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Effect of physiological and pharmacological stress on heart rate, blood pressure, and echocardiographic measurements in healthy Warmblood horses

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Abstract

Background: Echocardiographic measurements are important prognostic indicators but might be influenced by heart rate and blood pressure. This is particularly important when comparing repeated examinations.

Hypothesis: To determine the effect of physiological stress at mildly increased heart rates and pharmacological challenge using IV administration of *N*-butylscopolammonium bromide and metamizol sodium on heart rate, blood pressure, and echocardiographic measurements.

Animals: Twenty healthy Warmblood horses.

Methods: Randomized crossover study. Horses were examined echocardiographically by 2-dimensional, M-mode, pulsed wave (PW) Doppler, and PW tissue Doppler imaging with simultaneous ECG recording and noninvasive blood pressure measurements during rest, physiological stress, and pharmacological challenge. Cardiac dimensions and functions were measured by a blinded observer. Data were analyzed using repeated-measures analysis of variance.

Results: Mean heart rate and arterial blood pressure were significantly higher during physiological stress (46 ± 2 bpm, 93 ± 16 mm Hg) and pharmacological challenge (62 ± 13 bpm, 107 ± 17 mm Hg) compared with rest (34 ± 3 bpm, 86 ± 12 mm Hg; $P < .05$). Compared with rest, physiological stress resulted in increased left atrial

Abbreviations: 2D, 2-dimensional; 4C, 4 chamber; A_m, peak radial wall motion velocity during late diastole; Ao, aorta; AoDd, aortic diameter at sinotubular junction at end-diastole; AoDs, aortic diameter at sinotubular junction at peak-systole; $Ao_{sx}D$, aortic short axis diameter of the Ao at end-systole; AoV, aortic valve; BP, blood pressure; BSI, British Standards Institution; CH, chordal level; CO, cardiac output; CV, coefficient of variation; DAP, diastolic arterial blood pressure; E_m, peak radial wall motion velocity during early diastole; ET, ejection time; FAC, fractional area change; FS, fractional shortening; HR, heart rate; IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; L, left; LA FAC, left atrial fractional area change; LA, left atrium/atrial; LAAa, left atrial area at maximal atrial contraction; LAAd, left atrial area at end-diastole; LAAp, left atrial area at the onset of the P wave; LAAs, left atrial area at end-systole; LADd, left atrial diameter at end-diastole; $LADd_{max}$, maximal left atrial diameter at end-diastole; LADs, left atrial diameter at end-systole; $LADs_{max}$, maximal left atrial diameter at end-systole; $LA_{sx}D_{max}$, short axis diameter of the left atrium at end-systole; LAX, long-axis view; LV FAC, left ventricular fractional area change; LV FS, fractional shortening of the left ventricle; LV, left ventricle/ventricular; LVA, left ventricular area; LVFWd, LV free wall thickness at end-diastole; LVFWs, LV free wall thickness at end-systole; LVIDd, LV internal diameter at end-diastole; LVIVd, LV end-diastole volume; LVIDs, LV internal diameter at end-systole; LVIVs, LV end-systolic volume; LVOT, left ventricular outflow tract; M, M-mode Doppler ultrasonography; MAP, mean arterial blood pressure; NBB, *N*-butylscopolammonium bromide; NBBM, *N*-butylscopolammonium bromide and metamizol sodium; NIBP, Non-invasive blood pressure; PA, pulmonary artery; PADd, PA diameter parallel to sinotubular junction at end-diastole; PADs, PA diameter parallel to sinotubular junction at peak-systole; PEP, pre-ejection period; PP, Pulse pressure; PW TDI, pulsed wave tissue Doppler imaging; PW, pulsed wave; PWD, pulsed wave Doppler echocardiography; R, right; RC, repeatability coefficient; RV FS, fractional shortening of the RV; RV, right ventricle; RVIDd, RV internal diameter at end-diastole; RVIDs, RV internal diameter at end-systole; RWT, relative wall thickness; SAP, systolic arterial blood pressure; S_m, peak radial wall motion velocity during peak-systole; SV, stroke volume; SW, within-subject standard deviation; SX, short-axis view; TDI, tissue Doppler imaging; Vel_{max}, maximum velocity; VTI, velocity time integral.

fractional area change (34.3 ± 7.5 vs 27.3 ± 5.1%; $P = .01$) and left ventricular late diastolic radial wall motion velocity (13 ± 3 vs 10 ± 2 cm/s; $P = .01$) but had no significant effect on most other echocardiographic variables. Compared with rest, pharmacological challenge led to significantly decreased left atrial and diastolic ventricular dimensions (left ventricular internal diameter: 10.3 ± 0.9 vs 10.7 ± 0.8 cm; $P = .01$), increased aortic and pulmonary diameters, and ventricular wall thickness.

