groups using a linear mixed model analysis of variance using ANOVA. Significance was set at $P < 0.05$.

Results

Blood samples (50/Grp; 200 samples) from all horses (50) completed the study as assigned.

Horses- There was no significant effect of gender or age on any of the outcome assessments, including pH, Ca^{2+}, Mg^{2+}, or the Mg^{2+}/Ca^{2+} ratio. Breed distribution was varied but representative of adult horse populations. (Table 1.)

Table 1. Frequency of horse breed by age range and gender.

a. Breeds with more than on horse enrolled

			Gender		
Age range (years)	Breed	Number	Male Castrate	Female	Male
0-5	QH	1	1		
	TB	-			
	SB	1		1	
	WB	1	1		
	Trakehner	-			
	Appaloosa	-			
6-10	QH	7	1	6	
	TB	1	1		
	SB	2		2	
	WB	2	2		
	Trakehner	-			
	Appaloosa	-			
11-15	QH	3	1	1	1
	TB	3	2	1	
	SB	5		5	
	WB	7	4	3	
	Trakehner	2	2		
	Appaloosa	1		1	
16-20	QH	1		1	
	TB	4	2	2	
	SB	-			
	WB	-			
	Trakehner	-			
	Appaloosa	1	1		
20+	QH	-			
	TB	1	1		
	SB	3	2		1
	WB	-			
	Trakehner	-			
	Appaloosa	-			

Continued

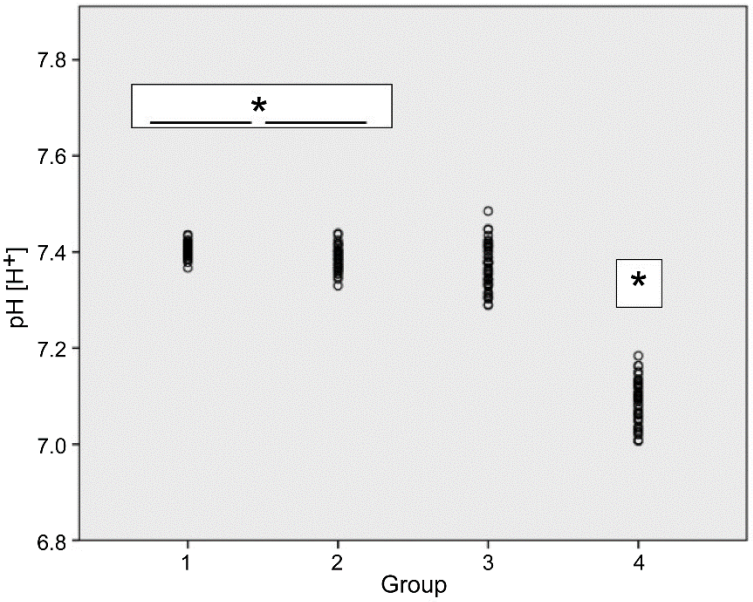
Table 1. Continued

QH—Quarter Horse
TB—Thoroughbred
SB—Standardbred
WB—Warmblood (e.g., Hanoverian, Holsteiner, Oldenburg)

b. Breeds with one horse enrolled.

Breed	Age (years)	Number	Gender
Arabian	13	1	Mare
American Saddlebred	14	1	Gelding
Rocky Mountain Horse	10	1	Gelding
Tennessee Walking Horse	19	1	Gelding

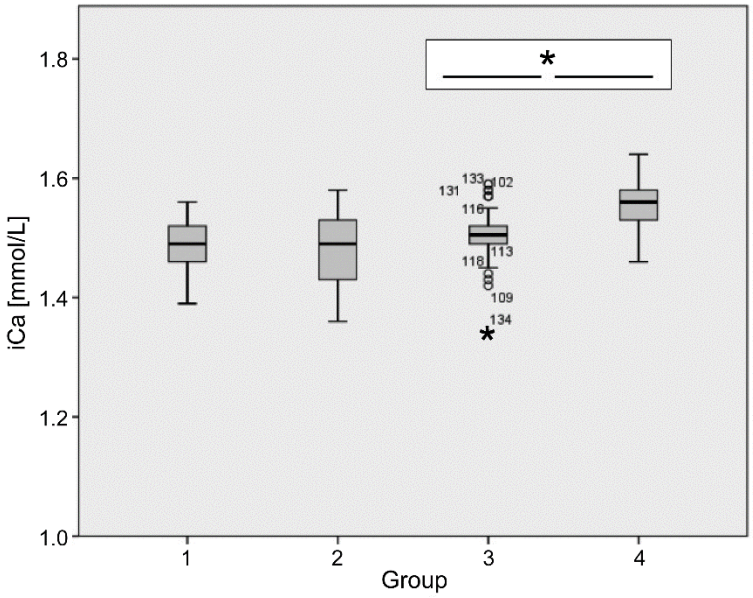
Analyses – Raw data of pH, Ca^{2+}, Mg^{2+} and Mg^{2+}/Ca^{2+} ratio are illustrated in Figs 1-4. Mean plasma pH differed among groups, with Gp 1 having the greatest pH (7.405), significantly greater than Gp 2 pH (7.387) ($P<0.037$), and Gp 3 pH (7.370) ($P<0.001$). The pH of Gp 2 and 3 did not differ due to a greater coefficient of variation in Gp 3 (CV =0.61%) than Gp 2 (CV =0.31%). The mean pH of Gp 4 (7.084) was significantly decreased compared to all other groups ($P<0.001$) and had a greater coefficient of variation (CV=0.63%) than other groups. In general, the CV significantly increased as group number increased and is demonstrated in the scatter plot. ($P<0.001$; Fig 1.) Subsequent freezing for 90 days and thawing prior to analysis significantly decreased pH a mean of 0.3 units below physiologic pH.



Group 1, 3cc heparinized syringe with blood analyzed on day of collection at clinical laboratory, Group 2, plasma separator tube and plasma analyzed on day of collection at clinical laboratory, Group 3, plasma separator tube refrigerated overnight and shipped to regulatory laboratory and plasma analyzed on day 2, and Group 4, plasma separator tube refrigerated overnight and shipped to regulatory laboratory and plasma frozen then thawed and plasma analyzed on day 90. * Differs from other groups (P<0.001), ___*___ Differs between groups (P<0.037).

Figure 1. Scatterplot of plasma pH in the four groups.

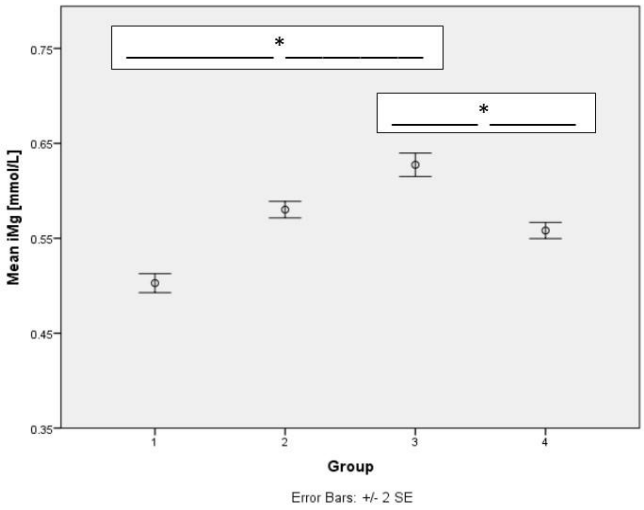
Plasma Ca^{2+} was not normally distributed for Gp 3 and, as noted in the boxplot, this appeared as multiple outliers creating a biphasic distribution with a few samples tending to be low or high (Fig. 2). Mean Ca^{2+} for data transformed to log10 demonstrated no significant difference among Gp 1 (1.480 mmol/L), Gp 2 (1.477 mmol/L) and Gp 3 (1.506 mmol/L). However, mean of Gp 4 (1.56 mmol/L) was significantly increased as compared to Gp 3 (Fig 2; P<0.001). Ca^{2+} was modestly variable (mean CV 3.03% for all groups) regardless of methods of processing.



Boxplot of raw data for plasma ionized calcium (Ca^{2+}) showing within group variability and lack of normal distribution for Gp 3. The box indicates the interquartile range (25–75%), and the line in the box denotes the median value. Statistical analysis with log transformation of data demonstrated a significant difference between Gp 3 and Gp 4. ___*___ ;P<0.001), Groups are the same as described in Figure 1.

Figure 2. Boxplot of raw data for plasma ionized calcium (Ca^{2+})

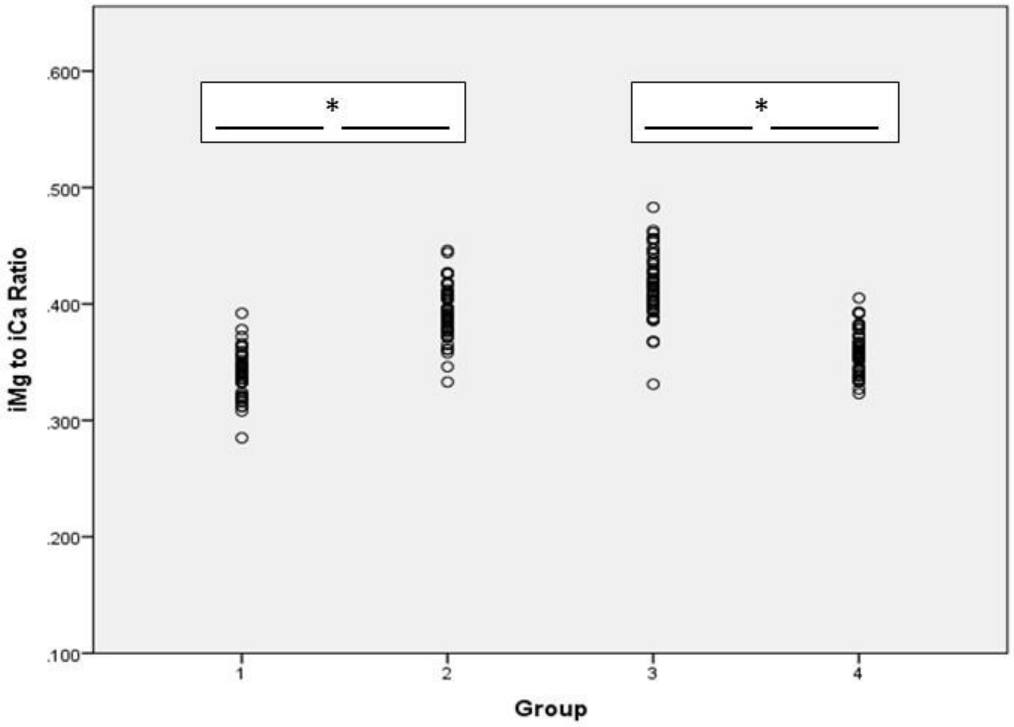
Mean plasma Mg^{2+} significantly differed among groups (P<0.001) with the Mg^{2+} increasing as group number increased; Gp1 (0.503 mmol/L), Gp 2 (0.580 mmol/L), Gp3 (0.627 mmol/L), except for Gp 4 (0.558 mmol/L) which was significantly decreased compared to Gp 3 (Fig. 3). Mean Mg^{2+} CV was 6.18% and did not differ among groups. These data demonstrated that Mg^{2+} increased with the use of the plasma separator tube, shipping, and storage. Subsequent freezing of the plasma for 90 days and thawing prior to analysis significantly decreased the Mg^{2+} compared to the sample analyzed just before freezing (Gp 3).



Mean [±SEM] of plasma ionized magnesium (Mg^{2+}) in the four groups; Ionized Mg increased as group number increased (P<0.001) except Group 4 which was significantly decreased to Group 3. (P<0.001) Groups are the same as described in Figure 1. ___*___ Differs between Groups.

Figure 3. Mean [±SEM] of plasma ionized magnesium (Mg^{2+})

Mean plasma Mg^{2+}/Ca^{2+} ratio was significantly different among all groups (Gp 1-4; 0.339, 0.393, 0.417, and 0.358, respectively), increasing as group number increased except for Gp 4 that was significantly decreased to Gp 3. (Fig. 4). Results for Mg^{2+}/Ca^{2+} was highly correlated with Mg^{2+} pH (r=-.933; P<0.01). These data demonstrated that Mg^{2+}/Ca^{2+} ratio increased with use of the plasma separator tube and shipping, and decreased with freezing mimicking the change in Mg^{2+}.



Ratio of the plasma ionized magnesium to ionized calcium in the four groups; Groups did not differ except Group 4 which was significantly decreased to Group 3. ___*___ Differs between Groups. (P<0.01) Groups are the same as described in Figure 1. The data for the Mg^{2+} (Fig 3) mimics the pattern seen with this Mg^{2+}/Ca^{2+} ratio.

Figure 4. Ratio of the plasma ionized magnesium to the ionized calcium in the four groups

Discussion

Our evaluation of Ca^{2+} and Mg^{2+} is directly relevant to the administration of $MgSO_4$ to performance horses. It is well known that even normal blood levels of Mg^{2+} and Ca^{2+} and their ratio are closely associated with each other and have important physiologic effects[11]. Our study is the first to report the comparison of pH, Ca^{2+}, Mg^{2+}

and Mg^{2+}/Ca^{2+} in horses using different collection methods that included the transportation of samples, and the effects of long-term frozen storage. Our methods were different than previous experiments[12,13] but consistent with the collection and analysis of post competition blood samples utilized for regulatory control of forbidden substances and prohibited methods. Our data demonstrated that early assessment and use of the plasma separator tube, centrifugation to isolate the plasma, and shipping, minimally and insignificantly affected Ca^{2+}, but did significantly decrease pH and increase Mg^{2+} and Mg^{2+}/Ca^{2+}. Freezing of samples significantly further decreased pH and increased Ca^{2+}, but significantly decreased Mg^{2+} and Mg^{2+}/Ca^{2+}.

While the collection of blood samples utilizing a heparinized syringe and the immediate analysis of samples is ideal, these are not possible in all circumstances. It is important to consider the influence of sample collection, short-term storage prior to shipping, shipping of samples in refrigerated states and the stability of samples following long-term frozen storage before appropriate regulatory thresholds can be determined. The consideration of long-term frozen storage is necessary as the adjudication of infractions can involve the confirmatory analysis of a sample that was collected at the same time as the original sample under the same circumstances but analyzed at a much later date. The original sample is commonly referred to as the A sample and analyzed by normal SOP upon arrival at an equine drug-testing laboratory. The additional sample is considered the B sample and is retained undisturbed unless it is necessary to confirm the results of the A sample. Depending upon the jurisdiction or regulatory body, this period can vary between 15 days and 60-90 days.

The Mg^{2+}/Ca^{2+} is a unit-less measure reflective of the co-dependent changes in Ca^{2+} and Mg^{2+}. Different laboratories report the Ca^{2+} and Mg^{2+} as either mg/dl or mmol/L making it difficult to directly compare values among laboratories but the ratio remains unit-less and therefore, can be comparable. Unpublished pilot studies from our team suggested that intravenous administration of $MgSO_4$ increased the Mg^{2+} to Ca^{2+} ratio, therefore, understanding the variation in Ca^{2+} and Mg^{2+} and their relationship with respect to the alterations in pH following long-term storage is critical to developing regulatory thresholds. It has been reported that pH influences the measurement of Ca^{2+}[14] and that changes in pH are more impactful on measured levels of Ca^{2+} than Mg^{2+}[12]. Our data demonstrated a relationship of the collection method (heparinized syringe in whole blood vs the plasma separator tube (Gp 1 vs Gp 2,) with significant differences in Mg^{2+} and pH. Due to the impact of pH on ionized values these variables were interrelated. The pH effect on the binding of Ca and Mg to albumin and protein[15,16] has likely impacted the changes we observed and mirrored the changes in pH. Point of care devices to date for blood analyses cannot yet measure the ionized component of Mg^{2+} [Abbott i-Stat®]. The heparinized syringe served as the gold standard in our study since it is the most common method employed to obtain blood samples for the determination of Ca^{2+} and Mg^{2+} and since the sample was not subjected to any substantive storage or shipping process[17]. Samples collected into a plasma separator tube and shipped overnight provided baseline values for most regulatory purposes as it is consistent with the processes commonly utilized to regulate the use of forbidden substances.

Our study demonstrated that the most consistent and relevant changes were between Gp 3 and Gp 4, and suggested that increases in Ca^{2+} and Mg^{2+} were associated with a decrease in pH. Both Ca^{2+} and Mg^{2+} concentrations increased as the pH in the specimen decreased, indicating the decreased binding of these ions with proteins in the more acidic environment[14]. In addition, our data permitted the comparison Ca^{2+}, Mg^{2+}, Mg^{2+}/Ca^{2+}, and pH values associated with freezing and storage to non-stored blood samples thereby identifying differences associated with the B sample compared to the A sample. Gp 3 and Gp 4 samples were handled consistent with the typical processing and long-term storage of a drug testing regulatory laboratory.

Changes in pH are known to be impacted by centrifugation, storage time and storage temperature[18-21]. In our study, the impact of storage and shipping increased the variability of pH measurements in groups 3 and 4, and produced a significant decrease in pH in Gp 4. Potential reasons for pH changes with our processing and storage include an accumulation of CO_2 within the plasma. We did not determine CO_2 in the samples that were transported (Gp 3, 4) but continued anaerobic metabolism may have resulted in CO_2 production from the red blood cells that would be more slowly separated in the plasma separator tube without centrifugation of the blood sample[13]. Increased CO_2 in conjunction with the lysis of red blood cells (intracellular pH approximately 7.0) can lead to a decrease in pH[22,23]. As shipping and refrigeration time increased, the variation in pH increased and the decrease in pH for group 4 was significantly different from groups 1 and 2. The more marked drop in pH recorded at 90 days of freezing is also likely due to the potential leak of red and white blood cell contents through the separator gel[24].

Regardless, the frozen sample for 90 days had a significantly different pH, Mg^{2+}, Ca^{2+} and Mg^{2+}/Ca^{2+} ratio. The similarity in the pattern of change in the Mg^{2+} and the Mg^{2+}/Ca^{2+} ratio as group number increased (Gp1-Gp4), and the more variable and generally non-significant differences in Ca^{2+}, suggests Mg^{2+} is a dominant and more reliable indicator of blood sample and plasma changes with collection, processing and frozen storage of samples.

Based on our findings to date, the consistency in these changes with processing and storage suggest that $Mg^{2+}$and the Mg^{2+}/Ca^{2+} ratio may serve as a biomarker for exogenous administration of magnesium in competition horses. The finding that the Mg^{2+} and Mg^{2+}/Ca^{2+} both increase in a similar manner with processing and then both decrease with freezing and storage, combined with the anticipated increase in iMg after administration of $MgSO_4$, support the use of the Mg^{2+}/Ca^{2+} ratio as the measurement of choice in the development of regulatory thresholds. Notably, the decrease in Mg^{2+} and Mg^{2+}/Ca^{2+} in B samples found with freezing and storage in Gp 4 samples are below values in Gp 3 samples. Therefore, any changes in Mg^{2+}/Ca^{2+} ratio associated with freezing would error in favor of the competitor. Threshold values for the A sample (Gp 3) should be established and used to call positives. Further studies to determine appropriate adjustments in Mg^{2+} and Mg^{2+}/Ca^{2+} in stored samples obtained from competition horses and horses administered $MgSO_4$ may be necessary to further validate these findings. Additional studies should include attempts to identify interlaboratory differences in analysis. Our data could be used to provide regulatory baseline values for Ca^{2+}, Mg^{2+} and Mg^{2+}/Ca^{2+} using both 95/95[25,26] methodology and reporting the mean

±SDs. Utilizing these standard regulatory methodologies, and the conservative k factor of 3 for 95/95 determination, the baseline values (95/95, mean ±SD) were for Ca^{2+} (1.651; 1.506 ±0.0458 mmol/L), for Mg^{2+} (0.774; 0.627 ±0.0438 mmol/L) and Mg^{2+}/Ca^{2+} (0.511; 0.417 ±0.0283 mmol/L). Prior publications in equine medicine have reported the inverse ratio (Ca^{2+}/Mg^{2+}) for clinical use. Using the data from this study, Ca^{2+}/Mg^{2+} baseline values (95/95, mean ±SD) were (1.957; and 2.41 ±0.169). This is the first report and analysis of normal Ca^{2+}, Mg^{2+}, and Mg^{2+}/Ca^{2+}, in a population of healthy horses using regulatory methodology. Additionally, in a field population study of 57 client-owned horses and using similar sample handling as in Gp 3 (regulatory methodology), of this study, the Ca^{2+}/Mg^{2+}mean and ±SD was calculated (1.991; and 2.41 ± 0.150) and essentially the same as in this study.

Conclusion

Our study is relevant to clinical pharmacology and regulatory medicine in general because of the potential for many laboratories to vary storage times or processing methods. This may be practically relevant to clinicians, scientists and regulatory bodies that may be limited to the use of plasma separator tubes, which are required to ship or store blood samples. Clinical applications often faced with these conditions include pre-purchase examination blood collection and sample holding as buyers determine if they wish to send the sample for testing. In clinical practice, blood samples might need to be collected in fields or barns and stored prior to analysis at laboratories. In conclusion, these data suggest that the Mg^{2+}/Ca^{2+} mirrors alterations in Mg^{2+} regardless of storage

and collection methods. This understanding can facilitate the development of a regulatory threshold for the control of the nefarious use of magnesium sulfate in competing horses, and an understanding of potential changes to Mg^{2+}/Ca^{2+} with storage of B samples.

Chapter 3. Pharmacokinetics and pharmacodynamics associated with experientally induced hypermagnesemia in horses

Abstract

The objectives of this study were to describe the pharmacokinetic and pharmacodynamic changes as a result of the intravenous administration of a single bolus of magnesium sulfate ($MgSO_4$). $MgSO_4$ is a magnesium salt that has been used to calm horses in equestrian competition and is difficult to regulate because magnesium is an essential constituent of all mammals. Six healthy adult female horses were administered a single bolus of $MgSO_4$ at a dose of 60 mg/kg of body weight. Blood, urine, and cerebrospinal fluid (CSF) samples were collected, and cardiovascular parameters were monitored and echocardiograms performed at predetermined times. Noncompartmental pharmacokinetic analysis was applied to plasma concentrations of ionized magnesium (Mg^{2+}). Objective data were analyzed using the Wilcoxon Rank-Sum test with $P < 0.05$ used as a determination for significance. Plasma concentrations of Mg^{2+} increased nearly 5-fold, ionized calcium (Ca^{2+}) decreased by nearly 10%, and the Ca^{2+} to Mg^{2+} ratio declined more than 3.5-fold and remained different than baseline until 24 hours ($p < 0.05$). Significant changes were seen with urinary fractional excretion of electrolytes, cardiovascular parameters and echocardiographic measurements. No changes were detected in CSF electrolyte concentrations. Alterations detected in plasma electrolyte

concentrations, fractional excretion and hormone concentrations may serve as biomarkers for regulatory control for the nefarious administration of $MgSO_4$.

Introduction

In equestrian pursuits, intravenous magnesium sulfate ($MgSO_4$) has been used to calm horses with the goal to make them perform better in the subjective environment of the show ring[9,10] and to minimize pre-race excitement in race horses that is counterproductive to performance[27]. Little is known of the effects of intravenous $MgSO_4$ administration on plasma or cerebrospinal fluid (CSF) electrolytes, the urinary fractional excretion of electrolytes, cardiovascular or behavioral variables, as well as endocrine changes related to calcium homeostasis (calcitonin, parathyroid hormone) in the horse; nor the pharmacokinetics of large doses of magnesium (Mg).

