ATRIAL AND AV-NODAL PHYSIOLOGY IN HORSES:
ELECTROPHYSIOLOGIC AND ECHOCARDIOGRAPHIC CHARACTERIZATION
AND PHARMACOLOGIC EFFECTS OF DILTIAZEM

DISSERTATION

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By
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ABSTRACT

Atrial fibrillation (AF) is considered the most common arrhythmia affecting exercise performance in horses. Quinidine is an effective therapy for conversion of AF to sinus rhythm, but treatment is associated with a number of adverse clinical effects. Furthermore, residual electrical and mechanical abnormalities of the equine atria may account for the recurrence of AF following successful quinidine therapy. Relative to these issues, we sought to better understand the physiology of the in vivo equine atria and the associated sinoatrial and atrioventricular (AV) nodal tissues.

In treating horses with AF using the drug quinidine, a common adverse effect is marked acceleration of the ventricular response rate prompting early discontinuation of treatment. Diltiazem effectively controls ventricular response to AF in other species, but has not been evaluated in horses. In our first series of experiments we studied the pharmacodynamic and pharmacokinetic effects of the calcium channel blocker diltiazem on the cardiovascular system in healthy horses in sinus rhythm. To further support these studies we characterized the clinical electrophysiology of the normal equine atria and then quantified the electrophysiologic effects of quinidine and diltiazem in a pacing model of atrial tachycardia. Lastly, we established echocardiographic techniques to assess mechanical function of the equine left atrium noninvasively and applied these methods to a subset of horses recently converted from AF to normal sinus rhythm.
The first study describes the effects of diltiazem on cardiac rate and rhythm (assessed by electrocardiography), left ventricular (LV) function and central hemodynamics (by cardiac catheterization), as well as peripheral blood flow (by Doppler sonography) in normal, standing, unsedated horses. Cardiac effects of diltiazem included intermittent depression of the sinus and atrioventricular nodes and mild impairment of systolic and diastolic LV function. Vascular effects of diltiazem included arterial vasodilatation, increased limb blood flow, and decreased systemic vascular resistance. Baroreceptor reflex-mediated sympathetic activation increased the sinus node rate, and presumably blunted the depressive effects of diltiazem on myocardial and nodal tissues.

The following two studies describe the pharmacokinetics and the plasma protein binding characteristics of diltiazem in horses. The disposition of diltiazem after intravenous administration to healthy horses was characterized by rapid distribution and elimination and a terminal half-life shorter than that reported in humans and dogs. Plasma diltiazem metabolite concentrations were not considered clinically important. The extent of protein binding of diltiazem in horses was slightly higher than protein binding reported for humans and other species.

The fourth study in this series describes the electrophysiologic effects of quinidine and the combination of quinidine and diltiazem in horses subjected to rapid atrial pacing. We were able to identify a rate-dependence of diltiazem effects on AV nodal conduction. Diltiazem was effective for ventricular rate control during treatment with quinidine in our equine pacing model of supraventricular tachycardia. The results showed again the
potential adverse effects of diltiazem, including hypotension and suppression of the sino-atrial nodal discharge leading to sinus arrhythmia or sinus pauses.

Based on these studies demonstrating inhibitory effects on AV nodal conduction, diltiazem is likely to be useful for ventricular rate control in horses with naturally occurring AF undergoing quinidine treatment. Diltiazem appeared relatively safe in healthy horses, but dosage may be limited by hypotension from vasodilatation and direct suppression of sinus node discharge. The results of our studies will allow developing accurate and effective dosing guidelines for diltiazem in the treatment of AF in horses.

Atrial enlargement and atrial mechanical dysfunction are important sequelae of atrial fibrillation that may persist even after successful treatment and conversion to sinus rhythm. However, atrial mechanical function has been incompletely studied in horses. The goals of the fifth study were to describe the methods and determine the reliability of transthoracic echocardiography for characterization of left atrial (LA) size and mechanical function in horses. The results showed that LA size and mechanical function can be assessed in standing, unsedated horses by use of 2D echocardiography, transmitral blood flow velocity profiles, and analyses of LA wall motion by tissue Doppler imaging.

In the last study we applied the echocardiographic methods established in the previous study to detect LA mechanical dysfunction in horses with cardiac disease. Specifically, we were able to show that LA mechanical dysfunction persists after successful conversion of naturally occurring AF to sinus rhythm in horses. Our findings were most consistent with the presence of atrial stunning, although we could not preclude underlying myocardial disease or residual treatment effects.
In conclusion, we developed preliminary guidelines for echocardiographic assessment of LA size and mechanical function that are likely to be useful to study LA function in horses with cardiac disease. Specifically, echocardiography may serve to investigate the clinical relevance of atrial remodeling and atrial mechanical dysfunction in horses with AF. Further studies will be required to establish the clinical value of echocardiographic assessment of LA mechanical function in horses with regards to prognosis, treatment, and outcome.
Dedicated to my parents and to my grandparents.
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INTRODUCTION

Atrial fibrillation (AF) is one of the most commonly encountered pathologic arrhythmias in horses.\textsuperscript{115} It is characterized by a rapid, irregular, and seemingly chaotic electrical activation of the atrial tissue, with an irregularly irregular ventricular response rate that is controlled by the atrioventricular node. While not life threatening, AF limits athletic performance due to its negative impact on ventricular filling and cardiovascular performance at higher levels of exercise.

In horses, AF often occurs without evidence of underlying structural disease, a condition also referred to as ‘lone’ AF. The large atrial mass and the high vagal tone are considered risk factors for AF in horses. Conversely, in people and in small animals, AF usually occurs secondary to predisposing disorders, including congestive heart failure, dilated cardiomyopathy, mitral valve disease, coronary artery disease, or hypertension.

Mechanisms of atrial fibrillation have been studied in a variety of species, including goats, sheep, dogs, and humans. Three principle mechanisms have been proposed: rapidly discharging atrial ectopic foci, single-circuit reentry, and multiple-circuit reentry.\textsuperscript{94} While the latter has been the predominant model of AF in the past, more recent investigations showed that all three mechanisms might be involved in AF. Intrinsic determinants such as action potential duration (which is dependent on autonomic input) and structural substrates such as myocardial fibrosis (which may occur in association with ischemic
damage, atrial dilation, or heart failure) may predispose to the onset and persistence of AF. Atrial fibrillation may be triggered by rapidly discharging ectopic foci and manifested as a single-circuit reentry, if the intrinsic determinants support a reentry mechanism. While in the early stages of AF the triggering mechanism may be crucial for persistence of AF, the arrhythmia may become self-perpetuating over time. Rapid atrial tachycardia, either due to ectopic beats or reentry, is known to cause electrophysiologic and structural changes in the atrial tissue, also referred to as tachycardia-induced electrical and structural remodeling.4,94,122,154 These changes are largely responsible for the self-perpetuating, progressive, and recurrent nature of AF. After electrical and structural remodeling has occurred, multiple-circuit reentry is likely the predominant mechanism and common ‘final pathway’ of AF, irrespective of the initiating mechanism.94

Despite the clinical relevance of AF in horses, the pathophysiological basis of this arrhythmia is largely unknown in this species. The knowledge of atrial structure, electrophysiology, and mechanical function in horses is limited, although a multitude of diagnostic techniques for invasive and noninvasive evaluation of cardiovascular function are available to date and could be applied to the equine species. Furthermore, few treatment options are available. Transvenous electrical cardioversion has been promoted recently,80,81 and other therapeutic options such as amiodarone or flecainide have been suggested. However, the clinical experience with these newer treatments is still limited, and quinidine sulfate remains the preferred treatment despite the relatively high incidence of adverse effects. Over half of all horses treated with quinidine sulfate develop sustained, rapid ventricular response rate during the course of treatment.115 In some cases,
differentiation of a quinidine-induced rapid ventricular response rate from ventricular tachycardia is challenging. Digoxin is usually recommended for rate control in horses with rapid ventricular response rate. However, digoxin is often not effective for ventricular rate control and is considered contraindicated in cases with suspected ventricular tachycardia. Therefore, quinidine-induced severe tachycardia often leads to early termination of treatment in horses with AF.

The first goal of this project was to evaluate the potential value of diltiazem for ventricular rate control in horses with AF undergoing quinidine treatment. In our first study, we used a variety of invasive and non-invasive diagnostic methods to study the effects of diltiazem on atrio-ventricular conduction, ventricular function, central hemodynamics, and peripheral blood flow. Subsequently, we investigated the pharmacokinetic and protein binding characteristics of diltiazem in healthy horses. Finally, we determined the electrophysiologic effects of quinidine and diltiazem in an equine pacing model of supraventricular tachycardia, in an attempt to characterize the rate-dependence of diltiazem effects on AV nodal conduction and to identify doses of diltiazem that were effective in controlling the ventricular response rate during rapid atrial pacing under the influence of quinidine.

The in vivo electrophysiologic methods used in this study not only allowed us to study drug effects, but also provided important insights into the basic SA nodal, atrial, and AV nodal electrophysiology in healthy horses. While the use of in vivo electrophysiologic studies is standard for evaluating the pathogenesis of arrhythmias in human patients, these techniques largely have been ignored in the horse. Elucidation of the pathogenic mechanisms responsible for AF in horses is fundamental to advancing
clinical management and refining prognostic indicators. The methods described in this study may therefore also be used for future investigations on the pathophysiologic basis and the electrophysiologic consequences of AF in horses.

Undoubtedly, the pathogenic mechanisms of AF involve altered atrial cell electrophysiology. However, atrial electrophysiology is closely tied to atrial mechanical function, and the assessment of atrial contractility is pivotal an understanding of atrial physiology. The gold standard of assessment of LA mechanical function and all its components is the invasive recording of pressure-volume loops.\textsuperscript{104} However, this approach requires direct LA pressure recordings, which precludes its routine use in a clinical setting. Our last two studies were devoted to the non-invasive assessment of left atrial (LA) size and LA mechanical function. We studied a variety of two-dimensional, flow Doppler, and tissue Doppler echocardiographic variables, determined their reliability, and developed preliminary imaging guidelines. Finally, we applied these techniques to investigate alterations of LA size and LA mechanical function in horses with naturally occurring AF after conversion to sinus rhythm.
Atrial fibrillation (AF) is the most common arrhythmia affecting performance in horses. Quinidine sulfate is the drug of choice for conversion of AF to sinus rhythm. However, anticholinergic effects of quinidine facilitate impulse conduction across the atrioventricular (AV) node independent of plasma concentration. This action often results in an increased ventricular response rate in horses with AF. Sustained tachycardia due to an accelerated ventricular response is the most common severe adverse effect of quinidine therapy, affecting over 50% of all treated horses. Digoxin can be administered IV for heart rate control in horses with high pre-treatment heart rate or when tachycardia develops during quinidine treatment. Digoxin exerts a parasympathetic action that increases the functional refractory period of the AV node, decreases AV nodal conduction, and reduces ventricular response rate. However, the value of digoxin treatment in the conversion of AF to sinus rhythm in horses is undetermined. Heart rate control with digoxin may fail during stress or excitement, when high sympathetic tone overrides the vagomimetic effect of digoxin. Furthermore, digoxin may not be optimal for the acute control of heart rate in patients with AF, because this drug has a delayed onset of action, carries a low toxic-to-therapeutic ratio, can precipitate ventricular ectopy, and may delay recovery from tachycardia-induced atrial remodeling.
Diltiazem is a calcium-channel blocker, which is used in humans and dogs for the treatment of supraventricular arrhythmias and for heart rate control in the treatment of AF, atrial flutter, AV nodal reentry, and atioventricular reciprocating tachycardia.\textsuperscript{4, 12} Diltiazem inhibits AV nodal conduction regardless of the level of sympathetic tone,\textsuperscript{6, 7, 13} and results in a faster and more efficacious ventricular rate control compared to digoxin.\textsuperscript{8, 9, 14} The cardiovascular effects of diltiazem have not been reported in horses.

The aim of the present study was to investigate the effects of graded doses of diltiazem on cardiac rate and rhythm, AV nodal conduction, systolic and diastolic left ventricular (LV) function, central hemodynamics, and peripheral blood flow in normal, standing, non-sedated horses.

**Material and Methods**

*Horses:* Ten Standardbred horses (5 geldings, 5 mares) with a mean age of 12.5 years (range, 6 to 23 years) and mean body weight of 485 kg (range, 426 to 531 kg) were used. Six horses were used for both the initial dose-finding study and the hemodynamic study that followed, allowing a drug-free time interval of at least 2 weeks between the 2 studies. Two horses were used for the dose-finding study only, and 2 horses were used for the hemodynamic study only. All horses were considered healthy based upon physical examination, cardiac auscultation, electrocardiogram, echocardiographic examination, CBC, serum chemistry, and plasma fibrinogen concentration. The left carotid artery of each horse was surgically elevated to a subcutaneous location at least 3 weeks before beginning the experiments.\textsuperscript{15} All diltiazem experiments, including instrumentation, were performed under local anesthesia\textsuperscript{a} in non-sedated horses standing in stocks. The studies
were approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.

**Dose-finding study:** The aim of this first part of the investigation was to determine a diltiazem dose that produced a decrease of mean carotid artery pressure of at least 10 mmHg, prolongation of the PR-interval, and consistent second degree AV block. Considering the frequency-dependent effects diltiazem exerts in other species, these end-points may not be appropriate measures for the therapeutic effects of diltiazem during rapid supraventricular arrhythmias such as AF in horses. However, they were considered adequate to assess the effects of diltiazem in healthy horses in sinus rhythm.

Eight horses were used for the dose-finding study. Two 14G catheters were aseptically placed under local anesthesia in either jugular vein for drug administration and blood sampling, respectively. A 19G catheter was placed aseptically under local anesthesia in the elevated carotid artery for measurement of arterial blood pressure using a fluid filled system. A base-apex electrocardiogram (ECG) was used to determine the atrial rate (AR), ventricular rate (VR), cardiac rhythm, and PR interval.

Diltiazem (5 mg/mL) was administered IV over 2 min at dosages of 0.05, 0.125, 0.25, 0.5, and 1.0 mg/kg every 15 min, in order to achieve cumulative dosages of 0.0 (baseline), 0.05, 0.175, 0.425, 0.925, and 1.925 mg/kg. The dose regimen was chosen based on published data in dogs. All physiologic data were recorded simultaneously at a sampling rate of 200 Hz by a digital data acquisition system and stored in a raw-format computer file for later offline analysis. Data were analyzed before drug administration (baseline) and at the midpoint of each treatment period (7.5 min after each dose of diltiazem).
The ECG tracing and pressure wave forms were analyzed by visual inspection and by the computer software of the data acquisition system. Systolic, diastolic, and mean arterial blood pressure, and PR interval were averaged over 10 consecutive beats. The AR and VR were averaged from a 1-min recording interval. Blood samples were collected at the beginning of each data collection period for later determination of diltiazem plasma concentrations.

_Hemodynamic study:_ Eight horses were used to assess the effects of IV diltiazem. Two 8F catheter introducers were aseptically placed under local anesthesia in the right jugular vein approximately 20 cm apart. A Swan-Ganz thermodilution catheter was inserted through the proximal introducer and advanced until the tip of the catheter was positioned in the pulmonary artery. A polyethylene catheter was inserted through the distal introducer and advanced into the right atrium. These two catheters were used for measurement of right atrial and pulmonary artery pressures and for determination of the cardiac output. A 9F catheter introducer was aseptically placed under local anesthetic in the elevated left carotid artery. A custom-made dual-tipped pressure-sensing catheter then was inserted into the introducer and advanced until the proximal sensor was positioned in the aorta and the distal sensor was positioned in the left ventricle. This catheter allowed simultaneous recording of aortic and LV pressures. Catheter placement was guided by observation of characteristic pressure waveforms during continuous pressure monitoring. The zero-pressure reference point for the fluid-filled catheters was the point of the shoulder. Both transducers were calibrated to 0 and 50 mmHg using a mercury manometer. A base-apex ECG was monitored.
Pre-baseline measurements were obtained (time -40 min) after an equilibration period of 30 min, followed by administration of a 50 mL bolus of 0.9% sodium chloride over 5 min (time -25 to -20 min). Baseline measurements were obtained 5 min after completion of the saline administration (time -15 min). The saline bolus allowed steady baseline values to be established and ruled out any influence on the measured variables of instrumentation, physiologic changes over time, and drug administration procedures. After baseline recordings, diltiazem (5 mg/mL) was administered IV at a dosage of 1 mg/kg over 5 min (time 0 to 5 min), followed by 2 additional doses of 0.5 mg/kg administered over 5 min every 30 min (time 30 to 35 min and 60 to 65 min), in order to achieve cumulative dosages of 1 mg/kg, 1.5 mg/kg, and 2 mg/kg, respectively. These dosages were based on the results of the dose-finding study. All drugs were injected into the jugular vein through the side-port of the proximal catheter introducer. A programmable infusion pump was used for controlled drug delivery.

All physiologic data were acquired simultaneously at a sampling rate of 200 Hz by a digital data acquisition system and stored for later offline analysis. Hemodynamic data were analyzed for the following time periods: Pre-baseline (PB; - 40 min), baseline (B; -15 min), diltiazem 1 mg/kg (D1; 15 min), diltiazem 1.5 mg/kg (D1.5; 45 min), and diltiazem 2 mg/kg (D2; 75 min). At the beginning of each data collection period, blood samples were collected for later determination of plasma drug concentrations. Analysis of the ECG recordings was performed by visual analysis and with the computer software of the data acquisition system. The rate of sinus node discharge (atrial rate, AR), ventricular rate (VR), the number of second degree AV blocks, and the occurrence of sinus arrhythmia were determined by visual inspection during a 1-min period. The duration of
the PR interval was averaged over 10 consecutive beats. All pressure measurements and derived variables were averaged over a 1-min interval for each time period. Systolic, diastolic, and mean aortic pressures (SAP, DAP, MAP), left ventricular end-diastolic pressure (LVEDP), mean right atrial pressure (RAP), and mean pulmonary arterial pressure (PAP) were determined. The maximal rate of increase (+dp/dt\text{max}) and decrease (-dp/dt\text{max}) in LV pressure, and the time constant of isovolumetric relaxation (\textit{tau}) were derived from the LV pressure recordings. \textit{Tau} was calculated using a semilogarithmic model during the initial 40 ms of isovolumetric relaxation starting at \textit{t0} = t(-dp/dt\text{max}).^{16}

Cardiac output (CO) was determined by thermodilution\textsuperscript{1,17} Five thermodilution determinations were made at each time period. Quality of the recordings was assessed by visual evaluation of the thermodilution curves. The 4 closest values then were averaged to give the final value for that time period. Stroke volume (SV = CO / HR) and systemic vascular resistance (SVR = [MAP – RAP] × 80 / CO) were calculated. Pulmonary vascular resistance (PVR) was estimated as PVR = (PAP - LVEDP) × 80 / CO, using LVEDP as a surrogate for pulmonary capillary wedge pressure, assuming unrestricted pulmonary venous and mitral blood flow.

Echocardiographic and vascular ultrasonographic examinations were performed at the end of each data collection period and recorded on videotape for offline analysis.\textsuperscript{9} All echo measurements were performed on 3 individual cardiac cycles and averaged for each time point. The first beats after sinus- or atrioventricular block were excluded from the analysis. The fractional shortening (FS) of the left ventricle was determined using standard 2D- and M-mode echocardiographic techniques.\textsuperscript{18} The junction between the brachial and the median artery with the branching common interosseus artery was imaged.
from the medial aspect of the right forelimb at the level of the proximal radius.

Measurements were performed in 2 distinct vessels in order to obtain optimal axial resolution for 2D measurements and optimal alignment of blood flow with the ultrasound beam for Doppler measurements. The diameter (D) of the brachial artery was determined immediately proximal to the common interosseus artery. Peripheral vascular blood flow was measured in the common interosseus artery using pulsed-wave Doppler technique.19 The instantaneous pulse rate (PR) and the velocity-time integral (VTI), mean velocity (MV), peak systolic velocity (PSV), and end-diastolic velocity (EDV) of the spectral Doppler tracing were determined. The pulsatility index (PI = [PSV - EDV] / MV) and the resistive index (RI = [PSV - EDV] / PSV) were calculated.19 Blood flow is commonly calculated as Flow = \( \pi (D/2)^2 \times VTI \times PR \). However, in this study, the product of VTI and PR (VTI \times PR) was calculated as a surrogate of vascular blood flow; the vascular diameter (D) was not included in this calculation and was assessed separately, because D was measured in a different vessel. Blood collection, pressure and ECG recordings, determination of cardiac output, echocardiography, and vascular ultrasonography were performed in consecutive order at each data collection period.

**Adverse effects:** In both experiments, the horses were monitored for potential adverse effects during drug administration periods, and for a duration of 8 hours after the last dose of diltiazem. Attention was focused on the development of bradycardia and other cardiac rhythm disturbances, hypotension, peripheral edema, coughing, increase in respiratory rate and signs of respiratory distress, colic, diarrhea, neurologic abnormalities, and laminitis. A serum chemistry profile\(^9\) was performed before and 24 hours after the dose-finding study.
**Determination of plasma drug concentrations (Cp):** Blood samples were collected into evacuated glass tubes containing EDTA\(^{f}\). All samples were centrifuged at 4000g for 15 min. Plasma then was transferred into cryovials\(^{g}\), frozen, and stored at -80\(^\circ\)C until analysis. Plasma diltiazem plasma concentrations were determined by a commercial laboratory\(^{f}\) using gas chromatography.\(^{20}\) The lower limit of detection was 5.0 ng/mL.

**Statistical analysis:** One-way repeated-measures analysis of variance (ANOVA) with Dunnett’s post-hoc test was used to compare variables across treatment periods to baseline\(^{u}\). Friedman repeated-measure ANOVA on ranks was used for nonparametric variables. The serum biochemical results before and after diltiazem treatment were compared using a paired t-test\(^{u}\). The relationships between Cp and drug dosage, PR interval, and MAP, respectively, were examined by regression analyses using linear mixed model techniques with random intercept and random slope\(^{v}\). The first-order autoregressive structure was identified as the best fitting covariance structure by using the likelihood methodology. The level of significance was defined as \( P < 0.05 \).

**Results**

**Dose-finding study:** A significant quadratic relationship was identified between drug dosage and plasma concentration, \( \text{Cp} = 29.96 + [899.58 \times \text{dose}] + [-268.58 \times \text{dose}^2] \); Figure 1.1). Considerable individual variation in plasma concentrations was noted, especially at the 2 highest doses. Diltiazem caused a significant dose-dependent decrease in mean arterial blood pressure (Figure 1.2) and slight, but not significant prolongation in PR interval (repeated-measures ANOVA with Dunnett’s test, \( 1-\beta = 0.5 \) with \( \alpha = 0.05 \)).

Mean arterial blood pressure (MABP) was inversely and linearly related to plasma
diltiazem concentrations, with a mean decrease in MABP of 0.0304 mmHg for every 1 ng/mL increase in Cp (MABP = - 0.0304 Cp + 109.15; p < 0.0001). The relationship between PR interval and Cp was not statistically significant. The AR and VR increased with increasing dosages of diltiazem from 0.05 mg/kg to 0.925 mg/kg, and the frequency of AV blocks decreased (Figure 1.3). At higher diltiazem dosages (1.425 mg/kg and 1.925 mg/kg) the frequency of AV blocks increased again (leading to a decrease in VR).

Sinus arrhythmia was observed in 3 horses. At the 2 highest doses, plasma diltiazem concentrations averaged 720 ± 43 ng/mL and 874 ± 113 ng/mL, respectively. The desired effects of diltiazem (decrease of mean carotid blood pressure of at least 10 mmHg, prolongation of PR interval, second degree AV block) were achieved at doses above 0.925 mg/kg, corresponding to plasma diltiazem concentrations higher than 646 ± 246 ng/mL.

