ABSTRACT

JOHANSSON, ANNA MARIA. FUROSEMIDE CONTINUOUS RATE INFUSION IN THE HORSE: EVALUATION OF ENHANCED EFFICACY AND REDUCED SIDE EFFECTS AND HYPOMAGNESEMIA IN THE HORSE – A RETROSPECTIVE STUDY OF 401 CASES (Under the direction of Dr. Sarah Young Gardner.)

Continuous rate infusion (CRI) of furosemide is considered a superior method of administration to intermittent administration (IA) in humans. This study examined whether furosemide CRI, compared to IA, would increase diuretic efficacy with decreased fluid and electrolyte fluctuations and activation of the renin-angiotensin-aldosterone system (RAAS) in the horse.

Five mares were used in a crossover design study. During a 24-hour period each horse received a total of 3 mg/kg furosemide by either CRI (0.12 mg/kg/h preceded by a loading dose of 0.12 mg/kg IV) or IA (1mg/kg q8h IV). Urine volume and concentrations of electrolytes, aldosterone, and furosemide in urine were recorded. Serial blood samples were obtained and analyzed for hematocrit, total solids, electrolytes, and furosemide.

Although we were not able to demonstrate a statistically significant difference in urine volume over 24 hours between methods, this study demonstrated that CRI of furosemide produces a more uniform urine flow, and decreases fluctuations in plasma volume and suppresses renal concentrating ability throughout the infusion period. Importantly, there was significantly greater urine output after CRI in the first 8 hours. More K, Ca and Cl were excreted after CRI. There was no significant difference in aldosterone excretion between methods.

The furosemide disposition data conformed to a two-compartment model with elimination half-lives of 1.35 and 0.47 hours for CRI and IA, respectively. The area under the excretion rate curve, indicating exposure of the renal tubules to furosemide, was 1,285.7 and 184.2 ml*mg/ml for CRI and IA, respectively.

The second study was initiated to identify the signalment and clinical variables potentially associated with hypomagnesemia in horses evaluated at the NCSU-CVM veterinary teaching hospital between January 1999 and May 2001. A nested case reference study (nested case-control study) was conducted to examine the potential relationship between hypomagnesemia and signalment, serum chemistry panel analyses, number of hospitalization days, discharge status, and diagnosis.[1] A series of independent and multivariable logistic regression models were used to assess the potential association of each variable with low total serum magnesium values.

Of all horses included in the study, 48.7% had total serum magnesium values below the normal reference range. Hypomagnesemia was more likely to occur in horses older than one month of age. Colic, acute diarrhea, other gastrointestinal disease, infectious respiratory disease, and multi-organ system disease were associated with hypomagnesemia in adult horses, while diarrhea in foals reduced the risk of hypomagnesemia. Overall there was no relationship between hypomagnesemia and mortality, but horses with colic and hypomagnesemia were more likely to survive than horses with colic and normal or elevated total magnesium. Among horses that survived, hypomagnesemia at admission was associated with a longer hospitalization period.

FUROSEMIDE CONTINUOUS RATE INFUSION IN THE HORSE: EVALUATION OF ENHANCED EFFICACY AND REDUCED SIDE EFFECTS

AND

HYPOMAGNESEMIA IN THE HORSE – A RETROSPECTIVE STUDY OF 401 CASES

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BIOGRAPHY

Anna Maria Johansson was born in Malmö, Sweden on September 8, 1973. She attended schools in the Malmö public school system and graduated from Heleneholm in 1992.

Anna received her Doctor of Veterinary Medicine degree in 1997 from the Swedish University of Agricultural Sciences. She received a stipend from the Sweden-America Foundation in 2000 and enrolled in the graduate school at North Carolina State University in 2000 and began conducting her research in Dr. Sarah Gardner's laboratory at the College of Veterinary Medicine.

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FUROSEMIDE

INTRODUCTION

Furosemide is a potent high-ceiling loop diuretic that is used in equine medicine to remove fluid from the body. It is commonly used in the treatment of pulmonary edema secondary to congestive heart failure, but is effective in any type of edema in the horse.[1-3] Furosemide is used to increase urine flow rate in horses with acute renal failure and oliguria or anuria.[4] The most common use of furosemide in the horse is however to prevent exercise induced pulmonary hemorrhage in racing horses, although the rationale and effects of furosemide in this context are highly debated.[5, 6]

Physical properties

Furosemide is an anthracillic acid derivative with a molecular weight equal to 330.74 g/mole, and a pKa of 3.9. The white to slightly yellow crystalline power is odorless and practically tasteless. The compound is unstable to light.[7]

Mechanism and site of action

Furosemide is actively secreted to the renal tubule by a non-specific organic acid pump in the proximal tubule. The drug acts by reversibly blocking a NaK2Cl-cootransporter in the thick ascending limb of the loop of Henle (TAL).[7-9]

Mechanism of secretion

The exact mechanism of secretion of anionic compounds via the secretory pathway in the proximal tubule is not completely understood. Most likely citric acid cycle intermediates enter the epithelial cells of the proximal tubule at the basolateral membrane by cotransport with Na down the favorable chemical gradient for Na. These citric acid cycle intermediates then provide energy for transport of organic anions into the cell by an intermediate-organic anion exchanger. Further, secretion of organic anionic molecules from the cells to the tubule lumen may be by carrier-mediated diffusion or an exchange with a luminal anion (e.g. Cl).[8]

Electrolyte transport in the loop of Henle

Furosemide has its site of action at the luminal membrane of the TAL, where it reversibly blocks the NaK2Cl-cootransporter.[7-9] The TAL is responsible for absorption of 15-20% of filtered sodium in primary urine. No water is absorbed in this segment, and therefore it dilutes the urine. This segment is responsible for establishment of the hypertonic interstitium of the renal medulla.

The NaKATP:ase located at the basolateral membrane of the epithelial cells in the tubules mainly drives absorption of ions in all segments of the kidney. This transport protein uses energy from ATP to provide transport of 3 Na out of the cell in exchange for 2 K into the cell. This keeps the intracellular concentration of Na low and the electrical potential within the cell interior negative versus outside, and therefore enhances further uptake of sodium at the luminal membrane.

A ClK-cotransporter is located at the basolateral membrane of the TAL. This protein uses the favorable chemical gradient for K and electrical gradient for Cl created by the

NaKATP:ase to transport K and Cl out of the cell. Cl can also exit the cell at the basolateral membrane via a selective Cl -channel. This provides a driving force for further absorption of Cl at the luminal side of the cell.[8]

At the luminal membrane of the tubule cells in the TAL, ions can be absorbed from the urine via the NaK2Cl -cotransporter or the NaH-exchanger. Both of these use the favorable electrochemical gradients for Na and/or Cl mentioned above for transportation. There is a recycling mechanism for K through a K -channel, thus the absorption of Na and Cl is not limited by lower levels of K in primary urine. Since the luminal membranes of epithelial cells in the TAL have conductive pathways only for K, the apical membrane voltage is determined by the equilibrium potential for K (E_K). In contrast, the basolateral membrane has channels for both K and Cl, so that the basolateral membrane voltage is less than E_K. This creates a transepithelial potential difference of approximately 10 mV. This lumen–positive potential difference provides an important driving force for the paracellular flux of cations, such as Na, Mg, and Ca.[8, 9]

The NaK2Cl -cotransporter and its interaction with furosemide

Furosemide increases diuresis by blocking the NaK2Cl -cotransporter in the TAL, and therefore this protein will be described more thoroughly. Almost all cells in the body have NaK2Cl -cotransporting proteins in their membrane. Two different forms of this protein have been identified, and it is type 2 that is active in the TAL. Further, there are three isoforms of type 2 NaK2Cl -cotransporters that have been identified, and they appear to be distributed at different sites in the TAL, suggesting that there might be different absorption of ions at different parts of the TAL.[10] The protein has a molecular weight of 120-130kDa

(unglycosylated). A central hydrophobic region of 50kDa is flanked by an amino-terminal of 20-30kDa and a carboxyl-terminal of 50kDa. These large intracellular terminal domains have several potential phosphorylation sites. At least two different kinases are known to be involved in regulation of the transport activity of the type 1 protein.[10, 11]

The hydrophobic region spans the plasma membrane 12 times and has several loops that provide binding sites for ions on both sides of the plasma membrane.

Binding of ions to the NaK2Cl -cotransporter occurs in a specific order, namely Na firstly, Cl secondly, K thirdly and finally the second Cl ion. Most likely, furosemide binds to the second Cl -binding site. It has been shown that furosemide can bind to the protein only if Na, K and Cl are available, but very high concentrations of Cl will inhibit binding of the drug.[10]

Furosemide binds the NaK2Cl -cotransporter reversibly but with high affinity. A study with bumetanide, another member of the loop-diuretic family, demonstrated a saturable component of reversible binding to apical membranes of TAL cells. A Scatchard analysis of the binding data was consistent with one bumetanide molecule per binding site. Higher doses of furosemide replaced bumetanide, but bumetanide was shown to have somewhat higher affinity for the receptor than furosemide[12]

Diuretic mechanism of furosemide

The primary effect of blocking the NaK2Cl -cotransporter is decreased reabsorption of Na and Cl. It also affects the potential difference between the loop lumen and the interstitium described earlier, so that there is a reduction in reabsorption of Mg and Ca. The excretion of H is increased, by increased activity in the NaHexchanger.[10] Increased ionic delivery to

distal segments of the nephron, coupled with decreased hypertonicity in the interstitium, increases the volume of urine produced enormously. The urine is nearly iso-osmotic with plasma regardless of the hydration state of the individual, i.e., the individual loses the ability to excrete either concentrated or dilute urine.[8] The diuretic response is determined by the amount of drug that reaches the renal tubule, not the concentration in plasma. Further, the diuretic response is dependent on the time course of delivery of furosemide to the renal tubule.[13, 14]

Pharmacokinetic properties of furosemide in the horse

Furosemide is usually administered intravenously (IV) or intramuscularly (IM) as boluses of 1-2 mg/kg q 6-12h to horses. Long-term therapy can be administered IM or orally (PO) at a dose of 0.5-2 mg/kg q 8-12h[15] It has a short half-life ($t_{1/2}$). One study used a three-compartment model and reported α-, β- and γ- $t_{1/2}$ of 5.8, 24.1 and 177.2 minutes, respectively, after administration of 1 mg/kg furosemide IV.[16] Similar results were obtained in another study where the data were analyzed by a two-compartment model; α- and β-$t_{1/2}$ were 5.0 and 38.6 minutes respectively.[17] After IM administration the drug is almost completely absorbed and the β-$t_{1/2}$ is prolonged to 86.0 minutes.[17] Furosemide is approximately 95% protein bound in equine plasma.[17] The volume of distribution is 241.1 ml/kg and renal clearance is 503.8 ml/kg/hour.[16] One hour after IV administration of 1mg/kg, 36% of the dose can be recovered in urine, and after 4 hours about 60% can be recovered.[17] This is a high excretion rate because 95% of the drug is protein bound, and therefore depends on active secretion in the proximal tubule.[17] The excretion rate of furosemide is prolonged by substances that compete for transport by the organic acid

transport system, such as probenecid.[18, 19] In horses with bilateral urether ligation, elimination of furosemide was markedly reduced, but not completely eliminated, indicating that extrarenal metabolism exists.[20] Metabolism by conjugation to glucoronide is known to occur for furosemide in other species.[7]

Pharmacodynamic properties of furosemide

Effect on body fluid

The diuretic effect of furosemide in the horse is rapid and short. Furosemide administration results in a profound diuresis. After 1 mg/kg furosemide IM, the average urine production in 8 hours was 14.3 L compared to 2.6 L in untreated controls.[21] The diuretic effect is realized as early as 5-10 minutes after intravenous administration of 1 mg/kg furosemide in the horse. The peak effect occurs 15 to 30 minutes after injection, and after 2 hours urine volume has returned to baseline values.[22] Decreased plasma volume can be detected as early as 5-10 minutes after IV injection. After 2 hours there is a decrease is plasma volume of about 13% and after 4 hours 8%.[23] The decrease in plasma volume after 4 hours is approximately 5 ml/kg, which is considerably less than the urine production of about 24 ml/kg during the same period. This indicates that fluid is shifted into the vascular space during this period. The source of this fluid is unclear, but it is reasonable to hypothesize that it originates from extravascular extracellular fluid, intracellular fluid, or from the gastrointestinal tract.[23]

Effect on electrolyte balance

Plasma concentrations of K, Cl, and Ca decrease after injection of furosemide in the horse.[21, 22, 24] Plasma concentrations of Mg have been shown to decrease in humans, and occasionally in horses.[2, 9] Plasma Na concentration is not affected by furosemide, while PCO_2, HCO_3 and venous pH increase.[21]

Furosemide increases urinary excretion of Na, Cl, and Ca.[21] Urine K excretion is unaffected in horses.[21, 22] Urinary pH decreases.[21]

The significant decrease in plasma K, despite an unchanged excretion in urine is explained by the normally high dietary intake of K in horses, which causes a constantly high excretion rate of K in urine, masking the increase caused by furosemide. The main reason for the hypokalemia in horses after furosemide administration appears to depend on redistribution of K from extracellular to intracellular fluid, rather than increased excretion in urine. This is partly due to the mild alkalosis that develops after treatment with furosemide.[21]

Both Cl and Na excretion increase after furosemide administration, but while serum concentration of Cl decreases, serum Na concentration is unaffected. This is explained by making a quantitative assessment of electrolyte changes. While about 15.6% of the estimated extracellular Cl content is lost, only 9.7% of the estimated extracellular Na content is lost.[21]

The increase in venous pH may be an effect of the compensatory mechanisms that are activated in response to decreases in plasma concentrations of Cl and K, and decreased plasma volume. These compensatory mechanisms activate reabsorption of HCO3 in the renal proximal tubule. Increased pH can further be an effect of increased renal secretion of

H.[21] Another explanation is that hypochloremia, in combination with normal Na concentrations, increases the plasma strong ion difference and thereby causes a decrease in H-concentration.[25]

Effect on the renin-angiotensin-aldosterone system

Furosemide activates the renin-angiotensin-aldosterone system (RAAS) in the horse as well as in other species.[26] The RAAS is activated when blood volume is decreased. When blood pressure falls the juxtaglomerular cells lining the arterioles in the kidney relax. This stimulates baroreceptors on their surface, and intracellular levels of Ca decrease, enhancing renin release. Renin cleaves angiotensinogen to angiotensin I, which is further cleaved to angiotensin II, the active substance. The physiologic function of angiotensin II is to maintain blood pressure at a sufficient level by vasoconstriction and stimulation of retention of Na and water in the proximal tubule. This increased absorption is mediated via a NaH - antiporter by a process in which adenylate cyclase is inhibited and cAMP levels reduced.[27] Angiotensin II also increases release of aldosterone by binding to a G_p-protein coupled receptor in the zona glomerulosa cells of the adrenal gland. Activation of the G_p-protein leads to activation of phospholipase C, which cleaves phosphatidyl inositol ($PIP_{2)}$ to inositol triphosphate (IP_3) and diacylglycerol (DAG). DAG mediates a rise in protein kinase C activity that leads to biosynthesis of aldosterone.[28] Aldosterone stimulates retention of sodium and water further by both increasing activation and number of NaKATP:ases at the basolateral membrane, and by opening Na channels at the luminal membrane of principal cells of the collecting ducts.[27]

During treatment with furosemide, the RAAS acts as an antagonist to the drug by conservation of salt and water. Additionally, excessive activation of the RAAS has several other negative effects. Aldosterone induces pathologic hypertrophy with increased fibrosis in the cardiac muscle. Aldosterone binds to its cytosolic receptor in fibroblasts in heart and arteries and the hormone receptor complex diffuses into the nucleus where it binds to DNA and increases the transcription rate of mRNA coding for collagen I and III. This accumulation of fibrillar collagen is a major determinant for impaired stiffness and pump dysfunction in the heart. In arteries, stiffness of the vessel wall inhibits the stretching that is necessary for baroreceptor mediated negative feedback on the sympathetic nervous system.[28, 29] In addition, angiotensin II has direct toxic effects on cardiocytes by altering sarcolemmal permeability.[28] Angiotensin II activates the angiotensin II receptor type 1 (AT1) and protein kinase C, which leads to apoptosis of myocytes.[30] Angiotensin II is also involved in cell-to-cell communication in cardiac muscle. Administration of angiotensin II to the extracellular fluid reduces gap junction conductance within seconds. This causes slow conduction and reentry, two major factors involved in the generation of cardiac arrhythmias.[30]

Taken together, these effects of the RAAS increase the risk for arrhythmias and enhance progression of heart disease. Blunting of the RAAS and sympathetic nervous system is a primary aim in the management of heart failure in all species.

Effect on renal blood flow

Renal blood flow increases after furosemide injection as a result of vasodilation mediated by increased production of prostaglandin E_2. This effect can be blocked by prior administration

of non-steroidal anti-inflammatory drugs (NSAIDs).[31] The clinical effect of minimizing the diuretic efficacy of furosemide by simultaneous treatment with NSAIDs is minimal, and is probably a concern only in situations in which renal vasoconstriction exists prior to furosemide administration.[32]

Loop diuretics block tubulo-glomerular feedback, probably by inhibition of salt transport into the macula densa, so that the macula densa can no longer "sense" the NaCl concentration in the tubular fluid. Therefore, furosemide does not decrease glomerular filtration rate.[9]

Extrarenal effects

The beneficial effect of furosemide in patients with congestive heart failure is generally believed to be due primarily to the rapid diuresis. In addition, furosemide has vascular effects that probably contribute to the acute clinical effects. Human patients with congestive heart failure show a marked decrease in left ventricular filling pressure, associated with increased venous compliance, and this effect precedes the induced diuresis.[33] The mechanisms for the extrarenal effects of furosemide are not clear. Inhibition of the NaKCl-cootransporter causes a hyperpolarization of endothelial and smooth muscle cell membrane in vitro. Hyperpolarization of the membrane inhibits voltage sensitive Ca-channels, resulting in lowered intracellular Ca concentrations and relaxation of cells lining the vessels. However, the concentration required to establish this effect in vitro is substantially higher than the concentrations obtained in vivo, especially for the effects on arteries.[33] Stimulation of vasodilatory prostaglandin synthesis in the kidney or in the endothelium is another possible mechanism.[33] NSAIDs attenuate the vasodilatory effect, indicating a dependency

on normal prostaglandin synthesis. However, furosemide does not increase plasma concentrations of vasodilatory prostaglandin $F_{1\alpha}$.[34] The vasodilatory effect is further dependent on an intact endothelium.[34] Furosemide-induced activation of the RAAS can be associated with arterial vasoconstriction.[33] Occasionally, human patients with congestive heart failure experience paradoxical effects of furosemide; blood pressure and vascular resistance increases while stroke volume decreases. These effects are most likely due to activation of rennin.[31]

A great deal of research has focused on changes in blood pressure in the horse after treatment with furosemide. Right atrial pressure, pulmonary arterial pressure and pulmonary arterial wedge pressure decrease. Cardiac output and stroke volume decrease, while total systemic vascular resistance and heart rate increase, compensatory, to maintain mean arterial pressure [35]. These effects are due primarily to the decrease in plasma volume that is a consequence of increased urine output in the horse,[35] however, an effect of furosemide on venous compliance, as mentioned above, cannot be excluded as contributing to the reduction in blood pressure.[36] It is known that in other species, at least in part, reduction in blood pressure is due to furosemide-induced increase in venous compliance and capacitance.[37]

Other extrarenal effects of furosemide that have been addressed include a mild negative inotropic effect that appears to be mediated by prostaglandins.[38] Pulmonary gas exchange improves in experimental studies of pulmonary edema due to clearance of edema fluid and a furosemide-mediated decrease in pulmonary shunting of blood.[39] Furosemide can prevent bronchoconstriction induced by indirect stimuli in humans,[40] but does not prevent bronchoconstriction after direct stimulation by inhaled histamine.[41] Furosemide

reduces pulmonary resistance in ponies with chronic obstructive pulmonary disease, but does not affect pulmonary mechanics in normal ponies.[42]

Clinical use of furosemide

Exercise induced pulmonary hemorrhage

The most common use of furosemide in the horse is administration prior to racing, in order to prevent exercise induced pulmonary hemorrhage (EIPH). About 75% of thouroughbred horses and 22% of standardbred horses in North America receive furosemide before racing.[5] Administration of 0.5-1mg/kg 4 hours before racing is a common regimen. The use of furosemide for this purpose is highly controversial.[5, 6] Studies have failed to prove a decreased incidence in EIPH after furosemide administration, but limited evidence indicates reduced severity of bleeding.[5] Furosemide improves racing time in both horses with and without EIPH.[5]

In exercising horses, the difference between alveolar pressure and pulmonary capillary pressure during inhalation creates a transmural pressure of about 125 mmHg. This high transmural pressure is believed to cause disruption of capillaries with resultant pulmonary bleeding.[6] The reduction in pulmonary pressure produced by furosemide has been reported to be in the range of 7-10 mmHg. This reduction is not sufficient to prevent all capillary disruptions, which could be an explanation for the reduction in severity of EIPH without a reduction in the incidence in horses treated with furosemide.[6] The most common theory behind the use of furosemide in preventing EIPH is the reduction in pressure in pulmonary vessels that occurs as a result of lowered plasma volume.[5] Since the effect of furosemide on plasma volume is greatest 15 - 30 minutes post administration,[23] furosemide

would be most effective in reducing blood pressure in the lung if administered at intervals less than 4 hours prior to exercise. However, one study showed that furosemide administered 2, 3, and 4 hours prior to exercise had more effect on pulmonary pressure than did furosemide administered 1 hour prior to racing, and these authors believe that furosemide exhibits a direct effect on pulmonary vessels.[43] However, since the half-life of furosemide is only about 30 minutes[16 17] most of the drug will be cleared from the circulation 2 - 3 hours after administration.

Aside from the reduced severity of EIPH, there are several possible explanations for the improved racing times seen after administration of furosemide. Furosemide has a bronchodilatory effect on ponies with chronic obstructive pulmonary disease, but the effect on normal horses is unclear.[42] Furosemide induces a mild, metabolic alkalosis in horses.[21] Alkalosis has been shown to enhance athletic capacity of human athletes performing short-term exercise, but the effect in horses remains unknown.[31] If the horse is denied access to water after administration of furosemide, body weight will decrease. This reduction in body weight is due to increased urine output. Four hours after administration of IV furosemide, plasma volume is restored due to compartmental shifts and the major losses are from extravascular extracellular fluid, intracellular fluid, and the gastrointestinal tract.[23] Decreased body weight results in an increase in the relative maximal rate of oxygen consumption, an important indicator of athletic capacity.[5]

Congestive heart failure

Congestive heart failure is not a common disorder in the horse. It is most often caused by myocardial disease, valvular heart disease, and arrhythmias. All horses with congestive heart

failure clearly will not be treated. A guarded prognosis for future exercise and economic concerns make euthanasia a reasonable alternative in many cases. However, there are cases that are worth treating. Congestive heart failure from supraventricular fibrillation can often be controlled. Some animal owners want to keep their horse as a pet, not requiring it to exercise.[44]

Congestive heart failure from any cause implies that the heart is unable to supply enough blood flow to meet the oxygen and nutrient needs of body tissues.[2] If cardiac output decreases, renal blood flow will decrease. This stimulates the release of renin, which activates the transformation of angiontensin I to angiotensin II, and increases release of aldosterone. Concentrations of antidiuretic hormone may also increase. As a result, more sodium and water will be reabsorbed in the kidney, resulting in a larger cardiac preload. However, if the cardiac muscle is already working over its capacity, it may not be able to effectively circulate the greater volume of blood. In addition, Na and hypertension results in signs of congestion.[2]

The treatment of congestive heart failure in the horse consists of digoxin combined with furosemide.[2, 3, 44, 45] Digoxin increases myocardiac contractility by increasing intracellular Ca- concentrations. Cardiac output and peripheral blood flow increases and the electrolyte and acid-base balance can be corrected. Removal of edema and enhanced diuresis is promoted.[2]

Digoxin has a narrow margin of safety.[2, 3] Manifestations of digitalis intoxication include changes in gut motility with anorexia, constipation and diarrhea, and cardiac arrhythmias.[2, 3] Clinical conditions such as hypokalemia, hypomagnesemia, dehydration, hypoproteinemia and decreased renal blood flow increase the risk for digitalis intoxication.[2]

Loop-diuretics are the only diuretic agents sufficiently effective in removing edema in horses.[2] In congestive heart failure, furosemide removes excessive body fluid and enhances cardiac work. In cases with severe congestive heart failure compensated by the RAAS, administration of large doses of furosemide can cause a fatal fall in cardiac output with lethargy, weakness, and fainting as a result. To prevent this furosemide is combined with digoxin.[2] Administration of furosemide can cause hypokalemia, hypomagnesemia, and dehydration, side effects that enhance the risk of digitalis intoxication.[2] Furosemide-induced hypocalcemia, on the other hand, impairs the action of digitalis.[46] Potassium-sparing diuretics might seem a better choice, but their diuretic effect is not sufficient.[3]

Other clinical uses

Furosemide is the drug of choice to remove any type of edema in the horse.[1] In addition to heart failure, pulmonary edema may be a life threatening complication to any disease that increases hydrostatic pressure in the lung vessels, such as renal failure, or rapid administration of intravenous fluids. Increased permeability of lung vessels in association with sepsis, disseminated intravasal coagulation, allergic reaction, aspiration pneumonia, near drowning, and inhalation of smoke, can all cause pulmonary edema.[47]

Acute renal failure is often associated with anuria or oliguria. To maintain sufficient urine flow furosemide is recommended. Even thought furosemide enhances diuresis in these cases it has not been shown to improve the prognosis.[4]

Continuous rate infusion of furosemide

Probenecid decreases renal clearance of furosemide by competition for secretion in the proximal tubule. Consequently, the concentration of furosemide in plasma increases if it is coadministered with probenecid. Despite increased plasma concentrations of furosemide, urine flow rate and fractional excretion of Na are lower in subjects pretreated with probenecid, which suggests that the amount of furosemide secreted into the renal tubule may be more important for efficacy than plasma concentrations.[48-50] Conversely, pretreatment with probenecid prior to furosemide administration increases the diuretic effect of furosemide significantly, without affecting the total amount of furosemide reaching the urine. Probenecid affects the overall response of furosemide by delaying the time course of delivery of the drug to its site of action.[13, 14, 19] Using pharmacokinetic-pharmacodynamic modeling, a maximal efficient excretion rate of furosemide of 21.5 μg/min has been calculated for humans.[13, 14]

Based on this knowledge, continuous rate infusion of furosemide should enhance efficacy of treatment. Continuous rate infusion has been demonstrated to increase diuresis and natriuresis in healthy humans as well as in humans with congestive heart failure and renal failure.[46, 51-53] As a consequence, lower doses of furosemide can be used. Furosemide has a short half-life, and as mentioned earlier, the diuretic effect persists for only approximately two hours after a bolus dose.[22] This regimen causes significant alterations in plasma concentrations of furosemide, and fluid and electrolyte balance, increasing the risk of negative side effects.[23, 52, 53] Continuous rate infusion of furosemide in humans decreases fluctuations in plasma concentrations of furosemide, creates a more uniform secretion of furosemide into the tubule lumen, and thereby a uniform effect on urine output and

electrolyte excretion.[51-53] Fewer alterations in plasma concentrations of furosemide and fluid and electrolyte balance decrease the risk of negative side effects.[51] Diuretic efficacy is improved, and the required dose can be lowered. Possible side effects of furosemide include alterations in electrolyte balance, metabolic alkalosis, hypokalemia, hyponatremia, hypomagnesemia, hypochloremia, hypocalcemia, and dehydration.[2, 21, 46, 51] In horses hypokalemia is a potential risk in horses with reduced food intake, since their diet normally contains large amounts of K.[22] Hypokalemia, hypocalcemia, hypomagnesemia, and dehydration are of specific importance when furosemide is combined with digoxin in congestive heart failure, as the furosemide-induced alterations increase the risk of digitialis intoxication.[2, 46] Other side effects reported in humans include ototoxicity and an increase in uric acid.[51] Continuous rate infusion of furosemide is considered a superior alternative to traditional intermittent administration in human medicine due to the decreased fluctuations in fluid and electrolyte balance, the reduction in peak plasma concentrations of furosemide, and more effective diuresis and natriuresis.[46, 51, 53] In many clinical situations, an acute diuretic effect is needed. In these cases a loading dose preceding the infusion is recommended to bring the plasma concentration to steady state as rapidly as possible.[46, 53]

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FUROSEMIDE CONTINUOUS RATE INFUSION IN THE HORSE: EVALUATION OF ENHANCED EFFICACY AND REDUCED SIDE EFFECTS

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Abstract

Continuous rate infusion (CRI) of furosemide is considered a superior method of administration to intermittent administration (IA) in humans. This study examined whether furosemide CRI, compared to IA, would increase diuretic efficacy with decreased fluid and electrolyte fluctuations and activation of the renin-angiotensin-aldosterone system (RAAS) in the horse.