In the extracellular compartment, total magnesium (tMg) exists in three different forms: bound to circulating proteins, complexed with weak acids and unbound, free, or ionized (Mg^{2+})[6]. Of these, Mg^{2+} is the active form. The impact of large doses of $MgSO_4$ on the plasma concentrations of Mg^{2+} has not been thoroughly evaluated even though Mg is a physiologically important element. Mg is among the 5 most abundant cations in mammals and is essential to countless enzymatic reactions including energy metabolism, neuronal and cardiac excitation, enzymatic activation, nucleic acid metabolism, protein synthesis, skeletal muscle contraction, and vasomotor tone[1,7]. Unlike calcium (Ca), plasma tMg concentrations are dependent upon the balance of gastrointestinal absorption, renal excretion and its exchange with bone[7,28-31]. It is difficult to assess the Mg status because it is widely distributed throughout all tissues of the body, it is extensively stored

in bone and muscle, and its highly interrelated with Ca[32]. As opposed to Mg, extracellular Ca is tightly controlled by a homeostatic system that includes three organs (intestine, kidney, bone) and three hormones (parathyroid hormone [PTH], calcitonin, and 1,25 dihydroxyvitamin D_3). However, endocrine regulation of Mg balance is weak and poorly understood[2,3]. Depending on its concentrations, Mg can be synergistic or antagonistic to calcium functions.

In human medicine, $MgSO_4$ is used to treat preeclampsia[33,34], before surgical removal of pheochromocytomas[35], to treat asthma[36,37], to treat arrhythmias[38], and to prevent hypoxia-induced injury in cerebral ischemic events[39-41]. Additionally, $MgSO_4$ has been used to treat asthma in horses[42]. Magnesium functions as an N-methyl-D-aspartate (NMDA) antagonist and protects against chronic moderate glutamate receptor stimulation[41]. The mechanism of action for Mg vary from vasodilation, to NMDA receptor antagonism[43]. Recently, $MgSO_4$ has also been explored as a treatment for trigeminal-mediated neuritis in the horse[44]. It is worth noting that $MgSO_4$ was a constituent of Chloropent® (Fort Dodge), one of the first intravenous anesthetic agent for domestic animals, which also contained chloral hydrate and pentobarbital.

The goal of this study was to evaluate pharmacokinetic and pharmacodynamic changes associated with single intravenous bolus of $MgSO_4$ in healthy horses in a controlled environment while restrained in stocks. Physiologic parameters measured included electrolytes (plasma, urine and CSF), cardiovascular variables, behavioral indices, urinary fractional excretion of electrolytes, as well as calcitonin and PTH concentrations. For regulatory purposes, the intent was to identify potential biological

markers serving as indications of nefarious $MgSO_4$ administration. Our hypothesis was that a single bolus administration of $MgSO_4$ would result in an increase in plasma, urine and CSF Mg^{2+}, a change in the Ca^{2+} to Mg^{2+} ratio, perturbations in the fractional excretion of electrolytes, and changes in cardiovascular variables and head height above ground (HHAG).

Materials and Methods

Animals- Six healthy adult female horses (4 - 18 years of age; mean ± SD weight of 509 ± 66 kg) were included in the study. All horses were part of the university-owned teaching and research herd. For the duration of this study, horses were housed in box stalls (3.6 x 3.6 meter) in the Ohio State University Veterinary Medical Center. Horses were fed grass hay and water *ad libitum*. All horses were vaccinated and dewormed as per USDA standards at least 1 month prior to the study. Mares were included to facilitate urine collection. This study was approved by the University Institutional Animal Care and Use Committee and fulfilled ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines) for the humane use of animals in research. Included horses had not received any medications for at least one month prior to the study. Horses were determined to be healthy and free of disease by complete physical examination, hematology, and serum chemistry analysis.

Experimental Design and Drug Administration- Six female horses were acclimated in individual box stalls for a minimum of 3 days prior to restraint in stocks for instrumentation and the collection of baseline samples. Subsequently, observational data were obtained serially over a 24-hour period following $MgSO_4$ administration. Outcome

included hematologic, cardiovascular, urine, and cerebrospinal fluid (CSF) variables, and behavioral indices such as head height. All horses were administered a single intravenous dose of $MgSO_4$ (60 mg/kg) (Magnesium Sulfate 50% solution, Wedgewood Pharmacy, Swedesboro, NJ, USA) over 5 minutes. This dose was chosen, as it is most equivalent to the dosing strategy commonly reported from the equine show industry, which is 60 ml for a typical horse (approximately 30 grams of Mg for a 500 kg horse). Depending on the variable, serial samples or measurements were made. Initially, quite frequently, but ultimately for 24 hours.

Instrumentation

To limit the effects of sedation on horses, a percutaneous, lumbosacral spinal catheter was placed 1 day prior to study start. All other instrumentation occurred on the day of the study without sedation. Horses were instrumented and baseline examinations and samples obtained between day -1 and time 0, prior to the administration of $MgSO_4$.

Lumbosacral catheter placement. The day prior to the experiment, mares were sedated with xylazine (0.4 mg/kg, IV). The lumbosacral area (10 cm × 20 cm) was clipped, aseptically prepared, and blocked with 2% mepivacaine at 1 mL/100 kg body weight (Carbocaine, Zoetis). Following sedation and local analgesia, an 18 Gauge × 15 cm Tuohy needle (Mila International, Florence, KY) was introduced into the lumbosacral subarachnoid space with the bevel facing cranially. A 20 Gauge intrathecal catheter (Mila International) was introduced through the needle, 5 ml of CSF was obtained by syringe, submitted for cytological examination and to measure electrolytes (Na^+, Cl^-, K^+, Ca^{2+} and Mg^{2+}) and one aliquot stored at -70 C. The needle was removed and the catheter was

secured in place with sutures and bandage material. Catheters remained patent in all horses, for the duration of the study.

External jugular catheter placement. The day of the experiment, two 14-guage catheters were placed aseptically; one in each external jugular vein. The right jugular catheter was used solely for the administration of $MgSO_4$, and the left jugular catheter for blood collection. The right jugular catheter was removed after administration of the total dose of $MgSO_4$.

Arterial catheter placement. An 18 Gauge x 2" over the needle catheter was placed aseptically into the transverse facial artery. A fluid filled pressure monitoring line was connected to a pressure monitoring transducer (Edwards Lifesciences TruWave, Irvine, CA). The transducer was suspended with tape at the level of the shoulder, approximately the level of the right atrium, and zeroed opened to room air while connected to a patient monitor (Datascope Mindray, Mahwah, NJ).

Central venous catheter placement. A 16 Gauge x 48" peel away single lumen polyurethane catheter (MILA International) was placed aseptically into the left jugular vein and advanced until the tip was in the right atrium, confirmed by physiologic waveform using the patient monitor.

Urinary catheter placement. A 28 F Foley urinary catheter was placed aseptically through the urethra into the bladder. A connector allowed for intermittent evacuation of urine and the bladder was completely emptied at each time point following collection.

Electrocardiogram (ECG) leads. ECG leads were attached to provide a base apex trace. Cardiac electrical activity was monitored continuously using a patient monitor

(Datascope, Mindray). A temperature probe (Fisher Scientific, Waltham, MA) was placed rectally and maintained in place using a tail wrap.

Head height above ground. A metric measuring tape was affixed to the halter and used to determine the head height.

Sample Collection.

Physical examinations. Temperature, heart rate and respiration rates were all recorded just prior to blood and CSF collections.

Cerebrospinal Fluid. The first samples obtained were CSF collected at the time of catheter placement, at baseline (before $MgSO_4$ administration), and serially (0, 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, and 150 minutes, and at 3, 4, 5, 6, 12 and 24 hours) to evaluate potential changes in CSF electrolyte concentrations following $MgSO_4$ administration. Cerebrospinal fluid was collected under anaerobic conditions to be analyzed for electrolytes and to be stored.

Blood. Blood samples were collected anaerobically using heparinized syringes and immediately transported to the veterinary clinical pathology laboratory for analysis of electrolytes, Ca^{2+} and Mg^{2+}. Additional blood samples were collected and transferred to plain tubes and EDTA tubes; plain tubes were allowed to clot for 1 h, centrifuged at 1000 g for 5 min at 4 °C. Serum and plasma samples were aliquoted and stored at -80°C for endocrine analysis. Blood samples were collected prior to $MgSO_4$ administration, and at same time as CSF collection.

Blood pressure and ECG monitoring. All invasive arterial and central venous pressure readings were obtained with a patient monitor and recorded on day 0, prior to $MgSO_4$

administration, and at 0, 2, 5, 8, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min post administration.

Urine. Urine was collected with a 60 cc catheter tip syringe connected to the Foley catheter, prior to $MgSO_4$ administration (time 0), and at 5, 10, 30, 60 min, and 2, 3, 4, 5 and 6 hours. Urine was analyzed for osmolality, electrolyte and creatinine concentrations. The fractional excretion of electrolytes was calculated as ([Ux/Sx]/[Ucr/Scr])×100, where U=urine, S=serum, x=each electrolyte, and cr=creatinine concentration. Results are expressed as a fraction (%) of the urinary excretion of creatinine.

Echocardiogram. Echocardiographic studies were performed by one of the investigators (BAS) using a dedicated echocardiographic system (General Electric Vivid 7 Dimension with EchoPAC software package, version BT09, GE Medical Systems, Waukesha, WI, USA) and a phased array cardiac transducer with a nominal frequency of 3 MHz. Echocardiographic recordings were made with a simultaneous base-apex ECG and all raw data were captured for off-line analysis at a digital workstation.

Processing and Analysis

Cerebrospinal fluid. CSF samples were analyzed for pH, pCO_2, Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}, glucose, lactate, Ca^{2+}/Mg^{2+}, HCO_3, and osmolality (Nova pHox Ultra Analyzer, Nova Biomedical, Waltham, MA, USA) in an accredited veterinary clinical pathology laboratory (The Ohio State University). The analyzer was maintained and calibrated according to manufacturer's recommendation, which included daily calibration and multiple internal levels of Quality Control.

Blood. The syringe containing the heparinized whole blood sample was analyzed for pH, pCO_2, Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}, glucose, lactate, Ca^{2+}/Mg^{2+}, HCO_3, and osmolality (pHOx Ultra Analyzer).

Pharmacokinetic analysis. All plasma concentrations for Mg^{2+} were converted to mmol/L, and total dose was converted from milligrams elemental Mg^{2+} and then to mmol/L. Prior to pharmacokinetic analysis, plasma concentrations were normalized by subtracting the baseline (T=0) concentrations of Mg^{2+} from the post administration values from each horse. Pharmacokinetic parameters were calculated by a commercially available pharmacokinetic software (Phoenix WinNonlin, Version 8.0, Pharsight Corporation, Cary, NC, USA) using noncompartmental analysis. The determination of the elimination rate constant (k_{el}) for Mg^{2+} was determined from the slope of the terminal portion of the plasma concentration x time curve. To determine terminal half-life (HL_Lamda_z), the natural log of 2 (ln 2) was divided by λz. The maximum plasma concentration (C_{max}) and the time to maximum concentration for Mg^{++} were directly determined from the plasma concentration of Mg^{2+}. The area under the curve (AUC_{last}) was calculated using the log-linear trapezoidal rule.

Urine. The collected urine was frozen (-80C) and analyzed at a later time. Urine creatinine and electrolytes were measured using an automated system (Cobas C501, Roche Diagnostics, Indianapolis, IN) within 1 week of collection.

Echocardiogram. Echocardiographic examinations were conducted on day 0 of the experiment prior to $MgSO_4$ administration (baseline) and at 20 minutes following the start of the administration. Standard imaging planes[45] were acquired from the right

parasternal imaging window with the horses in a standing position. All of the following measurements were performed in triplicate for each variable at each time point from at least three separate cardiac cycles and the average value utilized for analysis. Linear measurements of left ventricular internal diameter in diastole (LVIDd) and systole (LVIDs) were obtained from M-mode recordings of the left ventricle acquired in a short-axis imaging plane bisecting the left ventricular papillary muscles. The left ventricular percent fractional shortening (%FS) was calculated using the formula: %FS = 100 x (LVIDd – LVIDs) / LVIDd. Linear measurements of left atrial size (LA) were obtained at the end of ventricular systole, just prior to mitral valve opening from the long-axis imaging plane with a line drawn to bisect the middle of the left atrium, parallel to the mitral annulus, from the blood-tissue interface of the interatrial septum to the posterior left atrial wall. Left ventricular volumes were estimated from the long-axis imaging plane using a single plane Simpson's method of disks by tracing the endocardial border of the left ventricle at both end-diastole (EDV), defined as the R wave on the ECG, and end-systole (ESV), defined as the frame prior to mitral valve opening. Left ventricular stroke volume (SV) was derived from these measurements as SV = EDV – ESV. Ejection fraction was derived from the measurement of SV / EDV and expressed as a percentage. Heart rate was measured from the simultaneous ECG and cardiac output (CO) was then calculated as CO = SV x HR. Pulsed wave tissue Doppler imaging was used to obtain a spectral Doppler measurement of the velocity of systolic contraction (S') from the left ventricular free wall in a short-axis imaging plane[46]. Left ventricular systolic function was therefore estimated in three separate ways: %FS, CO, and S'[45]

Calcitonin and Parathyroid Hormone (PTH). Calcitonin concentrations were determined by batch analysis using a commercial human-specific calcitonin radioimmunoassay (Calcitonin Radioimmunoassay, Beckman Coulter, Webster, TX) previously validated for horses[47]. Parathyroid hormone (PTH) concentrations were determined by batch analysis using a human-specific immunoradiometric assay (Scantibodies Laboratory, Santee, CA, USA), previously validated for horses[48].

Statistical Analysis

Data were analyzed across time for each variable using commercially available software (SPSS®, IBM). Raw data were graphed and the non-parametric Wilcoxon-Rank sum test was used to determine the difference from baseline for each time point. The coefficient of variation (standard deviation/mean x 100) was calculated for each group for each variable. Significance was set at $P < 0.05$.

Results

Results of plasma chemistry are reported in Table 3.1. Pharmacokinetic parameters are reported in Table 2. Data for the urine electrolytes are reported in Table 3. and for fractional excretion of electrolytes are reported in Table 4. Data from arterial pressure variables are reported in Table 5. Data from echocardiography variables are reported in Table 6. Most hematological variables that were significantly different were not outside of their respective reference ranges.

CSF. No site complications occurred and no CSF cytological abnormalities were identified during or after the study. There were no statistically significant changes in the electrolytes analyzed in the CSF (Appendix A.).

Plasma electrolytes—Mg^{2+} increased approximately 5-fold ($p<0.05$) within 5 min and rapidly fell to near 1.8-fold by 60 min and slowly returned to baseline by 24 hours. Following the initial rise in Mg^{2+}, Ca^{2+} began to decline at 20 min and reached its lowest point at 45 min. Subsequently, Ca^{2+} returned to baseline value at 330 min but continued to increase above baseline through 24 hours. Plasma Mg^{2+} and Ca^{2+}, and their ratio (Ca^{2+}/Mg^{2+}), significantly and dramatically changed within the first thirty minutes following the administration of $MgSO_4$ and steadily returned toward baseline in the patterns demonstrated in (Fig. 5 a-b). Specifically, Ca^{2+}/Mg^{2+} ratio was generally the mirror image of Mg^{2+} values and reflected the dominant changes in Mg^{2+}. The Ca^{2+}/Mg^{2+} ratio, similarly to Mg^{2+}, returned to baseline in 24 hours.

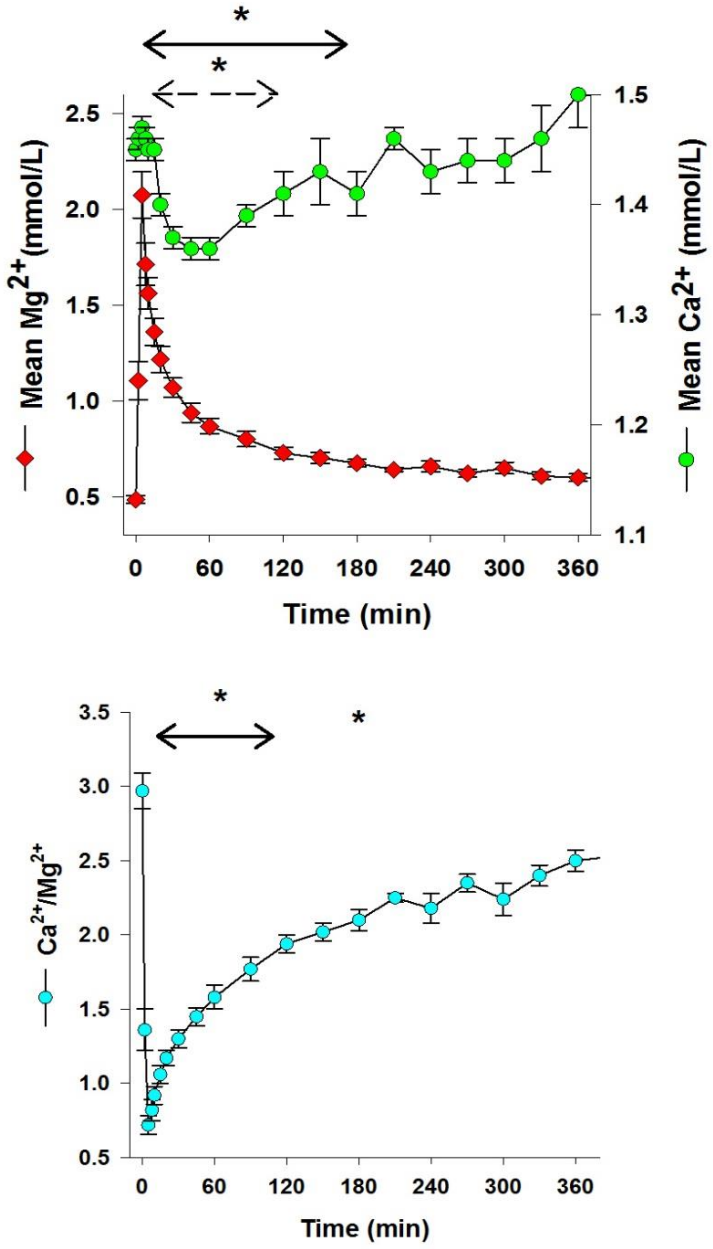


Figure 5. (a) Plot of plasma Mg^{2+} and Ca^{2+}; (b) plot of Ca^{2+} to Mg^{2+} ratio.

Table 2. Plasma electrolytes in healthy horses following MgSO4 administration. Values presented as mean ±SE.

	0 min	2 min	5 min	8 min	10 min	15 min	20 min	30 min	45 min	60 min	90 min
pH	7.491 ± 0.0062	7.402 ± 0.0088	7.397 ±0.0082	7.400 ± 0.0069	7.400 ± 0.0068	7.405 ± 0.0092	7.406 ± 0.0070	7.412 ± 0.0067	7.414 ± 0.0059	7.418 ± 0.0042	7.414 ± 0.0032
pCO2 (mmHg)	39.3 ± 0.99	41.4 ± 0.50	41.8 ± 0.48	41.8 ± 0.88*	41.4 ± 0.44†	42.0 ± 0.34	41.2 ± 0.64	41.5 ± 0.38†	40.9 ± 0.89	41.7 ± 1.43	42.0 ± 1.59
Na^+ (mmol/L)	137.5 ± 0.40	137.0 ± 0.47	136.5 ± 0.48*	136.7 ± 0.45*	137.0 ± 0.47*	136.8 ± 0.45*	137.0 ± 0.45†	137.1 ± 0.61	137.2 ± 0.42	136.6 ± 0.57*	136.8 ± 0.68†
K^+ (mmol/L)	3.60 ± 0.25	3.32 ± 0.08	3.32 ± 0.08	3.30 ± 0.06	3.28 ± 0.08	3.33 ± 0.04	3.42 ± 0.05	3.38 ± 0.08	3.29 ± 0.07	3.28 ± 0.08	3.33 ± 0.08
Cl^- (mmol/L)	104.0 ± 0.57	103.1 ± 0.94	103.3 ± 0.60*	103.1 ± 0.87	102.9 ± 0.74*	102.5 ± 0.59*	102.8 ± 0.67†	102.7 ± 0.71*	102.9 ± 0.69	103.0 ± 0.99	102.8 ± 1.00
Ca^{2+} (mmol/L)	1.45 ± 0.01	1.46 ± 0.01	1.47 ± 0.01	1.46 ± 0.01	1.45 ± 0.01	1.45 ± 0.01	1.40 ± 0.01†	1.37 ± 0.01*	1.36 ± 0.01*	1.36 ± 0.01*	1.39 ± 0.01*
Mg^{2+} (mmol/L)	0.49 ± 0.02	1.11 ± 0.10†	2.08 ± 0.12*	1.71 ± 0.11*	1.56 ± 0.08*	1.36 ± 0.07*	1.22 ± 0.07*	1.07 ± 0.05*	0.94 ± 0.05*	0.87 ± 0.04*	0.80 ± 0.04*
Glucose (mg/dl)	88.5 ± 4.33	89.4 ± 3.88	88.0 ± 5.18	90.7 ± 5.69	91.3 ± 5.42	95.2 ± 5.62†	92.7 ± 5.61†	95.0 ± 6.30†	96.8 ± 6.37*	101.0 ± 7.41*	101.8 ± 6.26*
Ca^{2+}/Mg^{2+} (mol/mol)	2.99 ± 0.12	1.32 ± 0.14*	0.71 ± 0.06*	0.85 ± 0.07*	0.93 ± 0.06*	1.07 ± 0.06*	1.15 ± 0.05*	1.28 ± 0.06*	1.45 ± 0.06*	1.57 ± 0.08*	1.73 ± 0.08*
Osmolality (mOsm/kg)	274.6 ± 0.77	274.0 ± 1.08	272.6 ± 1.09*	273.1 ± 0.98*	273.8 ± 1.07	273.6 ± 0.87	273.8 ± 0.94	274.0 ± 1.53	274.2 ± 0.88	273.5 ± 1.13	273.8 ± 1.31