Conclusions and Clinical Importance: Physiological stress at mildly increased heart rates significantly enhanced atrial pump function. Larger heart rate and blood pressure increases during pharmacological challenge resulted in altered cardiac dimensions. This should be taken into account when evaluating echocardiographic measurements at increased heart rates.

KEYWORDS

equine, hypertension, *N*-butylscopolammonium bromide, pharmacological challenge test, physiological stress, tachycardia

1 | INTRODUCTION

Echocardiography is the diagnostic method of choice to investigate cardiac structures and to determine or compare cardiac dimensions.[1-6] The assessment of cardiac dimensions during repeated exams has an important prognostic value as progressive cardiac dilatation suggests progressive cardiac disease.[3,7,8] Prerequisites to minimize measurement variability are well-defined imaging guidelines and adequate operator training.[6] Between-day intraobserver and interobserver variability should be taken into account when evaluating alterations in cardiac dimensions during longitudinal follow-up.[1,6] Generally, the interobserver measurement variability appears to be a more important factor than the intraobserver variability, which emphasizes that preferably the same operator should perform sequential recordings and measurements in the same patient to minimize test-retest variability.[6] However, slight changes in heart rate (HR) or blood pressure (BP) might influence measurements or measurement repeatability in case of follow-up examinations, among other related and determinative factors, such as preload, afterload, contractility, hydration, or cardiovascular status of the patient.[4] The general advice is to perform echocardiographic examinations in adult horses at physiological HR ranging from 28 to 48 beats per minute (bpm) in the absence of external stressors. However, HR can be higher because of disease or stress, especially at the start of the examination, and often decreases as the echocardiographic exam continues.[9,10]

Sedation of the horse is usually contraindicated for echocardiography because of drug effects on HR, rhythm, contractility, preload, and afterload.[4] Administration of detomidine hydrochloride resulted in decreases in HR and cardiac output (CO) with a tendency to increases in left atrial and ventricular dimensions.[11-15] Cardiac dimensions and function were also altered during physiological (treadmill exercise, lunging exercise) or pharmacological stress testing with atropine (parasympatholytic drug), or dobutamine (sympathomimetic drug), with high HR above 80 bpm and important increases in BP.[16-23] Postexercise evaluation of cardiac variables and pharmacological stress testing demonstrated decreases in left ventricular dimensions with increases in interventricular septum and left ventricular free wall thicknesses as well as an increased left ventricular fractional shortening (FS).[18-20,23-27] However, the influence of slight changes within the normal physiological range of HR on echocardiographic variables of cardiac function has not been investigated yet. Heart rate and BP might vary during the echocardiographic exam as a result of stress because of an unknown environment. This effect might also be imitated by pharmacological challenge. *N*-butylscopolammonium bromide (NBB) is a routinely used anticholinergic drug with an antagonistic effect on muscarinic receptors of the parasympathetic nervous system.[28] NBB is commonly used for the treatment of abdominal pain and bronchoconstriction in horses and has important cardiovascular effects as transient tachycardia and hypertension.[28-30]

Aim of this study was to compare echocardiographic variables including 2-dimensional (2D), M-mode, pulsed wave (PW), and PW tissue Doppler imaging (PW TDI) in healthy, trained Warmblood horses at different physiological heart rates and during transient mild tachycardia and hypertension after IV administration of NBB and metamizol (NBBM). We hypothesized that cardiac dimensions would be decreased at higher HR and BP.