Five mares were used in a crossover design study. During a 24-hour period each horse received a total of 3 mg/kg furosemide by either CRI (0.12 mg/kg/h preceded by a loading dose of 0.12 mg/kg IV) or IA (1mg/kg q8h IV). Urine volume and concentrations of electrolytes, aldosterone, and furosemide in urine were recorded. Serial blood samples were obtained and analyzed for hematocrit, total solids, electrolytes, and furosemide.

Although we were not able to demonstrate a statistically significant difference in urine volume over 24 hours between methods, this study demonstrated that CRI of furosemide produces a more uniform urine flow, and decreases fluctuations in plasma volume and suppresses renal concentrating ability throughout the infusion period. Importantly, there was significantly greater urine output after CRI in the first 8 hours. More K, Ca and Cl were excreted after CRI. There was no significant difference in aldosterone excretion between methods.

The furosemide disposition data conformed to a two-compartment model with elimination half-lives of 1.35 and 0.47 hours for CRI and IA, respectively. The area under the excretion rate curve, indicating exposure of the renal tubules to furosemide, was 1,285.7 and 184.2 ml*mg/ml for CRI and IA, respectively.

Key words:

Loop diuretic, equine, electrolyte, fluid balance, frusemide, Lasix

Furosemide is the most widely used diuretic in the horse. Furosemide is secreted in the proximal tubule in the kidney, and acts by inhibition of the NaK2Cl -cotransporter on the luminal side in the thick ascending limb of the loop of Henle (TAL).[1, 2] The diuretic effect is seen 5-10 minutes after intravenous administration of 1mg/kg furosemide in the horse. The peak effect is 15 to 30 minutes after injection, and after 2 hours urine volume has returned to baseline values.[3] After 2 hours there is a decrease in plasma volume of 13% and after 4 hours 8%.[4] Serum concentrations of potassium (K), chloride (Cl), calcium (Ca), and sometimes magnesium (Mg), decrease and venous pH increases after IV administration of furosemide.[3, 5-7]

Adverse side effects can result from loop diuretic therapy. With recurrent furosemide injections, marked fluctuations in intravascular volume and electrolyte balance increase the risk of negative side effects, such as hypovolemia, metabolic alkalosis, and electrolyte disturbances.[3, 4, 8-10] In addition, high peak concentrations of furosemide are associated with ototoxicity in humans[10] and a relationship between loop-diuretics and increased incidence of arrhythmic death in humans has been shown.[11] Furthermore, dehydration, hypomagnesemia, and hypokalemia, induced by furosemide, increase the risk for intoxication by digitalis, a drug routinely used concurrently for treatment of congestive heart failure.[5, 12] Furosemide activates the renin-angiotensin-aldosterone system (RAAS) by decreasing plasma volume.[13] This is an undesirable side effect for most diuretic indications, because the RAAS acts as an antagonist to furosemide by stimulating retention of sodium and water in the kidney. Activation of the RAAS also increases activity of the sympathetic nervous system, induces harmful cardiac remodeling and myocardial cell apoptosis, and interferes with cell-to-cell communication in the heart.[14-16] These effects increase the risk for arrhythmias and enhance

progression of heart disease in humans.[14-16] For these reasons, blunting of the RAAS and the sympathetic nervous system is a primary aim in the management of heart failure in all species, and its activation is considered detrimental in these circumstances.

Recently studies in man have shown that continuous rate infusion (CRI) of furosemide can increase urine volume and sodium excretion, sometimes without increasing potassium loss.[8, 9, 17-19] CRI has also proven to be more efficient in diuresis than traditional bolus intermittent administration (IA) in human patients with congestive heart and renal failure, and in healthy controls. With CRI, fluctuations in electrolyte concentrations and plasma volume decrease, and dangerous peak concentrations of furosemide are avoided, even though the total diuretic effect is improved.[8, 9, 18, 19] Therefore, continuous administration of furosemide is considered a superior method for human patients.[9, 18, 19] CRI of furosemide is often preceded by a loading dose to allow more rapid attainment of therapeutic blood levels.[8, 9]

The purpose of this study was to investigate whether CRI of furosemide, compared to IA, would improve diuresis and natriuresis, decrease fluctuations in electrolyte and fluid balance, reduce potential arrhythmias, and reduce activation of the RAAS, in the horse. An additional aim was to determine and compare pharmacokinetic parameters for furosemide after both methods of administration.

Materials and Methods

Experimental design

This study was approved by the Animal Care and Use Committee at North Carolina State University. Five adult mares, weighing 370-533 kg (median, 460 kg), were used in an open-label, randomized, crossover study. Horses were determined to be healthy prior to the study by physical examination, complete blood count, equine blood chemistry panel, urine analysis, indirect blood pressure, and electrocardiography (ECG). Two weeks prior to the study, all horses underwent an 8-hour mock-up of this study to acquaint them with equipment, handling and procedures, thereby lessening the potential effects of stress on the experiment.

The mares were brought into stalls 48 hours prior to each experiment and had free access to coastal bermuda hay and water. Twelve hours prior to the start of the experiment they where brought into in a climate-controlled barn where the study was performed. During the experiment the horses had free access to coastal bermuda hay and were offered water at a volume of 12.5 ml/kg every 6 hours beginning at time 0, enough for maintenance. They were catheterized with indwelling 28F Foley urinary catheters[1] to allow for constant collection of urine into collection bags[2]. IV catheters[3] were placed in both jugular veins; furosemide[4] was administered on one side and blood samples drawn from the contralateral side.

During a 24-hour period each horse received a total of 3 mg/kg furosemide by either CRI (0.12 mg/kg/hr, preceded by a loading dose of 0.12 mg/kg) or 3 intermittent IV bolus

[1] Foley Catheter 28 F (C.R. Bard Inc., Covington, GA)
[2] 4L Urine drainage bag (C.R. Bard Inc., Covington, GA)
[3] Short-term angiocath 14 Ga (Beckton Dickinson Infusion Therapy Systems Inc., Sandy, UT)
[4] Furosemide (Lasix 50mg/ml, Akorn Inc., Decatur, IL)

administrations at a standard clinical dosage (IA; 1mg/kg q8h). The dose chosen for CRI, based on a total 24-hour dosage of 3 mg/kg including a loading dose to bring the plasma concentration to steady state as rapidly as possible, was developed from the following formula:

$Q=Cl*Css$ and $L=Vd*Css$, rearranged to: $L=Vd*Q/Cl$,

where Q = infusion rate, Cl = clearance, L = loading dose, Vd = Volume of distribution, Css = concentration at steady state.[20] Assuming Cl = 500 ml/kg/hour and Vd = 250 ml/kg for furosemide in the horse,[21] a loading dose of 0.12 mg/kg, followed by an infusion rate of 0.12 mg/kg/hour was deemed most appropriate.

For CRI, furosemide was diluted in 1L 0.9% NaCl and administered via a digital infusion pump[5]. The drug was protected from light during infusion to prevent photochemical degradation. IA horses also received 1L 0.9% NaCl by infusion with a digital infusion pump. Two horses received CRI first and three received IA first, with a period of one week between studies, when the procedures were reversed (crossover design).

Urine specific gravity[6] and volume were recorded hourly. Urine samples were collected hourly for the first 8 hours for furosemide analysis. Concentrations of sodium (Na), K, and Cl were analyzed[7] from well-mixed samples of total urine produced after 8 hours. Concentrations of Ca, Na, K, Cl, HCO_3, Mg, creatinine, pH[g] and aldosterone[8] were analyzed from well-mixed pooled 24-hour urine samples. Blood samples were drawn at time (t) 0, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours during each of the three 8- hour periods, for analysis of hematocrit; hemoglobin; blood glucose concentration; blood urea nitrogen (BUN), Na, K,

[5] F10-Gard G200 Volumetric Infusion Pump (Baxter Healthcare Corp., Deerfield, IL)
[6] Uricon-NE 2722 (NSG Precision Cells INC., Farmingdale NY)
[7] Hitachi 912 (Roche Diagnostics Corp., Indianapolis, IN)
[8] RIA In-house Procedure (Esoterix Endocrinology, Calabasas Hills, CA)

Cl, and HCO_3 concentrations; TCO_2; Anion Gap; pCO_2; base excess; pH[9]; and total solids[f]. Analysis of serum Ca and Mg concentrations were performed at 0, 8, 16 and 24 hours[g]. Blood samples were drawn at t 0, 10 min., 20 min., 30 min., and 1, 1.5, 2, 3, 4, 6, and 8 hours for the first 8 hours for analysis of plasma furosemide concentrations. Indirect blood pressure and heart rate were monitored hourly throughout the study[10]. ECGs were obtained at 0, 12 and 24 hours[11]. Body weight, hay and water consumption, and fecal production were monitored for each horse.

The relative change in plasma volume at any timepoint (t=x) from initial values (t=0) was calculated from total solids according to the formula:

$$\%\Delta\ PV = [(TS_{t=0} / TS_{t=x}) - 1] * 100$$.[22]

HPLC Assay

Furosemide in plasma and urine was analyzed by reverse-phase high performance liquid chromatography (HPLC) and fluorescence detection. The HPLC apparatus consisted of a pump[12], autosampler[13], fluorescence detector[14] and computer for data collection and analysis[15]. The column was a Zorbax SB-C8[16], 4.6 mm x 15cm[17], kept at 40 ºC. Furosemide was eluted with a mobile phase consisting of 65% 0.02 M phosphate buffer (pH 2.4) and 35% acetonitrile. The flow rate was 1.0 ml/min and a stream of helium was purged

[9] I-STAT System (Heska, Fortcollins, CO)
[10] Dinamap Veterinary Blood Pressure Monitor 8300 (Chriticon Inc., Tampa, FL)
[11] Passport (Datascope Corp., Paramus, NJ)
[12] Waters Model 600 Pump (Millipore Corp., Milford, MA)
[13] Agilent Technologies Series 1100 Autosampler (Agilent Technologies, Wilmington, DE)
[14] Agilent Technologies 1100 FL detector (Agilent Technologies, Wilmington, DE)
[15] Agilent 1100 series ChemStation software running on Windows 98
[16] Zorbax SB-C8, (MAC-MOD Analytical Inc., Chadds Ford, PA)
[17] Part # 883975906 (MAC-MOD Analytical Inc., Chadds Ford, PA)

through the mobile phase during analysis. Furosemide was detected with fluorescence detection using an excitation wavelength of 235 nm and an emission wavelength of 389 nm.

Preparation of calibration curve: Stock solutions of 1 mg/ml were prepared by dissolving pure reference standards of furosemide in HPLC grade methanol. The solutions were diluted to make spiking concentrations ranging from 1,000 µg/mL (undiluted) to 1 µg/mL. Urine and plasma calibration samples ranging from 10.0 µg/mL to 0.01 µg/mL were prepared by adding furosemide spiking solutions to blank equine urine, diluted 9:1 with phosphate buttered saline solution, and plasma.

A new calibration curve was prepared for each day's samples. Approximately 24 samples were analyzed each day. In order for the calibration curve to be accepted, it had to be linear with an r^2 value of at least 0.99 and the calibration standards had to be back-calculated to within 15% of the true value.

The plasma samples were prepared with a solid phase HLB extraction cartridge[18]. The cartridges were 30 mg capacity and 1.0 mL volume. They were first conditioned by mounting them on a manifold vacuum system, where first methanol (1.0 mL), then distilled water (1.0 mL), and finally plasma (500 µL) were added and drawn through. A washing solution of 95% water, 5% methanol was used to clean the cartridge. The drug was eluted with 1.0 mL of methanol. These tubes were dried in an evaporator using a temperature of 45º C for 10-15 minutes under a gentle stream of nitrogen (20 psi). The dried residue was reconstituted with 200 µL of mobile phase. This was loaded into brown autoinjector vials and 20 µL was injected into the HPLC system. Quality control samples were analyzed with each day's run.

[18] Oasis Extraction cartridge (Waters Corporation, Milford MA)

The urine samples were diluted 9:1 with phosphate buffered saline solution vortexed and centrifuged, and 200 μL of supernatant was used for analysis.

The retention time for furosemide was between 6 and 6.2 minutes in plasma and between 6.3 and 6.5 minutes in urine. There were no interfering compounds identified in any of the blank samples that corresponded to the window of the furosemide peak. The lower limit of quantification was 0.005 μg/mL. Concentrations of study samples were calculated from the calibration curve using the response value plotted against concentration. Final concentrations for urine samples were multiplied by 10x to account for the original dilution.

Pharmacokinetic Analysis

Furosemide plasma concentrations were plotted on a semilogarithmic graph to examine the shape of the curve for preliminary selection of an appropriate model. Computer analysis of the concentrations was performed with pharmacokinteic software[19]. A weighting factor was: $W = 1/y^2$ x plasma concentrations. A standard two-stage approach was used in which data for each animal was analyzed independently and average parameters calculated for the group. A pharmacokinetic model and plasma vs. time profile was developed for the group that reflected the mean disposition of furosemide for each dosing regimen. For the single IV dose, a simple bolus IV dose input was used. For the CRI, a model was used that allowed input of a single zero-order bolus dose (loading dose) followed by a constant rate IV infusion (CRI) and first-order output.

[19] WinNonlin (Version 3.1, Pharsight Corporation)

The most appropriate pharmacokinetic compartmental model was determined by (1) visual examination of the curves produced by predicted models, (2) using the Akaike's information criterion(AIC), in which the best model is determined to be the one with the lowest (minimum) AIC,[23] and (3) examination of residual plots. Elimination rates, half-lives, and intercepts were determined from exponents and coefficients of the best fit to an equation using weighted nonlinear least squares regression analysis.

Parameters derived from compartmental pharmacokinetic analysis included: total area under the plasma concentration vs. time curve (AUC), apparent volumes of distribution (Vc, apparent volume of distribution of central compartment; V_2, apparent volume of distribution of peripheral compartment; Vss, apparent volume of distribution at steady-state), microdistribution rate constants (K_{10}, K_{12}, and K_{21}), and systemic clearance (Cl). A noncompartmental analysis was used for the urine data. For the single IV dose, a model employing a bolus IV input was used. For the CRI, a model was used that accounted for the total amount of drug administered during the 8 hour infusion. Parameters calculated included the area under the urinary excretion curve ($AURC_{(0-last)}$, area under the urinary excretion rate curve from zero to the last time point; $AURC_{(0-\infty)}$, area under the urinary excretion curve form zero to infinity), cumulative amount excreted, percent of dose recovered, and excretion rate.

Statistical Analysis

Urine output and clinical values were initially examined graphically for outliers and normality. Urine output and clinical values of the horses were compared independently between treatment groups at specific times post-treatment with the Wilcoxon

signed-rank test.24 A P value of < 0.05 was considered statistically significant. Since the data were not considered normally distributed, results are presented as median (25th percentile, 75th percentile), unless otherwise noted.

Results

There was no difference in weight loss, fecal production, or food and water consumption between groups. None of the horses drank all of the water that was offered. Although four out of 5 horses produced more urine after CRI than IA during the 24-hour study period, the difference was not statistically significant (fig 1). Median total urine volume was 12.3 L (11.3, 14.4) after IA, and 14.3 L (13.3, 20.0) after CRI. There was significantly greater urine output after CRI during the first 8 hours of treatment [5.9 L (5.3, 6.0) for IA vs. 9.6 L (8.9, 14.4) for CRI], while IA horses produced significantly more urine during the second [3.8 L (3.4, 4.2) for IA vs. 2.3 L (2.3, 2.6) for CRI] and third [2.9 L (2.6, 3.1) for IA vs. 2.6 L (2.1, 2.9) for CRI] 8-hour periods (fig 1). Urine flow rate was greatest during the first hour of treatment for both groups and there was no difference in urine volume produced during the first hour between methods (fig 2). CRI gave a much more consistent urine flow rate throughout the study. IA horses produced 71.1% (65.7, 80.1) of the total urine volume collected within the first 8-hour period within the first hour of treatment, while CRI horses produced 35.9% (35.7, 37.9). For IA horses, diuretic efficacy decreased with each administration with urine volume in the 8-hour periods following the second and third administration only 64.1 % (60.4, 64.4) and 47.0 % (44.6, 57.9), respectively, of that produced in the first 8-hour period. CRI horses produced only 26.5% (22.7, 32.0) and 23.6% (20.0, 24.2) of the urine produced in the first 8-hour period in the second and third periods, respectively. Urine specific gravity decreased significantly after administration of furosemide with both regimens, but while it returned to basal values between injections for IA horses, it stayed low throughout the treatment period for CRI horses (fig 3).

The effects of treatment on urinary electrolyte excretion are presented in table 1. In the mixed 24-hour urine, Na concentration was significantly less and K and Ca concentrations significantly higher in horses that had been receiving CRI than in those receiving IA. In absolute amounts, horses lost more Na, and significantly more K and Cl after 8 hours on CRI than IA, and significantly more K and Ca after 24 hours on CRI.

Serum concentrations of electrolytes, additional serum chemistries, and hematologic parameters at selected time-points are presented in table 2. Blood samples were taken at t = 24h, but because of equipment failure, one sample at that time-point was lost and results are reported for t = 22h instead. Serum Na concentrations decreased similarly throughout the study for both IA and CRI, although significantly only for IA. Serum K concentrations dropped significantly within 30 minutes after the initial administration of furosemide for both IA and CRI horses, but while K concentrations stayed low throughout the study with CRI, they recovered somewhat between administrations with IA. For both methods, serum K concentrations were significantly lower at the end of the study as compared to the beginning. Serum Cl concentrations decreased continuously and similarly with both methods. Serum Ca concentrations decreased for both methods but significantly only for CRI at 8 hours. Values for both pH and HCO_3 varied markedly during the study, with both parameters increasing slightly, reaching significance only for HCO_3 in IA-horses.

Blood urea nitrogen increased steadily in a similar fashion for both methods throughout the study. Relative change in plasma volume, calculated from total solids, demonstrated a decrease of plasma volume of 9.4% (9.1, 9.4) for IA horses and 8.6% (6.5, 8.6) for CRI horses within 30 minutes after the first bolus administration or loading dose respectively (fig 4). The most pronounced decrease in plasma volume for IA horses occurred

30 minutes after each administration. Between IA doses, the plasma volume increased, approaching baseline. However, for CRI horses plasma volume remained significantly below baseline through 19 hours reaching a minimum at 6 hours when it was decreased by 10.8% (8.6, 11.1) (fig 4).

Heart rate and blood pressure were recorded hourly and results for selected time-points are presented in Table 3. Blood pressure decreased throughout the study for both methods.

Urinary aldosterone excretion over 24 hours was measured as an estimate of RAAS activation during treatment. Although 4 of the 5 horses excreted more aldosterone after IA than after CRI, this did not reach statistical significance. The median amount of aldosterone excreted was 22.8 μg (15.3, 35.0) and 14.3 μg (13.0, 28.9) for horses on IA and CRI, respectively.

To assess effects of electrolyte changes, ECGs were obtained at 0, 12 and 24 hours. P-R, Q-T and R-R intervals were measured and compared, in a blind and random fashion, between CRI and IA, and no differences or abnormalities were detected.

Results from the pharmacokinetic analysis are shown in tables 4 and 5, and in figures 5 and 6. Following the IV bolus dose, the AIC showed that for most horses, a two-compartment open model was the most appropriate for the plasma concentrations. For one horse the AIC was slightly smaller using a three-compartment model, but the difference was only slight. In order to analyze the entire group similarly the results reported for this horse represent a two-compartment analysis. Visual inspection of the predictive curves, and

residual plots showed that selecting this model for the group produced acceptable fitted curves. For urinary excretion of furosemide a noncompartmental model was used (table 5).

Discussion

Efficacy of furosemide is determined not only by the amount of drug that reaches the site of action in the TAL, but by the time course of delivery to the tubule lumen.[25, 26] Several clinical studies in humans have shown increased diuretic efficacy after CRI of furosemide as compared to traditional IA, both in healthy subjects and patients with congestive heart failure.[8, 9, 18] We evaluated CRI in the horse with the hypothesis that it would improve diuresis and natriuresis, decrease fluctuations in electrolyte and fluid balance, reduce potential arrhythmias, and reduce activation of the RAAS. In our study, although 4 of 5 horses produced more urine after CRI compared to IA of the same total dose of furosemide over 24 hours, there was no statistically significant difference between methods. However, there was a marked enhanced efficacy of CRI over the first 8 hours, with all 5 horses exhibiting enhanced diuresis.

For horses on IA, the effect on urine production lasted for 2-3 hours, thereafter the kidneys again began concentrating the urine (fig 2 and 3). These results are similar to previous reports describing the diuretic effect of furosemide after IA.[3] Secretion of furosemide in the proximal tubule is a rapid process, and after a bolus dose of furosemide the amount of drug available in the TAL presumably exceeds the number of NaK2Clcotransporters initially, but soon falls below therapeutic levels.[2] CRI provides constant delivery of furosemide to the site of action, illustrated by the uniform urine flow rate (fig 2), urine specific gravity (fig 3), and urine furosemide concentrations (fig 6).

For both methods, efficacy decreased with time. It has been previously reported that repeated doses of furosemide with short intervals decrease the diuretic response in normal

horses.[3] Acute tolerance to furosemide is a known phenomenon, and is a consequence of the decrease in fluid volume that occurs with furosemide-induced diuresis.[19, 27] Decreased plasma volume activates renal compensatory mechanisms, increasing reabsorption of NaCl and water in the distal and proximal tubules and collecting duct, thereby counteracting furosemide's diuretic effects.[19, 27] The more profound diuresis that occurred after CRI during the first 8 hours in the horses probably activated compensatory mechanisms to a greater degree than in horses receiving IA, explaining the decreased efficacy in the second and third 8-hour periods for horses on CRI.

Several studies demonstrate enhanced diuretic efficacy after CRI of furosemide in humans.[8-9] Van Meyel *et. al.* studied healthy volunteers for 8 hours, while Lahav *et. al.* used patients with congestive heart failure over 48 hours. Whether or not CRI of furosemide would have been more beneficial in horses with excessive fluid accumulation compared to healthy horses remains unproved. In both of the above mentioned studies, the fluid lost by urine was replaced volume for volume by IV fluids to prevent dehydration induced acute tolerance to furosemide.

Although concentrations of Na, K, and Cl were not significantly different between methods in urine at 8 hours, the total amount of Na, K and Cl lost after CRI was greater because the volume of urine produced was much greater after CRI during this period of time. Hence, CRI for 8-hours increased diuretic, natriuretic, and chloruretic efficacy of furosemide substantially. Even though 24-hour urine volume was similar between methods, CRI horses lost significantly more K and Ca in the urine than did IA horses, while the amount of Na was less, although insignificant (table 1). Furosemide acts by blocking a NaK2Cl cootransporter at the luminal membrane of epithelial cells in the TAL. The NaK2Cl

cootransporter normally transports Na, K, and Cl into the epithelial cells, where Na and Cl are further transported to the systemic circulation. At the luminal membrane of the TAL cells, there is a K-channel that normally allows recirculation of reabsorbed K to the tubule lumen. Thus only Na and Cl reabsorption is ultimately blocked by furosemide. Decreased reabsorption of Na and Cl causes a reduction in the potential difference between the lumen and interstitium that is normally responsible for the uptake of Mg and Ca. Therefore, treatment with furosemide causes increased concentrations of Na, Cl, Mg, and Ca (and to some extent increased K) to remain in the lumen of the TAL. In more distal segments, Na is reabsorbed in exchange for K. This mechanism is responsible for the hypokalemia seen in other species after furosemide treatment.[1] One explanation for the fact that Na concentration in the urine decreased while K concentration increased with CRI compared to IA is that furosemide IA caused a large amount of Na to be delivered to the distal tubule over a shorter period of time, overwhelming the Na/K exchanger, rendering it unable to reabsorb more than a small fraction of the excess Na. On the other hand, with CRI, the intraluminal concentration of Na was uniformly elevated throughout the treatment period and the Na/K exchanger was able to reabsorb a larger fraction of intraluminal Na.

It has been previously reported in horses that a furosemide dose of 0.01 mg/kg increased urine K excretion maximally, and higher doses of furosemide did not further increase K loss.[3] Since horses normally eat a K-rich diet and excrete large amounts of K, the effect of IA furosemide on K urinary loss clinically is relatively small, and decreased plasma concentrations are thought to be mainly due to compartmental shifts.[3, 7] In our study, CRI resulted in more K loss than IA, and serum concentrations of K after CRI were consistently lower.

The decrease in serum Cl and Ca concentrations were similar between methods in this study and similar to the findings in earlier studies with furosemide IA in the horse.[3, 7] One horse on IA and two horses on CRI experienced mild hypocalcemia during treatment. This calciuretic effect of CRI of furosemide can potentially be used in the treatment of horses with hypercalcemia. Furosemide also occasionally induces hypomagnesemia in horses,[5] but this was not observed in this study. In contrast to earlier studies,[3, 7] we found a decrease in serum Na concentration that was statistically significant for horses on IA, but not CRI.

The increase in BUN is likely a reflection of furosemide-induced dehydration, with resulting pre-renal azotemia. Hemoglobin and hematocrit were only slightly affected by furosemide with non-significant, transient increases, immediately after administration of loading or bolus doses of furosemide, similar to earlier studies,[3, 4] but splenic sequestration of red blood cells in the horse makes these measurements unreliable as indicators of plasma volume.[4] While plasma volume is most accurately estimated by Evans blue dye dilution, calculated values for the relative change in plasma volume using total solids gives a fair estimate.[4] The decrease in plasma volume after IA was similar to that reported from earlier studies.[4] Plasma volume fluctuated more after IA, decreasing after each IA dose, but remaining below baseline with CRI (fig 4). Our horses had access to water, potentially increasing variability in measurements.