Continued

Table 2. Continued

	120 min	150 min	180 min	210 min	240 min	270 min	300 min	330 min	360 min	12 hr	24 hr
pH	7.410 ± 0.0066	7.414 ± 0.0067	7.415 ± 0.0046	7.411 ± 0.0039	7.406 ± 0.0067	7.412 ± 0.0064	7.420 ± 0.0066	7.413 ±0.0052	7.412 ± 0.0045	7.413 ± 0.0158	7.424 ± 0.0094
pCO2 (mmHg)	43.7 ± 2.79	40.6 ± 1.51	39.9 ± 0.71	38.8 ± 0.33	40.8 ± 1.43	39.3 ± 1.29	39.1 ± 0.90	42.3 ± 3.26	37.6 ± 2.39	37.2 ± 1.90	38.6 ± 0.80
Na^+ (mmol/L)	136.4 ± 0.72	136.6 ± 0.72	137.0 ± 0.63	137.7 ± 0.29	138.2 ± 0.49	137.9 ± 0.47	137.2 ± 0.60	137.3 ± 0.37	137.2 ± 0.43	134.8 ± 1.00†	136.3 ± 0.76
K^+ (mmol/L)	3.35 ± 0.12	3.35 ± 0.09	3.31 ± 0.06	3.31 ± 0.12	3.50 ± 0.09	3.50 ± 0.03	3.42 ± 0.06	3.59 ± 0.12	3.60 ± 0.17	4.89 ± 0.10†	4.05 ± 0.10
Cl^- (mmol/L)	103.1 ± 1.20	103.2 ± 0.91	103.5 ± 0.53	103.7 ± 0.27	104.5 ± 0.34	104.2 ± 0.37	104.3 ± 0.40	104.6 ± 0.54	104.0 ± 0.78	104.7 ± 1.74	105.0 ± 1.17
Ca^{2+} (mmol/L)	1.41 ± 0.02†	1.43 ± 0.03	1.41 ± 0.02*	1.46 ± 0.01	1.46 ± 0.01*	1.44 ± 0.02	1.44 ± 0.02	1.46 ± 0.03	1.50 ± 0.03	1.49 ± 0.03	1.53 ± 0.02
Mg^{2+} (mmol/L)	0.73 ± 0.03*	0.70 ± 0.03*	0.67 ± 0.02*	0.64 ± 0.01†	0.66 ± 0.03†	0.62 ± 0.02†	0.65 ± 0.03†	0.61 ± 0.02	0.60 ± 0.02	0.53 ± 0.04	0.50 ± 0.02
Glucose (mg/dl)	103.2 ± 8.05†	106.2 ± 10.71†	103.3 ± 6.56*	107.5 ± 11.89	106.3 ± 12.20	103.0 ± 10.30	104.0 ± 8.27†	97.8 ± 9.50†	104.3 ± 11.49	90.8 ± 6.41	90.8 ± 6.06
Ca^{2+}/Mg^{2+} (mol/mol)	1.94 ± 0.06†	2.03 ± 0.06†	2.09 ± 0.07*	2.28 ± 0.03	2.17 ± 0.10†	2.32 ± 0.06	2.22 ± 0.11†	2.40 ± 0.07	2.50 ± 0.07	2.81 ± 0.17	3.05 ± 0.09
Osmolality (mOsm/kg)	273.2 ± 1.35	273.8 ± 1.56	274.4 ± 1.36	276.0 ± 1.08	276.7 ± 1.28	276.0 ± 1.25	272.8 ± 2.58	274.7 ± 1.10	274.9 ± 1.39	269.9 ± 1.53	272.5 ± 1.09

Values reported as mean ±SE

* Significant ($P \leq 0.05$)

† Near significance ($P \leq 0.07$)

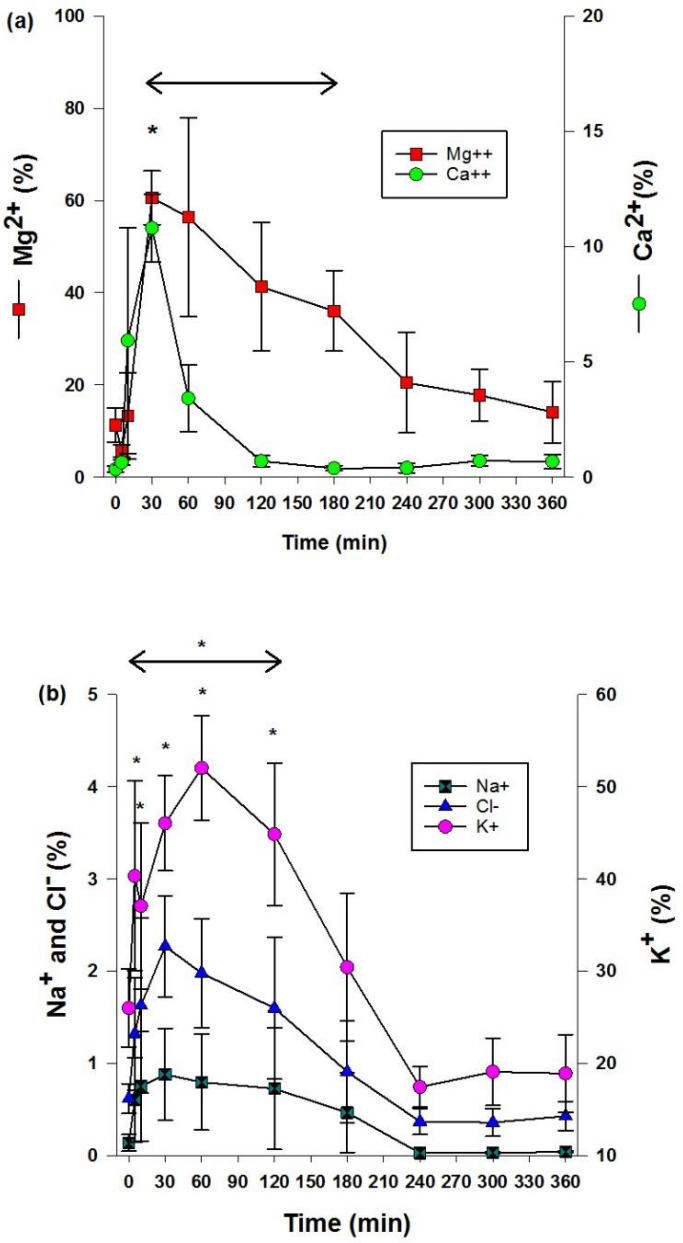
Pharmacokinetics— The selected pharmacokinetic parameters reported were all calculated following the subtraction of the baseline endogenous values for Mg^{2+} from each concentration time point. The noncompartmental analysis revealed an average maximal observed concentration (C_{max}) of 1.43 ± 0.35 mmol/L and average time of maximal observed concentration (T_{max}) of 6.6 ± 2.1 minutes. The observed average volume of distribution (Vz_obs) was 1.58 ± 0.65 L/kg , and average clearance (Cl) of 98 ± 29 L/hr. The terminal half-life (HL_Lambda_z) was 5.5 ± 0.7 hours, and the area under the curve for the plasma concentration time curve, from the time of dosing until the last measured concentration, was 2.77 ± 0.99 hr*mmol/L (Table 3).

Table 3. Pharmacokinetic parameters for plasma ionized Mg^{2+} after intravenous infusion of 60 mg/kg of MgSO4 over 5 minutes to healthy horses.kg of $MgSO_4$ over 5 minutes to healthy horses.

	Horse	1	2	3	4	5	6	Average	STDEV
Lambda_z (K_{el})	1/hr	0.13	0.12	0.12	0.12	0.11	0.16	0.13	0.02
HL_Lambda_z ($t_{1/2}$)	hr	5.23	6.00	5.56	5.77	6.17	4.39	5.52	0.65
T_{max}	min	7.8	4.8	4.8	10.2	7.8	4.8	6.6	2.06
C_{max}	mmol/L	1.3	1.86	1.88	1.21	1.17	1.15	1.43	0.35
AUC_{last}	hr*mmol/L	3.89	3.77	1.45	1.88	3.05	2.56	2.77	0.99
Vz_obs	L	513	518	999	1089	902	667	781	249
Vd	L/Kg	1.01	0.93	2.43	2.33	1.50	1.31	1.58	0.65
Cl_obs	L/hr	68	60	125	131	101	105	98	29

Urine values and fractional excretion—Urine concentration of Mg^{2+} increased nearly 4-fold, by 30 min, peaked by 180 min and but remained higher than baseline through 240 min ($P<0.05$), and then began to decline. Urine Mg^{2+} continued to decrease such that the 360 min value approached a 2-fold baseline corresponding to the decrease

of magnesium in the plasma. The urinary concentration of calcium remained relatively unchanged. Urine creatinine decreased earlier than urine Mg^{2+} and returned to baseline prior to the peak in magnesium concentration. Urine osmolality decreased from baseline to 30-120 min ($P<0.05$), and returned to baseline values by 360 min. Urine Na^{+} concentrations increased within 5 min through 10 min ($P<0.05$), and urine K^{+} decreased within 10 min until 120 min ($P<0.05$), and only approached baseline by 360 min. (Table 4). The urinary fractional excretion of Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, and Cl^{-} increased from 30 to 60 minutes after $MgSO_4$ administration to steadily return to baseline (Figure 6. a-b). The urinary fractional excretion of Mg^{2+} had a nearly 6-fold increase within 30 min to return to baseline by 360 min. Of interest, the fractional excretion of Ca^{2+} increased by 3-fold at 30 min to rapidly decline to baseline values by 180 min. The fractional excretion of K^{+} increased from 26% to 52% at 60 min ($p<0.05$) and returned to baseline level at 180 min; for Na^{+}, the fractional excretion increased from baseline more than 6-fold peaking around 30 min gradually decreasing to below baseline by 240 min; the fractional excretion of Cl^{-} increased more than 3-fold from baseline and peaked at 60 min before gradually returning to less than baseline by 240 min (Table 5).



Significance for Mg^{2+} (⟷) and Ca^{2+} (*); significance for, Cl^{-}(⟷), and K^{+}(*)*(P<0.05)*.

Figure 6. (a) Plot of fractional excretion of Mg2+ and Ca2+; (b) fractional excretion of Na+, Cl-, and K+.

Table 4. Urinary variables and electrolytes in healthy horses following MgSO4 administration.

	0 min	5 min	10 min	30 min	60 min	120 min	180 min	240 min	300 min	360 min
Mg^{2+}	38.1 ± 15.8	49.7 ± 20.0	89.5 ± 21.7	149.5 ± 41.8*	118.3 ± 28.8*	149.7 ± 29.7*	156.6 ± 30.3*	137.3 ± 32.0*	98.0 ± 17.4†	91.5 ± 12.3†
Ca^{2+}	5.9 ± 1.9	6.7 ± 2.2	18.7 ± 7.0*	49.0 ± 9.2*	13.6 ± 5.3	4.9 ± 1.1	5.2 ± 1.4	10.6 ± 6.5	19.9 ± 8.7*	26.5 ± 11.3*
Phosphorous	0.7 ± 0.18	0.4 ± 0.15	0.5 ± 0.18	0.9 ± 0.26	0.2 ± 0.08	0.4 ± 0.14	0.8 ± 0.22	0.3 ± 0.17	1.1 ± 0.68	1.4 ± 0.63
Creatinine	335 ± 62.5	189.0 ± 45.2*	251.6 ± 90.8*	99.1 ± 34.3*	93.3 ± 22.8*	190.1 ± 50.5	330.2 ± 84.5	394.9 ± 99.9	431.7 ± 136.7	606.6 ± 225.2
BUN	1169.8 ± 204.5	656.0 ± 128.4	971.2 ± 359.6	508.2 ± 172.3*	426.4 ± 110.0*	641.6 ± 167.4†	1041.8 ± 263	1201.7 ± 300.2	1201.0 ± 345.4	1461.6 ± 394.5
Na^{+}	37.7 ± 14.4	72.0 ± 27.1*	69.2 ± 15.0*	71.5 ± 20.1	71.4 ± 22.1	59.6 ± 23.8	42.7 ± 20.4	38.5 ± 18.6	38.0 ± 19.7	18.4 ± 1.1
K^{+}	269.2 ± 24.2	197.8 ± 31.2	191.3 ± 38.1*	128.4 ± 24.7*	152.8 ± 33.3*	208.4 ± 35.6	239.4 ± 33.8	232.1 ± 30.7	226.0 ± 34.7	255.2 ± 37.7
Cl^{-}	175.0 ± 21.1	161.5 ± 16.5	158.4 ± 16.1	160.4 ± 18.0	143.1 ± 13.7	147.5 ± 8.3	128.9 ± 7.5	138.1 ± 26.5	131.6 ± 36.1	132.7 ± 48.6
Osmolality	1292.0 ± 111.6	919.0 ± 102.7	997.8 ± 179.9	704.3 ± 101.42*	675.4 ± 74.0*	874.0 ± 112.3*	1093.5 ± 124.5	1136.3 ± 140.56	1173.7 ± 137.9	1369.6 ± 139.0

Values reported as mean ±SE
* Significant ($P \leq 0.05$)
† Near significance ($P \leq 0.07$

Table 5. Urinary fractional excretion of electrolytes $MgSO_4$ administration, expressed as % ±SE

%	0 min	5 min	10 min	30 min	60 min	120 min	180 min	240 min	300 min	360 min
Mg^{2+}	11.3 ± 3.7	5.7 ± 1.4	13.3 ± 9.3	60.6 ± 5.8*	56.4 ± 22.0*	41.3 ± 13.8*	36.1 ± 8.7	20.5 ± 10.9	17.9 ± 5.7	14.1 ± 6.7
Ca^{2+}	0.3 ± 0.1	0.6 ± 0.1	5.9 ± 4.9	10.8 ± 1.5*	3.4 ± 1.5	0.7 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0.71 ± 0.2	0.68 ± 0.3
Na^{+}	0.14 ± 0.09	0.60 ± 0.46	0.75 ± 0.60	0.88 ± 0.50	0.80 ± 0.52	0.73 ± 0.66	0.47 ± 0.43	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.01
K^{+}	26.0 ± 4.2	40.3 ± 10.3*	37.1 ± 9.0*	46.0 ± 5.2*	52.0 ± 5.7*	44.8 ± 7.7*	30.4 ± 8.0	17.4 ± 2.2	19.1 ± 3.7	18.9 ± 4.2
Cl^{-}	0.62 ± 0.16	1.32 ± 0.61*	1.63 ± 0.95*	2.27 ± 0.55*	1.98 ± 0.59*	1.60 ± 0.77*	0.91 ± 0.55	0.37 ± 0.14	0.36 ± 0.15	0.43 ± 0.16

Values reported as mean ±SE
* Significant ($P \leq 0.05$)
† Near significance ($P \leq 0.07$)

Cardiovascular. Mean arterial pressure decreased from 130.3 ± 11.1 to 116.0 ± 10.1 mm/Hg within 5 minutes of the start of $MgSO_4$ administration and remained significantly lower for 45 minutes ($P<0.05$) (Figure 7). Mean arterial pressure did not return to baseline levels for the 6 hours of pressure observation. Systolic arterial pressure decreased from baseline following administration, but did not achieve significance. Diastolic arterial pressure decreased from a baseline of 116.2 ± 4.6 to 98.0 ± 6.8 mm/Hg within 8 min and was significantly different by 10 min ($P<0.05$). Heart rate increased from baseline and trended higher, but failed to achieve significance (Table 6).

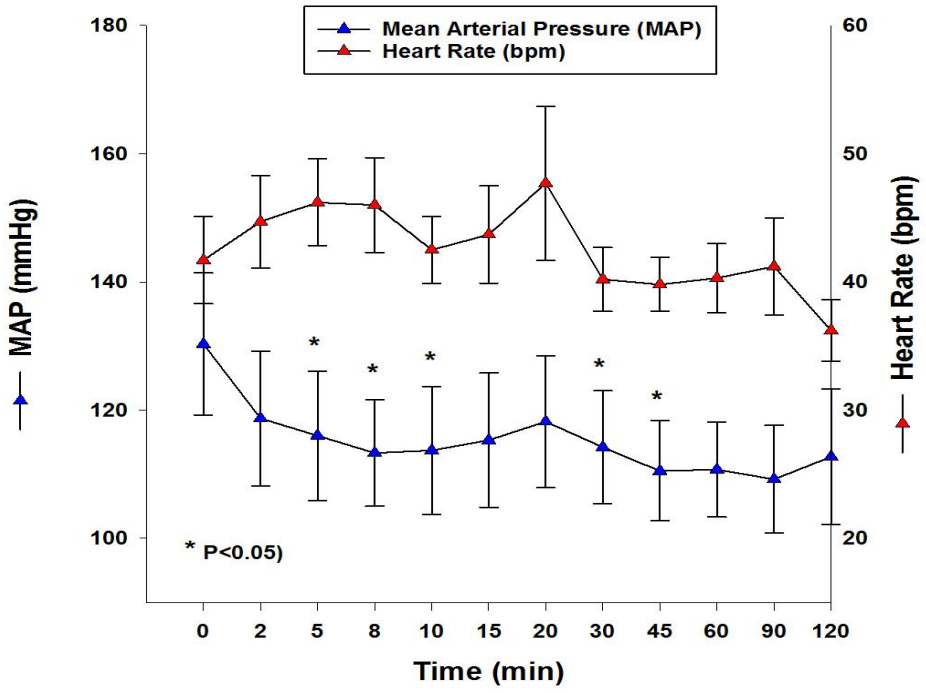


Figure 7. Plot of mean arterial pressure (MAP) and heart rate (HR).

Table 6. Arterial pressures and heart rate in healthy horses following $MgSO_4$ administration.

	0 min	2 min	5 min	8 min	10 min	15 min	20 min	30 min	45 min	60 min
Systolic (mmHg)	171.7 ± 19.0	161.7 ± 15.1	158.0 ± 13.9	158.0 ± 12.8	160.3 ± 16.1	158.3 ± 15.1	160.7 ± 14.1	152.7 ± 13.4	143.8 ± 10.4	147.5 ± 11.7
Diastolic (mmHg)	116.2 ± 4.6	104.0 ± 6.5	85.2 ± 15.7	98.0 ±6.8†	97.7 ± 6.4*	98.3 ± 5.9*	101.7 ± 6.6†	100.0 ± 3.7*	99.7 ± 4.8*	99.8 ± 5.2
Mean Arterial Pressure (mmHg)	130.3 ± 11.1	118.7 ± 10.5†	116.0 ± 10.1*	113.3 ± 8.3*	113.7 ± 10.0*	115.3 ± 10.5*	118.2 ± 10.3†	114.2 ± 8.8*	110.5 ± 7.8*	110.7 ± 7.4
Heart Rate (bpm)	38.5 ± 3.4	44.7 ± 3.6	46.2 ± 3.4	46.0 ± 3.7	42.5 ± 2.6	43.7 ± 3.8	47.7 ± 6.0	40.2 ± 2.5	39.8 ± 2.1	40.3 ± 2.7

Values reported as mean ±SE

* Significant (P ≤0.05)

† Near significance (P ≤0.07)

Echocardiogram. The indices of left ventricular function that significantly increased from baseline to the 20 min post $MgSO_4$ administration, included %FS, S' and %EF (Figure 8a-b). The increase in CO from 28.2 ± 1.7 to 33.0 ± 2.5 L/min approached significance (Table 7).

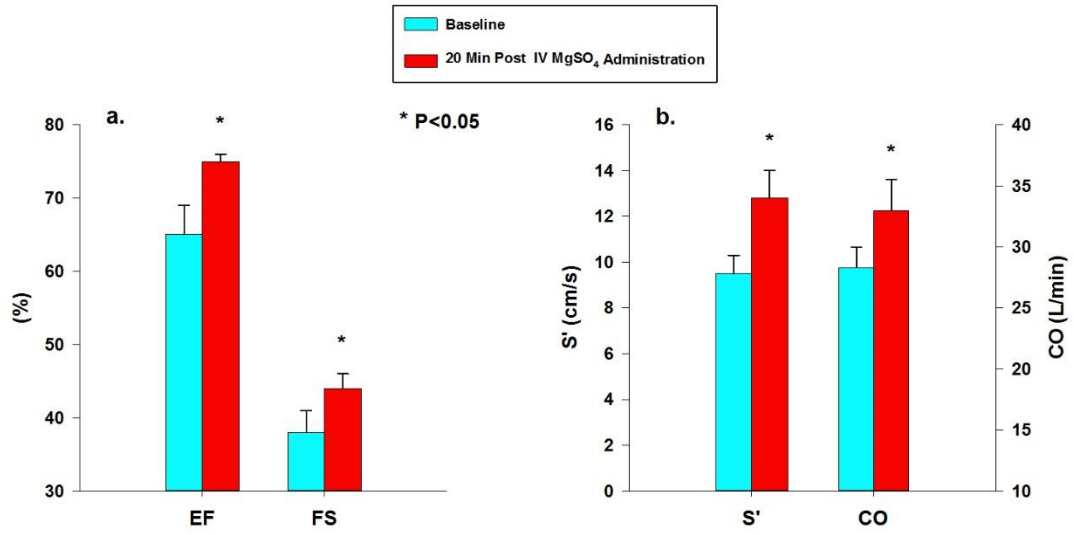


Figure 8. Echocardiographic variables for baseline and 20 min post IV MgSO4 administration plots of (a.) ejection fraction (EF) and fractional shortening (FS); (b.) peak systolic annular velocity (S') and cardiac output (CO).

Table 7. Echocardiographic variables for baseline (Pre) and 20 minutes post (Post) IV administration of MgSO4 to healthy horses.

	Pre	Post
EDV (ml)	1163.2 ± 136.9	1069.2 ± 101.8
ESV (ml)	427.3 ± 101.6	275.2 ± 48.2
%EF	65% ± 4%	75% ± 3%*
SV (ml)	736.3 ± 47.5	794.7 ± 65.9
HR (BPM)	39.2 ± 3.7	42.2 ± 3.3
CO (L/min)	28.2 ± 1.7	33.0 ± 2.5†
S' (cm/s)	9.5 ± 0.8	12.8 ± 1.2*
LVIDd (cm)	10.2 ± 0.4	10.6 ± 0.5
LVIDs (cm)	6.3 ± 0.3	5.9 ± 0.4
%FS	38% ± 1%	44% ± 2%*
LA (cm)	11.3 ± 0.3	11.5 ± 0.4

End diastolic volume (EDV); end systolic volume (ESV); percent ejection fraction (%EF); stroke volume (SV); heart rate (HR); cardiac output (CO); peak systolic annular velocity (S'); left ventricular internal diameter end diastole (LVIDd); left ventricular internal diameter end systole (LVIDs); percent fractional shortening (%FS); left atrial diameter (LA).

* Significant (P ≤0.05)

† Near significance (P ≤0.07)

Parathyroid hormone and Calcitonin. Serum calcitonin mildly increased from 10.4 ± 1.8 pg/ml to 12.7 ± 4.2 by 5 minutes, returning to baseline by 15 min and decreasing to below baseline for the duration. Serum PTH concentrations decreased from 15.7 pg/ml ± 3.6 to less than 0.1 pg/ml by 5 min and then rebounded to a peak level 6-fold of baseline and remained elevated for the duration (Figure 9). The changes in calcitonin mirrored the early rise of Mg^{2+}.

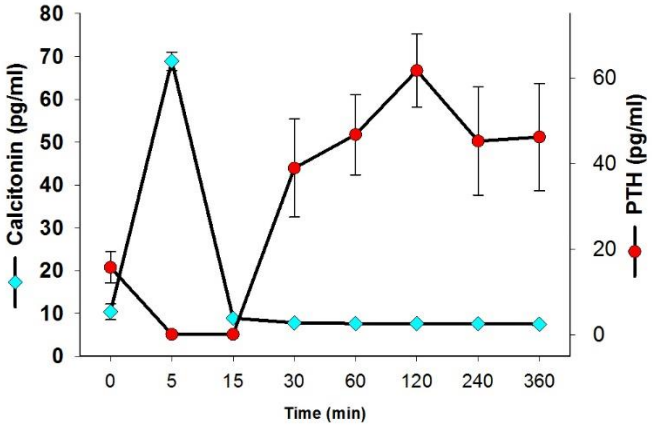


Figure 9. Plasma Calcitonin and parathyroid hormone (PTH) concentrations following IV administration of MgSO4 to healthy horses.

Head height above ground. The change in head height was determined by subtracting recorded measurements from the baseline value for each horse. The head height decreased significantly within 15 min following administration and remained significant until 240 min (Figure 10).