**Hemodynamic study:** Plasma diltiazem concentrations did not differ significantly among the treatment periods D1, D1.5, and D2, respectively (Table 1.1). The PR interval was significantly higher during treatment periods D1 and D1.5 (Figure 1.4). The VR increased insignificantly after diltiazem administration versus baseline. The rise in AR was more pronounced, consistent with the increased frequency of AV block after administration of diltiazem (Figure 1.5). Both frequency of second degree AV block and the degree of sinus arrhythmia increased during administration of diltiazem. Sinus arrhythmia was most pronounced during D1.5 and D2. However, considerable individual variation was observed in the effects on the sinus node and AV node among horses. Diltiazem caused high-degree sinus arrhythmia in 2 horses, whereas second degree AV block was predominant in 3 horses. One horse developed a large number of AV blocks.
during D1 and D1.5, and predominantly sinus arrhythmia during D2. In 1 horse neither sinus arrhythmia nor second degree AV block were noted.

The effects of diltiazem on hemodynamic parameters and indices of LV function are summarized in Table 1.1. Diltiazem caused SAP, DAP, and MAP to decrease, whereas PAP, RAP, and LVEDP increased significantly compared to baseline. The LV \( +\text{dp/dt}_{\text{max}} \) and \( -\text{dp/dt}_{\text{max}} \) decreased, FS decreased, and \( \tau \) increased. Significant decreases in SVR and PVR were detected. Changes in CO and SV were not significant throughout the study, but SV showed a tendency to decrease during D2. Statistical power was inadequate to detect small changes in these values (\( 1-\beta = 0.31 \) for CO and 0.05 for SV, with \( \alpha = 0.05 \)). The diameter of the brachial artery and VTI × PR in the common interosseus artery increased significantly during all treatment periods (Figures 1.6 and 1.7), whereas RI and the PI decreased (Figure 1.8). These findings were consistent with peripheral vasodilatation and increased limb blood flow.
Figure 1.1: Dose-finding study. Linear increase in diltiazem plasma concentrations with geometrical dosing (mean ± SD). Plasma diltiazem concentrations were determined 7.5 min after each diltiazem administration.
Figure 1.2: Dose-finding study. Diltiazem produced a dose-dependent decrease in mean arterial blood pressure (MAP) (mean ± SD; * significantly different from baseline, repeated-measures ANOVA with Dunnett’s test, p < 0.05). Measurements were performed 7.5 min after each diltiazem administration.
Figure 1.3: Dose-finding study. A dose-dependent increase in atrial rate (AR) and ventricular rate (VR) was observed with increasing doses of diltiazem. The difference between AR and VR equals the frequency of second degree AV blocks. SA indicates the occurrence of sinus arrhythmia in 3 horses (mean ± SD; * significantly different from baseline, repeated-measures ANOVA with Dunnett’s test, p < 0.05). Measurements were performed 7.5 min after each diltiazem administration.
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<sup>a</sup> values followed by the same letter do not differ significantly (Friedman repeated-measures ANOVA on ranks, p = 0.964)
<sup>b</sup> significantly different from baseline values (one-way repeated-measures ANOVA, Dunnett’s post-hoc, p < 0.05)

Table 1.1: Hemodynamic study: Effects of diltiazem on hemodynamic parameters and indices of left ventricular function (all values reported as mean ± SD)
Figure 1.4: Hemodynamic study. Diltiazem produced a slight but significant increase in PR interval (mean ± SD; * significantly different from baseline, repeated-measures ANOVA with Dunnett’s test, p < 0.05).
Figure 1.5: Hemodynamic study. Similar to the dose-finding study, the atrial rate (AR) and, to a lesser extent, the ventricular rate (VR) increased after administration of diltiazem. Considerable individual variation in AR and VR was present during the diltiazem treatment periods. The difference between AR and VR represents the frequency of AV blocks observed at each treatment period. SA indicates the occurrence of pronounced sinus arrhythmia in 3 horses (mean ± SD; * significantly different from baseline, repeated-measures ANOVA with Dunnett’s test, p < 0.05).
Figure 1.6: The diameter of the brachial artery was significantly increased during all treatment periods (mean ± SD; * significantly different from baseline, repeated-measures ANOVA with Dunnett’s test, p < 0.05).
Figure 1.7: The product of VTI and PR, a surrogate of the blood flow in the common interosseus artery, increased significantly during treatment periods (mean ± SD; * significantly different from baseline, repeated-measures ANOVA with Dunnett’s test, p < 0.05).
Figure 1.8: Diltiazem caused a significant decrease in resistive index (RI) and pulsatility index (PI) of blood flow in the common interosseus artery (mean ± SD; repeated-measures ANOVA with Dunnett’s test, p < 0.05).
Adverse effects: Two horses developed bouts of high-grade sinus arrest with severe hypotension and clinical signs of near-syncope during the diltiazem administration period. In the first horse, these effects occurred in both the dose-finding and the hemodynamic study at cumulative dosages of 1.8 mg/kg and 1 mg/kg, leading to plasma diltiazem concentrations of 1500 ng/mL and 760 ng/mL, respectively. On both occasions, drug administration was discontinued immediately and treatment with lactated Ringer’s solution, calcium gluconate, and dobutamine was initiated. This horse was excluded from final data analysis in both studies due to incomplete data. The second horse was used in the hemodynamic study only. It showed similar but somewhat milder adverse effects at a diltiazem dosage of 2 mg/kg and a plasma diltiazem concentration of 930 ng/mL. No emergency treatment was necessary and data collection was not affected by the event. This horse therefore was included in the final data analysis. Both horses developed marked sinus tachycardia immediately after the events, and the clinical signs of hypotension resolved within 1 min. No signs of bradycardia or tachycardia, rhythm disturbances, or hypotension occurred during the 8-hour monitoring period after the end of the trial. No signs of laminitis, respiratory, gastrointestinal, or neurologic compromise were noted during or after drug administration. No behavioral changes were observed.

Significant increases in liver enzyme activities were not detected 24 h after the dose-finding study. Statistically significant decreases were found in alkaline phosphatase activity (from 133 ± 14 U/L to 128 ± 14 U/L; p = 0.022, paired t-test [normal range: 80 – 187 U/L]), gamma-glutamyltransferase (from 11 ± 3 U/L to 9 ± 2 U/L; p = 0.005, paired t-test [normal range: 9 – 24 U/L]), and serum urea nitrogen concentration (from 22 ± 4 mg/dL to 19 ± 2 mg/dL; p = 0.014, paired t-test [normal range: 13 – 27 mg/dL]).
A statistically significant increase in serum phosphate concentration was detected (from 2.3 ± 1.0 mg/dL to 3.4 ± 0.5 mg/dL; p < 0.001, paired t-test [normal range: 1.2 – 4.8 mg/dL]).

Discussion

Diltiazem is a selective inhibitor of voltage-sensitive L-type calcium channels that control calcium influx into vascular smooth muscle cells and cardiac myocytes and play a major role in excitation-contraction coupling, control of sinoatrial pacemaker activity, and regulation of AV conduction in the AV node. Vascular effects of diltiazem include vasodilatation and reduction of systemic vascular resistance with subsequent lowering of systemic blood pressure. In the present study, the expected decrease in arterial blood pressure was shown to be dose-dependent and was inversely related in a linear fashion to plasma diltiazem concentrations. Dilatation of the brachial artery and decreased RI and PI in the common interosseus artery clearly demonstrated the vasodilatory effects of diltiazem. Because the vascular diameter and the indices of blood flow were determined in 2 distinct (but adjacent) vessels, a surrogate of peripheral blood flow (VTI × PR) was calculated, thereby ignoring changes in the vascular diameter. However, assuming that the vascular effects of diltiazem were similar in both vessels, and considering the concomitant increase in D and VTI × PR, we inferred that the peripheral blood flow (flow = \pi(D/2)^2 \times VTI \times PR) to the limb increased following administration of diltiazem. Effects on other peripheral arterial beds were not examined, but likely are similar.
In the intact animal, the net effects of diltiazem on cardiac function are dictated by the sum of the direct cardiac effects and the opposing indirect effects resulting from baroreceptor reflexes triggered by a decrease in systemic blood pressure.\textsuperscript{4,12,21-25} In the present study, increase in atrial rate, and to a lesser degree in ventricular rate, were consistent with increased sympathetic drive due to baroreceptor reflex activation, similar to findings in normal dogs and humans.\textsuperscript{23,26-28} The direct effects on sinus node and AV node varied among horses and were expressed by the occurrence of sinus arrhythmia, high-degree sinus arrest, slight prolongation of the PR interval, and second degree AV block, despite the presence of increased sympathetic tone. The inhibitory effect on AV nodal conduction is the basis for the use of calcium channel blockers in the treatment of supraventricular arrhythmia. Diltiazem typically acts in a frequency-dependent manner, hence the effects of diltiazem on the AV node are more pronounced at faster pacing rates and the slowing of AV conduction is enhanced in the presence of supraventricular arrhythmias.\textsuperscript{22,29} This study does not allow any conclusions to be made about the frequency-dependent effects of diltiazem in horses. However, it is likely that effective dosages for heart rate control in horses with AF will be lower than those used in the present study in normal horses. The dosages used in this study are considerably higher than those used clinically in dogs and humans with supraventricular arrhythmias. For acute heart rate control in humans with AF, an initial dose of 0.25 mg/kg IV over 2 min is recommended, followed by a second dose of 0.35 mg/kg IV or an infusion of 5 to 15 mg/h for up to 24 h to effect.\textsuperscript{4,30,31} Mean plasma diltiazem concentrations between 80 and 290 ng/mL are required to produce a 20 to 40% reduction in heart rate in these patients.\textsuperscript{31} In dogs with supraventricular arrhythmias, multiple boluses of 0.05 to 0.25 mg/kg IV are
administered every 5 min to effect.\textsuperscript{5,12} Total cumulative doses of 0.44 to 0.94 mg/kg leading to mean plasma concentrations of 60 to 120 ng/mL were found to be optimal for treatment of iatrogenic AF in dogs.\textsuperscript{6}

The effects of calcium channel blockers on systolic and diastolic ventricular performance in intact animals are complex and depend on the specific drug, the dose, the experimental design, existing pathologic conditions, concomitant effects of other cardiovascular drugs or anesthetics, adrenergic tone, and changes in loading conditions.\textsuperscript{23} Diltiazem exhibits negative inotropic actions on isolated myocardial tissue \textit{in vitro}\textsuperscript{32,33} and after intracoronary administration \textit{in vivo}.\textsuperscript{23} The slight decreases in +dp/dt\textsubscript{max} and FS found in the present study were consistent with decreased LV systolic function under direct influence of diltiazem. Reflex increase in adrenergic tone and changes in ventricular loading probably attenuated the direct negative inotropic effects of diltiazem.\textsuperscript{23} The degree of these effects was considered clinically irrelevant in the present study. However, decreased LV systolic function may be more pronounced in the presence of chronic volume overload and heart failure, and might therefore have important clinical implications.\textsuperscript{28,34} The use of calcium channel blockers generally is contraindicated in patients with untreated heart failure. However, effective treatment of AF in patients with congestive heart failure is clinically important, and rate control with diltiazem or a combination of digoxin and diltiazem was shown to be beneficial and relatively safe in humans with heart failure associated with AF and high ventricular response rate.\textsuperscript{9,35} In this context, the negative inotropic effects of diltiazem may be offset by reductions in heart rate and peripheral vascular resistance, leading to increased coronary perfusion,
reduced myocardial oxygen consumption, improved ventricular performance, and often to a reduction of clinical signs.\textsuperscript{9,35}

Diltiazem also is used for treatment of patients with diastolic heart failure caused by LV hypertrophy, hypertension, or ischemic heart disease.\textsuperscript{4,36-40} In the diseased ventricle, calcium channel blockers enhance ventricular relaxation, most likely due to improved myocardial blood flow and favorable effects on ventricular loading conditions.\textsuperscript{36-40} Conversely, calcium entry blockade directly impairs LV relaxation in conscious dogs and humans with normal ventricular function.\textsuperscript{41,42} This direct effect is closely linked to the negative inotropy of calcium channel blockers.\textsuperscript{41} It may be attenuated or even reversed during systemic administration due to concomitant reflex sympathetic stimulation,\textsuperscript{41,43} changes in inotropic state,\textsuperscript{43,44} effects on ventricular loading conditions,\textsuperscript{45-47} and alterations in loading sequence.\textsuperscript{47-49} The variables used to evaluate LV relaxation in the present study, \(-\frac{dp}{dt}_{\text{max}}\) and \(\tau\),\textsuperscript{50-52} indicated that diltiazem caused a slight, but clinically irrelevant decrease in ventricular relaxation in horses with normal ventricular function.

The increases in RAP and LVEDP indicated increased left and right heart filling pressures. Similar increases were reported in dogs, swine, and humans after IV administration of calcium channel blockers.\textsuperscript{42,53,54} Although calcium channel blockers relax arterial smooth muscle, they seem to have little effect on most venous beds, and do not affect preload significantly.\textsuperscript{21,55} Conversely, baroreceptor reflex-mediated sympathetic constriction of venous capacitance vessels may lead to an increase in venous return. Concurrent impairment of ventricular contractile or diastolic function, tachycardia, and the resulting incomplete emptying of the ventricles may cause an increase in end-diastolic volume and increase in filling pressures.\textsuperscript{42}
The elimination half-life of diltiazem is approximately 3 h (2.2–4 h) in dogs\textsuperscript{56-58} and 2–6 h in humans.\textsuperscript{4, 22, 31} The pharmacokinetic properties of diltiazem in horses were unknown at the time our study was conducted. Assuming a half-life similar to that of dogs and humans, the cumulative dosing regimen used in the hemodynamic study was expected to produce increasing plasma diltiazem concentrations, because the amount of diltiazem eliminated over the time of drug administration would be minimal.\textsuperscript{6} However, plasma diltiazem concentrations did not increase significantly between the time points D1, D1.5, and D2 of the hemodynamic study. This finding indicates that the plasma half-life of diltiazem may be considerably shorter in horses than reported in dogs and humans, either due to higher rate of elimination or redistribution into extravascular compartments. These findings are consistent with results of a pharmacokinetic study performed by the authors, indicating that diltiazem after IV administration to healthy horses has a median terminal half-life of 1.5 h (Chapter 2). Differences in drug effects between the dose-finding and the hemodynamic study, and among D1, D1.5, and D2, respectively, may be explained by differences in dosing intervals, time-dependent actions, presence of active metabolites, or redistribution of the drug. Metabolite concentrations were not determined in the present study.

Diltiazem is considered a safe and effective drug to rapidly lower heart rate in humans with AF and atrial flutter. SA or AV nodal disturbances, preexisting hypotension, and most forms of ventricular tachycardia are considered contraindications for the use of calcium channel blockers.\textsuperscript{4, 7, 21, 22} Although ventricular rate control is important in patients with supraventricular tachyarrhythmia and heart failure, diltiazem should be used with caution in patients with severe ventricular systolic dysfunction. The most prominent
adverse effects of calcium antagonists in humans, dogs, and cats are bradycardia, hypotension, and rhythm disturbances.\textsuperscript{21, 59} Whereas similar adverse effects also were found in the present study, other known adverse effects, such as gastrointestinal distress, constipation, central nervous system effects, peripheral edema, coughing, wheezing, pulmonary edema, or increases in liver enzyme activities were not observed after short-term administration of diltiazem. The decrease in serum alkaline phosphatase, gamma-glutamyl transferase, and serum urea nitrogen concentration as well as the increase in serum phosphate concentration in the present study were not considered clinically relevant. The IV use of diltiazem in healthy horses therefore is considered relatively safe, but the dosage is critical and its use may be limited by hypotension due to vasodilatation and transient high-degree sinus arrest leading to severe bradycardia. Due to the frequency-dependence of diltiazem’s effects, effective dosages for heart rate control in horses with AF may be considerably lower than those used in this study, and reduced dosages may reduce the risk of adverse effects. Diltiazem should be administered to effect under ECG and blood pressure monitoring. No inferences can be made regarding the long-term use of diltiazem in horses. Specific diltiazem antagonists are not available. Treatment of adverse effects consists of correction of hypotension and arrhythmia by IV administration of fluids, calcium gluconate or calcium chloride, inotropic agents (dobutamine), and vasopressors (norepinephrine, phenylephrine).\textsuperscript{4}

In conclusion, the results of this study in normal horses in sinus rhythm show that cardiac effects of diltiazem, at dosages between 1 and 2 mg/kg IV, include intermittent depression of the sinus and AV nodes and mild impairment of systolic and diastolic LV function. Vascular effects of diltiazem include arterial vasodilatation and decreased
systemic vascular resistance leading to reduced afterload. The decrease in arterial blood pressure seemingly invokes the baroreceptor reflex, causing sympathetic activation that increases sinus node rate, and presumably blunts the depressive effects of diltiazem on myocardial and nodal tissues. Due to its inhibitory effects on AV nodal conduction, diltiazem is likely to prove useful for heart rate control in horses with AF, presumably at dosages lower than those used in our study. Additional studies are required to determine the pharmacokinetic profile of diltiazem, the potential frequency-dependence of diltiazem effects on nodal tissues, and the effects and safety of combined treatment with diltiazem and quinidine in horses with AF.
Footnotes

a Bupivacaine HCl, Sensorcaine, AstraZeneca LP, Wilmington, DE.

b Angiocath 14G IV catheter, Becton Dickinson, Sandy, UT.

c Intracath 19G/30.5cm IV catheter, Becton Dickinson, Sandy, UT.

d Pressure Monitoring Kit (PX36N), Edwards Lifesciences LCC, Irvine, CA.

e Diltiazem HCl, D-2521, Sigma Chemical Co, St. Louis, MO; in Sterile Water for Injection, Vedco Inc., St. Joseph, MO.

f Ponemah Physiology Platform, Gould Instrument Systems Inc., Valley View, OH.

g 8F Percutaneous catheter introducer, Maxxim Medical, Athens, TX.

h 7F 110 cm Swan-Ganz thermodilution catheter, Edwards Lifesciences, Irvine, CA.

i Intramedic polyethylene 240 tubing (90 cm), Clay Adams, Parsippany, NJ.

j 9F Percutaneous catheter introducer, Maxxim Medical, Athens, TX.

k 8F 180-cm Mikro-Tip catheter (Model SPR-872), Millar Instruments Inc, Houston, TX.

l Marquette Series 7010 Monitor, Marquette Electronics Inc., Milwaukee, WI.

m 0.9% Sodium Chloride Injection USP, Baxter, Deerfield, IL.

n Quinidine Gluconate Q-5001, Sigma Chemical Co, St Louis, MO.

o PHD2000 Infusion Pump, Harvard Apparatus Inc., Holliston, MA.

p Megas ES, Biosound Esaote, Indianapolis, IN.

q Roche/Hitachi 911, Roche Diagnostics Corporation, Indianapolis, IN.

r BD Vacutainer K3 EDTA, 10 mL, Becton Dickinson, Franklin Lakes, NJ.

s Fisherbrand Cryovial 4.0mL, Fisher Scientific, Pittsburg, PA.

l National Medical Services, Willow Grove, PA.
References


CHAPTER 2

PHARMACOKINETICS OF THE CALCIUM CHANNEL BLOCKER DILTIAZEM AFTER A SINGLE INTRAVENOUS DOSE IN HORSES

Diltiazem is a calcium-channel blocker, which is used in humans and dogs for the treatment of supraventricular arrhythmias and for heart rate control in patients with atrial fibrillation, atrial flutter, and atrioventricular nodal reentry.\textsuperscript{1,2} Atrial fibrillation (AF) is the most common arrhythmia affecting performance in horses.\textsuperscript{3} In contrast to humans and dogs with AF, the resting heart rate in horses with AF is usually within normal limits and no underlying structural heart disease can be identified. Quinidine sulfate, the preferred drug for conversion of atrial fibrillation to sinus rhythm in horses, often increases impulse conduction through the atrioventricular (AV) node and results in an increased ventricular response rate.\textsuperscript{3-5} Sustained, rapid tachycardia due to an accelerated ventricular response is the most common severe side effect of quinidine therapy, affecting over 50% of all treated horses.\textsuperscript{3,4} The underlying mechanisms leading to an increase in ventricular response rate during quinidine treatment include vagolytic drug effects and increased organization of atrial impulses causing a decrease in AV nodal concealment.\textsuperscript{6} An increase in sympathetic tone secondary to development of drug-related abdominal pain may further facilitate AV nodal conduction. Traditionally, digoxin has been used to control heart rate in horses with AF.\textsuperscript{3} However, digoxin has a narrow therapeutic index, a
delayed onset of action, and is only partially effective for heart rate control in the presence of elevated sympathetic tone.\textsuperscript{7-9} Recently, we proposed that diltiazem may be used as an alternative to digoxin for heart rate control in horses treated with quinidine (Chapter 1).\textsuperscript{10} Our studies investigated the hemodynamic effects of diltiazem administered to healthy horses and demonstrated that the cardiac effects of diltiazem, at doses between 1 and 2 mg/kg IV, include intermittent depression of the sinus and AV nodes, mild impairment of systolic and diastolic left-ventricular function, arterial vasodilation, decreased systemic vascular resistance, and decreased systemic blood pressures.\textsuperscript{10} Due to its inhibitory effects on AV nodal function, diltiazem may prove useful for controlling heart rate in horses with AF treated with quinidine.

The aim of the present study was to identify and characterize the plasma concentration time relationship of diltiazem and its metabolites after a single intravenous dose in healthy horses. The results of this study should facilitate the development of dosing guidelines for diltiazem in horses.

**Material and Methods**

*Horses:* Eight Standardbred horses (5 geldings, 3 mares) with a mean age of 11 years (range: 6 to 16 years) and a mean body weight of 481 kg (range: 426 to 563 kg) were the subjects of these experiments. All horses were considered healthy based upon physical examination, cardiac auscultation, electrocardiogram, echocardiogram, complete blood count, serum chemistry profile, and plasma fibrinogen concentration. The horses were dewormed and vaccinated against equine influenza, tetanus, equine herpes virus, eastern and western equine encephalomyelitis, rabies, and West Nile virus. None of the horses
received medications during the 2 weeks preceding entry into the study. The studies were approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.

*Study design:* The skin over both jugular veins was clipped, surgically prepared, and infiltrated with 2% bupivacaine HCl. Two 14G catheters were aseptically placed in each jugular vein for administration of the drug (left jugular vein) and for blood sampling (right jugular vein). An aqueous solution of diltiazem HCl (5 mg/mL) was freshly prepared before each experiment. Diltiazem HCl was administered intravenously over 5 minutes at a dose of 1 mg/kg (equivalent to 0.919 mg/kg diltiazem base) at a constant rate. Venous blood samples were collected before diltiazem administration and 0, 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480, 720, and 1440 minutes after the end of the infusion period. Samples were collected into tubes containing EDTA and were stored on ice (maximum 20 minutes) until centrifugation. Blood samples were centrifuged at 2,000g and 4°C for 10 minutes, plasma was apportioned into 4-mL aliquots, transferred into cryovials, snap-frozen in liquid nitrogen, and kept on dry ice until transferred to a freezer (maximum 6 hours). The samples were then stored at -80°C until analyzed.

*Adverse effects:* The horses were monitored for adverse effects during and for 24 hours after drug administration. Attention was focused on the development of cardiac rhythm disturbances, peripheral edema, coughing, increase of respiratory rate, signs of respiratory distress, colic, diarrhea, neurologic abnormalities, and laminitis.

