Effect Of Magnesium Sulfate On Acute Bronchoconstriction In The Equine Asthma Model

Caitlin Jael Wenzel

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Effect of magnesium sulfate on acute bronchoconstriction in the equine asthma model

By

Caitlin Jael Wenzel

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Effect of magnesium sulfate on acute bronchoconstriction in the equine asthma model

By

Caitlin Jael Wenzel

Approved:

________________________
Jacquelyn E. Bowser
(Co-Major Professor)

________________________
Cyprianna E. Swiderski
(Co-Major Professor/Graduate Coordinator)

________________________
Committee Member's Name
(Committee Member)

________________________
Amelia R. Woolums
(Committee Member)

________________________
Lais R. Costa
(Committee Member)

________________________
Mark L. Lawrence
Associate Dean
College of Veterinary Medicine
Asthma is a chronic disease of airway hyper-responsiveness, airway inflammation and episodic bronchoconstriction. With asthma forecasted to increase by an additional 100 million cases by 2025, there is a critical and immediate need to address new asthma therapies. Guidelines for asthma treatment in the emergency department conditionally recommend intravenous magnesium sulfate (MgSO₄). However, some investigations have failed to demonstrate beneficial effects. Ethical constraints limit evaluation of the bronchodilatory effects of MgSO₄ alone in patients with acute asthma exacerbation, independent of other conventional therapeutics. To address this ethical dilemma, this study consisted of two phases: 1) quantification of the independent pulmonary effect of three doubling doses of MgSO₄ in the spontaneous equine model of asthma during naturally occurring exacerbations of bronchoconstriction, and 2) evaluation of arterial blood gas parameters in response to administration of MgSO₄ at a dose identified in phase 1 that yielded greatest efficacy without deleterious side effects.
DEDICATION

My Largos-Louise - you fuel my passion.

Thank You for guiding my heart and hands into the veterinary world.

To the parents who grew me up, dust me off when I get ‘bucked off,’ and support me on each and every one of my adventures I saddle up for.

I owe it all to you Mom & Dad
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The biggest shout out to my family: my Sister Sledge, Grandma & Grandpa B, my Aunts & Uncles, the clan Wenzel, and all my friends both near and far: JayMoo, Stephro, Sarah (“Cinbad”), Edmonton, my KCK, you’ve dealt me with on my outstanding days, and the ones I’d rather not relivet. Thank you for joining me, hands up! on this rollercoaster ride.

We did it you guys!!!. On to the next one.
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CHAPTER I

INTRODUCTION

Magnesium is often deemed the forgotten mineral or electrolyte (Elin 1994). Involved in more than 300 enzymatic reactions in the body, magnesium is essential for the regulation of muscular contraction, blood pressure (Jee 2002), cardiac excitability (Turlapaty 1980), vasomotor tone (Laurant 1997), nerve transmission and neuromuscular conduction (Fleming 1972, Castillo 1954). Due to magnesium’s immense biological activity, it plays an important role in prevention and treatment of many diseases (Gröber 2015, Volpe 2013) such as (pre-)eclampsia (Lucas 1995), Alzheimer’s disease (Barbagallo 2011, Andrási 2000), insulin resistance, type-2 diabetes mellitus (Paolisso 1990), hypertension (Jee 2002), cardiovascular disease (Turlapaty 1980), migraine headaches (Mauskop 1995, 1996), attention deficit hyperactivity disorder (Guran 2011), osteoporosis (Rude, 2009) and asthma (Griffiths 2016, Kew 2014, Rowe 2000).

Asthma is a chronic inflammatory disease in which airway smooth muscle (ASM) contracts and maintains sustained contraction around the airways due to a propagation of calcium (Ca\(^{2+}\)) oscillations into the ASM cell and cytosol (Sanderson 2007, Prakash 1997). Intravenous magnesium sulfate (MgSO\(_4\)) is an adjunctive therapy (Griffiths 2016, Kew 2014, Rowe 2000) for emergency asthmatic treatment and is thought to compete with Ca\(^{2+}\) receptor activation resulting in ASM relaxation (Gourgoulianis 2001, Haury 1938, Iseri 1984) through a mechanism independent from more standard bronchodilators.
such as β₂-adrenoceptor agonists and anticholinergics. In addition, magnesium has been found to reduce airway inflammation (Bois 1963, Cairns 1996), airway remodeling (Bichara 2009, Murdoch 2010), and clinical symptoms (Alamoudi 2000, Bichara 2009). Furthermore, ex-vivo use of magnesium induces vascular smooth muscle relaxation (Howell 1986) and thus, may act directly to increase pulmonary blood flow and reverse hypoxic pulmonary vasoconstriction.

Despite these noted capabilities and a strong safety profile (Bichara 2009, Kowal 2007, Rowe 2007), IV MgSO₄ remains only conditionally recommended in the emergency department. Recommendation of MgSO₄ for use in emergency treatment of asthma is limited to patients with life-threatening asthma exacerbation or patients whose exacerbations remain severe after 1 hour of intensive conventional treatments including β₂-adrenoceptor agonists, corticosteroids and anticholinergics (NHLBI 2007). Lack of implementation of IV MgSO₄ into the standard of care for treatment of asthma in the emergency department directly results from conflicting reports of efficacy when used with other standard-of-care therapies (Goodacre 2014, Bradshaw 2008, Hirashima 2016). To date, the ethical inability to withhold other standard treatments in the emergency setting obstructs determining the sole effect of IV MgSO₄ therapy on patients with asthma.

This project addressed this ethical dilemma by utilizing the Mississippi State University College of Veterinary Medicine’s Translational Respiratory Research Laboratory (TRRL) herd of horses suffering from a spontaneous asthma-like disease. These horses experience a clinical syndrome that parallels human asthma (Couëtil 2007, Seahorn 1993, Mair 1996, Costa 2000), have analogous key clinicopathologic features

With our translational model, we were able to answer the knowledge gap “does magnesium sulfate safely and effectively relieve bronchoconstriction and improve gas exchange during asthma exacerbation?”
CHAPTER II
LITERATURE REVIEW

Asthma Significance

Asthma impacts nearly 300 million people globally (WHO 2007) and accounts for about 1 in every 250 deaths worldwide (Masoli 2004). In the United States, 1 in 12 (approximately 25 million) persons have been diagnosed with asthma and there are an associated 10 asthma related deaths daily (CDC 2014). Interestingly, in the most recent data from the Centers for Disease Control, the state of Mississippi ranks first in child severe asthma (CDC (C) 2015), 3rd in adult severe asthma (CDC (A) 2015), and 4th in overall asthma mortality nationally (CDC 2016).

Asthma presents a serious health and economic concern in the United States. In the last decade, the proportion of people with asthma in the United States grew by nearly 15% (CDC (B)). This disease served as one of the leading causes for activity limitation (Barnett 2011) in the population. In 2009, asthma caused 479,300 hospitalizations and 1.9 million emergency department visits (CDC (B)). Asthma exacerbations requiring emergency treatment account for approximately 5.4% or $2.7 billion of the $50.1 billion in direct health care costs attributed to asthma (Wang 2014, NHLBI 2007). Direct costs included inpatient care, emergency visits, physician visits, nursing services, ambulance use, drugs and devices, diagnostic tests, research and education (Bahadori 2009). From 2000 to 2010, one of those direct costs, average cost per asthma-related hospital stay in
adults, underwent a noted increased from $5,200 to $6,600 per stay (Barrett 2014). Direct
costs tend to exceed indirect costs, with the major components of direct medical costs
being pharmacological expenditures and hospital admissions (Bahadori 2009).

With forecasted increases in the proportion of people living in urban
environments to increase from 45% to 59% by 2025 and the knowledge that the rate of
asthma increases as communities become urbanized (Lin 2001, Weinberg 2000), it is
estimated that there may be an additional 100 million asthmatics by 2025 (Masoli 2004).

In order to reduce the asthma burden, there is a critical and immediate need to
address new asthma therapies, especially for asthmatics non-responsive to conventional
treatments. Developing cost-effective approaches to treat and manage asthmatics has
been deemed a priority, not just to defer direct health care costs associated with the
disease, but more importantly, to ensure the availability of optimal treatment to as many
people as possible suffering from asthma worldwide (Masoli 2004).

**Asthma Pathophysiology**

Asthma is a chronic, progressive obstructive lung syndrome of the lower airways
characterized by airway inflammation (Murdoch 2010), episodic constriction of ASM
(Doeing 2013) and airway hyper-responsiveness (Murdoch 2010, Doeing 2013),
culminating in clinical episodes of tachypnea, dyspnea, coughing, shortness of breath,
and anxiety (WHO 2013, Doeing 2013). The severity of asthma can range from mild
manageable symptoms, to severe asthmatic exacerbations in which airways can
completely close, resulting in life-threatening circumstances (NHLBI 2007, Jain 2006).

Despite its classification as a chronic obstructive condition, asthma is not
considered a chronic obstructive pulmonary disease, such as emphysema, due to the

Airway Inflammation

Inflammation results from the immune system’s response to airway insult. Under normal circumstances this response helps in identifying and removing airway irritants and pathogens, but an asthmatic’s overzealous response leads to chronic unchecked inflammation. It is important to note that airway inflammation contributes to airway hyper-responsiveness, airflow limitation, respiratory symptoms, and disease chronicity. As the inflammation progresses, factors like edema may additionally occlude airflow (NHLBI 2007).

Episodes of acute inflammatory reactions often result from a response of the innate immune system. However, these acute inflammatory episodes are often accompanied by underlying chronic inflammation from the adaptive immune system, even in the absence of irritant exposure (Murdoch 2010). The diverse immunologic response of asthma includes inflammatory cell infiltration of a combination of
neutrophils (particularly in acute, fatal exacerbations and occupational asthma (Sur 1992), eosinophils and lymphocytes, as well as mast cell activation (NHLBI 2007).

Historically, asthma has been categorized as a T-helper type 2 disease with increased IgE levels and eosinophilic inflammation (Lloyd 2010, Britar 2014). Eosinophilic asthma includes both allergic and nonallergic phenotypes and is mostly responsible for mild-to-moderate asthma (Moore 2014). This disease phenotype is typically mediated by a hypersensitive adaptive immune response, however airway eosinophilia can also be associated with other relevant innate immune responses (Lee 2016). The recognition of alternative asthma subtypes occurred when a sub-group of asthmatics did not respond to conventional corticosteroid therapy (Schwartz 1968, Szelfer 1997, Green 2002). The T-helper 2 asthma disease pathogenesis was found to not suit all patients, and an alteration in the eosinophilic asthma paradigm was born (Kudo 2013, Lambrecht 2015).

In one type of non-eosinophilic asthma, patients can present with predominantly neutrophilic airway inflammation with an absence of T helper 2 cytokines (Bousquet 2000, Lambrecht 2015). Clinical cluster analysis from the Severe Asthma Research Program identified several severe asthma sub-phenotypes (Moore 2014). Representing a severity spectrum of early-onset allergic asthma, late-onset severe asthma, and severe asthma with chronic obstructive pulmonary disease characteristics, these subjects presented with eosinophilic (≥2%) and neutrophilic (≥40%) inflammation, and demonstrated clinical symptoms of very severe asthma (Moore 2014). This and other investigations have indicated that neutrophils are a dominant leukocyte in moderate-to-severe asthmatics (Moore 2014, Wenzel 1999). However, other investigators have found conflicting results, with eosinophilic airway inflammation correlating to more severe
disease (ten Brinke 2004, Lemière 2006, Bittar 2015). In lung tissue sections from some neutrophilic asthma patients, there is an overexpression of IL-17A and IL-17F (Al-Ramli 2009). These interleukins seem to correlate with asthma severity, and steroid resistant disease (Al-Ramli 2009, Kudo 2012, Chesné 2014). Newly accepted as a heterogeneous condition, patients with asthma that do not respond satisfactorily to standard therapy with inhaled corticosteroids often undergo disease phenotyping (Bittar 2015, Groot 2015, Lambrecht 2015, Chesné 2014).

Airway Remodeling

Airway dysfunction is influenced by many factors including inflammation. With chronicity, asthma leads to structural changes in the airways termed remodeling. These alterations, commonly attributed to an underlying chronic inflammatory process (Bergeron 2010) can include: goblet cell metaplasia with mucus hypersecretion (Aikawa 1992), increased airway smooth muscle (Bai 2005, Lloyd 2007), subepithelial fibrosis (Lloyd 2007), increased susceptibility to oxidative damage and epithelial injury (Evans 1999), angiogenesis (Lloyd 2007), in addition to others. Each of these factors increases airway wall thickening, and decreases airway caliber. Airway remodeling is suggested to be a factor in airway hyperresponsiveness as well as a significant contributor to declining lung function over time. This manifests as airway obstruction during asthma attacks that are incompletely reversible with the administration of bronchodilators (Lloyd 2007).

Subepithelial fibrosis reflects the deposition of extracellular matrix proteins such as collagens, laminin and tenascin (Lloyd 2007). Increases in airway smooth muscle reflect several events including metaplasia of peribronchiolar fibroblasts into
myofibroblasts (Evans 1999), myofibroblast proliferation and recruitment as well as cellular hypertrophy with enhanced extracellular matrix deposition (Bai 2005, Lloyd 2007). Asthmatic’s airway susceptibility to oxidant injury (Holgate 2007, Bucchieri 2002) and inability to repair rapidly leads to damaged epithelial cells and attenuated myofibroblast sheaths (Holgate 2007, Evans 1999). Epithelial shedding and hypertrophy are indicative of remodeled airways (Evans 1999).

Increased bronchial vascularity has also been documented (Lloyd 2007) as a component of airway remodeling. Vascular engorgement, vasodilatation, and microvascular leakage increase airway wall thickness (Elias 1999, Davies 2009). This can also facilitate infiltration of inflammatory cells into airway tissues (Bergeron 2010). Furthermore, neovascularization may sustain the metabolic demands of the increased ASM found in asthmatic airways and consequentially increase or sustain the force of contraction (Simcock 2008, Bai 2005).

Goblet cell metaplasia and mucus hypersecretion also contribute to the changes in airway architecture and may be a specific feature in fatal asthma (Aikawa 1992). Goblet cells are glandular secreting cells that produce mucins- the major components of mucus (Voynow 2009). Found inside the trachea, bronchi, and larger bronchioles of the respiratory tract, goblet cells secrete mucus in order to protect the mucous membranes.

Airway mucus serves critical functions in host defense. Mucous cell metaplasia is induced in response to harmful insults and provides protection from airway irritants and cellular debris (Voynow 2009, Fahy 2014). In chronic airway diseases, mucous gland hyperplasia leads to excessive mucus secretion and can, in severe cases, lead to occlusion of the airways (Fahy 2014). Hypersecretion of mucus plays a central role in the
Pathogenesis of severe airway obstruction, asphyxiation in fatal asthma attacks, and contributes significantly to the morbidity and mortality of the disease (Aikawa 1992, Curran 2010). Furthermore, extremely viscous inflammatory exudate may also result in a patient's poor response to inhaled bronchodilators (Bousquet 2000).

**Airway Hyper-responsiveness**

Airway hyper-responsiveness to aeroallergens and irritants is considered asthma’s phenotypic hallmark (Murdoch 2010). The magnitude of airway hyper-responsiveness manifests as airway sensitivity and reactivity to a given stimulus such as a methacholine challenge (Scope 1999), other bronchoconstrictors or irritants (Murdoch 2010, Juniper 1981).

**Bronchoconstriction**

Recurrent and sustained airway bronchoconstriction marks the final physiological dysfunction of asthma. In asthma, the dominant physiological event leading to clinical symptoms or an asthma attack is constriction of the conducting airways and consequent restriction of airflow. This is known as bronchoconstriction (NHLBI 2007).