The RAAS is activated in horses treated with furosemide in response to decreased extracellular fluid volume.[13] We hypothesized that by diminishing the abrupt fluctuations in plasma volume by administration of furosemide by CRI instead of traditional IA, activation of the RAAS would be lessened. To assess the activity of the RAAS over the treatment period, aldosterone excretion in urine was measured. Twenty-four hour urinary excretion of

aldosterone is considered a reliable method to diagnose aldosteronism in humans, eliminating the variability that occurs throughout the day in plasma concentrations.[28] Although 4 out of the 5 horses excreted more aldosterone after IA than CRI, there was no significant difference between methods. The low number of observations and high variability between horses in our study make it difficult to draw any conclusions about the activity of the RAAS during furosemide administration.

The half-life ($t_{1/2}$) of furosemide of approximately 30 minutes reported here, and in other papers,[29] seems to coincide with the duration of therapeutic effect.[4] Ninety percent of drug concentrations are eliminated in 3.3 half-lives, and 95% eliminated in 5 $t_{1/2}$. Therefore, in four hours, (duration of effect cited in various studies) most of the drug will have disappeared from the plasma. This was confirmed by our plasma concentration vs. time curve (fig 5), shown here and in other studies.[21, 29] The half-life of the elimination phase ($t_{1/2\beta}$) was similar in both studies, as were the volumes of distribution and Cl. In this study, goodness of fit criteria using the minimum AIC, showed that compared to a one- or three-compartment pharmacokinetic open model, the two-compartment model provided the best fit. In a previous study Roberts *et. al.* (1978) demonstrated that a two-compartment model similar to ours provided the best fit.[29] However, in a study by Chay and colleagues (1983), a three compartment model was used.[21] However their □ phase of the curve, corresponded with our □ phase as they yielded very similar $t_{½}$s. We did not detect a terminal gamma-phase in our study. The Cl of 617.4 ml/kg/h indicates that active tubular secretion must play a role in the excretion of unchanged drug.

Because of this rapid elimination, a constant rate infusion was able to maintain diuretic concentrations for the full duration of the measured infusion (8 hr). The Cl was very similar

(617.4 ml/kg/h vs. 662.6 ml/kg/h) and the Vc was similar (147.7 ml/kg vs. 159.0 ml/kg) between IA and CRI, respectively. The half life ($t_{½\square}$) was almost identical, but the $t_{½\beta}$ was highly variable for the CRI dosing and the average longer.

The urine data demonstrates the pharmacodynamic differences between IA and CRI. For the first 8 hours, virtually the same total amount of furosemide was administered (1 mg/kg for IA, and 1.08 mg/kg for CRI) and urine recovery of the total dose also was similar, but urine volume was almost twice as high for CRI, compared to IA (9.6 liters vs. 5.9 liters, respectively) (fig 1).

The explanation for the difference observed between the two regimens during the first 8 hours can be seen in the AURC (Table 5). This parameter measures the exposure of the renal tubules to furosemide. For CRI, the median was 1,285.7 mL*mg/mL, and for IA it was 184.2 mL*mg/mL. Comparing figures 2 and 6, although the amount of furosemide excreted in the urine during the first hour after IA is much higher than during the first hour of CRI, the urine volumes produced in this interval were similar. This suggests that a maximal diuretic response was already being produced from the initial bolus of 0.12 mg/kg with CRI, and a higher dose of 1 mg/kg did not further increase urine output with IA. Also, as seen in figure 6, most of the furosemide was excreted into the renal tubules during the first hour after IA, but CRI produced a more sustained and consistent exposure to the tubular target sites. The high, but transient renal excretion from IA is attributed to a higher maximum rate of excretion with this regimen, (211,802mL*µg/mL/hr) compared to the CRI regimen (60,750mL*µg/mL/hr).

In people, approximately half the administered dose is excreted unchanged in the urine.[1] We found similar results from the urine analysis of this study. The percent of dose

recovered, 58% and 65% for CRI and IA, respectively (table 5), is almost the same as measured in humans. After IA, the elimination rate of furosemide in urine was almost identical compared to the measurement obtained from the IV plasma kinetics (tables 4 and 5). This indicates that, although other mechanisms of furosemide elimination may exist in the horse, the rate-determining process for elimination of the parent drug is renal tubular secretion.

In conclusion, we found that CRI with furosemide provided better diuresis than traditional IA for the first 8 hours of treatment, and although it was not significantly more effective over 24 hours; it produced a more uniform urine flow rate, with less fluctuation in plasma volume. CRI of furosemide appears to be a safe and reliable method of administration. It is slightly more labor-intensive since an infusion pump is necessary and infusion sets have to be protected from light to prevent photochemical degradation of the drug during infusion. If profound diuresis is needed acutely in horses, we recommend furosemide CRI (0.12 mg/kg/h), preceded by a loading dose (0.12 mg/kg) for 8 hours to provide a more vigorous effect than traditional administration of a bolus dose (1mg/kg). Attention should be paid to blood pressure during infusion, and serum concentrations of K and Ca should be monitored and supplementation provided as needed.

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Table 1. Urinary electrolyte concentration and total excretion of electrolytes after 8 hours and 24 hours following administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses.

		Electrolyte concentration in urine (mmol/l)				Total electrolyte excretion in urine (mmol)			
		8 hour urine		24 hour urine		8 hour urine		24 hour urine	
		Median	(25^{th}, 75^{th})	Median	(25^{th}, 75^{th})	Median	(25^{th}, 75^{th})	Median	(25^{th}, 75^{th})
Na	IA	96	(86, 100)	91∗	(82, 92)	564	(460, 602)	1178	(991, 1245)
	CRI	91	(88, 95)	66 ∗	(65, 70)	909	(778, 1227)	959	(916, 1399)
K	IA	52	(44, 66)	65 ∗	(56, 74)	315 ∗	(298, 345)	764 ∗	(709, 904)
	CRI	55	(55, 59)	82 ∗	(79, 92)	537 ∗	(528, 701)	1133 ∗	(1110, 1229)
Cl	IA	138	(124, 133)	129	(125, 130)	723 ∗	(693, 866)	1596	(1457, 1767)
	CRI	140	(140, 141)	124	(122, 125)	1330 ∗	(1245, 1918)	1776	(1657, 2378)
Ca	IA			6.0 ∗	(5.1, 6.5)			73.3 ∗	(65.0, 73.5)
	CRI			6.5 ∗	(6.4, 8.5)			102.7 ∗	(96.0, 117.2)
Mg	IA			2.7	(2.3, 3.9)			33.7	(29.6, 58.5)
	CRI			2.8	(2.5, 2.8)			34.4	(33.4, 57.6)

Data presented as median (25^{th}, 75^{th} percentiles).
IA, Intermittent administration of furosemide (1mg/kg q8 IV); CRI, Continuous Rate Infusion of furosemide (0.12 mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV); Conc, concentration; Na, sodium; K, potassium; Cl, chloride, Mg, magnesium; Ca, calcium.
∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

Table 2. Hematological findings at selected time-points after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses.

		Time (h)					
		0		8		22(24 for Mg and Ca)	
		Median	(25^{th}, 75^{th})	Median	(25^{th}, 75^{th})	Median	(25^{th}, 75^{th})
Na (mmol/L)	IA	141.0	(141, 142)	139 †	(138, 140)	138 †	(137, 139)
	CRI	141.0	(139, 142)	140	(138, 141)	139	(137, 140)
K (mmol/L)	IA	3.7	(3.7, 3.8)	3.6 ∗	(3.4, 3.7)	3.1 †	(2.8, 3.3)
	CRI	3.6	(3.4, 3.8)	2.7 † ∗	(2.6, 2.9)	3.0 †	(2.5, 3.2)
Cl (mmol/L)	IA	107	(106, 108)	105 †	(102, 107)	101 †	(101, 103)
	CRI	106	(105, 108)	102 †	(101, 103)	100	(99, 100)
Mg (mg/dL)	IA	1.9	(1.8, 2.0)	2.0	(2.0, 2.1)	2.1	(2.0, 2.1)
	CRI	1.8	(1.7, 1.9)	2.1	(2.0, 2.1)	2.0	(1.9, 2.1)
Ca (mg/dL)	IA	12.1	(11.9, 12.2)	12.5	(12.0, 12.5)	12.0	(11.8, 12.2)
	CRI	11.5	(11.5, 11.5)	12.0 †	(11.8, 12.2)	11.4	(10.3, 11.4)
BUN (mg/dL)	IA	19	(17, 19)	21 †	(21, 22)	29 †	(26, 33)
	CRI	18	(17, 26)	22 †	(19, 29)	30 †	(23, 38)
Hb (g/dL)	IA	11	(11, 12)	12	(12, 12)	11	(11, 12)
	CRI	11	(10, 13)	13	(13, 14)	12	(12, 13)
Htc (%)	IA	33	(31, 34)	34	(34, 34)	33	(32, 34)
	CRI	31	(28, 37)	37	(37, 40)	36	(35, 38)
TS (g/dL)	IA	7.3	(7.1, 7.7)	7.2	(7.1, 7.8)	7.3	(7.2, 7.7)
	CRI	7.3	(7.1, 7.4)	8.0 †	(7.8, 8.3)	7.7	(7.6, 7.7)
PH	IA	7.413	(7.410, 7.422)	7.413	(7.412, 7.416)	7.434	(7.426, 7.464)
	CRI	7.423	(7.390, 7.426)	7.419	(7.412, 7.449)	7.449	(7.415, 7.458)
HCO_3 (mmol/L)	IA	27	(25, 27)	28	(28, 30)	31 †	(31, 34)
	CRI	27	(27, 29)	30	(28, 31)	32	(30, 36)

Data presented as median (25^{th}, 75^{th} percentiles).
IA, Intermittent administration of furosemide (1mg/kg q8 IV); CRI, Continuous Rate Infusion of furosemide (0.12 mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV); Na, sodium; K, potassium; Cl, chloride, Mg, magnesium; Ca, calcium; BUN, blood urea nitrogen; Hb, hemoglobin; Htc, hematocrit; TS, total solids; HCO_3, bicarbonate.
∗ Significant difference between methods, $p<0.05$; † Significant from value at time 0, $p<0.05$ (Wilcoxon signed-rank test).

Table 3. Heart rate and blood pressure prior to, and after 24 hours treatment with furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses.

		Time (h)					
		0		8		24	
		Median	(25^{th}, 75^{th})	Median	(25^{th}, 75^{th})	Median	(25^{th}, 75^{th})
HR (bpm)	IA	33	(33, 36)	36	(34,38)	38	(34, 42)
	CRI	37	(31, 34	41	(36,41)	33	(30, 38)
BP mean (mm Hg)	IA	76	(72, 81)	68	(63,69)	59 †	(54, 63)
	CRI	82	(69, 86)	65 †	(52,67)	60 †	(50, 62)
BP systolic (mm Hg)	IA	106	(105, 112)	94	(89,97)	88 †	(82, 90)
	CRI	107	(97, 121)	90	(82,97)	93 †	(85, 93)
BP diastolic (mm Hg)	IA	54	(46, 55)	49	(43,50)	38	(38,39)
	CRI	58	(48, 59)	42	(36,45)	39 †	(36, 43)

Data presented as median (25^{th}, 75^{th} percentiles).
IA, Intermittent administration of furosemide (1mg/kg q8 IV); CRI, Continuous Rate Infusion of furosemide (0.12 mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV). HR, heart rate; bpm, beats per minute; BP, blood pressure.
† Significant from value at time 0, $p<0.05$ (Wilcoxon signed-rank test).

Table 4. Pharmacokinetic parameters for plasma concentrations after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses.

		IA		CRI	
Parameter	Units	Median	(25th, 75th)	Median	(25th, 75th)
Cmax	ug/mL	6.77	(6.70, 8.02)		
A	ug/mL	5.79	(5.66, 7.62)	0.60	(0.46, 1.17)
B	ug/mL	0.40	(0.39, 0.91)	0.04	(0.03, 0.24)
□	1/hr	5.60	(4.65, 6.51)	4.24	(3.75, 10.01)
β	1/hr	1.48	(0.81, 1.62)	0.51	(0.26, 4.23)
$t_{½□}$	hr	0.12	(0.11, 0.15)	0.16	(0.07, 0.18)
$t_{½□}$	hr	0.47	(0.43, 0.85)	1.35	(0.16, 2.70)
K_{10}	1/hr	4.18	(4.12, 4.66)	4.24	(1.93, 6.66)
K_{12}	1/hr	0.64	(0.61, 1.27)	1.58	(1.21, 1.69)
K_{21}	1/hr	1.95	(0.92, 2.30)	0.77	(0.50, 4.23)
Vc	mL/kg	147.7	(124.7, 149.2)	159.0	(99.5, 186.6)
V_2	mL/kg	64.06	(63.3, 93.0)	398.8	(14.8, 590.0)
Vss	mL/kg	217.8	(212.5, 244.1)		
CL	mL/hr/kg	617.4	(496.1, 875.1)	662.6	(454.2, 673.4)
MRT	hr	0.35	(0.28, 0.40)		
AUC	hr*ug/mL	1.62	(1.14, 2.02)		
AUMC	hr*hr*ug/mL	0.64	(0.29, 0.72)		

Data presented as median (25^{th}, 75^{th} percentiles). IA, Intermittent administration of furosemide (1mg/kg q8 IV); CRI, Continuous Rate Infusion of furosemide (0.12 mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV); C_{MAX}, maximum plasma concentration; A and B, Intercepts for distribution and elimination phases of curve, respectively; □ and □, rate constants for distribution (alpha) and elimination (beta) phases of plasma curve, respectively; $t_{½□}$ and $t_{½□}$, corresponding half-lives for alpha and beta; K_{10}, K_{12}, and K_{21}, compartmental microdistribution rate constants; Vc, apparent volume of distribution of central compartment; V_2, apparent volume of distribution of peripheral compartment; Vss, apparent volume of distribution at steady-state; CL, systemic clearance; MRT, mean residence time; AUC, area under the plasma concentration vs. time curve; AUMC, area under the 1^{st} moment curve.

Table 5. Pharmacokinetic parameters for urinary excretion of furosemide after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses.

		IA		CRI	
Parameter	Units	Median	(25th, 75th)	Median	(25th, 75th)
Elimination rate	1/hr	0.38	(0.33, 0.49)	0.05	(0.04, 0.06)
$t_{1/2}$	hr	1.83	(1.40, 2.13)	ND	
Maximum rate of excretion	mL*µg/mL/hr	211,802	(156,216, 300,200)	60,750	(58,104, 61,568)
Last measurable rate of excretion	mL*µg/mL/hr	800	(627, 1097)	39,694	(27,892, 39,780)
Sum of urine volumes	mL	5,880	(5,345, 6,015)	9,570	(8,280, 14,435)
Amount furosemide recovered	mL*µg/mL	236,382	(175,714, 326,870)	328,745	(220,348, 338,089)
Percent of dose recovered	%	65.0	(42.7, 74.6)	58.0	(52.1, 60.5)
$AURC_{(0 - last)}$	mL*µg/mL	183,177	(138,165, 251,506)	292,321	(197,179, 293,827)
$AURC_{(0 - \infty)}$	mL*µg/mL	184,210	(141,829, 252,769)	1,285,746	(792,434, 1,356,307)

Data presented as median (25^{th}, 75^{th} percentiles). IA, Intermittent administration of furosemide (1mg/kg q8 IV); CRI, Continuous Rate Infusion of furosemide (0.12 mg/kg/h, preceded by a loading dose 0.12 mg/kg IV); $t_{1/2}$, half-life; ND, not determined; $AURC_{(0 - last)}$, area under the urinary excretion rate curve from zero to the last time point; $AURC_{(0 - \infty)}$, area under the urinary excretion curve form zero to infinity.

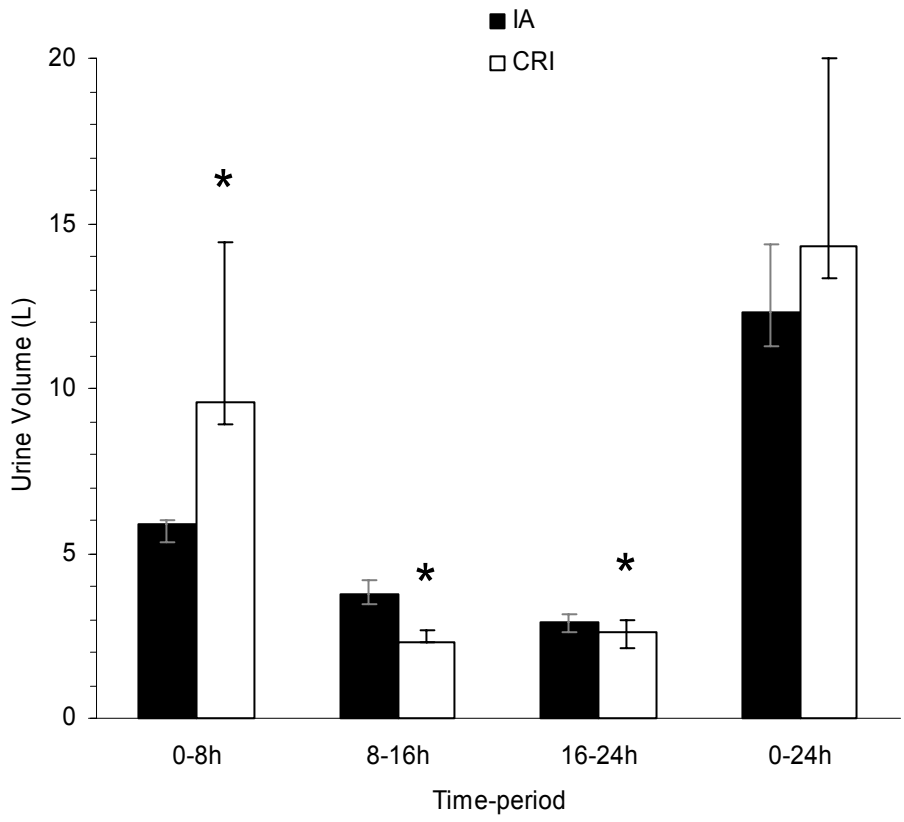

Figure 1. Urine volume produced in each of three 8-hour periods and during the total treatment period (24 hours) following administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

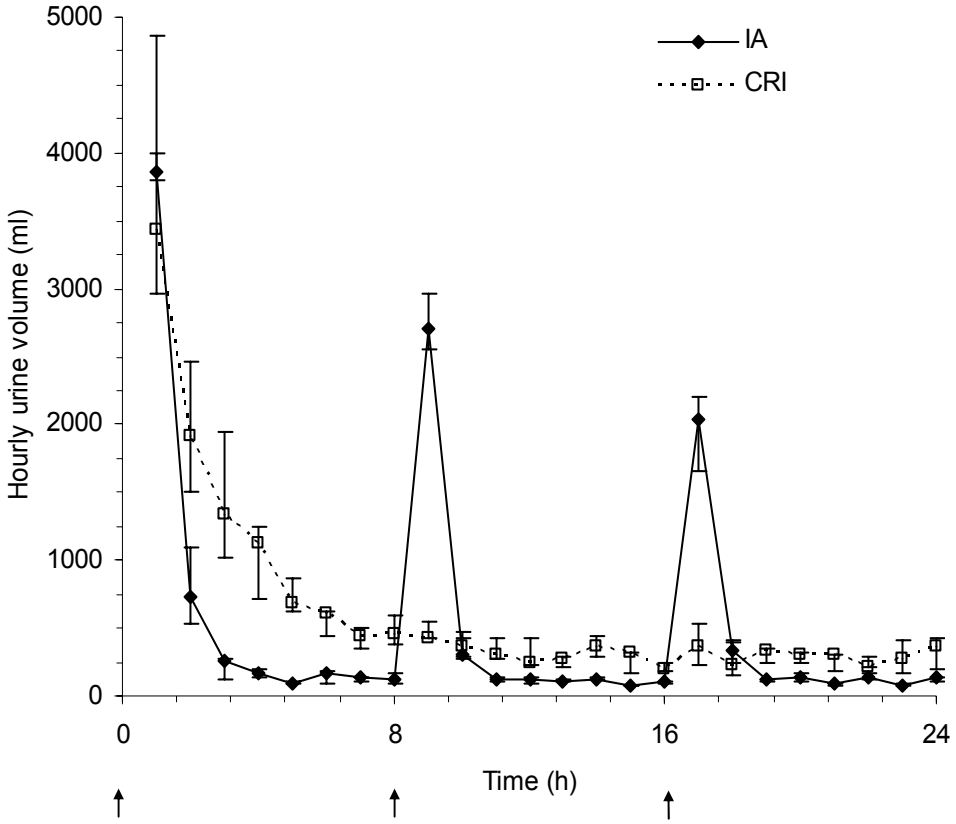

Figure 2. Hourly urine flow after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Arrows indicate time points of furosemide administration for horses receiving IA. Median (25^{th}, 75^{th} percentile).

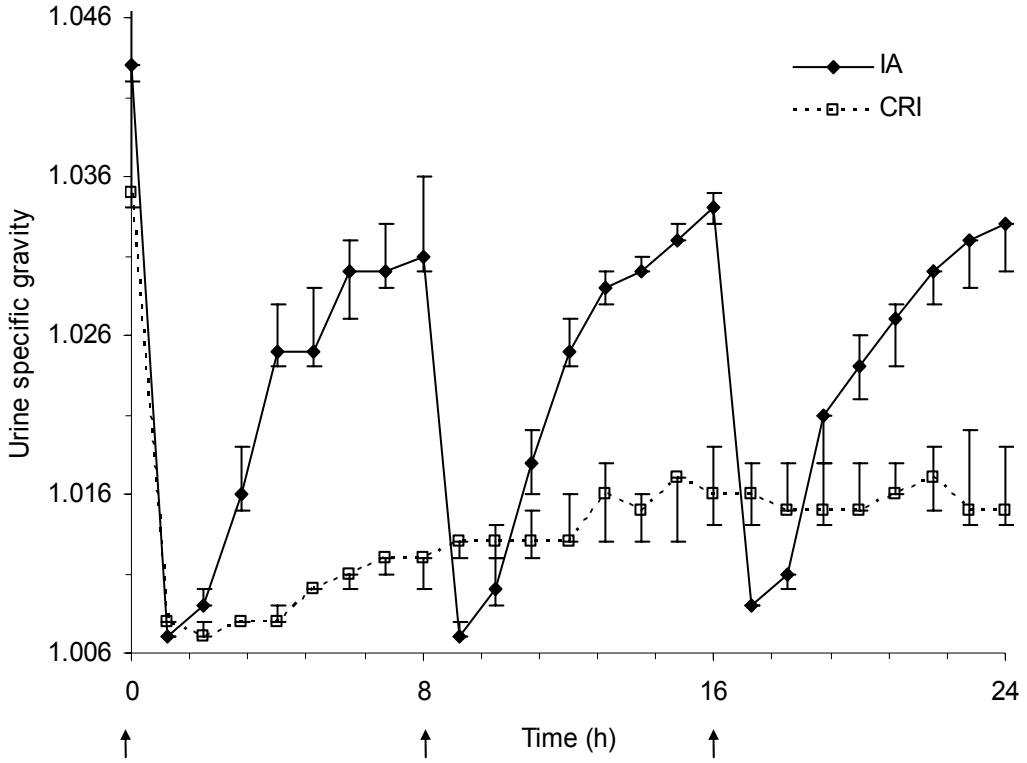

Figure 3. Urine specific gravity after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Arrows indicate time points of furosemide administration for horses receiving IA. Median (25^{th}, 75^{th} percentile).

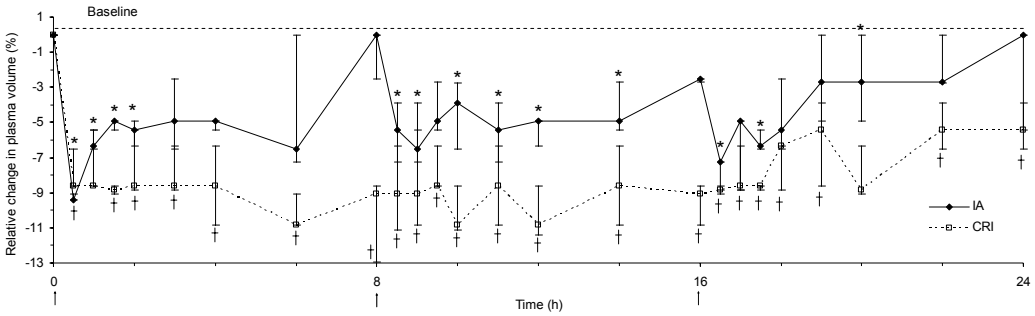

Figure 4. Relative changes in plasma volume, calculated from total solids, after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Arrows indicate time points of furosemide administration for horses receiving IA. Median (25^{th}, 75^{th} percentile).

∗ IA, significant difference from value at t=0, $p<0.05$ (Wilcoxon signed-rank test).

† CRI, significant difference from value at t=0, $p<0.05$ (Wilcoxon signed-rank test).

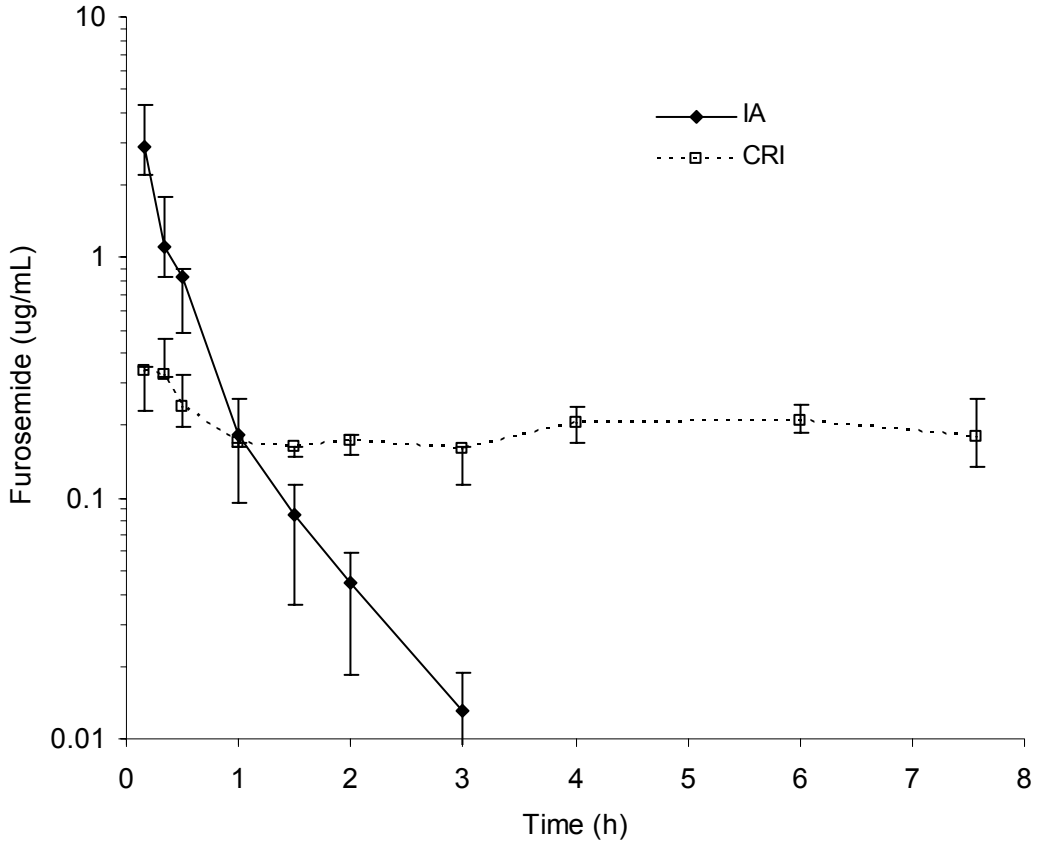

Figure 5. Plasma furosemide concentrations during 8 hours following administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses.

