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ORIGINAL ARTICLE

Pharmacokinetics of thiamine (vitamin B1) in adult horses after administration of three single intravenous doses

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Abstract

Thiamine is a vital co-factor for several anti-inflammatory and antioxidant processes that are critical for mitigation of sepsis-associated inflammation, but pharmacokinetic (PK) analysis has not been reported in horses. We hypothesized that IV thiamine hydrochloride (TH) at increasing dosages would result in corresponding increases in plasma thiamine concentrations without causing adverse effects. A randomized cross-over study was performed in 9 healthy horses that each received TH at 5, 10, and 20 mg/kg IV. Blood was collected immediately prior to drug administration and at several time points thereafter. High-performance liquid chromatography with mass spectrometry was used to quantify thiamine concentrations at each time point. Non-compartmental PK methods showed that IV TH resulted in supraphysiologic plasma concentrations with a short half-life (0.77–1.12 h) and no adverse clinical signs were observed. The terminal rate constant decreased as the dosage increased ($p < .0001$) and clearance significantly decreased at the 20 mg/kg dosage ($p = .0011$). The area under the curve (AUC) increased in a non-linear fashion. These findings suggest that thiamine follows non-linear elimination kinetics in horses, which is likely due to saturation of renal elimination. Future studies are needed to identify therapeutic plasma concentrations and develop thiamine dosing recommendations for horses.

KEYWORDS
horse, metabolic, pharmacokinetics, thiamine, vitamin B1

1 | INTRODUCTION

Sepsis continues to represent a common cause of morbidity and mortality in neonatal foals and adult horses (Arroyo et al., 2017; Macias-Rioseco et al., 2020; Peek et al., 2006; Wong et al., 2018). The dysregulated host response to microbial invasion, termed systemic inflammatory response syndrome (SIRS), can lead to rapid deterioration in the face of pathogen control. Current therapy focuses on broad-spectrum antimicrobial treatment and fluid resuscitation, but this is often inadequate in reversing the catastrophic effects of SIRS. During SIRS, pro-inflammatory cytokines are produced in excess and trigger release of acute phase proteins from the liver. Collectively, these inflammatory mediators act on endothelial cells to cause vasodilation and increased vascular permeability, which lead to tissue hypoxia and lactic acidosis (Joffre et al., 2020; Peters et al., 2003). Activation of the immune system is accompanied by a complex chain of redox events that include generation of reactive oxygen species (ROS), particularly by neutrophils (Woodfin et al., 2016). Thiamine (Vitamin B1) is a vital co-factor for many cellular metabolic processes, including aerobic metabolism and ROS clearance (Costa et al., 2014; Frank et al., 2007). Thiamine deficiency is common in septic humans and is associated with refractory

lactic acidosis and death (Woolum et al., 2018). Thiamine supplementation improves neutrophil phagocytosis in sheep (Olkowski et al., 1990) and reduces the risk of renal damage in septic humans (Moskowitz et al., 2017). Furthermore, thiamine administration within 24 h of admission in human patients with sepsis is associated with improved lactate clearance and survival compared to controls (Woolum et al., 2018).

Depletion of endogenous thiamine and ascorbic acid (Vitamin C) has been documented in septic humans, (Attaluri et al., 2018; Donnino et al., 2010; Lima et al., 2011; Schorah et al., 1996; Wilson, 2009) and endogenous serum cortisol concentrations are often inadequate for the degree of illness (Marik, 2007; Soni et al., 1995). Therefore, administration of thiamine hydrochloride (TH), ascorbic acid, and hydrocortisone has been shown to dampen inflammation and improve survival in septic humans (Kim et al., 2018; Marik et al., 2017). This combination therapy, termed "metabolic resuscitation," might also benefit septic horses, but data are lacking. In horses, endogenous ascorbic acid depletion has been reported after IV lipopolysaccharide (LPS) infusion in the majority of subjects, (Anderson et al., 2020) and critical illness-related cortisol insufficiency is common in septic horses; (Anderson et al., 2020; Hart & Barton, 2011; Stewart et al., 2019) however, it is unknown whether or not thiamine depletion occurs in sick horses.