Bronchoconstriction is due to over-activation of the parasympathetic nervous system (Canning 2006) and is detailed in Figure 2.1 (Perez-Zoghi 2009). Parasympathetic nerve impulses travel via the vagus nerve and generate action potentials in postganglionic nerves; these nerves travel along short postganglionic fibers to the ASM and submucosal glands. Acetylcholine stimulates $M_3$ muscarinic receptors on smooth muscle and mucus glands. Although $M_2$ receptors are also present on ASM and $M_1$ receptors are on submucosal glands, $M_3$ cholinergic receptor signaling is considered...
the principle mechanism for bronchoconstriction and mucus secretion (Scott 2012, Perez-Zoghbi 2009).

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**Figure 2.1** Pathway of airway smooth muscle (ASM) contraction

Acetylcholine (Agonist), M3 type cholinergic receptor (Receptor), phospholipase C-β (PLC), inositol 1,4,5-trisphosphate (PI3), diacylglycerol (DAG), arachidonic acid (AA), inositol triphosphate receptors (IP3R), sarcoplasmic reticulum (SR), sarcoplasmic reticulum Ca2+ ATPase (SERCA), store-operated Ca2+ channel (SOC), receptor operated Ca2+ channel (ROC), voltage-gated L-type Ca2+ channels (VDC), stromal interaction molecule one (STIM1), stretch-activated channels (SA), potassium channel (K+).

Source: Perez-Zoghbi et al. (2009)

During Ca2+ mobilization for contraction, postganglionic acetylcholine is released next to the ASM cell, stimulating muscarinic receptors. Muscarinic receptors are G-protein coupled receptors that activate the phospholipase C pathway to synthesize inositol trisphosphate and diacylglycerol from membrane lipids (Scott 2012, Pelaiàa 2008).

Inositol trisphosphate diffuses throughout the cell and binds to the inositol trisphosphate receptors on the sarcoplasmic reticulum, sensitizing them to Ca2+. This in turn stimulates the opening of the inositol trisphosphate receptor to allow the release of Ca2+ from the
sarcoplasmic reticulum into the cytosol. The released $\text{Ca}^{2+}$ stimulates a $\text{Ca}^{2+}$ induced $\text{Ca}^{2+}$ release from adjacent, sensitized inositol trisphosphate receptors on the membrane of the sarcoplasmic reticulum, henceforth propagating a $\text{Ca}^{2+}$ wave (Perez-Zoghbi 2009).

The cytosolic increase in $\text{Ca}^{2+}$ promotes binding of $\text{Ca}^{2+}$ to a secondary site on the inositol trisphosphate receptors, inactivating and closing the receptor for termination of $\text{Ca}^{2+}$ release. Additionally, the decrease in the sarcoplasmic reticulum’s $\text{Ca}^{2+}$ contributes to the termination release so $\text{Ca}^{2+}$ can be re-accumulated in the sarcoplasmic reticulum via sarcoendoplasmic reticulum $\text{Ca}^{2+}$ ATPase (SERCA). These SERCA channels are the major mechanism for replenishing $\text{Ca}^{2+}$ stores in the sarcoplasmic reticulum. It is suggested that the increase in ASM $\text{Ca}^{2+}$ that is observed in asthma may be facilitated by suppressed SERCA channel expression and function (Prakash 2009), as ASM from asthmatics expresses a decreased number of SERCA channels, but greater proliferation and secretion (contributing to airway remodeling) when compared with normal patients (Mahn 2009).

The $\text{Ca}^{2+}$ deficit in the sarcoplasmic reticulum resulting from the extrusion of $\text{Ca}^{2+}$ requires restoration of adequate $\text{Ca}^{2+}$ for subsequent contraction. The process of replenishing the $\text{Ca}^{2+}$ of the sarcoplasmic reticulum to maintain calcium induced ASM contraction is achieved via the influx of calcium through store-operated calcium entry, receptor-operated channels, and voltage-gated L-type $\text{Ca}^{2+}$ channels (Kume 2015). Voltage-dependent $\text{Ca}^{2+}$ influx, via activation of L-type $\text{Ca}^{2+}$ channels, plays a significant role in store-refilling and these channels have long been viewed as the primary channel for voltage-dependent $\text{Ca}^{2+}$ influx (Hirotta 2007).
During the Ca\(^{2+}\) oscillations, Ca\(^{2+}\) stores are depleted; this is sensed by stromal interaction molecule-1 (STIM1) in the sarcoplasmic reticulum. This molecule translocates through the sarcoplasmic reticular membrane to combine with and activate store-operated Ca\(^{2+}\) entry or channels (Perez-Zoghibi 2009). Diacylglycerol and its byproduct (arachidonic acid) also move in the membrane to activate receptor-operated Ca\(^{2+}\) channels to replenish internal Ca\(^{2+}\) stores. Ca\(^{2+}\) entry via these receptor-operated channels may complement or precede Ca\(^{2+}\) influx via store-operated channels (Perez-Zoghibi 2009).

At the molecular level, smooth muscle contraction is controlled by the antagonistic behavior of the myosin light-chain kinase and myosin light-chain phosphatase; the net action of these enzymes determines the phosphorylation state. The regulation of myosin light-chain kinase activity is a function of the calcium ion as it is activated by binding Ca\(^{2+}\) to the calmodulin complex (Vetterkind 2012). In ASM cells, agonists initiate, but cannot maintain, contraction in Ca\(^{2+}\) free conditions, indicating that Ca\(^{2+}\) oscillations are mediated by repetitive release of Ca\(^{2+}\) from internal stores that require refilling by Ca\(^{2+}\) influx (Dai 2006, Berridge 2007).

Calcium overall has been shown to influence the magnitude of ASM cell contraction in the short term, and in the long term it is believed to influence smooth muscle cell proliferation, production and secretion of pro-inflammatory factors that collectively contribute to airway hyper responsiveness (Perez-Zoghibi 2009).

With respect to vasculature, activation of M\(_3\) receptor on vascular endothelial cells causes increased synthesis of nitric oxide. This diffuses to adjacent vascular smooth muscle cells and causes their relaxation and vasodilation, thereby explaining the
paradoxical effect of parasympathomimetics on vascular tone and bronchiolar tone (Scott 2012).

The inflammatory component of asthma is responsible for tissue injury, atelectasis and severe bronchoconstriction, and leads to airway obstruction and gas exchange abnormalities (Papiris 2002, Rodriguez-Roisin 1997). Measures of arterial blood gas determine the proficiency of gas exchange in asthmatics and often indicate extreme hypercapnia and hypoxemia during asthma exacerbations (Higgins 2003). Hypercapnia occurs with increasing severity of airway obstruction, often with tiring of the respiratory muscles that may indicate impending respiratory failure (Higgins 2003). Hospitalization for asthmatic patients is implemented in patients whose post-treatment arterial oxygen saturation is less than 90 percent, who maintain respiratory acidosis, or patients with severe obstruction that does not improve (or worsens) with the administration of sympathomimetic agents (Higgins 2003).

Airway obstruction results in decreased alveolar ventilation, but maintenance of perfusion can create a ventilation/perfusion mismatch (Papiris 2002). The physiologic phenomenon of hypoxic pulmonary vasoconstriction ordinarily aids in preventing ventilation/perfusion mismatches by redistributing pulmonary blood flow from areas with poor alveolar airflow to those that are receiving better ventilation. In severe asthma, generalized bronchoconstriction reduces ventilation in all small airways, leading to a vicious cycle of poor ventilation, prolonged vasoconstriction and further diminished perfusion (Voelkel 1986). While the management of hypoxic vasoconstriction indicates treating the underlying bronchoconstrictive condition, patients with ventilation-perfusion mismatch usually also benefit from supplemental oxygen therapy (Higgins 2003).
**Emergency Asthma Therapeutics**

Emergency department management and treatment guidelines for asthma from the National Heart, Lung, and Blood Institute (NHLBI) National Asthma Education and Prevention Program Expert Panel Report 3 (NHLBI 2007) address rapid reversal of bronchospasm and resolution of airway inflammation as priority. Recommendations for emergency asthma therapy include oxygen therapy for most patients, short acting β₂-adrenoceptor agonists for all patients, systemic corticosteroids for most patients, and inhaled anticholinergics (NHLBI 2007). These guidelines also conditionally recommend heliox and/or intravenous magnesium sulfate (MgSO₄) for patients with life-threatening asthma exacerbation and patients whose exacerbations remain severe after 1 hour of intensive conventional treatment (NHLBI 2007).

**Oxygen**

Oxygen therapy is delivered to most patients to relieve hypoxemia in moderate or severe asthma exacerbations (NHLBI 2007). However, the degree of bronchoconstriction and airway obstruction impact the efficacy of this treatment, as oxygen must reach the alveolus for gas exchange to occur (Fink 2006).

**Short Acting β₂-Adrenoceptor Agonists**

All patients receive short acting β₂-adrenoceptor agonists to relieve airflow obstruction. The NHLBI Expert Panel recommends the use of short acting β₂-adrenoceptor agonists as the most effective medication for relieving acute bronchoconstriction (NHLBI 2007). Examples include albuterol, clenbuterol and terbutaline (Figure 2.2) (Hilal-Dandan 2012). The sympathetic nervous system does not
directly innervate the lung (Canning 2006). Sympathetic signaling occurs via the
adrenergic receptors of the sympathetic nervous system which include α-adrenergic
receptors (α-receptors) and β-adrenergic receptors (β-receptors). In the lung, β₂-receptors
on airway smooth muscle mediate bronchodilation. β₂-receptor agonist therapeutics elicit
this effect. β₂-adrenergic receptors are G-protein coupled receptors. Upon their
extracellular activation, the intracellular G-protein subunit stimulates adenyl cyclase
production which increases intracellular cyclic 3',5'-adenosine monophosphate (cAMP)
concentrations (Barisone 2010). Increased cAMP activates protein kinase A, which
inactivates myosin light-chain kinase and activates myosin light-chain phosphatase. This
ultimately leads to dephosphorylation of the the regulatory light chain of myosin II in
smooth muscle tissue, and consequent ASM relaxation. In addition, β₂-agonists open
large calcium-activated potassium channels by hyperpolarizing the ASM cells, and they
are sent into a refractory period. The combination of myocyte hyperpolarization and
decreased myosin light chain kinase are synergistic events in smooth muscle relaxation
and bronchodilation (Barisone 2010).
β2-agonist therapeutics are rapid and highly effective bronchodilatory agents, but there are cons to this drug class. β2-agonists do not control inflammation. Additionally, they are accompanied with a black box warning (Salpeter 2006). This warning cautions the potential increase in asthma severity, exacerbations, and asthma related deaths with long-term use, especially when used without corticosteroids (NHLBI 2007). The main β2-agonists in use today (i.e., albuterol, levalbuterol, and pirbuterol) possess few negative cardiovascular effects in contrast to former β2-agonists isoprenaline and fenoterol, which were associated with severe and fatal attacks of asthma (NHLBI 2007). Furthermore, frequent use of nebulized β2-agonists reduces serum potassium, phosphate, and magnesium (Bodенхамер 1992).
Systemic Corticosteroids

Though inhaled corticosteroids are the hallmark of asthma prevention, systemic corticosteroids are also administered to most asthma patients that require emergency management in order to decrease airway inflammation (NHLBI 2007). Corticosteroids block late-phase reactions to allergens, inhibit inflammatory cell migration and pro-inflammatory transcription factors (NFκB, AP-1) (Barnes 2003), reverse histone acetylation of activated inflammatory genes (Barnes 2006), decrease vascular permeability via vasoconstriction (Hoshino 2001) and interfere with chemotaxis (NHLBI 2007). Medications such as dexamethasone, prednisolone, fluticasone are commonly used.

Despite their tremendous effectiveness in reducing inflammation and thus secondary bronchoconstriction, administration of corticosteroids can take 4-6 hours for full effect and is not without significant short and long-term potential side effects (Medicines for Children 2015). Side effects can include, but are not limited to: immunosuppression, hypertension, growth suppression, osteoporosis, decreases in linear growth and bone mineral density, disseminated varicella, dermal thinning and increased ease of skin bruising, hypothalamic-pituitary-adrenal axis function, and effects on glucose metabolism (NHLBI 2007, Cornelisse 2004).

Inhaled Anticholinergics

The last standard treatment of asthma exacerbation in the emergency department is inhaled anticholinergics (NHLBI 2007). Known by a variety of names (parasympatholytics, anticholinergics, muscarinic receptor antagonists), this drug class inhibits muscarinic cholinergic receptors of the airway smooth muscle and as a result,
reduces cholinergic bronchomotor tone and hypersecretion of airway mucus (Buels 2012, Soler 2014).

Anticholinergic agents such as atropine, glycopyrrolate, and scopolamine derivatives are nonselective muscarinic receptor antagonists that block the parasympathetic nervous system’s functions. Therefore, this drug class possesses the possibility for undesirable systemic side effects. Sequelae to use of these drugs can include ileus, tachycardia, nausea, decreased mucociliary clearance and resultant increase in airway infections, dry mouth, nervousness, dizziness and palpitation (NHLBI 2007, Soler 2014) and may not be recommended for older patients (Feinberg 1993). These agents potently desiccate the respiratory secretions, which can be detrimental to resolving mucus plugging of the distal airways that commonly occurs in asthma.

Heliox

Helium is a biologically inert and insoluble gas in human tissues having no bronchodilator or anti-inflammatory effect. Its effect is as a temporizing agent, working while conventional treatments have time to act. Due to helium’s low specific gravity, it also possesses a low density, and thus a higher rate of flow than oxygen (Gluck 1990). Combining helium and oxygen gas (Heliox) results in a gas mixture with a similar viscosity to air but with a substantially lower density. Density and efficiency of flow are directly related. By lowering the resistance to flow within the airways via converting turbulent flow into more efficient laminar flow and decreasing the pressure gradient needed to achieve turbulent flow, heliox reduces the work of breathing (Reuben 2004, Gluck 1990).
To be effective at reducing airway resistance, the concentration of helium must be high, ideally greater than 70% of the inhaled gas mixture. This limits the amount of oxygen that can be delivered simultaneously. Patients with high oxygen requirements may not be amendable to this therapeutic modality. Additionally, helium’s high thermal conductivity, 6x higher than that of oxygen, may lower body temperature when used for prolonged periods (Reuben 2004).

**Intravenous Magnesium Sulfate**

Intravenous magnesium sulfate (MgSO₄) is a conditionally recommended therapy for emergency department treatment of asthma that is refractory to inhaled β₂-agonist therapy (2007 NHLBI guidelines). Support for the efficacy of MgSO₄ as a bronchodilatory agent in asthma originated over a century ago, when its bronchodilatory effects were first described in bovine trachea (Trendelenburg 1912). Twenty years later, bronchodilatory effects were noted in asthmatic patients treated with MgSO₄, both intravenously (Rosello 1936) and intramuscular (Haury 1940). Since the publication of the 2007 NHLBI guidelines for emergency treatment of asthma, a meta-analysis investigating the effect of a single infusion of MgSO₄ to adult asthmatics failing to respond to conventional therapy (oxygen, short acting β₂-agonists and corticosteroids) in emergency departments identified significant reduction (7.5%) in hospital admissions and significant improvement in Forced Expiratory Volume (Kew 2014). In pediatric asthma, a recent meta-analysis demonstrated a 68% decrease in hospital admission in patients treated with MgSO₄, but lacked the power to determine effects on treatment duration, intensive care admissions, hospital stay, and clinical parameters (Griffiths 2016). In the past three decades, individual reports of emergency department IV MgSO₄ use in adults

There is a strong rational for the further implementation of magnesium sulfate in asthma therapy; the growing body of clinical evidence favors its efficacy in emergency therapy (Griffiths 2016, Kew 2014, Rowe 2000). Magnesium competes with Ca²⁺ to relax airway smooth muscle (Gourgoulianis 2001, Haury 1938, Iseri 1984) and provides additional benefit by reducing airway inflammation (Bois 1963, Cairns 1996) and airway remodeling (Cairns 1996, Bichara 2009, Murdoch 2010).