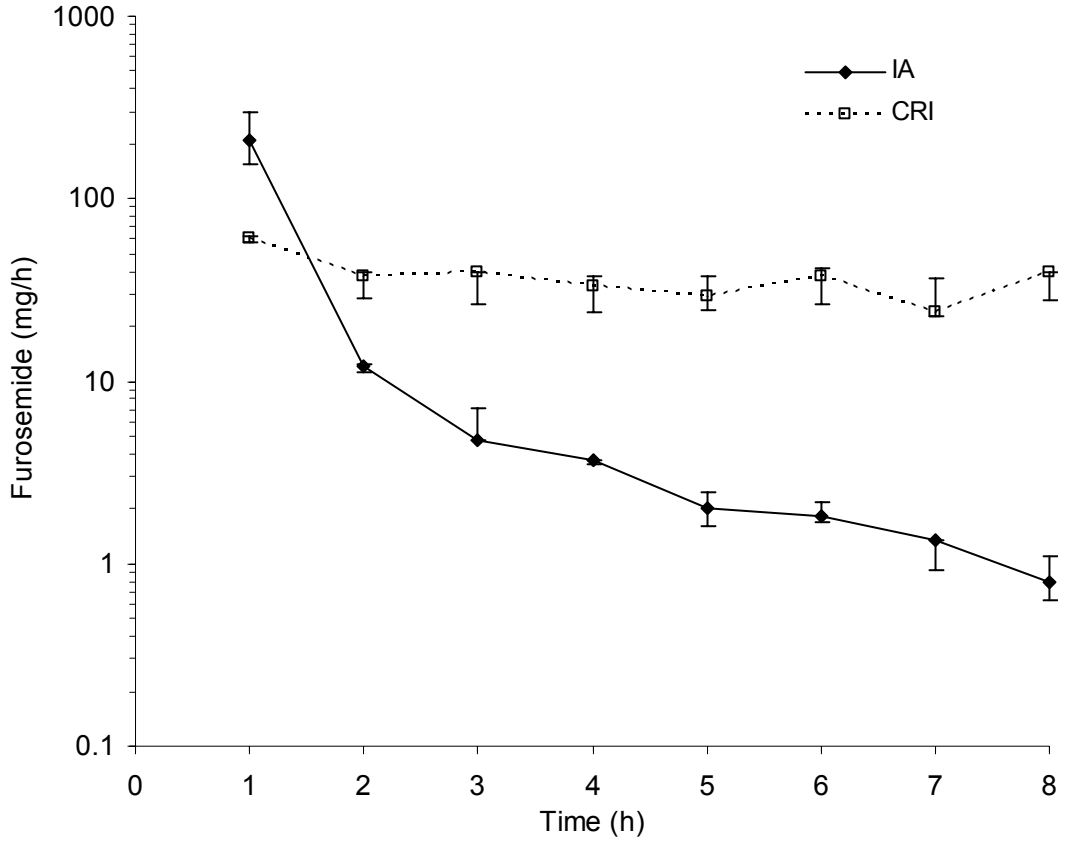

Figure 6. Furosemide excretion in urine during 8 hours following administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses.

CONCLUSIONS

Summary of results

We found that CRI with furosemide provided better diuresis than traditional IA for the first 8 hours of treatment, and although it was not significantly more effective over 24 hours; it produced a more uniform urine flow rate, with less fluctuation in plasma volume. CRI of furosemide appears to be a safe and reliable method of administration. It is slightly more labor-intensive since an infusion pump is necessary and infusion sets have to be protected from light to prevent photochemical degradation of the drug during infusion. If profound diuresis is needed acutely in horses, we recommend furosemide CRI (0.12 mg/kg/h), preceded by a loading dose (0.12 mg/kg) for 8 hours to provide a more vigorous effect than traditional administration of a bolus dose (1mg/kg). Attention should be paid to blood pressure during infusion, and serum concentrations of K and Ca should be monitored and supplementation provided as needed.

Our study was limited by the low number of observations, hence the significant differences we found are very strong. Contrary, for the results that were close to significant, a larger number of observations probably would have been beneficial. Specific examples of this is 24-hour urine volume and aldosterone secretion. Further, acute tolerance to furosemide, induced by the dehydration that inevitably follows treatment probably limited our study. Horses on CRI may have become dehydrated earlier based on the more pronounced effect early on during treatment. By replacing fluids volume for volume with IV fluids we could have avoided this limitation, and maybe created an experimental model more similar to a patient with fluid retention.

Recommendations for further research

Since dogs are commonly affected with congestive heart failure, a similar study evaluating the efficacy of furosemide CRI in dogs would be indicated. By replacing fluids lost continuously the limitations mentioned above could be avoided. A randomized clinical trail evaluating efficacy of CRI compared to IA of furosemide in dogs with congestive heart failure secondary to mitral regurgitation, with emphasis on urine production, electrolyte abnormalities, arrhythmias, clinical response, and RAAS-activity, would assist clinicians in optimizing treatment for such animals.

MAGNESIUM

INTRODUCTION

Magnesium (Mg) intracellular concentrations are high, second in intracellular cation concentrations only to potassium (K). Magnesium is the fourth most common cation in the body after K, sodium (Na) and calcium (Ca). It plays a crucial role in energy metabolism because it is required as a cofactor for most enzymes involving ATP. Mg is also a constituent of chlorophyll and is therefore present in virtually all food sources.[1] About 99% of total body Mg is found intracellularly; approximately 60% in the skeleton, 20 % in skeletal muscle, and 20 % in the heart, liver, and other organs.[2] Consequently, Mg in serum consists of only 1% of total body Mg. Mg in serum is divided into three fractions: Mg bound to protein (20-30%), in particular to albumin and to a lesser extent globulins; ionized Mg (70-80%); and Mg complexed with anions (1-2%) such as phosphate, bicarbonate, and citrate.[3] Mg is a small ion, but it attracts water molecules and is therefore functionally a large ion, making it difficult for it to pass through narrow channels. Mg binding to proteins is generally weaker than that for Ca.[3]

Normal function and regulation of magnesium

Mg is an essential activator for more than 300 enzymes.[4-6] Mg activates ATP:ase enzymes involved in establishing and maintaining intracellular electrolyte content. One example is the ouabain-sensitive Mg-(Na-K)-ATP:ase that regulates intracellular sodium and potassium concentrations. Additionally, Mg activates proton pumps that are involved in mitochondrial ATP generation and Ca pumps that preserve intracellular Ca-levels.[4] Mg is necessary for

enzymes involved in nucleic acid synthesis and degradation, DNA transcription, glucose metabolism, fatty acid metabolism, and mitochondrial oxidative metabolism. It plays a role in regulation of signal transduction, because Mg ion is essential for adenylyl cyclase activity. Further, Mg regulates ion-channels including Ca-channels and is essential for cell cycle activity and the cytoskeleton.[4-6]

Absorption and excretion of magnesium

Absorption of Mg in the gastrointestinal (GI) tract is thought to be primarily a passive paracellular mechanism, but recent studies have shown a Na-dependent transport mechanism at the basolateral membrane of intestinal epithelial cells. This may be by a 2Na1Mg antiporter, but it cannot be excluded that Na is necessary for the NaK2Clcootransporter to provide Cl to an Mg2Cl symporter for example.[3]

Fifteen percent of Mg that is filtered in the glomerulus is reabsorbed in the proximal tubule and 50-60 % in the thick ascending limb of the loop of Henle (TAL). Both trans- and paracellular mechanisms are involved. No specific transporter has been identified, but a 2Na1Mg-antiporter or Mg2Clsymporter might be involved. The mechanism of paracellular transport of Mg remains elusive, however recently a gene that codes for a protein located in the tight junctions of the TAL and that is mutated in people with a rare genetic disease with Mg wasting and renal hypomagnesemia, has been identified.[3]

Magnesium homeostasis

No specific endocrinologic control for blood levels of Mg has been identified. Regulatory organs include the kidney, GI tract, and bone. Renal regulation seems to be the most

important. A number of hormones have been reported to influence Mg homeostasis. Parathyroid hormone (PTH) stimulates reabsorption of Mg both in the loop of Henle and distal tubule, mediated by activation of adenylyl cyclase and an increase in cAMP levels. PTH may also increase Mg absorption in the intestines and release Mg from bone.[3] Vitamin D enhances GI absorption of Mg.[3] Cyclical variation of Mg has been seen in women, indicating a role for estrogen and progesterone in Mg homeostasis.[3]

Chronic administration of aldosterone causes Mg wasting, and there is some evidence that Mg may modulate aldosterone production in the adrenal cells in vitro.[3] Patients with diabetes mellitus have a higher prevalence of hypomagnesemia, and there is a correlation between hypomagnesemia and the severity of hyperglycemia. Glucose administration does not affect Mg homeostasis in healthy people. In vitro studies demonstrate that insulin enhances cellular uptake of Mg, where insulin may also inhibit reabsorption of Mg in the loop of Henle.[3] Glucagon increases reabsorption of Mg in the loop of Henle and in the distal tubule, mediated by adenylyl cyclase and cAMP.[3] Studies on the adrenergic effects on plasma Mg levels have given contradictory results. Studies in conscious humans or sheep infused with catecholamines resulted in varying degrees of hypomagnesemia.[7] One explanation for this phenomenon has been that catecholamines stimulate lipolysis with intracellular chelation of Mg by free fatty acids.[8] More recent studies in which infusions of increasing doses of isoproterenol, epinephrine, and norepinephrine were administered to anaesthetized rats resulted in an increase in circulating Mg detectable within 10 minutes, and at maximum after 20 minutes.[7]

Presently no specific signaling mechanism for plasma Mg has been identified. It appears that Mg can inhibit hormone-stimulated, cAMP-mediated reabsorption of Mg in the kidney, and thereby provide a self-regulatory mechanism.[3]

Mg can prevent norepinephrine-induced increases in mean arterial pressure and increased systemic vascular resistance. This indicates a role for Mg as an endogenous modulator of catecholamine release and activity and may suggest that an increase in plasma Mg following sympathetic stimulation contributes to improved blood flow to the heart at a time when an increase in energy production is expected.[7]

Hypomagnesemia

Causes of hypomagnesemia

Causes of hypomagnesemia can be either renal or gastrointestinal. Reabsorption of Mg filtered in the kidney is normally near maximal transport capacity, and therefore, small changes in plasma concentrations of Mg are accompanied with rather rapid increases or decreases in urinary Mg excretion.[1] Increased urine production associated with administration of diuretics, ketoacidotic diabetes, alcohol consumption, fluid therapy, or hypercalciuria can contribute to increased excretion of Mg. In addition to the diuretics, other drugs, including aminoglycosides, cyclosporines, and cisplatin are known to cause hypomagnesemia.[1, 4] Further, hyperaldosteronism and hyperparatyroidism may increase renal loss of Mg.

Gastrointestinal causes of hypomagnesemia include states of dietary Mg deficiency, impaired absorption, vomiting, diarrhea, and decreased food intake.[1, 4] In disorders like

steatorrhea and chronic pancreatic insufficiency, non-absorbable Mg-fatty acid soaps may be formed.[3]

Clinical relevance of hypomagnesemia

Determination of Mg concentration in serum has earned a growing interest among intensive care unit (ICU) clinicians for several years,[5] because there have been several reports identifying a high incidence of hypomagnesemia in patients admitted to ICUs with prevalence ranging from 20-65%.[9-13] In addition, patients with severe hypomagnesemia are reported to have a higher rate of both hypokalemia and death.[5, 9, 13]

Clinical signs

Clinical signs of hypomagnesemia include tremor, tetany, weakness, anorexia, apathy, and rarely seizures.[1] The increase in excitability of muscles and nerves during Mg deficiency is related to increased acetylcholine release from nerve terminals as well as increased intracellular calcium content in skeletal muscle.[14] Hypomagnesemia, with and without accompanying hypocalcemia, causes tetany, while hypomagnesemia and concomitant hypokalemia cause generalized weakness.[14] Cardiac arrhythmias are associated with hypomagnesemia, particularly arrhythmias of ventricular origin.[1, 4, 13] Increased risk for digoxin toxicity and hypertension also occur.[1, 4] Migraine headaches have been associated with Mg depletion.[5] If severe Mg depletion develops, paresthesia, muscular cramps, irritability, decreased attention span, and mental confusion often occur.[3]

Magnesium and calcium

Hypomagnesemia is the most common cause of hypocalcemia in humans. Decreased plasma levels of Mg inhibit release of parathyroid hormone (PTH) and thereby lower plasma Ca levels.[1, 14] Hypomagnesemia also increases end-organ resistance to PTH.[14]

Magnesium and potassium

Forty percent of patients with hypomagnesemia also have hypokalemia. If the intracellular concentration of one of these cations decreases, the other one will follow.[1] Mg deficiency leads to decreased function of the Na-K-ATP:ase, and thereby lower intracellular levels of K. Hypomagnesemia may also lead to inappropriate kaliuresis.[14] A primary depletion of K, leading to a decrease in intracellular K concentrations, causes a decrease in intracellular Mg concentrations, but plasma concentrations of Mg may not be affected. Hypokalemia secondary to hypomagnesemia can usually not be corrected without concomitant Mg-administration.[1]

Cardiac disease

Accumulating evidence suggests that dietary Mg deficiency plays an important role in the pathogenesis of ischemic heart disease, congestive heart failure, sudden cardiac death, cardiac arrhythmias, and hypertension.[3] Since Mg is necessary for normal function of Na-K-ATP:ase, hypomagnesemia may increase resting membrane potential and increase excitability.

Hypomagnesemia is associated with digitalis toxicity and cardiac arrhythmias, such as ventricular premature contractions.[4] Mg deficiency induces vascular damage in the heart

and kidney, accelerates development of atherosclerosis, causes vasoconstriction of coronary arteries, increases blood pressure, and induces thrombocyte aggregation. Infusion of Mg results in vasodilation of systemic vasculature and coronary arteries, platelet inhibition, and antiarrhythmic effects.[3] Hypomagnesemia alters myocardial electrophysiologic features, contributing to loss of myocardial K and increasing the Ca:Mg ratio.[5] Mg is recommended in treatment of ventricular tachyarrhythmias if it is the cause of K depletion because of the relationship of hypomagnesemia to refractory ventricular fibrillation and to refractory potassium repletion.[4] Both hypomagnesemia and hypermagnesemia have been causally related to sudden death in patients with CHF.[4] Intravenous administration of Mg in patients with acute myocardial infarction reduces mortality.[4]

Hypertension

Mg deficiency can contribute to hypertension. Mg regulates Ca channels, and a decrease in Mg concentrations will cause an increase in intracellular Ca that enhances contraction of smooth muscles in blood vessels. A decreased Mg:Ca ratio enhances the vasoconstrictive effects of both norepinephrine and angiotensin II.[4]

Diabetes mellitus

Diabetes mellitus is associated with hypomagnesemia, apparently caused by osmotic diuresis. Although it most likely doesn't play a primary role in the pathogenesis, hypomagnesemia can predispose diabetics to complications such as retinopathy, hypertension, and cardiovascular disease. There is a strong relationship between

hypomagnesemia and insulin resistance. Mg supplementation is recommended in patients with diabetes and documented hypomagnesemia.[3, 4]

Eclampsia

Mg sulfate is widely used as a routine therapy to prevent eclamptic seizures in pregnant women with hypertension.[3]

Stroke

There is an inverse association between dietary Mg intake and the risk of stroke. Mg has been shown to be neuroprotective in several models of experimental ischemic or toxic brain injury. Currently, the role of $MgSO_4$ treatment after acute stroke is under investigation.[3]

Asthma

Dietary Mg intake has been related to lung function, airway reactivity, and respiratory symptoms in the general population. Theories regarding these effects include Ca competition in the cell, inhibition of cholinergic transmission, stimulation of synthesis of nitric oxide and prostacyclin, and stabilization of mast cells and T lymphocytes. $MgSO_4$ has been shown to cause bronchodilation and improve lung function, but the use of Mg in asthma treatment remains to be determined.[3]

Treatment of hypomagnesemia

Formulations of Mg for treatment of hypomagnesemia include: 50% magnesium sulfate heptahydrate I.V. ($MgSO_4 \bullet 7\ H_2O$), magnesium oxide P.O.., Mg lactate P.O., or Mg antacids P.O.[2-3] Side effects are rare. Diarrhea and abdominal cramps may occur.[3]

Measurement of magnesium

In most clinical laboratories total serum Mg concentration is measured. Because 99% of total body Mg is intracellular, and because Mg is believed to exert its major function as an intracellular biochemical cofactor, the relevance of serum concentrations have been questioned.[3, 4] Despite some limitations, total serum Mg is most commonly used for evaluating Mg deficiency and is thought to give a reasonable estimate of total body status.[4] A patient with hypomagnesemia usually has some degree of magnesium depletion. A patient with a normal Mg level must be evaluated along with their clinical condition. If such patients also have diabetes, cardiac arrhythmias, chronic diarrhea, or are taking diuretics, they should be evaluated carefully for Mg depletion.[5]

The free ionic form of Mg is the active form and hence that seems to be the ideal quantity to measure in both serum and cells, but until recently no methods for routine measurement of ionized Mg have been available. A difficulty encountered in analyzing ionized Mg is that calcium ions interfere with Mg selective electrodes. About 50% of Ca in blood is ionized, and the relative difference between ionized and total Ca can be significant. For Na and K, 99% is ionized and the clinical relevance of ionized:total ratios for these ions is limited. For Mg, 65-70% is free ionized so the clinical value of ionized compared with total is probably of less importance than for Ca.[5] However, assaying ionized Mg is gaining

popularity since there are reports on the relevance of ionized Mg in different clinical situations and the superiority of this parameter over total Mg.[3]

A recent study on the relationship between total Mg, ionized Mg, Mg in red blood cells, and Mg in mononuclear blood cells and clinical outcome in critical care patients showed no association between intracellular or extracellular levels of Mg and clinical outcome.[15] According to this study based on total serum Mg, 51.3% of the patients were hypomagnesemic, but if only ionized Mg was considered, then only 14.4% were classified as hypomagnesemic. The intracellular concentrations were normal in all patients. No correlation between intracellular and extracellular concentrations of Mg was found, and no correlation between serum Mg parameters and albumin was found. There was a negative correlation between the calculated free fraction of Mg and albumin. Total Mg correlated positively with ionized Mg, but not strongly enough to be used as a clinical predictor of decreased ionized Mg. An explanation for these results according to the authors was that the decrease in total serum Mg reflected a shift of Mg to the intracellular compartment rather than total body depletion, and that the shift did not affect the ionized fraction to the same extent. Since Mg is much more abundant in the intracellular compartment, this influx did not cause a significant percentage increase inside the cells.

The best method for determination of Mg deficiency currently is the Mg loading test.[4, 14, 15] In this test Mg is administered intravenously and a 24-hour urine sample is collected. A healthy person will excrete 75% or more of the Mg load.[4] However, this method is not routinely used in clinical practice since it is rather complicated.

Magnesium in horses

Mg is primarily absorbed in the jejunum and ileum. Horses require about 13 mg of Mg/kg body weight per day for maintenance.[16]

Recently a study on Mg status in horses with colic was published.[17] Thirty-five horses over one year of age that underwent colic surgery participated in the study. Blood samples for ionized Mg, total Mg, ionized Ca, and total Ca were drawn at admission and 1, 3, 5, and 7 days post-surgery. At admission 17% of horses had total serum Mg concentrations below reference values and 54% had serum concentrations of ionized Mg below reference values. According to the authors, this difference could have been due to the fact that changes in pH or albumin concentrations affected total Mg more than ionized Mg. Low pH decreases Mg protein binding. Ionized Mg was, according to the authors, considered a more accurate indicator of Mg concentrations in the systemic circulation since it was less affected by other parameters. Ionized Mg was lower at admission in horses with strangulating intestinal lesions and horses that were euthanatized during surgery. Horses that developed postoperative ileus had lower ionized Mg values after surgery than horses that did not develop ileus. Both decreased total and ionized Mg at admission were correlated to increased heart rate and ECG changes, such as prolongation of the QT interval. No correlation between Mg and hospitalization time, complications, or survival was found. The authors concluded that early recognition and correction of Mg deficiencies may help to reduce morbidity and mortality in horses with colic, and that studies of the potential benefits of Mg supplementation are warranted.[17]

Mg sulfate can be effective in the treatment of ventricular tachycardia in horses with both normo- and hypomagnesemia. It can be administered either as a bolus dose or as an

intravenous drip at 1g/minute until effect up to a total dosage of 25g for a 450-500 kg horse.[18] Supplementation of Mg may be necessary in horses with duodenitits-proximal jejunitis that are treated over longer periods with intravenous fluids, since commercial polyionic fluids do not always contain sufficient Mg.[19] Mg sulfate slurries can be used as oral cathartics to clear the carbohydrate from the large intestine before fermentation and thereby prevent absorption of endotoxins in horses that have accidentally overeaten.[20] Further, Mg can be used in the treatment of primary large colon impaction.[21] Mg is a component of struvite enteroliths (Mg ammonium phosphate) that can cause intestinal obstruction in the horse.[21] Equine sweat contains large amounts of Mg, and hypomagnesemia is common in endurance horses.[22]

Magnesium supplementation to horses can be provided either by a slow IV infusion of 25-50 mg/kg of magnesium sulfate, or oral magnesium oxide, magnesium carbonate, or magnesium sulfate.[22]

Magnesium in dogs

One study showed an incidence of hypomagnesemia in 33.6% of dogs and cats admitted to ICUs. Seventy-three percent of the hypomagnesemic dogs also had hypokalemia. Another study showed that 31% of critically ill dogs had concurrent hypomagnesemia and hypokalemia.[14]

Mg supplementation has been recommended in animals with refractory hypokalemia, in diabetic ketoacidotic patients, and in the treatment of cardiac arrhythmias refractory to conventional antiarrhythmics, or those associated with digoxin toxicity. It has also been used in critically ill animals with total serum Mg below 1.2 mg/dl.[14]

Hypomagnesemia has been recognized with protein-losing enteropathy in dogs. This may be due to intestinal loss, malabsorption, and abnormalities of vitamin D and parathyroid hormone metabolism associated with the disease.[8]

In a retrospective study, all dogs admitted to a small animal hospital, with at least one blood chemistry panel, in a two-year period were evaluated.[23] The dogs were considered hypomagnesemic if they had at least one total serum Mg concentration below reference range (1.7-2.5mg/dl). One hundred and eighty-eight hypomagnesemic patients and 2,914 normals were included. Data from the most recent hospital visit were evaluated. The following parameters were collected:

- Blood chemistry panel: ALP, amylase, CK, albumin, total bilirubin, total protein, BUN, Mg, Na, anion gap, osmolality, K, total CO_2, creatinine, uric acid, Cl, glucose, phosphorus, and Ca.
- Age, breed, gender, disease code for system affected and discharge status.

Continuous variables were compared between groups with a student t-test. For non-parametric variables, the Wilcoxon sum-rank test was used. Categorical variables were analyzed with a λ^2-test or a two-tailed Fisher's test where needed. Significant variables from the univariate model were then used in building two models for conditional logistic regression (multivariate), one for the hypomagnesemic patients and one for the controls. An overall prevalence of hypomagnesemia of 6.1% was noted. In the multivariate model, the following variables were significant: albumin, BUN, K, total CO_2, disease of the cardiovascular system, and being a Collie or German shepherd. A strong association between hypomagnesemia and hypocalcemia was seen in the univariate model, but because of collinearity between Ca and albumin, Ca was excluded from the final model.[23]

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HYPOMAGNESEMIA IN THE HORSE – A RETROSPECTIVE STUDY OF 401 CASES

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Abstract

This study was initiated to identify the signalment and clinical variables potentially associated with hypomagnesemia in horses evaluated at the NCSU-CVM veterinary teaching hospital between January 1999 and May 2001. A nested case reference study (nested case-control study) was conducted to examine the potential relationship between hypomagnesemia and signalment, serum chemistry panel analyses, number of hospitalization days, discharge status, and diagnosis.[1] A series of independent and multivariable logistic regression models were used to assess the potential association of each variable with low total serum magnesium values.

Of all horses included in the study, 48.7% had total serum magnesium values below the normal reference range. Hypomagnesemia was more likely to occur in horses older than one month of age. Colic, acute diarrhea, other gastrointestinal disease, infectious respiratory disease, and multi-organ system disease were associated with hypomagnesemia in adult horses, while diarrhea in foals reduced the risk of hypomagnesemia. Overall there was no relationship between hypomagnesemia and mortality, but horses with colic and hypomagnesemia were more likely to survive than horses with colic and normal or elevated total magnesium. Among horses that survived, hypomagnesemia at admission was associated with a longer hospitalization period.