*Determination of plasma drug concentrations (Cp):* Plasma concentrations of diltiazem and its major metabolite desacetyldiltiazem were determined using high-
performance liquid chromatography (HPLC). Stock standard solutions of diltiazem\(^{h}\) and desacetyldiltiazem\(^{h}\) were dissolved in water\(^{i}\) for preparation of calibrators at a concentration of 0.0010 g/L. Stock standard solutions of diltiazem\(^{c}\) and desacetyldiltiazem\(^{h}\) were prepared in water at a concentration of 0.0010 g/L for preparation of positive control samples. All standard solutions were stored in the dark at 2 – 8 °C.

Calibrators of diltiazem and desacetyldiltiazem were prepared in drug-free, pooled equine plasma at concentrations of 5.00, 10.0, 20.0, 30.0, 50.0, 100, 200, and 300 ng/mL. Positive control samples of diltiazem and desacetyldiltiazem were prepared in drug-free, pooled plasma at concentrations of 6.00, 50.0, 150, and 300 ng/mL. Both calibrators and control samples were diluted with 1.0 mL of 0.1M ammonium acetate (pH 5.0), and 1.0 mL of drug-free horse plasma\(^{j}\) was added. All calibrators and controls were prepared on the day of analysis. All reagents used were ACS reagent grade or better.

Aliquots of 0.5 mL of the experimental samples were diluted with drug-free horse plasma to produce a total volume of 1.0 mL. These plasma samples were then diluted with 1.0 mL of 0.1M ammonium acetate (pH 5.0). Smaller aliquots of the experimental samples were used when the initial analyses indicated that drug concentrations exceeded the upper limit of the calibration curve. Dilution factors were adjusted accordingly for calculation of the final drug plasma concentrations.

Prior to sample preparation, the stopcocks and collection needles of the vacuum manifold were rinsed sequentially with 4 mL each of water and methanol\(^{i}\) and the SPE columns\(^{b}\) were preconditioned with 2.0 mL each of acetonitrile\(^{i}\) and 0.1 M ammonium acetate (pH 5.0). Diluted plasma samples (calibrators, positive control samples, and
Experimental samples) were applied to conditioned columns in not less than 2 minutes. The columns were then washed with 2.0 mL of water, 2.0 mL of acetonitrile/water (20:80, v/v), and 1.0 mL of acetonitrile/water (40:60, v/v). The columns were dried under full vacuum for 5 minutes. The analytes were eluted with 1.0 mL of elution solvent (methanol/0.1M ammonium acetate, 95:5, v/v). The vacuum was briefly increased and the analytes were eluted with a 0.75 mL-aliquot of elution solvent. The eluates were combined and then evaporated to dryness at 45 ± 5 °C under a stream of nitrogen. The residues were dissolved in 250 µL of mobile phase by sonicatation for 30 seconds and brief vortex-mixing. The dissolved residues were transferred to autosampler vials and 75 µL aliquots were analyzed by HPLC.

The liquid chromatographic system consisted of a reciprocating pump, ultraviolet absorption detector, and autosampler. The detector was set at a wavelength of 237 nm and the detector output was recorded on a reporting integrator. Chromatograms were recorded at a chart speed of 0.25 cm/min. The chromatographic column was 100 mm x 3.0 mm packed with 3.5 µm chromatographic medium. A 20 mm x 2 mm guard column was packed with 30-40 µm particle size pellicular reverse phase chromatography medium. The column temperature was maintained at 30°C. The mobile phase consisted of acetonitrile and 0.05 M ammonium acetate containing 0.2% triethylamine adjusted to pH 5.0 with glacial acetic acid (28:72, v/v). The flow rate was 0.75 mL/min and the mobile phase composition was unchanged throughout each run.

Calibration curves for both diltiazem and desacetyldiltiazem were constructed for concentration ranges of 5.00 to 50.0 ng/mL (applied for samples with drug concentrations < 50 ng/mL) and 5.00 to 300 ng/mL (applied for samples with drug concentrations 5.00 to 300 ng/mL (applied for samples with drug concentrations
≥ 50 ng/mL), respectively. The calibration curves showed excellent linearity between 5.00 and 300 ng/mL ($r^2 > 0.99$). The accuracy and precision of the assay were determined by replicate assay of control samples of known concentration. Accuracy was expressed bias, calculated as the percent ratio between the absolute measurement error and the nominal concentration of each control sample and summarized as the 95% confidence interval of the mean of all measurements for each concentration. Precision was expressed as the intra-day and inter-day coefficient of variation, using estimates for the within-day and between-day variability determined by one-way analysis of variance. The lower limit of quantitation for diltiazem and desacetyldiltiazem was 5.0 ng/mL. At the time of analysis, analytical standards for diltiazem metabolites other than desacetyldiltiazem were not available. Quantification of the second, unidentified substance, assumed to be a putative metabolite, was based on the diltiazem calibration curves.

**Pharmacokinetic calculations and data analysis:** The time course of plasma diltiazem concentrations was visually assessed by use of a semi-logarithmic graphic presentation. Data for each horse were then analyzed by nonlinear least squares regression analysis with relative weighting ($1/y^2$), using a monoexponential and a biexponential decay model, respectively. The preferred model was chosen based on the Akaike’s information criterion (AIC). A biexponential equation $C_p(t) = R \cdot e^{-\alpha t} + S \cdot e^{-\beta t}$, representing a two-compartment model with first-order drug elimination, best described the data for each horse. Because drug administration was performed as a short-term continuous rate infusion over 5 min, the intercepts $R$ and $S$ were corrected according to the following equations: $A = [R \cdot \text{Dose} \cdot \alpha] / [k_0 \cdot (1 - e^{-\alpha T})]$, $B = [S \cdot \text{Dose} \cdot \beta] / [k_0 \cdot (1 - e^{-\beta T})]$, where
Dose is the total administered amount of diltiazem base, T is the infusion time (5 min), and \( k_0 \) is the zero-order infusion rate (\( k_0 = \text{Dose} / T \)).

Pharmacokinetic variables were then calculated using the corrected intercepts (A, B) and slopes (\( \alpha, \beta \)) of the best-fit equation\(^8\) of each horse.\(^12\) All dosages were expressed as diltiazem base for the calculations. The plasma diltiazem concentration at time 0 (\( C_0 \)) was calculated as \( C_0 = A + B \). The distribution half-life (\( t_{1/2\alpha} \)) and the elimination (terminal) half-life (\( t_{1/2\beta} \)) were calculated as \( t_{1/2\alpha} = 0.693/\alpha \) and \( t_{1/2\beta} = 0.693/\beta \), respectively. The area under the plasma concentration versus time curve (AUC) was calculated as \( \text{AUC} = A/\alpha + B/\beta \). The area under the first moment curve (AUMC) was calculated as \( \text{AUMC} = A/\alpha^2 + B/\beta^2 \). Total plasma clearance (\( \text{Cl}_t \)) was calculated as \( \text{Cl}_t = \text{Dose} / \text{AUC} \). The volume of distribution of the central compartment (\( V_c \)), the volume of distribution during the terminal phase (\( V_{d\beta} \)), and the volume of distribution at steady state (\( V_{ss} \)) were calculated as follows: \( V_c = \text{Dose} / (A + B) \), \( V_{d\beta} = \text{Dose} / (\text{AUC} \cdot \beta) \), and \( V_{ss} = \text{Dose} \cdot \text{AUMC} / \text{AUC}^2 \). Mean residence time (MRT) was calculated as \( \text{MRT} = \text{AUMC} / \text{AUC} \). The distribution rate constants (\( k_{12}, k_{21} \)) and the elimination rate constant (\( k_{10} \)) were calculated as follows: \( k_{21} = (A \cdot \beta + B \cdot \alpha) / (A + B) \), \( k_{10} = \alpha \cdot \beta / k_{21} \), and \( k_{12} = \alpha + \beta - k_{21} - k_{10} \).

The concentrations of diltiazem and desacetyldiltiazem were plotted over time and the areas under the plasma concentration time curve were determined for the parent drug and its metabolite using the trapezoid method (\( \text{AUC}_t \)).\(^5\) The ratio of the \( \text{AUC}_t \) of desacetyldiltiazem to the \( \text{AUC}_t \) of the parent drug was calculated and normalized for the difference in molecular weight (\( \text{MW}_{\text{DZ}} = 414.53 \text{ g/mol} \), \( \text{MW}_{\text{desacetyl-DZ}} = 372.49 \text{ g/mol} \)). No quantitative analysis was performed for the second unidentified metabolite.
Results

_Determination of plasma drug concentrations by HPLC:_ The accuracy and precision of the assay are summarized in Table 2.1. The mean bias was less than 10% at all concentrations for both, diltiazem and desacetyldiltiazem. The within-day and between-day coefficients of variation were below 15% except for diltiazem at a nominal concentration of 6.0 ng/mL.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Parameter</th>
<th>Nominal concentration (ng/mL)</th>
<th>6.0&lt;sup&gt;a&lt;/sup&gt;</th>
<th>50.0&lt;sup&gt;a&lt;/sup&gt;</th>
<th>50.0&lt;sup&gt;b&lt;/sup&gt;</th>
<th>150&lt;sup&gt;b&lt;/sup&gt;</th>
<th>300&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>Diltiazem</td>
<td>N</td>
<td></td>
<td>21</td>
<td>20</td>
<td>20</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Mean (ng/mL)&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>5.8 to 6.5</td>
<td>45.5 to 47.6</td>
<td>45.7 to 47.5</td>
<td>142.4 to 148.4</td>
<td>288.2 to 305.9</td>
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<tr>
<td></td>
<td>%Bias&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>-3.8 to 7.6</td>
<td>-9.1 to -4.7</td>
<td>-8.6 to -5.0</td>
<td>-5.1 to -1.0</td>
<td>-4.0 to 2.0</td>
</tr>
<tr>
<td></td>
<td>%CV</td>
<td>Within days</td>
<td>10.0</td>
<td>3.8</td>
<td>3.7</td>
<td>4.5</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Between days</td>
<td>19.8</td>
<td>8.3</td>
<td>6.0</td>
<td>5.8</td>
<td>10.1</td>
</tr>
<tr>
<td>Desacetyl-</td>
<td>N</td>
<td></td>
<td>21</td>
<td>19</td>
<td>19</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>diltiazem</td>
<td>Mean (ng/mL)&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>5.8 to 6.2</td>
<td>47.8 to 49.1</td>
<td>47.4 to 48.7</td>
<td>144.9 to 150.8</td>
<td>291.5 to 302.8</td>
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<tr>
<td></td>
<td>%Bias&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>-3.4 to 3.4</td>
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<td>-5.3 to -2.6</td>
<td>-3.4 to 0.6</td>
<td>-2.8 to 1.0</td>
</tr>
<tr>
<td></td>
<td>%CV</td>
<td>Within days</td>
<td>4.2</td>
<td>3.5</td>
<td>2.4</td>
<td>3.2</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Between days</td>
<td>14.0</td>
<td>3.7</td>
<td>4.5</td>
<td>7.0</td>
<td>7.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> concentration determined from calibration curve: 5.00 - 50.0 ng/mL  
<sup>b</sup> concentration determined from calibration curve: 5.00 - 300 ng/mL  
<sup>c</sup> reported as 95% confidence interval of the mean

Table 2.1: Accuracy (expressed by bias) and precision (expressed by intra- and inter-day coefficient of variation) for diltiazem and its major metabolite desacetyl-diltiazem determined by replicate assay of control samples of known concentration by HPLC.
**Pharmacokinetics:** Median peak plasma diltiazem concentration after intravenous administration of 1 mg/kg diltiazem HCl was 727 ng/mL (range 539 to 976 ng/mL). Plasma diltiazem concentration fell below the lower limit of quantitation of 5.0 ng/mL within 12 hours in 7 horses and within 24 hours in one horse. The time courses of diltiazem and desacetyldiltiazem plasma concentrations are displayed in Figure 2.1. A third substance was detected, but could not be identified due to the lack of an authentic standard. This substance was assumed to be a putative metabolite, due to the similar molar absorptivity compared to diltiazem and the time course of its plasma concentrations, characterized by an increase over the first 3 hours after drug administration followed by a decrease parallel to the diltiazem and desacetyldiltiazem concentrations. Pharmacokinetic parameters describing the time course of diltiazem plasma concentration are summarized in Table 2.2. The median AUC\textsubscript{t} of desacetyldiltiazem was 5070 ng·min/mL (3460 to 8740 ng·min/mL), the median AUC\textsubscript{t} of diltiazem was 63,300 ng·min/mL (range 47,100 to 84,500 ng·min/mL), and their median normalized ratio was 0.088 (0.062 to 0.179).

**Clinical observations:** Intravenous administration of diltiazem HCl at a dose of 1 mg/kg over 5 minutes was well tolerated by the horses. No adverse effects were noted during and for the duration of 24 hours after drug administration.
Figure 2.1: Time course of the plasma concentrations (Cp) of diltiazem, desacetyldiltiazem, and a putative second metabolite after a single intravenous injection to 8 healthy horses (mean ± SD). Note that the concentration of the second, unidentified substance was estimated based on the diltiazem calibration curves, assuming that it represents a metabolite with a similar molar absorptivity compared to the parent drug.
<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (ng/mL)</td>
<td>298</td>
<td>99</td>
<td>478</td>
</tr>
<tr>
<td>α (min⁻¹)</td>
<td>0.062</td>
<td>0.030</td>
<td>0.111</td>
</tr>
<tr>
<td>B (ng/mL)</td>
<td>444</td>
<td>285</td>
<td>545</td>
</tr>
<tr>
<td>β (min⁻¹)</td>
<td>0.008</td>
<td>0.004</td>
<td>0.01</td>
</tr>
<tr>
<td>k₁₂ (min⁻¹)</td>
<td>0.019</td>
<td>0.006</td>
<td>0.039</td>
</tr>
<tr>
<td>kₛ₁ (min⁻¹)</td>
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<td>0.022</td>
<td>0.065</td>
</tr>
<tr>
<td>k₁₀ (min⁻¹)</td>
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<td>0.015</td>
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<tr>
<td>C₀ (ng/mL)</td>
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<td>931</td>
</tr>
<tr>
<td>t₁/₂a (min)</td>
<td>12</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>t₁/₂β (min)</td>
<td>93</td>
<td>73</td>
<td>161</td>
</tr>
<tr>
<td>AUC (ng·min/mL)</td>
<td>6.54 × 10⁴</td>
<td>4.94 × 10⁴</td>
<td>8.86 × 10⁴</td>
</tr>
<tr>
<td>AUMC (ng·min²/mL)</td>
<td>8.35 × 10⁶</td>
<td>5.27 × 10⁶</td>
<td>15.5 × 10⁶</td>
</tr>
<tr>
<td>Cl₁ (mL/kg/min)</td>
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<td>10.4</td>
<td>18.6</td>
</tr>
<tr>
<td>Vₑ (L/kg)</td>
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</tr>
<tr>
<td>V₄₉ (L/kg)</td>
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<td>1.58</td>
<td>2.83</td>
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<tr>
<td>Vₛₛ (L/kg)</td>
<td>1.84</td>
<td>1.46</td>
<td>2.51</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>125</td>
<td>99</td>
<td>206</td>
</tr>
</tbody>
</table>

A, B, corrected intercepts of the best-fit equation; α, β, slopes of the best-fit equation; k₁₂, kₛ₁, distribution rate constants; k₁₀, elimination rate constant; C₀, extrapolated plasma diltiazem concentration at time 0; t₁/₂a, distribution half-life; t₁/₂β, elimination half-life; AUC, area under the plasma concentration versus time curve; AUMC, area under the first moment curve; Cl₁, total plasma clearance; Vₑ, volume of distribution of the central compartment; V₄₉, volume of distribution during the terminal phase; Vₛₛ, volume of distribution at steady state; MRT, mean residence time.

Table 2.2: Pharmacokinetic variables of diltiazem following a single intravenous injection at a dose of 1 mg/kg to 8 horses.
Discussion

Our study suggests that the pharmacokinetics of IV diltiazem in healthy horses are best described by a two-compartment model with first-order drug elimination. Diltiazem HCl, 1 mg/kg IV, is rapidly distributed and cleared from the central compartment. The terminal half-life of diltiazem in healthy horses ranges between 1.2 and 2.7 h and is shorter than the half-life reported for both humans (2–6 h)\textsuperscript{2,13,14} and dogs (2.2–4 h).\textsuperscript{15-17}

A comparison of the metabolism of diltiazem in humans, dogs, rabbits, and rats showed that species differences are mainly quantitative rather than qualitative.\textsuperscript{18} Although not specifically investigated, this is also likely to be true for horses. Diltiazem is primarily metabolized by the liver.\textsuperscript{14} The majority of the total body clearance can therefore be attributed to hepatic clearance. Less than 5% of the dose is excreted unchanged in the urine, regardless of the species.\textsuperscript{18,19} Therefore, impairment of renal function does not significantly influence diltiazem elimination. The total plasma clearance of diltiazem determined in the present study corresponds to estimates of liver plasma flow in resting horses (15 – 20 mL/kg/min).\textsuperscript{20,21} Assuming that total plasma clearance equals hepatic clearance, the hepatic extraction ratio of diltiazem, calculated as the ratio between hepatic plasma clearance and hepatic plasma flow, would be very high, predicting a substantial first-pass effect.\textsuperscript{20-22} Furthermore, a change in liver blood flow, but not liver function, is likely the most important determinant of hepatic clearance of diltiazem, assuming that extrahepatic clearance is minimal. In particular, the profound vascular effects of diltiazem itself could alter liver blood flow and thereby reduce hepatic clearance, an effect which may become important during long-term administration of diltiazem.\textsuperscript{23} Significant accumulation of diltiazem has been reported after prolonged oral
administration in human patients, presumably due to saturation of the hepatic elimination pathways.\textsuperscript{17} Moreover, prolonged administration of diltiazem in people can result in progressive accumulation of metabolites, leading to inhibition of diltiazem biotransformation and non-linear kinetics.\textsuperscript{14} Prolonged use of diltiazem may rarely be indicated in horses with AF. Nevertheless, diltiazem administration should be accompanied by close monitoring of drug effects and adverse effects, particularly if diltiazem is used over extended periods of time in horses.

The major metabolic pathways responsible for hepatic clearance of diltiazem in humans are O-deacetylation, N-demethylation, and O-demethylation.\textsuperscript{14,19} The two major active metabolites in humans and dogs are desacetyl-diltiazem and N-desmethyldiltiazem. Due to their low plasma concentrations and their low pharmacologic activity compared to the parent drug, neither is considered clinically important.\textsuperscript{14,19} Our data indicate that the plasma metabolite concentrations are low compared to plasma diltiazem concentrations (Fig. 1). Quantitative analysis revealed that desacetyl-diltiazem represented only 6 to 18% of the diltiazem plasma concentration over time. Analytical standards for other metabolites were not available at the time of analyses. Therefore, we were unable to identify the exact nature of the second substance detected by HPLC. Presentation of the data for this putative second metabolite was limited to graphical description, suggesting that the plasma concentration over time profile was equally low. Although determination of plasma concentrations of this second substance was based on the diltiazem calibration curves, the error introduced by this procedure was considered to be small, assuming that this substance represented a diltiazem metabolite with a similar molecular structure and a similar chromophore compared to the parent compound, leading to comparable molar
absorptivity at the measurement wavelength. Based on our data, and considering the low pharmacologic activity of metabolites in other species,\textsuperscript{14} we do not consider drug metabolites to be clinically relevant in horses after short-term IV administration of diltiazem.

Interactions between diltiazem and other drugs administered to horses have not been reported, but are likely similar to those described in other species. While the pharmacokinetic interactions may depend on characteristics of drug metabolism and elimination, the cardiovascular effects of combined drug use can often be predicted from the characteristic actions of each individual drug. Clinically, diltiazem will most likely be used for ventricular rate control in horses with AF treated with quinidine. Diltiazem significantly decreases the clearance and prolongs the half-life of quinidine in people, whereas diltiazem kinetics are not significantly influenced by pretreatment with quinidine.\textsuperscript{24} Both quinidine and diltiazem show dose-dependent cardiodepressant and vasodilatory effects and may produce a reduction in cardiac output and hypotension. Consideration of these potential interactions between diltiazem and quinidine is therefore important when using the drug in horses.

Among other drugs that may be coadministered with calcium channel blockers in horses, digoxin and beta blockers warrant special consideration. To date, digoxin is the drug of choice for heart rate control in horses with a high ventricular response rate secondary to AF. Diltiazem appears to have no effects on digoxin pharmacokinetics in healthy individuals, but it may increase plasma digoxin concentrations in patients with cardiac insufficiency due to a reduction in renal and extrarenal clearance.\textsuperscript{14} Although rarely administered to horses, beta blockers are occasionally used in other species to
inhibit AV nodal conduction in patients with rapid supraventricular arrhythmias. Diltiazem increases plasma propranolol concentrations, either by inhibition of biotransformation or due to effects on hepatic plasma flow rates. The combined use of calcium antagonists and beta blockers can cause marked cardiac depression, excess bradycardia, prolongation of atrioventricular conduction, high-grade AV block, and hypotension. These effects are dose dependent, and dose reduction will likely decrease the incidence of adverse effects. Close monitoring of drug effects and adverse reactions is mandatory when diltiazem is coadministered with other cardiovascular drugs. Monitoring of systemic blood pressure and ECG is strongly recommended in order to detect hypotension, serious bradycardia, and the occurrence of drug-induced dysrhythmias. Reduction of drug doses or dosing intervals and dose titration may reduce the incidence of adverse effects related to drug interactions.

Pharmacodynamic data were not collected in the present study, because a clinically relevant dose-effect relationship could not be established in this population of normal horses in sinus rhythm and normal AV nodal conduction. However, the plasma concentrations achieved in the present study were similar to those required in healthy horses to produce consistent hemodynamic and electrocardiographic effects. In dogs, the plasma diltiazem concentration is closely related to myocardial drug concentrations and myocardial tissue is neither a site of preferential distribution nor retention of the drug. Hypotensive effects and inhibitory effects on the AV node are closely related to plasma diltiazem concentrations. However, the effects of diltiazem on AV nodal conduction also depend on the rate of activation of the nodal tissue and are more pronounced at faster rates of excitation. Due to individual variations the degree of
tachycardia, differences in AV nodal conduction, and variability in action of diltiazem, safe and effective therapy of the individual patient will likely require individual dose regimens guided by clinical response to treatment. The results of the present pharmacokinetic study together with the data of previous and ongoing studies on the pharmacologic effects of diltiazem in horses with a rapid atrial rhythm will provide the basis for development of accurate and effective dosing guidelines for diltiazem in the treatment of AF in horses.
Footnotes

a Sensorcaine®, AstraZeneca LP, Wilmington, DE.

b Angiocath 14G IV catheter, Becton Dickinson, Sandy, UT.

c (+)-cis-diltiazem HCl, D2521, Sigma Chemical Co, St. Louis, MO.


e PHD2000 Infusion Pump, Harvard Apparatus Inc., Holliston, MA.

f BD Vacutainer K3 EDTA, 10 mL, Becton Dickinson, Franklin Lakes, NJ.

g Fisherbrand Cryovial 4.0 mL, Fisher Scientific, Pittsburg, PA.

h USPC, Rockville, MD.

i Burdick & Jackson Laboratories, Muskegon, MI.

j Cleveland Scientific, Bath, OH.

k Bond Elut® - C18, 100 mg, Varian, Walnut Creek, CA.

l Model 515 HPLC pump, Waters, Milford, MA.

m Spectroflow model 783, Kratos, Ramsey, NJ.

n WISP model 717 plus, Waters, Milford, MA.

o Spectra Physics model 4270, Thermo Separation Products, San Jose CA.

p Zorbax SB-C18, Agilent Technologies, Palo Alto, CA.

q Perisorb RP-18, Upchurch Scientific, Oak Harbor, WA.

r GraphPad Prism, v4.00 for Windows, GraphPad Software, San Diego, CA.

s Microsoft® Office Excel 2003, Microsoft Corporation, Redmond, WA.
References


23. McAllister RG, Jr., Hamann SR, Blouin RA. Pharmacokinetics of calcium-entry blockers. Am J Cardiol 1985;55:30B-40B.


Diltiazem is a calcium-channel blocker that is used in humans and dogs (extra-label) for the treatment of supraventricular arrhythmias and for heart rate control in patients with atrial fibrillation, atrial flutter, and atrioventricular nodal reentry.\textsuperscript{1,2} Recently, we proposed that diltiazem may be used as an alternative to digoxin for heart rate control in horses with atrial fibrillation treated with quinidine and we described the hemodynamic effects (Chapter 1) and pharmacokinetic properties (Chapter 2) of diltiazem in healthy horses.\textsuperscript{3,4} To further characterize the pharmacology of diltiazem in this species, we conducted an \textit{in vitro} study to determine the extent of plasma protein binding of diltiazem at various concentrations. The results of this study would allow us to assess the potential of protein binding displacement interactions with other drugs and the possible influence of dysproteinemia on the concentration-effect relationship. Furthermore, the ability to estimate free drug concentrations at therapeutic concentrations was considered useful for future \textit{in vitro} studies on the effects of diltiazem on equine cardiomyocytes.
Material and Methods

One hundred milliliters of venous blood were collected from each of 8 horses (3 geldings, 5 mares; age 2 to 19 years) into tubes containing EDTA. All horses were considered healthy based upon physical examination, complete blood count, serum chemistry profile, and plasma fibrinogen concentration. None of the horses received medications during the 2 weeks preceding entry into the study. Plasma was harvested after centrifugation of the blood samples for 15 min at 2,000g and pooled for each horse. Plasma protein binding of diltiazem was determined by ultrafiltration methodology.