Magnesium is a cofactor that participates in over 300 enzymatic reactions including all reactions involving the formation and utilization of ATP (Kies 1995) and is vital to maintenance of cell function, physical stability, and transformation of ribosomes into polysomes (Pasternak 2010). The exceptional biochemical activity of magnesium is a
result of its low ionic radius in relationship to its nucleus (0.86, versus a ratio of 1.14 for calcium). As a result of these physicochemical properties, intracellular magnesium easily binds to the nucleus, ribosomes, cell membranes and other macromolecules occurring in the cell's cytosol (Pasternak 2010).

**Magnesium Inhibits Calcium-Mediated ASM Contraction**

Magnesium has been described as ‘nature’s physiologic calcium blocker’ (Naithani 2014). Magnesium salts dissolve easily in water and are much more soluble than their respective calcium salts. As a result, magnesium is more readily available to organisms (Maguire 2002). As a ‘natural calcium antagonist’ magnesium competes with calcium for similar binding sites (Walser 1967, Hunter 1976) such as at motor endplates, where magnesium antagonizes the calcium-dependent release of acetylcholine (Wacker 1980). Furthermore, magnesium also has an anticonvulsive effect (Ramanathan 1988, Euser 2009).

Magnesium’s primary bronchodilatory effect is via direct relaxation of ASM from its antagonist effect on the Ca²⁺ influx channels that maintain muscular contraction (Connolly 1999, Rowe 2008). Although ASM cells can initiate contraction in Ca²⁺ free conditions, maintenance of contraction is mediated by Ca²⁺ oscillation (repetitive release of Ca²⁺ from internal stores and refilling by further cellular Ca²⁺ influx) (Dai 2006, Berridge 2007). Calcium transport across the cellular membrane is the most important regulator of intracellular calcium content. This is mainly attained through the action of a calcium/magnesium dependent membrane ATPase, and voltage and receptor operated calcium channels (Reinhart 1988).
Magnesium works to inhibit maintenance of calcium-induced muscle contraction. This is achieved by decreased cellular entry of Ca$^{2+}$ through the high-voltage L-type Ca$^{2+}$ channels (Bara 2001). In addition, magnesium hinders calcium entry and release from the sarcoplasmic reticulum (Spivey 1990) and vies for Ca$^{2+}$ binding sites on troponin C and myosin (Iseri 1984, Gourgoulianis 2001).

Enzyme classes that regulate phosphorylation-dephosphorylation reactions of the ASM are protein kinases and phosphoprotein phosphatases. The first group includes myosin kinases, which phosphorylate myosin chains, and the second group includes myosin phosphatases that dephosphorylate light chains of myosin (Domínguez 1998). Myosin kinases are magnesium-dependent enzymes, whereas myosin phosphatases are calcium-dependent enzymes (Reinhart 1988, Altura 1984). Due to magnesium’s involvement in calcium transport across the cellular membrane (Levine 1984, Iseri 1984), both enzyme types are directly or indirectly influenced by intracellular magnesium levels (Reinhart 1988, Altura 1984). Due to this, it is hypothesized that magnesium deficiency can lead to an increased excitability of bronchial smooth muscle with consequent bronchoconstriction (McLean 1994).

**Magnesium Also Moderates Bronchial Tone in a Calcium-Independent Manner**

In addition to the proposed direct bronchodilatory effects via Ca$^{2+}$ inhibition of muscular bronchospasm, magnesium also yields indirect bronchodilatory effects by means of: inhibition of cholinergic transmission (Castillo 1954), stimulation of nitric oxide and prostacyclin synthesis, attenuation of neutrophilic burst (Cairns 1996), inhibition of mediator release from mast cells (Bois 1963), stabilization of T-lymphocytes (Connolly 1999, Rowe 2008) and potentially increasing $\beta_2$-agonist receptor affinity.
Calcium antagonists have also been found to inhibit synthesis of leukotrienes in rat lungs and cyclooxygenase products in sheep, possibly by inactivating phospholipase A2 and/or 5-lipoxygenase, suggesting that this may also be an effect that is mediated by magnesium (Ahmed 1988).

Magnesium also activates adenylate cyclase which increases intracellular cAMP synthesis. Consequently, the accessibility of magnesium to adenylate cyclase can modulate cyclic nucleotide metabolism and its associated relaxation pathway in ASM (Touyz 2004, Pasternak 2010). Also, high intracellular cyclic adenosine monophosphate and cyclic guanosine monophosphate concentrations slow down or stop degranulation of mast cells which are known contributors to airway inflammation and mast cell derived ASM spasmogens (Touyz 2004, Pasternak 2010) (i.e. histamine).

Magnesium also produces anxiolytic effects through N-methyl-D-aspartate (NMDA) receptor blocking (Akhtar 2011, Apan 2004) which may result in central nervous system depression and forms of sedation.

**Magnesium Effects on Vascular Smooth Muscle**

Magnesium also has potential beneficial effects on hypoxic vasoconstriction. In addition to magnesium’s direct ASM relaxing effect (Voelkel 1986), magnesium induces vascular smooth muscle relaxation ex-vivo (Howell 1986) and may act directly to increase pulmonary blood flow. There is also evidence that elevations in extracellular magnesium relax vascular smooth muscle by decreasing myoplasmic calcium, even in the absence of increased myoplasmic magnesium (Gilbert D'Angelo 1992). Furthermore, magnesium has been successfully used as a monotherapy to treat persistent pulmonary hypertension in neonates that failed to achieve normal cardiopulmonary post-partum
adaptation with conventional vasodilator therapies (Chandran 2004).

Weigand et al. (2005) suggests that the major cause of hypoxic pulmonary vasoconstriction during acute hypoxia is increased intracellular calcium concentration in distal pulmonary arteriolar smooth muscle cells, by influx and entry through store-operated Ca\(^{2+}\) channels and voltage-gated channels. As a result, this condition may be reversible by introduction of a competitive cation such as magnesium. Magnesium has a good safety profile with appropriate monitoring (Alter 2000, Irazusta 2016, Egelund 2013, Kokotajlo 2014) as patients receiving an effective dose of MgSO\(_4\) or MgCl\(_2\) for the emergency treatment of asthma rarely, if ever, had life-threatening side effects (Kowal 2007). However, adverse effects for any dose of magnesium may include burning or warmth at the site of infusion and facial flushing (Rowe 2000, Bloch 1995). If serum magnesium is greater than 3 mg/dl, patients may begin to experience CNS depression, and electrocardiographic changes such as increased PR interval and widened QRS-duration (McDonnel 2010, Reinhart 1988). If serum magnesium rises above 5 mg/dl, patients may experience decreased deep tendon (patellar) reflexes, hypotension, nausea, and urinary retention (Lu 2000, Attygale 2002, Reinhart 1988). With serum magnesium of greater than 10 mg/dl, absence of the deep tendon reflex, somnolence, muscle weakness, hypotension, diarrhea, and abdominal cramping may occur (Saade 2010, Reinhart 1988). At serum magnesium levels of greater than 15 mg/dl, patients are likely to undergo respiratory depression, paralysis, and cardiac conduction abnormalities such as complete heart block (Lu 2000). Finally, cardiac arrest due to asystole may occur with serum magnesium levels of greater than 20 mg/dl (Saade 2010). Side effects of magnesium administration can be rapidly reversed with calcium gluconate infusion.
Among cations of biologic importance, magnesium seems to be the forgotten member, and is often labeled as the body's 'orphan ion' because of an apparent lack of any specific endocrine control. As a result, hypomagnessemia is the most common electrolyte disturbance in patients with chronic/acute asthma (Mohammada 2014, Emelyanov 1999). This electrolyte disturbance may be exacerbated by prolonged β-adrenoceptor agonist therapy (Bodenhamer 1992).

Translational Asthma Models

Animal models of asthma include rodents, guinea pigs, pigs, dogs, ruminants, primates, horses and cats (Zosky 2007, Meurs 2008, Allen 2009). However, not all models replicate the major characteristics of the human disease such as airway hyper-responsiveness (Meurs 2008). In many species, early asthmatic reactions are observed only after repeated allergen challenges, and a late asthmatic reaction is rarely observed (Padrid 1992). Horses (neutrophilic) and cats (eosinophilic) are the only two animal models that spontaneously development asthmatic features including hyper-responsiveness in relation to early and late asthmatic responses (Norris 2004, Leclere 2011).

Equine Asthma Models

In horses, airway hyper-reactivity, chronic inflammation and bronchoconstriction have been identified in a spontaneous asthma-like disease known as recurrent airway obstruction (Robinson 1996). Recent nomenclature has proposed that this disease, by virtue of its overt but reversible respiratory distress at rest, be renamed severe equine
asthma (Pirie, 2016). However, the term severe asthma is specifically reserved for human asthmatics that are refractory to management with inhaled corticosteroids and is reflective of longitudinal measures of asthma severity (GINA 2017). Two forms of ‘severe equine asthma’/RAO are recognized. One is triggered by inhaled barn dust, occurring in horses housed indoors in temperate climates (Robinson, 1996). The second form of this disease is triggered by inhaled particulates from pasture during summer months (Seahorn 1993). Etiological evidence for the role of aerosolized allergens, particularly molds, particulates and endotoxin from hay and bedding is quite strong for barn dust asthma (Pirie 2014). This work employs the latter, termed equine pasture asthma, to align to nomenclature for animal asthma models which signify the inciting agent. The clinical hallmarks of horses with both barn dust and pasture induced asthma mimic those of human asthma. Signs include exacerbations of reversible airway obstruction that cause respiratory distress resulting from bronchospasm, mucus accumulation, and airway inflammation (Couëtil 2007, Seahorn 1993, Mair 1996, Costa 2000, Leclere 2011).

Equine pasture asthma is a spontaneous progressive disease estimated to affect 3-5% of grazing horses in the southeastern US (Costa 2006) with no known breed or sex predilection and a mean age of disease onset of 10-14 years (Seahorn 1993, Mair 1996, Costa 2000, Costa 2006). Equine pasture asthma is characterized by clinicopathologic factors similar to that of human asthmatics: airway hyper-reactivity, reversible bronchoconstriction, and goblet cell metaplasia with mucus accumulation and neutrophilic lower respiratory tract inflammation (Beadle 1993, Seahorn 1993, Costa 2000, Costa 2006). Clinical signs of equine pasture asthma occur from late spring to early
autumn and affect pastured horses in the Southeastern United States, Great Britain and Scotland (Seahorn 1993, Mair 1996, Costa 2000, Dixon 1995). Clinical exacerbation of equine pasture asthma is associated with increases in temperature, humidity, aerosolized fungal spores, and grass pollens (Seahorn 1993, Mair 1996, Costa 2006). Typical signs include varying degrees of expiratory dyspnea, tachypnea, coughing, and auscultable wheezes and crackles (Seahorn 1993, Mair 1996, Costa 2000). In his report describing horses with pasture asthma in Great Britain, Mair suggested that the equine pasture asthma disease tended to be more clinically severe and acute in onset relative to barn dust asthma in that locale (Mair 1996).

Barn dust asthma and pasture asthma share well described key features of human asthmatic airway remodeling such as goblet cell hyperplasia/metaplasia with mucus hypersecretion and increased ASM (Pirie 2014, Costa 2000, Reed 2009, Barton 2016, Leclere 2011). They also share similar airway inflammatory profiles with non-eosinophilic asthma phenotypes described in human asthma (Barton 2016), specifically as it applies to the neutrophilic airway inflammation that is characteristic of these two equine diseases (Costa 2000, Leclere 2011, Pirie 2014).

Acute treatment and chronic management of equine barn dust asthma and pasture asthma are congruent with that of human asthma and include allergen avoidance, β₂-adrenoeceptor agonists and corticosteroid administration (Pollart 2011, Cornelisse 2004, Derksen 1999, Tesarowski 1994, Salpeter 2006, Reed 2009).

**Project Rationale**

The efficacy of IV infusion of MgSO₄ as a bronchodilator in acute asthma exacerbation merits more research. In order to characterize its independent
bronchodilatory effects, it is necessary to treat patients experiencing bronchoconstriction with MgSO₄ in the absence of other therapies. However, ethical constraints prevent evaluating intravenous MgSO₄ as a sole therapeutic agent in human subjects as it is unethical to withhold drugs known to reverse bronchoconstriction from patients during an asthma attack.

Furthermore, despite the clear importance of airway homeostasis to equine health, emergency therapeutic options for control and reversal of airway bronchospasm in equine airways are limited. Intravenous MgSO₄ as a therapy for acute equine bronchoconstriction has not been evaluated in horses to date.

Based upon a hypothesis that MgSO₄ would cause rapid bronchodilation, the effects of three doubling doses of intravenous MgSO₄ on measures of pulmonary function were determined during spontaneous exacerbation of equine pasture asthma using conventional pulmonary mechanics. From this data, an optimal intravenous dose of MgSO₄ was identified and employed in Phase 2 of the investigation to determine the efficacy of this treatment on pulmonary gas exchange.
CHAPTER III
MATERIALS AND METHODS

Phase 1

Animals

To investigate our hypothesis, six horses were studied during naturally occurring equine pasture asthma exacerbations elicited by pasture-associated aeroallergens (Costa 2000, Costa 2006, Leclere 2011, Pirie 2014). This group of horses consisted of 4 geldings (2 American Quarter Horses, 1 Appaloosa, and 1 Tennessee Walking Horse) and 2 mares (1 Tennessee Walking Horse, 1 Quarab) ranging in age from 16 to 24 years (average age 21 +/- 4.7 years). The horses were in good body condition; the average weight of the horses was 480.5 +/- 39.4 kg. These animals were maintained in the TRRL Herd at Mississippi State University for 1-5 years and had a documented history of reversible airway obstruction, chronic neutrophilic airway inflammation (>25% neutrophils in BALF), persistent airway hyper-responsiveness to methacholine, and normal serum biochemistries and complete blood counts. Protocols were approved by the Mississippi State University Institutional Animal Care and Use Committee.

Respiratory effort was graded each morning using a clinical score of respiratory effort (CSRE) (Costa 2000). Briefly, CSRE scores are calculated by evaluating magnitude of medial and lateral nostril flaring during inspiration and expiration, and amount of abdominal muscle contraction during expiration. Scores are based on a scale of
0 (least severe) to 4 (most severe), and total CSRE score was calculated by averaging medial and lateral nostril flare scores and adding the abdominal effort score (Costa 2000). Horses scoring ≥ 5, equivalent to a maximal change in pulmonary pressure (ΔPpl_max) of > 15cm H2O (Costa 2000), were removed from pasture, brought into the TRLL, weighed for drug dose calculation, and instrumented without sedation for continuous ECG and conventional pulmonary mechanic (esophageal balloon) evaluation. Total resistance of the lung (R_L), dynamic compliance (C_d), maximum change in pleural pressure (ΔPpl_max) respiratory frequency (f) and minute volume (MV) were determined using Buxco Electronics, Biosystems XA software (Hoffman 2002, Jean 1999). Intravenous catheters were also placed in both jugular veins.

**Magnesium Sulfate Dose Calculation**

The dose of MgSO₄ for administration to horses in this study was extrapolated from human emergency department management guidelines for asthma in the National Heart, Lung, and Blood Institute National Asthma Education and Prevention Program Expert Panel Report 3 (NHLBI 2007), as there are currently no published guidelines for dosage of MgSO₄ in horses for relief of bronchoconstriction. Allometric calculation (FDA 2005) was performed using the equation:

\[
ED_H = ED_A \times (K_{m_A} / K_{m_B})
\]

Where \( ED_H \) was the human equivalent dose of 2g MgSO₄ (20-30mg/kg) effective for treatment of acute asthma based on literature review (Rowe 2008, Kew 2014). \( K_{m_A} \) was the conversion factor for weight to body surface area of the animal model (65 for a 450kg horse) and \( K_{m_B} \) was the conversion factor for weight to body surface area of
humans (37 for a 70kg individual). The equation was solved for \( EDA \), defined as the animal equivalent dose for horses (Knottenbelt 2006).