Key words:
Magnesium, equine, electrolyte, fluid balance

Magnesium (Mg) is involved in over 300 enzymatic reactions in mammals and thus is essential for normal cellular function and replication. Intracellular concentrations of Mg are high, second only to potassium (K). Magnesium activates ATP:ases such as the NaK-ATP:ase that regulates cell membrane potential. Magnesium regulates calcium (Ca) channels and pumps that regulate intracellular Ca concentrations. Magnesium is also involved in nucleic acid synthesis, signal transduction, and metabolism of protein, fatty acids, and glucose.[2] Mg in serum consists of only 1% of total body Mg. Magnesium in serum is divided into three fractions: Mg bound to protein (20-30%), in particular to albumin and to a lesser extent to globulins; ionized Mg (70-80%); and Mg complexed with anions (1-2%) such as phosphate, bicarbonate, and citrate.[3]

Clinical reports during the 1990s describing a high incidence of hypomagnesemia in patients admitted to intensive care units (ICU) prompted human clinicians to routinely measure serum concentrations of magnesium.[4-8] Prevalence of hypomagnesemia in ICUs has been reported to range from 20 to 65%.[5-9] This is of clinical importance because patients with hypomagnesemia are also reported to have a higher death rate.[4, 7, 9] In addition to these striking findings, low serum Mg concentrations are associated with hypocalcemia due to impaired release of parathyroid hormone and hypokalemia due to improper functioning of NaK-ATP:ases in cell membranes.[10] Hypomagnesemia has been associated with a number of disease processes including diabetes mellitus, eclampsia, stroke, and asthmatic disease.[3] Accumulating evidence demonstrates that Mg depletion increases the risk of developing ischemic heart disease, congestive heart failure, sudden cardiac death, cardiac arrhythmias, and hypertension in humans.[3] Intravenous administration of Mg in patients with acute myocardial infarction reduces mortality.[2]

Clinical signs of hypomagnesemia include tremor, tetany, weakness, anorexia, apathy, and rarely seizures.[10] The increased excitability of muscles and nerves during Mg deficiency is due to increased acetylcholine release from nerve terminals as well as increased intracellular calcium content in skeletal muscle.[11] Hypomagnesemia, with and without accompanying hypocalcemia, causes tetany, while hypomagnesemia and concomitant hypokalemia causes generalized weakness.[11] Cardiac arrhythmias are associated with hypomagnesemia, particularly arrhythmias of ventricular origin.[2, 9, 10]

Hypomagnesemia can occur due to decreased absorption in the small intestine or to impaired renal reabsorption. Dietary deficiency in the horse seems to be rare, but in humans, intestinal resection and diarrhea impair absorption of Mg.[12, 13] Increased renal losses can be secondary to renal tubular acidosis, acute renal failure, extensive fluid therapy, hypercalciuria, diabetes mellitus (osmotic diuresis), diuretic therapy, and aminoglycoside treatment.[12, 13] Hypomagnesemia can be seen after increased cellular uptake of Mg after insulin administration, endotoxemia, and hypothermia.[12, 13]

In one study total hypomagnesemia was observed in 54% and ionized hypomagnesemia was observed in 17% of horses with surgical colic.[14] Horses with strangulating lesions and horses that were euthanized during surgery had lower ionized Mg concentrations at admission than horses with non-strangulating lesions and horses that survived surgery, respectively. Horses that developed ileus had lower ionized Mg concentrations postoperatively than horses that did not develop ileus.[14] Another study reported that 78% of horses with enterocolitis had ionized hypomagnesemia.[15]

The purpose of this study was to determine: 1) the frequency of hypomagnesemia in horses admitted to the large animal clinic at North Carolina State University; College of

Veterinary Medicine (NCSU- CVM); 2) whether or not there was an association between hypomagnesemia and diagnosis; 3) the clinical outcome of animals with hypomagnesemia; and 4) if hypomagnesemia was associated with other serum chemistry irregularities.

Materials and Methods

Data were obtained for all horses admitted to the Veterinary Teaching Hospital of North Carolina State University, College of Veterinary Medicine (NCSU-CVM) between January 1999 and May 2001. All horses that had a blood chemistry panel analyzed within 24 hours after arrival were included in the study. If more than one panel was analyzed in a horse, only the first one was evaluated. Each horse was only considered once for the study; if a horse had visited the clinic several times, data from the most recent visit was collected, unless it was a recheck.

Information about the signalment (breed, age, and gender) was collected for each animal. Horse breed was classified as Quarter Horse-type (Quarter Horse, Appaloosa or American Paint Horse), Thoroughbred, Arabian, Warmblood, or other. Age was categorized as neonate (less than 1 month), foal (1 month to 1 year), young horse (1 to 3 years), adult (3 to 15 years), and geriatric (older than 15 years). Gender was defined as male (stallions and geldings) or female (mares).

Results from the equine blood chemistry panel taken within 24 hours after arrival to the clinic were obtained from the medical record. Blood chemistry analysis was performed at the NCSU-CVM Laboratory of Clinical Pathology. Before March 2000, the instrument used for chemistry analysis was a Monarch 2000[20], while more recent samples were analyzed on a Hitachi 912[21]. Both instruments used the Xylidyl blue method to determine total Mg. Categorization of blood chemistry parameters were based on the "normal" reference ranges for each instrument established according to laboratory standards. Clinical parameters were recorded as below, within, or above the "normal" reference range for

[20] Monarch 2000 (Instrumentation laboratory, Lexington, MA)
[21] Hitachi 912 (Roche Diagnostics Corp., Indianapolis, IN)

albumin/globulin ratio (A/G), albumin, alkaline phosphatase (ALP), anion gap (AnGap), aspartate aminotransferase (AST), total bilirubin, total Ca, chloride (Cl), creatine kinase (CK), creatinine, gamma glutamyltransferase (GGT), globulin, glucose, total Mg, osmolality, phosphorus, K, sodium (Na), total protein, and blood urea nitrogen (BUN).

For each case, the organ system affected and the final diagnosis were recorded, and horses were grouped according to a predefined list of diagnoses (Table 3). The diagnosis was defined as "Multi-organ system disease" if multiple organ systems were affected or if it was not possible to localize the problem according to the list. Horses that were determined to be healthy, except for a localized non-infectious injury that did not affect systemic health, were categorized as "Medically healthy". Examples of horses included in this group were those affected with minor fractures and fissures, degenerative joint disease, and horses admitted for pre-purchase examinations. To evaluate clinical response, the discharge status and number of hospitalization days were recorded from the medical records. Discharge status was recorded as alive or dead at the time of discharge. The number of hospitalization days was classified as less than 4 days, 4 to 7 days, and greater than 7 days.

A series of independent and multiple logistic regression models were evaluated to assess the potential association of signalment, blood chemistry parameters, disease process, morbidity, and mortality with low total magnesium values,16, 17 using a commercial software.[22] Each variable was initially examined independently and then later included in a multivariable model to identify confounding variables. Maximum likelihood coefficients (MLC) β-coefficient were used to estimate odds ratios and corresponding 95% confidence

[22] Egret, for Windows, Software for the Analysis of Biomedical and Epidemiological Studies. 1999 edition. (Cytel Software Corporation, Cambridge, MA)

intervals. Separate multivariable models were initially built for signalment, blood chemistry results, and clinical response. All variables were initially added to a base model in a forward fashion. The numeric stability of each model when a variable was added was evaluated by examining changes in the estimated standard errors of the MLCs. Variables were deleted to improve precision or added to improve validity based on changes in adjusted odds ratios and corresponding confidence intervals. Odds ratios greater than one with corresponding confidence intervals above one indicated that an animal with hypomagnesemia was more likely to also be in the studied category (e.g. a certain age group) compared to an animal with normo- or hypermagnesemia, while odds ratios less than one with corresponding confidence intervals below one indicated that an animal with hypomagnesemia was less likely to be in that category.

Results

Of all horses admitted to NCSU-CVM during the study period, 823 matched the inclusion criteria. Quarter horses made up the majority of the horses in the study population (37.4%) and horses ranged from 0 to 31 years of age. The study population included more males (53.8%) than females (46.2%).

Hypomagnesemia was observed in 48.7% of the horses. Only 2.9% of the horses in the study population were hypermagnesemic. Total serum Mg concentrations ranged from 0.6 - 1.6 mg/dl in hypomagnesemic horses, and 1.7 - 5.4 mg/dl in horses with normo- or hypermagnesemia. Horses with hypomagnesemia ranged in age from 0 to 30 years (median: 8 years; 25^{th} percentile: 4 years; 75^{th} percentile: 14 years), and horses with normo- or hypermagnesemia ranged in age from 0 to 31 years (median: 7 years; 25^{th} percentile: 1.25 years; 75^{th} percentile: 13 years).

The most common disease process was colic (32.1%); other categories from the recorded diagnoses that were common included other gastrointestinal disease (6.3%), other foal disease (6.1%), multi-organ system disease (5.6%), medically healthy horses (5.1%), and ophthalmic disease (4.7%).

Hypomagnesemia was initially identified to be associated with a variety of individual signalment, clinical and diagnosis related variables (Tables 1-4). In the initial univariate analysis, hypomagnesemia was more likely to occur among thoroughbreds than other breeds (Table 1). Animals with hypomagnesemia were more likely to be older than one month of age. Gender was not associated with hypomagnesemia in the study population. Horses with hypomagnesemia were more likely to have hypoalbuminemia, hypoglobulinemia, hypocalcemia, hypophosphatemia, hypokalemia; or elevated serum concentrations of total

bilirubin and glucose (Table 2). Horses with hypomagnesemia were less likely to have elevated serum concentrations of ALP, AnGap, Ca, phosphorous, K, creatinine; or low serum concentrations AST (Table 2). Upon investigating diagnoses, horses with hypomagnesemia were more likely to have colic, acute diarrhea, other gastrointestinal disease, infectious respiratory disease, and multi-organ system disease, while foals with hypomagnesemia were less likely to have diarrhea (Table 3). In the overall population there was no relationship between mortality and total Mg, but among horses that survived, horses that had hypomagnesemia at admission were more likely to be hospitalized longer than horses with normo- or hypermagnesemia at admission (Table 4).

Age was the sole signalment variable retained in the final multivariable analysis. The initial suggestion that hypomagnesemia was associated with breed and specifically thoroughbreds, was discounted in subsequent models because thoroughbreds were more likely to be older horses with lower ALP and elevated bilirubin. Consequently the apparent association between breed and hypomagnesemia was merely driven by the association of the effect of modifying variables such as age, low ALP and elevated bilirubin. The effect of these modifying variables was adjusted for in the final multivariable model. The final model included ALP, bilirubin, Ca, creatinine, globulin, and phosphorus (Table 5). Animals with hypomagnesemia were more likely to have acute diarrhea, colic, other gastrointestinal disease, infectious respiratory disease or multi-organ system disease and less likely to be foals with diarrhea (Table 6). Additional stratified analysis was conducted to assess the relationship of each diagnostic category with survival. Animals with colic and hypomagnesemia were more likely to survive than horses with colic and normo- or hypermagnesemia (Table 7). Among horses that survived until discharge, horses that had

hypomagnesemia at admission were more likely to be hospitalized more than 7 days (Table 8).

Discussion

Almost 50% of the horses tested at the hospital were hypomagnesemic. Since this study was conducted at a tertiary care institution the proportion of animals with hypomagnesemia in our study population may not reflect the actual frequency with which hypomagnesemia is observed in the primary care veterinary hospital. However, we observed an association between gastrointestinal disease and hypomagnesemia and animals with gastrointestinal disease are frequently treated in primary care equine clinics. Many horses with gastrointestinal disease have acidosis, and low pH will decrease the protein-binding for Mg, so that even if the horse has total hypomagnesemia, ionized Mg may still be normal.[18] However, changes in serum total Mg often parallel changes in ionized Mg,[18] and reports from horses indicate that ionized hypomagnesemia is common among horses with gastrointestinal disease.[14, 15]

Hypomagnesemia was less likely to occur in young horses, especially foals, than in adult and old horses. Serum Mg concentrations in healthy horses do not vary with age and healthy foals have values that are within the normal reference range.[19] Based on estimations of the daily Mg requirements of adult horses (13 mg/kg body weight),[20] and the concentration of Mg in mares' milk in early lactation (90 mg/kg),[13, 21] nursing foals do not appear to receive excessive amounts of Mg in their diets. However, young animals are able to mobilize 30-60% of their bone Mg when dietary intake is reduced, and the ability for adult animals to mobilize Mg from the skeleton is significantly less.[21]

In the final model we found that horses with hypomagnesemia were less likely to have an elevated ALP. Since young horses normally have markedly elevated ALP

concentrations,[22] the relationship between high ALP and hypomagnesemia most likely reflects the relationship between age and hypomagnesemia .

Hypomagnesemia was associated with elevated serum bilirubin concentrations. The most common cause of elevated bilirubin in the horse is fasting.[23] Many horses admitted to the hospital are anorexic, and elevated bilirubin is a frequent finding. Mg is absorbed in the small intestine,[20] and hypomagnesemia can result from decreased dietary intake, and since an elevation in serum bilirubin is associated with fasting, it was not surprising that there was a relationship between increased bilirubin and hypomagnesemia.

Horses with "normal" or elevated concentrations of magnesium were more likely to have elevated serum creatinine concentrations. Chronic renal failure is associated with hypermagnesemia, but renal capacity to excrete Mg is great, and a substantial decrease in creatinine clearance occurs before hypermagnesemia results.[24] Pre-renal azotemia indicates a decrease in the glomerular filtration rate, and it appears that absolute Mg excretion falls as glomerular filtration declines.[24, 25] Osmolality also increases with dehydration.

Horses with elevated Ca were less likely to be hypomagnesemic and this association has been well documented previously.[10] Hypomagnesemia impairs release of parathyroid hormone (PTH) and decreases end-organ receptor sensitivity for PTH, thereby often resulting in hypocalcemia.[10] We found a similar relationship between phosphorus and Mg, and it has been reported that hypophosphatemia and hypomagnesemia often occur concurrently in humans.[26, 27] It has been demonstrated that experimentally induced hypomagnesemia causes phosphaturia in rats.[28] However, this may not always be the case, since horses with enterocolitis often have decreased serum Ca and Mg, but elevated phosphorous.[15] Low K is common in humans with hypomagnesemia.[10] We found a

potential relationship between low K in our independent assessment but when K was adjusted for the other clinical parameters in the multivariable model, hypomagnesemia did not appear associated with low K.

The finding that hypomagnesemia was more likely in horses with colic, acute diarrhea, and other gastrointestinal disease was not surprising. Hypomagnesemia can result from decreased absorption or increased secretion into the gastrointestinal tract.[2, 10] It has previously been reported that horses with colic requiring surgery or enterocolitis have a high prevalence of hypomagnesemia.[14, 15] Many of the horses with multi-organ system disease had infectious disease (e.g. septicemia, esophageal obstruction with secondary aspiration pneumonia and fever with signs of infection of undetermined origin). Similarly horses with septic diseases such as pneumonia, pleuropneumonia, and strangles made up most or the animals in the infectious respiratory disease category. Sepsis can affect Mg homeostasis by causing an extracellular to intracellular shift of Mg and thereby lower serum concentrations.[13] Septic human patients often have very low serum Mg concentrations.[29] The diseases that we found associated with hypomagnesemia often result in endotoxemia in the horse.[30] The diseases that we found associated with hypomagnesemia often result in endotoxemia in the horse.[30] It has been demonstrated that rats that received a Mg-deficient diet three to six weeks prior to endotoxin administration had higher morbidity and mortality compared to Mg-sufficient animals, and Mg replacement significantly increased survival in the Mg deficient rats.[31]"

Much attention has been paid to Mg in critically ill humans since reports suggested that hypomagnesemia was an independent factor that affected mortality.[7, 9] We found no association between hypomagnesemia and the likelihood of mortality, except for in the

group of horses with colic where we actually found that horses with hypomagnesemia were more likely to survive than horses that had normo- or hypermagnesemia. The relationship between Mg concentration and survival in colicky horses is not intuitively obvious. A potential explanation may be that in the initial independent assessment, hypomagnesemia was less likely to occur in horses with elevated anion gap, creatinine, and osmolality. Horses with these characteristics are dehydrated and have decreased peripheral perfusion, and they might have Mg in the normal range or above due to hemo-concentration and decreased glomerular filtration.[24, 25] The group that we refer to as normo- or hypermagnesemic might therefore include systemically unaffected horses, as well as severely dehydrated or hypovolemic horses. Earlier reports regarding Mg concentrations in horses with colic demonstrated that total serum Mg concentrations were not associated with survival, however horses with low ionized serum Mg concentrations at admission more often had strangulatory lesions and were more often euthanized during surgery.[14]

In this study, and in most clinical laboratories, total serum Mg concentration is measured. Because 99% of total body Mg is intracellular, and because Mg is believed to exert its major function as an intracellular biochemical cofactor, the relevance of serum concentrations have been questioned.[2, 3] Despite some limitations, total serum Mg is most commonly used for evaluating Mg deficiency and is thought to give a reasonable estimate of total body status.[2] The free ionic form of Mg is the active form and hence that seems to be the ideal quantity to measure in both serum and cells, but until recently no methods for routine measurement of ionized Mg have been available. A difficulty encountered in analyzing ionized Mg is that calcium ions interfere with Mg selective electrodes. About 50% of Ca in blood is ionized, and the relative difference between ionized and total Ca can be

significant. For Na and K, 99% is ionized and the clinical relevance of ionized:total ratios for these ions is limited. For Mg, 65-70% is free ionized so the clinical value of ionized compared with total is probably of less importance than for Ca.[4]

It should be noted that the present study investigates a population with a wide range of disease entities, many of which would not be described as life threatening. Analysis of a population of equine critical care patients may have given different results.

In conclusion, we found that hypomagnesemia is common in horses treated by our clinic, especially if the gastrointestinal system is involved. Concurrent electrolyte disturbances occur frequently, and young horses appear to be able to regulate serum total Mg concentrations more efficiently. Horses with hypomagnesemia were more likely to be hospitalized longer, but they did not have higher mortality.

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Table 1. Initial independent assessment of the potential association of signalment with the presence of hypomagnesemia.

	Hypomagnesemia		Normal or high Mg		Odds ratio	95 % CI		
Signalment	No.	%	No.	%		Lower	-	Upper
Breed								
Other	117	29	126	30	1.00			
Quarter Horse	131	33	177	42	0.80	0.57	-	1.12
Thoroughbred	83	21	55	13	1.66	1.06	-	2.48
Arab	45	11	32	8	1.51	0.90	-	2.54
Warmblood	25	6	32	8	0.84	0.47	-	1.50
Age								
0-1month	14	3	68	16	1.00			
1month-1year	14	3	23	5	2.96	1.23	-	7.12
1-3years	55	14	52	12	5.14	2.58	-	10.23
3-15 years	243	61	210	50	5.62	3.07	-	10.28
>15 years	75	19	69	16	5.28	2.72	-	10.23
Gender								
Female	188	47	192	45	1.00			
Male	213	53	230	55	0.95	0.72	-	1.24

Abbreviations: CI, confidence interval.

Table 2. Initial independent assessment of the potential association of blood chemistry variables with the presence of hypomagnesemia.

	Hypomagnesemia		Normal or high Mg		Odds ratio	95 % CI		
Parameter	No.	%	No.	%		Lower	-	Upper
AG								
Normal	283	70.6	307	72.7	1.00			
Below	20	5.0	16	3.8	1.36	0.69	-	2.69
Above	98	24.4	99	23.5	1.07	0.77	-	1.48
ALB (g/dL)								
Normal	266	66.3	292	69.2	1.00			
Below	96	23.9	75	17.8	1.40	1.00	-	1.99
Above	39	9.7	55	13.0	0.78	0.50		1.21
ALP (IU/L)								
Normal	308	76.8	272	64.5	1.00			
Below	20	5.0	16	3.8	1.10	0.96	-	1.33
Above	73	18.2	134	31.8	0.48	0.35	-	0.69
AnGap								
Normal	327	81.5	336	79.6	1.00			
Below	53	13.2	39	9.2	1.40	0.90	-	2.17
Above	21	5.2	47	11.1	0.46	0.29	-	0.79
AST (IU/L)								
Normal	264	65.8	262	62.1	1.00			
Below	30	7.5	53	12.6	0.56	0.35	-	0.91
Above	107	26.7	107	25.4	0.97	0.72	-	1.36
BILI total (mg/dL)								
Normal	103	25.7	176	41.8	1.00			
Below	1	0.2	1	0.2	1.71	0.11	-	27.60
Above	297	74.1	244	58.0	2.08	1.57	-	2.80
Ca (mg/dL)								
Normal	117	29.2	226	53.6	1.00			
Below	277	69.1	151	35.8	3.54	2.63	-	4.78
Above	7	1.7	45	10.7	0.30	0.13	-	0.69
Cl (mmol/L)								
Normal	318	79.3	331	78.4	1.00			
Below	31	7.7	39	9.2	0.83	0.50	-	1.36
Above	52	13.0	52	12.3	1.04	0.69	-	1.58
CK (IU/L)								
Normal	226	56.4	247	58.5	1.00			
Below	30	7.5	21	5.0	1.56	0.87	-	2.81
Above	145	36.2	154	36.5	1.03	0.77	-	1.38
Crea (mg/dL)								
Normal	312	77.8	260	61.6	1.00			
Below	18	4.5	19	4.5	0.79	0.41	-	1.54
Above	71	17.7	143	33.9	0.41	0.30	-	0.58

Table 2 (continued)

G G T (I U / L)							
Normal	339	84.5	340	80.6	1.00		
Below	6	1.5	1	0.2	6.02	0.72 -	50.25
Above	56	14.0	81	19.2	0.69	0.48 -	1.01
Glob (g/dL)							
Normal	175	43.6	230	54.5	1.00		
Below	193	48.1	157	37.2	1.62	1.21 -	2.16
Above	33	8.2	35	8.3	1.24	0.74 -	2.07
Gluc (mg/dL)							
Normal	139	34.7	177	42.0	1.00		
Below	4	1.0	11	2.6	0.46	0.14 -	1.49
Above	258	64.3	234	55.6	1.40	1.06 -	1.89
Osm (mOsm/L)							
Normal	281	70.1	292	69.2	1.00		
Below	104	25.9	82	19.4	1.32	0.95 -	1.84
Above	16	4.0	48	11.4	0.37	0.19 -	0.62
Phos (mg/dL)							
Normal	260	64.8	242	57.3	1.00		
Below	80	20.0	32	7.6	2.33	1.49 -	3.63
Above	61	15.2	148	35.1	0.39	0.27 -	0.54
K (mmol/L)							
Normal	292	72.8	348	82.5	1.00		
Below	108	26.9	61	14.5	2.11	1.40 -	3.00
Above	1	0.2	13	3.1	0.09	0.01 -	0.71
Na (mmol/L)							
Normal	264	65.8	294	69.7	1.00		
Below	105	26.2	85	20.1	1.38	0.99 -	1.92
Above	32	8.0	43	10.2	0.82	0.51 -	1.35
TP g/dL)							
Normal	245	61.1	266	63.0	1.00		
Below	130	32.4	119	28.2	1.19	0.88 -	1.61
Above	26	6.5	37	8.8	0.76	0.45 -	1.30
BUN (mg/dL)							
Normal	158	39.4	207	49.1	1.00		
Below	48	12.0	37	8.8	1.70	1.06 -	2.77
Above	195	48.6	178	42.2	1.44	1.07 -	1.92

Abbreviations: CI, confidence interval; AG, albumin/globulin ratio; ALB, albumin; ALP, alkaline phosphatase; AnGap, anion gap, AST, aspartate aminotransferase; BILI, bilirubin; Ca, calcium; Cl, chloride; CK, creatine kinase; crea, creatinine; GGT, gamma glutamyltransferase; glob, globulins; gluc, glucose; osm, osmolality; phos, phosphorus; Na, sodium; K, potassium; TP, total protein; BUN, blood urea nitrogen.

Table 3. Initial independent assessment of the potential association of diagnose with the presence of hypomagnesemia.

	Hypomagne-semia		Normal or high Mg		Odds ratio	95 % CI		
Diagnosis	No.	%	No.	%		Lower	-	Upper
Medically healthy horses	15	3.7	27	6.4	1.00			
Cardiovascular disease	1	0.2	3	0.7	0.60	0.06	-	6.29
Dermatologic disease	1	0.2	4	0.9	0.45	0.05	-	4.40
Endocrinologic disease	2	0.5	5	1.2	0.72	0.12	-	4.17
Hemolymphatic disease	6	1.5	4	0.9	2.70	0.66	-	11.10
Anterior enteritis	8	2.0	8	1.9	1.80	0.56	-	5.77
Acute diarrhea	20	5.0	6	1.4	6.00	1.98	-	18.20
Colic	165	41.1	99	23.5	3.00	1.52	-	5.91
Gastroduodenal ulcers	9	2.2	8	1.9	2.03	0.65	-	6.35
Other gastrointestinal diseases	28	7.0	24	5.7	2.10	0.91	-	4.84
Muscle disease	5	1.2	8	1.9	1.12	0.31	-	4.06
Neurologic disease	11	2.7	18	4.3	1.10	0.41	-	2.93
Neonatal septicemia, peripartum asphyxia syndrome, premature	8	2.0	19	4.5	0.76	0.27	-	2.14
Foal diarrhea	1	0.2	16	3.8	0.11	0.01	-	0.93
Rhodococcus pneumonia	4	1.0	6	1.4	1.20	0.29	-	4.93
Foal musculoskeletal disease	4	1.0	13	3.1	0.55	0.15	-	2.00
Other foal diseases	13	3.2	37	8.8	0.63	0.26	-	1.54
Reproduction	8	2.0	15	3.6	0.96	0.33	-	2.79
Infectious respiratory disease	20	5.0	7	1.7	5.14	1.77	-	14.95
Recurrent airway obstruction	4	1.0	7	1.7	1.03	0.26	-	4.09
Other respiratory diseases	5	1.2	14	3.3	0.64	0.19	-	2.14
Septic artritis/ foot abscess	9	2.2	13	3.1	1.25	0.43	-	3.59
Laminitis	8	2.0	10	2.4	1.44	0.47	-	4.43
Other skeletal diseases	1	0.2	3	0.7	0.60	0.06	-	6.29
Urinary tract disease	1	0.2	7	1.7	0.26	0.03	-	2.29
Opthalmologic disease	18	4.5	21	5.0	1.54	0.63	-	3.76
Multi-organ system disease	26	6.5	20	4.7	2.34	0.99	-	5.53

Abbreviations: CI; confidence interval.

Table 4. Initial independent assessment of the potential association of clinical response with the presence of hypomagnesemia.

Clinical response	Hypomagne-semia		Normal or high Mg		Odds ratio	95 % CI		
	No.	%	No.	%		Lower	-	Upper
Hospitalization days								
0-3days	165	41	216	51	1.00			
4-7days	118	29	108	26	1.43	1.03	-	1.99
>7days	118	29	98	23	1.58	1.13	-	2.21
Survivial								
Survived	319	80	336	80	1.00			
Died	82	20	86	20	1.00	0.72	-	1.41

Abbreviations: CI, confidence interval.

Table 5. Final logistic regression model for assessment of the potential association of blood chemistry variables with the presence of hypomagnesemia.

	Odds ratio	**95**	**%**	**CI**
Parameter		Lower	-	Upper
ALP (IU/L)				
Normal	1.00			
Below	1.04	0.47	-	2.27
Above	0.68	0.43	-	1.06
BILI total (mg/dL)				
Normal	1.00			
Below	1.17	0.05	-	26.4
Above	1.95	1.37	-	2.76
Ca (mg/dL)				
Normal	1.00			
Below	4.03	2.87	-	5.66
Above	0.27	0.11	-	0.66
Crea (mg/dL)				
Normal	1.00			
Below	1.23	0.57	-	2.65
Above	0.36	0.24	-	0.53
Globulin (g/dL)				
Normal	1.00			
Below	1.61	1.14	-	2.28
Above	1.34	0.75	-	2.39
Phos (mg/dL)				
Normal	1.00			
Below	2.23	1.34	-	3.72
Above	0.37	0.23	-	0.59

Abbreviations: see table 2.

Table 6. Final logistic regression model for assessment of the potential association of diagnosis with the presence of hypomagnesemia.

	Odds ratio	95 % CI		
Diagnosis		Lower	-	Upper
Medically healthy horses	1.00			
Acute diarrhea	5.91	2.32	-	15.06
Colic	2.96	2.14	-	4.08
Other gastrointestinal diseases	2.07	1.15		3.71
Foal, diarrhea	0.11	0.01	-	0.84
Infectious respiratory disease	5.07	2.09	-	12.28
Multi-organ system disease	2.31	1.24	-	4.28

Abbreviations: CI, confidence interval.