Thiamine hydrochloride has been used as adjunctive treatment for several conditions in the horse, including neurological and renal diseases, but specific therapeutic effects and therapeutic plasma thiamine concentrations have not been determined (Holbrook et al., 2007; Loew, 1973; Steele, 1948; Wilkins et al., 1994). In addition, an appropriate dosage of TH for use in the horse has not been established. Finally, TH has been reported to cause anaphylactic reactions in ruminants and humans when administered IV, (Cebra & Cebra, 2004; McLaughlin et al., 2003; Stephen et al., 1992; Thomson et al., 2019) but adverse effects of IV TH administration have not been adequately studied in horses. Therefore, the objectives of the study reported here were to evaluate the pharmacokinetic profile of thiamine in healthy adult horses after single IV dose administration at three dosage levels (5, 10, and 20 mg/kg) and to investigate the potential adverse effects after IV TH administration.

2 | MATERIALS AND METHODS

2.1 | Animals and experimental design

Nine adult horses from a university teaching herd were determined to be healthy based on physical examination, complete blood count (CBC), and serum biochemical analysis (SBA). A repeated Latin square design was used to ensure that each horse received each drug dosage (5, 10, and 20 mg/kg). Horses were randomly assigned a number (1 through 9) and divided into groups of 3. Each dosage was randomly assigned to each horse. Each of the 3 treatment trials was staggered over the course of 3 days. All horses were fed free-choice grass hay during each treatment trial and turned out on pasture between trials. The horses consisted of 6 geldings and 3 mares that ranged in age from 13 to 18 years, with a mean of 16 ± 2 years. Five breeds were represented, including 3 Quarter Horses, 2 Thoroughbreds, 2 Warmbloods, 1 Saddlebred and 1 Standardbred. The horses weighed 445–641 kg and were weighed immediately prior to the start of each treatment trial to ensure accurate dosing. All horses gained weight over the course of the study, with a mean weight gain of 32 ± 21 kg from the first to the last treatment trial. This was attributed to access to lush pasture grass between treatment trials. Demographic data for individual horses are presented in Table S1. All procedures were approved by the Institutional Animal Care and Use Committee at Purdue University.

2.2 | Drug administration

Thiamine hydrochloride (Neogen Vet, 500 mg/ml, Lexington, KY, USA) was administered IV at 5, 10, and 20 mg/kg. These dosages were chosen based on anecdotal reports in large animals and extrapolation from human sepsis studies (Apley, 2015; Maiti et al., 1990; Marik et al., 2017). Drug was administered over a 10 min period for each treatment trial. A washout period of ≥1 week was chosen since this was >7 times the expected elimination half-life of approximately 5 h (Smithline et al., 2012; Tallaksen et al., 1993). Bilateral IV jugular catheters were aseptically placed the night before the start of each treatment trial. One jugular vein was used for drug administration and the other for blood collection, alternating sides in subsequent trials. Heparinized blood samples were collected immediately prior to drug administration (T0; baseline) and at 5, 10, 15, 20, 30, 45, 60, and 90 min and 2, 4, 6, 8, 10, 12, 24, and 48 h. The T5m sample was collected 5 min after the end of the 10 min drug administration. Blood tubes were light-protected and stored on ice prior to centrifugation. Plasma was collected following centrifugation at 1,300 g for 5 min within 2 h of collection and stored at −80°C until analysis (Tashirova et al., 2013). For each horse during each trial, the jugular catheter used for drug administration was removed within 2 h of drug administration and the catheter used for blood collection was removed immediately following blood collection at T24 h. All T48 h blood samples were collected via jugular venipuncture.

2.3 | Adverse effects

Horses were continually monitored for the first 12 h of each treatment trial, with complete physical examinations performed at T0 (baseline), 6, 12, 24, and 48 h. A CBC and SBA were performed immediately prior to the start of the study to ensure overall systemic health prior to the start of the study. Subjective assessments were made regarding changes in appetite, behavior, and fecal consistency. Jugular catheter insertion sites and jugular veins were assessed for swelling, heat, and pain.