Aqueous solutions of diltiazem HCl, 1 mg/mL, were freshly prepared. The solutions were then diluted in the fresh plasma of each horse and in phosphate buffered saline (PBS) for protein-free negative controls, respectively, to achieve end-concentrations of 1000, 500, 250, 125, and 62.5 ng/mL. A 1-mL aliquot of each plasma dilution and of the negative control solution was then transferred into a Cryovial, incubated at 37°C for 20 minutes, snap-frozen in liquid nitrogen, and placed in a freezer at -80°C until analyzed. Another 1-mL aliquot of each sample was transferred into the sample reservoir of the Centrifree ultrafiltration device and incubated at 37°C for 10 minutes to allow drug-plasma protein binding equilibrium. The samples were then centrifuged at 2,000 g and 37°C for 15 min. After centrifugation, the filtrate cup was disconnected from the filtration device, sealed with a cap, snap frozen, and stored in a freezer at -80°C until analyzed. Concentrations of diltiazem and its metabolite desacetyldiltiazem in the unfiltered samples (total concentration; \( C_t \)) and the filtered samples (concentration of free drug; \( C_f \)), respectively, were determined by use of high-performance liquid chromatography (HPLC) as described elsewhere (Chapter 2). Calibrators and controls
for plasma samples were prepared in drug-free pooled equine plasma, while those for ultrafiltrates and protein-free negative control samples were prepared in phosphate buffered saline (pH 7.4). The lower limit of quantitation for diltiazem and desacetyl diltiazem was 5.0 ng/mL.

The concentration of protein-bound drug \( (C_b) \) was calculated as \( C_b = C_t - C_f \). The percent plasma protein binding \( (PB) \) was calculated as \( PB = C_b/C_t \times 100 \). Similarly, the degree of nonspecific adsorption \( (A) \) of diltiazem to the filtration device was determined based on the PBS diluted, protein-free negative control samples as \( A = C_b/C_t \times 100 \).

Binding constants were obtained by non-linear regression analysis using the following equation: \( C_b = B_{\text{max}} \times C_f/(K_d + C_f) \), where \( B_{\text{max}} \) is the maximal protein binding (in ng/mL), and \( K_d \) is concentration of free diltiazem required to reach half-maximal binding.

Differences in plasma protein binding between different nominal diltiazem concentrations were assessed using one-way repeated measures ANOVA with Tukey’s post-hoc test. All analyses were performed using commercial statistical software. The level of significance was defined as \( p < 0.05 \).

**Results**

The concentrations [mean ± SD] of total serum protein (6.5 ± 0.5 g/dL), serum albumin (3.4 ± 0.2 g/dL), serum globulin (3.1 ± 0.5 g/L), and plasma fibrinogen concentrations (280 ± 55 mg/dL) in the blood samples used for the study were all within normal limits. The protein binding of diltiazem was characterized by the following binding constants [mean (95% confidence interval)]: \( B_{\text{max}} = 1648 (1276 – 2020) \) ng/mL, and \( K_d = 130.4 (72.8 – 188) \) ng/mL. The data for the percent plasma protein binding of
diltiazem at nominal plasma concentrations between 62.5 and 1000 ng/mL are
summarized in Table 3.1.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N</th>
<th>Units</th>
<th>Nominal plasma diltiazem concentration (ng/mL)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>62.5</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>8</td>
<td>%</td>
<td>91.2 ± 2.9</td>
</tr>
</tbody>
</table>

a, b, c Different letters indicate significant differences between means (p < 0.05).

Table 3.1: Percent protein binding (mean ± SD) of diltiazem in plasma of 8 healthy horses.

The percent plasma protein binding of diltiazem decreased significantly with
increasing plasma drug concentrations. The extent of nonspecific adsorption was -3.2 %
(95% CI: -16.7 to 10.4 %), indicating that adsorption of diltiazem to the filtration device
was negligible. The metabolite desacetyldiltiazem was not detected in any of the plasma
samples or ultrafiltrates, ruling out significant hydrolysis of diltiazem by plasma esterases
during sample preparation.

Discussion

The results of our study showed that the extent of protein binding of diltiazem in
horses is slightly higher than protein binding reported for humans (77 to 86%), dogs
(70.2 ± 1.0%), rats (81.6 ± 0.6%), and monkeys (52.4 ± 3.8%). In cats, protein binding
ranges between 45 and 65%, and is higher after IV administration (65%) compared to PO
administration (45 to 60%). Although protein binding in humans was unrelated to
plasma diltiazem concentrations ranging from 30 to 2060 ng/mL, we found that protein binding is concentration-dependent in horses, suggesting a potential saturation of protein binding sites at higher plasma drug concentrations. However, this effect is not likely to be clinically relevant, as therapeutic total plasma diltiazem concentrations for treatment of supraventricular arrhythmias in horses are expected to be considerably lower than 500 ng/mL (see Chapter 4).

We did not specifically investigate the influence of diltiazem metabolites or other drugs on protein binding of diltiazem. In other species, including humans and dogs, diltiazem binding does not seem to be altered in the presence of desacetyldiltiazem. Drugs such as digoxin, propranolol, phenylbutazone, salicylic acid, and warfarin do not displace diltiazem from its protein binding sites, nor does diltiazem alter the binding of propranolol and digoxin.

The results of the present study together with the data of previous and ongoing studies on the pharmacologic effects of diltiazem in horses will provide the basis for development of accurate and effective dosing guidelines for diltiazem in the treatment of atrial fibrillation in horses.
Footnotes

a  BD Vacutainer K3 EDTA, 10 mL, Becton Dickinson, Franklin Lakes, NJ.

b  D2521, Sigma Chemical Co, St. Louis, MO; dissolved in water, Burdick & Jackson Laboratories, Muskegon, MI.

c  Fisher Scientific, Pittsburg, PA.

d  Millipore Corp, Bedford, MA.

e  Microsoft Office Excel 2003, Microsoft Corporation, Redmond, WA.

f  GraphPad Prism v4.03 for Windows, GraphPad Software, San Diego, CA.
References


Atrial mechanical function is responsible for late-diastolic ventricular filling and contributes to ventricular preload, stroke volume, and overall cardiovascular performance. Normal atrial pump function is crucial for maintaining adequate cardiac output at higher levels of exercise. Conversely, atrial dysfunction can significantly impair exercise capacity in athletes.\(^1\) Atrial fibrillation (AF), leading to a loss of atrial pump function, is considered the most common pathologic arrhythmia causing poor performance in sports horses.\(^2\)

The sodium channel blocker quinidine is the drug of choice for conversion of AF or atrial flutter to sinus rhythm in horses.\(^2\) Quinidine prolongs the cycle length of the atrial impulses and increases the fibrillation threshold of the myocardium. However, anticholinergic effects of quinidine can enhance AV nodal conduction. The resulting rapid ventricular response rate is a common reason for aborting therapy before conversion to sinus rhythm, and is reported as the most common severe adverse effect of quinidine therapy in horses.\(^3\) In one study, heart rates exceeding 100 beats/min were observed during quinidine sulfate therapy in 22 of 41 horses with AF.\(^2\) Importantly, the effect is not necessarily dose dependent and tachycardia has been reported to occur at
plasma quinidine concentrations between 1.7 and 4.2 μg/mL, hence at concentrations well within the therapeutic range of 2 to 5 μg/mL.²

Diltiazem is a calcium-channel blocker that is effective in humans and dogs for ventricular rate control in the treatment of supraventricular tachyarrhythmias.⁴⁻⁶ Recently, we proposed that diltiazem might be used as an alternative to digoxin for rate control in horses undergoing treatment with quinidine (Chapter 1).⁷ We were able to demonstrate that diltiazem, administered to healthy horses at doses of 1 to 2 mg/kg IV, causes intermittent depression of the sinus and AV nodes, mild impairment of systolic and diastolic LV function, arterial vasodilation, decrease in systemic vascular resistance, and dose-dependent decrease in systemic blood pressures.⁷ However, the doses required to produce consistent and significant electrocardiographic and hemodynamic effects in healthy horses in sinus rhythm are higher than the therapeutic doses recommended for dogs and people with supraventricular arrhythmias.⁸⁻¹⁰ This difference in dose response may be explained by the rate-dependence of diltiazem effects. In other species it has been shown that the AV nodal effects are more pronounced at faster atrial rates and depression of atroventricular conduction is enhanced in the presence of supraventricular tachyarrhythmias.¹¹⁻¹³

Electrophysiologic studies have provided important insights into the pathogenesis of AF in humans and in a variety of animal models. However, despite the clinical relevance of atrial arrhythmias and atrial dysfunction in horses, electrical and functional characteristics of the equine atria are incompletely studied to date. Accordingly, we attempted to apply standard electrophysiologic methods to horses and demonstrate that drug effects on supraventricular electrophysiology could be measured by these methods.
Our first goal was characterization of normal \textit{in vivo} atrial, SA nodal, and AV nodal electrophysiology in healthy horses using programmed electrical stimulation methods routinely used in humans for diagnosis and characterization of cardiac arrhythmias. The second study goal involved application of these techniques for determining the electrophysiologic effects of quinidine and diltiazem in horses using a clinically relevant sequence of drug administration. In this study, we hypothesized that quinidine would prolong the atrial effective refractory period, but also shorten the functional refractory period of the AV node leading to an undesirable acceleration of ventricular response rate in an equine pacing model of supraventricular tachycardia. We further hypothesized that diltiazem would increase the FRP of the AV node and control the ventricular response rate during atrial pacing in a rate-dependent manner when administered after pre-treatment with quinidine. Finally, we hypothesized that diltiazem would cause potentially adverse and dose-dependent suppression of SA nodal function and decrease in systemic blood pressure.

To address our study objectives, we determined the electrophysiologic effects of quinidine and the combination of quinidine and diltiazem in horses subjected to rapid atrial pacing. Sinus nodal, atrial, and AV nodal function were measured and compared to baseline values obtained from these healthy horses. By applying progressively shorter pacing cycles, we attempted to identify any rate dependence of diltiazem effects on AV nodal conduction. Finally, we identified doses of diltiazem effective in controlling the ventricular response rate during rapid-atrial pacing under the influence of quinidine.
Material and Methods

Horses: 14 horses (11 geldings, 3 mares; 9 Standardbreds, 3 Thoroughbreds, 2 Quarter Horses) with a median age of 10 years (range, 8 to 17 years) and a median body weight of 535 kg (range, 468 to 646 kg) were used. All horses were considered healthy based on history, physical examination, cardiac auscultation, electrocardiogram, and echocardiographic examination. The horses were equipped with carotid loops to facilitate intra-arterial catheter placement. None of the horses received medications during the 2 weeks preceding entry into the study. The studies were approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.

Study design: All studies were conducted under local anesthesia in standing, non-sedated horses restrained in a stock. Two 14G catheters were placed in the right jugular vein for drug administration and blood sampling, respectively. A 19G catheter was placed in the elevated carotid artery for measurement of arterial blood pressure using a fluid-filled system. The zero-pressure reference point for the fluid-filled catheter was the point of the shoulder. The transducer was calibrated to 0 and 100 mmHg using a mercury manometer. An 8F catheter introducer was placed in the left jugular vein. A quadripolar electrode catheter was then introduced through the catheter introducer and advanced into the right atrium, so that the catheter electrodes were located in the high right atrium. Catheter placement was guided by analysis of the surface ECG and intracardiac electrogram. The catheter was positioned so that the A wave of the intracardiac electrogram appeared in close temporal association with the beginning of the P wave on the surface ECG, consistent atrial capture was achieved during atrial pacing at a cycle length of 1000 ms, and the paced P’ wave morphology was similar to the P wave.
morphology during sinus rhythm. A surface ECG was recorded using standard base-apex leads. The signals of the surface ECG, the right atrial (RA) electrogram, and the arterial blood pressure were recorded simultaneously at a sampling rate of 200 Hz by a digital data acquisition system and stored for subsequent offline analysis.

Baseline mean arterial blood pressure (MAP), surface ECG, and right atrial electrogram were recorded over 1 min during sinus rhythm. The MAP was averaged over 1 min. The electrophysiologic intervals were averaged over 5 consecutive beats. The following intervals were determined: Sinus cycle length (SCL), measured as the interval between successive A waves measured from the right atrial electrogram; and RR interval, PQ interval, QRS duration, and QT interval measured from the surface ECG (subsequently called ‘basic ECG variables during sinus rhythm’). The AV conduction ratio was calculated as SCL/RR. The QT interval was corrected for heart rate using Fridericia’s correction \( QT_{cf} = QT/\sqrt[3]{RR} \).

Right atrial electrical stimulation was then performed using the 2 distal electrodes of the electrophysiology catheter. The threshold for stimulation was determined by pacing at a cycle length of 1000 ms and a pulse width of 2 ms. The lowest current amplitude which consistently captured the atrium was identified as the threshold for stimulation. Basic electrophysiologic parameters were determined using programmed electrical stimulation at twice threshold amplitude.

The atrial effective refractory period (AERP) was determined by pacing the atrium with 8 conditioning stimuli (S1) at pacing cycle lengths (PCL) of 1200, 1000, 800, 600, 400, and 300 ms, respectively, followed by an extra stimulus (S2) with a coupling interval of 350 ms. The S1-S2 interval was decreased by 10 ms intervals and the...
procedure repeated until capture was lost. The longest S1-S2 interval that did not capture the atrium was the AERP for the respective conditioning cycle length (S1-S1).  

For assessment of AV nodal conductive function, the atrium was paced for 1 min at cycle lengths of 1200, 1000, 800, 600, 400, and 300 ms, respectively, and the ventricular response to atrial pacing was observed. Pacing was not preceded by a conditioning period. A 1 min recovery period was allowed between the pacing periods. The mean RR interval during atrial pacing was determined by averaging all consecutive RR intervals over each 1-min pacing period using the automated peak detection feature of the analysis software. Accurate peak detection was assured by beat-to-beat visual inspection by the operator. The mean RR interval and the AV conduction ratio (PCL/mean RR) were used as indices of AV conductive function. The functional refractory period (FRP) of the AV node was determined as the shortest RR interval observed during 1 min of rapid atrial pacing at a cycle length of 300 ms. The ventricular response rate (VRR) during the 1 min pacing period at 300 ms PCL was determined at the time of the recording by counting the QRS complexes for rapid determination of the target rate for ventricular rate control (see below). The MAP was recorded during each pacing period and averaged over 1 min.

The duration between the last pacing spike and the first spontaneous sinus node discharge was determined after each 1-min pacing period at all cycle lengths. The sinus node recovery time (SNRT) was defined as the longest interval measured in each series. The corrected sinus node recovery time (cSNRT) was calculated by subtracting the SCL (determined as the average of 5 AA intervals immediately before the
pacing cycle) from the SNRT. The ratio of SNRT and SCL (SNRT / SCL x 100) was calculated.

Aqueous solutions of quinidine gluconate (40 mg/ml)\textsuperscript{i} and diltiazem HCl (5 mg/mL)\textsuperscript{j} were freshly prepared before each experiment. After baseline recordings, quinidine gluconate was administered IV using a programmable infusion pump\textsuperscript{k}. Intravenous administration was selected to allow consistent dosing, thereby avoiding differences due to variable drug absorption and bioavailability. Seven horses received a total dose of 10 mg/kg IV, administered at a rate of 0.333 mg/kg/min over 30 min (low-dose [LD] group). This total dose was chosen based on published dosing recommendations for horses with AF\textsuperscript{22,23} Although this dose was effective at increasing the ventricular response rate during rapid atrial pacing and allowed investigation of the AV nodal effects of diltiazem, the resulting plasma quinidine concentrations were below the recommended therapeutic range of 2 to 5 $\mu$g/mL (see results). Therefore, the remaining seven horses received a higher total dose of quinidine, consisting of a loading dose of 12 mg/kg at a rate of 2.4 mg/kg/min IV over 5 min, immediately followed by a constant rate infusion of 5 mg/kg/h for the remaining duration of the study (high-dose [HD] group). This dose was chosen based on unpublished clinical data and pilot studies with the goal of expanding the range of achieved plasma quinidine concentrations to concentrations above 2 $\mu$g/mL while avoiding toxic peak plasma concentrations. After the end of administration of the total dose (LD group) and the loading dose (HD group), respectively, an equilibration period of 30 min was granted in order to minimize effects due to redistribution and rapidly changing plasma quinidine concentrations. Subsequently, electrophysiologic recordings were performed as described above in the following order (recording period ‘Quinidine’
= Q): (1) AERP (at conditioning pacing cycle lengths of 600, 400, and 300 ms); (2) mean RR during atrial pacing, AV nodal FRP, SNRT, VRR, and MAP (6 periods of 1-min pacing at various PCL and 1-min recovery); and (3) SCL, basic ECG variables, and MAP during sinus rhythm (1-min recording). Venous blood samples were collected for later determination of plasma quinidine concentrations.

Subsequently, diltiazem was administered at a dose of 0.125 mg/kg IV over 2 min. After an equilibration period of 5 min, basic ECG variables during sinus rhythm were determined and rapid atrial pacing was performed for 1 min at a pacing cycle length of 300 ms to determine the effect of diltiazem on ventricular response rate. Diltiazem administration was repeated every 12 min until the effective dose (ED) was reached. The ED was defined as that which returned the VRR during rapid atrial pacing at 300 ms PCL to less than 110% of the baseline response rate previously determined (see above). This target dose was arbitrarily chosen by the investigators assuming that this degree of ventricular rate control (to achieve ventricular rates close to baseline values) represents the maximal rate control which would be desirable in clinical situations.

When the effective dose was reached, venous blood samples were collected for later determination of plasma quinidine and diltiazem concentrations, respectively, and electrophysiologic recordings were performed in the following order (recording period ‘Quinidine/Diltiazem’ = Q/D): (1) SCL, basic ECG variables, and MAP during sinus rhythm (1-min recording); (2) mean RR during atrial pacing, AV nodal FRP, SNRT, VRR, and MAP (6 periods of 1-min pacing at various PCL and 1-min recovery); and (3) AERP (at conditioning pacing cycle lengths of 600, 400, and 300 ms).
**Determination of plasma drug concentrations (Cp):** Blood samples were collected into tubes containing K$_3$ EDTA (for determination of diltiazem concentrations) and sodium heparin (for determination of quinidine concentrations), respectively. Samples were stored on ice until centrifugation at 4,000g and 4°C for 15 min. Plasma was then apportioned into 4-ml aliquots, transferred into cryovials, frozen, and stored at -80°C until analyzed. Plasma diltiazem concentrations were determined in a commercial laboratory (National Medical Services, Willow Grove, PA) using chromatographic methods. The lower limit of detection was 5.0 ng/mL. Quinidine plasma concentrations were determined in the clinical laboratory of the School of Veterinary Medicine of the University of Pennsylvania, Kennet Square, PA using a commercial fluorescence polarization immunoassay. The lower limit of detection was 0.2 μg/mL.

**Adverse effects:** The horses were monitored for potential adverse effects during drug administration and for the next 24 hours. Attention was focused on the development of severe hypotension (MAP < 60 mmHg or decreasing MAP associated with clinical signs of weakness or syncope), cardiac rhythm disturbances, peripheral edema, coughing, increased respiratory rate, signs of respiratory distress, colic, diarrhea, ataxia, bizarre behavior, convulsions and laminitis.

**Data analysis and statistics:** Descriptive summary statistics and graphical analyses were performed. All results were reported as mean ± SD unless stated otherwise. The distribution of the data was assessed using scatter plots, histograms, and normal probability plots. The level of significance was defined as P < 0.05 for all statistical analyses.
For assessment of the effects of quinidine and diltiazem on SCL, basic ECG variables during sinus rhythm, AV nodal FRP, SNRT, and MAP, one-way repeated-measures analysis of variance (ANOVA) or Friedman repeated-measures ANOVA on ranks were used to compare variables across treatment periods. The combined effects of treatment and PCL on AERP and mean RR during atrial pacing, respectively, were examined by two-way repeated-measures ANOVA. Where the F-statistics indicated significant differences, all pairwise comparisons were performed using Tukey’s post-hoc test. Separate analyses were performed for the LD group and the HD group, respectively.

The quinidine-induced degree of RR shortening at 300 ms PCL (dRR) was calculated as the difference of the mean RR at 300 ms PCL between baseline and quinidine treatment periods. The relationship between dRR and plasma quinidine concentrations was assessed by simple linear regression analysis. The relationship between the ED of diltiazem, plasma quinidine concentrations, and dRR was assessed using backward stepwise linear regression analysis with plasma quinidine concentrations and dRR as independent variables. For these analyses, data from the LD and HD groups were pooled.

Results

The baseline resting heart rate was 35 ± 3 min⁻¹ with an AV conduction ratio of 1.0 ± 0.0, corresponding to a SCL and an RR interval, respectively, of 1721 ± 177 ms. Baseline ECG parameters were: PQ interval, 345 ± 41 ms; QRS duration, 119 ± 11 ms; QT interval, 529 ± 34 ms; QT<sub>cf</sub> interval, 442 ± 24 ms. Mean arterial blood pressure at baseline was 115 ± 16 mmHg.
The mean threshold for atrial pacing was 3.5 ± 1.3 mA. At a PCL of 1200 ms, the baseline AERP was 231 ± 37 ms. Shortening of the AERP was observed with increasing pacing rates, reaching 196 ± 15 ms at a PCL of 300 ms (Figure 4.1). Baseline SA nodal function was characterized by a SNRT of 2263 ± 559 ms, a cSNRT of 633 ± 466 ms, and a ratio of SNRT to BCL of 1.39 ± 0.26. The FRP of the AV node was 824 ± 131 ms. The mean RR during atrial pacing, a surrogate of AV nodal function that determines the ventricular response rate, was relatively independent of pacing rate at cycle lengths between 1200 and 300 ms. Consequently, the AV conduction ratio decreased with shortening of the PCL (Figure 4.2). At a pacing cycle length of 300 ms, ventricular response rate was 41 ± 8 min⁻¹, corresponding to a mean RR interval of 1532 ± 275 ms and an AV conduction ratio of 0.20 ± 0.04.