Table 7. Stratified analysis for assessment of the potential association of mortality with the presence of hypomagnesemia within specific diagnoses.

	Odds ratio	95 % CI		
Diagnosis		Lower	-	Upper
All horses				
Alive	1.00			
Dead	1.00	0.72	-	1.41
Acute diarrhea				
Alive	1.00			
Dead	0.25	0.04	-	1.74
Colic				
Alive	1.00			
Dead	0.53	0.30	-	0.95
Other gastrointestinal disease				
Alive	1.00			
Dead	0.66	0.19		2.34
Foal, diarrhea	N/A			
Infectious respiratory disease	N/A			
Multi-organ system disease				
Alive	1.00			
Dead	0.86	0.24	-	3.12

Abbreviations: N/A, insufficient number of observations to assess relationship.

Table 8. Stratified analysis for assessment of the potential association of hospitalization days with the presence of hypomagnesemia within specific horses. Only horses that survived until discharge are included in this analysis.

	Odds ratio		**95%**	**CI**
Diagnosis		Lower	-	Upper
All horses that survived				
0-3days	1.00			
4-7days	1.33	0.92	-	1.92
>7days	1.45	1.00	-	2.11
Acute diarrhea	N/A			
Colic				
0-3days	1.00			
4-7days	1.19	0.59	-	2.42
>7days	1.42	0.67	-	3.03
Other gastrointestinal disease				
0-3days	1.00			
4-7days	0.80	0.18	-	3.46
>7days	1.60	0.30	-	8.49
Foal, diarrhea	N/A			
Infectious respiratory disease				
0-3days	1.00			
4-7days	0.40	0.05	-	3.42
>7days	1.60	0.10	-	24.70
Multi-organ system disease				
0-3days	1.00			
4-7days	7.00	1.04	-	46.95
>7days	7.00	1.04	-	46.95

Abbreviations: N/A, insufficient number of observations to assess relationship.

CONCLUSIONS

Summary of results

We found that hypomagnesemia is common in horses treated by our clinic, especially if the gastrointestinal system is involved. Concurrent electrolyte disturbances occur frequently, and young horses appear to be able to regulate serum total Mg concentrations more efficiently. Horses with hypomagnesemia were more likely to be hospitalized longer, but they did not have higher mortality.

Recommendations for further research

The knowledge that hypomagnesemia is common among critically ill horses is important to clinical management of these cases. Considering the multiple processes and regulatory mechanisms in the body where Mg is required it appears that more attention should be paid to its importance. The presented study neither claims to investigate a cause-effect relationship, nor can conclusions be drawn about the importance of hypomagnesemia in sick horses. However, it does point out the importance of further research in the area. Many of the disease categories that demonstrated significant relationships with hypomagnesemia are also associated with endotoxemia in horses. Does this mean that hyopmagnesemic horses are more prone to endotoxemia, or does endotoxemia contribute to the development of hypomagnesemia? Would Mg supplementation to theses horses decrease morbidity and mortality? Magnesium is known to increase gastrointestinal motility. The presented study demonstrates that hypomagnesemia is common in horses with colic. Hence, further investigation of this relationship to determine if there is a difference in serum Mg concentrations in horses with different types of colic would be indicated.

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APPENDIX

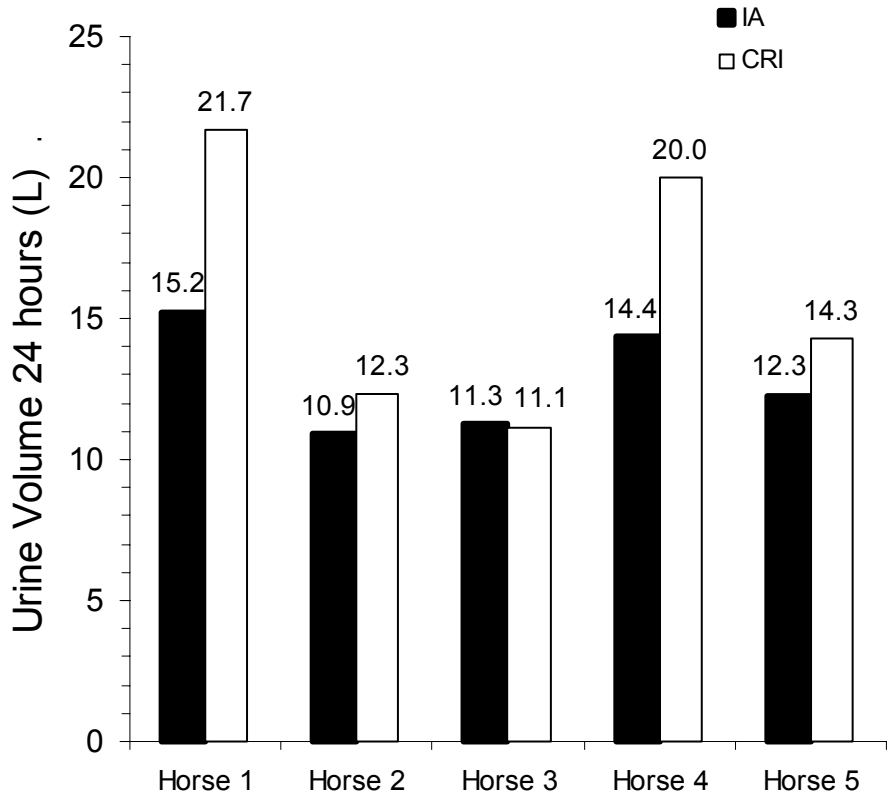

Figure 1. Urine volume produced during 24 hours following administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses.

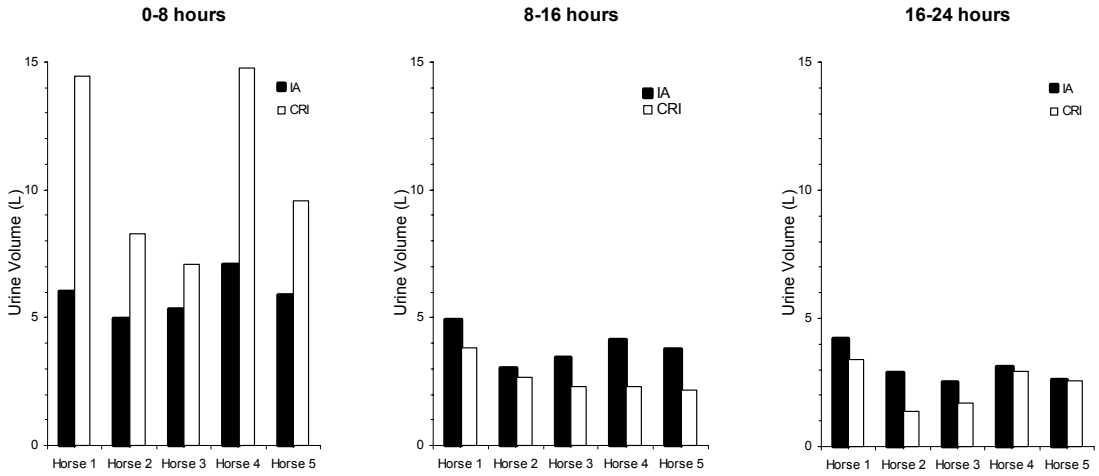

Figure 2. Urine volume produced in each of three 8-hour periods following administration of furosemide to five horses. IA= intermittent administration (1mg/kg q 8h IV), CRI= continuous rate infusion (0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV). Results differ significantly between methods during each period (Wilcoxon signed-rank test).

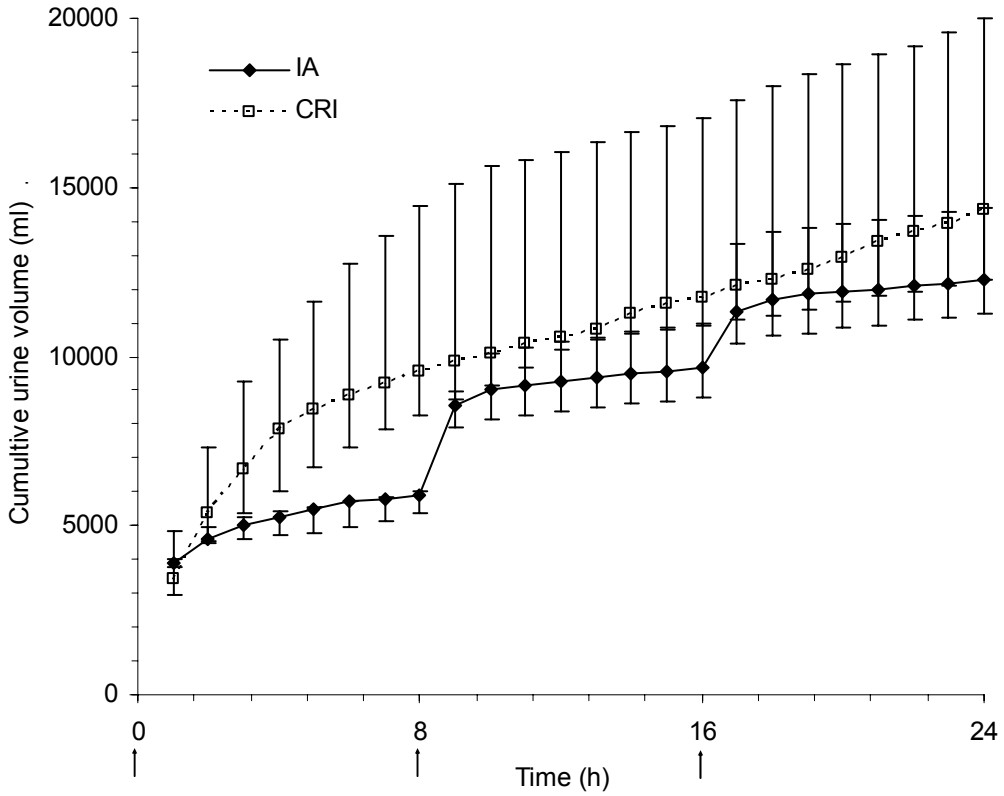

Figure 3. Cumulative urine volume after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Arrows indicate time points of furosemide administration for horses receiving IA. Median (25^{th}, 75^{th} percentile).

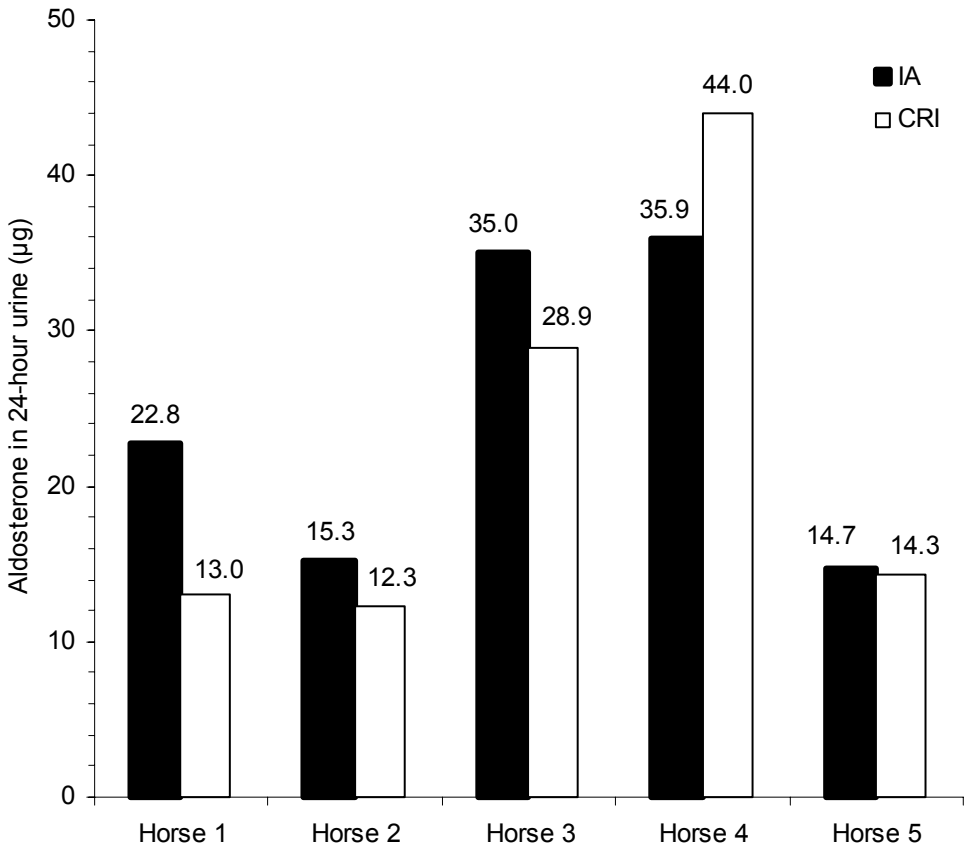

Figure 4. Urine aldosterone secretion during 24 hours following administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses.

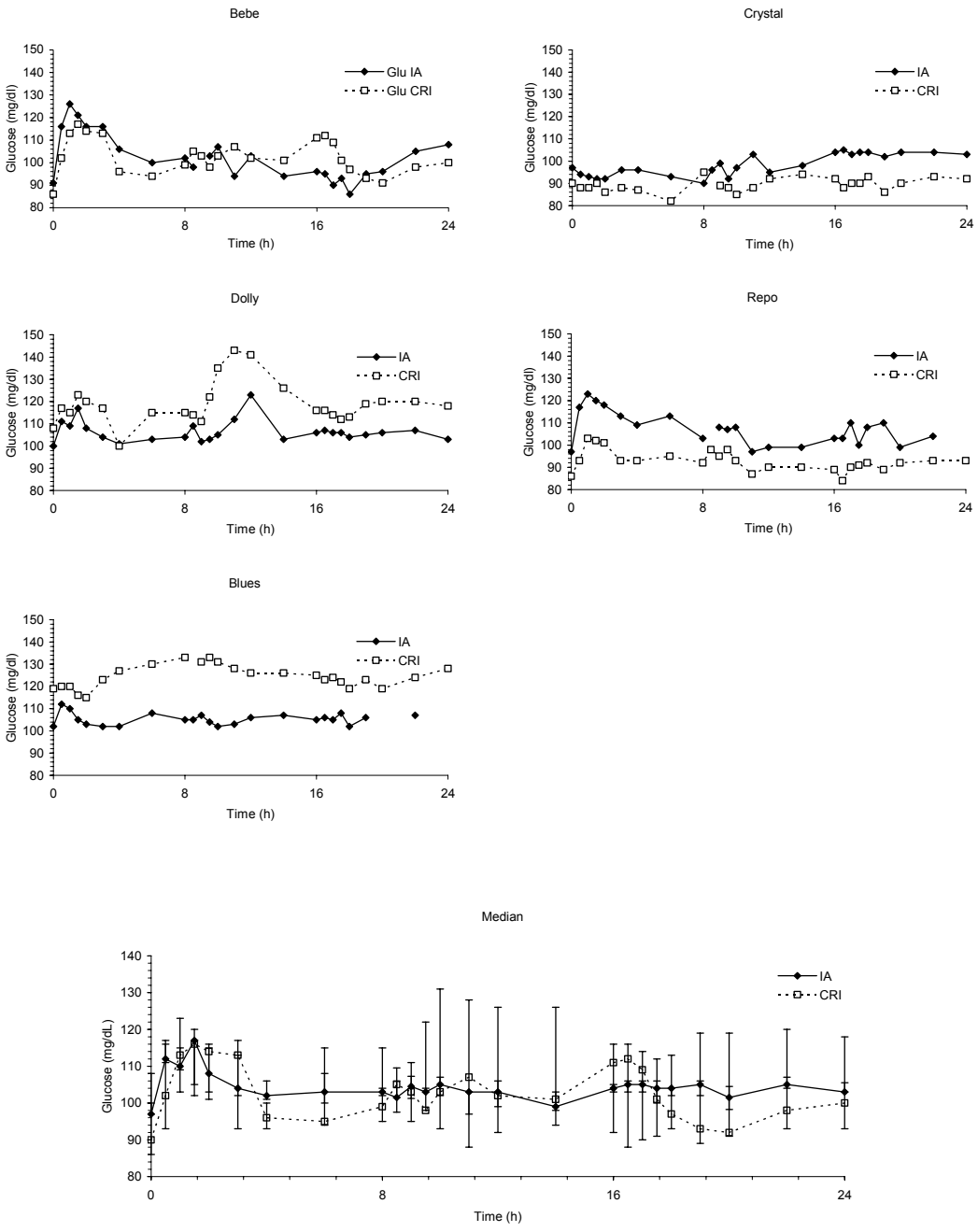

Figure 5. Serum glucose concentrations after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

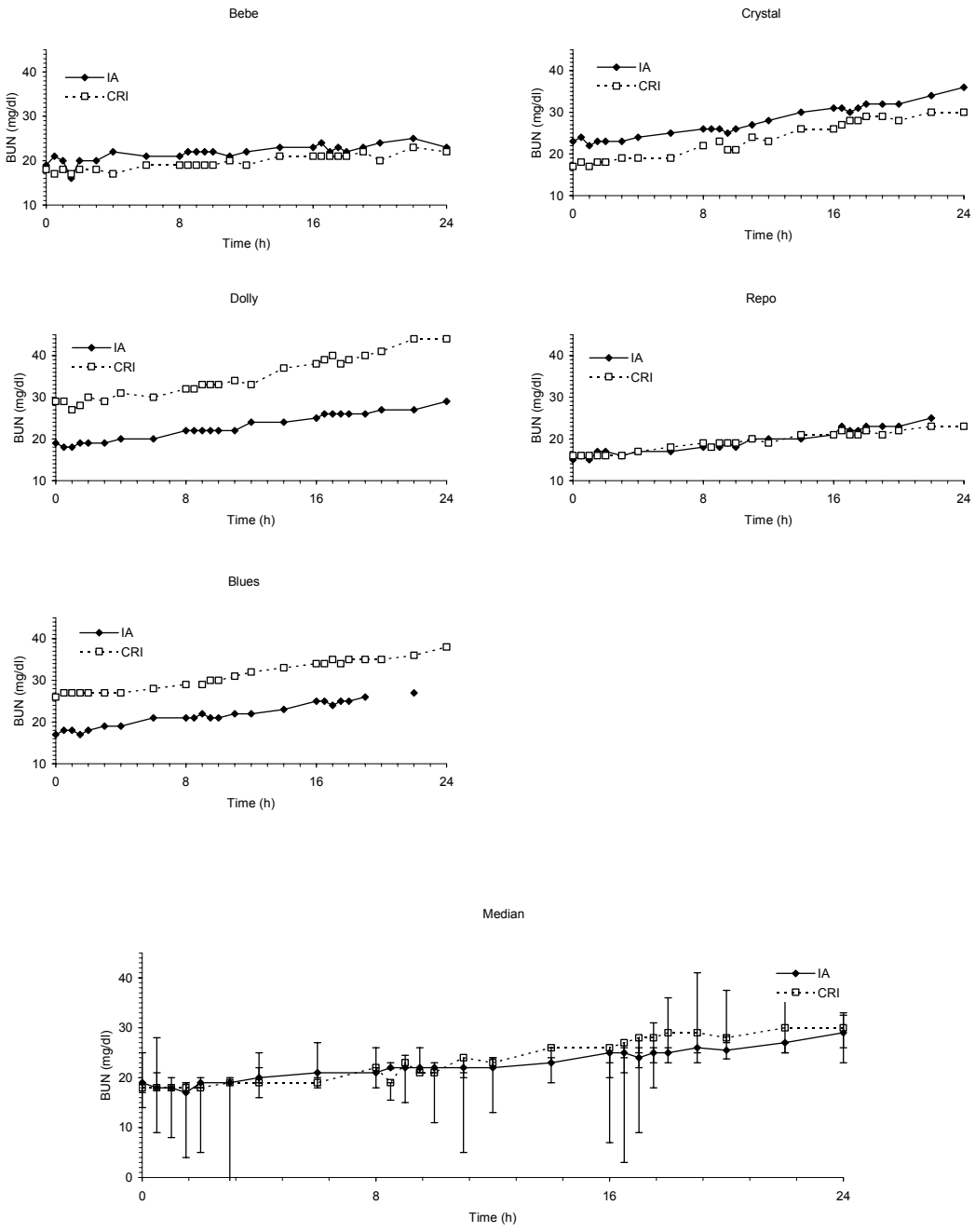

Figure 6. Serum blood urea nitrogen concentrations after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

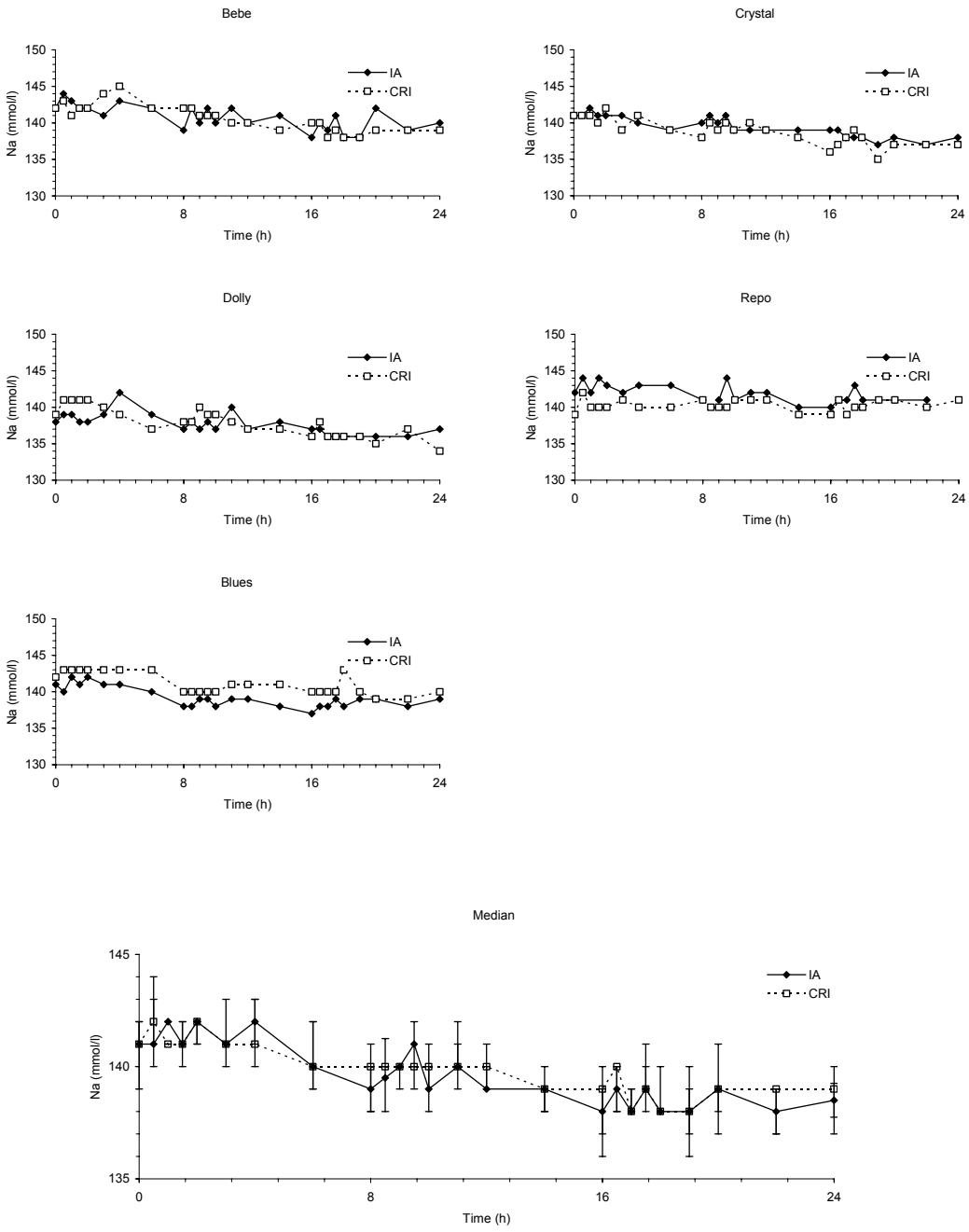

Figure 7. Serum sodium concentrations after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

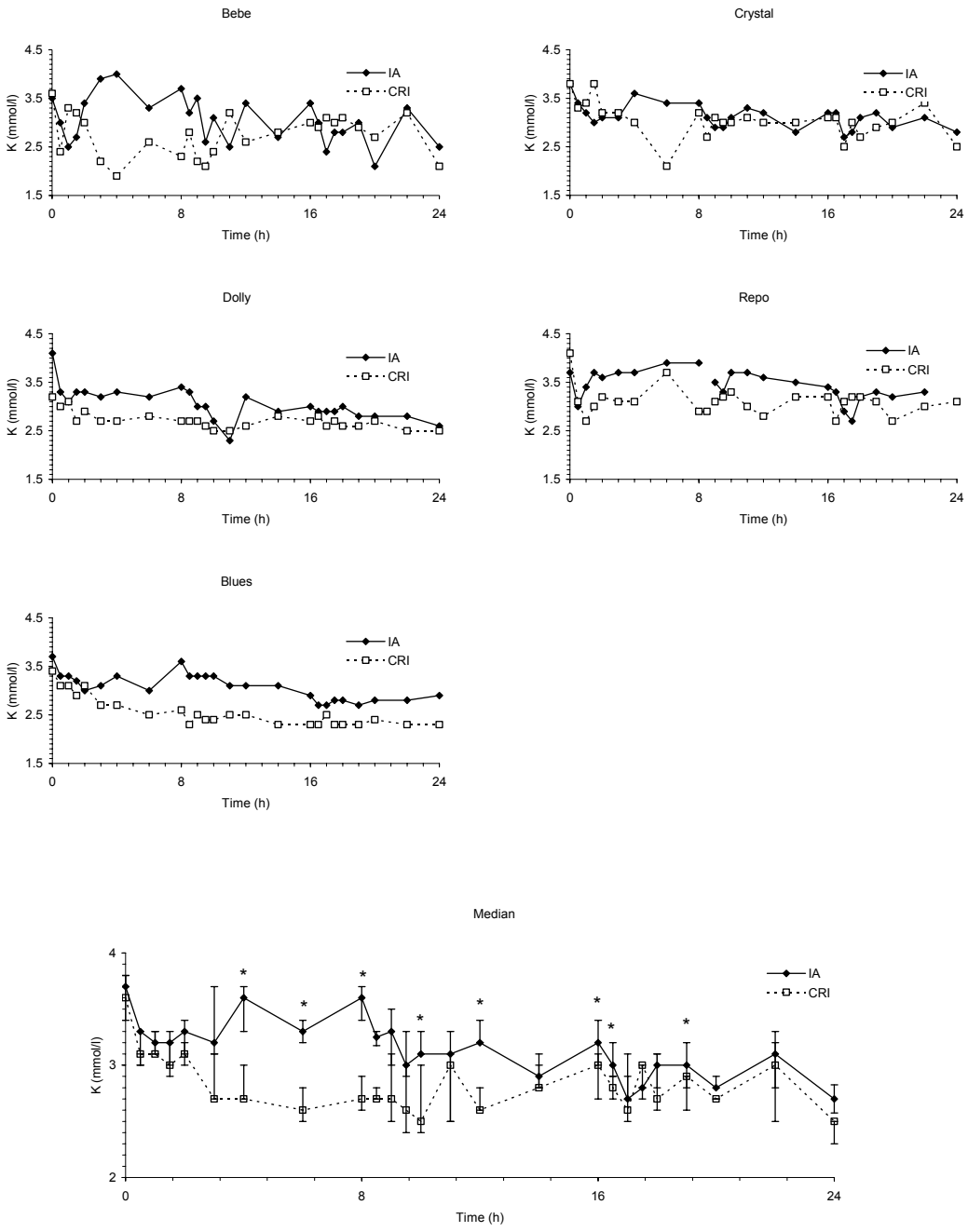

Figure 8. Serum potassium concentrations after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