2.4 | Sample analysis

2.4.1 | Sample preparation

Frozen horse plasma was stored at −80°C until analysis. The extraction protocol was based on modifications from Al-Attas and McCann (Al-Attas et al., 2012; McCann et al., 2017). Each sample was thawed and a 0.1 ml aliquot was transferred to microcentrifuge tube for extraction. Isotopically labeled ($^{13}C_3$) thiamine was used as an internal standard and for quantification of endogenous thiamine in each sample (Toronto Research Chemicals, North York, ON). Each sample aliquot was spiked with 100 ng of $^{13}C_3$-thiamine prior to the extraction process. To extract each sample, 25 µl of 10% trichloroacetic acid (TCA) was added and the sample placed on ice for 15 min. Samples were then vortexed for 3 min and centrifuged at 15,000 g for 10 min to precipitate proteins. The supernatant was collected and pellet discarded. The supernatants were stored at −20°C until ready for analysis on a LC/MS/MS system. At the time of analysis, each sample was diluted 20-fold with a solution of 50% water/50% acetonitrile, vortexed, and transferred to an autosampler vial.

2.4.2 | HPLC/MS-MS analysis

An Agilent 1260 Rapid Resolution liquid chromatography (HPLC) system coupled to an Agilent 6470 series triple quadrupole mass spectrometer (MS/MS) was used to analyze thiamine (Agilent Technologies, Santa Clara, CA). A Waters Acquity BEH HILIC (2.1 mm × 100 mm, 1.7 µm) column was used for HPLC separation (Water Corp, Milford, MA). The buffers were (A) acetonitrile: isopropyl alcohol:200 mM ammonium formate at pH 3 (90:5:5 v/v) and (B) water: acetonitrile:200 mM ammonium formate at pH 3 (90:5:5 v/v). The linear HPLC gradient was as follows: time 0 min, 0% B; time 1 min, 0% B; time 7 min, 40% B; time 8 min, 90% B; time 11 min, 0% B; and time 15 min, 0% B. The flow rate was 0.3 ml/min. Multiple reaction monitoring was used for MS analysis. Data were acquired in positive electrospray ionization (ESI) mode. The calibration curve ranged from 100 to 0.001 µg/ml. Quantitation was based on the ion transition of thiamine 265.5→122.4 and $^{13}C_3$-thiamine 268.5→122.4 and qualifier ions 265.5→144.4 and 268.5→147.4. Agilent Masshunter Quantitative Analysis software was used for data analysis (version 8.0).

2.5 | Statistical analysis

Data are expressed as mean ± standard deviation (range). To account for endogenous plasma thiamine, for each horse at each dosage level (5, 10, and 20 mg/kg), plasma thiamine concentrations were normalized by subtracting the endogenous thiamine concentration at time 0, prior to TH administration. Any negative values were entered into the analysis as 0 µg/ml. For each IV TH dosage level, non-compartmental pharmacokinetic analysis was performed using commercially available software (Phoenix WinNonLin 8.1, Certara, Princeton, NJ). Calculated parameters included the terminal rate constant (λ_z), terminal half-life ($t_{1/2}$), concentration at time 0 (C_0), observed area under the curve (AUC_{obs}), area under the curve extrapolated to infinity ($AUC_{0-\infty}$), percent of $AUC_{0-\infty}$ extrapolated ($AUC_{\%extrap}$), volume of distribution by the area method (V_z), clearance (Cl), observed area under the moment curve ($AUMC_{obs}$), area under the moment curve extrapolated to infinity ($AUMC_{0-\infty}$), percent of $AUMC_{0-\infty}$ extrapolated ($AUMC_{\%extrap}$), mean residence time (MRT), and volume of distribution at steady state (V_{ss}). To determine whether thiamine is eliminated in a linear fashion in the horse or is affected by dosage, λ_z and Cl were each compared between dosage levels. First, data normality was assessed using the Kolmogorov–Smirnov test. Then, each parameter (λ_z and Cl) was compared between dosages using a repeated measures ANOVA and Tukey's *post hoc* test for multiple comparisons. Increases in $AUC_{0-\infty}$ with dosage were also visually assessed for linearity in individual horses. Statistical comparisons were performed using commercially available software (Prism 9; GraphPad Software, LLC., San Diego, CA). Significance was set at $p < .05$.