Intravenous administration of quinidine and of diltiazem was well tolerated by all horses. Rapid infusion of quinidine gluconate at a dose of 12 mg/kg over 5 min caused transient rapid sinus tachycardia in all horses. Mild diarrhea associated with frequent defecation was noted after quinidine administration in 3 horses. In all cases, the diarrhea resolved without treatment after completion of the study. Soft respiratory stridor, suggesting mild nasal edema, was observed in 3 horses. No other clinically relevant adverse effects were noted during and for 24 hours after drug administration.

The effective dose of diltiazem for ventricular rate control during rapid atrial pacing at 300 ms PCL was 0.25 (0.125 – 0.75) mg/kg [median (range)] and 0.875 (0.125 – 1.125) mg/kg, respectively, in the LD and HD group. Backward stepwise linear regression indicated that the dose of diltiazem required for establishment of a near-normal ventricular response rate during rapid atrial pacing (effective dose) was
Figure 4.1: Rate adaptation curve for the atrial effective refractory period (AERP) determined in 14 normal, non-sedated horses at rest. PCL, pacing cycle length.

Figure 4.2: Ventricular response to atrial pacing at various pacing cycle lengths (PCL) in 14 normal, non-sedated horses at rest. Mean RR, RR intervals averaged over 1 min of atrial pacing; PCL/mean RR, AV conduction ratio.
linearly related to the quinidine-induced degree of RR shortening at 300 ms PCL (Figure 4.3). Inclusion of plasma quinidine concentrations in the equation did not significantly improve the ability of the model to predict the effective dose of diltiazem (data not shown). Also, dRR was not significantly related to plasma quinidine concentrations when assessed by simple linear regression analysis ($r^2 = 0.09$, $p = 0.290$).

Figure 4.3: Relationship between the effective dose (ED) of diltiazem and the quinidine-induced degree of RR shortening (dRR) at PCL 300 ms. Linear regression analysis; dotted lines represent the 95% confidence band of the regression line (inner band) and the 95% prediction band (outer band), respectively.
The time between recordings, plasma drug concentrations, and effects of quinidine and diltiazem on basic ECG variables during sinus rhythm are summarized in Table 4.1. The time between the quinidine and quinidine/diltiazem recording periods varied depending on the number of diltiazem doses required to reach the target effect. However, plasma quinidine concentrations were relatively stable throughout both recording periods. The RR interval was significantly decreased during both treatment periods compared to baseline, consistent with a mild increase in ventricular rate during sinus rhythm. Co-administration of diltiazem did not have a significant effect on the RR interval during sinus rhythm. No atrio-ventricular block was observed and the AV conduction ratio during sinus rhythm indicated 1:1 AV nodal conduction throughout all treatment periods. A slight but statistically non-significant shortening of the PQ interval was observed after administration of quinidine. After administration of diltiazem, the PQ interval was not different from baseline values. A slight but statistically non-significant QRS prolongation was detected during both treatment periods in the HD group. Slight prolongation of QTcF was observed during the quinidine treatment period, with subsequent shortening during the quinidine/diltiazem treatment period.

The effects of quinidine and diltiazem on the AERP are shown in Figure 4.4. The AERP shortened significantly with decreasing PCL. Quinidine caused significant prolongation of the AERP that was slightly, but not significantly attenuated by diltiazem. A rate-dependent effect of diltiazem on the AERP was not evident based on graphical and statistical analyses.
<table>
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<th>Statistics†</th>
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<td>Quinidine^b</td>
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RR, RR interval; PQ, PQ interval; QRS, QRS duration; QTcf, Fridericia-corrected QT interval

† LD, low-dose group (n=7); HD, high-dose group (n=7)

† One-way repeated-measures ANOVA with Tukey’s post-hoc test for multiple comparisons

(ab baseline vs. quinidine, ac baseline vs. quinidine/diltiazem, bc quinidine vs. quinidine/diltiazem)

‡ Median (range)

Table 4.1: Time between recordings, plasma drug concentrations, and effects of quinidine and diltiazem on basic ECG variables during sinus rhythm.
Figure 4.4: Effects of quinidine and diltiazem on AERP at 3 different pacing cycle lengths (PCL). There was no significant interaction between treatment effects and PCL. Hence, the p values refer to effects of PCL and treatment, respectively. Two-way repeated-measures ANOVA with Tukey’s post-hoc test. LD, low-dose group; HD, high-dose group.
The drug effects on AV nodal conductive function are illustrated in Figures 4.5 and 4.6. Quinidine significantly decreased the mean RR at all PCL. Effective doses of diltiazem (defined as those that returned the ventricular response rate during rapid atrial pacing at 300 ms PCL to less than 110% of baseline response rate) did not significantly prolong the mean RR at 1200 ms PCL. However, diltiazem caused a progressive increase in RR mean with shortening of the PCL, consistent with a rate-dependence of diltiazem effects.

The drug-induced alterations in SNRT are shown in Figure 4.7. The drug effects were identical when assessed by cSNRT and SNRT/SCL (data not shown). Although statistical significance was not reached, prolongation of the SNRT after diltiazem administration was evident in 5 horses. After administration of diltiazem at doses above 0.625 and 1.0 mg/kg, respectively, 2 of the horses in the HD group developed marked sinus arrhythmia and intermittent sinus pauses during spontaneous rhythm. Effective diltiazem doses in these horses were 0.875 and 1.125 mg/kg. Both horses had low mean arterial blood pressures (76 and 85 mmHg) but had normal average heart rates (mean RR over 1 min, 1591 and 1628 ms) and were clinically asymptomatic. In both horses, diltiazem at effective doses caused severe prolongation of the SNRT up to 6125 ms and 7984 ms, respectively.
Figure 4.5: Effects of quinidine and diltiazem on the functional refractory period (FRP) of the AV node during rapid atrial pacing at a pacing cycle length of 300 ms. One-way repeated-measures ANOVA with Tukey’s post-hoc test. LD, low-dose group; HD, high-dose group; Q, Quinidine; Q/D, Quinidine/Diltiazem.
Figure 4.6: Effects of quinidine and diltiazem on AV nodal conductive function, expressed as the mean RR interval in response to atrial pacing at various pacing cycle lengths (PCL). The interaction between treatment effects and PCL was statistically significant. Hence, the p values for comparison of treatment effects are reported separately for each PCL (table). Two-way repeated-measures ANOVA with Tukey’s post-hoc test. LD, low-dose group; HD, high-dose group; B, Baseline; Q, Quinidine; Q/D, Quinidine/Diltiazem.
Figure 4.7: Effects of quinidine and diltiazem on the sinus node recovery time (SNRT). The asterisks indicate 2 horses with severe sinus arrhythmia and sinus pauses after administration of diltiazem. Friedman repeated-measures ANOVA on ranks. LD, low-dose group; HD, high-dose group; Q, Quinidine; Q/D, Quinidine/Diltiazem.
Baseline arterial blood pressure during rapid atrial pacing at 300 ms PCL was 110 ± 6 mmHg in the LD group and 108 ± 12 mmHg in the HD group. Quinidine did not significantly decrease mean arterial pressure compared to baseline (LD, p = 0.162; HD, p = 0.337). However, diltiazem significantly decreased blood pressures to 88 ± 15 mmHg (LD, p < 0.001) and 87 ± 10 mmHg (HD, p = 0.006). No clinical signs of hypotension were noted.

Discussion

This investigation demonstrates that variables characterizing atrial, SA nodal, and AV nodal electrophysiologic function can be determined in conscious, unsedated horses using standard methods similar to those routinely used in human patients. Furthermore, we demonstrated that these methods can be used to study the effects of drugs on cardiac electrophysiology in horses. Finally, we identified a potential benefit of diltiazem for control of ventricular rate response during atrial tachyarrhythmia, although additional studies are needed to validate these results.

Atrial and AV nodal electrophysiologic characteristics are of major importance in patients with AF. In our study population, the AERP ranged between 231 ± 37 ms and 196 ± 15 ms, at pacing cycle lengths of 1200 and 300 ms, respectively. Van Loon et al. reported values between 287 ± 29 ms (at 1000 ms PCL) and 234 ± 20 ms (at 333 ms PCL) in a group of 4 healthy ponies. These somewhat higher values may be explained by differences in breed, pacing amplitude, and recording techniques between the 2 studies. Specifically, Van Loon et al. determined AERP using a permanent dual-chamber pacemaker and an incremental S1-S2 interval (as opposed to decremental pacing used in
this study), which may have led to somewhat higher values. The rate-dependent shortening of the AERP (Figures 4.1 and 4.4) is a normal finding in non-rodent mammals and reflects the abbreviation of the action potential at higher rates of stimulation.\textsuperscript{25-29} The AERP is an important determinant of atrial impulse conduction and plays a crucial role in the development of supraventricular arrhythmias. It is well known that atrial flutter and atrial fibrillation lead to time-dependent changes in atrial electrophysiologic characteristics, including a shortening and a loss of normal rate adaptation of the AERP. These changes, also referred to as electrical remodeling, contribute to the progressive, self-perpetuating, and recurrent nature of AF in many species.\textsuperscript{20,27,30-32} A 5-day period of repeated AF induction in an equine pacing model of chronic AF increased the duration of AF episodes, shortened the fibrillation cycle length, and increased the complexity of the atrial electrogram morphology, presumably due to atrial electrical remodeling.\textsuperscript{18} However, AF-induced atrial remodeling has not been specifically studied in the horse. The electrophysiologic methods used in the present study might be used in future investigations to elucidate the electrophysiologic basis of AF in horses.

Electrophysiologic characteristics of AV nodal function in horses have been described previously in the literature.\textsuperscript{16,33,34} In agreement with these investigations, our results show that the mean RR interval during rapid atrial pacing is relatively independent of pacing cycle length and that the AV conduction ratio decreases linearly with shortening of the atrial cycle length, consistent with AV nodal conduction block at higher rates of stimulation (Figure 4.2). These findings indicate that intrinsic AV nodal properties, but not the rate of atrial stimulation, are the primary determinants of ventricular response rate at higher rates of atrial stimulation. However, this may not be
true in patients with atrial fibrillation in which the impulse cycle length is shorter and AV nodal stimulation occurs irregularly. Gelzer et al. investigated the temporal organization of the ventricular rate in response to spontaneous atrial fibrillation in horses and studied the effect of quinidine on AV nodal function. They reported a quinidine-induced shortening of the FRP of the AV node which was partly related to the drug’s vagolytic effects. Furthermore, quinidine administration led to a gradual prolongation of cycle length and increase in spatio-temporal organization of the atrial impulses, which presumably reduced the degree of impulse concealment in the AV node and contributed to the increase in ventricular response rate. These results suggest that ventricular response during AF is to some degree determined by the input rate into the AV node. In the present study, quinidine induced a shortening of the FRP that was associated with an increase in ventricular response rate during rapid atrial pacing independent of the rate of atrial stimulation (Figures 4.5 and 4.6).

AV nodal conduction during normal sinus rhythm, assessed by PQ interval and AV conduction ratio, was not significantly affected by quinidine and diltiazem at the doses used here, although a tendency for PQ shortening after quinidine administration (vagolytic effect) and PQ prolongation after diltiazem treatment (calcium channel blocking effect) were noted. These findings were in agreement with previous studies performed by the authors, showing that higher doses of diltiazem (i.e., 1 to 2 mg/kg IV) are required to produce significant electrocardiographic effects in healthy horses in sinus rhythm (Chapter 1). However, the quinidine-induced acceleration of AV nodal conduction during rapid atrial pacing allowed further investigation of diltiazem effects on AV nodal function, which was the main focus of the second part of this study. In
agreement with our hypothesis, the effects of diltiazem on AV nodal conduction were rate-dependent. In contrast to the baseline and quinidine treatment periods, during which the ventricular response rate was largely independent of PCL, diltiazem caused a gradual prolongation of the mean RR interval with shortening of the PCL (Figure 4.6). Hence, although diltiazem at doses used here was not effective for rate control during normal sinus rhythm (Table 4.1) and during slow atrial pacing rates (e.g., 1200 ms PCL), it allowed complete control of the ventricular response rate during rapid atrial pacing (e.g., 300 ms PCL) in horses pre-treated with quinidine. Consequently, diltiazem doses and resulting plasma diltiazem concentrations effective for rate control during rapid atrial pacing were lower than those required to produce significant effects in healthy horses in sinus rhythm, but corresponded well to those recommended in people and dogs for rate control in patients with supraventricular arrhythmias. Although the effective diltiazem dose in our study was significantly related to the quinidine-induced degree of RR shortening, the width of the 95% prediction band shown in Figure 4.3 indicates that the response to diltiazem is variable and that accurate prediction of the effective dose in an individual horse cannot be made based on ventricular response rate. Therefore, doses should be individualized based on effect.

We observed a prolongation of the AERP after administration of quinidine, consistent with the classic effects of type IA antiarrhythmic agents (Figure 4.4). Although not statistically significant, the prolongation of the AERP appeared slightly attenuated during the quinidine/diltiazem treatment period in the LD group. In the HD group, the effects of diltiazem on the AERP appeared variable and were most pronounced in the HD group at a PCL of 300 ms, although there was no clear evidence of a rate-dependent effect based
on graphical and statistical analyses. A transient diltiazem-induced shortening of the AERP, most likely resulting from the effects of calcium current inhibition on action potential duration, has also been reported in dogs.\textsuperscript{37} Hence, calcium channel blockers may blunt the antiarrhythmic effects of quinidine and thereby increase atrial vulnerability to reentry arrhythmias.\textsuperscript{38-40} However, the inhibitory effects of calcium channel blockers on AV nodal function provide good efficacy for ventricular rate control in patients with AF and rapid ventricular response rate. Clinically, these effects may outweigh potential adverse effects of calcium channel blockers on atrial electrophysiology in patients with AF.

We propose that diltiazem may be useful for acute ventricular rate control when rapid supraventricular tachycardia occurs during standard quinidine treatment in horses with AF. However, diltiazem is not approved for use in horses, and any administration demands careful consideration of potential adverse effects and drug interactions. Quinidine and diltiazem exert hypotensive and cardiodepressant effects, and combined treatment may cause severe bradycardia and hypotension.\textsuperscript{7,13,41} The decrease in mean arterial blood pressure and the suppression of SA nodal function by diltiazem in this study were consistent with previous findings in horses.\textsuperscript{7} However, marked SA nodal suppression seemed to occur primarily at higher doses of diltiazem, and neither the decrease in blood pressure nor the SA nodal depression was associated with any clinical signs in these horses. Co-administration of quinidine and diltiazem at the doses used here appears safe in healthy horses undergoing adequate physiologic monitoring. Nonetheless, idiosyncratic adverse effects cannot be predicted from the small number of horses used in the present study and the results of this study may not be directly applicable to horses.
with naturally occurring AF. Short-term rapid atrial pacing in horses with normal atria is likely to be different from natural AF in patients with remodeled or diseased atrial tissue. A pacing cycle length of 300 ms was chosen to simulate AF, based on the observation that an increase in ventricular response rate during quinidine treatment often coincides with the transition from atrial fibrillation to a flutter-like activation pattern with a cycle length of approximately 300 ms. However, atrial cycle lengths in chronic, untreated AF are usually shorter, ranging between 150 and 200 ms. The concept of rate-dependence suggests that diltiazem effects may be even more pronounced at cycle lengths shorter than 300 ms. Furthermore, quinidine may reach higher plasma concentrations than detected in this study, and other factors such as hemodynamic status and autonomic balance may vary in individual patients with AF. Diltiazem effects and dose responsiveness may therefore differ in patients with naturally occurring AF, and monitoring heart rate, rhythm, systemic blood pressure, and clinical signs of hypotension and weakness are mandatory when using diltiazem in conjunction with quinidine in such patients.

In conclusion, variables of SA nodal, atrial, and AV nodal electrophysiologic function can be determined in standing, unsedated horses by use of standard diagnostic techniques. These methods can be used to characterize physiologic and pathologic atrial electrical function and to investigate drug effects on specific electrophysiologic variables. In this study, we were able to show that diltiazem, at cumulative doses between 0.125 and 1.125 mg/kg IV, is effective for ventricular rate control during treatment with quinidine in an equine pacing model of supraventricular tachycardia. Potential adverse effects of diltiazem include hypotension and suppression of sino-atrial nodal discharge leading to
sinus arrhythmia or sinus pauses. The results of this study should be useful in future clinical trials to establish effective and safe doses of diltiazem for treatment of horses with naturally occurring AF.
Footnotes

a  Bupivacaine HCl, Sensorcaine®, AstraZeneca LP, Wilmington, DE.

b  Angiocath 14G IV catheter, Becton Dickinson, Sandy, UT.

c  Intracath 19G/30.5cm IV catheter, Becton Dickinson, Sandy, UT.

d  BIOPAC Systems, Inc., Goleta, CA.

e  Argon Medical Corp, Athens, TX.

f  EPT-DX™ Steerable Diagnostic Catheter, 6F/110cm, Model M00411030, Boston Scientific/EP Technologies, San Jose, CA.

g  AcqKnowledge v.3.5.7., BIOPAC Systems, Inc., Goleta, CA.

h  Medtronic 5325 Programmable Stimulator, Minneapolis, MN.

i  Q5001, Sigma Chemical Co, St Louis, MO; dissolved in Sterile Water for Injection, Vedco Inc., St. Joseph, MO.

j  D2521, Sigma Chemical Co, St. Louis, MO; dissolved in Sterile Water for Injection, Vedco Inc., St. Joseph, MO.

k  PHD2000 Infusion Pump, Harvard Apparatus Inc., Holliston, MA.

l  BD Vacutainer, 10 ml, Becton Dickinson, Franklin Lakes, NJ.

m  Fisherbrand Cryovial 4.0ml, Fisher Scientific, Pittsburg, PA.

n  TDx/TDxFLx Quinidine Assay System, Abbott Laboratories, Abbott Park, IL.

o  Microsoft Office Excel 2003, Microsoft Corporation, Redmond, WA.

p  GraphPad Prism v4.00 for Windows, GraphPad Software, San Diego, CA.

q  SigmaStat Version 3.0, SPSS Inc., Chicago, IL.
References


CHAPTER 5

ECHOCARDIOGRAPHIC ASSESSMENT OF LEFT ATRIAL SIZE AND LEFT ATRIAL MECHANICAL FUNCTION IN HORSES: METHODOLOGY AND RELIABILITY

Atrial mechanical function facilitates the transition between the almost continuous flow through the venous circulation and the intermittent filling of the ventricles and exerts a profound effect on overall cardiovascular performance.¹⁻³ Many clinical conditions are associated with atrial remodeling and dilation and can affect the passive and active components of atrial function.²⁴ Two of them, mitral regurgitation and atrial fibrillation, are of particular interest in horses.

Chronic mitral regurgitation (MR) results in chronic volume overload and progressive LA dilatation that may eventually result in an elevation of LA pressures, LA contractile dysfunction, and a reduction in LA emptying fraction.² The degree of LA enlargement is related to the chronicity and severity of regurgitation, the extent of fluid retention, and the degree of ventricular dysfunction, and plays a role as prognostic indicator in MR in humans and animals.⁵⁶

Atrial fibrillation (AF) leads to a loss of atrial pump function, associated with a decrease in cardiac output during higher levels of exercise. Atrial enlargement is thought to be a major independent risk factor of development of AF in patients with MR and is an
important prognostic indicator, predicting restoration of sinus rhythm and risk of AF recurrence after cardioversion. Conversely, AF itself may cause progressive atrial enlargement, most likely as a consequence of contractile dysfunction of atrial myocytes and an increase in atrial compliance. In other species, including man, it has been shown that atrial enlargement and atrial contractile dysfunction persist for a certain time period after conversion of AF to sinus rhythm. This condition has been termed ‘atrial stunning’ and has been attributed to AF-induced atrial remodeling that goes hand in hand with electrical and structural changes in the atrial myocardium. The clinical relevance of atrial stunning in horses is unknown to date. However, active pump function is particularly important during exercise, when diastole shortens and ventricular filling time is limited, and atrial mechanical dysfunction can significantly reduce cardiac output and impair exercise capacity in human athletes. Therefore, it seems likely that atrial stunning may have a negative impact on performance in athletic horses. Furthermore, the degree of atrial contractile dysfunction and the time course of recovery may have prognostic relevance and may predict maintenance of SR after cardioversion.

Despite the clinical relevance of atrial enlargement and atrial mechanical dysfunction in horses, structural and functional characteristics of the equine atria are incompletely studied to date. Pressure-volume loops are considered the gold standard of assessment of LA mechanical function. However, this approach requires invasive measures which precludes its use in clinical practice. A variety of two-dimensional, flow Doppler, and tissue Doppler echocardiographic variables have been used to assess LA size and mechanical function noninvasively in dogs and people. In horses, assessment of LA size has traditionally been limited to subjective evaluation and measurement of the
left atrial diameter.\textsuperscript{6,22} However, the methods commonly used may not provide accurate measurements of the LA chamber at its widest dimensions and may not accurately reflect changes in LA geometry and actual LA size.\textsuperscript{3,22} Moreover, LA mechanical function is usually not specifically assessed during routine examination.

Accordingly, the goals of this study were to demonstrate the feasibility, describe the techniques, and determine the reliability of transthoracic echocardiography for characterization of LA size and LA mechanical function in horses using 2D, flow Doppler, and tissue Doppler imaging methods. Finally, we assessed the potential diagnostic value of each variable for clinical applications and formulated preliminary recommendations for echocardiographic assessment of LA size and mechanical function in horses.

**Material and Methods**

*Study population:* Six horses (4 geldings, 2 mares; 3 Standardbreds, 3 Thoroughbreds) aged 9.8 ± 2.2 years (mean ± SD) and with a body weight of 548 ± 32 kg were studied prospectively. All horses were part of the hospital teaching herd and were considered healthy based upon physical examination, cardiac auscultation, electrocardiogram, and routine echocardiographic examination. None of the horses was in athletic condition, and none of them received medications during the 2 weeks preceding entry into the study. The studies were approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.
Echocardiography: All studies were conducted in unsedated horses standing in a quiet room, restrained by an experienced handler. Transthoracic echocardiography was performed using a GE Vivid 7 echocardiograph with a M3S phased array transducer at a frequency of 1.9/4.0 MHz (octave harmonics). A single lead echocardiogram was recorded simultaneously. Recordings were stored as still frames or cine-loops in digital raw format for offline analyses. Three representative, non-consecutive cardiac cycles were measured and averaged for each variable. Cycles immediately following a sinus pause or 2nd degree atroventricular block were precluded from analysis. The minimal resolution of measurements, hence the smallest possible increments of measurement given by the software, were < 0.07 cm for two-dimensional spacial measurements; < 0.07 cm/s (velocity) and 4 ms (time intervals) for pulsed-wave tissue Doppler recordings; < 0.03 cm/s and 1 ms for color tissue Doppler recordings; and 0.01 m/s and 4 ms for pulsed-wave Doppler recordings of transmitral flow velocity profiles.