This equation yielded a starting dose range of 11.2 to 18.5 mg/kg MgSO\(_4\). Based upon evidence of dose dependent improvements in respiratory function (Cirollo 2000) doubling doses of 15, 30 and 60 mg/kg were selected.

**Experimental Design**

Using a three-way crossover design, each of the 6 horses received doses of 15, 30 and 60 mg/kg in the left jugular catheter as a constant rate infusion over 20 minutes.

Treatment boluses were separated by a minimum 48-hour washout. Indices of pulmonary function (\( R_L \), \( C_{dyn} \), \( \Delta P_{\text{pl max}} \), \( F \), \( MV \)), ECG monitoring, heart rate (HR), respiratory rate (RR) and thoracic (TA) and tracheal (TR) auscultation scores (adapted from Jensen 1987, Table 3.1 and 3.2) were performed every 5 minutes beginning just prior to bolus initiation (\( T_0 \)) and ending 30 minutes following bolus administration (\( T_{50} \)). Thoracic auscultation was performed in four lung fields (cranioventral, central, dorsal, caudodorsal) for 3-5 breaths per field bilaterally to obtain scoring data. Whole blood was collected from the right jugular catheter into lithium heparin evacuated tubes at 0, 20, 50 minutes and 24 hours after initiating the MgSO\(_4\) bolus; total serum magnesium was quantified using an ACE Alera clinical chemistry analyzer.
Table 3.1  Thoracic Auscultation (TA) Scale (scale out of 10)

<table>
<thead>
<tr>
<th>Auscultation Scale:</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheezes</td>
<td>None</td>
<td>Low Pitch</td>
<td>Intermediate</td>
<td>High Pitch</td>
</tr>
<tr>
<td>Sound Intensity</td>
<td>Normal</td>
<td>Increased</td>
<td>Reduced</td>
<td>Absent/Faint</td>
</tr>
<tr>
<td>Distribution</td>
<td>None</td>
<td>1 Field</td>
<td>≥ 2 Fields</td>
<td>All Fields</td>
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<tr>
<td>Crackles</td>
<td>No</td>
<td>Yes</td>
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<td></td>
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</tbody>
</table>

Source: Adapted from Jensen et al. (1987).

Table 3.2  Modified Tracheal Auscultation (TRA) Scale (scale out of 7)

<table>
<thead>
<tr>
<th>Tracheal Scale:</th>
<th>0</th>
<th>1</th>
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<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sound Intensity</td>
<td>Normal</td>
<td>Increased</td>
<td>Reduced</td>
<td>Faint</td>
</tr>
<tr>
<td>Phlegm</td>
<td>None</td>
<td>Mild</td>
<td>Moderate</td>
<td>Marked</td>
</tr>
<tr>
<td>Wheeze</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Adapted from Jensen et al. (1987).

Following the 50-minute treatment protocol, 1.25mg of levalbuterol was administered via nebulization to reverse bronchoconstriction. All instrumentation was removed and horses were returned to pasture or placed into a climate controlled stalled based on disease severity, and entered a ≥ 48 hour washout period between dose administrations.

Statistical Analysis

Mixed models using PROC MIXED in SAS for Windows 9.4b analyzed pulmonary function, physical parameters and auscultation scores. Bolus phase (T₀–T₂₀), post-bolus phase (T₂₀–T₅₀) and entire protocol (T₀–T₅₀) data were analyzed separately with the same model. Treatment, time, and the treatment*time interaction were fixed effects, with baseline value of the dependent variable included as a covariate. Repeated
measures of horses were accounted for using an autoregressive one covariance structure. Differences in least squares means between treatments were calculated at each time point and compared to the initial time point within each treatment. Simulate adjustment for multiple comparisons was used for outcomes with significant main effects or interaction terms. P < 0.05 was considered statistically significant.

Phase 2

Animals

To determine the efficacy of MgSO₄ to improve ventilation, five horses with pasture asthma in the Mississippi State University’s TRRL were used. This group consisted of 2 geldings (American Quarter Horses) and 3 mares (1 Tennessee Walking Horse, 1 Quarab, 1 American Quarter Horse) ranging in age from 17 to 25 years (average age 20.8 +/- 3 years). Horses were in good body condition; their average weight was 474 +/- 43.7 kg. Documentation of equine pasture asthma, criteria for entry into treatment protocol, and all instrumentation was identical to phase 1. Additional instrumentation of an arterial catheter in the auricular artery was placed 1.5-4 hours prior to sampling with the aid of sedation if necessary.

Experimental Design

Each of the 5 horses received 30 mg/kg MgSO₄ in the left jugular catheter as a constant rate infusion over 20 minutes. Indices of pulmonary function (Rl, Cdyn, APplmax, F), ECG monitoring, HR, RR, TA and TRA were preformed every 5 minutes beginning just prior to bolus initiation (T₀) and ended 30 minutes post bolus administration (T₅₀). Whole blood was collected from the right jugular catheter into lithium heparin evacuated
tubes every 5 minutes from T₀ to T₅₀ and at 24 hours after initiating the MgSO₄ bolus. Whole blood underwent serum chemistry analysis using a Stat Profile® pHOx® Ultra machine from Nova Biomedical. At the time of sampling for this project, the required protocol for arterial blood gas at the Mississippi State University College of Veterinary Medicine Diagnostic Laboratory required submission of arterial blood into lithium heparin evacuated tubes. Arterial blood gases were collected and analyzed at 0, 20 and 50 minutes after initiating the MgSO₄ bolus using a Stat Profile® pHOx® Ultra machine from Nova Biomedical.

Following the 50-minute treatment protocol, levalbuterol was administered and all instrumentation removed as described in phase 1.

Statistical Analysis

Mixed models using PROC MIXED in SAS for Windows 9.4 (SAS Institute, Inc., Cary, NC) were fit for each outcome. Models were fit for data that included time points from 0 to 20 minutes, where data was limited to 20 to 50 minutes, and for data including time points from 0 to 50. Time and the baseline value of the dependent variable were fixed effects in the models. Horse identity was included as a random effect with variance components covariance structure. Repeated measures within horses were accounted for in a repeated statement using an autoregressive one covariance structure. If time was significant, differences in least squares means for each time point compared to the initial time point were determined. The simulate adjustment for multiple comparisons was used for outcomes with a significant time effect. The distribution of the conditional residuals was evaluated for each outcome to ensure the assumptions of the statistical method had
been met. An alpha level of 0.05 was used to determine statistical significance for all methods.
CHAPTER IV

RESULTS

Phase 1

Serum Magnesium

Technical difficulties unrelated to the study precluded the administration of the 15 mg/kg bolus to a single horse. Overall, all MgSO₄ boluses were well tolerated by all horses. Average total serum magnesium for each MgSO₄ bolus are listed in Table 4.1. All horses were normomagnasemic at baseline (T₀); peak serum magnesium levels corresponded with T₂₀, completion of the bolus phase. By 30 minutes following bolus conclusion (T₃₀), serum magnesium levels had decreased an average of 22.8% (15 mg/kg), 24.0% (30 mg/kg) and 28.1% (60 mg/kg), and returned to baseline by 24 hours post-bolus.

Table 4.1 Serum total Mg average and 95% confidence interval for 6 horses bolused IV MgSO₄ over 20 minutes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline (mg/dL)</th>
<th>20 mins (mg/dL)</th>
<th>50 mins (mg/dL)</th>
<th>24 hours (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mg/kg</td>
<td>1.6(1.4,1.7)</td>
<td>2.4(1.8,3.0)</td>
<td>1.9(1.6,2.1)</td>
<td>1.7(1.4,2.0)</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>1.6(1.5,1.7)</td>
<td>3.2(3.0,3.5)</td>
<td>2.5(2.2,2.7)</td>
<td>1.7(1.4,2.0)</td>
</tr>
<tr>
<td>60 mg/kg</td>
<td>1.6(1.4,1.9)</td>
<td>4.6(4.3,5.0)</td>
<td>3.4(3.1,3.8)</td>
<td>1.8(1.5,2.0)</td>
</tr>
</tbody>
</table>

Example: (Average(lower limit, upper limit)
Normal reference range for horses is 1.5 – 2.5 mg/dL
Toxicity Monitoring

At any MgSO₄ bolus dosages, continuous ECG found no abnormalities in cardiac electrical activity in any of the horses. Clinical signs of magnesium toxicosis, defined as: agitation, sweating, fine muscle tremors, excessive tachypnea (respiratory rate >12bpm from baseline respiratory rate) or tachycardia (Stewart 2011), were not noted at any time point throughout the study period. However, at the highest dose of 60 mg/kg, depression and decreased responsiveness to external stimuli were noted in 4 of the 6 horses.

Pulmonary Function

Baseline values for ΔPpl_{max}, C_{dyn}, R_{L} and MV varied among horses (Table 4.2).
Table 4.2  Average and 95% confidence interval for pulmonary function data of horses administered a 20-minute bolus of IV MgSO₄

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose</th>
<th>T0</th>
<th>T5</th>
<th>T10</th>
<th>T15</th>
<th>T20</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔP_plmax</td>
<td>15mg/kg</td>
<td>43.9(19.8,68.0)</td>
<td>34.2(10.2,58.1)</td>
<td>37.5(16.6,58.5)</td>
<td>31.4(12.0,50.8)</td>
<td>33.8(15.9,51.7)</td>
</tr>
<tr>
<td></td>
<td>30mg/kg</td>
<td>40.3(20.0,60.6)</td>
<td>28.6(14.4,42.7)</td>
<td>29.0(15.8,42.3)</td>
<td>22.4(11.2,33.7)</td>
<td>23.5(13.1,33.8)</td>
</tr>
<tr>
<td></td>
<td>60mg/kg</td>
<td>41.9(29.2,54.5)</td>
<td>29.4(19.7,39.1)</td>
<td>25.4(18.0,32.8)</td>
<td>18.3(12.5,24.0)</td>
<td>19.0(14.1,23.9)</td>
</tr>
<tr>
<td>R_l</td>
<td>15mg/kg</td>
<td>3.0(1.6,4.5)</td>
<td>2.7(1.2,4.3)</td>
<td>2.7(1.3,4.0)</td>
<td>2.3(0.9,3.8)</td>
<td>2.5(1.2,3.7)</td>
</tr>
<tr>
<td></td>
<td>30mg/kg</td>
<td>3.3(2.0,4.6)</td>
<td>2.7(1.6,3.9)</td>
<td>2.5(1.5,3.5)</td>
<td>2.2(1.2,3.2)</td>
<td>2.2(1.4,3.1)</td>
</tr>
<tr>
<td></td>
<td>60mg/kg</td>
<td>3.0(2.1,3.9)</td>
<td>2.6(1.7,3.6)</td>
<td>2.2(1.5,3.0)</td>
<td>2.0(1.1,3.0)</td>
<td>1.9(1.3,2.5)</td>
</tr>
<tr>
<td>C_dyn</td>
<td>15mg/kg</td>
<td>0.6(-0.2,1.5)</td>
<td>0.9(-0.2,2.0)</td>
<td>0.8(-0.3,1.8)</td>
<td>0.9(-0.1,1.8)</td>
<td>0.8(-0.2,1.7)</td>
</tr>
<tr>
<td></td>
<td>30mg/kg</td>
<td>0.5(0.2,0.8)</td>
<td>0.6(0.3,0.8)</td>
<td>0.6(0.3,0.9)</td>
<td>0.8(0.4,1.1)</td>
<td>0.7(0.4,1.0)</td>
</tr>
<tr>
<td></td>
<td>60mg/kg</td>
<td>0.3(0.1,0.5)</td>
<td>0.5(0.3,0.7)</td>
<td>0.5(0.3,0.7)</td>
<td>0.8(0.4,1.1)</td>
<td>0.6(0.5,0.8)</td>
</tr>
<tr>
<td>MV</td>
<td>15mg/kg</td>
<td>94.8(78.4,111.2)</td>
<td>86.2(71.8,100.5)</td>
<td>96.8(76.7,116.8)</td>
<td>98.1(73.1,123.2)</td>
<td>99.2(77.0,121.4)</td>
</tr>
<tr>
<td></td>
<td>30mg/kg</td>
<td>85.2(65.8,104.6)</td>
<td>80.7(60.0,101.4)</td>
<td>85.5(63.0,108.0)</td>
<td>77.3(55.9,98.6)</td>
<td>81.0(59.5,102.4)</td>
</tr>
<tr>
<td></td>
<td>60mg/kg</td>
<td>101.2(87.3,115.0)</td>
<td>89.6(75.6,103.6)</td>
<td>96.2(80.2,112.3)</td>
<td>85.2(68.9,101.5)</td>
<td>90.2(72.8,107.7)</td>
</tr>
</tbody>
</table>

Example: (Average(lower limit, upper limit))
*denotes a significant change from baseline from time 0-50, † denotes a significant change from baseline from time 0-20
Δ denotes a significant change from T20 after treatment bolus: time 20-50
### Table 4.2 (continued)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T20</th>
<th>T25</th>
<th>T30</th>
<th>T35</th>
<th>T40</th>
<th>T45</th>
<th>T50</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta P_{l\max}$</td>
<td>33.8(15.9,51.7) *†</td>
<td>31.0(13.1,148.8) *Δ</td>
<td>36.1(18.1,154.0) **</td>
<td>32.1(14.6,49.6) **</td>
<td>36.0(17.4,54.6) **</td>
<td>32.7(16.1,49.3) **</td>
<td>37.1(18.6,55.6) **</td>
</tr>
<tr>
<td>(cm H$_2$O)</td>
<td>23.5(13.1,33.8) *†</td>
<td>23.7(10.8,36.5) **</td>
<td>28.1(13.7,42.5) **</td>
<td>27.2(10.3,44.1) **</td>
<td>30.8(15.6,46.0) **</td>
<td>27.4(15.0,39.9) **</td>
<td>29.7(16.8,42.6) **</td>
</tr>
<tr>
<td>$R_L$ (cmH$_2$O x L$^{-1}$)</td>
<td>19.0(14.1,23.9) *†</td>
<td>19.7(14.2,25.2) **</td>
<td>26.6(17.3,35.8) **</td>
<td>24.7(16.1,33.3) **</td>
<td>30.4(22.3,38.4) **</td>
<td>28.0(18.9,37.1) **</td>
<td>30.7(21.0,40.3) **</td>
</tr>
<tr>
<td>$C_{dyn}$ (cmH$_2$O L$^{-1}$)</td>
<td>2.5(1.2,3.7) *†</td>
<td>2.5(1.0,3.9) **</td>
<td>3.1(1.7,4.5) **</td>
<td>2.4(1.2,3.7) **</td>
<td>2.7(1.4,4.0) **</td>
<td>2.6(1.1,4.1) **</td>
<td>2.7(1.6,3.9) **</td>
</tr>
<tr>
<td>$MV$ (L x min$^{-1}$)</td>
<td>2.2(1.4,3.1) *†</td>
<td>2.2(1.2,3.2) **</td>
<td>2.6(1.4,3.7) **</td>
<td>2.4(1.2,3.7) **</td>
<td>2.8(1.8,3.9) **</td>
<td>2.6(1.6,3.7) **</td>
<td>2.8(1.7,3.8) **</td>
</tr>
<tr>
<td>$MV$ (L x min$^{-1}$)</td>
<td>1.9(1.3,2.5) *†</td>
<td>2.3(1.2,3.4) **</td>
<td>2.1(1.4,2.9) **</td>
<td>2.5(1.5,3.5) **</td>
<td>2.8(1.9,3.6) **</td>
<td>2.8(1.6,4.1) **</td>
<td>2.9(1.8,4.0) **</td>
</tr>
<tr>
<td>$MV$ (L x min$^{-1}$)</td>
<td>0.8(-0.2,1.7) *†</td>
<td>0.8(-0.1,1.8) *</td>
<td>0.7(-0.1,1.6) *</td>
<td>1.1(-0.3,2.4) *</td>
<td>0.8(-0.2,1.8) *</td>
<td>1.0(-0.2,2.1) *</td>
<td>0.8(-0.3,1.9) *</td>
</tr>
<tr>
<td>$MV$ (L x min$^{-1}$)</td>
<td>0.7(0.4,1.0) *†</td>
<td>0.7(0.4,3.0) *</td>
<td>0.6(0.3,1.0) *</td>
<td>0.7(0.4,1.0) *</td>
<td>0.6(0.3,0.9) *</td>
<td>0.6(0.4,0.9) *</td>
<td>0.6(0.3,0.9) *</td>
</tr>
<tr>
<td>$MV$ (L x min$^{-1}$)</td>
<td>0.6(0.5,0.8) *†</td>
<td>0.6(0.4,0.8) *</td>
<td>0.5(0.3,0.7) *</td>
<td>0.5(0.3,0.8) *</td>
<td>0.4(0.2,0.7) *</td>
<td>0.5(0.2,0.8) *</td>
<td>0.4(0.2,0.7) *</td>
</tr>
<tr>
<td>$MV$ (L x min$^{-1}$)</td>
<td>99.2(77.0,121.4)</td>
<td>88.5(68.1,108.9)</td>
<td>98.4(69.7,127.0)</td>
<td>93.3(67.9,118.8)</td>
<td>94.0(69.5,118.6)</td>
<td>96.2(69.1,123.2)</td>
<td>93.7(74.0,113.3)</td>
</tr>
<tr>
<td>$MV$ (L x min$^{-1}$)</td>
<td>81.0(59.5,102.4)</td>
<td>82.2(60.3,104.1)</td>
<td>81.6(60.8,102.4)</td>
<td>80.4(58.8,102.0)</td>
<td>80.0(59.3,100.8)</td>
<td>78.9(59.4,98.4)</td>
<td>80.1(59.4,100.8)</td>
</tr>
<tr>
<td>$MV$ (L x min$^{-1}$)</td>
<td>90.2(72.8,107.7)</td>
<td>79.1(62.0,96.3) *</td>
<td>86.2(65.0,107.4) *</td>
<td>81.1(65.3,96.8) *</td>
<td>87.7(72.1,103.3) *</td>
<td>87.8(61.8,95.7) *</td>
<td>86.3(66.4,106.1) *</td>
</tr>
</tbody>
</table>