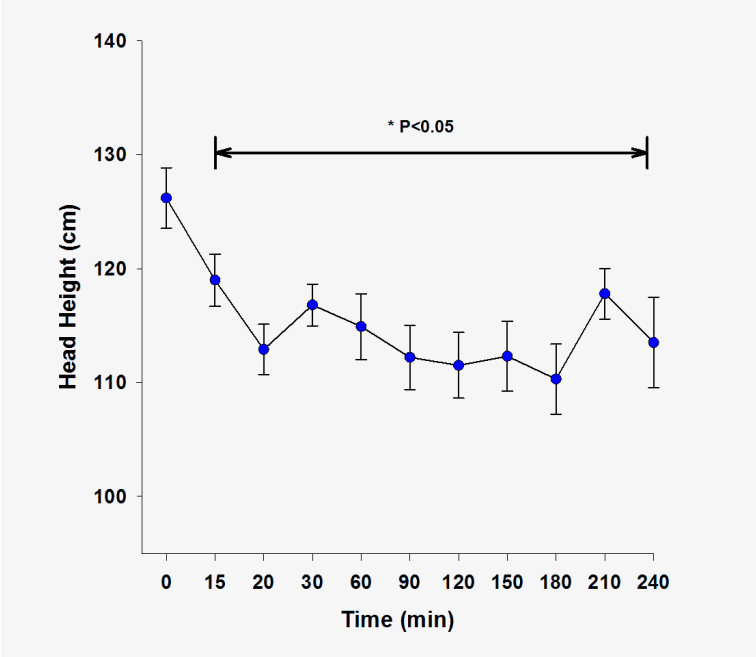


Figure 10. Plot of head height above ground (cm).

Discussion

In the present study, we showed that experimental hypermagnesemia alters the plasma and urinary concentrations of various electrolytes, calcium-regulating hormones, as well as selected cardiovascular variables. To our knowledge, this is the first study to assess the dynamics of magnesium in healthy horses as well as its interactions with other electrolytes. These results demonstrated a rapid and convincing influence of a single intravenous bolus of $MgSO_4$ over a multitude of variables. Considering that magnesium circulates in high concentrations in healthy horses, it can be difficult to regulate its illegal use to enhance performance. Magnesium is regulated mainly through intestinal absorption, renal reabsorption, bone resorption, and shifts from the intracellular compartment, however, its control is not as precise as that of. Our data demonstrate that

four variables may serve as biomarkers for regulatory control of exogenous $MgSO_4$ administration; plasma Mg^{2+}, Ca^{2+}/Mg^{2+}, the fractional excretion of Mg^{2+} and alterations in PTH and calcitonin concentrations.

As anticipated, plasma concentration and the urinary fractional excretion of Mg^{2+} rapidly increased after intravenous $MgSO_4$ administration. Although this reflected exogenous $MgSO_4$ administration, Mg^{2+} alone is less likely to be as predictive as also inclusion of associated plasma changes in Ca^{2+}. The increase in plasma Mg^{2+} coupled with the decrease in Ca^{2+} resulted in a significant decrease in the Ca^{2+}/Mg^{2+} ratio. This ratio mirrored the Mg^{2+} concentration and reflected a broader physiologic response. The ratio of Ca^{2+} to Mg^{2+}, and its inverse, have been suggested as a biological marker for regulatory control of $MgSO_4$[49]. The rapid decrease of the Ca^{2+}/Mg^{2+} ratio to less than one third of the baseline values within 5 min and not returning to near baseline values until 12 hours suggest a robust marker for $MgSO_4$ administration. Horses are able to clear Ca^{2+} faster than Mg^{2+}[47,50]. Alone, the increase in Mg^{2+} was significant, but coupled with a marked change in Ca^{2+}/Mg^{2+}, would further support the detection of $MgSO_4$ administration. Initially, plasma Ca^{2+} concentrations followed a similar pattern of Mg^{2+} concentrations to later on decrease to values below baseline (Fig. 5a). Plasma Ca^{2+} significantly differed from baseline for nearly 120 minutes. The decrease in plasma Ca^{2+} coincided with the increase in fractional excretion of urinary Ca^{2+}.

Changes in serum calcitonin and PTH concentrations occurred rapidly in response to administration of $MgSO_4$. Calcitonin is produced by the parafollicular cells of the thyroid gland in response to hypercalcemia to reduce extracellular calcium

concentrations. PTH is produced in the parathyroid gland chief cells in response to hypocalcemia to restore normocalcemia. PTH increases renal reabsorption of calcium and promotes osteoclast-mediated bone resorption to maintain calcium concentrations within narrow limits. The timing of these rapid changes in calcium-regulating hormones is compelling as a mechanism to normalize plasma electrolytes after $MgSO_4$ administration. Further physiologic investigations could provide, not only understanding, but evidence that calcitonin and PTH could be used as additional biomarkers for nefarious $MgSO_4$ administration in horses. Furthermore, while Mg^{2+} and Ca^{2+} have been shown to be sensitive to pH changes[14] and storage conditions[6,49], calcitonin and PTH have been shown to be stable in long term storage (R. Toribio, personal communication). This could be advantageous as a biomarker, particularly for confirmation of an initial positive test with a stored "B" sample.

Changes in the fractional excretion of electrolytes in response to hypermagnesemia were evident and expected considering the close relationship of Mg^{2+} to the other electrolytes. It has been proposed that Mg^{2+}, similar to Ca^{2+}, activates the calcium-sensing receptors (CaSR) in the kidney to promote electrolyte excretion and diuresis[50]. The activation of these CaSR in the renal tubules would decrease renal reabsorption of the electrolytes, which would increase their fractional excretion. While all examined electrolytes increased in their fractional excretion, besides Mg^{2+}, Ca^{2+} had the greatest increase in excretion initially, that quickly resolved. It is possible that the activation of the CaSR, by the excessive Mg^{2+} blocked the reabsorption of Ca^{2+} by a mechanism similar to furosemide, increasing its elimination. The activation of other

CaSR in the endocrine system could be responsible for halting the excessive excretion of Ca^{2+} seeking to reverse the perceived initial loss. This pattern of increased fractional excretion of electrolytes could also serve as an additional biomarker for $MgSO_4$ administration.

The significant physiologic changes that occurred to mean arterial blood pressure (MAP) and left ventricular (LV) function were both immediate and directly related to the timing of the administration. A decrease in MAP has been shown to be associated with an increase in LV function to accommodate and maintain blood pressure[51,52]. The significant increase in %EF, S' and % FS all indicate an increase in LV function. It cannot be determined from this study if this increase was only due to the decrease in MAP or could have also involved the influence of increased Mg^{2+} on cardiac muscle; however, the peripheral dilatory effect of Mg^{2+}, through Ca^{2+} blocking effects[53], is suggestive of vasodilation precipitating an increase in LV function. The reduction in MAP could be associated with the concurrent postural change as seen with the decreasing head height. Additional studies are being completed that will evaluate these relationships more closely.

In humans, $MgSO_4$ is most commonly administered as a constant rate infusion or serial intravenous boluses when treating preeclampsia in women[54], and as a single bolus when treating acute asthmatic attacks[36,55]. The authors are not aware of any pharmacokinetic studies conducted using a single intravenous bolus of $MgSO_4$ in healthy horses. This study provides the first look at the effect of a single intravenous bolus of $MgSO_4$ in horses, and reports a volume of distribution of 1.58 L/kg, which is higher than

the 0.31 L/kg reported in a human study[56]. However, that study evaluated a 20 mmol intravenous infusion of $MgSO_4$ administered over one hour and subjects were followed for 12 hours. The volume of distribution for Mg^{2+} is challenging to evaluate as most Mg^{2+} is complexed in bone. Blood collections were conducted more frequently immediately following the beginning of the administration with the intention of capturing the peak of the plasma concentration. The rapid time to T_{max} (mean 6.6 min; 4.8-10.2 min) would be expected with an intravenous bolus, and the C_{max} (mean 1.43 mmol/L; 1.15-1.88 mmol/L) was more than a 3-fold increase. These have not been previously reported in the horse. The terminal half-life for Mg^{2+} of 5.5 hours is slightly longer than expected considering it has been reported in humans at 3.01 hours. It is not discernible from this study when the plasma Mg^{2+} concentration returned to baseline values as there were no blood collections between 12 and 24 hours, but it's important to note that by 24 hours these levels were consistent with baseline values, whereas at 12 hours, they were not.

It was anticipated that hypermagnesemia will increase Mg^{2+} concentration in the CSF; however, no changes were noted. As Mg^{2+} serves as a natural Ca^{2+} channel blocker[53,57], in addition to its peripheral effect on small vessels, it was questioned whether its effects could be exerted in the central nervous system at NMDA receptors and related voltage-dependent calcium channels. A lack of changes in electrolyte concentration in CSF suggests it is unlikely that there was a central effect from the bolus of $MgSO_4$. However, one potential limitation of this analysis is due to the distance of the collection site in the lumbrosacral area to the most likely transport site of Mg^{2+} across the

blood brain barriers in the choroid plexus, as this might create a delay in potential changes in the distal CNS. Another potential reason for the lack of change for Mg^{2+} in the CSF is that magnesium concentration in the CSF is actively maintained above that of plasma and changes in CSF magnesium trail behind changes in the plasma due to active transport mechanisms[58]. Additionally, in human patients, $MgSO_4$ is more commonly administered using an initial bolus followed by a constant rate infusion. As our study only examined a single bolus intravenous administration, it is possible the increase in fractional excretion of Mg^{2+} along with extensive distribution, rapidly decreased the plasma level of Mg^{2+} quickly enough to minimize any change in the CSF.

Head height above ground (HHAG) is a commonly used method to evaluate depth of sedation in horses[59], and as a passive measurement, does not include response to stimulation but can be assessed objectively. The head height decreased in initial readings following $MgSO_4$ administration to then become no different than baseline. The dropping of the head was evident, but the horses did not appear obtunded or tranquilized. True sedation is not the intent of $MgSO_4$ administration in competition horses, as they are still required to perform athletically. The intent of the administration of $MgSO_4$ prior to competition is to "take the edge off", and this is difficult to assess clinically; however, the use of HHAG provides an objective measurement of a change in behavior/posture in response to $MgSO_4$. The obvious decrease in HHAG was directly related to the administration; however, is not a reliable means to assess the use of $MgSO_4$ prior to competition.

Conclusion

In conclusion, administration of a single intravenous bolus of $MgSO_4$ to healthy horses alters plasma and urinary electrolytes, cardiovascular variables, head height, changes in the fractional excretion of electrolytes, and calcium-regulating hormones. This is the first study to provide pharmacokinetic and pharmacodynamic information of intravenous administration of $MgSO_4$ in the horse, which could have regulatory implications for it illicits use. The biological markers available for documenting the nefarious use of intravenous $MgSO_4$ include plasma ionized Mg^{2+}, Ca^{2+}/Mg^{2+}, and the fractional excretion of electrolytes. Calcitonin and PTH may serve as additional biomarkers for control, but additional work is necessary.

Chapter 4. Radio-telemetric assessment of cardiac variables and locomotion with experimentally induced hypermagnesemia in horses using chronically implanted catheters

Abstract

Objective--To characterize the pharmacokinetics and pharmacodynamics of intravenous administration of magnesium sulfate to horses using a novel radio-telemetry system for physiologic signal capture.

Animals—5 adult horses.

Procedures—Horses were surgically implanted with a radio-telemetric carotid catheter. Implants were paired with a non-invasive telemetric unit which acquired a six lead ECG and 3-axis acceleration to assess activity acquired wirelessly in real-time for future analysis. Horses were exposed to a new stall environment before (baseline) and after 60 mg/kg (30 mL) of magnesium sulfate ($MgSO_4$), or the same volume of 0.9% saline, administered intravenously in a blinded, random crossover design. Blood for pharmacokinetics, telemetric data, and body temperature were recorded serially for 24 hours. Data were analyzed across time and between treatments. Significance was set at $P<0.05$.

Results- Ionized magnesium concentration (Mg^{2+}) increased and the Ca^{2+} to Mg^{2+} ratio decreased and persisted for 5 hrs after $MgSO_4$ administration. Heart rate (HR) increased and mean arterial blood pressure (MAP) decreased for at least 6 hrs. Electrocardiogram (ECG) intervals (RR) decreased and (PR and QTc) increased in duration compared to

controls indicating an increase in heart rate, and slower myocardial conduction in the $MgSO_4$ group. Acceleration in all planes was less in the $MgSO_4$ group compared to controls indication decreased locomotion.

Conclusions and Clinical Relevance—This novel method permitted collection of physiologic signals without interference by handlers or animal restraint. An intravenous bolus of $MgSO_4$ produced cardiac variable changes associated with the reduction of locomotion in these horses, and in a direction that may be causal. Locomotion was decreased when horses were first introduced into a new environment which reflects the calming effect desired in sport horses. Telemetric monitoring can be used as a model to elucidate the behavior and physiologic effects of other drugs. The administration of $MgSO_4$ may be detected for regulatory purposes with the monitoring of Mg^{2+} and Ca^{2+} concentrations and their ratio.

Introduction

The abuse of magnesium sulfate ($MgSO_4$) is a regulatory issue in equestrian sport. The Federation Equestre Internationale (FEI), the international governing body of equestrian sport, has made the administration of $MgSO_4$ a prohibited practice and has listed $MgSO_4$ on the Equine Prohibited Substances List (EPSL)[a] due to its potential for calming and abuse, but evidence is predominantly anecdotal. The United States Equestrian Federations (USEF) Equine Drugs and Medications Rules prohibit the use of injections within the 12 hours prior to competition, but at this time does not prohibit $MgSO_4$. The USEF is the recognized national governing body of equestrian sport. If

evidence is identified that the administration of $MgSO_4$ is a behavior modifying substance, USEF will likely reevaluate its stance on the substance. However, the detection of $MgSO_4$ administration is difficult because the endogenous nature of magnesium and its active form, ionized magnesium (Mg^{2+}). To introduce regulatory control requires a method to differentiate between normal Mg^{2+} and related electrolyte concentrations and changes in these concentrations because of $MgSO_4$ administration. Magnesium is very important for countless physiologic functions and one of the most abundant elements in all mammals. Magnesium is considered a calcium channel blocker, both centrally blocking Ca^{2+} at NMDA receptors in the CNS and in peripheral vessels[60]. $MgSO_4$ is commonly used in humans for the treatment of preeclampsia in women[54,61,62], post stroke prevention of hypoxia induced glutamate excitotoxicity[41], the perioperative management of pheochromocytomas[35], and the treatment of acute asthma[36,37,63]. In horses, $MgSO_4$ is used to treat large colon impactions[64,65] and there has been recent investigation into its use for the treatment of trigeminal neuritis[44].

In previous work, a decrease in mean arterial pressure (MAP) from baseline was detected immediately following the administration of $MgSO_4$; concurrently, there was an increase in heart rate. The horses included in previous work were confined in stocks for 6 hrs for the duration of the experiment. There was no control group due to the difficulty with restraining the horses, as instrumented, for the 6 hrs without the administration of $MgSO_4$. It is difficult to assess the pharmacodynamic effects of drugs on animals when restraint of the animal is required. To address these limitations and prove a physiologic effect on blood pressure and heart rate as well as behavior calming, a telemetric

assessment of these parameters to study horses in a free and natural environment was necessary.

The goals of this experiment were to evaluate the effects of intravenous $MgSO_4$ on arterial pressure, identify changes in the electrocardiogram, and locomotion in unrestrained horses. Our hypotheses were that the pharmacokinetics and decrease in blood pressure, compared to the 0.9% NaCl control, would be similar to our prior work; and utilizing sensitive accelerometers, a decrease in locomotion would be detected in the $MgSO_4$ treatment group compared to controls. We will also confirm that plasma subjected to the

standard collection, storage and shipping methods of the United States Equestrian Federation's Equine Drugs and Medications Program will have similar Mg^{2+} and Ca^{2+} as previously published[49].

Materials and Methods

Animals—Six healthy adult (median age 8 yrs [range, 4 to 9 yrs]) university-owned Quarter Horse (n = 4) and Standardbred (n = 2) mares with a mean weight of 1247 lbs were included in the study. This study was approved by the University Institutional Animal Care and Use Committee (IACUC) and fulfilled ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines) for the humane use of animals in research. All horses were deemed healthy following physical exam. All horses were vaccinated and dewormed at least one month prior to inclusion. Housing for the duration of the study was in the Ohio State University Medical Center, in box stalls (3.6 x 3.6 meter), and horses were fed grass hay and water ad libitum.

Study Design—This study was a blinded, randomized crossover study with each horse receiving a 60 mg/kg intravenous administration of $MgSO_4$, or, with a minimum one-week washout period, an equivalent volume dose of 0.9% NaCl. All administrations were infused over 5 minutes. Physiologic signals and plasma samples were collected serially (5, 15, 30 minutes, and at 1, 2, 3, 4, 5, 6, 12, and 24 hours).

Surgical Implantation of arterial catheters—Implantation was conducted at least two weeks prior to experimental data collection to allow time for recovery from the implantation procedure. Horses were premedicated with 1.1 mg/kg of xylazine (AnaSed®; Akorn, Lake Forest, IL), and then placed under general anesthesia using a

mixture of 2.2 mg/kg ketamine (VetaKet®; Akorn, Lake Forest, IL) and 0.06 mg/kg of midazolam (Novaplus®; West-Ward, Eatontown, NJ). Horses were intubated and maintained under general anesthesia using isoflurane (Akorn, Lake Forest, IL). During the surgical procedure, heart rate, respiration rate, electrocardiogram and depth of anesthesia were monitored to ensure appropriate plane of anesthesia. Horses were placed in right lateral recumbency with limbs secured. Following appropriate sterile technique to prepare the surgical site, a skin incision (~4in) was made in the caudal-neck longitudinally along the jugular furrow. The left carotid artery was identified and exteriorized with umbilical tape securing the vessel above the plane of the skin. A purse string suture pattern was preplaced around the site identified for catheter insertion using 5-0 nonabsorbable nylon suture. A #11 scalpel blade was used to create a small stab incision in the carotid artery, and a 16g gel filled 35cm long arterial telemetric catheter (easyTEL +_L_PT g35, Emka Technologies, Falls Church, VA) was threaded into the carotid artery and advanced toward the subclavian artery. Approximately 8cm remained outside of the vessel. The catheter was secured with a tightening of the preplaced purse-string suture. The transducer/battery pack were secured in a subcutaneous pocket of muscle and subcutaneous tissue lateral to by 2-0 nonabsorbable nylon suture. Implant functionality was confirmed by recovery of arterial pressure waveforms. The subcutaneous fascia was closed using a continuous pattern and 2-0 absorbable suture. The skin was closed with 0 non-absorbable suture in a simple interrupted pattern. Horses were recovered from general anesthesia and continuously monitored until they could stand. Implant functionality was again confirmed and horses were observed every 6

hours for the following 48 hours. Phenylbutazone (Vetone®,Boise, ID) was administered intravenously once daily for 4 days at a dose of 4.4 mg/kg, and procaine penicillin (PenOne Pro™;Vetone®, Boise, ID) was administered intramuscularly for 4 days at a dose 6600 units/kg. Skin sutures were removed on day 14 post surgery.

External jugular catheter placement—On the day of the experiment, two 14-guage catheters were placed aseptically; one in each external jugular vein. All placement of jugular catheters took place in the horse's regular stall and not in the treatment stall. The right jugular catheter was used solely for the administration of $MgSO_4$, and the left jugular catheter for blood collection. The right jugular catheter was removed after administration of the total dose of $MgSO_4$ or 0.9% NaCl.

Instrumentation for Radio-telemetric data Collection—On the day of the experiment, following the placement of the jugular catheters, horses were placed in a novel stall used for the experiment. Surface leads were attached to the horse using foam monitoring ECG electrodes (3M Maplewood, MN), and placed with the (green;RL) caudal to the olecranon, the (red; LL) superior to the (green) lead. The (white; RA) lead was placed along the cranial sternum with the neutral (black; LL) lead placed at the point of the shoulder. The surface leads for the ECG were directly connected to the (emkaPACK_4G_TR+_2ECG, EMKA Technologies, Falls Church, VA), which was connected via cable to the base transmitter (emkaPACK_4G_TR_base, EMKA Technologies, Falls Church, VA). The telemetric catheters, which were in dormant mode, were activated by placing a magnet over the subcutaneously placed transducer/battery pack. Battery life for catheter and transducer is approximately 200 hours, which allows

for numerous experiments if the catheter is turned off in between treatments/experiments. A lightweight, spandex collar was placed on the horses. The collar had been placed on each of the horses for an hour a day for five days the week prior to get them familiar with the process. This collar had pockets designed to hold the modules used for signal acquisition and relaying telemetric readings (Figure 11.). The implant MANAGER module (emkaPACK_4G_TR_iMNG, EMKA Technologies, Falls Church, VA), which received signals from the implant was located at the point of the shoulder in close proximity to the subcutaneous transducer/battery pack. The signal from the implanted telemetric catheter and transducer was radio-transmitted to implant MANAGER module which was connected by a hard-wire to the base transmitter. The base transmitter was located in a pocket on the withers, and also housed the accelerometers responsible for monitoring activity. The electrocardiograms were obtained by surface electrodes placed in a base apex configuration. The base transmitter connected, via unique frequency, to the Bluetooth receiver (emkaPACK_4G_RE_16 Receiver, EMKA Technologies, Falls Church, VA). The Bluetooth receiver was capable of collecting unique signals from up to 12 base transmitters, but in our experiment, the receiver collected the signals for two horses on each day of the experiment. The Bluetooth receiver was attached to the top of the stall wall and was connected via Ethernet cable to the acquisition computer. Once the horses were properly instrumented, baseline readings were taken.