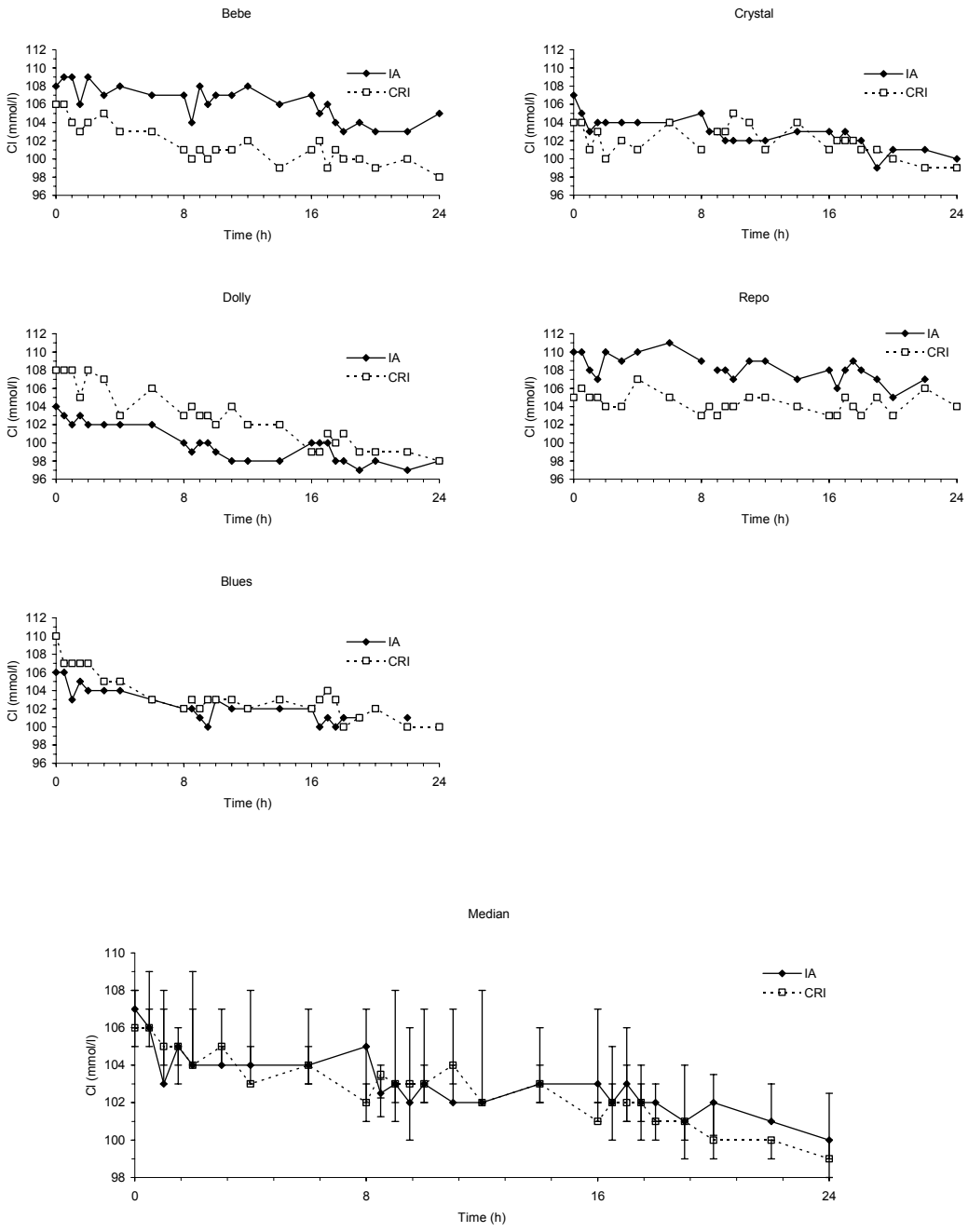

Figure 9. Serum chloride concentrations after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

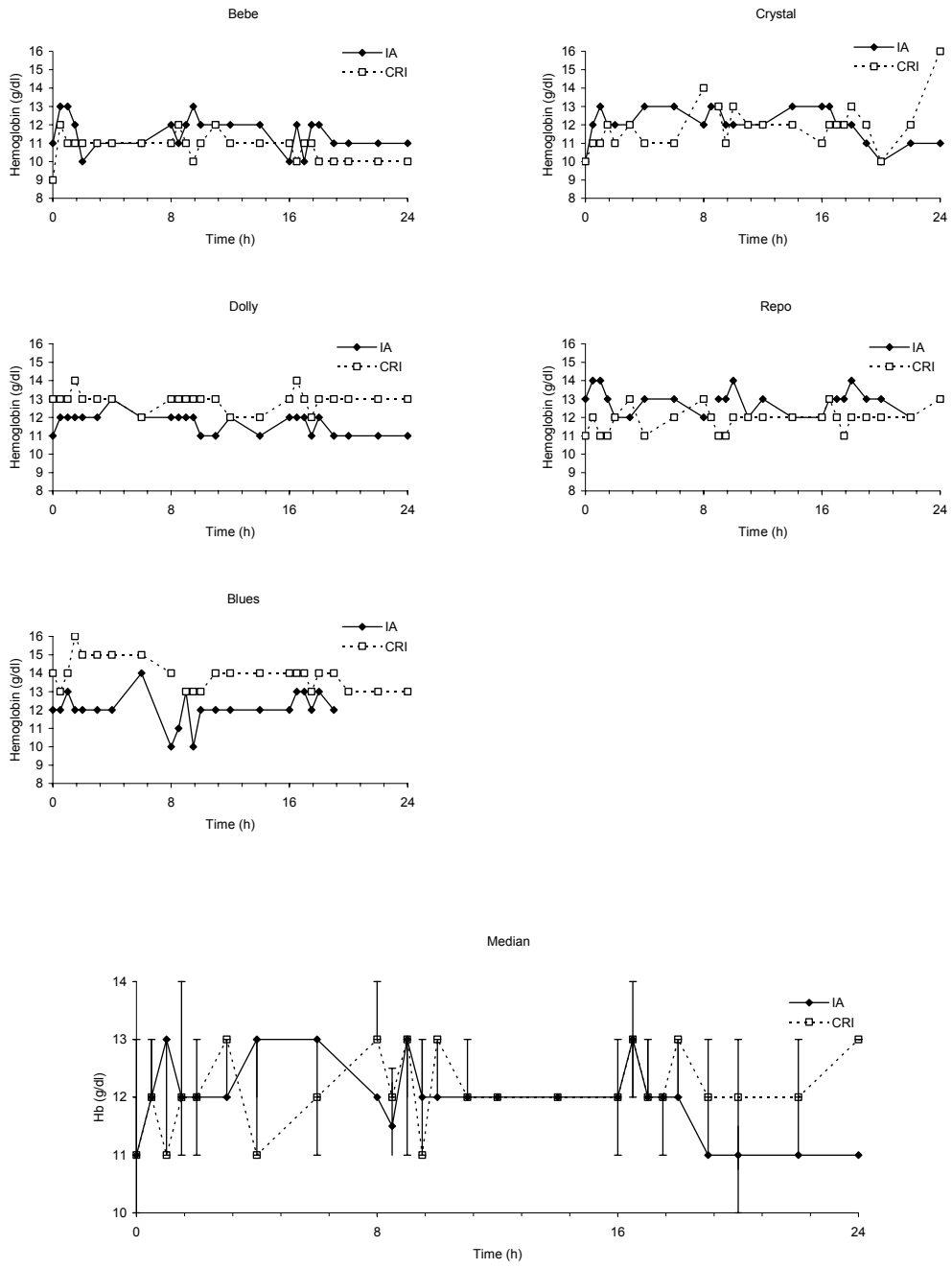

Figure 10. Serum hemoglobin concentrations after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

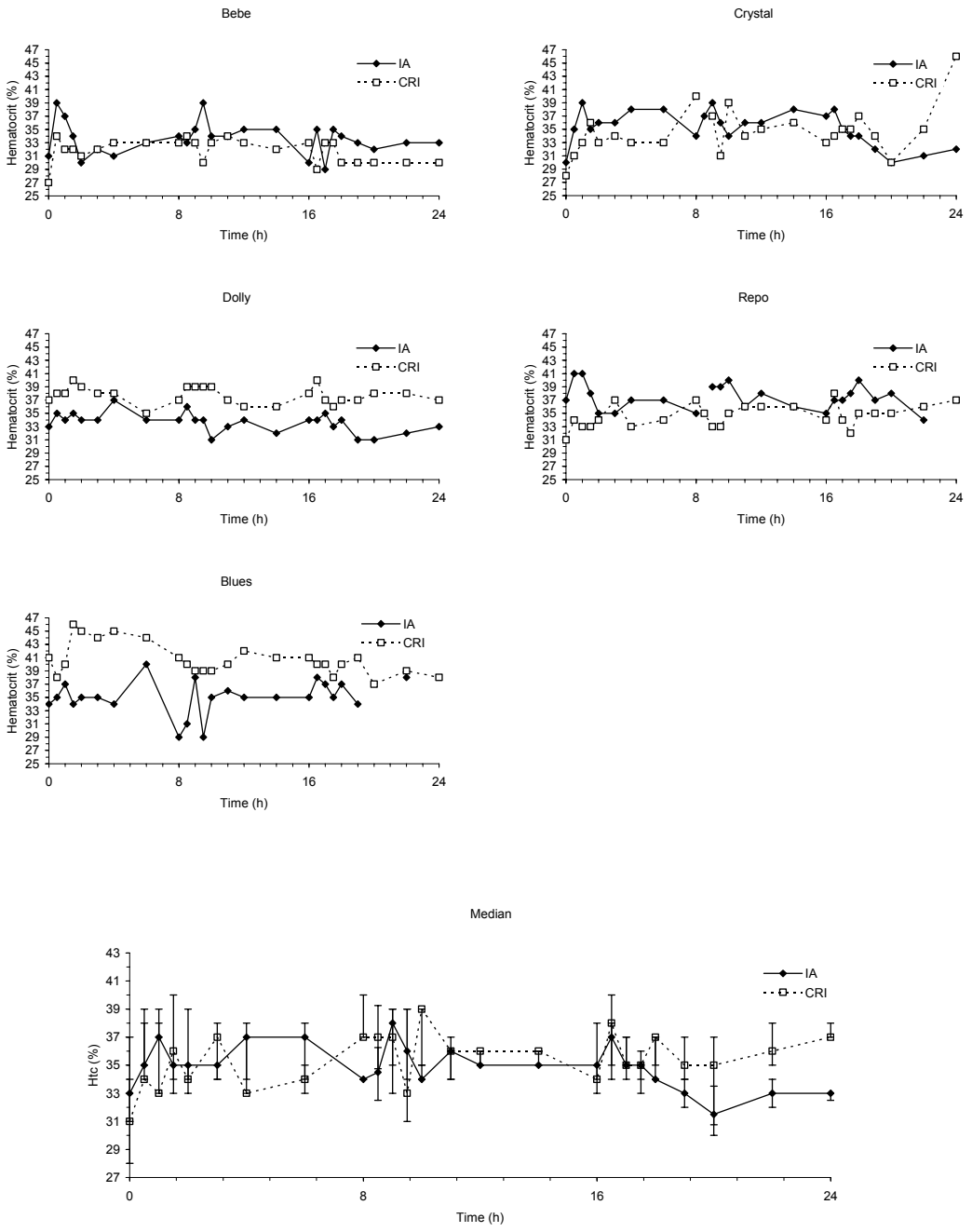

Figure 11. Serum hematocrit after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

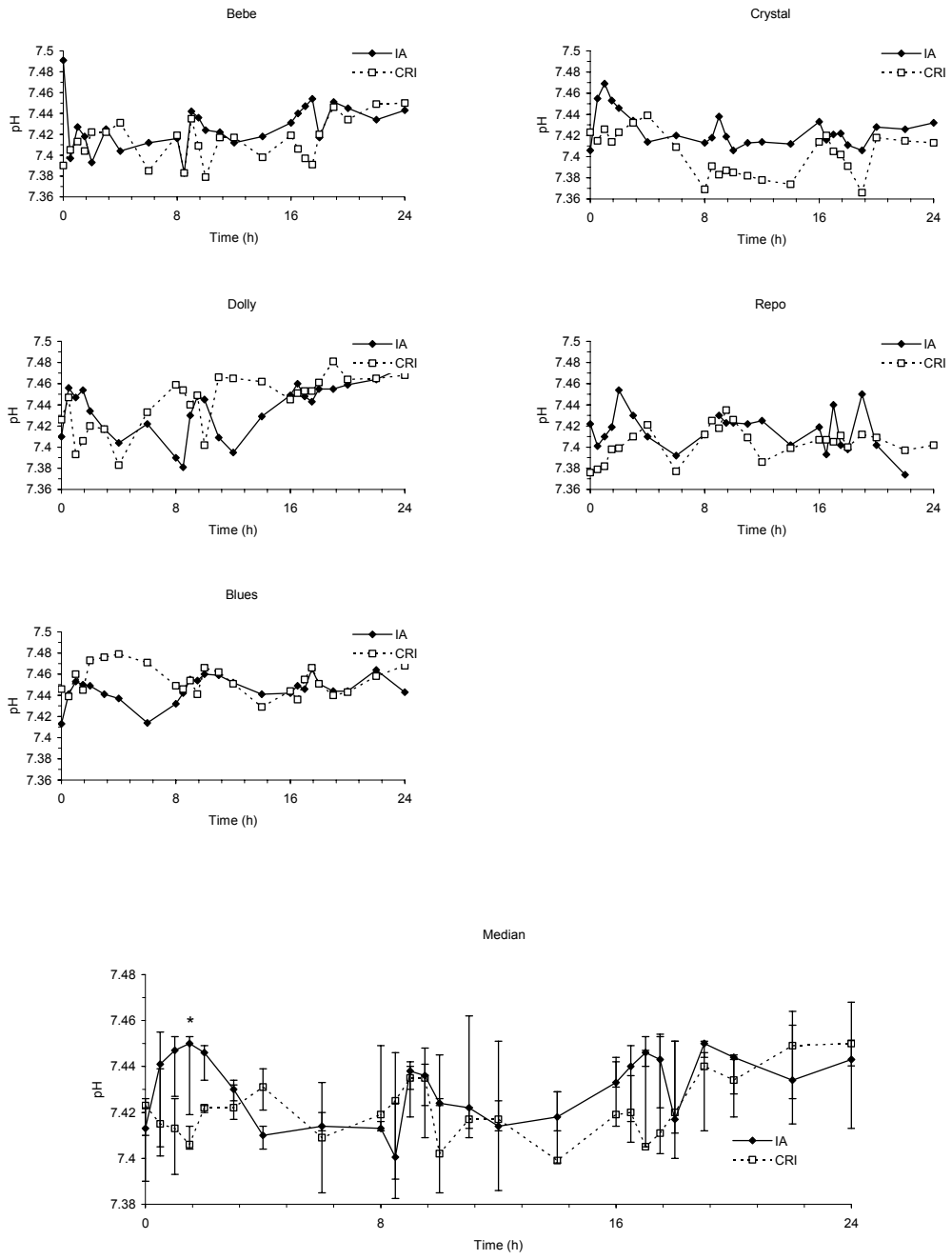

Figure 12. Serum pH after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

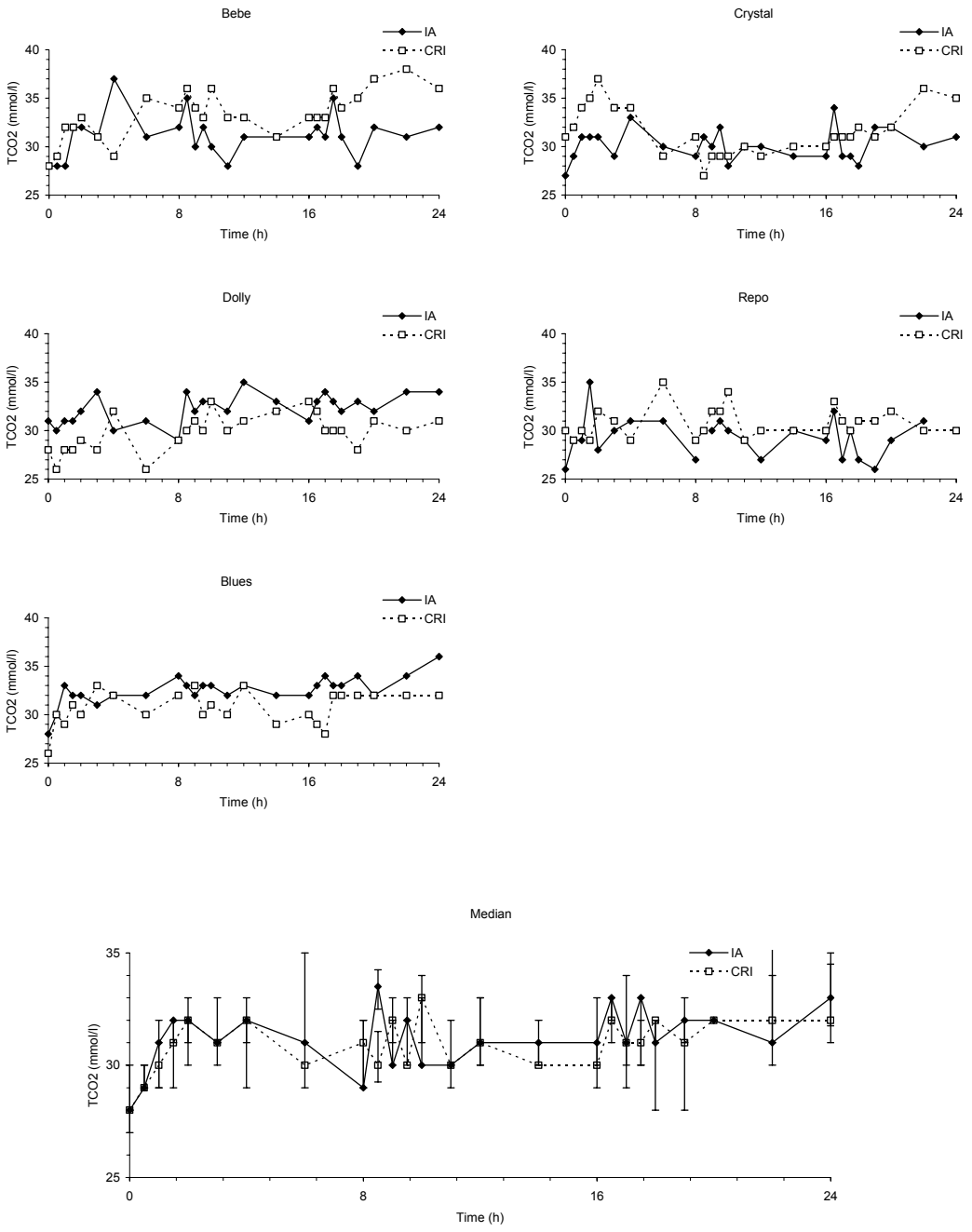

Figure 13. Serum TCO_2 concentrations after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

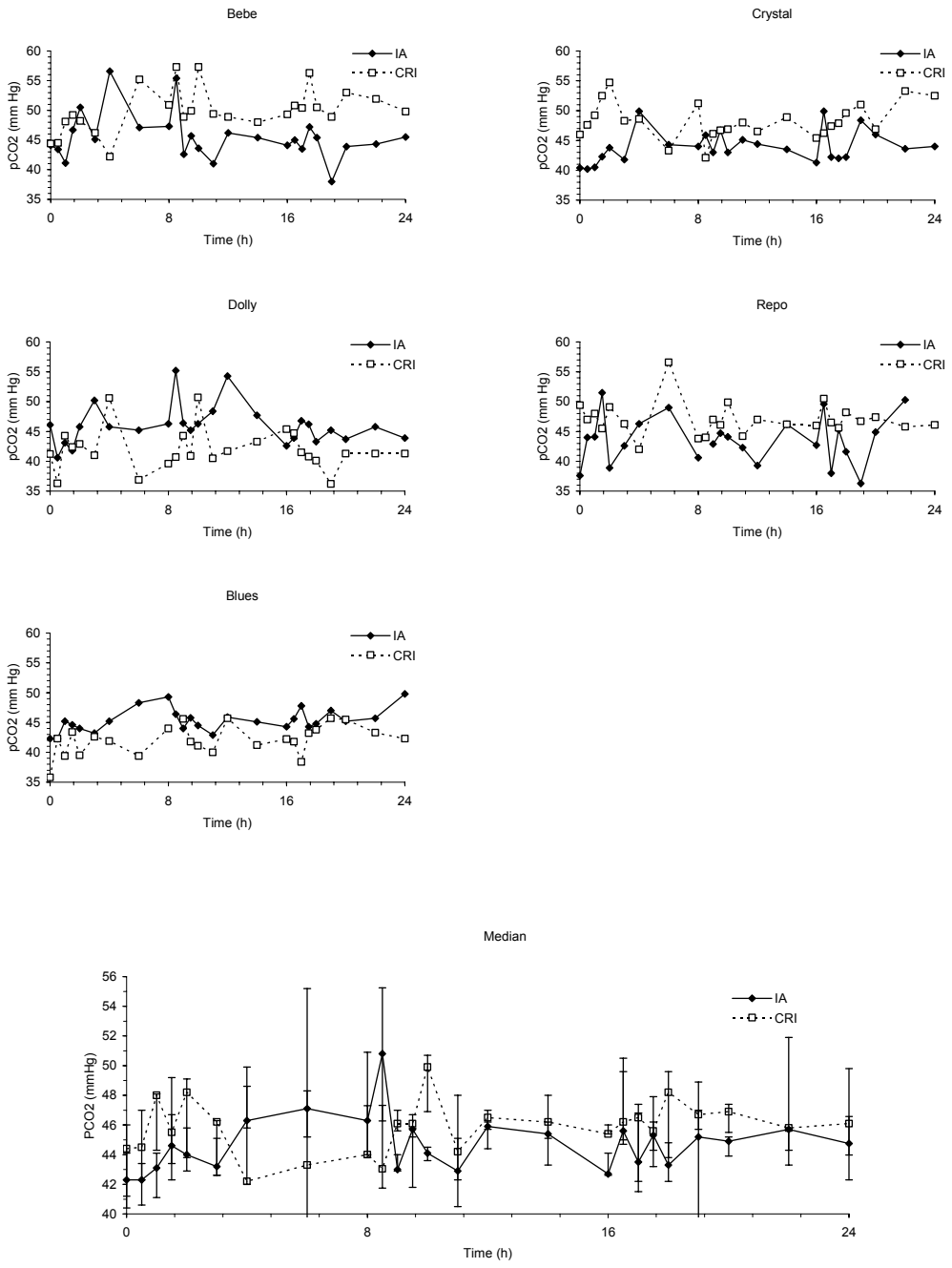

Figure 14. Serum PCO_2 concentrations after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

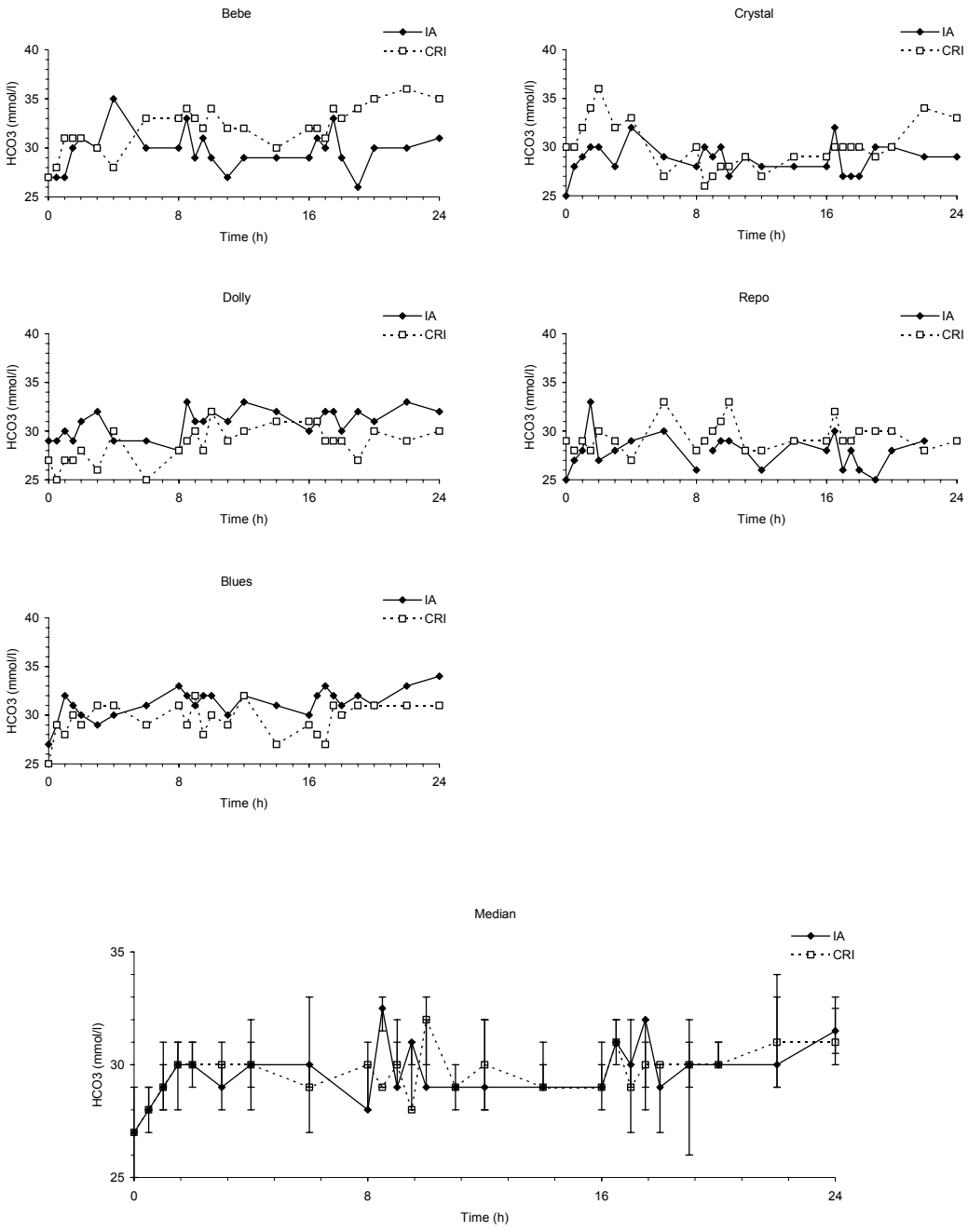

Figure 15. Serum HCO_3 concentrations after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