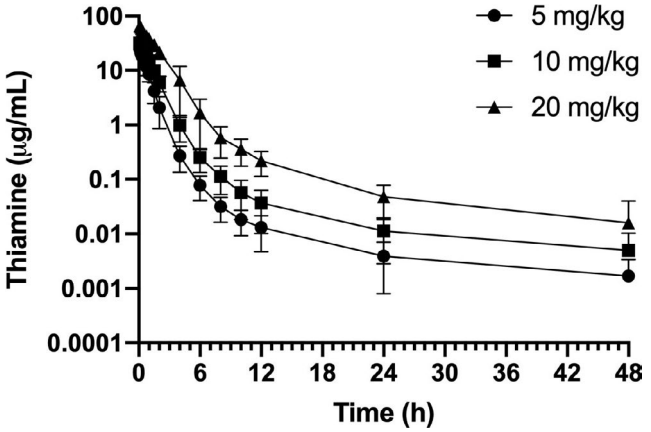

FIGURE 1 Plasma concentration–time relationship of thiamine hydrochloride after 5, 10, and 20 mg/kg IV dosing in healthy horses ($n = 9$)

3 | RESULTS

3.1 | Pharmacokinetics

Plasma thiamine concentration vs. time plots are presented in Figure 1. Pharmacokinetic parameters for horses administered TH at 5, 10, and 20 mg/kg IV are presented in Tables 1–3. Individual horse plasma thiamine concentrations and pharmacokinetic parameters for all three dosages assessed are presented in Tables S2 and S3, respectively.

To determine whether thiamine kinetics are linear in horses, λ_z and Cl were assessed for changes between dosage levels. λ_z significantly decreased as dosage increased ($p < .0001$) as seen

TABLE 1 Non-compartmental pharmacokinetic parameters for thiamine hydrochloride in healthy horses (n = 9) after a single dosage of 5 mg/kg

Parameter	Mean	SD	Min	Max
λ_z (h^{-1})	0.905	0.055	0.803	0.964
Half-life (h)	0.77	0.05	0.72	0.86
C_0 (μg/ml)	29.81	4.26	25.51	38.79
AUC_{obs} (h*μg/ml)	23.82	4.97	16.90	30.56
$AUC_{0-\infty}$ (h*μg/ml)	23.83	4.97	16.91	30.56
$AUC_{\%extrap}$ (%)	0.02	0.02	0.00	0.08
V_z (ml/kg)	240.0	41.4	179.5	309.0
Cl (ml/kg/h)	218.4	46.4	163.6	295.8
$AUMC_{obs}$ (h^2*μg/ml)	24.49	9.56	13.69	38.84
$AUMC_{0-\infty}$ (h^2*μg/ml)	24.62	9.62	13.75	38.99
$AUMC_{\%extrap}$ (%)	0.51	0.23	0.19	0.82
MRT (h)	1.00	0.20	0.69	1.33
V_{ss} (ml/kg)	212.7	31.4	174.5	259.9

Abbreviations: λ_z, terminal rate constant; terminal half-life ($t_{1/2}$); $AUC_{\%extrap}$, percent of $AUC_{0-\infty}$ extrapolated; $AUC_{0-\infty}$, area under the curve extrapolated to infinity; AUC_{obs}, observed area under the curve; $AUMC_{\%extrap}$, percent of $AUMC_{0-\infty}$ extrapolated; $AUMC_{0-\infty}$, area under the moment curve extrapolated to infinity; $AUMC_{obs}$, observed area under the moment curve; C_0, concentration at time 0; Cl, clearance; MRT, mean residence time; V_{ss}, volume of distribution at steady state; V_z, volume of distribution by the area method.

TABLE 2 Non-compartmental pharmacokinetic parameters for thiamine hydrochloride in healthy horses (n = 9) after a single dosage of 10 mg/kg