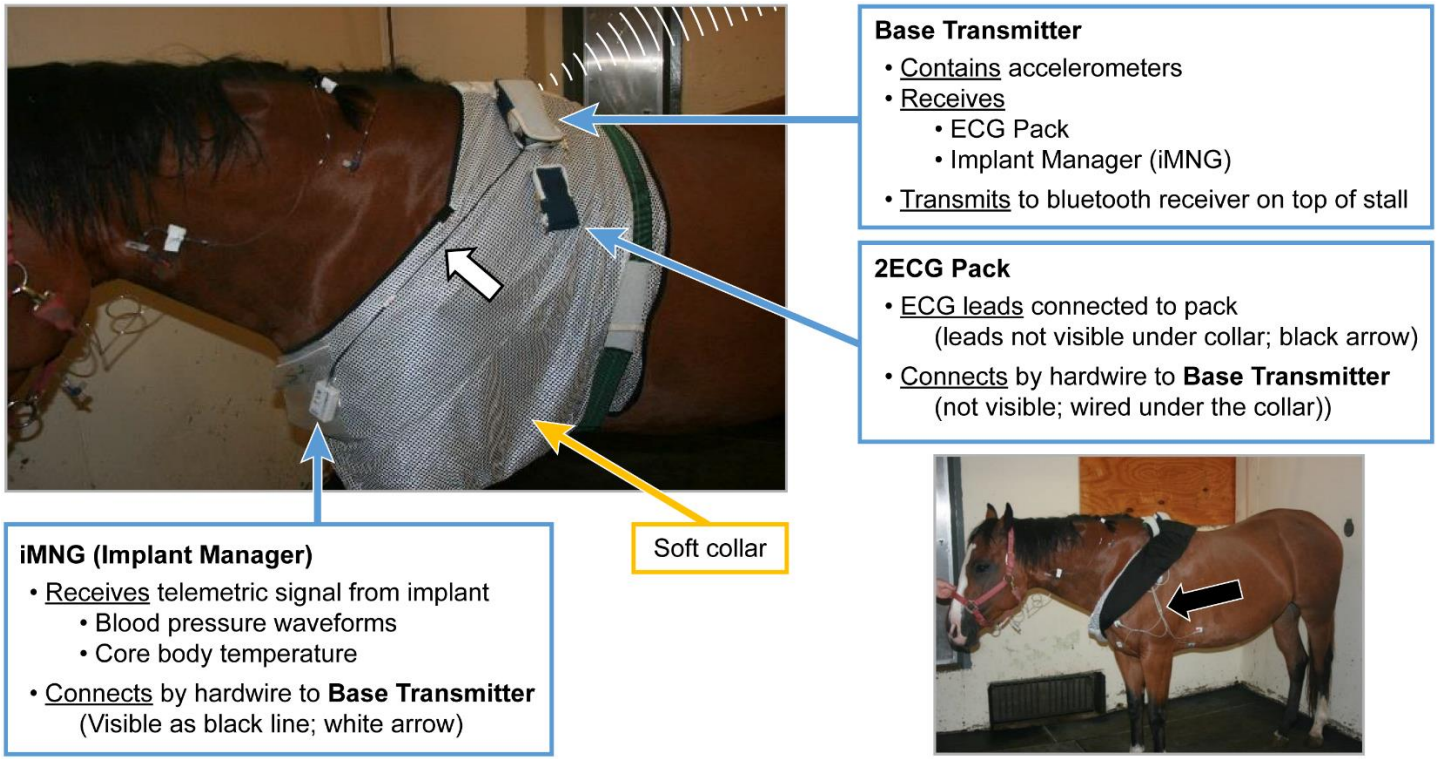


Figure 11. Picture of horse with soft collar applied and the connections of iMNG (Implant Manager), and the 2ECG Pack, to the Base Transmitter.

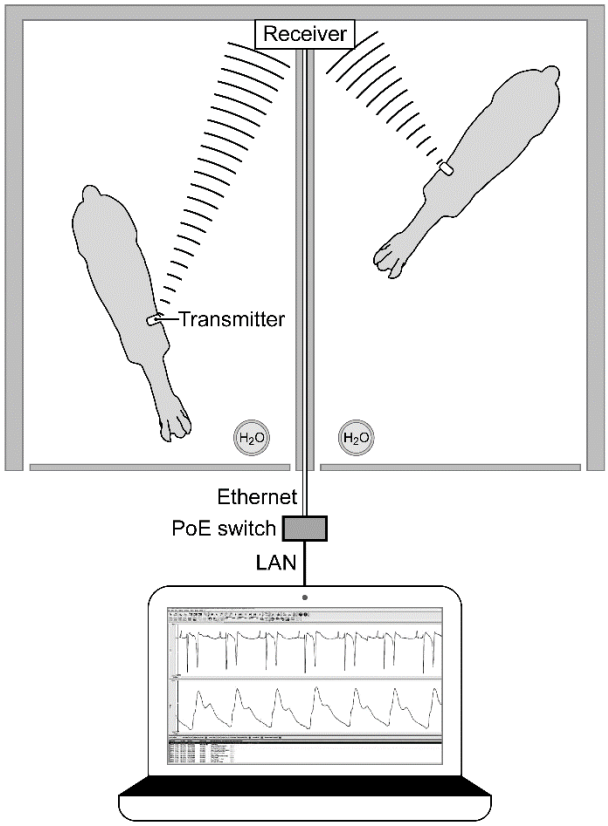


Figure 12. Overhead diagram of Bluetooth receiver mounted on the top of the stall wall with Ethernet connection to computer for acquisition of signals. Bluetooth receiver collecting signals from the base pack on the withers of each horse.

Sample Collection

Horses were placed in a new stall for the first time approximately 30 minutes prior to the experiment, and baseline physiologic signals and plasma samples were collected. The stalls were not bedded and water was ad libitum during the first 6 hours of continuous monitoring. Following $MgSO_4$ or 0.9% NaCl (control) administration; both infused over 5 minutes, all physiologic signals and plasma samples were collected serially (5, 15, 30 minutes, and at 1, 2, 3, 4, 5, 6, 12, and 24 hours). At each of the collection time points, blood pressure, ECG, and acceleration data were acquired for 2

minutes prior to study personnel entry into the stall for collection of plasma samples. With the exception of the time to collect plasma samples, horses were loose in the stall (3.6 x 3.6 meters). All interaction with the horses during the active experiment was limited to the collection of plasma samples from the jugular catheter.

***Blood Collection*—**Plasma samples were collected anaerobically at each of the time points described above. A 10cc syringe was used to collect waste blood to assure a fresh blood sample; the waste was discarded. Blood was collected using a 30cc syringe and divided into 2 plasma separator tubes (8.0 ml PST^{TM} Gel and Lithium Heparin, BD Vacutainer, Franklin Lakes, NJ). The remaining blood was transferred to plain tubes and EDTA tubes; plain tubes were allowed to clot for 1 h, centrifuged at 1000 g for 5 min at 4 °C. Serum and plasma samples were aliquoted and stored at -80°C for later endocrine analysis.

Electrocardiogram— ECG signals were transmitted from the base to the Bluetooth receiver. Standard ECG intervals (RR, PR, QRS, and QT) were measured. The value for the QT interval was corrected for HR using Bazett's formula[66] and provided a corrected QT interval (QTc). The monitor was evaluated by an observer to detect any failure to transmit or arrhythmias. Post experiment, ECG's were analyzed using ECG auto software package (ECG_AUTO_FULL, EMKA Technologies, Falls Church, VA).

Blood pressure measurements—All blood pressure measurements were acquired using the implanted telemetric catheters (easyTEL + implant). Real time arterial pressure waveforms were acquired and analyzed post experiment for diastolic (DAP), systolic (SAP) and mean arterial pressures (MAP). Analysis was completed by a cardio analyzer

software (ECG_AUTO_Cardio1+, EMKA Technologies, Falls Church, VA). Arterial waveforms were also analyzed for heart rate (HR), and +dP/dt (the maximum rate of rise in left ventricular pressure).

***Temperature*—**The implanted blood pressure telemetric catheter, described above, also acquired core body temperature data and transmitted to the computer concurrently with the blood pressure data.

***Locomotion*—**The base (emkaPACK_4G_TR_base) included accelerometers that measure in 3-axes (X, Y and Z); the x-axis measured acceleration from side to side, the y-axis measured acceleration up and down, and the z-axis measured acceleration moving forward and backward. The overall measure of locomotion was taken as the square root of the sum of the squares for each of the 3-axes. This analysis was completed using the ECG_AUTO_Slow+ analyzer (EMKA Technologies, Falls Church, VA).

Observational behavior assessment—All horses were observed for head elevation, ear movement and overall movement. Head elevation was assessed on a scale range of 1-4; 1 was below the level of the neck, 2 was at the level of the neck, 3 was natural head position, and 4 was elevated head height. Ear movements were counted over 30 seconds at each time period, and movement was assessed as a binary function with ear movement given a score of 1 and ear non-movement given a score of 0.

Data Analysis

***Blood*—**Plasma samples were refrigerated, then shipped by commercial shipper within 48 hours to the United States Equestrian Federation's Equine Drug Testing and Research Laboratory (EDTRL) in Lexington, Kentucky to duplicate the process used in

the collection and analysis of regulatory samples[49]. A Nova pHox Ultra Analyzer (Nova Biomedical, Waltham, MA) was used to analyze plasma samples for pH, TCO_2, Mg^{2+}, and Ca^{2+}, and provided the Ca^{2+}/Mg^{2+} ratio.

***Post experiment review of physiologic data*—**All physiologic data (blood pressure, ECG, locomotion, and temperature) were analyzed by a contract research organization (QTest Labs, Columbus, OH) using ECG_AUTO_Cardio1+ cardio analyzer, ECG_AUTO_FULL, and ECG_AUTO_Slow+ software (EMKA Technologies, Falls Church, VA). For all objective measures, including blood pressure, heart rate, ECG intervals, and activity, data were recorded every 500ms. Post study analysis consisted of averaging four consecutive 30 second intervals at each time point for a full two minutes of data.

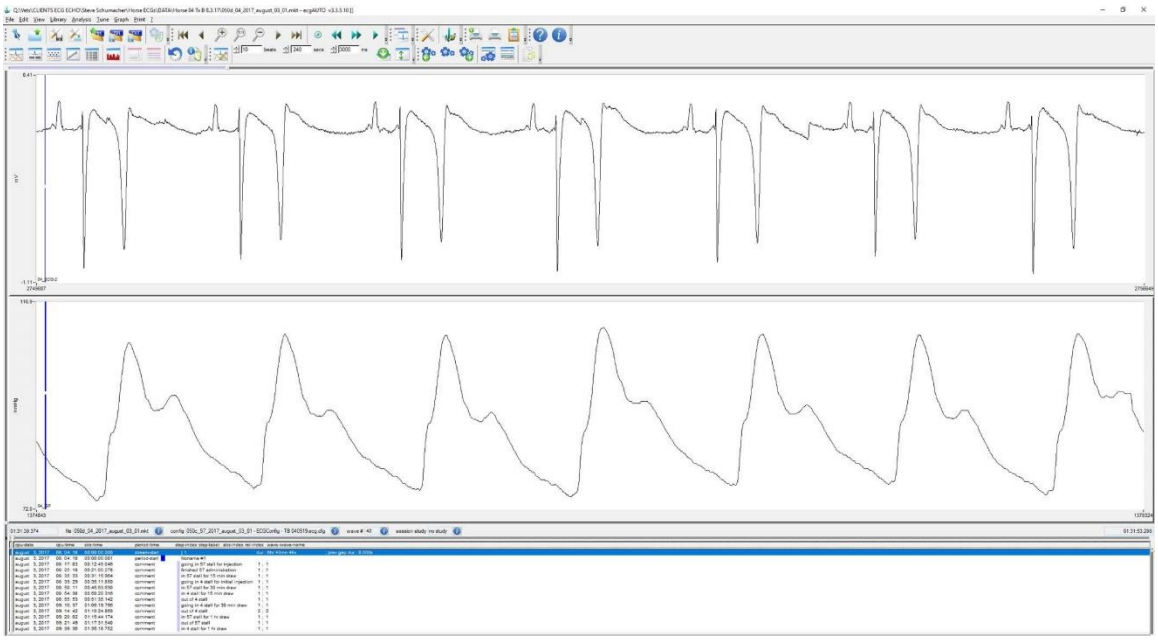


Figure 13. Screen shot of ECG and arterial blood pressure waveform during acquisition.

***Pharmacokinetic analysis*—**A commercially available pharmacokinetic software package was used to calculate the pharmacokinetic parameters (Phoenix WinNonlin, Version 8.0, Pharsight Corporation, Cary, NC, USA) using noncompartmental analysis. All plasma concentrations for Mg^{2+} were reported in mmol/L and the total dose administered was converted to mmol from milligrams elemental Mg^{2+}. To normalize plasma concentrations, prior to pharmacokinetic analysis, the Mg^{2+} concentration from baseline (T=0) was subtracted from each of the time points. The elimination rate constant (k_{el}) was determined from the slope of the terminal portion of the plasma concentration x time curve. The terminal half-life (HL_Lamda_z) was determined by dividing the natural log of 2 (ln 2) by the terminal elimination rate constant (λz). The maximum plasma concentration (C_{max}) and the time to maximum concentration for Mg^{2+} were directly determined from the plasma concentration of Mg^{2+}. The area under the curve (AUC_{last}) was calculated using the log-linear trapezoidal rule.

Statistical analysis—Data were analyzed across time using a commercially available software (R 3.5 Statistical Software). Raw data were tested for normality using Shapiro-Wilk test and graphed using SigmaPlot. The paired t-test was used to analyze for significance for each ECG and blood pressure variable, and the electrolyte concentrations across the two treatment groups. A linear mixed effects model was used to determine significance across time points. For observational behavior data, ANOVA, poisson, and logistic models were fit based on the observations for head elevation (treated as a likert scale), ear movement (count data), and action (binary). Each of these

responses were fitted using a repeated measures design, since each horse was recorded over time. Significance was set at $P<0.05$.

Results

Plasma—There was a significant difference of treatment between the control group and the $MgSO_4$ group or Ca^{2+}, Mg^{2+} and Ca^{2+}/Mg^{2+}. The plasma concentration of Mg^{2+} increased rapidly following the administration of $MgSO_4$ and remained significantly elevated from 5 min until 2 hours; plasma Mg^{2+} did not return to baseline levels until 24 hours. Plasma Ca^{2+} decreased by 30 min and remained lower until the 3rd hour ($P<0.001$) as compared to the baseline concentration, The ratio of Ca^{2+} to Mg^{2+} declined immediately from a baseline ratio of 2.77 ±0.12 SE to a ratio of 0.76 ±0.0.02 SE at 5 min and then gradually increased, but remained significantly lower than baseline until 5 hours ($P<0.05$) (Figure 14. a-c; Table 8;Appendix B.).

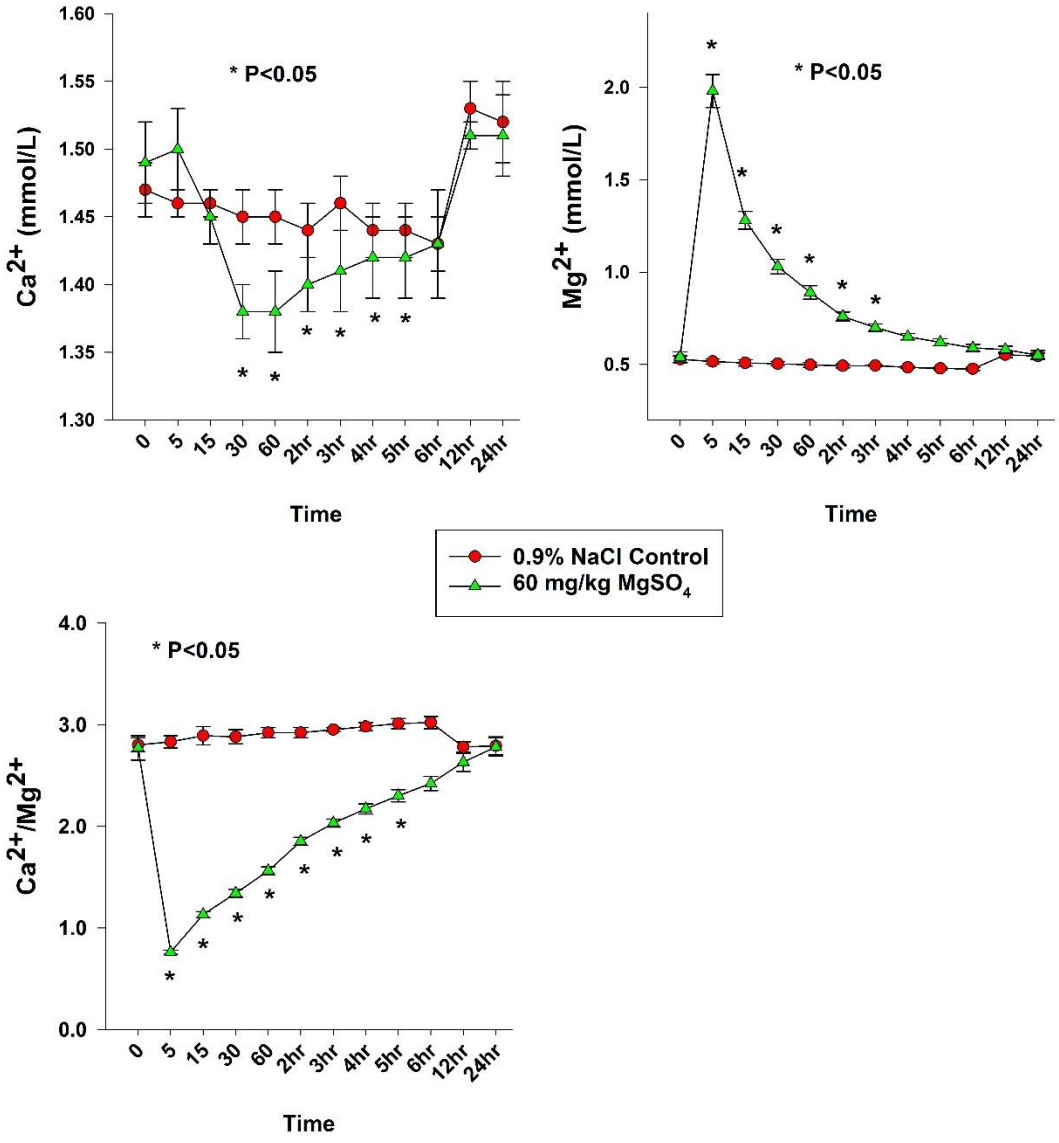


Figure 14. (a) Plasma Ca^{2+} concentration, (b) plasma Mg^{2+} concentration, and (c) ratio of plasma Ca^{2+} to plasma Mg^{2+} concentrations from horses administered intravenously 60 mg/kg $MgSO_4$ (●) and equivalent volume of 0.9% NaCl control horses (▲).

Table 8. Plasma electrolytes in horses administered 0.9% NaCl (control) and horses administered 60Mg/kg $MgSO_4$. Values presented as mean ±SE.

0.9% NaCl Control	**Baseline**	**5 Min**	**15 Min**	**30 Min**	**60 Min**	**2 Hr**	**3 Hr**	**4 Hr**	**5 Hr**	**6 Hr**	**12 Hr**	**24 Hr**
pH (H^+)	7.3 ± 0.05	7.31 ± 0.03	7.3 ± 0.04	7.28 ± 0.04	7.3 ± 0.03	7.31 ± 0.02	7.31 ± 0.03	7.31 ± 0.04	7.32 ± 0.03	7.32 ± 0.03	7.28 ± 0.03	7.27 ± 0.02
Ca^{2+} (mmol/L)	1.47 ± 0.02	1.46 ± 0.01	1.46 ± 0.01	1.45 ± 0.02	1.45 ± 0.02	1.44 ± 0.02	1.46 ± 0.02	1.44 ± 0.02	1.44 ± 0.02	1.43 ± 0.02	1.53 ± 0.02	1.52 ± 0.03
Mg^{2+} (mmol/L)	0.53 ± 0.02	0.52 ± 0.01	0.51 ± 0.02	0.5 ± 0.01	0.5 ± 0.01	0.49 ± 0.01	0.49 ± 0	0.48 ± 0.01	0.48 ± 0.01	0.48 ± 0.01	0.55 ± 0.01	0.55 ± 0.01
TCO_2 (mmol/L)	29.68 ± 0.62	28.18 ± 0.48	27.84 ± 0.88	28.84 ± 0.69	28.94 ± 0.59	28.92 ± 0.37	28.82 ± 0.44	28.16 ± 0.58	27.92 ± 0.31	28.68 ± 0.2	26.48 ± 0.6	27.52 ± 0.76
Ca^{2+}/Mg^{2+} (mol/mol)	2.8 ± 0.07	2.83 ± 0.06	2.89 ± 0.09	2.88 ± 0.07	2.92 ± 0.05	2.92 ± 0.05	2.95 ± 0.02	2.98 ± 0.04	3.01 ± 0.05	3.02 ± 0.06	2.78 ± 0.05	2.79 ± 0.09
60mg/kg $MgSO_4$	**Baseline**	**5 Min**	**15 Min**	**30 Min**	**60 Min**	**2 Hr**	**3 Hr**	**4 Hr**	**5 Hr**	**6 Hr**	**12 Hr**	**24 Hr**
pH	7.28 ± 0.02	7.3 ± 0.02	7.28 ± 0.03	7.28 ± 0.02	7.28 ± 0.02	7.28 ± 0.02	7.3 ± 0.03	7.31 ± 0.04	7.33 ± 0.03	7.32 ± 0.03	7.31 ± 0.02	7.32 ± 0.02
Ca^{2+} (mmol/L)	1.49 ± 0.03	1.5 ± 0.03	1.45 ± 0.02	1.38 ± 0.02*	1.38 ± 0.03*	1.4 ± 0.02*	1.41 ± 0.03*	1.42 ± 0.03*	1.42 ± 0.03*	1.43 ± 0.04†	1.51 ± 0.01	1.51 ± 0.03
Mg^{2+} (mmol/L)	0.54 ± 0.03	1.98 ± 0.09*	1.28 ± 0.05*	1.03 ± 0.04*	0.89 ± 0.04*	0.76 ± 0.02*	0.70 ± 0.02*	0.65 ± 0.02	0.62 ± 0.02	0.59 ± 0.02	0.58 ± 0.02	0.55 ± 0.02
TCO_2 (mmol/L)	28.48 ± 1.04	26.58 ± 0.7*	27.42 ± 0.69*	27.98 ± 0.61	28.72 ± 0.58	28.38 ± 0.4	28.22 ± 0.64	28.68 ± 0.63	27.64 ± 0.57*	28.48 ± 0.37	27.78 ± 1.1*	27.22 ± 0.73*
Ca^{2+}/Mg^{2+} (mol/mol)	2.77 ± 0.12	0.76 ± 0.02*	1.13 ± 0.03*	1.34 ± 0.04*	1.56 ± 0.04*	1.85 ± 0.04*	2.03 ± 0.04*	2.17 ± 0.05*	2.3 ± 0.06*	2.42 ± 0.07	2.63 ± 0.09	2.78 ± 0.09

* Significant (P ≤0.05)

† Near significance (P ≤0.07)

Cardiovascular

***ECG*—**The heart rate increased significantly with $MgSO_4$ administration ($P<0.05$). As compared to baseline in the $MgSO_4$ group, the HR increased by 5 min and remained higher than the control group until 30 min (Figure 15.b). Intervals for RR, PR, and QTc differed between groups ($P<0.05$). The RR interval decreased from a baseline value of 1739 ±127.4 to 1671 ±151.4 ms by 15 min and returned to baseline values by 60 min (Figure 15.c).