2 | MATERIALS AND METHODS

2.1 | Animals and experimental design

A randomized crossover study was performed. The study sample included 20 healthy, trained adult Warmblood horses with a regular heart rhythm and no audible cardiac murmur. The study was approved by the ethical committee of the Faculty of Veterinary Medicine and Bioscience Engineering (EC2020/100), and written owner informed consent was obtained. Body condition score was estimated based on the Henneke

Body condition score scale.[31] Height at the withers, body length, and chest circumference were measured for all horses. Body length was determined from the most cranial point of the shoulder to the tuber ischiadicum; chest circumference was measured caudal of the elbow. Body weight (BW) was estimated using the following formula[32-34]:

$$BW = \text{chest circumference}^2 \times \text{body length}/11900$$

Serum was collected by jugular venipuncture before the examination at rest, centrifugated, and stored at −80°C upon analysis. Cardiac troponin I concentrations were determined by the Alinity i STAT High-Sensitive Troponin-I assay (chemiluminescent microparticle immunoassay) on the Alinity i analyzer (Abbott, Illinois) to check for absence of myocardial disease. All cardiac troponin I concentrations were within normal limits (<0.06 ng/mL) as assumed for a normal health state (inclusion criterion for this study). A resting and exercise ECG was performed to confirm absence of pathological dysrhythmias.[35]

Data collection occurred between April and July 2021. Echocardiographic examination was performed 4 times for each horse in a random order: twice at rest, once during physiological stress, and once during pharmacological challenge. For each examination, image acquisition was performed from the left and right side in a random order. Bilateral cardiac auscultation was performed immediately before image acquisition. A continuous ECG was recorded throughout the entire echocardiographic exams to determine HR during image acquisition. Repetitive, consecutive, and noninvasive BP measurements were performed starting on and simultaneously with the acquisition of ultrasonographic images to determine diastolic arterial BP (DAP), mean arterial (MAP), and systolic arterial BP (SAP). An indirect oscillometric BP device (Cardell Veterinary Monitor, 9401, Midmark, Florida), with the cuff placed around the base of the tail (coccygeal artery), was used as previously reported by Vera et al (2020),[36,37] with a cuff width of 9 cm to achieve the ideal cuff width-to-tail circumference ratio between 0.4 and 0.6. Measurements for NIBP were taken according to the running time of the oscillometric BP device: approximately every 2 to 3 minutes at lower heart rates and approximately every minute at higher heart rates. Only measurements where the HR of the device approximated the true HR were taken into account. A minimum of 15 BP measurements per scan were performed, and the average value was used for further analysis. One cardiac examination at rest included a full echocardiographic exam to evaluate cardiac dimensions, function, and valvular regurgitation.[8,38] The other cardiac examination at rest was performed to assess measurement repeatability and was performed either on the same or another day. Target HR at rest ranged between 30 and 35 bpm. Both exams at rest were performed in the stable of the horse within its familiar environment. The cardiac exam under physiological stress at mildly increased physiological HR was performed by examination of the horse in an unknown environment and, if needed, immediately after forward or backward walking of the horse to introduce slight stress. Target HR for the physiological stress exam was 40 to 45 bpm. The resting period between the rest and physiological stress examinations was at least 1 hour, which allowed the horse to return to its resting state. The pharmacological challenge exam was performed after IV administration of 0.2 mg/kg NBB and 25 mg/kg metamizol sodium (NBBM, Buscopan compositum, Boehringer Ingelheim Vetmedica GmbH, Germany). Image acquisition was started 2 minutes after administration, when tachycardia and hypertension were present, and finished within 25 minutes.[28] The wash-out period between the pharmacological challenge and other examinations was at least 7 days to clear the body from the drug taking into account an elimination half-life of 6 hours for NBB.