Parameter	Mean	SD	Min	Max
λ_z (h^{-1})	0.774	0.063	0.679	0.862
Half-life (h)	0.90	0.07	0.80	1.02
C_0 (μg/ml)	34.40	3.72	30.37	42.45
AUC_{obs} (h*μg/ml)	42.82	8.71	32.46	55.69
$AUC_{0-\infty}$ (h*μg/ml)	42.83	8.71	32.46	55.71
$AUC_{\%extrap}$ (%)	0.02	0.01	0.00	0.04
V_z (ml/kg)	310.8	41.3	264.4	369.7
Cl (ml/kg/h)	242.3	48.9	179.5	308.0
$AUMC_{obs}$ (h^2*μg/ml)	60.59	22.77	32.03	97.00
$AUMC_{0-\infty}$ (h^2*μg/ml)	60.94	23.00	32.09	97.80
$AUMC_{\%extrap}$ (%)	0.52	0.44	0.03	1.40
MRT (h)	1.38	0.28	0.99	1.76
V_{ss} (ml/kg)	325.8	50.7	288.5	448.1

Abbreviations: λ_z, terminal rate constant; terminal half-life ($t_{1/2}$); $AUC_{\%extrap}$, percent of $AUC_{0-\infty}$ extrapolated; $AUC_{0-\infty}$, area under the curve extrapolated to infinity; AUC_{obs}, observed area under the curve; $AUMC_{\%extrap}$, percent of $AUMC_{0-\infty}$ extrapolated; $AUMC_{0-\infty}$, area under the moment curve extrapolated to infinity; $AUMC_{obs}$, observed area under the moment curve; C_0, concentration at time 0; Cl, clearance; MRT, mean residence time; V_{ss}, volume of distribution at steady state; V_z, volume of distribution by the area method.

in Figure 2. Although Cl also significantly changed with dosage ($p < .0001$), these changes were dosage specific. Cl did significantly decrease from the 5 mg/kg to the 20 mg/kg dosage ($p = .0011$). However, Cl at the 10 mg/mg dosage was significantly higher than 5 mg/kg ($p = .0388$) and 20 mg/kg ($p = .0004$) as shown in Figure 3. For most horses, AUC appeared to increase in a non-linear fashion (Figure 4).

3.2 | Adverse effects

There were no significant changes in physical examination parameters for any horse during the study. There was no evidence of swelling, heat or pain at the jugular catheter insertion sites and jugular vein refill remained normal for all horses throughout the study period.

TABLE 3 Non-compartmental pharmacokinetic parameters for thiamine hydrochloride in healthy horses (n = 9) after a single dosage of 20 mg/kg

Parameter	Mean	SD	Min	Max
λ_z (h^{-1})	0.632	0.094	0.482	0.726
Half-life (h)	1.12	0.18	0.95	1.44
C_0 (μg/ml)	69.38	7.61	59.53	82.68
AUC_{obs} (h*μg/ml)	123.64	26.85	96.42	171.28
$AUC_{0-\infty}$ (h*μg/ml)	123.68	26.86	96.43	171.32
$AUC_{\%extrap}$ (%)	0.03	0.04	0.00	0.11
V_z (mL/kg)	264.7	22.9	223.8	297.5
Cl (mL/kg/h)	167.9	32.4	116.7	207.4
$AUMC_{obs}$ (h^2*μg/ml)	244.97	95.73	139.15	404.09
$AUMC_{0-\infty}$ (h^2*μg/ml)	246.61	96.62	139.31	405.78
$AUMC_{\%extrap}$ (%)	0.60	0.68	0.11	2.25
MRT (h)	1.94	0.39	1.44	2.38
V_{ss} (ml/kg)	317.0	52.1	276.5	452.9

Abbreviations: λ_z, terminal rate constant; terminal half-life ($t_{1/2}$); $AUC_{\%extrap}$, percent of $AUC_{0-\infty}$ extrapolated; $AUC_{0-\infty}$, area under the curve extrapolated to infinity; AUC_{obs}, observed area under the curve; $AUMC_{\%extrap}$, percent of $AUMC_{0-\infty}$ extrapolated; $AUMC_{0-\infty}$, area under the moment curve extrapolated to infinity; $AUMC_{obs}$, observed area under the moment curve; C_0, concentration at time 0; Cl, clearance; MRT, mean residence time; V_{ss}, volume of distribution at steady state; V_z, volume of distribution by the area method.