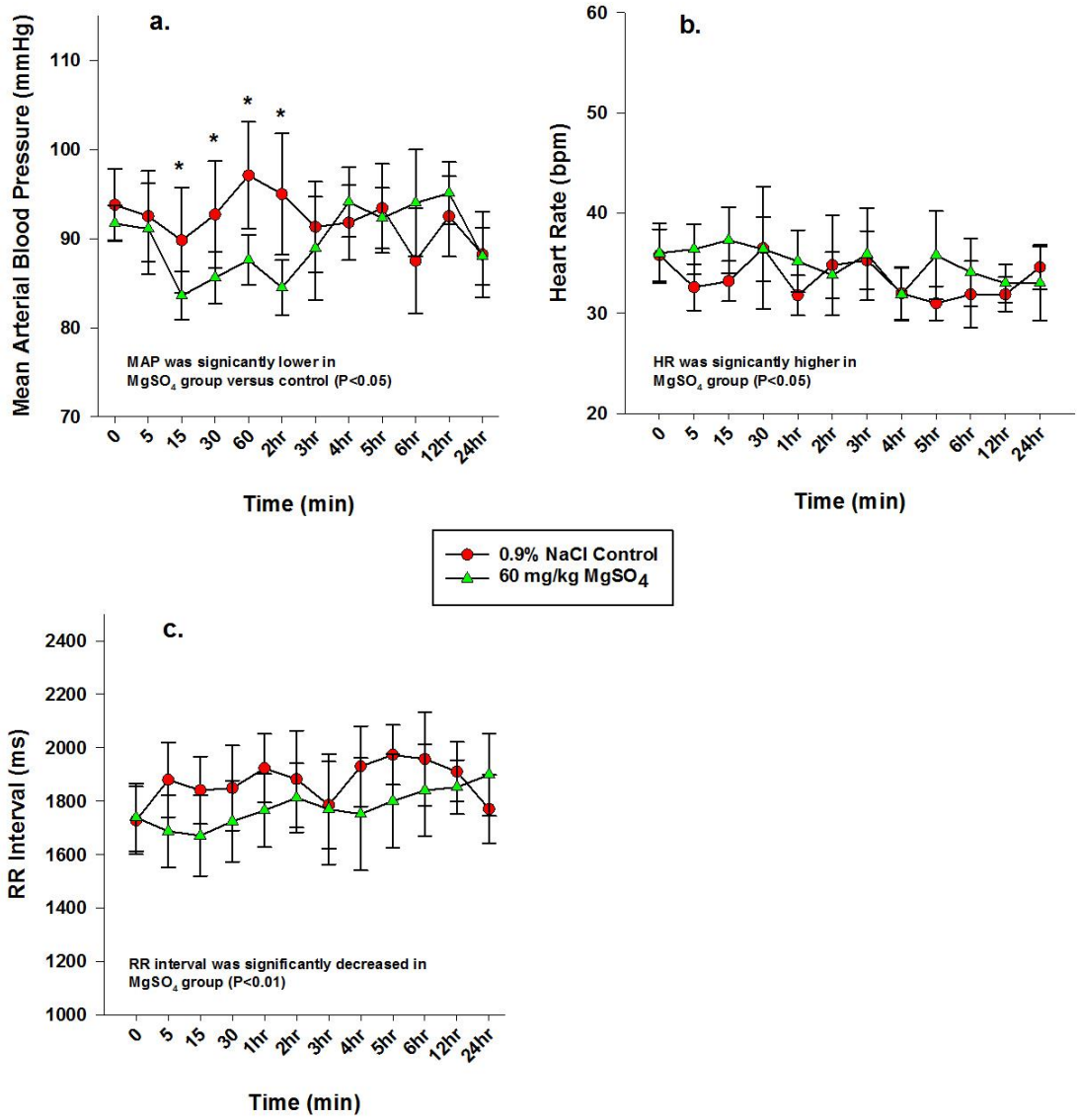


Figure 15. (a) Mean arterial pressure (mmHg), (b) heart rate (bpm), and (c) electrocardiographic RR interval (ms) from horses administered intravenously 60 mg/kg $MgSO_4$ and equivalent volume of 0.9% NaCl control horses.

The PR interval significantly increased over the first 15 min and remained significantly increased through 2 hrs, and was significant between groups (Figure 16.a). The QTc interval was significantly increased in the $MgSO_4$ treatment group as compared to the control group. As compared to baseline, the QTc interval was longer at 30 min, 1, 3, 4, 5, 6 hr time points ($P<0.05$), and at the 2hr time point, was nearly significant ($P<0.055$) compared to the control group (Figure 16.b).

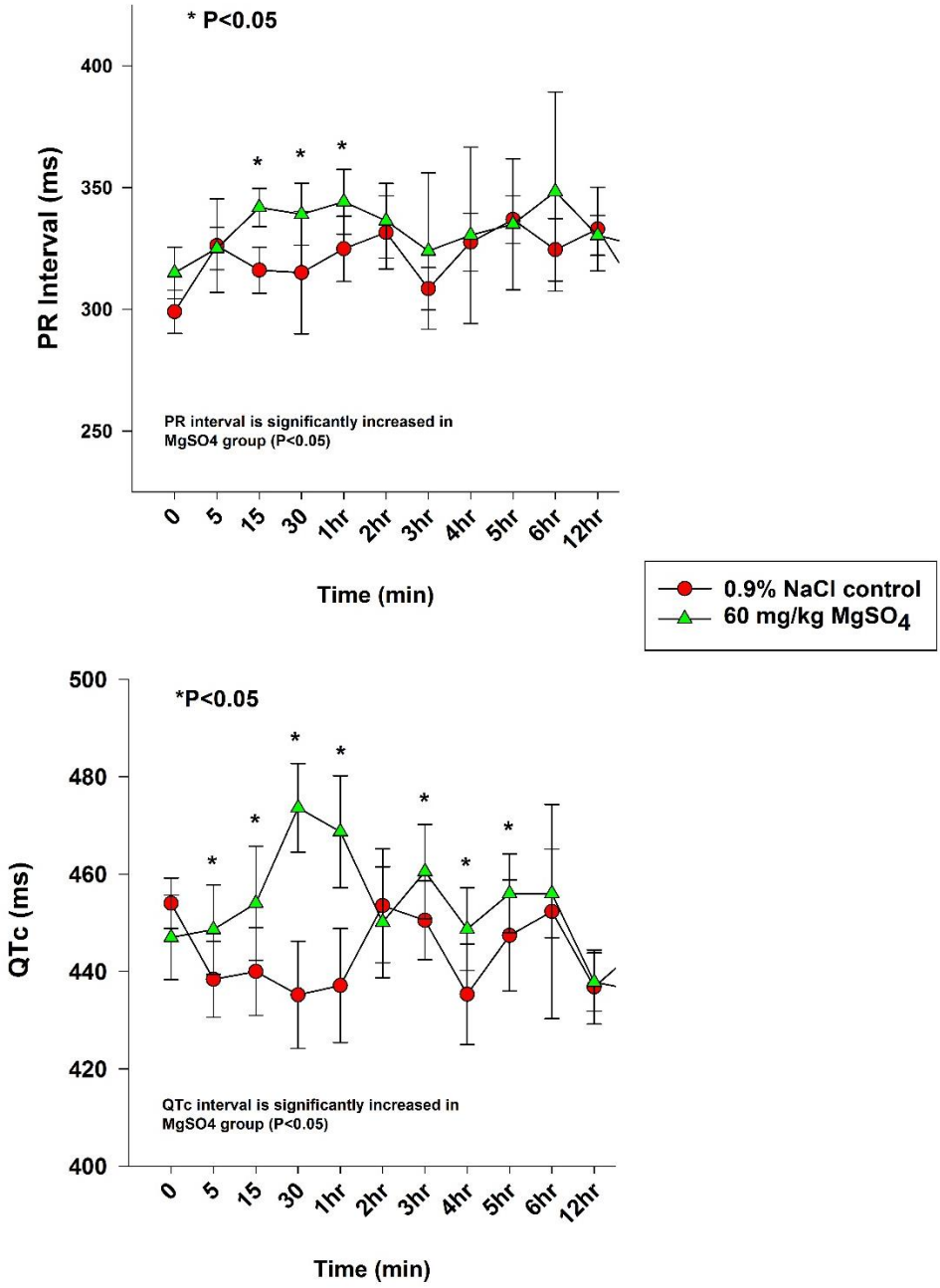


Figure 16. (a) PR interval (ms) and (b) QTc intervals (ms) from the analysis of electrocardiograms from horses administered intravenously 60 mg/kg $MgSO_4$ and equivalent volume of 0.9% NaCl control horses.

Table 9. ECG Intervals in horses administered 0.9% NaCl (control) and horses administered 60Mg/kg $MgSO_4$. Values presented as mean ±SE.

0.9% NaCl Control	Baseline	5 Min	15 Min	30 Min	60 Min	2 Hr	3 Hr	4 Hr	5 Hr	6 Hr	12 Hr	24 Hr
HR (bpm)	36 ± 2.6	33 ± 2.3	33 ± 2.0	36 ± 6.1	32 ± 2.0	35 ± 5.0	35 ± 2.9	32 ± 2.6	31 ± 1.7	32 ± 3.3	32 ± 1.7	35 ± 2.2
PR (ms)	299 ± 8.9	326 ± 19.2	316 ± 9.5	315 ± 25.1	325 ± 13.4	332 ± 15.0	309 ± 8.7	328 ± 11.9	337 ± 9.8	324 ± 12.8	333 ± 17.2	306 ± 7.9
QRS (ms)	136 ± 5.2	138 ± 4.8	134 ± 4.4	133 ± 4.9	136 ± 3.8	140 ± 5.3	138 ± 5.1	139 ± 6.1	141 ± 6.0	141 ± 6.8	137 ± 5.2	136 ± 4.4
RR (ms)	1,728 ± 126.7	1,880 ± 139.7	1,841 ± 126.3	1,849 ± 159.2	1,923 ± 128.9	1,882 ± 181.3	1,785 ± 163.1	1,930 ± 149.9	1,973 ± 111.5	1,958 ± 175.2	1,910 ± 111.5	1,771 ± 127.7
QT (ms)	590 ± 21.0	598 ± 24.1	595 ± 23.4	586 ± 41.2	604 ± 27.7	613 ± 26.5	593 ± 25.6	602 ± 25.9	624 ± 20.4	625 ± 17.6	602 ± 18.9	586 ± 21.2
QTcB (ms)	454 ± 5.2	438 ± 7.8	440 ± 9.0	435 ± 11.0	437 ± 11.7	454 ± 11.7	450 ± 8.1	435 ± 10.3	447 ± 11.4	452 ± 22.0	437 ± 7.6	444 ± 12.5

60 mg/kg $MgSO_4$	Baseline	5 Min	15 Min	30 Min	60 Min	2 Hr	3 Hr	4 Hr	5 Hr	6 Hr	12 Hr	24 Hr
HR (bpm)	36 ± 3.0	36 ± 2.5	37 ± 3.3	36 ± 3.2	35 ± 3.1	34 ± 2.3*	36 ± 4.6	40 ± 7.9	36 ± 4.4	34 ± 3.4	33 ± 1.9	33 ± 3.7
PR (ms)	315 ± 10.6	325 ± 8.7	342 ± 7.8*	339 ± 12.7*	344 ± 13.3*	336 ± 15.4*	324 ± 32.1	330 ± 36.2	335 ± 26.9	348 ± 40.9	330 ± 8.2	326 ± 13.3
QRS (ms)	143 ± 4.7	143 ± 4.2	142 ± 5.1	144 ± 5.5	142 ± 2.9	144 ± 6.0	143 ± 6.0	143 ± 2.9	145 ± 5.6†	143 ± 7.4	140 ± 3.5	144 ± 4.0
RR (ms)	1,739 ± 127.4	1,688 ± 135.2	1,671 ± 151.4	1,725 ± 152.1	1,765 ± 136.3	1,813 ± 128.9*	1,770 ± 206.1	1,752 ± 210.2	1,801 ± 175.3	1,841 ± 171.5	1,852 ± 101.3	1,899 ± 154.3
QT (ms)	581 ± 22.0	579 ± 17.2	581 ± 26.6	615 ± 27.5	618 ± 23.3*	604 ± 28.3	608 ± 45.1	587 ± 46.8	604 ± 32.9	613 ± 28.7	592 ± 13.9	593 ± 24.4
QTcB (ms)	447 ± 8.7	449 ± 9.2	454 ± 11.7	474 ± 9.1*	469 ± 11.5*	450 ± 11.4†	460 ± 9.7*	449 ± 8.5*	456 ± 8.1*	456 ± 9.1*	438 ± 6.0	436 ± 20.6

*Significant (P ≤0.05)

† Near significance (P ≤0.07)

Blood Pressure—The mean arterial blood pressure (MAP) decreased from 92 ±2.0 mmHg to 84 ±2.7 mmHg within the first 15 min after administration of $MgSO_4$, not seen with 0.9% saline, and slowly returned to baseline levels by 3 hours (Figure 15.a). MAP differed and was lower than control ($P<0.05$), and was lower than baseline from 15 min to the 2 hr time period ($P=0.029$). Both systolic arterial blood pressure (SBP) and diastolic arterial blood pressure (DBP) differed between the two treatment groups ($P<0.05$). SBP decreased from baseline from 15 min to the 2 hr time period ($P<0.05$) and DBP decreased from baseline at 15 min, 30 min and 2 hr ($P<0.05$) (Appendix C.) after $MgSO_4$ administration.

Locomotion—There was a significant decrease in activity in x, y and z-axis and in overall acceleration axes for the $MgSO_4$ treatment group compared to the control group. (Figure 17. a-d) (Appendix D.).

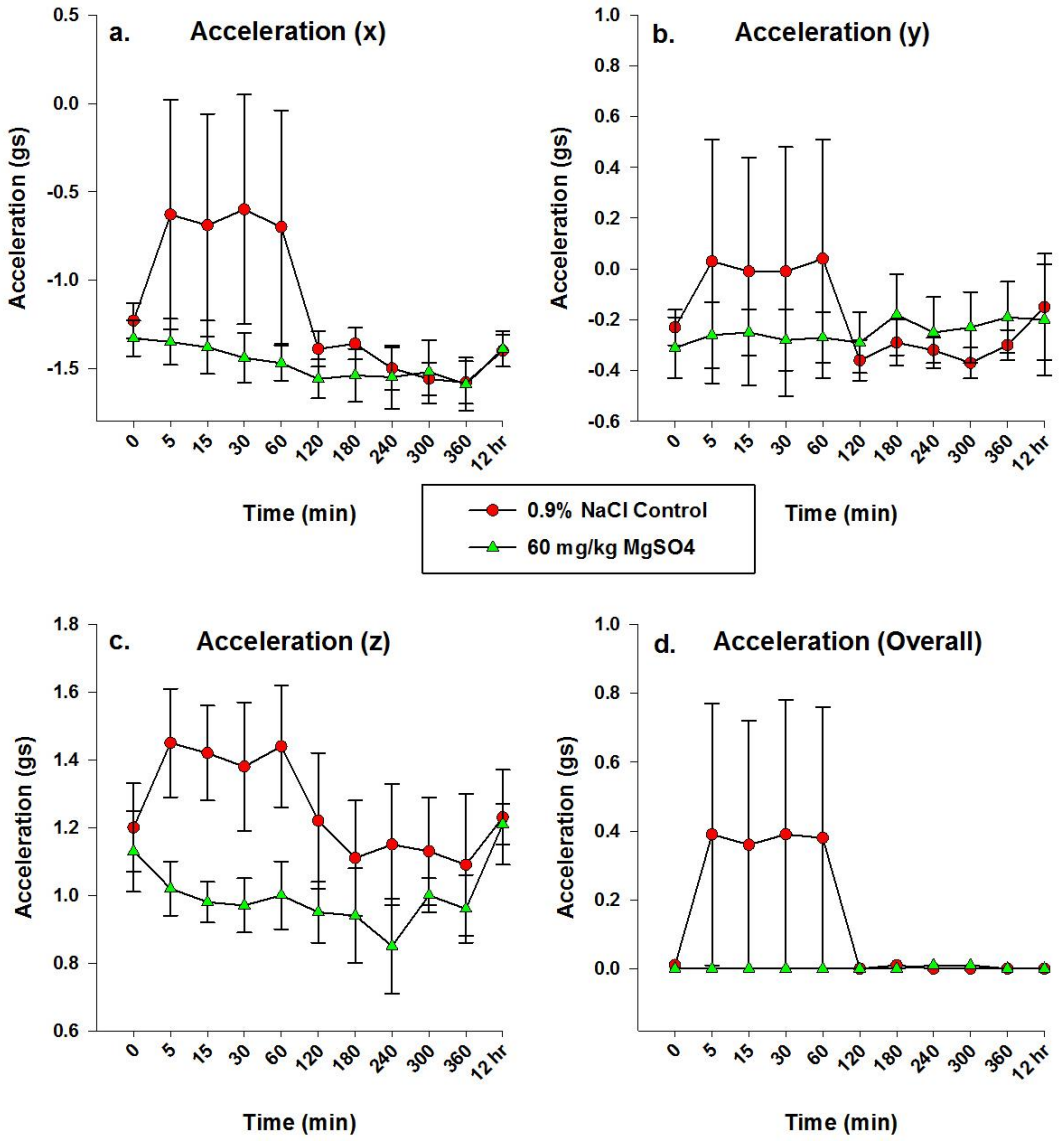


Figure 17. Locomotion as measured in three planes (x, y and z) by acceleration (gs), using accelorometers housed in the base transmitter located on the withers; (a) x-vector plane, (b) y-vector plane, (c) z-plane, and (d) overall locomotion.

Observational behavior data—Ear movement was significantly less, 4.2 ±0.65 movements over 30 seconds, in the $MgSO_4$ treatment group compared to 5.8 ±1.55 ear movements over 30 seconds in the control group ($P<0.01$).

***Pharmacokinetic*—**The selected reported pharmacokinetic parameters were calculated with the endogenous baseline values for Mg^{2+} subtracted prior to analysis. This normalization was done for each horse at each time point within the $MgSO_4$ treatment group. Noncompartmental analysis provided a maximum concentration (C_{max}) of 1.44 ±0.19 mmol/L and an average time of maximal observed concentration (T_{max}) of 5.0 min. The average clearance (CL) was 164.7 ±62.7 L/min and the observed average volume of distribution (Vss_obs) was 1.2 ±0.38 L/kg.

Temperature—There was no significant change from baseline in either group or across time (36.4 ±0.2 °C) (Appendix E.).

***Instrumentation*—**All instrumentation functioned properly for the duration of the study, and no horses experienced any complications from the implanted catheters One issue that occurred was that the ECG electrodes would occasionally detach from the horse. Electrodes were reaffixed as necessary, but did not there were no lapses in data collection. Horses appeared to be undisturbed by the placement of the collar.

Discussion

The most important findings of our study was that the bolus IV administration of $MgSO_4$ resulted in changes in electrophysiology of the heart, reduced blood pressure, and reduced locomotion, and may be responsible for the nefarious use for the calming behavior in horses. This is the first report documenting these pharmacodynamic effects and which may be directly related to the inappropriate use of $MgSO_4$ in horses.

The direct arterial blood pressure is the most accurate means for detecting changes in the cardiovascular tone of mammals[67]. Our study confirmed, with appropriate

controls, that mean arterial blood pressure decreased in an amount similar to reported values for beta-adrenergic antagonists that are also clinically effective to not only reduce blood pressure, and in humans can cause fatigue[68]. In a review of 56 randomized controlled trials examining the blood pressure lowering effects of beta-1 selective blockers, the average reduction in blood pressure was 10 and 8 mmHg in systolic and diastolic blood pressure, respectively in patients with mild to moderate hypertension[69]. An associated side effect of the beta-blocker propranolol is fatigue[70]. In our study, the observed changes in systolic and diastolic blood pressures were 9 and 8 mmHg, respectively at the 15 minute time point, which is similar to the reports in humans treated with beta-1 selective blockers. This change in blood pressure upon administration of $MgSO_4$ could be causing a similar sensation of fatigue, possibly noted as calming in horses.

The use of telemetric monitoring to detect physiologic changes is not a novel approach in animal research, this method has been used to measure changes in hemodynamic parameters and physiologic responses to drug administrations in other species[71-74]. A previous study examined the wireless invasive blood pressure monitoring in ponies, but this study did not use the implanted devices for more than 48 hours[75]. To our knowledge, ours is the first study to implant horses with invasive chronic telemetric catheters to serially measure changes in arterial blood pressure, core temperature, locomotion, and ECG changes in response to drug administration. This system allowed for the direct monitoring of the desired variables in horses while allowing them freedom of movement to assess their activity. The use of this invasive technology limited the

number of test subjects as it was not practical to implant a larger number of horses, but the increased accuracy and decreased variability associated with handling, reduced the number of animals needed to show significant effects. This method appeared to be reliable, accurate, and most importantly, successful with the horse being allowed free movement. This novel method could be used in future studies to evaluate the effect of drugs on the cardiovascular physiologic variable and locomotion. The unit also can be used without the implanted telemetric catheters to gather only ECG and locomotion data.

In previous work by these authors, changes in blood pressure and heart rate could not be compared to a control group because of the difficulty in restraining instrumented horses without the benefit of the test article. The data in our current study, as to be expected, was similar to our prior data, but in this study we did inject an equivalent volume of saline as a control. We did not think the lack of a control affected our prior data, but now we have confirmed the effect. The use of a control group allowed for analysis of the effect of treatment which identified significant changes in HR and MAP, and ECG intervals for PR, QRS and QTc. These findings are consistent with reported results following MgSO4 administration to a variety of species[76,77]. Following the administration of $MgSO_4$ the decrease in MAP was less significant, but the pressure waveforms were acquired from a more central artery than the previously used facial artery. There are inherent differences in the placement of the catheter for monitoring with respect to the size of the artery versus the size of the catheter tip, as well as, the height of the monitoring position above the heart, and the use of fluid filled catheters versus get tips. However, as Mg^{2+} has been shown to lower blood pressure[60,78,79], and act

as a peripheral vasodilator by serving as a Ca^{2+} antagonist in vascular smooth muscle[57,80,81], and has been shown to effect vasodilation of coronary vessels[60,82,83], these changes were not unexpected. Little is known about its effect on larger arterial vessels, but a direct effect has been demonstrated on the peripheral vasculature where Mg^{2+} has been shown to lower arterial pressure by causing significant vasodilation of intact arterioles and venules[80]. There is an important association between blood pressure and heart rate, as MAP declines, a baroreceptor-mediated reflexive increase in HR to maintain cardiac output is the usual result[84]. As opposed to the administration of beta 1-selective blockers, with the administration of $MgSO_4$, there was no decrease in heart rate, but rather an increase in heart rate; which is consistent with a baroreceptor mediated reflex. There was an increase in heart rate at the 5 min time point and nearly significant increase in heart rate at the 15 min time point, and an increase in heart rate across treatment, but not as significant as anticipated. This diminished effect on heart rate could be due to the fact $MgSO_4$ has also been shown to have a sympatholytic effect by inhibiting norepinephrine release[85], and this mechanism could also be contributing to decreasing blood pressure independent of peripheral vasodilation.

While heart rate was mildly increased, there were very relevant changes identified with the ECG intervals. It's important to note that the ECG interval analysis was conducted using a very sensitive analysis program, capable of more sensitive analysis of interval duration than by visual observation or manual measurement using an ECG ruler. This software is capable of measuring to the thousands of a second as compared to the tenths of a second common with manual measurement. When viewing the ECG

waveforms in real time, no differences were noticeable to any of the investigators. It was only through the high resolution and sensitivity of the ECG analysis program that these significant interval conduction time changes were detectable. These ECG intervals represent the conduction time of electrical impulses across the atria, through the atrioventricular node (AV) and the bundle of His, and across the ventricles. Changes to these intervals can be representative of a delay in the transmission of the electrical impulses through the heart.