Routine transthoracic two-dimensional (2D), M-mode, and color Doppler echocardiography was performed to assess cardiac structures, valvular competence, chamber dimensions, and left-ventricular systolic function, using standard right-parasternal long-axis and short-axis views. The main attention was then directed to the assessment of LA size and mechanical function. Details on machine settings, imaging planes, measurements, variables, calculations, and abbreviations are summarized in Tables 5.1 to 5.3. The echocardiographic variables used in this study were chosen based on published data from the medical literature and based on our own pilot studies (data not shown). Briefly, the left atrium was imaged in a right-parasternal four chamber view, optimized to obtain an image of the entire atrium at its maximal dimensions (Figure 5.1).
The left atrial linear dimensions, area, and volume, respectively, were measured or calculated during maximal atrial filling, immediately prior to mitral valve opening, and indexed to the size of the aorta.\textsuperscript{3,16,17,24} For this purpose, the aortic annulus diameter (AAD) was determined from a right-parasternal long-axis view by measuring the inner distance between the opened aortic valve leaflets during peak systole. Left atrial passive and active emptying were characterized using calculated ejection-phase indices, including passive, active, and total LA fractional shortening, fractional area change, and emptying fraction (Table 5.1).\textsuperscript{16} Active emptying was further assessed by calculating the ratio of active-to-total area change and emptying volume, respectively. Finally, the reservoir function was assessed by calculating the LA reservoir index, derived from the LA area and volume measurements, respectively.\textsuperscript{16,18}

The LA, left atrial appendage, and aorta were also imaged in a right-parasternal short axis view. Atrial linear dimensions and atrial cross-sectional area were measured at end-systole and indexed to the dimensions of the aortic root (Table 5.1 and Figure 5.2).\textsuperscript{17,19} Functional indices were not determined in the short axis plane.

Transmitral flow velocity profiles were recorded from a left-parasternal long-axis view with the pulsed-wave (PW) Doppler cursor positioned between the opened tips of the mitral leaflets (Figure 5.3).\textsuperscript{25,26} The transducer was positioned as ventral as possible and angled dorsally to improve alignment with blood flow. No angle correction was used. Left atrial mechanical function was characterized by peak and integral velocities during atrial contraction (A wave), atrial systolic time intervals, A-wave acceleration, and LA ejection force (Table 5.2).\textsuperscript{1,12,16,20,21,27-34}
Finally, novel tissue Doppler imaging techniques were applied to evaluate active LA wall motion. Based on the results of our pilots studies, LA wall motion was quantified by measuring the following variables: Maximum and minimum radial wall motion velocity at the mid-point of the LA free wall in long- and short-axis, time intervals from the onset of the electrocardiographic P-wave to the onset and to the peak of the A\textsubscript{m} wave, and the duration of the A\textsubscript{m} wave (Table 5.3 and Figure 5.4).\textsuperscript{18,35-38} When the spectral Doppler tracing of the wall motion velocity was multiphasic (characterized by an early positive or negative wave, followed by a late positive-negative wave), only the more consistent late wave was considered for measurements. When the velocity curve did not allow clear distinction of the onset of the second wave, t\textsubscript{Am} and d\textsubscript{Am} were not measured (this was the case in 39 out of a total of 126 measured cardiac cycles from the short-axis color TDI recordings of the LA wall).
### Table 5.1: 2D echocardiographic variables used for assessment of LA size and mechanical function (continued)

<table>
<thead>
<tr>
<th>Measured:</th>
<th>Calculated:</th>
<th>Measurements:</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAD&lt;sub&gt;max, a, min&lt;/sub&gt;</td>
<td>LAV&lt;sub&gt;max, a, min&lt;/sub&gt;</td>
<td>Mitral annular diameter</td>
</tr>
<tr>
<td>LAD&lt;sub&gt;max, a, min&lt;/sub&gt;</td>
<td>LAD&lt;sub&gt;max&lt;/sub&gt;/AAD</td>
<td>Left atrial diameter</td>
</tr>
<tr>
<td>LAL&lt;sub&gt;max, a, min&lt;/sub&gt;</td>
<td>LAL&lt;sub&gt;max&lt;/sub&gt;/AAD&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Left atrial length</td>
</tr>
<tr>
<td>LAA&lt;sub&gt;max, a, min&lt;/sub&gt;</td>
<td>LAA&lt;sub&gt;max&lt;/sub&gt;/AAD&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Left atrial area</td>
</tr>
</tbody>
</table>

- **MAD**: diameter of the mitral valve annulus, atrial side, inner edge
- **LAD**: widest distance parallel to mitral valve annulus, inner edge (septal wall) to pericardial lining (free wall)
- **LAL**: ventro-dorsal dimension from the mid-point of the mitral annulus plane to the inner edge of the dorsal atrial roof, perpendicular to the MAD and LAD dimensions
- **LAA**: area tracing the inner edge of the septal wall and atrial roof and the outer edge (pericardial lining) of the free wall; the confluences of the pulmonary veins were excluded; the ventral border was represented by the plane of the mitral annulus
- **LAV**: automated calculation, single plane method of discs (Simpson)

#### Timing:
- **max**: one frame before opening of the mitral valve (maximum size)
- **a**: at the onset of the P wave (size at onset of active contraction)
- **min**: at closure of the mitral valve (minimum size)

#### Formulas:
- **FS, FAC, EF**:
  - Passive area change: \( \text{passive} = \frac{\text{max} - a}{\text{max}} \)
  - Active area change: \( \text{active} = \frac{a - \text{min}}{\text{a}} \)
  - Emptying fraction: \( \text{EF} = \frac{\text{max} - \text{min}}{\text{max}} \)
- **Active/total AC**: \( \frac{(\text{LAA}_{\text{max}} - \text{LAA}_{\text{min}})}{(\text{LAA}_{\text{max}} - \text{LAA}_{\text{min}})} \)
- **Active/total EV**: \( \frac{(\text{LA}_{\text{max}} - \text{LAV}_{\text{min}})}{(\text{LAV}_{\text{max}} - \text{LAV}_{\text{min}})} \)
- **LA-RI<sub>Area</sub>**: \( \frac{(\text{LAA}_{\text{max}} - \text{LAA}_{\text{min}})}{\text{LAA}_{\text{min}}} \)
- **LA-RI<sub>Vol</sub>**: \( \frac{(\text{LAV}_{\text{max}} - \text{LAV}_{\text{min}})}{\text{LAV}_{\text{min}}} \)

*View: Right-parasternal four-chamber view, optimized to obtain the largest dimensions of the LA (Figure 5.1)*
Table 5.1: Continued

<table>
<thead>
<tr>
<th>View: Right-parasternal short-axis view of the aorta and LA, optimized to image the LA and the LA appendage (Figure 5.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Measured:</strong></td>
</tr>
<tr>
<td>$L_{Ax}A_{\text{max}}$</td>
</tr>
<tr>
<td>$L_{Ax}D_{\text{max}(1)}$</td>
</tr>
<tr>
<td>$L_{Ax}D_{\text{max}(2)}$</td>
</tr>
<tr>
<td>$A_{0x}A$</td>
</tr>
<tr>
<td>$A_{0x}D$</td>
</tr>
<tr>
<td><strong>Calculated:</strong></td>
</tr>
<tr>
<td>$L_{Ax}D_{\text{max}/A_{0x}}$</td>
</tr>
<tr>
<td>$L_{Ax}A_{\text{max}/A_{0x}}$</td>
</tr>
<tr>
<td><strong>Timing:</strong></td>
</tr>
</tbody>
</table>
Figure 5.1: The left atrium imaged in a right-parasternal four-chamber view, optimized to obtain an image of the entire atrium at its maximal dimensions. The mitral annular diameter (MAD), left atrial diameter (LAD), left atrial length (LAL) were measured, and the LA area was traced as indicated. The LA volume was calculated automatically based on the traced area. All measurements were performed in zoom mode. Detailed measurement guidelines are provided in Table 5.1.

Figure 5.2: The left atrium, left atrial appendage, and aorta imaged in a right-parasternal short-axis view. Left atrial linear dimensions (LA_{x1}D_{(1)}, LA_{x2}D_{(2)}) and aortic diameter (Ao_{x3}D) were measured, and the left atrial area and aortic area were traced as indicated. Measurements were performed at end-systole. Detailed measurement guidelines are provided in Table 5.1.
<table>
<thead>
<tr>
<th>Measured:</th>
<th>Calculated:</th>
<th>Measurements:</th>
<th>Formulas:</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{max}}$</td>
<td>$E_{\text{max}}/A_{\text{max}}$ ratio</td>
<td>Peak $E$ wave velocity</td>
<td>$FA_{\text{max}} = A_{\text{max}} / (E_{\text{max}} + A_{\text{max}}) \times 100$</td>
</tr>
<tr>
<td>$E_{\text{VTI}}$</td>
<td>$E_{\text{VTI}}/A_{\text{VTI}}$ ratio</td>
<td>Velocity-time integral $E$ wave</td>
<td>$FA_{\text{VTI}} = A_{\text{VTI}} / (E_{\text{VTI}} + A_{\text{VTI}}) \times 100$</td>
</tr>
<tr>
<td>$A_{\text{max}}$</td>
<td>$FA_{\text{max}}$</td>
<td>Peak $A$ wave velocity</td>
<td>$LA \text{ ejection force} = \frac{1}{2} \times \rho \times MVA_{A} \times A_{\text{max}}^{2}$</td>
</tr>
<tr>
<td>$A_{\text{VTI}}$</td>
<td>$FA_{\text{VTI}}$</td>
<td>Velocity-time integral $A$ wave</td>
<td>with $\rho = \text{density of blood} = 1.06 \text{ g/cm}^{3}$</td>
</tr>
<tr>
<td>$t_{A_{\text{max}}}$</td>
<td>$dv/dt_{A}$</td>
<td>Time from $P$ wave to peak $A$ wave</td>
<td>and $MVA_{A} = \text{Mitrval valve area} = \frac{1}{2} \times \pi \times MAD_{A}^{2}$</td>
</tr>
<tr>
<td>$\text{PEP}_{A}$</td>
<td>$\text{PEP}<em>{A}/ET</em>{A}$ ratio</td>
<td>Left atrial pre-ejection period</td>
<td>($MAD_{A} = \text{mitral annular diameter at onset of atrial contraction}$)</td>
</tr>
<tr>
<td>$ET_{A}$</td>
<td>$LA \text{ ejection force}$</td>
<td>Left atrial ejection time</td>
<td></td>
</tr>
<tr>
<td>$AT_{A}$</td>
<td></td>
<td>Acceleration time of $A$ wave</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.2: PW Doppler variables of transmitral blood flow used for assessment of LA mechanical function. 1,12,16,20,21,27-34
Figure 5.3: Transmirtal flow velocity profile recorded from a left-parasternal long-axis view with the pulsed-wave Doppler cursor positioned between the open tips of the mitral valve leaflets. No angle-correction was used. $E_{\text{max}}$, peak E wave velocity; $E_{\text{VTI}}$, velocity-time integral E wave; $A_{\text{max}}$, peak A wave velocity; $A_{\text{VTI}}$, velocity-time integral A wave; $t_{A_{\text{max}}}$, time to peak A wave; $\text{PEP}_A$, left atrial preejection period; $\text{ET}_A$, left atrial ejection time; $\text{AT}_A$, A wave acceleration time. Detailed measurement guidelines are provided in Table 5.2.
**Table 5.3: Tissue Doppler imaging variables used for assessment of LA mechanical function**

<table>
<thead>
<tr>
<th>Pulsed-wave and color TDI:</th>
<th>Recordings:</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_{e_{max}}$</td>
<td><strong>PW TDI:</strong></td>
</tr>
<tr>
<td>$v_{e_{min}}$</td>
<td>Sample location: LA free wall, at the mid-point between the mitral annulus and the pulmonary vein (long axis) and at the mid-point between the atrial appendage and the pulmonary vein (short axis)</td>
</tr>
<tr>
<td>$t_{e_{max}}$</td>
<td>Sample volume: 5.9 – 8.8 mm</td>
</tr>
<tr>
<td>$t_{A_m}$</td>
<td>Velocity scale: ± 7.5 cm/s (if necessary adjusted to avoid aliasing)</td>
</tr>
<tr>
<td>$d_{A_m}$</td>
<td>Color TDI:</td>
</tr>
<tr>
<td>$t_{A_m}/d_{A_m}$</td>
<td>Narrowest sector angle and minimum imaging depth chosen to achieve frame rates above 120 FPS (range: 143.7 to 147.3 frames/second)</td>
</tr>
</tbody>
</table>

**Measurements:**

**PW TDI:**

- Sweep speed: 100 mm/s
- The outer edge of the strongest echo was measured at standard gain settings.

**Color TDI:**

- Measurements performed using the system-integrated Q-Analysis software in trace mode.
- Sweep speed: maximized to display the PQ interval
- Sample size: 40 x 20 mm
- Sample location: Same as for PW TDI recordings. The sample area was placed so that it covered the entire atrial wall thickness, including the pericardial lining at the outer border of the sample area.
- Smoothing filter: 30 ms
- Time intervals: Begin and end of the $A_m$ wave was defined as the points where velocity tracing crossed the zero baseline.

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*References: 18, 35-38*
Figure 5.4: Left atrial wall motion, quantified by measuring the maximum ($v_{\text{max}}$) and minimum ($v_{\text{min}}$) radial wall motion velocity at the mid-point of the LA free wall in long-axis (lx) and short-axis (sx). Time intervals from the onset of the electrocardiographic P-wave to the onset ($t_{\text{Am}}$) and the peak of the $A_m$ wave ($t_{\text{vmax}}$), respectively, and the duration of the $A_m$ wave ($d_{\text{Am}}$) were determined. Only the more consistent late wave was considered for measurements.

Detailed measurement guidelines are provided in Table 5.3.

Top – Pulsed-wave tissue Doppler imaging.
Bottom – Color-coded tissue Doppler imaging, analyzed with Q-Analysis in trace mode.
Reliability of echocardiographic variables: All horses underwent repeated 
echocardiographic examinations by two experienced examiners, according to the 
previously determined imaging guidelines. One echocardiographer (CCS) examined each 
horse 3 times at an interval of 2 days. On one occasion, a second, independent 
echocardiographer (KES) examined each horse immediately before (3 horses) or after (3 
horses) the other echocardiographer. All recordings were labeled with random codes, 
allowing subsequent offline measurements in a blinded fashion.

The intraobserver measurement variability was determined by a single observer 
(CCS) measuring the same six studies (one study of each horse) repeatedly on three 
different days, thereby averaging the same set of three cardiac cycles for each variable. 
For determination of the interobserver measurement variability, a second observer (KES) 
measured the same cardiac cycles on the same six studies, independently of the first 
observer. For determination of the interobserver within-day variability, one observer 
(CCS) measured the 2 studies of each horse that were recorded consecutively on the same 
day by the two observers. The intraobserver between-day variability was determined by 
one blinded observer (CCS) measuring each horse’s three studies that were recorded by 
CCS on different days. The interobserver between-day variability was determined by one 
blinded observer (CCS) measuring two studies of each horse that were recorded by the 
two observers two days apart; the studies were chosen so that 3 horses were examined by 
CSS first and 3 horses were examined by KES first. All measurement were performed in 
random order and with the observers blinded to signalment and previous measurements.
The test reliability was quantified using the within-subject variance for repeated measurements (residual mean square) determined by one-way analysis of variance with the horses being the ‘groups’. The within-subject standard deviation \( (s_w) \) was calculated as the square root of the residual mean square. Measurement variability and recording variability were reported in two ways: (1) The within subject coefficient of variation \( (CV) \) expressed as a percent value was calculated as \( CV = s_w / \text{mean} \times 100 \). The degree of variability was arbitrarily defined as follows: \( CV < 5\% \), very low variability; \( 5 - 15\% \), low variability; \( 15 - 25\% \), moderate variability; \( > 25\% \), high variability. (2) In addition to the \( CV \), the absolute value below which the difference between two measurements will lie with 95\% probability was estimated following the British Standards Institution (BSI) recommendations: \( BSI = 1.96 \times \sqrt{2} \times s_w = 2.77 \times s_w \). Summary statistics (mean ± SD) of each variable were calculated based on the first study of each horse (n=6) and were reported for comparison. All statistical and graphical analyses were performed using standard computer software.
Results

Echocardiographic assessment of LA size and LA mechanical function by 2D echocardiography and tissue Doppler imaging was possible in all horses using right-parasternal long-axis and short-axis views. Transmitral flow velocity profiles could be recorded in all horses by PW Doppler echocardiography in a left-parasternal long-axis view.

Reliability data of all echocardiographic variables of LA size and mechanical function are summarized in Tables 5.4 to 5.7. Briefly, most 2D variables of LA size showed very low to low variability in long-axis and short-axis imaging planes, respectively. Exceptions were the indexed measures of LA area and LA volume in long-axis view. Among the 2D indices of LA mechanical function, the fractional shortening (based on linear measurements of the LA diameter) showed the highest variability for all three components (passive, active, total). Conversely, variability of area-based and volume-based variables, respectively, was considerably lower. Generally, variability was higher for the indices of active LA function compared to the indices of passive function and reservoir function. Variables of LA function derived from PW Doppler imaging of transmitral blood flow were characterized by very low to low measurement variability, low to moderate within-day variability, and low to high between-day variability. TDI variables were characterized by very low to low measurement variability and low to high within-day and between-day variability. Wall motion velocities were more variable than TDI-derived time intervals.
For an explanation of variables see Table 5.1.

Mean ± SD – summary statistics based on first study of each horse (n = 6)

CV – Coefficient of variation (%)

BSI – Absolute value below which the difference between two measurements will lie with 95% probability

Variables recommended for assessment of LA size and LA mechanical function are reported in **bold print**.

Table 5.4: Reliability of 2D echocardiographic variables used for assessment of LA size.
For an explanation of variables see Table 5.1. For other abbreviations see Table 5.4.

Table 5.5: Reliability of 2D echocardiographic variables used for assessment of LA mechanical function.
For an explanation of variables see Table 5.2. For other abbreviations see Table 5.4.

Table 5.6: Reliability of PW Doppler variables of transmitral blood flow used for assessment of LA mechanical function.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Units</th>
<th>Mean ± SD</th>
<th>Measurement variability</th>
<th>Within-day variability</th>
<th>Between-day variability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intra-observer</td>
<td>Inter-observer</td>
<td>Intra-observer</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>cm/s</td>
<td>58.5 ± 18.4</td>
<td>1.8</td>
<td>3.0</td>
<td>3.5</td>
</tr>
<tr>
<td>$E_{\text{v}i}$</td>
<td>cm</td>
<td>11.5 ± 3.7</td>
<td>4.8</td>
<td>1.6</td>
<td>3.8</td>
</tr>
<tr>
<td>$A_{\text{max}}$</td>
<td>cm/s</td>
<td>37.1 ± 8.8</td>
<td>1.6</td>
<td>1.7</td>
<td>4.4</td>
</tr>
<tr>
<td>$A_{\text{v}i}$</td>
<td>cm</td>
<td>4.6 ± 1.0</td>
<td>2.3</td>
<td>0.29</td>
<td>8.4</td>
</tr>
<tr>
<td>$E_{\text{max}}/A_{\text{max}}$</td>
<td>-</td>
<td>1.62 ± 0.42</td>
<td>2.3</td>
<td>0.10</td>
<td>1.9</td>
</tr>
<tr>
<td>$E_{\text{v}i}/A_{\text{v}i}$</td>
<td>-</td>
<td>2.58 ± 0.81</td>
<td>3.5</td>
<td>0.25</td>
<td>3.9</td>
</tr>
<tr>
<td>$F_{\text{A}_{\text{max}}}$</td>
<td>%</td>
<td>39 ± 7</td>
<td>1.3</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td>$F_{\text{A}_{\text{v}i}}$</td>
<td>%</td>
<td>30 ± 6</td>
<td>2.7</td>
<td>2.1</td>
<td>3.4</td>
</tr>
<tr>
<td>$t_{\text{A}_{\text{max}}}$</td>
<td>ms</td>
<td>170 ± 27</td>
<td>4.4</td>
<td>23.1</td>
<td>7.4</td>
</tr>
<tr>
<td>PEP$_{A}$</td>
<td>ms</td>
<td>89 ± 20</td>
<td>4.9</td>
<td>12</td>
<td>10.5</td>
</tr>
<tr>
<td>ET$_{A}$</td>
<td>ms</td>
<td>207 ± 19</td>
<td>3.4</td>
<td>20</td>
<td>11.3</td>
</tr>
<tr>
<td>AT$_{A}$</td>
<td>ms</td>
<td>92 ± 17</td>
<td>5.1</td>
<td>13</td>
<td>7.1</td>
</tr>
<tr>
<td>dv/dt$_{A}$</td>
<td>cm/s²</td>
<td>434 ± 162</td>
<td>3.8</td>
<td>46</td>
<td>3.4</td>
</tr>
<tr>
<td>PEP$<em>{A}$/ET$</em>{A}$</td>
<td>-</td>
<td>0.44 ± 0.12</td>
<td>8.3</td>
<td>0.10</td>
<td>18.7</td>
</tr>
<tr>
<td>LA ejection force</td>
<td>kdynes</td>
<td>53.3 ± 20.9</td>
<td>4.6</td>
<td>7.1</td>
<td>6.4</td>
</tr>
</tbody>
</table>

*View: Left-parasternal long-axis view optimized for the inflow tract.*
For an explanation of variables see Table 5.3. For other abbreviations see Table 5.4.

Table 5.7: Reliability of tissue Doppler variables used for assessment of LA mechanical function (continued).
For an explanation of variables see Table 5.3. For other abbreviations see Table 5.4.
† Values affected by selection bias. 39 of 126 cycles could not be measured because the velocity curve did not allow clear distinction of the onset and/or the end of the second velocity wave. Therefore, there was a bias towards including cycles of higher quality, leading to falsely high reliability.
Discussion

This investigation showed that LA size and LA mechanical function can be assessed by transthoracic echocardiography in standing, unsedated, adult horses. Furthermore, it provides data on imaging techniques and reliability using a variety of standard 2D and transmitral flow Doppler measurements as well as tissue Doppler imaging variables.

Assessment of the LA dimensions by measurement of its diameter is part of any routine echocardiogram in horses. However, the two methods traditionally used to measure the LA diameter have significant limitations. M-mode recordings performed in a right-parasternal short-axis view at the level of the heart base largely depend on the placement of the cursor line and often provide a measure of the left atrial appendage rather than the left atrial body. Two-dimensional echocardiography in a left-parasternal long-axis view often does not allow imaging of the LA in its entirety due to interference with the ventral lung border; this often results in measurements of the LA diameter that are not parallel to the mitral valve annulus or that are made too close to the annulus and thereby underestimate the true maximal atrial diameter. Conversely, the right-parasternal window used in this study offers a view of the entire LA and provides sufficient anatomical landmarks for consistent measurements of the largest LA dimensions in any direction and at any instance of the cardiac cycle.

One-dimensional (linear) measurements such as the LA diameter are easy to measure. However, they tend to be less sensitive to detect changes in LA size compared to two- or three-dimensional measurements, because LA enlargement may not occur in a uniform fashion and LA geometry may change over time. Anatomical limitations in adult horses prevent the use of the standard biplane measures of LA volume that are commonly
used in people.\textsuperscript{3,41} Therefore, in addition to LA diameter and LA length, we chose to measure LA area in right-parasternal long-axis and short-axis views. We also calculated the LA volume using a single-plane method of discs in the long-axis plane, realizing that single-plane estimates of LA volume may not provide significant additional information compared to area measurements, and may actually be less reliable due to amplification of small measurement errors.

The timing of LA measurements is often not specified in the equine literature, leading to uncertainties when comparing results from different studies. According to the recommendations of the American Society and the European Association of Echocardiography, we measured the LA size at the end of the ventricular systole, one frame prior to mitral valve opening, when the LA chamber was at its greatest dimension.\textsuperscript{3} In order to account for potential differences in body size, the measures of LA size were indexed to the dimensions of the aortic root according to the principles of allometric scaling, assuming that the aortic size correlates with body mass.\textsuperscript{24,42} We did not attempt to prove that these aortic ratios were actually independent of body size. Rather, we intended to investigate the influence on test reliability when indexing a variable to another, independently measured reference variable.