Example: (Average (lower limit, upper limit))

* denotes a significant change from baseline from time 0-50, † denotes a significant change from baseline from time 0-20
Δ denotes a significant change from T20 after treatment bolus: time 20-50
Relative to T₀, MgSO₄ caused a dose dependent decrease in ΔPpl_max at all time points (T₃-T₅₀, p<0.0001) (Figure 4.1). The largest decrease in average ΔPpl_max from T₀ occurred at T₂₀, corresponding to the end of IV MgSO₄ administration. From this nadir, ΔPpl_max increased significantly by T₃₀, 10 minutes following the conclusion of bolus administration. Pairwise comparisons of the effect of treatment (dose) on indices of pulmonary mechanics identified a significant decrease in ΔPpl_max (p=0.009) and increase in Cdyn (p=0.001) in horses treated with 60 mg/kg dose relative to the 15 mg/kg dose. Comparisons of Cdyn between 60 mg/kg & 30 mg/kg, and R₄ between 30 mg/kg & 15 mg/kg were also significantly different (p=0.007 and p=0.03, respectively). The remaining pairwise comparisons of dose effect on ΔPpl_max, Cdyn, and R₄ were not significantly different from each other.
Phase 1:
Maximal Change in Pleural Pressure ($\Delta P_{Pl_{max}}$)

Figure 4.1  Box & Whisker plot of: Maximal change in pleural pressure ($\Delta P_{Pl_{max}}$) of horses administered a 20-minute bolus of IV MgSO$_4$

* Denotes $p < 0.0001$ difference from baseline $\Delta P_{Pl_{max}}$ (time 0) for all dosages. The 15mg/kg treatment is not different from 30mg/kg (A, $p = 0.2217$), 30mg/kg is not different from 60mg/kg treatment ($p = 0.3597$), but 60mg/kg improvement is statistically different from 15mg/kg treatment (B, $p = 0.0090$).

Relative to $T_0$, MgSO$_4$ caused a dose dependent increase in $C_{dyn}$ by 10 minutes after bolus initiation until 15 minutes following bolus termination ($T_{10-T_{35}}$, $p<0.001$) (Figure 4.2). The largest increase in average $C_{dyn}$ from $T_0$ occurred at $T_{20}$, corresponding to the end of IV MgSO$_4$ administration. From this zenith, $C_{dyn}$ decreased significantly by $T_{40}$, 20 minutes following the conclusion of bolus administration.
Figure 4.2   Box & Whisker plot of Dynamic Compliance ($C_{dyn}$) of horses administered a 20-minute bolus of IV MgSO$_4$ (15mg/kg, 30mg/kg, 60mg/kg)

* Denotes $p \leq 0.01$ difference from baseline $C_{dyn}$ (time 0) for all dosages. Overall, 15mg/kg treatment is not different from 30mg/kg (A, $p = 0.9719$), and 60mg/kg is different from both 30mg/kg (A, $p = 0.0076$), and 15mg/kg treatment (B, $p = 0.0090$).

Relative to $T_0$, MgSO$_4$ caused a dose dependent decrease in $R_L$ at all time points ($T_5$-$T_{50}$, $p<0.03$) (Figure 4.3). The largest decrease in average $R_L$ from $T_0$ occurred at $T_{20}$, corresponding to the end of IV MgSO$_4$ administration. From this nadir, $R_L$ increased significantly by $T_{25}$, 5 minutes following the conclusion of bolus administration.
Figure 4.3  Box & Whisker plot of: Pulmonary Resistance ($R_L$) of horses administered a 20-minute bolus of IV MgSO$_4$ (15mg/kg, 30mg/kg, and 60mg/kg)

* Denotes $p \leq 0.009$ difference from baseline $R_L$ (time 0) for all dosages. Overall, treatment groups were not different from each other (A, $p = 0.0898$), but there was a trend for a dose-dependent reduction in $R_L$.

Minute Volume was the only pulmonary function parameter with mixed effects on pulmonary function by treatment group (Figure 4.4). The 15 and 30 mg/kg MgSO$_4$ dosages were not different from $T_0$ measurements at any time point ($p = 0.1192$). Relative to $T_0$, the 60 mg/kg dose of MgSO$_4$ caused decrease in MV by 15 minutes after bolus initiation and until the end of the 30 minute observation period ($T_{15}$-$T_{30}$, $p < 0.01$). The largest decrease in average MV from $T_0$ occurred at $T_{20}$, corresponding to the end of IV MgSO$_4$ administration. From this nadir, MV did not increased significantly toward baseline in the 30 minutes following the bolus administration.
**Phase 1:**

**Minute Volume (MV)**

- **Infusion**
- **Observation**

---

**Figure 4.4**  
Box & Whisker plot of: Minute Volume (MV) of horses administered a 20-minute bolus of IV MgSO₄ (15mg/kg, 30mg/kg, and 60mg/kg)

* Denotes $p \leq 0.01$ difference from baseline MV (time 0) for the 60 mg/kg dose. Overall, the 15mg/kg and 30 mg/kg treatment groups were not different from baseline or each other (A, $p = 0.32$), and 60mg/kg is different from the 15mg/kg treatment (B, $p = 0.0002$)

**Physical Parameters**

Heart rate for all MgSO₄ dosages was not different from $T_0$ measurements at any time point ($p = 0.8084$), and there was no difference between treatment groups ($p = 0.5160$) (Table 4.3).
Table 4.3  Average and 95% confidence interval for physical parameter data of horses administered a 20-minute bolus of IV MgSO₄

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose</th>
<th>T0</th>
<th>T5</th>
<th>T10</th>
<th>T15</th>
<th>T20</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR (Bpm)</td>
<td>15mg/kg</td>
<td>24.4(19.7,29.1)</td>
<td>22.0(15.9,28.1)†</td>
<td>23.2(16.6,29.8)†</td>
<td>20.0(12.6,27.4)‡†</td>
<td>22.8(15.9,29.7)‡†</td>
</tr>
<tr>
<td></td>
<td>30mg/kg</td>
<td>44.0(39.9,48.1)</td>
<td>43.5(38.5,48.5)†</td>
<td>41.4(37.6,45.2)†</td>
<td>40.5(35.9,45.1)‡†</td>
<td>41.0(36.6,45.4)‡†</td>
</tr>
<tr>
<td></td>
<td>60mg/kg</td>
<td>44.0(39.9,48.1)</td>
<td>43.5(38.5,48.5)†</td>
<td>41.4(37.6,45.2)†</td>
<td>40.5(35.9,45.1)‡†</td>
<td>41.0(36.6,45.4)‡†</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>15mg/kg</td>
<td>44.4(36.5,52.5)</td>
<td>40.8(34.1,47.4)</td>
<td>43.8(37.6,50.0)</td>
<td>42.0(36.4,47.6)</td>
<td>44.0(39.2,48.8)</td>
</tr>
<tr>
<td></td>
<td>30mg/kg</td>
<td>38.8(35.4,42.3)</td>
<td>41.4(34.5,48.3)</td>
<td>40.2(33.4,46.9)</td>
<td>41.2(34.1,48.3)</td>
<td>38.8(34.8,42.9)</td>
</tr>
<tr>
<td></td>
<td>60mg/kg</td>
<td>45.3(31.6,59.0)</td>
<td>44.2(37.9,50.5)</td>
<td>39.3(33.4,45.2)</td>
<td>40.6(38.5,42.7)</td>
<td>40.8(37.5,44.1)</td>
</tr>
<tr>
<td>TA</td>
<td>15mg/kg</td>
<td>7.2(5.4,9.0)</td>
<td>4.8(1.8,7.7)†</td>
<td>4.4(1.6,7.2)†</td>
<td>3.5(0.8,6.2)†</td>
<td>4.0(1.3,6.7)†</td>
</tr>
<tr>
<td></td>
<td>30mg/kg</td>
<td>6.2(4.5,7.9)</td>
<td>5.0(3.4,6.6)††</td>
<td>3.3(1.8,4.8)††</td>
<td>3.6(1.9,5.3)††</td>
<td>3.2(2.2,4.1)††</td>
</tr>
<tr>
<td></td>
<td>60mg/kg</td>
<td>7.7(6.4,9.0)</td>
<td>6.0(4.7,7.3)††</td>
<td>4.8(3.6,6.1)††</td>
<td>4.4(3.2,5.6)††</td>
<td>4.2(3.2,5.1)††</td>
</tr>
<tr>
<td>TRA</td>
<td>15mg/kg</td>
<td>3.6(1.9,5.3)</td>
<td>2.8(0.9,4.6)‡†</td>
<td>3.0(1.4,4.6)‡†</td>
<td>2.3(0.3,4.2)‡†</td>
<td>2.8(0.7,4.9)‡†</td>
</tr>
<tr>
<td></td>
<td>30mg/kg</td>
<td>2.5(1.4,3.6)</td>
<td>2.2(0.9,3.5)††</td>
<td>1.5(0.1,2.9)††</td>
<td>1.6(0.0,3.2)††</td>
<td>1.5(0.1,2.9)††</td>
</tr>
<tr>
<td></td>
<td>60mg/kg</td>
<td>3.0(2.0,4.0)</td>
<td>2.8(1.3,4.3)††</td>
<td>2.0(0.8,3.2)‡†</td>
<td>2.2(0.9,3.5)††</td>
<td>2.0(0.8,3.2)‡†</td>
</tr>
</tbody>
</table>

Example: (Average(lower limit, upper limit))
* denotes a significant change from baseline from time 0-50, † denotes a significant change from baseline from time 0-20
‡ denotes a significant change from T20 after treatment bolus: time 20-50
Table 4.3 (continued)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T20</th>
<th>T25</th>
<th>T30</th>
<th>T35</th>
<th>T40</th>
<th>T45</th>
<th>T50</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR (bpm)</td>
<td>22.8(15.9,29.7)*</td>
<td>21.8(14.6,28.9)*</td>
<td>22.4(15.2,29.6)*</td>
<td>21.5(13.0,30.0)*</td>
<td>24.0(15.9,32.1)</td>
<td>23.5(16.1,30.9)</td>
<td>23.6(16.3,30.9)</td>
</tr>
<tr>
<td></td>
<td>41.0(36.6,45.4)*</td>
<td>42.5(37.7,47.3)*</td>
<td>41.4(36.7,46.1)*</td>
<td>41.5(35.9,47.1)*</td>
<td>41.6(37.1,46.1)</td>
<td>44.0(40.2,47.8)</td>
<td>41.2(36.3,46.1)</td>
</tr>
<tr>
<td></td>
<td>41.0(36.6,45.4)*</td>
<td>42.5(37.7,47.3)*</td>
<td>41.4(36.7,46.1)*</td>
<td>41.5(35.9,47.1)*</td>
<td>41.6(37.1,46.1)</td>
<td>44.0(40.2,47.8)</td>
<td>41.2(36.3,46.1)</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>44.0(39.2,48.8)</td>
<td>42.3(36.2,48.3)</td>
<td>43.0(34.9,51.1)</td>
<td>40.0(35.7,44.3)</td>
<td>41.6(35.8,47.4)</td>
<td>39.8(35.2,44.3)</td>
<td>44.0(37.9,50.1)</td>
</tr>
<tr>
<td></td>
<td>38.8(34.8,42.9)</td>
<td>38.2(33.9,42.5)</td>
<td>37.0(33.2,40.8)</td>
<td>38.2(34.5,41.9)</td>
<td>39.8(35.6,44.1)</td>
<td>39.0(34.8,43.2)</td>
<td>39.3(34.4,44.3)</td>
</tr>
<tr>
<td></td>
<td>40.8(37.5,44.1)</td>
<td>41.0(36.2,45.8)</td>
<td>40.5(37.2,43.8)</td>
<td>44.0(37.2,50.8)</td>
<td>43.3(37.5,49.2)</td>
<td>42.6(37.1,48.1)</td>
<td>42.7(36.5,48.9)</td>
</tr>
<tr>
<td>TA</td>
<td>4.0(1.3,6.7)*†</td>
<td>3.3(0.6,6.5)*†</td>
<td>4.6(1.6,7.6)*†</td>
<td>4.3(0.9,7.6)*†</td>
<td>5.4(2.1,8.7)*†</td>
<td>4.5(1.3,7.7)†</td>
<td>5.8(2.4,9.2)†</td>
</tr>
<tr>
<td></td>
<td>3.2(2.2,4.1)*†</td>
<td>3.2(2.2,4.2)*†</td>
<td>3.7(2.6,4.8)*†</td>
<td>3.8(2.5,5.1)*†</td>
<td>4.0(2.9,5.1)*†</td>
<td>4.6(2.9,6.3)†</td>
<td>5.0(3.6,6.4)†</td>
</tr>
<tr>
<td></td>
<td>4.2(3.2,5.1)*†</td>
<td>4.2(3.2,5.2)*†</td>
<td>4.5(3.5,5.5)*†</td>
<td>5.4(4.2,6.6)*†</td>
<td>6.2(4.8,7.5)*†</td>
<td>6.4(4.7,8.1)†</td>
<td>6.7(5.3,8.1)†</td>
</tr>
<tr>
<td>TRA</td>
<td>2.8(0.7,4.9)*†</td>
<td>2.5(0.2,4.8)*†</td>
<td>2.8(0.7,4.9)***</td>
<td>2.5(0.2,4.8)***</td>
<td>3.2(0,8,5.6)***</td>
<td>3.3(1.3,5.2)***</td>
<td>3.8(1.8,5.8)***</td>
</tr>
<tr>
<td></td>
<td>1.5(0.1,2.9)*†</td>
<td>1.6(0.0,3.2)*†</td>
<td>1.5(0.1,2.9)***</td>
<td>1.6(0.0,3.2)***</td>
<td>1.7(0.3,3.1)***</td>
<td>2.0(0.5,3.5)***</td>
<td>2.0(0.7,3.3)***</td>
</tr>
<tr>
<td></td>
<td>2.0(0.8,3.2)*†</td>
<td>2.2(0.9,3.5)*†</td>
<td>2.3(0.8,3.9)***</td>
<td>2.6(0.9,4.3)***</td>
<td>2.3(0.8,3.9)***</td>
<td>3.0(1.3,4.7)***</td>
<td>2.7(1.0,4.3)***</td>
</tr>
</tbody>
</table>

Example: (Average(lower limit, upper limit))
* denotes a significant change from baseline from time 0-50, † denotes a significant change from baseline from time 0-20
Δ denotes a significant change from T20 after treatment bolus: time 20-50
Relative to T₀, all MgSO₄ treatments caused a decrease in respiratory rate (RR) by 15 minutes after bolus initiation. This remained reduced until 15 minutes after bolus termination (T₁₅-T₃₅, p<0.02). The largest decrease in average RR from T₀ occurred at T₁₅. From this nadir, RR did not increase significantly by T₃₀. Pairwise comparisons of the effect of treatment (dose) on RR were not different between treatment groups (p=0.516). Average peak reduction from baseline in RR at T₁₅ was 2.82 +/- 0.59 breaths per minute. Maximal reductions of RR for all doses (15, 30 and 60 mg/kg) as a percent change from baseline T₀ were -13.6% (T₁₅), -16.3% (T₁₅), and -14.40% (T₂₅), respectively (Table 4.3).