The shortening of the RR interval was statistically different between $MgSO_4$ and 0.9% saline for multiple early time points ($P<0.01$). The RR interval might be a more sensitive indicator of HR changes as it measures time from beat to beat as opposed to number of beats, which could be less sensitive due to the low heart rate of horses. While The RR interval decreased following $MgSO_4$ administration, the PR and QTc intervals increased. In similar studies involving $MgSO_4$, increased the PR and QRS intervals were detected[86-89]. The PR interval in the $MgSO_4$ treatment group increased from a baseline of 315 ±10.6 to 344 ±13.3 ms by 60 min, and reflected a change in the atrioventricular nodal conduction time[88]. It has been proposed that the mechanism of action for altering the conduction times could be direct, such as the blockage of calcium channels[81], which is time and voltage dependent[83], or as an indirect mechanism of altering peripheral vascular resistances and autonomic tone. The alteration of electrical conductance in the heart explains why $MgSO_4$ has been used as an antiarrhythmic in humans to treat torsade de pointes[90,91] and other ventricular arrhythmias[92,93]. $MgSO_4$ possesses many properties that effect the electrophysiological functioning of the heart, but potentially the most relevant

antiarrhythmic activity is the inhibition of calcium channels[94,95]. QT intervals typically shorten during tachycardia and extend during bradycardia, and represents ventricular depolarization and repolarization[96]. HR can create variability in measuring the QT interval; it is necessary to correct for HR, and in this study, Bazett's formula was utilized to generate a QT corrected value (QTc). The prolongation of the QTc in the $MgSO_4$ treatment group was most likely due to Mg^{2+} blocking the influx of Ca^{2+} into the ventricular cardiomyocytes and extending the rapid depolarization phase. As previously, stated similar studies in other species identified increases in PR and QRS intervals following $MgSO_4$ administration, with no increase in QTc intervals. In our study, the QTc interval was significantly increased in the $MgSO_4$ treatment group from a baseline of 447 ±8.7 to 474 ±9.1 ms then returned to baseline values by 60 min. While prolongation of the QTc can be congenital or acquired through drug induction, there were no visible ventricular arrhythmias or visual changes in duration that could be considered as dangerous or as a prelude to ventricular tachycardia or fibrillation. It has been reported from the field that some horses have collapsed and even succumbed to intravenous administration of $MgSO_4$ following very rapid infusions; for this reason, the 5 min duration of infusion was used for this study. However, it is possible that the changes in ECG intervals for a 5 min infusion could be even more pronounced and potentially fatal with a very rapid infusion, and account for the reports from the field.

As total magnesium is not active, only Mg^{2+} was monitored. The changes in Mg^{2+} and Ca^{2+} observed in this experiment were expected and consistent with previous research documenting experimentally induced hypermagnesemia[44,49]. As a result of the

administration of $MgSO_4$ in this study, there were obvious and expected changes in the plasma concentration of Mg^{2+}, Ca^{2+} and the ratio of Ca^{2+} to Mg^{2+}, some cardiovascular variables, as well as, changes in activity and observed behavior. The increase in Mg^{2+} remained significantly elevated for 3 hours, which allows for its use in regulatory control. The decrease in Ca^{2+} persisted for 5 hours and the ratio of Ca^{2+} to Mg^{2+} remained depressed for 5 hours. The increase in Mg^{2+} and decrease in Ca^{2+} both obviously contributed to the change in their ratio. In reported literature, the decrease in Ca^{2+} following $MgSO_4$ was not as significant as reported here, or if it was, did not decline for this extended period of time[44,97] One explanation is a lower dose of $MgSO_4$ was administered (40mg/kg)[44]. In dogs, the changes associated with concentrations of Mg^{2+} and Ca^{2+} have been found to be dependent upon the dose of $MgSO_4$ administered as a continuous infusion or as a bolus[76].

The $MgSO_4$ treatment group of horses demonstrated less activity with little variability, while the control group had increased activity coinciding with the end of the 0.9% NaCl injection. It is important to acknowledged that the increase in activity for the control group occurred immediately after the horses were turned loose following the 5 min administration. Members of the research team who were blinded to treatment, described the movement of the control group as "searching" or "exploring" in the context of a novel environment. Additionally, the control group of horses had a great deal of variability over the first hour of the experiment. The goal in observing the accelerometers was not to analyze differences between the separate planes, but to evaluate overall activity. Following the introduction to the novel environment, the

activity level for the control group decreased around 2 hours. Beyond the 2 hour time point, the activity levels between the two groups was very similar, so it was difficult to differentiate between the two groups. This timing is consistent with the desired effect competitors are looking for; 2 hrs following the administration of $MgSO_4$ the locomotion of the two treatment groups was not different.

The behavior of the horses was observed by research team members blinded to treatment. Of the three subjectively recorded observation categories, ear movement was the only one statistically significant with the control group having a more a greater number of ear movements over a 30 second observation period. While the research team was blinded to treatment group, it became obvious to them which horses had received $MgSO_4$. In humans, the intravenous administration of 6 grams of 4% $MgSO_4$ (over 6 min) resulted in patients reporting a sensation of increased warmth with flushing and sweating[98]. These clinical signs were associated with a transient decrease in blood pressure[98]. In our study, three of the five horses administered $MgSO_4$, were observed to exhibit a combination of licking and chewing behavior during the administration or immediately following the conclusion of the administration.

The use of a novel telemetric approach for the acquisition of physiologic signals had an impact on the analysis of the pharmacokinetics of Mg^{2+} following the administration of $MgSO_4$. In an attempt to limit human-horse interactions, fewer time points were assigned for blood collection. As a result, there were fewer data points available from the semi-log concentration-time plot. It is typical to fit at least three of the final data points to a linear regression; more is better if possible to maximize r^2. For this

study the geometric mean of data points used for the determination of the terminal elimination rate constant (Lambda_z; K_{el}) was 4.2 with a minimum of 3 and a maximum of 6. The K_{el} reported in this study was the geometric mean of 0.187 mmol/hr with a minimum of 0.119 and maximum of 0.29. This value is slightly higher than the 0.13 mmol/hr reported in previous work by these authors (submitted paper) and can be explained by the reduction in terminal data points. The terminal half-life of the terminal phase (HL_Lambda z; $t_{1/2}$) reported in this study was the geometric mean of 3.7 hours with a minimum of 2.4 and maximum of 5.8 hours, and previously reported values with the same administration strategy, but different collection time points, was 5.5 ±0.65SD hours. In humans the terminal half-life for intravenous Mg^{2+} has been reported to be around 3 hours[56,99]. As $t_{1/2}$ is dependent upon the clearance of the drug and the volume of distribution, the increased Cl observed for this study can account for part of the decrease in $t_{1/2}$; as clearance increases, there is less 'drug' in the plasma at each of the successive time points. With fewer time points available, the AUC_{last} is impacted and can represent an underestimation of the true value when using the log-linear trapezoidal method. As AUC and Cl are inversely related, a lower AUC can provide a higher Cl and therefore a lower $t_{1/2}$. Overall, the pharmacokinetic values reported for a single intravenous bolus of 60 mg/kg $MgSO_4$ are similar to previous work in horses.

Conclusion

This study identified a decrease in blood pressure as a result of a 60 mg/kg intravenous bolus of $MgSO_4$. Concurrently, increases in HR were most likely due the baroreceptor reflex. The changes in PR and QTc intervals were most likely due to a

direct effect of the plasma Mg^{2+} increase had on the reduction of cardiac electrical conduction through the blockade of Ca^{2+} channels. The decrease in locomotor activity and observed behavior were obvious and compelling considering the higher HR's were associated with the horses with the lower locomotor activity; horses in the control group demonstrated an increase in activity with greater variability but had lower HR's, particularly early on after exposure to the new environment.

Horses in this study did not appear to be depressed but rather had a diminished interest in their surroundings. It is likely the similar decrease in blood pressure, following intravenous $MgSO_4$ administration, could generate behavior changes or a resistance to the distraction of environmental stimuli as evidenced by the lack of locomotor activity in the $MgSO_4$ group. When the control horses were left loose following the administration of the identical volume of 0.9% NaCl, they exhibited a searching or investigatory behavior not observed with the $MgSO_4$ treatment group. From a competition standpoint, the ability of horses to ignore environmental distractions of stimuli would provide a competitive advantage. Additionally, the administration of $MgSO_4$ provided changes in Mg^{2+} concentration and the ratio of Ca^{2+} to Mg^{2+} that could provide support for regulatory control through the analysis of post competition plasma samples.

Our data supported that intravenous administration of $MgSO_4$, can induce a calming effect in the horse, and the potential mechanism could be a decrease in blood pressure, or sympathetic reduction[85], or a combination of mechanisms. In concert with our prior data, demonstrating no increase in Mg^{2+} in the CNS and its similarity in calming

seen with other vasodilators, the reduction in blood pressure and sympathetic tone, appear to be the most likely mechanism of calming seen in horses.

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Appendix A. Cerebrospinal fluid from horses administered 60 mg/kg $MgSO_4$.

	0 min	2 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min
pH (H+)	7.501 ± 0.02	7.541 ± 0.02	7.519 ± 0.02	7.538 ± 0.06	7.494 ± 0.02	7.526 ± 0.02	7.497 ± 0.01	7.500 ± 0.02	7.533 ± 0.03
pCO2 (mmHg)	24.3 ± 1.8	18.8 ± 1.8	21.7 ± 2.9	22.7 ± 5.2	25.1 ± 3.0	22.3 ± 2.8	25.4 ± 2.0	23.6 ± 1.4	21.8 ± 3.1
pO2 (mmHg)	181.7 ± 3.7	189.9 ± 1.9	175.6 ± 9.1	169.9 ± 14.9	161.7 ± 12.4	175.5 ± 9.7	163.9 ± 7.6	163.2 ± 7.9	178.9 ± 9.4
Na+ (mmol/L)	136.8 ± 0.62	137.1 ± 0.15	136.7 ± 0.54	136.6 ± 0.57	136.6 ± 0.58	136.7 ± 0.43	136.8 ± 0.47	136.8 ± 0.56	136.5 ± 0.47
K+ (mmol/L)	2.84 ± 0.03	2.87 ± 0.04	2.81 ± 0.03	2.98 ± 0.17	2.82 ± 0.03	2.82 ± 0.04	2.80 ± 0.03	2.81 ± 0.03	2.80 ± 0.02
Cl- (mmol/L)	118.1 ± 1.01	118.7 ± 0.57	117.6 ± 0.83	117.8 ± 1.06	117.7 ± 1.47	117.8 ± 1.15	117.4 ± 0.88	117.6 ± 1.03	117.7 ± 0.99
Ca++ (mg/dl)	4.03 ± 0.04	3.91 ± 0.05	4.04 ± 0.04	3.88 ± 0.12	4.01 ± 0.05	3.99 ± 0.03	4.02 ± 0.03	4.01 ± 0.04	3.99 ± 0.03
Mg++ (mg/dl)	1.38 ± 0.03	1.31 ± 0.03	1.36 ± 0.03	1.32 ± 0.04	1.33 ± 0.02	1.36 ± 0.03	1.37 ± 0.03	1.36 ± 0.03	1.37 ± 0.02
Glu (mg/dl)	53.5 ± 2.1	54.5 ± 3.1	54.3 ± 2.1	56.6 ± 2.3	55.3 ± 3.1	55.0 ± 2.4	55.3 ±2.4	54.8 ± 2.7	56.5 ± 2.7
Lac (mmol/L)	2.37 ± 0.14	2.38 ± 0.20	2.50 ± 0.14	2.60 ± 0.09	2.40 ± 0.20	2.47 ± 0.13	2.43 ± 0.13	2.38 ± 0.14	2.42 ± 0.12
BUN	10.4 ± 1.6	9.7 ± 2.4	10.3 ± 1.5	9.9 ± 1.8	12.0 ± 0.4	10.3 ± 1.5	10.3 ± 1.5	12.0 ± 0.3	10.3 ± 1.5
Creat	0.73 ± 0.03	0.75 ± 0.06	0.75 ± 0.02	0.70 ± 0.03	0.80 ± 0.04	0.77 ± 0.03	0.73 ± 0.03	0.76 ± 0.02	0.77 ± 0.02
nCa++ (mg/dl)	4.26 ± 0.07	4.24 ± 0.11	4.32 ± 0.07	4.20 ± 0.21	4.21 ± 0.06	4.28 ± 0.06	4.24 ± 0.05	4.25 ± 0.07	4.29 ± 0.05
nMg++ (mg/dl)	1.48 ± 0.05	1.45 ± 0.05	1.48 ±0.05	1.46 ± 0.08	1.41 ± 0.03	1.48 ± 0.04	1.46 ± 0.03	1.45 ± 0.03	1.50 ± 0.04
Ca++/Mg++	1.78 ± 0.05	1.83 ± 0.03	1.82 ± 0.05	1.78 ± 0.04	1.85 ± 0.03	1.78 ± 0.03	1.80 ± 0.04	1.82 ± 0.04	1.78 ± 0.03
BUN/Creat	16.3 ± 0.48	15.3 ± 0.73	15.8 ± 0.37	16.4 ± 0.23	15.3 ± 0.72	15.6 ± 0.44	16.0 ± 0.52	15.6 ± 0.40	15.4 ± 0.29
HC03 (mmol/L)	18.9 ± 0.79	16.1 ± 0.89	17.6 ± 1.52	17.8 ± 2.46	19.2 ± 1.48	18.2 ± 1.31	19.7 ± 1.07	18.6 ± 0.51	17.8 ± 1.47
Osm (mOsm)	270.6 ± 1.2	271.2 ± 0.3	270.5 ± 1.0	270.3 ± 1.0	270.5 ± 1.0	270.5 ± 0.8	270.7 ± 0.8	270.6 ± 1.0	270.2 ± 0.8

	90 min	120 min	150 min	180 min	240 min	300 min	360 min	12 hr	24 hr
pH (H+)	7.482 ± 0.02	7.511 ± 0.03	7.468 ± 0.04	7.530 ± 0.02	7.520 ± 0.02	7.494 ± 0.02	7.494 ± 0.01	7.487 ± 0.03	7.501 ± 0.03
pCO2 (mmHg)	26.6 ± 3.0	24.4 ± 3.6	30.9 ± 5.3	21.4 ± 2.5	22.8 ± 1.7	25.6 ± 2.0	26.0 ± 1.9	27.9 ± 6.9	25.5 ± 3.3
pO2 (mmHg)	153.0 ± 19.5	180.3 ± 31.1	166.3 ± 60.9	201.8 ± 14.5	192.9 ± 13.0	188.3 ± 18.1	194.6 ± 10.5	16.8 ± 34.5	146.5 ± 14.3
Na+ (mmol/L)	136.4 ± 0.63	135.2 ± 1.55	137.1 ± 0.50	136.8 ± 0.09	136.5 ± 0.60	136.3 ± 0.57	135.9 ± 0.71	134.6 ± 2.45	137.9 ± 0.76
K+ (mmol/L)	2.81 ± 0.03	2.82 ± 0.07	2.92 ± 0.16	2.78 ± 0.05	2.77 ± 0.04	2.75 ± 0.04	2.75 ± 0.04	2.93 ± 0.16	2.88 ± 0.04
Cl- (mmol/L)	117.5 ± 1.72	116.4 ± 2.28	114.7 ± 4.63	118.2 ± 0.59	117.1 ± 0.85	117.4 ± 1.00	117.6 ± 1.39	115.5 ± 4.35	118.4 ± 1.19
Ca++ (mg/dl)	3.98 ± 0.04	4.24 ± 0.26	4.49 ± 0.52	3.98 ± 0.07	4.02 ± 0.02	3.99 ± 0.02	3.99 ± 0.04	4.22 ± 0.21	4.10 ± 0.04
Mg++ (mg/dl)	1.34 ± 0.02	1.43 ± 0.06	1.43 ± 0.08	1.35 ± 0.03	1.38 ± 0.01	1.37 ± 0.02	1.36 ± 0.03	1.37 ± 0.0	1.40 ± 0.02
Glu (mg/dl)	57.3 ± 3.4	65.0 ± 8.5	78.0 ± 15.0	57.2 ± 3.3	58.7 ± 2.9	58.2 ± 3.4	58.2 ± 3.4	63.5 ± 5.5	54.7 ± 2.3
Lac (mmol/L)	2.30 ± 0.16	2.18 ± 0.23	1.93 ± 0.47	2.36 ± 0.13	2.48 ± 0.13	2.42 ± 0.12	2.42 ± 0.12	2.75 ± 0.05	2.42 ± 0.12
BUN	12.0 ± 0.4	10.6 ± 1.7	13.0 ± 1.2	9.9 ± 1.8	10.3 ± 1.5	11.6 ± 0.5	11.6 ± 0.2	12.0 ± 1.0	10.5 ± 1.6
Creat	0.75 ± 0.03	0.78 ± 0.03	0.77 ± 0.03	0.78 ± 0.04	0.80 ± 0.0	0.80 ± 0.03	0.78 ± 0.02	0.85 ± 0.15	0.82 ± 0.03
nCa++ (mg/dl)	4.16 ± 0.04	4.50 ± 0.22	4.64 ± 0.44	4.27 ± 0.08	4.29 ± 0.06	4.21 ± 0.05	4.21 ± 0.04	4.42 ± 0.14	4.33 ± 0.07
nMg++ (mg/dl)	1.42 ± 0.02	1.54 ± 0.04	1.47 ± 0.04	1.48 ± 0.03	1.50 ± 0.02	1.46 ± 0.03	1.45 ± 0.03	1.46 ± 0.03	1.50 ± 0.04
Ca++/Mg++	1.80 ± 0.04	1.80 ± 0.04	1.87 ± 0.12	1.78 ± 0.02	1.77 ± 0.02	1.76 ± 0.02	1.78 ± 0.04	1.90 ± 0.10	1.78 ± 0.03
BUN/Creat	15.8 ± 0.59	15.7 ± 0.84	17.3 ± 0.63	14.9 ± 0.79	14.9 ± 0.44	15.0 ± 0.65	14.9 ± 0.52	14.5 ± 1.30	14.9 ± 0.45
HC03 (mmol/L)	19.9 ± 1.43	19.0 ± 1.35	19.7 ± 1.65	17.7 ± 1.38	18.6 ± 0.60	19.7 ± 0.94	20.1 ± 0.97	20.9 ± 3.90	19.5 ± 1.51
Osm (mOsm)	270.1 ± 1.1	271.4 ± 1.0	273.0 ± 2.1	270.8 ± 0.2	270.1 ± 1.1	269.9 ± 1.2	269.2 ± 1.2	267.1 ± 3.8	272.8 ± 1.4

Appendix B. Plasma electrolytes and chemistry for horses administered 60 mg/kg $MgSO_4$ and equal volume of 0.9% NaCl (control).

0.9% NaCl Control	Baseline	5 Min	15 Min	30 Min	60 Min	2 Hr
pH	7.48 ± 0.01	7.49 ± 0.01	7.48 ± 0	7.49 ± 0.01	7.49 ± 0.01	7.5 ± 0.01
pCO_2	34.46 ± 0.91	33.5 ± 0.64	34.02 ± 1.11	34 ± 1.25	33.8 ± 1.28	34.36 ± 1.12
pO_2	101.8 ± 9.83	94.2 ± 7.08	90.5 ± 7.58	96.48 ± 10.92	107.9 ± 20.08	126.72 ± 13.8
$S0_2$%	97.78 ± 0.88	97.62 ± 0.7	97.14 ± 0.94	96.9 ± 1.68	97.52 ± 1.04	98.9 ± 0.36
Na^+	132.2 ± 1.4	131.7 ± 1.13	133.1 ± 0.77	132.62 ± 1.33	132.64 ± 0.72	131.84 ± 1.41
K^+	4.74 ± 0.17	4.31 ± 0.14	4.28 ± 0.14	4.18 ± 0.11	4.08 ± 0.08	4.27 ± 0.25
Cl^-	106.86 ± 0.57	107.56 ± 0.79	106.04 ± 1.02	106.65 ± 0.96	106.3 ± 1.23	106.82 ± 0.67
Ca^{2+} (mol)	1.42 ± 0.02	1.41 ± 0.01	1.41 ± 0.03	1.39 ± 0.02	1.39 ± 0.03	1.38 ± 0.01
Mg^{2+} (mol)	0.55 ± 0.02	0.53 ± 0.02	0.54 ± 0.03	0.53 ± 0.02	0.52 ± 0.02	0.49 ± 0.01
Ca^{2+} /Mg^{2+} (mol)	2.57 ± 0.08	2.69 ± 0.09	2.65 ± 0.12	2.66 ± 0.12	2.68 ± 0.09	2.8 ± 0.07
Glu	84.6 ± 4.45	87.4 ± 6.92	87.6 ± 6.63	87.6 ± 6.87	87.6 ± 6.9	88.4 ± 5.63
Lac	0.9 ± 0.1	0.82 ± 0.07	0.8 ± 0.07	0.82 ± 0.07	0.78 ± 0.08	0.7 ± 0.07
TCO_2	27.02 ± 0.3	26.78 ± 0.35	26.84 ± 0.76	27.06 ± 0.7	27.02 ± 0.76	28 ± 0.7
nCa (mol)	1.48 ± 0.03	1.48 ± 0.01	1.48 ± 0.03	1.46 ± 0.03	1.46 ± 0.03	1.46 ± 0.02
nMg (mol)	0.59 ± 0.02	0.56 ± 0.02	0.57 ± 0.03	0.56 ± 0.03	0.55 ± 0.02	0.53 ± 0.02
Ca^{2+} /Mg^{2+} (mol)	2.54 ± 0.08	2.66 ± 0.09	2.61 ± 0.11	2.63 ± 0.12	2.66 ± 0.09	2.77 ± 0.07
$HC0_3$	25.96 ± 0.28	25.72 ± 0.35	25.74 ± 0.73	26.02 ± 0.67	25.98 ± 0.72	26.94 ± 0.66
A	103.06 ± 1.36	104.2 ± 0.93	103.56 ± 1.56	103.58 ± 1.7	103.84 ± 1.57	102.94 ± 1.51
Tot Calcium (mg/dL)	11.66 ± 0.19	11.32 ± 0.17	11.34 ± 0.17	11.24 ± 0.17	11.24 ± 0.22	10.98 ± 0.18
Ca^{2+} (mmol/L)	2.92 ± 0.05	2.83 ± 0.04	2.84 ± 0.04	2.81 ± 0.04	2.81 ± 0.05	2.75 ± 0.05
Tot Magnesium (mg/dL)	1.82 ± 0.07	1.72 ± 0.12	1.72 ± 0.12	1.68 ± 0.09	1.64 ± 0.09	1.6 ± 0.07
Mg^{2+} (mmol/L)	0.75 ± 0.03	0.71 ± 0.05	0.71 ± 0.05	0.69 ± 0.04	0.67 ± 0.04	0.66 ± 0.03