\textit{Atrial mechanical function} is determined by the complex interrelation between atrial loading conditions, atrial and ventricular inotropic state, the rate and extent of atrial and ventricular relaxation, atrial and ventricular compliance, heart rate, and intraatrial electrical conduction.\textsuperscript{2} Noninvasive echocardiographic variables are unlikely to allow distinct separation and quantification of the individual factors causing alterations in LA mechanical function. Rather, they provide an overall assessment of the resulting atrial
mechanical performance. Nevertheless, atrial mechanical function can be divided into three distinct phases that, to a certain degree, can be assessed separately by means of echocardiography. During ventricular systole, the atria act as a ‘reservoir’ to receive blood from venous return. During early- and mid-diastole, the atria serve as a ‘conduit’, allowing emptying of the stored blood into the ventricles and continued passage of venous return from the large veins into the ventricles. During late diastole, the atria actively contract and serve as ‘booster-pumps’ that establish the final ventricular end-diastolic volume.

In this study, reservoir function was assessed using the area-based and volume-based LA reservoir index. Conduit function was not specifically assessed due to the lack of accurate methods for volumetric measurements of LA inflow and outflow during diastole. Hence, the majority of the variables used in this study were related to active LA pump function, including 2D ejection-phase variables (Table 5.5), PW Doppler variables of transmitral flow velocity (Table 5.6), and tissue Doppler variables of LA wall motion (Table 5.7). Due to technical limitations and anatomical restrictions leading to poor alignment with blood flow and insufficient image quality, we elected not to investigate pulmonary venous flow and left atrial appendage flow profiles in this study. Also, tissue Doppler based strain and strain rate, which have been used in humans to quantify LA mechanical function, were not studied because the image quality and the atrial wall thickness were insufficient to properly derive these variables from the radial (as opposed to longitudinal) velocity data recorded in the imaging planes available in horses.
The 2D indices of LA mechanical function are easily calculated from linear, area, or volume measurements of LA dimensions at different time points during the cardiac cycle. The active fractional shortening, fractional area change, and emptying fraction, respectively, directly quantify the active booster-pump function, while the active/total area change and volume change ratios relate the active emptying to the total emptying.

Transmitral blood flow velocities, determined by PW Doppler echocardiography, are commonly used for assessment of LV diastolic function and evaluation of LV filling pressures. However, the mitral flow velocity profile is also strongly influenced by LA mechanical function. Most importantly, LA active pump function (together with LV compliance) is a major determinant of the maximal transmitral A wave velocity. The velocity time integral of the A wave correlates well with LA stroke volume in people, while the E/A ratios (Emax/Amax, Evti/Avti) and the fractional active emptying (FAmax, FAvti) provide information on relative contribution of atrial pump function to ventricular filling. The LA systolic time intervals (PEP, ETA, PEP/ETA) may be useful indices of LA systolic function, with a lower PEP/ETA ratio indicating improved systolic function. Similarly, the time to peak A velocity (tAmax), the acceleration time of the A wave (AT), and the A wave acceleration rate (dv/dtA) have been used to characterize atrial electromechanical activation in humans and may serve the same purpose in horses. The LA ejection force is a composite variable based on the peak A velocity and the mitral valve area, calculated from a linear measurement of the mitral valve diameter (Table 5.2). LA ejection force provides a physiologic assessment the strength of the atrial contraction and has been widely used as a surrogate for LA systolic function.
function in people.\textsuperscript{12,33,34,38} The major limitation of transmitral Doppler flow recordings in adult horses is the lack of adequate alignment with the direction of transmitral blood flow. However, while the absolute measurements may not be accurate, velocity ratios, time intervals, and serial comparisons of $A_{\max}$ over time may still be valid, provided that they can be reliably measured.

Recently, novel tissue Doppler imaging techniques have been used to evaluate global and regional atrial contractile function in people using a variety of imaging planes and imaging modalities.\textsuperscript{18,36-38} However, the clinical value of this technique remains unclear to date. In humans, longitudinal wall motion recorded from apical imaging planes is usually studied. These views are not available in adult horses. Therefore, we elected to study radial motion of the LA free wall in a right-parasternal long-axis and short-axis view using PW and color TDI techniques, assuming that $v_{\max}$ and $v_{\min}$ directly reflect atrial contraction and relaxation, while TDI time intervals reflect global atrial systolic function in a similar fashion as systolic time intervals derived from transmitral Doppler flow profiles.

Similar to other diagnostic methods, echocardiographic measurements are subject to different sources of variability. Normal \textit{biological variability} can affect echocardiographic recordings in the short term (i.e. beat-to-beat) and long-term (i.e. hour-to-hour or day-to-day) and is influenced by physiologic effects, environmental factors leading to stress responses, and behavioral reactions.\textsuperscript{49} \textit{Recording variability} is caused by differences within and between observers in transducer placement, imaging planes, and machine settings. \textit{Measurement variability} largely depends on image quality and is related to the ability to identify anatomical landmarks (for spacial measurements) and
temporal events (for measurement of time intervals), machine settings, minimal resolution of measurements provided by the software, and adherence of operators to measurement guidelines. With contemporary equipment, calibration error is largely eliminated by the ability to store and analyze raw data in digital format.

In the current study, we attempted to minimize the sources of variability by performing the exams in a quiet environment, using high-end echocardiographic equipment with digital raw data storage, standardizing machine settings for recording and measurements, implementing strict imaging and measurement guidelines, and optimizing image planes to achieve adequate image quality. However, to avoid selection bias, we deliberately refrained from selecting small-frame, young horses in athletic condition that were more likely to provide ‘ideal’ images. Instead, our study population consisted of untrained horses of average size and varying body condition that were more likely to represent a typical patient population.

The reliability of a diagnostic test is the extent to which the test yields the same results on repeated trials. Hence, test reliability is directly related to the variability of the test results. Reliability data are important to assess the usefulness of echocardiographic variables to measure serial changes within an individual horse or differences between small groups of horses related to disease progression, treatment, age, exercise, training, or other factors. Many different methods have been used to quantify reliability of echocardiographic measurements. Unfortunately, there is no general consent on how to assess reliability of echocardiographic variables. The results of different studies may vary depending on study design and statistical analyses. Furthermore, the terminology and format used to report the results are not standardized. Therefore, direct
comparison between different studies has been difficult. In this study, we quantified reliability by calculating the standard deviation for repeated measurements on the same subjects. The results were then reported in two ways, as within subjects coefficient of variation (CV) and as the absolute value below which the difference between two measurements will lie with 95% probability. The coefficient of variation has the advantage that it is ‘standardized’ to the mean and therefore independent of the absolute values and the units of measurement. This feature simplifies comparison between different variables within a study. In the current study, echocardiographic variables and indices were classified based the magnitude of the CV. However, for clinical use, rigid cut-offs may be very misleading as they do not account for the magnitude of changes that are seen in clinical practice and that are thought to be clinically relevant. Therefore, we also provided absolute values below which the differences between two measurements will lie with 95% probability if caused by normal variability. These values allow, on a case-by-case basis, comparison with measured changes in echocardiographic variables, in order to decide whether the observations represent true changes or normal variability.

The intra-observer and inter-observer measurement variability was determined to assess the ability of observers to reliably measure the respective variables. In general, echocardiographic variables are unlikely to be useful if image quality and image planes do not allow reliable measurement of the variables. Our results showed that most variables had a low to very low measurement variability, with the exception of the 2D variables of LA mechanical function that were characterized by low to moderate variability (area- and volume-based variables) and high variability (fractional shortening).
Determination of the *within-day, inter-observer variability* allowed assessment of the error introduced by a second operator performing the examination while minimizing (but not excluding) the biological variability (by performing the exams consecutively on the same day) and measurement error (by having one observer measure all recordings). In order for a variable to be clinically useful, imaging guidelines and adequate training should allow two independent observers to record standardized images of adequate quality in order to reliably measure the variable. The results showed that this was true for most variables used in this study. Overall, variables of LA size were characterized by a very low to low recording variability, and most variables of LA mechanical function were characterized by a low to moderate recording variability. Exceptions were LA fractional shortening and $v_{\text{max}}$ determined by color TDI from a right-parasternal long-axis view.

Finally, we determined the *between-day, intra-observer and inter-observer variability*, respectively, to assess the day-to-day variability, including biological, recording, and measurement variability. These two measures of test reliability may be most useful for clinical applications. In general, true alterations in echocardiographic variables caused by disease or interventions in an individual patient would have to be larger than the potential changes caused by to day-to-day variability. Due to differences in patient population, equipment, observer experience, implementation of imaging guidelines, and image quality, the data of our study may not be directly applicable to all clinical situations. However, in situations where one experienced operator performs serial echocardiographic examinations on the same patient using contemporary equipment and adequate imaging guidelines, the data for the intra-observer, between-day variability may be helpful as a guide to decide whether observed alterations in echocardiographic
variables represent true changes. In cases where two observers perform serial examinations on the same horse, it is generally advisable that one observer measures both recordings for direct comparison of variables. In this situation, the data for the inter-observer, between-day variability can be used, provided that both observers are equally experienced and trained, use the same equipment, and follow standardized imaging guidelines. For many, less standardized situations, the variability of echocardiographic measurements may be higher than reported here.

Our results indicate that LA diameter, length, area, and volume can be reliably measured from a right-parasternal long-axis and short-axis view, respectively (Table 5.4). The two linear measurements performed in the short-axis plane may be somewhat redundant to the diameter measured in long-axis, as they are oriented in a similar direction. Furthermore, reliability data do not suggest superior performance of the short-axis measurements. Therefore, linear measurements during routine examination may be limited to LA diameter and length from a long-axis view. In addition to these traditional linear measurements, we suggest measuring the cross-sectional LA area in a right-parasternal long-axis and short-axis view, to assess changes of LA size in horses. Calculation of the LA volume based on a single-plane method may not provide significant additional information and might actually be slightly less reliable compared to the long-axis area measurements. The indexed measures of LA size were generally more variable than the native measurements, most likely due to the cumulative errors of the two independent measurements. Therefore correction to body weight (or body weight raised to the $n^{th}$ power) may be preferable, because indexing to a constant would not affect reliability of the variable.
The area-based and volume-based 2D indices of LA reservoir function and LA pump function performed similarly in regards to reliability of measurements, while all components of the LA fractional shortening were highly variable. Following our recommendations for assessment of LA size, we suggest using the area-based variables for assessment of LA reservoir function and LA pump function in horses. The active fractional area change and the active/total area change ratio were characterized by moderate day-to-day variability and may therefore not be very reliable indicators to detect minor alterations in LA pump function. The total fractional area change (FAC) was characterized by low variability and may therefore be a useful index for clinical applications, although it does not allow separation of the active pump function from reservoir and conduit function.

Variables derived from maximal transmitral flow velocities appeared to be slightly more reliable than integral variables. Overall, our results revealed a moderate to high day-to-day variability for these variables, suggesting that their accuracy to detect minor changes in individual patients may be suboptimal. Among the atrial systolic time intervals, PEP\textsubscript{A}/ET\textsubscript{A}, ET\textsubscript{A}, AT\textsubscript{A}, and t\textsubscript{Amax} had low to moderate variability, indicating a potential clinical value for assessment of LA mechanical function in horses. Conversely, the use of the dv/dt\textsubscript{A} is hampered by the high between-day variability. Similarly, the LA ejection force may not be useful for detection and serial evaluation of LA mechanical dysfunction due to its high intra-observer between-day variability.

Generally, recordings of adequate quality could be recorded in both long- and short-axis views and with both PW and color TDI methodology. The exception were the color TDI short-axis recordings that often did not allow distinct identification of the beginning
and the end of the wall motion velocity wave, in which case $t_{Am}$ and $d_{Am}$ could not be measured (Table 5.7). The results indicated poor day-to-day reliability for all velocity measures. Therefore, in individual patients wall motion velocity may at best serve to detect presence or absence of wall motion, but will not allow accurate quantification of minor changes in velocity over time. Among the TDI-derived time intervals, reliability was adequate only for the pulsed-wave TDI measurements in right-parasternal long-axis view (disregarding the short-axis color TDI measurements that were affected by selection bias). We believe that these time intervals may be a clinically useful tool for quantifying atrial electromechanical activation in horses. However, it remains unknown how much additional information is gained by measuring TDI time intervals in addition to 2D and transmitral flow velocity variables.

In conclusion, we were able to show that LA size and LA mechanical function can be evaluated noninvasively using 2D echocardiography, transmitral flow velocity profiles, and TDI-based wall motion analysis. Our imaging guidelines and recommendations should be regarded as preliminary and will have to be validated in clinical practice. Further studies will be required to establish normal values, considering potential differences related to breed, age, sex, body weight, and athletic condition. Investigation of the interrelationship between different variables and the influence of autonomic tone and loading conditions will help elucidating the physiological and pathophysiological meaning of the echocardiographic variables. Finally, and most importantly, the clinical value of these variables to assess disease-related alterations in LA function and their relation to severity of disease, exercise capacity, and prognosis will have to be established.
Footnotes

a GE Medical Systems, Milwaukee, WI.
b EchoPAC v3.1.3, GE Medical Systems, Milwaukee, WI.
c Microsoft Office Excel 2003, Microsoft Corporation, Redmond, WA.
d SigmaStat v3.01, SPSS Inc., Chicago, IL.

References


8. Van Loon G. Atrial pacing and experimental atrial fibrillation in equines. PhD Dissertation. Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, University of Gent, Belgium; 2001:268 pages.


CHAPTER 6

ECHOCARDIOGRAPHIC DETECTION OF LEFT ATRIAL MECHANICAL DYSFUNCTION AFTER CONVERSION OF ATRIAL FIBRILLATION TO SINUS RHYTHM IN 5 HORSES

Atrial fibrillation (AF) is common in horses and is typically associated with exercise intolerance at higher levels of performance.\textsuperscript{1} Although clinical and echocardiographic findings often do not reveal any underlying cardiac disease, ultrastructural and functional atrial changes may go undetected during routine examination. Atrial enlargement, if present, is thought to be a major independent risk factor for the development of AF.\textsuperscript{2,3} Conversely, AF itself may also cause progressive atrial enlargement in the absence of other underlying factors, most likely as a consequence of loss of contractility and increase in atrial compliance.\textsuperscript{3-6} In other species, including man, it has been shown that atrial enlargement and atrial contractile dysfunction persist for a certain time after conversion of AF to normal sinus rhythm (NSR). This condition has been attributed to AF-induced atrial remodeling that is associated with electrical, metabolic, and structural changes in the atrial myocardium.\textsuperscript{7,8} VanLoon et al. found evidence for atrial electrical remodeling and atrial contractile dysfunction in a pacing-model of equine AF.\textsuperscript{6,9} However, atrial dysfunction has not been demonstrated after cardioversion in horses with naturally occurring AF, and the clinical relevance of this condition is unknown to date.
Atrial pump function is crucial for normal exercise capacity in athletes.\textsuperscript{4,10} Therefore, it seems likely that alterations of atrial mechanical function may have some negative impact on performance in athletic horses. Furthermore, the degree of atrial contractile dysfunction and the time course of recovery after restoration of NSR may have prognostic relevance and may predict recurrence of AF after cardioversion.\textsuperscript{4,11-14} Recently, we investigated the feasibility of echocardiography for assessment of left atrial (LA) size and LA mechanical function in horses, and we were able to identify echocardiographic variables that may be clinically useful to detect and quantify atrial mechanical dysfunction in horses with cardiac disease (Chapter 5).\textsuperscript{a}

The goal of this study was to characterize LA mechanical function after successful treatment of horses with naturally occurring AF. Specifically, we hypothesized that echocardiography will be able to detect LA mechanical dysfunction in horses after conversion of AF to NSR, consistent with AF-induced atrial remodeling.

**Material and Methods**

*Study population:* The study population consisted of 6 healthy Standardbred horses (‘normal’ group) and 5 Standardbred horses diagnosed with atrial fibrillation (‘AF’ group). All horses were client-owned race horses in athletic condition. None of the normal horses received medications during the 2 weeks preceding entry into the study and all were considered healthy based upon physical examination, cardiac auscultation, electrocardiogram, and echocardiographic examination.

The horses in the ‘AF’ group were diagnosed with AF based on a surface base-apex electrocardiogram (ECG). Standard two-dimensional echocardiography (2DE) and
Doppler echocardiography (DE) served to rule out underlying structural cardiac disease prior to treatment. Four horses were treated pharmacologically with quinidine sulfate.\textsuperscript{1,15} One horse was treated by means of transvenous electrical cardioversion,\textsuperscript{16,17} after previous quinidine treatment had caused adverse reactions (accelerated ventricular response rate, colic) and had been discontinued. Follow-up phone calls were made to all owners to inquire about recurrence of AF. Details on signalment, clinical findings, treatment, and outcome are summarized in Table 6.1.

\textit{Echocardiography:} Echocardiography was performed in standing, unsedated horses by an experienced echocardiographer (CCS). All horses were in NSR at the time of the examination. ‘Normal’ horses were examined once, ‘AF’ horses were examined 24 hours (‘AF24’) and 72 hours (‘AF72’) after conversion to NSR. Transthoracic echocardiography was performed using a GE Vivid 7 echocardiograph\textsuperscript{b} with a M3S phased array transducer at a frequency of 1.9/4.0 MHz (octave harmonics). A single-lead ECG was recorded simultaneously for timing. Recordings were stored as still images or cine-loops in digital raw format for offline analyses\textsuperscript{c}. Exams were labeled with a random code and offline measurements were performed blinded to the horses’ signalment, treatment, health status, and time point of examination. Three representative, non-consecutive cycles were measured and averaged for each variable. Cycles immediately following a sinus pause or a 2\textsuperscript{nd} degree atrioventricular block were precluded from analysis.
Table 6.1: General characteristics of the study population. Numeric data are reported as median (min – max).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal horses</th>
<th>AF horses</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

**Signalment**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal horses</th>
<th>AF horses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td>Standardbred</td>
<td>Standardbred</td>
</tr>
<tr>
<td>Sex</td>
<td>4 mc, 1 m, 1 f</td>
<td>2 m, 3 mc</td>
</tr>
<tr>
<td>Age (years)</td>
<td>4 (3–7)</td>
<td>5 (2–8)</td>
</tr>
<tr>
<td>BWT (kg)</td>
<td>494 (437–540)</td>
<td>470 (427–498)</td>
</tr>
</tbody>
</table>

**History**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal horses</th>
<th>AF horses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of AF</td>
<td>–</td>
<td>3–10 days (n=3), unknown (n=2)</td>
</tr>
<tr>
<td>Recurrent AF</td>
<td>–</td>
<td>Two episodes over last 2 years (n=1; horse #3)</td>
</tr>
</tbody>
</table>

**Treatment**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal horses</th>
<th>AF horses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacologic cardioversion (n=4)</td>
<td>–</td>
<td>Quinidine, 22 mg/kg PO q2h; total dose 10 to 50 g Additional digoxin, 2 × 1 mg total dose IV q12h (n=1; horse #4)</td>
</tr>
<tr>
<td>Plasma quinidine concentration</td>
<td>–</td>
<td>24h post cardioversion: 0.37 (0.16 – 0.67) μg/mL 72h post cardioversion: 0.17 (0 – 0.26) μg/mL</td>
</tr>
<tr>
<td>Outcome</td>
<td>–</td>
<td>Follow-up for 10 to 12 months: No recurrent episodes of AF reported.</td>
</tr>
<tr>
<td>Electrical cardioversion (n=1; horse #2)</td>
<td>–</td>
<td>Transvenous approach, 8 shocks, maximum energy 225 J (biphasic) Recurrent AF reported 3 and 7 months after initial treatment. Second treatment successful, third treatment unsuccessful.</td>
</tr>
</tbody>
</table>

Table 6.1: General characteristics of the study population. Numeric data are reported as median (min – max).
Transthoracic 2DE, M-mode, and color DE was performed to assess cardiac structures, valvular competence, chamber dimensions, and left-ventricular systolic function using standard right-parasternal long-axis and short-axis views.\textsuperscript{18-20} The main attention was then directed to the assessment of LA size and LA mechanical function (Chapter 5).\textsuperscript{a} The LA was imaged in a right-parasternal four chamber view, optimized to obtain an image of the entire atrium at its maximal dimensions (Figure 5.1). The LA diameter (LAD) was measured as the widest diameter parallel to mitral valve annulus, from the inner edge of the septal wall to the pericardial lining of the free wall. The LA area (LAA) was measured by tracing the inner edge of the septal wall and atrial roof and the outer edge (pericardial lining) of the free wall. The confluences of the pulmonary veins were excluded and the ventral border was represented by the plane of the mitral annulus (Table 5.1).\textsuperscript{a} The LAD and LAA, respectively, were determined at three time points during the cardiac cycle: (1) during maximum atrial filling, one frame prior to mitral valve opening (LAD\textsubscript{max}, LAA\textsubscript{max}), (2) at the onset of active contraction, defined as the onset of the electrocardiographic P wave (LAD\textsubscript{a}, LAA\textsubscript{a}), and (3) during minimum atrial filling, at closure of the mitral valve (LAD\textsubscript{min}, LAA\textsubscript{min}) (Table 5.1).\textsuperscript{a,21,22} The LA (including the LA appendage) was also imaged in a right-parasternal short-axis view and its internal cross-sectional area was measured at end-systole, one frame after closure of the aortic valve (LA\textsubscript{sxAmax}). If necessary, the caudal edge of the LA was approximated by extending the visible edges in a curved fashion (Figure 5.2; Table 5.1).\textsuperscript{a,23}

The LA size was reported as LAD\textsubscript{max}, LAA\textsubscript{max}, and LA\textsubscript{sxAmax}. Left atrial mechanical function was characterized using calculated ejection-phase indices, including active fractional area change (active FAC (%) = \[\text{LAA}_a - \text{LAA}_{\text{min}}\] / LAA\textsubscript{a} × 100) and the ratio...
of active-to-total LA area change ([LAA_a – LAA_min] / [LAA_max – LAA_min]). Left atrial reservoir function was assessed by calculation of the reservoir index (LA RI (%) = [LAA_max – LAA_min] / LAA_min × 100) (Table 5.1).<sup>a,22-24-26</sup>

Transmitral flow velocity profiles were recorded from a left-parasternal long-axis view with the pulsed-wave (PW) Doppler cursor positioned between the tips of the open mitral leaflets (Figure 5.3).<sup>27,28</sup> The transducer was positioned as ventral as possible and angled dorsally to improve alignment with blood flow. No angle correction was used. Peak velocities during passive early inflow (E_max) and atrial contraction (A_max) were measured. The following atrial systolic time intervals were determined: The time from the onset of the electrocardiographic P wave to peak A wave velocity (t_Amax), the time from the onset of the P wave to onset of the A wave (preejection period, PEP_A), and the duration of the A wave (ejection time, ETA).<sup>a,22,26,29-31</sup> LA active mechanical function was characterized by the fractional active emptying velocity (FA_max (%) = A_max/[E_max+A_max] × 100), the PEP_A/ET_A ratio, and t_Amax (Table 5.2).<sup>a</sup>

Finally, the LA was imaged in right-parasternal long-axis view optimized to image the LA, using the PW tissue Doppler imaging (TDI) mode with the sample volume located at the mid-point of the LA free wall, halfway between the mitral annulus and the dorsal atrial roof (Figure 5.4).<sup>a</sup> The time intervals from the onset of the electrocardiographic P wave to the onset (t_Am) and to the peak of the A_m wave (t_vmax), and the duration of the A_m wave (d_Am) were measured, and the t_Am/d_Am ratio was calculated to characterize LA electromechanical function (Table 5.3).<sup>a,24,32-35</sup> When the spectral tracing of the wall motion velocity was multiphasic (characterized by an early positive or
negative wave, followed by a late positive-negative wave), only the more consistent late wave was considered for measurements (Figure 5.4).