Auscultation Scores

Baseline scores for thoracic auscultation (TA) and tracheal auscultation (TRA) varied among horses (Table 4.3). Relative to T₀, MgSO₄ caused a decrease in TA at all time points (p<0.0086 for T₅-T₅₀₃) (Figure 4.5). The largest decrease in average TA from T₀ occurred at T₂₅ corresponding to the end of IV MgSO₄ administration. From this nadir, TA increased significantly by T₃₀, 10 minutes following the conclusion of bolus administration. Pairwise comparisons of the effect of treatment (dose) on indices of auscultation identified no significant differences in TA (p=0.9819) or TRA (p=0.9083).
Figure 4.5  Thoracic Auscultation (TA) score of horses administered a 20-minute bolus of IV MgSO₄ (15mg/kg, 30mg/kg, and 60mg/kg)

* Denotes p <0.0086 difference from baseline TA (time 0) for all doses. Overall, treatment groups were not different from each other (p =0.9819). Solid line indicates average score of each group.

Relative to T₀, MgSO₄ caused a decrease in TRA by 5 minutes after bolus initiation until 20 minutes following bolus termination (T₃-T₄₀, p<0.03) (Figure 4.6). The largest decrease in average TRA from T₀ occurred at T₂₀ (T₁₅ 1mmg/kg). From this nadir, TRA increased significantly by T₄₅, 25 minutes following the conclusion of bolus administration.
Figure 4.6  Tracheal Auscultation (TRA) score of horses administered a 20-minute bolus of IV MgSO₄ (15 mg/kg, 30 mg/kg, and 60 mg/kg).

* Denotes p < 0.0086 difference from baseline TRA (time 0) for all doses. Overall, treatment groups were not different from each other (p=0.9083). Solid line indicates average score of each group.

Phase 2

Pulmonary Function

Baseline values for ΔPpₘₐₓ, Cdyn, Rₐ, and MV varied among horses (Table 4.4).
Table 4.4  Average and 95% confidence interval for pulmonary function and physical parameter data of horses administered a 20-minute, 30 mg/kg bolus of IV MgSO₄

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T0</th>
<th>T5</th>
<th>T10</th>
<th>T15</th>
<th>T20</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔPpₚₚₚₚₚₚₚₚₚₚₘₚₘ (cm H₂O)</td>
<td>39.9(30.1,49.8)</td>
<td>31.4(23.5,39.3) *†</td>
<td>28.3(20.4,36.2) *†</td>
<td>22.9(15.9,29.8) *†</td>
<td>24.9(16.1,33.7) *†</td>
</tr>
<tr>
<td>RL (cmH₂O x L⁻¹)</td>
<td>3.0(2.5,3.5)</td>
<td>2.7(2.1,3.4)</td>
<td>2.3(1.8,2.7) *†</td>
<td>2.1(1.6,2.5) *†</td>
<td>2.1(1.6,2.5) *†</td>
</tr>
<tr>
<td>Cᵥ (cmH₂O⁻¹)</td>
<td>0.44(0.1,0.8)</td>
<td>0.52(0.2,0.8)</td>
<td>0.59(0.2,1.0)</td>
<td>0.65(0.4,0.9)</td>
<td>0.56(0.3,0.9)</td>
</tr>
<tr>
<td>MV (L x min⁻¹)</td>
<td>88.6(58.7,118.5)</td>
<td>83.2(52.3,114.2)</td>
<td>91.5(59.5,123.5)</td>
<td>82.6(46.7,118.6)</td>
<td>93.1(54.1,132.0)</td>
</tr>
<tr>
<td>RR (bpm)</td>
<td>44.0(39.9,48.1)</td>
<td>43.5(38.5,48.5)</td>
<td>41.4(37.6,45.2)</td>
<td>40.5(35.9,45.1)</td>
<td>41.0(36.6,45.4)</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>44.0(39.9,48.1)</td>
<td>43.5(38.5,48.5)</td>
<td>41.4(37.6,45.2)</td>
<td>40.5(35.9,45.1)</td>
<td>41.0(36.6,45.4)</td>
</tr>
<tr>
<td>TA</td>
<td>6.8(6.1,7.5)</td>
<td>6.0(5.3,6.7) *†</td>
<td>3.8(2.8,4.8) *†</td>
<td>3.5(2.6,4.4) *†</td>
<td>3.4(2.6,4.2) *†</td>
</tr>
<tr>
<td>TRA</td>
<td>1.4(0.6,2.2)</td>
<td>0.5(0.0,1.0) *†</td>
<td>0.6(0.1,1.1) *†</td>
<td>0.5(0.0,1.0) *†</td>
<td>0.6(0.1,1.1) *†</td>
</tr>
</tbody>
</table>

Example: (Average(lower limit, upper limit))
* denotes a significant change from baseline from time 0-50, † denotes a significant change from baseline from time 0-20
Δ denotes a significant change from T₂₀ after treatment bolus: time 20-50
<table>
<thead>
<tr>
<th>Parameter</th>
<th>T20</th>
<th>T25</th>
<th>T30</th>
<th>T35</th>
<th>T40</th>
<th>T45</th>
<th>T50</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔPpl_{max} (cm H₂O)</td>
<td>24.9(16.1,33.7) * †</td>
<td>23.6(16.6,30.5) *</td>
<td>27.0(18.2,35.9) *</td>
<td>27.9(16.8,39.0) *</td>
<td>29.3(20.6,38.0) *</td>
<td>28.1(19.3,36.9) *</td>
<td>29.4(20.8,38.1) *</td>
</tr>
<tr>
<td>R_{L} (cmH₂O x L⁻¹)</td>
<td>2.1(1.6,2.5) * †</td>
<td>2.2(1.6,2.8) *</td>
<td>2.3(1.7,2.9) *</td>
<td>2.3(1.7,3.0) *</td>
<td>2.5(2.0,3.1)</td>
<td>2.6(1.9,3.3)</td>
<td>2.6(2.0,3.2)</td>
</tr>
<tr>
<td>C_{dyn} (cmH₂O⁻¹)</td>
<td>0.56(0.3,0.9)</td>
<td>0.59(0.3,0.9)</td>
<td>0.49(0.2,0.8)</td>
<td>0.53(0.2,0.8)</td>
<td>0.45(0.2,0.7)</td>
<td>0.53(0.3,0.8)</td>
<td>0.51(0.2,0.8)</td>
</tr>
<tr>
<td>MV (L x min⁻¹)</td>
<td>93.1(54.1,132.0)</td>
<td>83.9(49.0,118.7)</td>
<td>89.4(55.6,123.1)</td>
<td>82.9(51.2,114.5)</td>
<td>87.3(55.2,119.4)</td>
<td>81.8(47.7,116.0)</td>
<td>85.2(53.9,116.4)</td>
</tr>
<tr>
<td>RR (bpm)</td>
<td>41.0(36.6,45.4)</td>
<td>42.5(37.7,47.3)</td>
<td>41.4(36.7,46.1)</td>
<td>41.5(35.9,47.1)</td>
<td>41.6(37.1,46.1)</td>
<td>44.0(40.2,47.8)</td>
<td>41.2(36.3,46.1)</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>41.0(36.6,45.4)</td>
<td>42.5(37.7,47.3)</td>
<td>41.4(36.7,46.1)</td>
<td>41.5(35.9,47.1)</td>
<td>41.6(37.1,46.1)</td>
<td>44.0(40.2,47.8)</td>
<td>41.2(36.3,46.1)</td>
</tr>
<tr>
<td>TA</td>
<td>3.4(2.6,4.2) * †</td>
<td>3.3(2.4,4.1) *</td>
<td>3.6(2.8,4.4) *</td>
<td>4.0(2.8,5.2) *</td>
<td>4.8(3.2,6.4) *</td>
<td>5.5(3.4,7.6) Δ</td>
<td>6.0(4.2,7.8) Δ</td>
</tr>
<tr>
<td>TRA</td>
<td>0.6(0.1,1.1) * †</td>
<td>0.5(0.0,1.0) *</td>
<td>0.6(0.1,1.1) *</td>
<td>0.5(0.0,1.0) *</td>
<td>0.8(0.1,1.5) *Δ</td>
<td>1.0(0.3,1.7) *Δ</td>
<td>1.2(0.5,1.9) Δ</td>
</tr>
</tbody>
</table>

Example: (Average(lower limit, upper limit))

* denotes a significant change from baseline from time 0-50, † denotes a significant change from baseline from time 0-20
Δ denotes a significant change from T20 after treatment bolus: time 20-50
Relative to $T_0$, MgSO$_4$ caused a significant decrease in $\Delta P_{\text{pl}}$ at all time points ($T_5$-$T_{30}$, $p = 0.0001$) (Figure 4.7). The largest decrease in average $\Delta P_{\text{pl}}$ from $T_0$ occurred at $T_{20}$, corresponding to the end of IV MgSO$_4$ administration. From this nadir, $\Delta P_{\text{pl}}$ did not increased significantly by $T_{30}$, 30 minutes following the conclusion of bolus administration ($p=0.6028$).

Figure 4.7  Box & Whisker plot of: Maximal change in pleural pressure ($\Delta P_{\text{pl}}$) of horses administered a 20-minute bolus of 30mg/kg IV MgSO$_4$

* Denotes $p = 0.0001$ difference from baseline $\Delta P_{\text{pl}}$ (time0).

Relative to $T_0$, MgSO$_4$ caused an increase in $C_{\text{dyn}}$ by 5 minutes after bolus initiation until 30 minutes following bolus termination, however this change failed to reach significance ($p=0.1434$) (Figure 4.8). The average of individual differences in $C_{\text{dyn}}$ from baseline was greatest at $T_{20}$, corresponding to the end of IV MgSO$_4$ administration (Average of 30.78% increase above $T_0$).
Figure 4.8  Box & Whisker plot of: Dynamic Compliance ($C_{dy}$) of horses administered a 20-minute bolus of 30mg/kg IV MgSO₄.

Difference from baseline $C_{dy}$ (time 0) was not significant (p=0.1660)

Relative to T₀, MgSO₄ caused a significant decrease in $R_L$ at all time points (T₅-T₅₀, p=0.0002) (Figure 4.9). The largest decrease in average $R_L$ from T₀ occurred at T₂₀, corresponded to the end of IV MgSO₄ administration. From this nadir, $R_L$ had not increased significantly by T₅₀, 30 minutes following the conclusion of bolus administration (p=0.1843).
Figure 4.9  Box & Whisker plot of: Pulmonary Resistance ($R_L$) of horses administered a 20-minute bolus of 30mg/kg IV MgSO$_4$

* Denotes $p=0.0002$ difference from baseline $R_L$ (time 0)

Minute Volume for 30 mg/kg MgSO$_4$ treatment was not different from $T_0$ measurements at any time point ($p=0.3284$) (Figure 4.10).
Figure 4.10  Box & Whisker plot of Minute Volume (MV) of horses administered a 20-minute bolus of 30mg/kg IV MgSO₄. There was no significant difference from baseline MV (time 0). (p=0.3284)

There was no significant difference from baseline MV (time 0). (p=0.3284)

Physical Parameters

Baseline values for HR and RR varied among horses (Table 4.4). Heart rate was not different from T₀ measurements at any time point (p =0.4680). RR also did not demonstrate a significant change when compared to baseline (T₀) values at any time point (p=0.0970).

Auscultation Scores

Baseline scores for thoracic auscultation (TA) and tracheal auscultation (TRA) varied among horses (Table 4.4). Relative to T₀, MgSO₄ caused a decrease in TA by 5 minutes after bolus initiation until 20 minutes following bolus termination (T₃–T₄₀, p<0.0181) (Figure 4.11). The largest decrease in average TA from T₀ occurred at T₂₀, corresponding to the end of IV MgSO₄ administration. From this nadir, TA increased
significantly by T_{45}, 25 minutes following the conclusion of bolus administration.

![Thoracic Auscultation (TA) graph](image)

Figure 4.11 Thoracic (TA) auscultation scores of horses administered a 20-minute bolus of 30mg/kg IV MgSO₄.

* Denotes a p<0.02 difference from baseline TA. Solid line indicates average TA.

Relative to \( T_0 \), MgSO₄ caused a decrease in TRA by 5 minutes after bolus initiation until 45 minutes following bolus termination (\( T_5-T_{45}, p<0.04 \)) (Figure 4.12).