2.2 | Echocardiography

Echocardiography was performed using a 1.5 to 4.6 MHz phased array probe (M5Sc-RS) at a frequency of 1.7/3.3 MHz with simultaneous ECG recording (base-apex; Vivid IQ, GE Healthcare, Diegem, Belgium) by an experienced Diplomate of the European College of Equine Internal Medicine (ECEIM). All echocardiographic exams were performed by the same operator. One of the resting echocardiographic examinations included a full echocardiographic exam to check for normality of all cardiac structures including 2D-, color, M-mode, tissue, and PW Doppler techniques.[39] For the other exams, a shorter predetermined echocardiographic protocol was performed. Two-dimensional images included 2 right parasternal long-axis 4-chamber views (R-4C) with focus on either the ventricles or the atria, the right parasternal long-axis view of the left ventricular outflow tract (R-LVOT), the right parasternal short-axis view of aorta and the left atrium (R-LASAX/AoSAX), and the left parasternal long-axis view of left ventricle (LV), mitral valve, and left atrium (L-LVLAX). M-mode images were obtained from the right parasternal short-axis view of the LV at the chordal level (R-LVSAX$_{ch}$). PW TDI images were obtained from the R-LVSAX$_{ch}$ view. Left ventricular free wall velocity was assessed with the pulsed wave Doppler cursor positioned within the subendocardial region at end-diastole and a sample volume width of 5.0 mm. The blood flow velocity profile across the aortic valve was evaluated by pulsed wave Doppler echocardiography (PWD) obtained from the left parasternal long-axis left ventricular outflow tract view (L-LVOT). Color flow Doppler imaging was performed to check for valvular regurgitation. A minimum of 3 loops of 3 consecutive cardiac cycles were recorded for off-line analysis.

2.2.1 | Off-line analysis

Images were analyzed using EchoPAC Software Version 203 (GE Healthcare, Diegem, Belgium) by a single observer (Diplomate ECEIM) who was blinded for horse and scan (rest/physiological stress/pharmacological challenge). For each echocardiographic variable, the mean of 3 measurements from 3 different cardiac cycles was used for further analysis. Cycles following a second-degree atrioventricular or sinoatrial block were not used for analysis. Mean HR was determined from the RR intervals before the analyzed cardiac cycles. An overview of cardiac measurements is presented within Supplemental Table S1.[34,40] Measurements on the R-4C view included left atrial and ventricular dimensions.[34] Active left atrial fractional area change (LA FAC) was calculated as LA FAC =

([R-LAApLAX − R-LAAaLAX]/R-LAApLAX) × 100, as reported earlier,[40-42] whereby R-LAApLAX and R-LAAaLAX are considered as left atrial area at the onset of the P wave and during maximal atrial contraction, respectively. Fractional area change of LV area (LV FAC) was calculated as LV FAC = ([R-LVAd$_{end}$LAX − R-LVAs$_{end}$LAX]/R-LVAd$_{end}$LAX) × 100.[39]

On the R-LVOT view, the aortic diameter at the level of the sino-tubular junction and the pulmonary artery diameter in short-axis were measured.[34,39] The ratio of the pulmonary artery diameter and aortic diameter at peak-systole was calculated (R-LVOT PADs$_{peak}$/AoDs$_{peak}$).[34] Pulmonary artery diameter was measured along the direction of the ultrasound beam while ensuring that the diameter was not overestimated by measuring an oblique section of the pulmonary artery. On the right short-axis view of the aorta and the left atrium, aortic diameter (R-Ao$_{sx}$Ds$_{end}$) and LA diameter (R-LA$_{sx}$D$_{max}$) were measured at end-systole, and the ratio was calculated (R-LA$_{sx}$D$_{max}$/Ao$_{sx}$Ds$_{end}$) as described by De Clercq et al.[40] For R-Ao$_{sx}$Ds$_{end}$, the aorta calipers were placed along a line extending from the commissure between the noncoronary and right coronary aortic valve cusps. For R-LA$_{sx}$D$_{max}$, calipers were placed in a line extending from and parallel to the commissure between the non-coronary and left coronary aortic valve cusps to the distant margin of the left atrium, ensuring not to measure inside pulmonary vein ostium II.[43] M-mode measurements from the right short-axis LV view at chordal level were performed.[34,39] FS of the LV (LV FS), LV relative wall thickness (RWTd$_{M_ch}$), the LV internal volume at end-diastole (LVIVd) and end-systole (LVIVs), the left ventricular stroke volume (LV SV), ejection fraction (LV EF), and CO (LV CO) were calculated using the formulas below.[8,34,39,44] Volumetric assessment of the left ventricular dimensions was performed based on an area-length method.[8,44]

$$\text{LV FS}\,(\%) = ([\text{LVIDd}_{\text{M\_ch}} - \text{LVIDs}_{\text{M\_ch}}]/\text{LVIDd}_{\text{M\_ch}}) \times 100$$