*Plasma drug concentrations:* In the ‘AF’ group, echocardiographic assessment of atrial function was performed 24 and 72 hours after conversion to NSR. Assuming a quinidine half-life of 8 hours in horses, the drug effect on atrial function was not expected to be clinically significant by 72 hours after conversion. To assess potential influence of residual circulating drug concentrations on atrial function, quinidine plasma concentrations were determined at the time of the exams (‘AF24’, ‘AF72’) in all horses that underwent medical treatment of AF. Quinidine plasma concentrations were determined in the clinical laboratory of the School of Veterinary Medicine of the University of Pennsylvania, Kennet Square, PA using a commercial fluorescence polarization immunoassay. The lower limit of detection was 0.2 μg/mL.

*Data analyses and statistics:* Descriptive summary statistics and graphical analyses were performed using standard computer software. All results were reported as median (min – max). Data pertaining to LA mechanical function were presented using vertical scatter plots, including the median value. In addition, paired data points from the same horse in the ‘AF24’ and ‘AF72’ recording periods were connected by lines. Due to the low sample size, no attempts were made to test for normality, and non-parametric tests were chosen for analyses. Pairwise comparisons of echocardiographic variables between ‘normal’ and ‘AF24’ and between ‘normal’ and ‘AF72’ were performed using a Mann Whitney test. Echocardiographic variables were compared between ‘AF24’ and ‘AF72’ using a Wilcoxon signed rank test. Bonferroni correction was applied for 3 pairwise comparisons. Hence, the level of significance was defined as $\alpha = 0.05 / 3 = 0.0167$. 

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Results

The resting heart rate, cardiac dimensions, and left ventricular fractional shortening of both groups are summarized in Table 6.2. No significant differences were detected between ‘normal’ and ‘AF’ horses, with the exception of LAA$_{\text{max}}$, that was significantly larger in the ‘AF72’ group compared to normal horses. The 2DE indices of LA function are presented in Figure 6.1. The results showed that the indices of active LA function (active LA FAC and active/total LA AC) and the index of LA reservoir function (LA RI) were significantly decreased 24 hours after conversion to sinus rhythm compared to ‘normal’ horses. Although statistically not significant, the three echocardiographic indices increased between ‘AF24’ and ‘AF72’ in all horses, with the exception of horse #2 (treated by electrical cardioversion). The transmitral Doppler flow indices of LA function are displayed in Figure 6.2. No significant differences were detected between normal horses and horses after conversion of AF to NSR. The tissue Doppler indices of LA function are presented in Figure 6.3. The $t_{\text{vmax}}$ showed a non-significant trend towards prolongation in the AF groups, while the $t_{\text{Am}}/d_{\text{Am}}$ ratio was significantly prolonged in the ‘AF24’ group compared to ‘normal’. In all but one horse (horse #2), the $t_{\text{Am}}/d_{\text{Am}}$ decreased between ‘AF24’ and ‘AF72’.
Table 6.2: 2DE and M-mode echocardiographic findings.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>AF 24</th>
<th>AF 72</th>
<th>nl → AF24</th>
<th>nl → AF72</th>
<th>AF24 → 72</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR (min⁻¹)</strong></td>
<td>38 (29 – 41)</td>
<td>31 (28 – 50)</td>
<td>32 (25 – 49)</td>
<td>0.4286</td>
<td>0.3290</td>
<td>0.1875</td>
</tr>
<tr>
<td><strong>Left atrial dimensions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LADₘₐₓ (cm)</td>
<td>12.1 (10.4 – 13.6)</td>
<td>12.4 (12.3 – 13.3)</td>
<td>13.0 (12.9 – 13.6)</td>
<td>0.6623</td>
<td>0.3290</td>
<td>0.0625</td>
</tr>
<tr>
<td>LAAₘₐₓ (cm²)</td>
<td>95.0 (73.8 – 102.7)</td>
<td>99.2 (91.5 – 107.6)</td>
<td>107.6 (101.9 – 116.5)</td>
<td>0.2468</td>
<td>0.0087</td>
<td>0.1250</td>
</tr>
<tr>
<td>LAₘₐₓ (cm²)</td>
<td>115.2 (97.4 – 137.0)</td>
<td>128.8 (110.2 – 137.3)</td>
<td>127.9 (112.5 – 142.0)</td>
<td>0.2571</td>
<td>0.1775</td>
<td>0.8750</td>
</tr>
<tr>
<td><strong>Left ventricular dimensions and systolic function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVSₜ (cm)</td>
<td>3.6 (2.9 – 3.8)</td>
<td>3.2 (3.1 – 3.7)</td>
<td>3.4 (2.7 – 3.6)</td>
<td>0.6623</td>
<td>0.2468</td>
<td>0.6250</td>
</tr>
<tr>
<td>LVIDₜ (cm)</td>
<td>11.5 (10.8 – 12.4)</td>
<td>12.7 (11.4 – 13.4)</td>
<td>12.2 (11.7 – 13.1)</td>
<td>0.0519</td>
<td>0.0823</td>
<td>0.8125</td>
</tr>
<tr>
<td>LVFWₜ (cm)</td>
<td>2.8 (2.7 – 3.2)</td>
<td>2.5 (2.1 – 3.4)</td>
<td>2.5 (2.0 – 3.1)</td>
<td>0.4286</td>
<td>0.2468</td>
<td>0.8125</td>
</tr>
<tr>
<td>LV FS (%)</td>
<td>37.2 (28.9 – 39.3)</td>
<td>28.7 (25.9 – 41.3)</td>
<td>33.5 (24.5 – 39.0)</td>
<td>0.2468</td>
<td>0.6623</td>
<td>0.8125</td>
</tr>
<tr>
<td><strong>Dimensions of the large vessels</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AoD (cm)</td>
<td>7.3 (7.2 – 8.5)</td>
<td>8.0 (7.4 – 9.3)</td>
<td>7.5 (7.3 – 9.1)</td>
<td>0.0519</td>
<td>0.1775</td>
<td>0.1250</td>
</tr>
<tr>
<td>PAD (cm)</td>
<td>6.2 (5.9 – 6.9)</td>
<td>6.3 (6.0 – 6.8)</td>
<td>6.6 (6.0 – 6.8)</td>
<td>0.9307</td>
<td>1.0000</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

HR, heart rate; LADₘₐₓ, maximum left atrial diameter; LAAₘₐₓ, maximum left atrial area (long-axis); LAₘₐₓ, maximum left atrial area (short-axis); IVSₜ, interventricular septal thickness in diastole; LVIDₜ, left ventricular inner diameter in diastole; LVFWₜ, left ventricular free wall thickness in diastole; LV FS, left ventricular fractional shortening; AoD, diameter of the aortic sinus at end-diastole; PAD, diameter of the pulmonary artery sinus at end-diastole.

Normal, normal horses; AF24, horses with AF, 24 hours after conversion to NSR; AF 72, horses with AF, 72 hours after conversion to NSR.

# Two-tailed Mann-Whitney test.
† Two-tailed Wilcoxon signed rank test.
Values reported as median (min – max).
Figure 6.1: 2DE indices of LA function. Active LA FAC, active left atrial fractional area change; active/total LA AC, ratio of active-to-total left atrial area change; LA RI, left atrial reservoir index. The data points of horse #2 (electrical cardioversion) are connected by a continuous line. Median values are indicated by horizontal lines.
Figure 6.2: Transmitral Doppler flow indices of LA function. $F_{A_{\text{max}}}$, fractional active emptying velocity; $\text{PEP}_A/\text{ET}_A$, ratio of the left atrial preejection period to ejection time; $t_{A_{\text{max}}}$, time to peak A wave. The data points of horse #2 (electrical cardioversion) are connected by a continuous line. Median values are indicated by horizontal lines.
Figure 6.3: PW tissue Doppler indices of LA function. $t_{vmax}$, time to peak wall motion velocity; $t_{Am}/d_{Am}$, ratio of the time to onset over duration of the $A_m$ wave. The data points of horse #2 (electrical cardioversion) are connected by a continuous line. Median values are indicated by horizontal lines. Time intervals could not be measured in one horse in the AF24 group, because the $A_m$ wave was absent.
Discussion

We were able to show that transthoracic echocardiography can be used to study alterations in LA size and LA mechanical function in standing, unsedated, adult horses. To our knowledge, this is the first report indicating that LA mechanical dysfunction in horses may persist for a certain period of time after successful conversion of naturally occurring AF to NSR.

In humans, dogs and goats, the mechanisms primarily responsible for the self-perpetuating, progressive, and recurrent nature of AF have been attributed to AF-induced progressive changes in atrial electrophysiologic, contractile, and tissue characteristics, commonly also referred to as electrical, functional, and structural remodeling.7,38-40 Atrial contractile dysfunction goes hand in hand with electrical and structural changes in the atrial myocardium.7,8 It is usually reversible and improves progressively over time, if NSR is maintained.41 The factors affecting the severity of AF-induced atrial contractile dysfunction and the rate of recovery after restoration of NSR are incompletely understood, but are influenced by the duration of preceding AF, atrial size, and underlying structural heart disease.41,42

In people, atrial contractile dysfunction is considered an important risk factor of thromboembolism after conversion of AF to NSR.41 Furthermore, data suggest that it delays the improvement in exercise tolerance after cardioversion41 and may predict maintenance of NSR and recurrence of AF.4,11-14 In horses with naturally occurring AF, atrial mechanical dysfunction has not been investigated to date and the potential clinical significance is unknown. However, independent of treatment modalities, clinical experience in horses suggests that the success of treatment and the risk of recurrent or
persistent AF are related to the duration of AF at the time of presentation. This could be explained by atrial remodeling similar to that reported in other species. Van Loon and colleagues provided some evidence that atrial remodeling may occur in horses under experimental conditions. Specifically, they showed that pacing-induced AF of 6 months duration resulted in an increase in LA size and a loss of LA contractile function that persisted for 1 to 2 months after conversion to NSR.

Overall, our results indicate that LA contractile function and reservoir function are significantly depressed 24 hours after successful treatment of naturally occurring AF and may, in some horses, remain depressed for at least 72 hours after conversion. Echocardiographic indices of LA mechanical function improved between the first and the third day after cardioversion in most horses, suggesting that the alterations were reversible. However, we could not demonstrate complete recovery of LA function in all horses, most likely due to the limited follow-up period of three days.

The alterations in LA mechanical function were not reflected by all echocardiographic variables used in this study. The 2DE indices of LA contractile function (active LA FAC and active/total LA AC) and LA reservoir function (LA RI), and the TDI derived systolic time intervals (tvmax and tAm/dAm) appeared to be more useful for detection of altered LA mechanical function compared to transmitral Doppler flow variables (FAmx, PEPa/ETA, tAmx). Moreover, the results for the FAmax in horse #2 appeared to be somewhat conflicting. The low values of the 2DE variables of LA contractile function and the high values for the transmitral and the TDI-derived systolic time intervals suggested that LA mechanical performance in this horse was more severely and more persistently depressed compared to other horses. Conversely, the FAmax was
higher compared to most other horses, which would be more consistent with a stronger LA contractile function. However, the echocardiographic variables used in this study are likely influenced by a variety of factors, including atrial loading conditions and atrial and ventricular contractility, relaxation, and compliance. Currently, it is unknown to which extent these factors influence the respective echocardiographic variables, and changes in single variables and in single horses will have to be interpreted with caution.

Likewise, the effects of age, breed, sex, body weight, and underlying structural cardiac disease on echocardiographic indices of LA mechanical function in horses are unknown to date. However, our two study populations were well balanced with regards to age and the other factors, and the main difference between the two populations was the presence or absence of AF. Hence, we believe that AF-induced atrial remodeling is the most likely explanation of the persistent LA mechanical dysfunction in our study population. However, based on our data, we cannot preclude that atrial function was depressed for reasons other than atrial remodeling, including residual treatment effects or previously undetected, preexisting myocardial disease.

Due to the low number of horses, we were not able to study the effects of different treatment modalities on LA mechanical function. Although the plasma quinidine concentrations at the time of examination were well below the therapeutic concentration of 2 to 5 μg/mL, it is possible that residual drug effects were at least partly responsible for the depression of LA contractile function after treatment. Moreover, although atrial mechanical dysfunction occurs with all modes of conversion, some (but not all) studies in other species demonstrated that electrical cardioversion causes prolonged and more severe LA dysfunction than either spontaneous or pharmacological cardioversion.
Interestingly, most echocardiographic variables indicated that the horse that was electrically cardioverted (horse #2) appeared to have a more severe and more persistent depression of LA contractile function compared to the remaining horses and did not show any noticeable improvement between ‘AF24’ and ‘AF72’. However, the same horse also had failed pharmacologic conversion prior to entry into the study and experienced recurrent episodes of AF following successful electrical cardioversion. We were not able to determine the cause of the more pronounced, persistent depression of LA function and the potential influence of treatment or preexisting myocardial disease in this horse.

Some limitations of this study need to be acknowledged. First, although the reliability of the echocardiographic indices used in this study has been previously determined (Chapter 5), their use has not been thoroughly validated in horses and none of the variables has been compared to a ‘gold-standard’ of LA mechanical function. Hence, until more data becomes available, assessment of echocardiographic findings will need to be based on published data from other species and theoretical considerations. Second, as mentioned above, the low case number in this study does not allow drawing any conclusions on the relation between the severity and rate of recovery of atrial mechanical dysfunction and duration of AF, method of treatment, underlying (primary) myopathy, exercise capacity, and outcome (maintenance of NSR or recurrence of AF). Finally, some might argue that the statistical analyses should have been corrected for the family-wise error of all pairwise comparisons of echocardiographic variables of LA mechanical function, rather than just adjusting for three comparisons within each variable. However, we believe that this approach would have been too conservative, preventing the detection of potential differences in LA mechanical function in this small, preliminary study.
In conclusion, we were able to show that LA contractile function and LA reservoir function can be altered in horses with cardiac disease. Specifically, we showed that LA mechanical dysfunction is present in horses after conversion of AF to sinus rhythm and can be detected for at least 1 to 3 days after successful treatment by means of transthoracic echocardiography. These results are consistent with AF-induced atrial remodeling or possibly primary myopathy or residual treatment effects as underlying causes of LA mechanical dysfunction. Further studies will be required to determine the clinical relevance of LA mechanical dysfunction in the presence of cardiac disease in horses, and to relate the changes in echocardiographic variables to severity of disease, exercise capacity, or prognosis, using appropriate outcome measures.
Footnotes

a Schwarzwald CC, Schober KE, Bonagura JD. Echocardiographic assessment of left atrial size and mechanical function in horses: Methodology and reliability. Manuscript in preparation.

b GE Medical Systems, Milwaukee, WI.

c EchoPAC v3.1.3, GE Medical Systems, Milwaukee, WI.

d TDx/TDxFLx Quinidine Assay System, Abbott Laboratories, Abbott Park, IL.

e Microsoft Office Excel 2003, Microsoft Corporation, Redmond, WA.

f GraphPad Prism v4.00 for Windows, GraphPad Software, San Diego, CA.

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SUMMARY AND CONCLUSIONS

This project served two overall goals. First, we intended to study a new therapeutic option for ventricular rate control in horses with atrial fibrillation (AF) undergoing treatment with quinidine. The idea for these studies arose on grounds of the current inability to effectively and safely control the heart rate in horses that develop a rapid ventricular response rate during quinidine treatment. Therefore, we investigated the pharmacology and hemodynamic and electrophysiologic effects of the calcium channel blocker diltiazem in healthy horses, in order to lay the foundation for its future use for ventricular rate control in horses with naturally occurring AF.

Second, we intended to advance the knowledge on equine atrial physiology and pathophysiology by establishing methods to study atrial electrophysiology and atrial mechanical function in adult horses and applying these methods to investigate drug effects and effects of disease. Specifically, we investigated the SA nodal, atrial, and AV nodal electrophysiology in horses using standard procedures that are routinely used in humans. Finally, we studied the feasibility of transthoracic echocardiography for non-invasive assessment of left atrial (LA) size and LA mechanical function, determined the reliability of this technique, and applied the methods to study LA mechanical function in horses after successful treatment of AF.
The first study (Chapter 1) determined the effects of diltiazem on cardiac rate and rhythm, left-ventricular (LV) function, central hemodynamics, and peripheral blood flow in normal, standing, unsedated horses. A dose-finding study was performed. Afterward, 8 healthy horses were treated with diltiazem IV every 30 min to achieve cumulative dosages of 0 (saline control), 1, 1.5, and 2 mg/kg. Plasma diltiazem concentration, heart rate and rhythm (by electrocardiography), LV function and central hemodynamics (by cardiac catheterization), LV dimensions (by echocardiography), and forelimb blood flow (by Doppler sonography) were determined during each treatment period. Diltiazem plasma concentrations between 390 and 910 ng/mL were achieved, with considerable variation among horses. Cardiac effects of diltiazem, at doses between 1 and 2 mg/kg IV, included intermittent depression of the sinus and atrioventricular (AV) nodes and mild impairment of systolic and diastolic LV function. Vascular effects of diltiazem included arterial vasodilatation, increased limb blood flow, and decreased systemic vascular resistance. Baroreceptor reflex-mediated sympathetic activation increased sinus node rate, and presumably blunted the depressive effects of diltiazem on myocardial and nodal tissues. Two horses developed transient high-grade sinus arrest with severe systemic hypotension.

The pharmacokinetic characteristics of diltiazem were then determined in eight healthy horses (Chapter 2). Diltiazem HCl, 1 mg/kg IV, was administered over 5 min. Venous blood samples were collected at regular intervals after administration. Plasma concentrations of diltiazem and desacetyldiltiazem were determined by high-performance liquid chromatography. A second, putative metabolite was detected, but could not be identified due to the lack of an authentic standard. Data were analyzed by nonlinear least
squares regression analysis. The median (min-max) peak plasma concentration of diltiazem was 727 (539-976) ng/mL. Plasma diltiazem concentration versus time data were best described by a two-compartment model with first-order drug elimination. The distribution half-life was 12 (6-23) min, the terminal half-life was 93 (73-161) min, and mean residence time was 125 (99-206) min, total plasma clearance was 14.4 (10.4-18.6) mL/kg/min, and the volume of distribution at steady state was 1.84 (1.46-2.51) L/kg. The normalized ratio of the area under the curve (AUC) of desacetyldiltiazem to the AUC of diltiazem was 0.088 (0.062-0.179). The disposition of diltiazem in horses was characterized by rapid distribution and elimination and a terminal half-life shorter than reported in humans and dogs. Due to the reported low pharmacologic activity, plasma diltiazem metabolite concentrations were not considered clinically important. The average percent protein binding of diltiazem in horses ranged between 84 and 92% in healthy horses and was concentration-dependent at concentrations of 500 ng/mL and higher (Chapter 3). However, this effect was not considered to be clinically relevant, as therapeutic total plasma diltiazem concentrations for treatment of supraventricular arrhythmias in horses are expected to be considerably lower than 500 ng/mL.

In the final study (Chapter 4), standard electrophysiologic methods were applied to 14 unsedated, healthy, adult horses to study the effects of quinidine and diltiazem on AV nodal conduction during rapid atrial pacing, using a clinically relevant sequence of drug administration. Arterial blood pressure, surface electrocardiogram, and right atrial electrogram were recorded during sinus rhythm and during programmed electrical stimulation at baseline, after administration of quinidine gluconate (10 mg/kg IV over 30 min, n=7; and 12 mg/kg IV over 5 min, followed by 5 mg/kg/h CRI for the remaining
duration of the study, n=7), and after co-administration of diltiazem (0.125 mg/kg IV over 2 min repeated every 12 min to effect). Quinidine significantly prolonged the atrial effective refractory period, shortened the functional refractory period (FRP) of the AV node, and increased the ventricular response rate during rapid atrial pacing. Subsequent administration of diltiazem increased the FRP, controlled the ventricular rate in a rate-dependent manner, caused dose-dependent suppression of the sinoatrial node and a significant, but well tolerated decrease in arterial blood pressure. Due to the rate-dependence of diltiazem effects, effective doses were lower than in the previous hemodynamic study and ranged from 0.125 to 1.125 mg/kg.

The previous study not only provided important data on the effects of diltiazem on AV nodal conduction in horses, but also demonstrated that standard in vivo techniques can be used to study atrial electrophysiologic function in standing, unsedated horses. The last two studies in our project served to characterize LA mechanical function in horses. Specifically, we aimed to show the feasibility, describe the techniques, and determine the reliability of transthoracic echocardiography for noninvasive assessment of left atrial (LA) size and LA mechanical function (Chapter 5). Six standing, unsedated, healthy horses underwent repeated echocardiographic examinations performed by two independent observers using two-dimensional (2D) echocardiography, flow Doppler, and tissue Doppler imaging (TDI) techniques. Test reliability was determined by estimating measurement variability, within-day inter-observer variability, and between-day inter- and intra-observer variability of all echocardiographic variables. Most echocardiographic variables of LA size showed low overall variability (coefficient of variation (CV) < 15%). Among the 2D indices of LA mechanical function, area-based and volume-based
ejection phase indices showed moderate between-day variability (CV usually < 25%). Transmitral Doppler flow indices were characterized by low to high between-day variability (CV 6 – 35%). TDI wall motion velocities had high between-day variability (CV > 25%), while most TDI-derived time intervals had low variability (CV < 15%).

These results allowed formulation of preliminary recommendations for echocardiographic assessment of LA size and function in horses, which were subsequently applied to study the effects of AF on left atrial (LA) mechanical function in horses after successful treatment of AF (Chapter 6). Five Standardbreds with AF and 6 healthy Standardbreds with similar age, weight, and athletic condition were included in this study. Four horses were treated pharmacologically (quinidine) and one horse was treated by means of transvenous electrical cardioversion. Echocardiographic examinations were performed in normal horses (once) and in AF horses (24 hours and 72 hours after conversion to sinus rhythm) using two-dimensional (2D), transmitral flow Doppler, and tissue Doppler imaging (TDI) techniques. Echocardiographic indices of LA mechanical function were compared between normal horses and AF horses. 2D and TDI indices of LA mechanical function indicated significant depression of LA contractile function and LA reservoir function 24 hours after cardioversion. This depression was not statistically significant any more at 72 hours after cardioversion, although improvement in LA contractile function between 24 and 72 hours varied among horses. The results of this study are consistent with AF-induced atrial ‘stunning’, although residual treatment effects or influence of underlying primary myopathy cannot be precluded.

In conclusion, the results of our studies provide data on novel treatment options for AF in horses and give some insight into the atrial electrophysiology and mechanical
function in normal horses and horses with AF. Diltiazem has the potential to become a useful treatment option for ventricular rate control in horses with naturally occurring atrial fibrillation. Diltiazem effectively controlled AV nodal conduction in an equine pacing model of supraventricular tachycardia. It appeared relatively safe in healthy horses, although higher doses caused a decrease in systemic blood pressure and suppression of sino-atrial nodal discharge leading to sinus arrhythmia or intermittent sinus pauses. Therefore, diltiazem will have to be administered under close physiologic monitoring. Preferably, doses should be slowly increased to effect or until adverse reactions prevent further administration. Clinical trials will be necessary to establish the efficacy and safety diltiazem for treatment of horses with naturally occurring AF.

In addition to the exploration of new treatment options, we demonstrated that variables of SA nodal, atrial, and AV nodal electrophysiologic function can be determined in standing, unsedated horses by use of standard diagnostic techniques. We further showed that LA size and LA mechanical function can be reliably assessed in standing, unsedated horses by use of 2D echocardiography, transmitral Doppler flow velocity profiles, and analyses of LA wall motion by TDI. These techniques were able to detect LA contractile dysfunction in horses after pharmacological or electrical cardioversion of naturally occurring AF. The diagnostic methods used in our studies can be used in future investigations to elucidate pathophysiologic mechanisms, develop novel treatments, and refine prognosis indicators for horses with atrial diseases.
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