The largest decrease in average TRA from \( T_0 \) occurred at \( T_{20} \), corresponding to the end of IV MgSO₄ administration. From this nadir, TRA increased significantly by \( T_{30} \), 30 minutes following the conclusion of bolus administration.
Figure 4.12  Tracheal (TRA) auscultation scores of horses administered a 20-minute bolus of 30mg/kg IV MgSO₄

* Denotes a p<0.04 difference from baseline TRA. Solid line indicates average TRA

Venous Blood Chemistry Analysis

Technical difficulties unrelated to the study precluded the collection of venous blood in two horses at the 24-hour post-bolus administration time point. Baseline values for venous free calcium:magnesium ratio (vCa:Mg), free ionized magnesium (viMg²⁺), blood glucose (vBG), oxygen saturation (vsO₂), venous pH (vpH), partial pressure of carbon dioxide (vpCO₂), total carbon dioxide (vtCO₂), partial pressure of oxygen (vpO₂), free ionized calcium (viCa), lactate, total hemoglobin (vtHb), oxygenated hemoglobin (vO₂Hb), carbaminohemoglobin (vCO₂Hb), methemoglobin (vMetHb) and deoxyhemoglobin (vHHb) varied among horses (Table 4.5).
Table 4.5  Average and 95% confidence interval for venous blood chemistry data of horses administered a 20-minute, 30 mg/kg bolus of IV MgSO₄

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T0</th>
<th>T5</th>
<th>T10</th>
<th>T15</th>
<th>T20</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO₂ %</td>
<td>74.28(76.9,80.7)</td>
<td>74.60(72.0,77.2)</td>
<td>75.82(73.0,78.6)</td>
<td>75.30(73.1,77.6)</td>
<td>75.12(72.5,77.7)</td>
</tr>
<tr>
<td>pH</td>
<td>7.39(7.4,7.4)</td>
<td>7.39(7.4,7.4)</td>
<td>7.40(7.4,7.4)</td>
<td>7.39(7.4,7.4)</td>
<td>7.40(7.4,7.4)</td>
</tr>
<tr>
<td>pCO₂</td>
<td>46.66(42.2,51.1)</td>
<td>47.38(42.6,52.2)</td>
<td>46.16(42.4,49.9)</td>
<td>47.12(42.4,51.9)</td>
<td>46.16(41.1,51.2)</td>
</tr>
<tr>
<td>pO₂</td>
<td>38.80(34.9,42.7)</td>
<td>38.36(36.6,40.1)</td>
<td>39.44(38.0,40.9)</td>
<td>38.94(37.0,40.9)</td>
<td>39.20(37.4,41.0)</td>
</tr>
<tr>
<td>iMg</td>
<td>0.54(0.5,0.6)</td>
<td>0.68(0.6,0.7)</td>
<td>0.77(0.7,0.8)</td>
<td>0.85(0.8,0.9)</td>
<td>0.87(0.8,0.9)</td>
</tr>
<tr>
<td>iCa</td>
<td>1.56(1.5,1.6)</td>
<td>1.58(1.5,1.6)</td>
<td>1.56(1.5,1.6)</td>
<td>1.57(1.5,1.6)</td>
<td>1.51(1.5,1.5)</td>
</tr>
<tr>
<td>iCa/iMg</td>
<td>2.92(2.7,3.1)</td>
<td>2.34(2.2,2.5)</td>
<td>2.06(1.9,2.2)</td>
<td>1.86(1.7,2.0)</td>
<td>1.74(1.6,1.9)</td>
</tr>
<tr>
<td>Glu</td>
<td>87.40(77.8,97.0)</td>
<td>91.40(82.2,100.6)</td>
<td>91.40(83.4,99.9)</td>
<td>94.00(85.1,102.9)</td>
<td>89.80(84.9,94.7)</td>
</tr>
<tr>
<td>Lac</td>
<td>0.80(0.5,1.1)</td>
<td>0.72(0.6,0.8)</td>
<td>0.72(0.6,0.8)</td>
<td>0.72(0.6,0.8)</td>
<td>0.74(0.6,0.8)</td>
</tr>
<tr>
<td>TCO₂</td>
<td>30.18(27.8,32.6)</td>
<td>30.34(28.1,32.6)</td>
<td>30.10(28.2,32.0)</td>
<td>30.50(28.1,32.9)</td>
<td>29.86(27.6,32.1)</td>
</tr>
<tr>
<td>tHb</td>
<td>17.50(11.5,23.5)</td>
<td>13.78(13.0,14.5)</td>
<td>13.64(12.9,14.3)</td>
<td>13.78(13.1,14.5)</td>
<td>13.82(12.8,14.8)</td>
</tr>
<tr>
<td>O₂Hb</td>
<td>73.46(67.0,79.9)</td>
<td>73.88(71.3,76.5)</td>
<td>74.68(72.0,77.4)</td>
<td>74.40(72.3,76.5)</td>
<td>74.16(71.5,76.9)</td>
</tr>
<tr>
<td>CO₂Hb</td>
<td>0.44(0.2,0.6)</td>
<td>0.34(0.1,0.5)</td>
<td>0.66(0.5,0.8)</td>
<td>0.64(0.3,0.9)</td>
<td>0.60(0.4,0.8)</td>
</tr>
<tr>
<td>MetHb</td>
<td>0.66(0.5,0.9)</td>
<td>0.62(0.5,0.8)</td>
<td>0.84(0.7,1.0)</td>
<td>0.60(0.5,0.7)</td>
<td>0.68(0.5,0.8)</td>
</tr>
<tr>
<td>HHb</td>
<td>25.44(19.1,31.8)</td>
<td>25.16(22.6,27.7)</td>
<td>23.82(21.0,26.6)</td>
<td>24.34(22.1,26.6)</td>
<td>24.54(22.0,27.1)</td>
</tr>
<tr>
<td>A</td>
<td>91.18(85.7,96.6)</td>
<td>90.26(84.5,96.1)</td>
<td>91.76(87.2,96.3)</td>
<td>90.60(84.9,96.3)</td>
<td>91.78(85.7,97.9)</td>
</tr>
</tbody>
</table>

Example: (Average (lower limit, upper limit))
* denotes a significant change from baseline from time 0-50, † denotes a significant change from baseline from time 0-20
Δ denotes a significant change from T20 after treatment bolus: time 20-50
Table 4.5 (continued)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T20</th>
<th>T25</th>
<th>T30</th>
<th>T35</th>
<th>T40</th>
<th>T45</th>
<th>T50</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO2%</td>
<td>75.1(72.5,77.7)</td>
<td>75.5(69.4,81.5)</td>
<td>76.1(73.8,78.5)</td>
<td>72.9(70.5,75.3)</td>
<td>73.8(69.0,78.7)</td>
<td>72.9(69.6,76.3)</td>
<td>73.9(71.6,76.2)</td>
</tr>
<tr>
<td>pH</td>
<td>7.40(7.4,7.4)</td>
<td>7.39(7.4,7.4)</td>
<td>7.40(7.4,7.4)</td>
<td>7.39(7.4,7.4)</td>
<td>7.40(7.4,7.4)</td>
<td>7.40(7.4,7.4)</td>
<td>7.39(7.4,7.4)</td>
</tr>
<tr>
<td>pCO2</td>
<td>46.2(41.1,51.2)</td>
<td>47.6(43.9,51.4)</td>
<td>47.8(42.8,51.2)</td>
<td>47.9(43.4,52.4)</td>
<td>47.2(42.2,52.2)</td>
<td>47.2(42.3,52.1)</td>
<td>48.3(44.1,52.4)</td>
</tr>
<tr>
<td>pO2</td>
<td>39.2(37.4,41.0)</td>
<td>39.5(35.6,43.5)</td>
<td>40.0(38.4,43.1)</td>
<td>38.4(36.7,40.2)</td>
<td>38.6(36.3,40.8)</td>
<td>38.1(36.7,39.4)</td>
<td>38.6(37.2,40.0)</td>
</tr>
<tr>
<td>iMg</td>
<td>0.87(0.8,0.9)**</td>
<td>0.83(0.8,0.9)Δ</td>
<td>0.79(0.7,0.8)Δ</td>
<td>0.76(0.7,0.8)Δ</td>
<td>0.75(0.7,0.8)Δ</td>
<td>0.74(0.7,0.8)Δ</td>
<td>0.72(0.7,0.8)Δ</td>
</tr>
<tr>
<td>iCa</td>
<td>1.51(1.5,1.5)</td>
<td>1.54(1.5,1.6)</td>
<td>1.53(1.5,1.6)</td>
<td>1.53(1.5,1.6)</td>
<td>1.56(1.5,1.6)</td>
<td>1.55(1.5,1.6)</td>
<td>1.55(1.5,1.6)</td>
</tr>
<tr>
<td>iCa/iMg</td>
<td>1.74(1.6,1.9)**</td>
<td>1.86(1.7,2.0)Δ</td>
<td>1.96(1.8,2.1)Δ</td>
<td>2.02(1.9,2.2)Δ</td>
<td>2.06(1.9,2.2)Δ</td>
<td>2.12(1.9,2.3)Δ</td>
<td>2.16(2.0,2.3)Δ</td>
</tr>
<tr>
<td>Glu</td>
<td>89.8(84.9,94.7)</td>
<td>93.8(86.5,101.1)Δ</td>
<td>93.0(85.7,100.3)</td>
<td>93.6(86.0,101.2)*</td>
<td>94.8(86.8,102.8)*Δ</td>
<td>95.4(87.4,103.4)*Δ</td>
<td>95.6(87.2,104.0)*Δ</td>
</tr>
<tr>
<td>Lac</td>
<td>0.74(0.6,0.8)</td>
<td>0.72(0.6,0.8)</td>
<td>0.74(0.6,0.8)</td>
<td>0.78(0.7,0.9)</td>
<td>0.78(0.7,0.9)</td>
<td>0.78(0.7,0.9)</td>
<td>0.78(0.7,0.9)</td>
</tr>
<tr>
<td>TCO2</td>
<td>29.9(27.6,32.1)</td>
<td>30.8(29.0,32.6)</td>
<td>30.4(28.6,32.2)</td>
<td>30.9(29.1,32.8)</td>
<td>30.7(28.9,32.5)</td>
<td>30.5(28.7,32.4)</td>
<td>30.9(29.3,32.6)</td>
</tr>
<tr>
<td>tHb</td>
<td>13.8(12.8,14.8)</td>
<td>13.5(12.7,14.3)</td>
<td>13.3(12.1,14.5)</td>
<td>13.1(12.0,14.2)</td>
<td>13.2(12.1,14.2)</td>
<td>13.3(12.2,14.3)</td>
<td>13.2(11.9,14.5)</td>
</tr>
<tr>
<td>O2Hb</td>
<td>74.2(71.5,76.9)</td>
<td>74.7(68.6,80.9)</td>
<td>75.3(73.1,77.5)</td>
<td>72.2(69.6,74.7)</td>
<td>73.0(67.9,78.1)</td>
<td>72.2(68.8,75.6)</td>
<td>72.8(70.7,74.9)</td>
</tr>
<tr>
<td>CO2Hb</td>
<td>0.60(0.4,0.8)</td>
<td>0.44(0.2,0.7)</td>
<td>0.44(0.2,0.7)</td>
<td>0.68(0.6,0.8)</td>
<td>0.50(0.2,0.8)</td>
<td>0.52(0.2,0.8)</td>
<td>0.60(0.3,0.9)</td>
</tr>
<tr>
<td>MetHb</td>
<td>0.68(0.5,0.8)</td>
<td>0.52(0.3,0.7)</td>
<td>0.58(0.2,1.0)</td>
<td>0.38(0.2,0.6)</td>
<td>0.64(0.4,0.8)</td>
<td>0.52(0.2,0.8)</td>
<td>0.76(0.5,1.0)</td>
</tr>
<tr>
<td>HHb</td>
<td>25.4(19.1,31.8)</td>
<td>25.2(22.6,27.7)</td>
<td>23.8(21.0,26.6)</td>
<td>24.3(22.1,26.6)</td>
<td>24.5(22.0,27.1)</td>
<td>24.3(18.3,30.3)</td>
<td>23.6(21.3,26.0)</td>
</tr>
<tr>
<td>A</td>
<td>91.2(85.7,96.6)</td>
<td>90.3(84.5,96.1)</td>
<td>91.8(87.2,96.3)</td>
<td>90.6(84.9,96.3)</td>
<td>91.8(85.7,97.9)</td>
<td>89.0(85.4,94.5)</td>
<td>90.3(85.4,95.3)</td>
</tr>
</tbody>
</table>

Example: (Average (lower limit, upper limit))

* denotes a significant change from baseline from time 0-50, † denotes a significant change from baseline from time 0-20Δ denotes a significant change from T20 after treatment bolus: time 20-50
T₀ values for viMg²⁺ ranged from 0.48 to 0.59 mmol/L. Relative to T₀, MgSO₄ caused a significant increase in viMg²⁺ for all time points T₀—T₅₀ (p<0.0001) (Figure 4.13). The largest increase in average viMg²⁺ from T₀ occurred at T₂₀, corresponding to the end of IV MgSO₄ administration (Average 0.87 mmol/L). From this zenith, viMg²⁺ decreased significantly by T₃₀, 10 minutes following the conclusion of bolus administration. At the end of the 30 minute post-bolus phase viMg²⁺ remained significantly increased from T₀ (Average 0.72 mmol/L). By 24 hours post-bolus, viMg²⁺ had returned to T₀ values (Average 0.53 mmol/L).

![Figure 4.13 Box & Whisker plot of Venous free ionized magnesium (viMg²⁺) of horses administered a 20-minute bolus of 30mg/kg IV MgSO₄ *Denotes p<0.001 difference from baseline viMg²⁺ (time 0)

T₀ ratios for vCa:Mg ranged from 2.7 to 3.3 mmol/L (Average: 2.92). Relative to T₀, MgSO₄ caused a significant decrease in vCa:Mg at all time points (T₅—T₅₀, p<0.0001) (Figure 4.14). The largest decrease in average vCa:Mg from T₀ occurred at T₂₀.
corresponding to the end of IV MgSO₄ administration (Average 4.14). From this nadir, vCa:Mg increased significantly by T₂₅, 5 minutes following the conclusion of bolus administration. At the end of the 30 minute post-bolus phase vCa:Mg remained significantly decreased from T₀ (Average 2.16). By 24 hours post-bolus, vCa:Mg had not completely returned to T₀ values (Average 2.63).

![Box & Whisker plot of Venous free ionized magnesium (vCa:Mg) of horses administered a 20-minute bolus of 30mg/kg IV MgSO₄](image)

*Denotes p<0.0001 difference from baseline vCa:Mg (time)

T₀ values for vBG ranged from 74.0 to 103.0 mg/dL (Average: 87.4 mg/dL).

Relative to T₀, MgSO₄ caused a significant increase in vBG for most time points (T₁₅ [p<0.02], T₂₅ [p<0.02], T₄₀-T₅₀ [p<0.03]) (Figure 4.15). The largest increase in average vBG from T₀ occurred at T₃₀ (Average 95.6 mg/dL). By 24 hours post-bolus, vBG had returned to below T₀ values (Average 83.0 mg/dL).
Phase 2: 30 mg/kg

Venous Glucose (vBG)

Figure 4.15 Box & Whisker plot of Venous Blood Glucose (vBG) of horses administered a 20-minute bolus of 30mg/kg IV MgSO₄

*Denotes p<0.03 difference from baseline vBG (time 0)

Relative to T₀, MgSO₄ caused no significant change in the venous values of: vsO₂ (p=0.8757), vpH (p=0.7733), vpCO₂ (p=0.3063), vtCO₂ (p=0.1416), vpO₂ (p=0.94241), viCa (p=0.1330), lactate (p=0.6457), vtHb (p=0.2014), vO₂Hb (p=0.9088), vCO₂Hb (p=0.5443), vMetHb (p=0.2004) and vHHb (p=0.8640).

Arterial Blood Gas Analysis

Samples for arterial free ionized magnesium (aiMg²⁺), pH (apH), partial pressure of carbon dioxide (apCO₂), partial pressure of oxygen (apO₂), oxygenation index (pO₂/FiO₂), base excess (BE), bicarbonate (HCO₃⁻), total carbon dioxide (atCO₂), oxygen saturation (asO₂) and lactate (aLac) were taken. Values for aiMg²⁺ and aLac are reported (Table 4.6).
Table 4.6  Average and 95% confidence arterial blood gas data of horses administered a 20-minute, 30 mg/kg bolus of IV MgSO₄

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T0</th>
<th>T20</th>
<th>T50</th>
</tr>
</thead>
<tbody>
<tr>
<td>iMg</td>
<td>0.53(0.5,0.6)</td>
<td>0.86(0.8,0.9)*</td>
<td>0.72(0.7,0.8)*</td>
</tr>
<tr>
<td>Lac</td>
<td>0.43(0.2,0.6)</td>
<td>0.46(0.3,0.6)</td>
<td>0.45(0.2,0.7)</td>
</tr>
</tbody>
</table>

Example: (Average (lower limit, upper limit)

*Denotes statistically significant changes from T0

T₀ values for aiMg²⁺ ranged from 0.48 to 0.58 mmHg (Average: 0.53 mmol/L).

Relative to T₀, MgSO₄ caused a significant increase in aiMg²⁺ for all time points T₀ – T₅₀ (p=<0.0001) (Figure 4.16). The largest increase in average aiMg²⁺ from T₀ occurred at T₂₀, corresponding to the end of IV MgSO₄ administration (Average 0.86 mmol/L). At the end of the 30 minute post-bolus phase viMg²⁺ remained significantly increased from T₀ (Average 0.72 mmol/L).

![Phase 2: 30 mg/kg Arterial Mg²⁺ (aiMg²⁺)](image-url)

Figure 4.16  Box & Whisker plot of: Arterial free ionized magnesium (aiMg²⁺) of horses administered a 20-minute bolus of 30mg/kg IV MgSO₄

*Denotes p<0.0001 difference from baseline aiMg²⁺ (time 0
Relative to T₀, MgSO₄ caused no significant change in the arterial value of aLac (p=0.23).