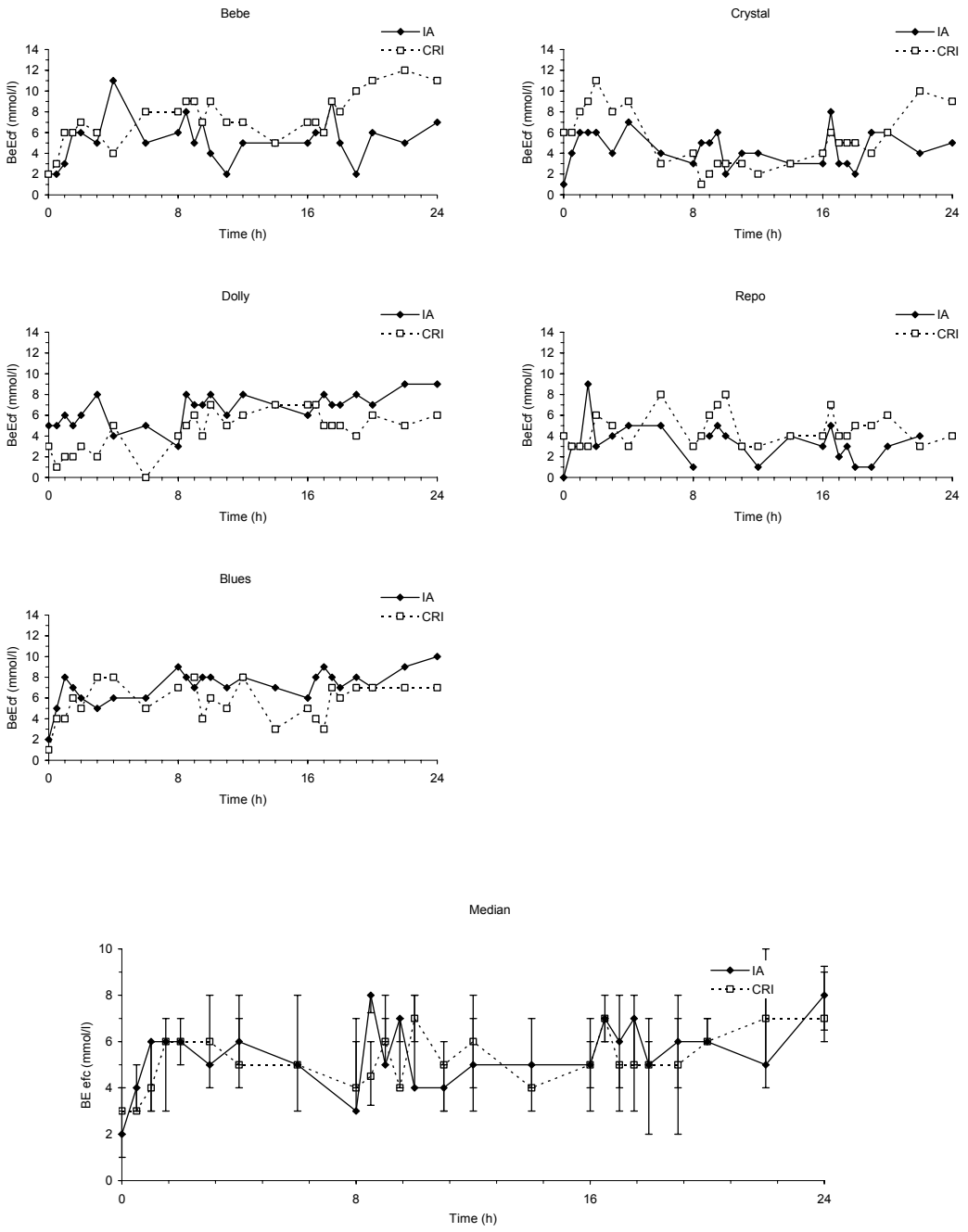

Figure 16. Serum base excess administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

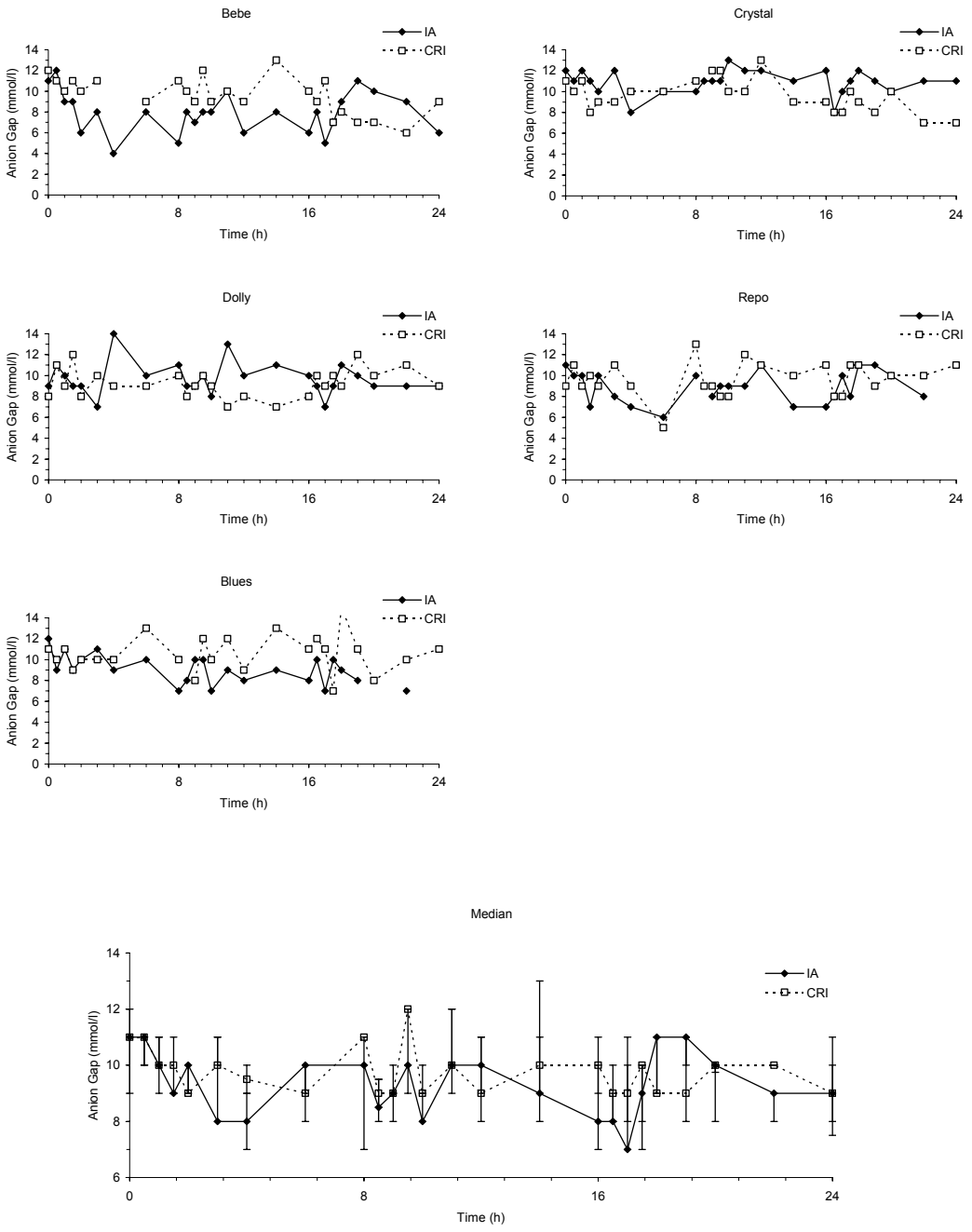

Figure 17. Serum anion gap after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

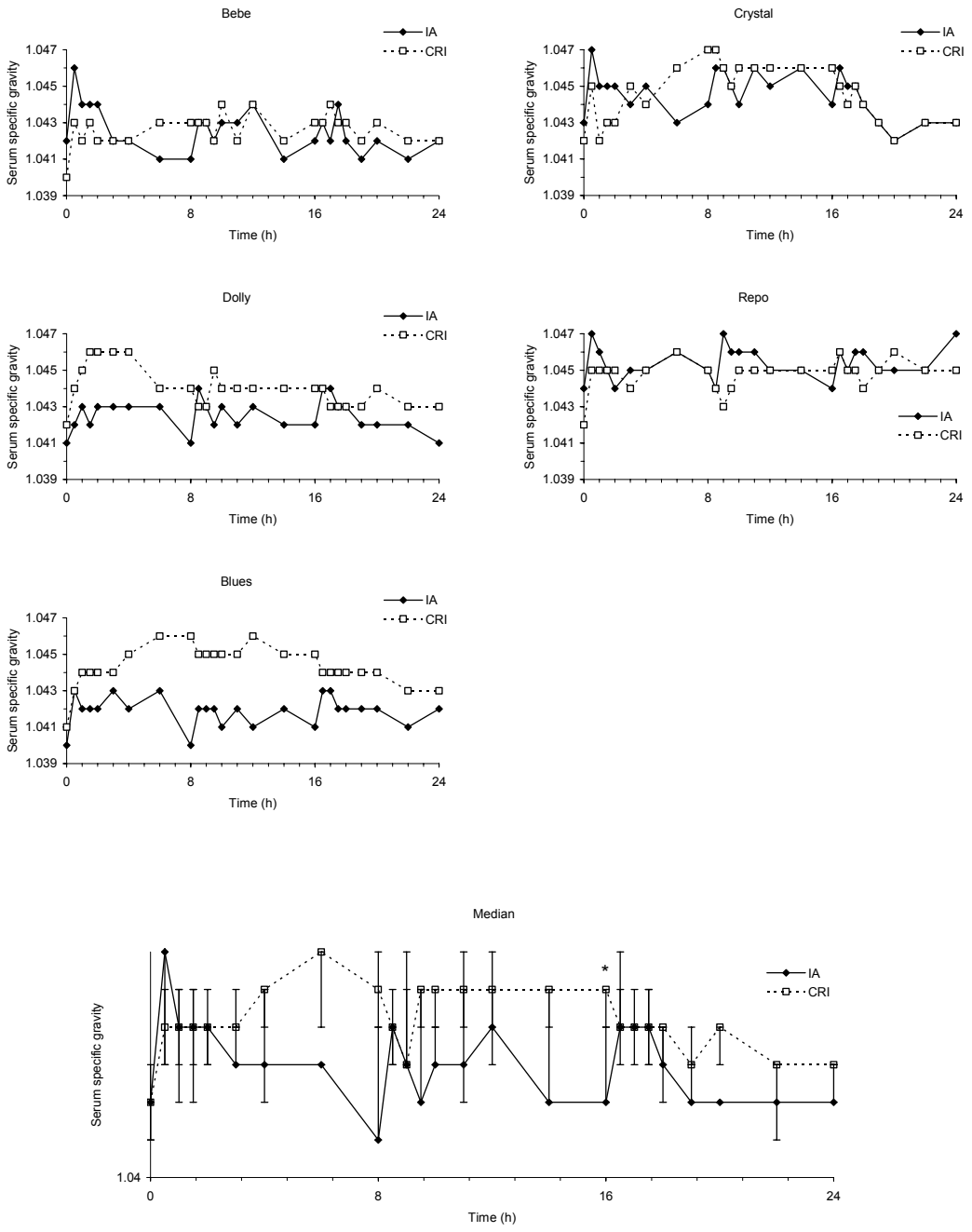

Figure 18. Serum specific gravity after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

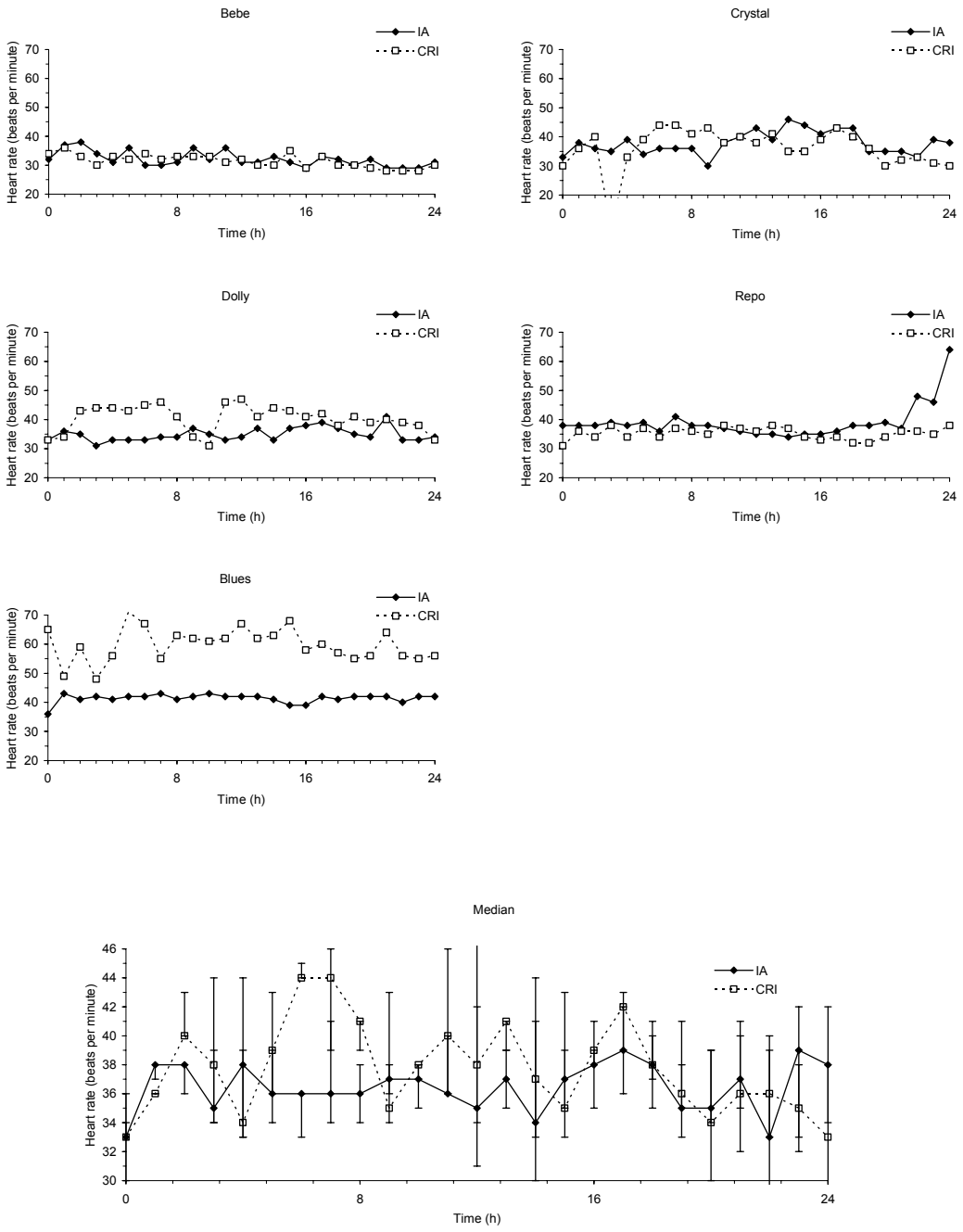

Figure 19. Heart rate after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

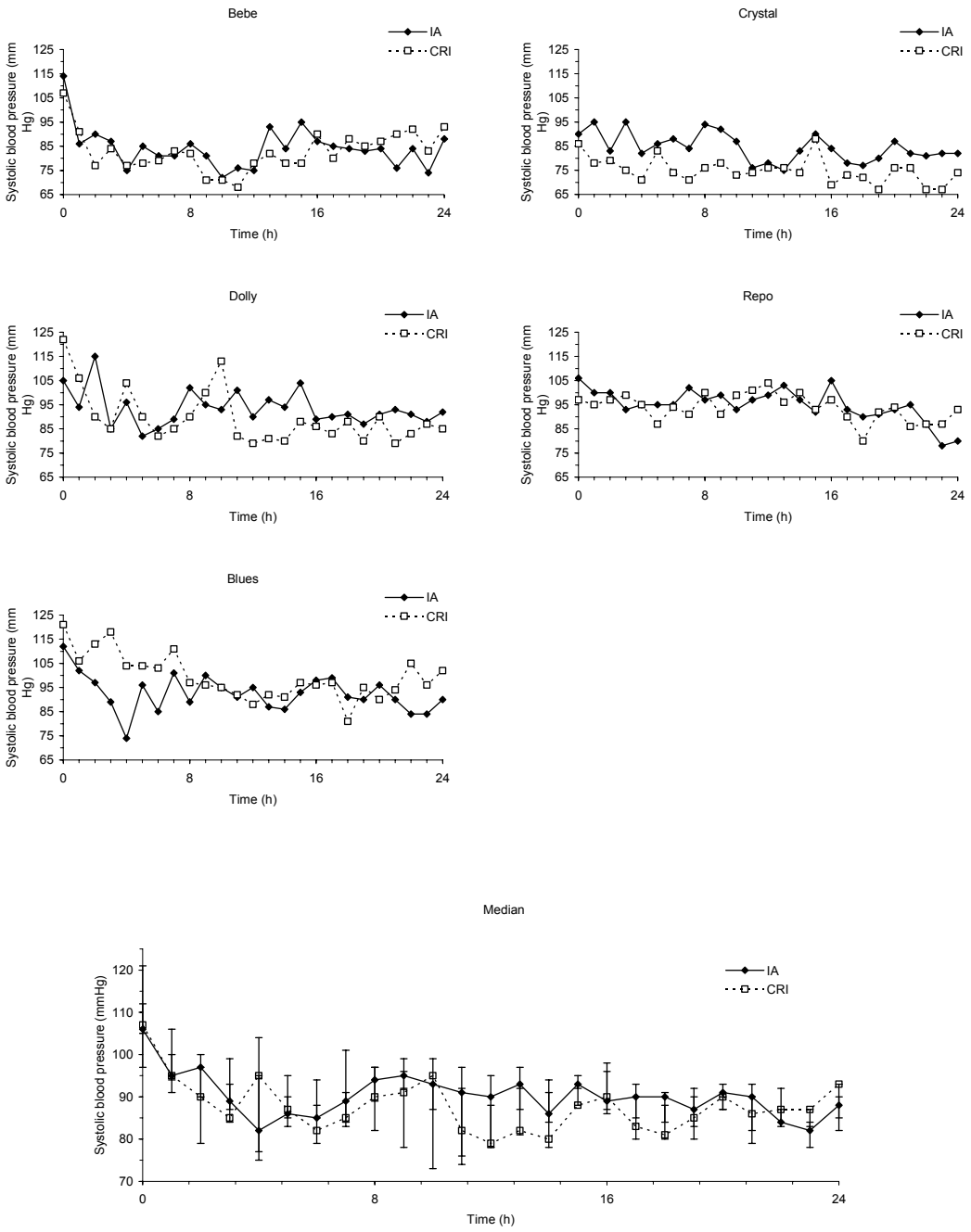

Figure 20. Systolic blood pressure after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

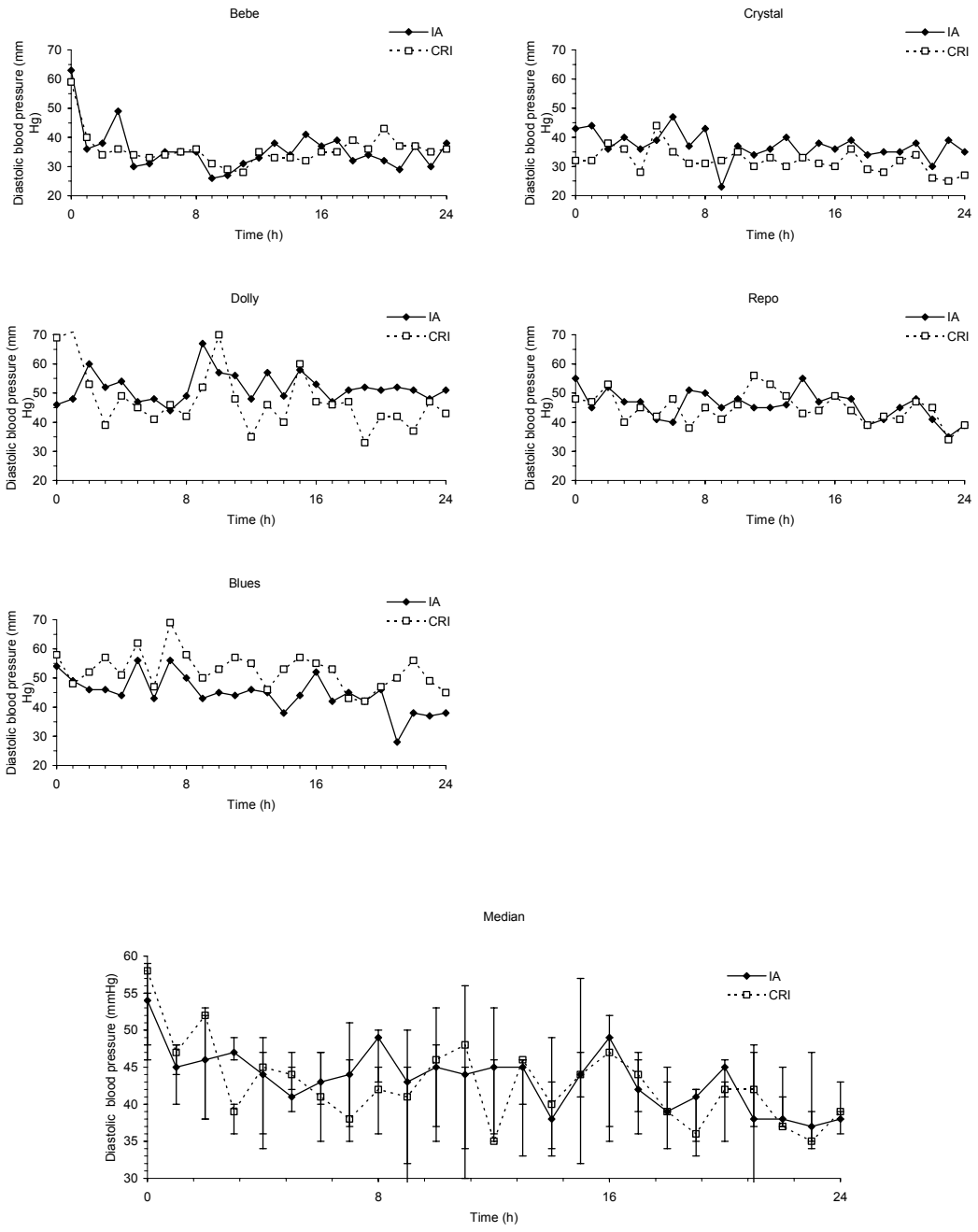

Figure 21. Diastolic blood pressure after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).

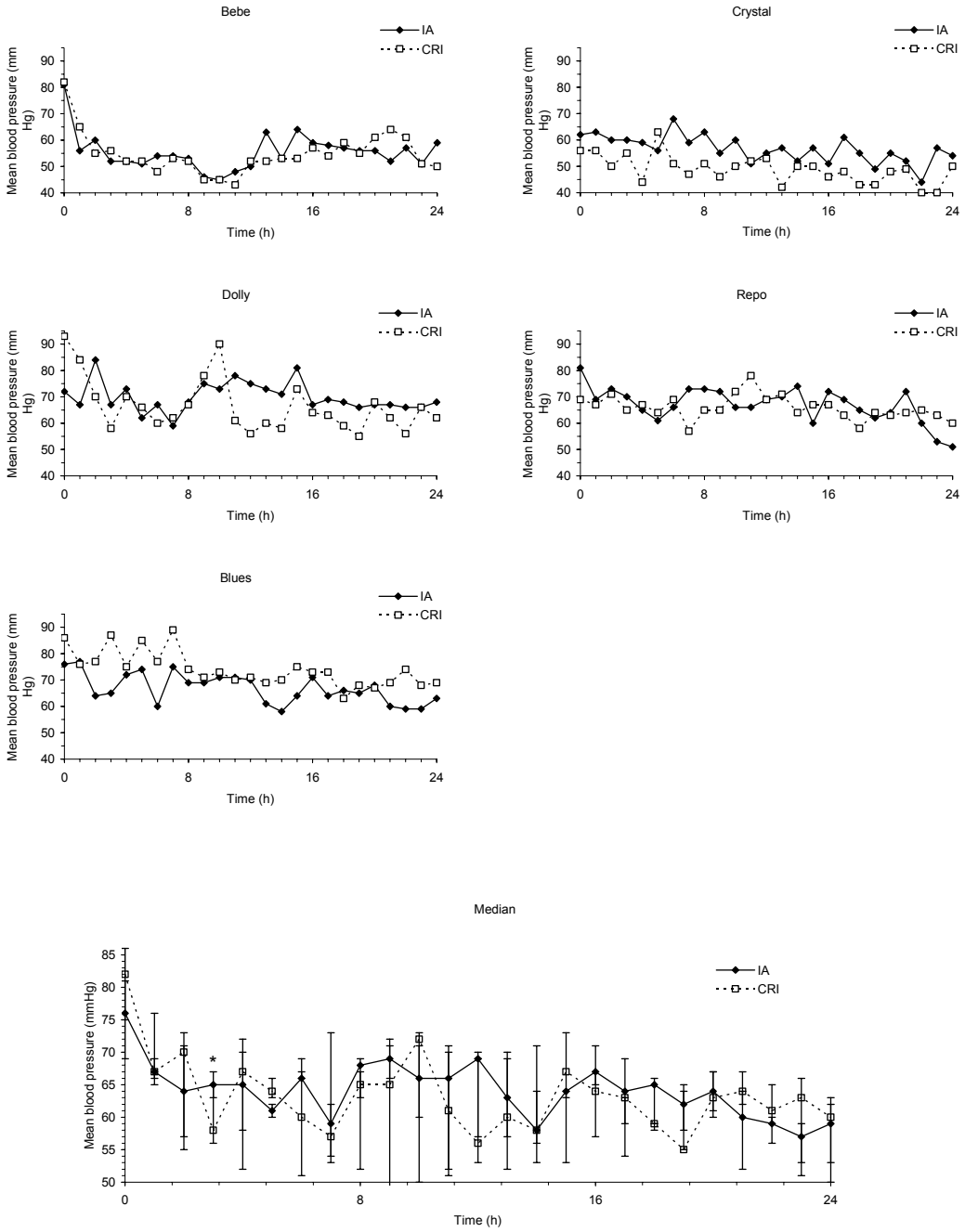

Figure 22. Mean blood pressure after administration of furosemide by intermittent administration (IA; 1mg/kg q 8h IV) or continuous rate infusion (CRI; 0.12mg/kg/h, preceded by a loading dose of 0.12 mg/kg IV) to five horses. Median (25^{th}, 75^{th} percentile). ∗ Significant difference between methods, $p<0.05$ (Wilcoxon signed-rank test).