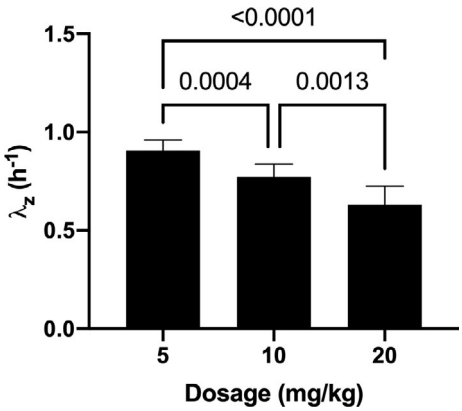

FIGURE 2 Terminal rate constant (λ_z) of thiamine hydrochloride after 5, 10, and 20 mg/kg IV dosing in healthy horses (n = 9)

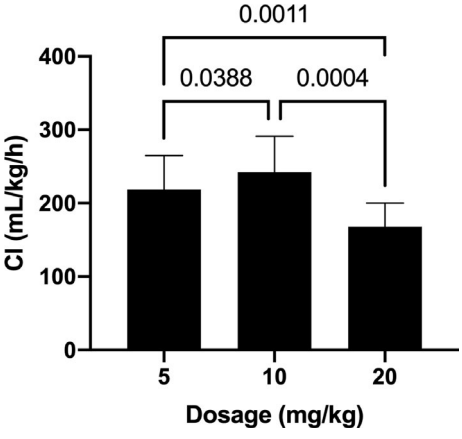

FIGURE 3 Clearance of thiamine hydrochloride after 5, 10, and 20 mg/kg IV dosing in healthy horses (n = 9)

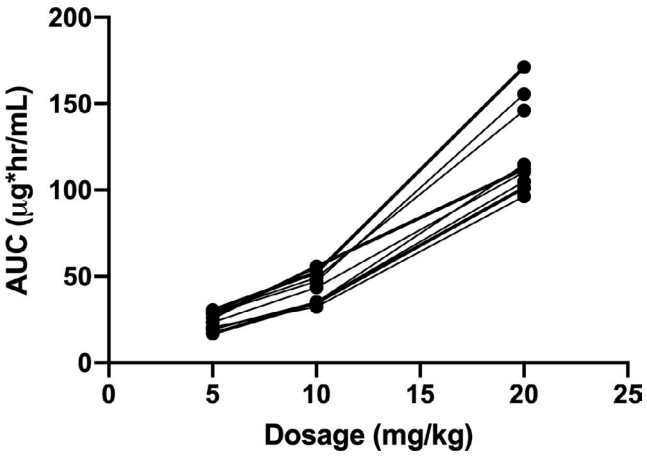

FIGURE 4 Area under the curve of thiamine hydrochloride after 5, 10, and 20 mg/kg IV dosing in healthy horses (n = 9)

4 | DISCUSSION

This is the first study to document the pharmacokinetics of IV TH administration in healthy horses or, to our knowledge, any large animal species. Despite common use of TH in small ruminants and cattle for treatment and prevention of polioencephalomalacia, pharmacokinetic studies have not been performed (Apley, 2015; Karapinar et al., 2008; Zhang et al., 2020). In our study, all three dosages administered resulted in supraphysiologic plasma thiamine concentrations with relatively short half-lives (0.77 ± 0.05–1.12 ± 0.18 h). We also documented that the terminal rate constant for thiamine significantly decreased with dosage. This, combined with non-linear increases in AUC with dosage, suggests that thiamine is eliminated in a non-linear, dosage-dependent fashion in horses.

Non-linear pharmacokinetics have also been reported for thiamine in humans. Weber et al. (1985) documented fourfold to10-fold differences in renal clearance between the initial and terminal phases of thiamine elimination (Weber & Kewitz, 1985). This phenomenon is attributed to handling by the kidney, which is the major route of thiamine excretion. As a small molecule, thiamine is freely filtered at the glomerulus and then either reabsorbed or secreted by tubular transporters in a concentration-dependent manner (Ott & Werneke, 2020). At low, physiologic concentrations, thiamine is reabsorbed by brush-border thiamine and organic cation transporters leading to minimal excretion and low renal clearance. Whereas, at high concentrations achieved by exogenous administration, thiamine is actively secreted, dramatically increasing renal clearance (Weber et al., 1990). Because thiamine secretion is transporter-mediated, it is also saturable which causes decreases in total plasma clearance at high concentrations. If these processes are similar in horses, they could partially explain the dosage-related differences in plasma clearance documented in the present study. The increase in Cl between 5 and 10 mg/kg could be due to a switch from renal reabsorption to secretion. However, in humans, this switch occurs around 200 nmol/L (0.053 μg/ml), which is well below plasma concentrations induced after IV administration of TH to horses. Thus, it is more likely that this difference in Cl is due to type II error, rather than renal transporter function. The lower thiamine Cl at 20 mg/kg compared to 5 and 10 mg/kg, however, could be evidence of transporter saturation.