0.9% NaCl Control	3 Hr	4 Hr	5 Hr	6 Hr	12 Hr	24 Hr
pH	7.49 ± 0.01	7.49 ± 0.01	7.49 ± 0.01	7.49 ± 0.01	7.45 ± 0.01	7.45 ± 0.01
pCO_2	34.24 ± 1.35	33.94 ± 0.97	33.36 ± 1.01	34.3 ± 1.24	35.08 ± 0.81	36.78 ± 1.15
pO_2	121.05 ± 18.19	118.78 ± 11.32	110.2 ± 21.69	112.6 ± 10.77	92.14 ± 10.28	92.88 ± 16.03
$S0_2$%	98.6 ± 0.36	98.74 ± 0.33	98.8 ± 0.41	98.58 ± 0.32	96.76 ± 1.07	94.18 ± 3.74
Na^+	132.38 ± 1.6	132.52 ± 1.28	133.34 ± 0.99	132.6 ± 1.43	132.4 ± 0.8	132.36 ± 1.13
K^+	4.15 ± 0.21	4.2 ± 0.25	4.14 ± 0.2	3.92 ± 0.12	4.88 ± 0.17	4.23 ± 0.22
Cl^-	106.32 ± 0.55	86.66 ± 19.06	105.42 ± 0.86	106.24 ± 0.91	106.16 ± 1.05	108.66 ± 0.9
Ca^{2+} (mol)	1.41 ± 0.02	1.38 ± 0.02	1.38 ± 0.02	1.37 ± 0.02	1.44 ± 0.02	1.46 ± 0.02
Mg^{2+} (mol)	0.51 ± 0.01	0.5 ± 0.01	0.5 ± 0.01	0.49 ± 0.01	0.58 ± 0.02	0.56 ± 0.02
Ca^{2+} /Mg^{2+} (mol)	2.79 ± 0.07	2.76 ± 0.05	2.77 ± 0.05	2.79 ± 0.08	2.5 ± 0.13	2.62 ± 0.12
Glu	89 ± 5.5	90.6 ± 5.87	91.8 ± 5.73	91.2 ± 5.54	97.2 ± 5.49	90.2 ± 5.59
Lac	0.66 ± 0.08	0.74 ± 0.12	0.72 ± 0.07	0.68 ± 0.07	1.03 ± 0.23	0.84 ± 0.07
TCO_2	27.26 ± 0.62	27.18 ± 0.44	26.52 ± 0.51	27.36 ± 0.41	25.62 ± 0.76	26.9 ± 0.58
nCa (mol)	1.48 ± 0.02	1.45 ± 0.02	1.45 ± 0.02	1.44 ± 0.02	1.48 ± 0.02	1.5 ± 0.02
nMg (mol)	0.54 ± 0.01	0.53 ± 0.01	0.53 ± 0.01	0.53 ± 0.01	0.6 ± 0.02	0.58 ± 0.02
Ca^{2+} /Mg^{2+} (mol)	2.76 ± 0.07	2.73 ± 0.04	2.74 ± 0.05	2.75 ± 0.07	2.48 ± 0.13	2.6 ± 0.11
$HC0_3$	26.22 ± 0.59	26.12 ± 0.45	25.48 ± 0.47	26.34 ± 0.39	24.58 ± 0.75	25.78 ± 0.57
A	103.06 ± 1.79	103.36 ± 1.31	104.04 ± 1.36	102.92 ± 1.61	101.8 ± 1.22	99.72 ± 1.51
Tot Calcium (mg/dL)	11.32 ± 0.21	11.16 ± 0.25	11.08 ± 0.24	11.12 ± 0.25	11.66 ± 0.08	11.78 ± 0.16
Ca^{2+} (mmol/L)	2.83 ± 0.05	2.79 ± 0.06	2.77 ± 0.06	2.78 ± 0.06	2.92 ± 0.02	2.95 ± 0.04
Tot Magnesium (mg/dL)	1.64 ± 0.04	1.56 ± 0.05	1.54 ± 0.04	1.54 ± 0.02	1.82 ± 0.07	1.82 ± 0.09
Mg^{2+} (mmol/L)	0.67 ± 0.02	0.64 ± 0.02	0.63 ± 0.02	0.63 ± 0.01	0.75 ± 0.03	0.75 ± 0.04

60 mg/kg MgSO4	Baseline	5 Min	15 Min	30 Min	60 Min	2 Hr
pH	7.48 ± 0.01	7.47 ± 0.01	7.48 ± 0.01	7.48 ± 0.01	7.48 ± 0.01	7.49 ± 0.01
pCO_2	34.32 ± 1.01	34.34 ± 0.7	34.2 ± 0.86	33.88 ± 0.6	33.82 ± 0.54	35.86 ± 0.92
pO_2	110.7 ± 19.99	109.46 ± 13.65	93.82 ± 11.46	82.7 ± 7.29	91.1 ± 10.65	88.94 ± 8.57
$S0_2$%	97.58 ± 0.94	97.84 ± 0.93	97.06 ± 0.92	96.6 ± 0.91	97 ± 0.92	96.84 ± 1.11
Na^+	131.66 ± 1.03	130.18 ± 1.1	130.44 ± 0.98	131.1 ± 1.11	131.32 ± 1.29	130.68 ± 0.92
K^+	4.63 ± 0.26	4.16 ± 0.22	4.11 ± 0.21	4.08 ± 0.19	3.91 ± 0.15	4.54 ± 0.39
Cl^-	106.9 ± 0.49	105.58 ± 0.48	106.4 ± 0.96	105.52 ± 1.02	105.2 ± 0.89	105.32 ± 1.14
Ca^{2+} (mol)	1.43 ± 0.03	1.43 ± 0.03	1.38 ± 0.03	1.32 ± 0.01	1.33 ± 0.03	1.35 ± 0.02
Mg^{2+} (mol)	0.56 ± 0.03	2.2 ± 0.05	1.38 ± 0.03	1.12 ± 0.03	0.96 ± 0.04	0.82 ± 0.01
Ca^{2+} /Mg^{2+} (mol)	2.56 ± 0.13	0.65 ± 0.02	1 ± 0.01	1.18 ± 0.03	1.39 ± 0.03	1.66 ± 0.02
Glu	85 ± 2.81	88.2 ± 2.4	90.4 ± 2.98	90.2 ± 2.84	92 ± 3.7	90.8 ± 3.65
Lac	0.88 ± 0.08	0.84 ± 0.11	0.84 ± 0.14	0.8 ± 0.11	0.92 ± 0.09	0.76 ± 0.14
TCO_2	26.98 ± 0.7	26.04 ± 0.62	26.58 ± 0.63	26.6 ± 0.31	26.42 ± 0.59	28.36 ± 0.62
nCa (mol)	1.5 ± 0.03	1.49 ± 0.03	1.44 ± 0.02	1.39 ± 0.02	1.39 ± 0.03	1.42 ± 0.02
nMg (mol)	0.6 ± 0.04	2.24 ± 0.04	1.46 ± 0.04	1.19 ± 0.04	1.01 ± 0.04	0.86 ± 0.01
Ca^{2+} /Mg^{2+} (mol)	2.53 ± 0.13	0.66 ± 0.01	0.99 ± 0.01	1.17 ± 0.03	1.38 ± 0.03	1.64 ± 0.02
$HC0_3$	25.9 ± 0.68	24.98 ± 0.61	25.58 ± 0.63	25.54 ± 0.31	25.38 ± 0.57	27.24 ± 0.59
A	103.14 ± 1.33	103.1 ± 0.86	103.22 ± 1.1	103.64 ± 0.8	103.74 ± 0.67	101.02 ± 1.17
Tot Calcium (mg/dL)	11.78 ± 0.24	11.3 ± 0.2	10.88 ± 0.2	10.38 ± 0.14	10.44 ± 0.17	10.48 ± 0.13
Ca^{2+} (mmol/L)	2.95 ± 0.06	2.83 ± 0.05	2.72 ± 0.05	2.6 ± 0.03	2.61 ± 0.04	2.62 ± 0.03
Tot Magnesium (mg/dL)	1.82 ± 0.11	7.5 ± 0.13	5.38 ± 0.18	4.32 ± 0.15	3.62 ± 0.12	2.94 ± 0.09
Mg^{2+} (mmol/L)	0.75 ± 0.04	3.08 ± 0.05	2.21 ± 0.07	1.78 ± 0.06	1.49 ± 0.05	1.21 ± 0.04

60 mg/kg MgSO4	3 Hr	4 Hr	5 Hr	6 Hr	12 Hr	24 Hr
pH	7.49 ± 0.01	7.49 ± 0.01	7.48 ± 0.01	7.48 ± 0	7.45 ± 0	7.47 ± 0.01
pCO_2	35.08 ± 0.76	34.24 ± 1.39	34.34 ± 1.46	34.72 ± 0.66	36.82 ± 0.9	35.42 ± 1.77
pO_2	109.08 ± 13.88	99.88 ± 17.54	112.36 ± 18.87	97.28 ± 13.89	104.66 ± 21.15	110.66 ± 15.63
$S0_2$%	98.1 ± 0.71	97.3 ± 0.81	97.86 ± 0.84	97.28 ± 0.88	96.66 ± 1.25	97.98 ± 0.74
Na^+	130.82 ± 1.32	131.14 ± 1.16	132.44 ± 0.81	132.86 ± 1.06	132.96 ± 0.73	132.04 ± 1.08
K^+	4.32 ± 0.39	4.45 ± 0.29	4.31 ± 0.23	4.05 ± 0.17	4.73 ± 0.2	4.32 ± 0.09
Cl^-	106.14 ± 1.09	105.82 ± 0.78	104.7 ± 0.79	105.14 ± 0.48	105.66 ± 0.75	107.06 ± 0.73
Ca^{2+} (mol)	1.35 ± 0.04	1.34 ± 0.03	1.36 ± 0.03	1.37 ± 0.04	1.45 ± 0.02	1.44 ± 0.03
Mg^{2+} (mol)	0.75 ± 0.02	0.69 ± 0.02	0.67 ± 0.02	0.63 ± 0.02	0.62 ± 0.02	0.57 ± 0.03
Ca^{2+} /Mg^{2+} (mol)	1.8 ± 0.03	1.95 ± 0.04	2.04 ± 0.05	2.16 ± 0.08	2.34 ± 0.05	2.54 ± 0.14
Glu	91.8 ± 3.92	94.4 ± 5.84	93.8 ± 5.96	92.8 ± 5.33	97.6 ± 5.19	92.2 ± 4.08
Lac	0.74 ± 0.1	0.76 ± 0.08	0.8 ± 0.08	0.8 ± 0.08	1 ± 0.2	0.84 ± 0.1
TCO_2	28.14 ± 0.47	27.48 ± 0.92	26.88 ± 0.84	27.24 ± 0.62	27.2 ± 0.73	26.8 ± 0.74
nCa (mol)	1.42 ± 0.04	1.41 ± 0.04	1.42 ± 0.03	1.43 ± 0.04	1.5 ± 0.02	1.49 ± 0.02
nMg (mol)	0.8 ± 0.02	0.73 ± 0.02	0.71 ± 0.01	0.67 ± 0.02	0.65 ± 0.02	0.6 ± 0.03
Ca^{2+} /Mg^{2+} (mol)	1.78 ± 0.03	1.92 ± 0.03	2.02 ± 0.04	2.13 ± 0.07	2.32 ± 0.05	2.52 ± 0.14
$HC0_3$	27.08 ± 0.46	26.44 ± 0.89	25.84 ± 0.79	26.14 ± 0.59	26.06 ± 0.71	25.72 ± 0.7
A	101.9 ± 1.04	102.9 ± 1.65	102.72 ± 1.74	102.28 ± 0.75	99.66 ± 1.25	101.23 ± 3
Tot Calcium (mg/dL)	10.82 ± 0.25	11.02 ± 0.18	10.92 ± 0.31	9.5 ± 1.83	11.75 ± 0.21	11.62 ± 0.09
Ca^{2+} (mmol/L)	2.71 ± 0.06	2.76 ± 0.04	2.73 ± 0.08	2.38 ± 0.46	2.35 ± 0.59	2.91 ± 0.02
Tot Magnesium (mg/dL)	2.68 ± 0.07	2.44 ± 0.05	2.26 ± 0.07	3.66 ± 1.56	1.95 ± 0.06	1.82 ± 0.09
Mg^{2+} (mmol/L)	1.1 ± 0.03	1 ± 0.02	0.93 ± 0.03	1.5 ± 0.64	0.64 ± 0.16	0.75 ± 0.04

Appendix C. Blood pressure variables for horses administered 60 mg/kg $MgSO_4$ and equal volume of 0.9% NaCl (control).

0.9% NaCl Control Group	Baseline	5 Min	15 Min	30 Min	60 Min	2 Hr
Beat to Beat (ms)	1,714 ± 119.7	1,879 ± 138.5	1,841 ± 128.1	1,849 ± 163.5	1,929 ± 128.8	1,925 ± 148.5
HR (bpm)	37 ± 2.8	33 ± 2.3	33 ± 2.1	37 ± 6.7	32 ± 2.1	32 ± 2.5
dp/dt+ (mmHg/s)	597 ± 132.4	569 ± 144.1	461 ± 54.7	454 ± 55.0	455 ± 47.7	414 ± 76.6
SBP (mmHg)	118 ± 5.9	116 ± 7.1	112 ± 8.2	115 ± 6.9	121 ± 7.5	119 ± 9.0
DBP (mmHg)	75 ± 3.5	74 ± 5.1	71 ± 5.7	75 ± 6.1	79 ± 5.9	77 ± 7.0
MBP (mmHg)	94 ± 4.0	92 ± 5.1	90 ± 5.9	93 ± 6.0	97 ± 6.0	95 ± 6.8
Pulse Pressure (mmHg)	42 ± 3.3	42 ± 3.8	41 ± 3.5	39 ± 2.4	42 ± 3.5	42 ± 3.8
D_DN time	893 ± 44.1	824 ± 29.4	868 ± 66.6	840 ± 49.9	889 ± 42.3	842 ± 53.3
Area under the curve from diastole to diastole	34 ± 3.5	36 ± 3.5	33 ± 3.2	32 ± 3.4	34 ± 2.9	35 ± 3.7
Presure at dicrotic notch (mmHg)	94 ± 2.3	96 ± 3.6	92 ± 3.9	96 ± 4.5	99 ± 4.3	99 ± 6.6
rate x pressure	4 ± 0.4	4 ± 0.5	4 ± 0.5	4 ± 1.0	4 ± 0.5	4 ± 0.4

0.9% NaCl Control Group	3 Hr	4 Hr	5 Hr	6 Hr	12 Hr	24 Hr
Beat to Beat (ms)	1,787 ± 159.0	1,925 ± 149.9	1,967 ± 110.5	1,928 ± 200.7	1,911 ± 110.3	1,768 ± 126.4
HR (bpm)	36 ± 3.0	32 ± 2.7	31 ± 1.7	33 ± 4.4	32 ± 1.6	35 ± 2.3
dp/dt+ (mmHg/s)	475 ± 60.4	436 ± 70.4	413 ± 57.3	711 ± 311.9	475 ± 53.1	514 ± 58.4
SBP (mmHg)	116 ± 6.2	116 ± 6.3	118 ± 6.9	110 ± 7.4	116 ± 6.3	112 ± 5.8
DBP (mmHg)	73 ± 5.5	74 ± 4.0	77 ± 4.7	71 ± 6.4	75 ± 4.5	69 ± 5.3
MBP (mmHg)	91 ± 5.1	92 ± 4.2	93 ± 5.0	88 ± 5.9	93 ± 4.5	88 ± 4.8
Pulse Pressure (mmHg)	42 ± 3.1	42 ± 3.7	41 ± 3.1	40 ± 2.9	41 ± 2.3	43 ± 1.3
D_DN time	878 ± 56.7	835 ± 44.1	945 ± 54.8	841 ± 49.5	823 ± 66.0	852 ± 60.3
Area under the curve from diastole to diastole	33 ± 3.1	34 ± 3.5	35 ± 2.9	35 ± 4.8	33 ± 2.5	32 ± 2.2
Presure at dicrotic notch (mmHg)	92 ± 4.1	95 ± 4.1	96 ± 4.0	92 ± 6.2	96 ± 3.8	89 ± 4.8
rate x pressure	4 ± 0.5	4 ± 0.4	4 ± 0.4	4 ± 0.3	4 ± 0.4	4 ± 0.4

60 mg/kg $MgSO_4$	Baseline	5 Min	15 Min	30 Min	60 Min	2 Hr
Beat to Beat (ms)	1,738 ± 130.2	1,685 ± 134.3	1,671 ± 152.6	1,728 ± 151.2	1,780 ± 123.6	1,816 ± 128.3
HR (bpm)	36 ± 3.2	37 ± 2.5	38 ± 3.4	37 ± 3.4	35 ± 2.7	34 ± 2.4
dp/dt+ (mmHg/s)	523 ± 84.1	588 ± 50.2	579 ± 78.1	503 ± 58.5	455 ± 59.6	489 ± 96.5
SBP (mmHg)	116 ± 3.0	116 ± 6.4	107 ± 4.1	108 ± 4.8	110 ± 4.2	107 ± 5.0
DBP (mmHg)	73 ± 2.8	71 ± 4.9	65 ± 2.9	68 ± 3.2	71 ± 3.3	68 ± 3.4
MBP (mmHg)	92 ± 2.0	91 ± 5.1	84 ± 2.7	86 ± 2.9	88 ± 2.8	85 ± 3.1
Pulse Pressure (mmHg)	43 ± 1.8	44 ± 2.9	42 ± 3.0	40 ± 3.2	39 ± 2.6	39 ± 3.3
D_DN time	889 ± 79.4	882 ± 64.3	811 ± 33.9	755 ± 33.6	782 ± 40.0	819 ± 34.7
Area under the curve from diastole to diastole	34 ± 2.3	33 ± 2.7	31 ± 2.2	30 ± 2.5	30 ± 1.9	30 ± 2.9
Presure at dicrotic notch (mmHg)	93 ± 3.4	91 ± 3.4	85 ± 2.0	88 ± 2.0	90 ± 2.3	87 ± 2.6
rate x pressure	4 ± 0.3	4 ± 0.5	4 ± 0.5	4 ± 0.5	4 ± 0.4	4 ± 0.3

60 mg/kg $MgSO_4$	3 Hr	4 Hr	5 Hr	6 Hr	12 Hr	24 Hr
Beat to Beat (ms)	1,769 ± 205.5	1,739 ± 219.8	1,797 ± 177.3	1,825 ± 182.8	1,855 ± 99.4	1,899 ± 153.4
HR (bpm)	36 ± 5.0	41 ± 8.9	36 ± 4.5	35 ± 4.1	33 ± 2.0	33 ± 3.8
dp/dt+ (mmHg/s)	438 ± 53.8	653 ± 267.8	471 ± 51.7	546 ± 132.3	463 ± 66.1	461 ± 82.3
SBP (mmHg)	111 ± 8.4	118 ± 6.1	115 ± 5.6	116 ± 8.0	118 ± 5.0	111 ± 4.2
DBP (mmHg)	72 ± 5.8	76 ± 4.1	74 ± 4.0	76 ± 6.2	78 ± 3.6	71 ± 4.1
MBP (mmHg)	89 ± 5.8	94 ± 3.9	92 ± 3.4	94 ± 6.0	95 ± 3.5	88 ± 3.2
Pulse Pressure (mmHg)	39 ± 4.4	42 ± 4.7	40 ± 3.3	40 ± 3.3	40 ± 2.5	40 ± 2.3
D_DN time	812 ± 44.6	839 ± 74.7	829 ± 65.6	850 ± 60.4	788 ± 29.7	828 ± 61.1
Area under the curve from diastole to diastole	30 ± 3.7	34 ± 3.4	38 ± 3.4	31 ± 3.0	32 ± 2.5	33 ± 3.2
Presure at dicrotic notch (mmHg)	90 ± 4.2	97 ± 4.6	95 ± 3.3	97 ± 6.4	99 ± 2.7	92 ± 2.9
rate x pressure	4 ± 0.8	5 ± 1.3	4 ± 0.6	4 ± 0.4	4 ± 0.4	4 ± 0.3

Appendix D. Locomotion data for horses administered 60 mg/kg $MgSO_4$ and equal volume of 0.9% NaCl (control).

0.9% NaCl Control	Baseline	5 Min	15 Min	30 Min	60 Min	2 Hr
Acceleration	0.01 ± 0.00	0.39 ± 0.38	0.36 ± 0.36	0.39 ± 0.39	0.38 ± 0.38	0.00 ± 0.00
Accel(x)	-1.23 ± 0.10	-0.63 ± 0.65	-0.69 ± 0.63	-0.60 ± 0.65	-0.70 ± 0.66	-1.39 ± 0.10
Accel(y)	-0.23 ± 0.07	0.03 ± 0.48	-0.01 ± 0.45	-0.01 ± 0.49	0.04 ± 0.47	-0.36 ± 0.08
Accel(z)	1.20 ± 0.13	1.45 ± 0.16	1.42 ± 0.14	1.38 ± 0.19	1.44 ± 0.18	1.22 ± 0.20

60 mg/kg MgSO4	Baseline	5 Min	15 Min	30 Min	60 Min	2 Hr
Acceleration	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Accel(x)	-1.33 ± 0.10	-1.35 ± 0.13	-1.38 ± 0.15	-1.44 ± 0.14*	-1.47 ± 0.12*	-1.56 ± 0.11*
Accel(y)	-0.31 ± 0.12	-0.26 ± 0.13	-0.25 ± 0.09	-0.28 ± 0.12	-0.27 ± 0.12	-0.29 ± 0.12
Accel(z)	1.13 ± 0.12	1.02 ± 0.08*	0.98 ± 0.06*	0.97 ± 0.08*	1.00 ± 0.08	0.95 ± 0.09*

0.9% NaCl Control	3 Hr	4 Hr	5 Hr	6 Hr	12 Hr	24 Hr
Acceleration	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Accel(x)	-1.36 ± 0.09	-1.50 ± 0.12	-1.56 ± 0.09	-1.58 ± 0.12	-1.40 ± 0.09	-1.27 ± 0.06
Accel(y)	-0.29 ± 0.09	-0.32 ± 0.05	-0.37 ± 0.06	-0.30 ± 0.06	-0.15 ± 0.21	-0.42 ± 0.08
Accel(z)	1.11 ± 0.17	1.15 ± 0.18	1.13 ± 0.16	1.09 ± 0.21	1.23 ± 0.14	1.18 ± 0.11
60 mg/kg MgSO4	3 Hr	4 Hr	5 Hr	6 Hr	12 Hr	24 Hr
Acceleration	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Accel(x)	-1.54 ± 0.15†	-1.55 ± 0.18*	-1.52 ± 0.18*	-1.59 ± 0.15*	-1.39 ± 0.10*	-1.42 ± 0.12*
Accel(y)	-0.18 ± 0.16	-0.25 ± 0.14	-0.23 ± 0.14	-0.19 ± 0.14†	-0.20 ± 0.22	-0.15 ± 0.21
Accel(z)	0.94 ± 0.14†	0.85 ± 0.14*	1.00 ± 0.05	0.96 ± 0.10*	1.21 ± 0.06	1.24 ± 0.14

Appendix E. Temperatures of for horses administered 60 mg/kg $MgSO_4$ and equal volume of 0.9% NaCl (control).

0.9% NaCl Control	Baseline	5 Min	15 Min	30 Min	60 Min	2 Hr
Temp °C	36.06 ± 0.39	36.20 ± 0.35	36.14 ± 0.36	36.14 ± 0.29	36.18 ± 0.26	36.03 ± 0.38

60 mg/kg MgSO4	Baseline	5 Min	15 Min	30 Min	60 Min	2 Hr
Temp °C	36.39 ± 0.20	36.43 ± 0.19	36.35 ± 0.18	36.36 ± 0.18	36.46 ± 0.17	36.35 ± 0.27

0.9% NaCl Control	3 Hr	4 Hr	5 Hr	6 Hr	12 Hr	24 Hr
Temp °C	36.09 ± 0.41	36.16 ± 0.35	36.16 ± 0.25	36.09 ± 0.33	36.24 ± 0.30	36.10 ± 0.24
60 mg/kg MgSO4	3 Hr	4 Hr	5 Hr	6 Hr	12 Hr	24 Hr
Temp °C	36.24 ± 0.26	36.35 ± 0.27	36.49 ± 0.22	36.39 ± 0.23	36.63 ± 0.19	36.18 ± 0.33