Blood gas data was determined to be erroneous due to the sampling method imposed by the laboratory and is therefore not reported.
CHAPTER V
DISCUSSION

This study, utilized horses with exacerbation of equine pasture asthma to measure the effects of three doubling doses of intravenous MgSO₄ on conventional pulmonary mechanics and physical parameters (phase 1). Among the three doses, a dose of 30 mg/kg administered over 20 minutes optimized pulmonary mechanics with minimal sedative effects.

At all doses, the onset of improvement in pulmonary function during infusion of MgSO₄ was rapid. Maximal change in intrapleural pressure (ΔPp_{max}), pulmonary compliance (C_{dyn}) and pulmonary resistance (R_L) significantly improved at all bolus dosages within the first 5-10 minutes of bolus initiation. Furthermore, decrease in ΔPp_{max} and R_L, as well as increase in C_{dyn} were dose dependent, with the 60 mg/kg group yielding a 52.6%, 139.7%, and 35.56% peak improvement in average individual change from baseline, respectively, at the conclusion of the bolus phase. Pulmonary function changes observed are in agreement with Skobeloff et al. (1989) and Bijani et al. (2001) who found that at the conclusion of adjunctive MgSO₄ bolus therapy in emergency treatment of human asthmatics, improvements from baseline peak expiratory flow rate (PEFR) or maximum speed of expiration were approximately 83% and 62% respectively. In addition, Bloch et al. (1995), Ciarallo et al. (1996) and Silverman et al. (2002) found improvements from baseline for the volume of air forcefully exhaled in 1 second (FEV1)
in MgSO₄ groups with severe asthma between 17%, and 34%. Due to the nature of our model, PEFR and FEV1 could not be performed. Although a forced expiration maneuver in horses is described (Couëtil 2000), no standard equipment is available and the technique necessitates standing anesthesia and is not suited to repeated maneuvers.

During the 30-minute post-bolus observational period, significant improvement from baseline was maintained for 30 minutes with ΔPpmax and for 15 minutes with Cdyn and Rl. Despite this, all three parameters returned close to baseline by 30 minutes following the end of bolus. Our results are in agreement with Okayama et al. (1987) who also found that MgSO₄ bronchodilator effect was rapid in onset with improvement seen within 2 minutes, but plateaued at the end of the bolus and was significantly reduced by 10 minutes after bolus conclusion. Interestingly, this study was performed on mild asthmatics that had not received any other adjunct therapy in the previous 12 hours. In contrast, several studies with severe asthmatics given MgSO₄ have noted continued improvement in FEV1 and PEFR statistically different from placebo groups at time points up to 7 hours following discontinuation of the bolus (Bloch 1995, Devi 1997, Ciarallo 2000, Bijani 2001, Silverman 2002, Kumar 2008). These studies all provided standard adjunctive therapeutics of nebulized β₂-agonists (all studies), Methylprednisolone IV (Bloch 1995, Ciarallo 2000, Bijani 2001, Silverman 2002, Kumar 2008), oxygen therapy (Bijani 2001, Silverman 2002) and ipatropium bromide (Kumar 2008). Based on our observations of the effect of MgSO₄ as a monotherapy, the use of MgSO₄ in concurrence with standard adjunctive asthma therapy is synergistic, and may help augment the effects of standard therapy beyond the duration achieved by magnesium alone.
Improved pulmonary function noted in this study is likely a result of competitive inhibition of calcium induced ASM contraction by the magnesium ion (Mg$^{2+}$) (Spivey 1990). Magnesium blocks calcium (Ca$^{2+}$) binding sites required for continuation of ASM contraction by interfering with Ca$^{2+}$ entry and release from sarcoplasmic reticulum. Magnesium also partially blocking receptor mediated Ca$^{2+}$ channels, blocking entry of Ca$^{2+}$ through voltage gaited ion channels and competing for similar binding sites on troponin C and myosin, as well as inhibiting Ca$^{2+}$ dependent phosphorylation/dephosphorylation enzymes necessary for ASM contraction (Sprvey 1990, Dominguez 1998, Perez-Zoghibi 2009). Additionally, Mg$^{2+}$ may cause greater increase in pulmonary perfusion due to direct effect on pulmonary vascular smooth muscle (Howell 1986).

Unexpectedly, minute volume (MV), the product of RR and tidal volume, was no different from baseline (T0) for the 15 mg/kg and 30 mg/kg treatment groups. In addition, MV became significantly reduced from baseline in the 60mg/kg treatment group from T25-T45. The average of individual changes in MV for each horse relative to baseline was greatest at T45, a 19% reduction. For all MgSO4 dosages in phase 1, there was a small, but significant reduction in RR from baseline from time 15-35 minutes, however no statistical difference between treatment groups was noted. Average peak reduction in RR was 2.82 +/- 0.59 breaths per minute and occurred at 15 minutes into the bolus. In addition, there was a trend for continued reduction in RR longer into the clearance phase in only the 60mg/kg group; this may have contributed to reductions observed in MV.

As a divalent cation, magnesium blunts the autonomic, somatic and endocrine reflexes by acting as a calcium channel blocker and non-competitive N-methyl-D-
aspartate (NMDA) receptor antagonist (Akhtar 2011). At serum magnesium levels greater than 3 mg/dl, patients may first begin to experience CNS depression (Reinhart 1988). In a case report by McDonnell et al. (2010), accidental overdose of MgSO4 led to depression of ventilation and decreased tidal volumes, although serum magnesium levels were not reported. In our study, serum magnesium levels for the 60mg/kg bolus group peaked at 4.8 +/- 0.554 mg/dL (T20). This is above the 3 mg/dl threshold of potential CNS depression, but is the lower range of other reported hyper-magnesemic clinical signs in people (4.8 to 6.2 mg/dL) (McDonnel 2010). These signs may include ventilatory changes, drowsiness, sedation, slurred speech, nausea, reduced deep tendon reflexes (patellar), and double vision. Interestingly, in addition to the reduction on MV, subjective notations of a sedation effect were noted in 4 of the 6 horses during their 60 mg/kg treatment. All observations of this sedation effect began approximately 10 minutes into the bolus phase and lasted for varying durations of time into the 30-minute post-bolus time-period. Although patellar reflexes are commonly used to monitor patients in the emergency room undergoing MgSO4 bolus (Lu 2000, Attygale 2002), it is not possible to elicit a patellar reflex in a standing conscious horse (de Lahunta 2014). The clinical relevance of observed mild reductions in MV at the higher bolus dose in this study is unknown. However, the changes observed in MV at the 60 mg/kg dose is likely to negatively impact pulmonary gas exchange in the absence of oxygen supplementation. Due to this significant reduction in MV observed in the 60 mg/kg treatment group, the dose of 30 mg/kg was chosen for phase 2 of this study.

Despite the fact that extracellular Mg2+ can reduce myocardial excitability (Hall 1992), no changes in HR or EKG were noted for all MgSO4 dosages in either phase of
This study. This is similar to findings by Okayama et al. (1987) who also noted no change in heart rate throughout the bolus and for 30 minutes following when administering 2.46g MgSO₄ over 20 minutes. Conversely, significant reductions in HR were noted by Kumar et al. (2008) at 50 minutes after the conclusion of a 20 minute infusion of 2g MgSO₄. While adjunct therapeutics of nebulized β₂-agonists, IV methylprednisolone and ipatropium bromide were administered in the Kumar study, in addition to MgSO₄ after baseline HR was obtained. However, the reported baseline heart rates in the Kumar study were well above adult normal reference range (127.97 +/- 10.19 BPM). Baseline heart rates in the Okayama study were at the upper limit of normal adult reference range (80 BPM) and more closely mimicked conditions in our study (normal equine HR: 30-40 BPM; average baseline observed: 41.30 +/- 6.2 BPM).

Thoracic auscultation scores (TA) significantly improved in all treatment groups, but a dose effect between treatment groups was not observed. Improvement in TA was characterized by an increase in audible air flow, a decrease in crackles and wheezes, and a return to normal sounding bronchovesicular sounds. Tracheal scores (TRA) were evaluated on similar grounds of fluid sounds, wheeze and intensity. Decreases in TA and TRA paralleled observations made in pulmonary function, as airways became less constricted and were better able to move larger quantities of air, and deliver air to the peripheral airways. Improvement in RR as well as TA and TRA scores noted in our study are in agreement with Okayama et al. (1987) who also noted significant reduction in RR and dyspnea scores within 2-5 minutes of beginning MgSO₄ bolus. Furthermore, piping rales observed in some patients disappeared and two patients were subsequently able to expectorate sputum easily. Similar to the Okayama study, the beneficial effects on
RR, TA and TRA in our study did not last for more than 30 minutes following cessation of the bolus. A primary deficit of the scoring was that the person doing the scoring was not blinded.

Asthma attacks generate significant psychological anxiety amongst patients and anxiety disorders are more common in asthmatics, considerably influencing asthma management due to individual perception of symptoms (Thoren 2000). Interestingly, animals in this study appeared less anxious and quieter during infusion of all bolus dosages. Investigation into role of Magnesium in reducing anxiety during treatment of asthma exacerbation may be warranted.

In phase 2, we measured the effects of 30 mg/kg intravenous MgSO4 on conventional pulmonary mechanics, serum chemistry and arterial blood gas parameters during acute exacerbation of disease. Assessment of conventional pulmonary mechanics in phase 2 confirmed the same rapid, repeatable improvement in pulmonary function during infusion of MgSO4 that was elicited in phase 1. Phase 2 bolus administration yielded a 38.14%, 30.78% and 31.03% peak improvement in the average of individual change from baseline for ΔPpl\text{max}, C\text{dyn} and R\text{L} respectively, all at the conclusion of the bolus phase. This is very similar to maximal improvements noted with administration of 30 mg/kg in phase 1 (ΔPpl\text{max}: 36.87%, C\text{dyn}: 59.76% and R\text{L}: 31.68%) with the exception of approximately 30% less improvement in C\text{dyn} achieved in phase 2 compared to phase 1. This is likely the reason for the lack of statistical significance achieved for C\text{dyn} in phase 2 comparisons.

Horses again tolerated the 30 mg/kg dose, showing no recognizable side effects such as ECG abnormalities or symptoms of CNS depression. Similar to phase 1, phase 2
administration of 30 mg/kg produced comparable decreases in TA and TRA, and no significant change in MV. The dose dependent overall results confirm the efficacy of MgSO₄ and repeatability of the drug action during acute asthma exacerbations.

Significant increases in both serum total Mg²⁺ (tMg²⁺) and venous and arterial iMg²⁺, as well as reduction in vCa:Mg ratio were noted in our study. The reduced vCa:Mg ratio is due directly to increasing serum magnesium, as venous calcium was unaffected by our study (Table 6). With increasing biologically available Mg, competitive inhibition of Ca²⁺ available for sustaining ASM contraction should occur. Changes observed in pulmonary function in our study suggest this mechanism.

The physiologically active form of magnesium is free or ionized (iMg²⁺) and makes up approximately 55-70% of total serum magnesium (Jahnen-Dechent 2012). Although the most accurate way of measuring true magnesium status is by measuring serum iMg²⁺, there is significant correlation between total Mg²⁺ and iMg²⁺ in healthy individuals (Greenway 1996). Furthermore, correlations between ionized and total magnesium levels have been confirmed as clinically acceptable for assessing magnesium status during administration of MgSO₄ for both preterm labor and preeclampsia (Yoshida 2005), thus warranting either evaluation mechanism as a means to determine active magnesium during treatment. This is congruent with our findings that both venous and arterial iMg²⁺ levels during phase 2 closely mimicked changes seen in venous tMg²⁺ in phase 1.

Serum tMg²⁺ and iMg²⁺ monitoring in each phase of this study confirmed that intravenous bolus of MgSO₄ does rapidly increase circulating total Mg²⁺ and iMg²⁺. Furthermore, the serum levels of magnesium closely paralleled the rapid improvement
and waning of pulmonary and physical parameters observed during the study period. In humans, magnesium is rapidly eliminated by glomerular filtration and urinary excretion (Blaine 2014), with horses additionally utilizing an alternate pathway of gastrointestinal excretion (Quamme 1989, Meyer 1977). The physiologic ability to rapidly remove excess circulating magnesium explains the rapid waning of serum tMg\(^{2+}\) and iMg\(^{2+}\) noted in the 30-minute post bolus phase, although serum magnesium did not completely return to baseline (T\(_0\)).

It is well documented that hypomagnesemia exacerbates insulin sensitivity in both diabetics and asthmatics (Kim 2010, Higashihara 2005) and oral magnesium supplementation improves insulin sensitivity, even in non-diabetics (Guerrero-Romero 2004). Congruent with this, rapid intravenous administration of MgSO\(_4\) and subsequent improvement in insulin sensitivity should enhance glucose cellular uptake, decreasing circulating blood glucose overall. However, a small, but significant rise in serum glucose (BG) was observed during phase 2 of this study. The clinical significance of this change in serum BG is unknown as all serum BG measurements of all horses, at all time points throughout the study period (Table 6) remained within the normal equine range of 72-115 mg/dL (Smith 2014). The trend was for BG to steadily increase from T\(_0\) to T\(_{50}\) with an average rise in BG of 8.2 mg/dL over the entire study period (Figure 15). Another possible explanation for this trend would be a very mild stress response (Smith 2014) to the study conditions. Although our horses are positively conditioned to receive pulmonary function testing, horses in this study were in disease exacerbation and participated in an intensive data-collection protocol that necessitated every 5-minute pulmonary and physical data collection with catheterized blood collections and ECG...
monitoring. This would also explain the 4.4mg/dl average decrease in BG from T₀ at the 24-hours post-study time point when the horses were either stalled or released back to pasture.

Due to sample mishandling of the arterial blood for blood gas analysis, results were invalid and are not reported. Arterial blood was collected via indwelling auricular artery catheter into a 3mL heparinized syringe, and then handled according to the established protocol of the Mississippi State University College of Veterinary Medicine Diagnostic Laboratory. Briefly, blood was transferred into 3 mL lithium heparinized evacuated tubes on ice and delivered to the laboratory for prompt sample analysis. Shortly after completion of this study, the MSU-CVM Laboratory modified their protocol. A proper blood sample for arterial blood gas analysis is collected anaerobically and left in the collection syringe in the absence of head gas, and fitted with a small-bore needle, cork, or properly vented syringe cap (Bowen 2010). Any air bubbles inadvertently introduced during sampling must be promptly evacuated (Trulock 1990). With the use of evacuated tubes, despite filling them to their maximum capacity, there was the opportunity for gas to diffuse across the blood air barrier, and thus potentially alter blood gas results.
CHAPTER VI

CONCLUSIONS

This study utilizes a spontaneous model of severe asthma to validate the beneficial therapeutic effect of MgSO₄ in acute bronchoconstriction by delivering it as a monotherapy. Intravenous MgSO₄ in the absence of adjunctive therapeutics, was not only well tolerated across all treatment groups as predicted, but delivered a dose dependent improvement in pulmonary function, thoracic and tracheal auscultation scores, as well as reducing respiratory rate and anxiety. Although improvement in pulmonary and physical parameters were repeatable and a rise in biologically active magnesium was found during administration, analysis of the benefit to pulmonary gas exchange could not be determined.

During MgSO₄ administration, improvement is rapid in onset, but duration of effect is short. MgSO₄ is readily available, inexpensive, and safe within the treatment ranges utilized in this study. While physicians usually limit MgSO₄ use to patients with life threatening exacerbations or severe exacerbations unresponsive to conventional therapeutics (Rowe 2008, NHLBI 2007), results of this study suggest that MgSO₄ therapy earlier in the standard emergency asthma treatment protocol, at all levels of exacerbation severity, may be beneficial. Furthermore, the effective and rapid ability of MgSO₄ to reverse bronchoconstriction through a mechanism independent from standard
bronchodilators such as β2-agonists and anticholinergics may aid in penetration of these inhaled therapeutics to the distal airways
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