Non-linear kinetics of thiamine in horses makes plasma concentrations difficult to predict for dosages outside of those studied (5, 10, and 20 mg/kg IV). Plasma concentrations during chronic therapy may also be difficult to predict, although thiamine's short half-life makes significant drug accumulation unlikely with intermittent dosing. However, before dosage and dosing frequency recommendations can be made, therapeutic targets must be established. Metabolic resuscitation protocols for sepsis in humans include a standard TH dosage of 200 mg (approximately 2.0–3.0 mg/kg) administered IV q 12 h (Donnino et al., 2016; Fujii et al., 2020; Marik et al., 2017). The rationale for this dosage is unclear but is centered around replacing thiamine that has been depleted during sepsis (Donnino et al., 2010).

No adverse reactions were observed after administration of IV TH in this study. Anecdotal reports of collapse and sudden death have been reported in small ruminants and cattle after IV administration, which has led to a general recommendation to dilute TH in 0.9% sodium chloride or sterile water and administer slowly (Apley, 2015; Cebra & Cebra, 2004). The horses in this study were administered IV TH over a 10 min period, so it is possible that more rapid administration might result in adverse effects.

As with most "first-in-species" pharmacokinetics reports, the small sample size of our study is a potential limitation. Inter-individual variation for many pharmacokinetic parameters was actually fairly low among our study participants; however, these values are unlikely to capture the full range of potential variability within the equine population. Similarly, our study design precluded assessing the effect of patient factors such as age, breed, disease status, or renal function on thiamine pharmacokinetics. Future population pharmacokinetic studies should be considered to address these factors, particularly since thiamine may have benefit in critically ill patients (Donnino et al., 2010; Marik et al., 2017). Another limitation is that we only quantified plasma-free thiamine in this study. Thiamine exists in several forms in the body including free thiamine, several phosphorylated forms, and thiamine bound to adenosine di- and triphosphate. Proportions of these moieties differ between plasma, red blood cells, and tissue with free thiamine and thiamine-monophosphate predominating in the plasma and the biologically active form, thiamine-diphosphate, predominating intracellularly (Ott & Werneke, 2020). Thus, quantification of the various forms of thiamine in both plasma and red blood cells would enhance understanding of thiamine distribution, metabolism, and potential for biologic effects in the horse. However, in humans, a single IV administration of TH increases plasma-free thiamine to a much greater degree than plasma thiamine-monophosphate or red blood cell thiamine-diphosphate so, for the purposes of this initial study, plasma-free thiamine quantification is likely sufficient (Tallaksen et al., 1993). Finally, endogenous thiamine from absorption from diet and hindgut microflora production (Carroll et al., 1949) may have interfered with determining the kinetics of exogenously administered thiamine. Because baseline (T0) concentrations and previously reported equine endogenous thiamine concentrations (Cymbaluk et al., 1978; Loew & Bettany, 1973) were so much lower than concentrations achieved after IV administration, this is unlikely to significantly affect results. Therefore, we chose to normalize the data by simply subtracting baseline concentrations.

In summary, a single IV TH injection in healthy horses achieved plasma thiamine concentrations well above endogenous levels and demonstrated a relatively short half-life. Thiamine hydrochloride appears to possess non-linear kinetics in the horse, likely due to complex renal elimination processes. Future pharmacodynamic studies are needed to identify therapeutic plasma concentrations of thiamine so rational dosage regimens can be established. Subsequent studies to investigate the effects of TH administration in septic horses can then be pursued.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

ANIMAL WELFARE AND ETHICS STATEMENT

This study was approved by the university's Institutional Animal Care and Use Committee (Protocol # 2003002025, Approval date: April 30, 2020).

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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