$$\text{RWTd}_{\text{M\_ch}}\,(\text{cm}) = (\text{IVSd}_{\text{M\_ch}} + \text{LVFWd}_{\text{M\_ch}})/\text{LVIDd}_{\text{M\_ch}}$$

$$\text{LVEDV}_{\text{al}} = (0.85 \times \text{LVEDA}_{\text{lx}}{}^2)/\text{LVEDL}$$

$$\text{LVESV}_{\text{al}} = (0.85 \times \text{LVESA}_{\text{lx}}{}^2)/\text{LVESL}$$

$$\text{LV SV}_{\text{al}}\,(\text{mL}) = \text{LVEDV}_{\text{al}} - \text{LVESV}_{\text{al}}$$

$$\text{LV EF}_{\text{al}}\,(\%) = ([\text{LVEDV}_{\text{al}} - \text{LVESV}_{\text{al}}]/\text{LVEDV}_{\text{al}}) \times 100$$

$$\text{LV CO}_{\text{al}}\,(\text{L/min}) = \text{HR} \times \text{SV}/1000$$

PW TDI analysis of the LV free wall enabled to determine the peak radial wall motion velocity during early (E_m) and late diastole (A_m) as well at peak-systole (S_m). The E_m/A_m ratio was calculated.[38,39,45,46] On the PWD trace of aortic flow, the pre-ejection period (L-PWD AoV PEP) and the ejection time (L-PWD AoV ET) were measured. The ratio of L-PWD AoV LVPEP/LVET was calculated.[8] Velocity time integral (L-PWD AoV VTI) and maximal velocity (L-PWD AoV Vel$_{max}$) of aortic blood flow were measured. LV SV (L-PWD AoV SV) and CO (L-PWD AoV CO) were calculated using following formulas[8]:

$$\text{L-PWD AoV SV}\,(\text{mL}) = (\text{R-LVOTs}_{\text{peak}})^2 \times 0.785 \times \text{L-PWD AoV VTI}$$

$$\text{L-PWD AoV CO}\,(\text{L/min}) = ([\text{R-LVOTs}_{\text{peak}}]^2 \times 0.785 \times \text{L-PWD AoV VTI} \times \text{HR})/1000$$

2.2.2 | Data analysis

Based on a power analysis using G*Power, a minimum sample size of 20 horses was required to detect a 5% to 10% difference in cardiac chamber dimensions[12] ($\alpha = 0.025$ to account for multiple comparisons between groups, $1 - \beta = 0.80$). Statistical analysis was performed using SPSS Statistics 28 (IBM, Brussels, Belgium). Mean HR, BP, and echocardiographic variables were compared between rest (short protocol), physiological stress, and pharmacological challenge using a repeated-measures analysis of variance with post hoc Bonferroni correction for multiple comparisons. Normality of the distribution of the variables was checked by visual inspection of the QQ plots and histograms, the Kolmogorov-Smirnov test, and Shapiro-Wilk test. Normally distributed data are presented as mean ± standard deviation (SD). Non-normally distributed data are presented as median [range]. The overall significance level for the general linear model for repeated measures (univariate analysis of variance) was ≤.001 to correct for multiple comparisons. For each variable, familywise P values <.05 were considered significant (post hoc Bonferroni correction for multiple comparisons). Mean differences and 95% confidence intervals (CI) of the differences of means between physiological stress and rest as well as between pharmacological challenge and rest were calculated and reported.

2.2.3 | Reliability of echocardiographic variables

To assess image acquisition and measurement variability, both exams at rest were measured by the same observer, and the within-subject variance for repeated measurements (residual mean square) was determined by statistical analysis. The within-subject SD (sw) was calculated as the square root of the residual mean square.[47] Measurement variability was reported as the within-subject coefficient of variation (CV) and the repeatability coefficient (RC, 95% intermeasurement difference according to British Standards Institution [BSI] recommendations).[47] The within-subject CV was calculated by the formula CV = (within-subject SD (sw)/mean) × 100, expressed as a percent value. The RC, the absolute value below which the difference between the mean of 2 measurements at rest will lie with a 95% likelihood, was calculated by the formula: $1.96 \times \sqrt{2} \times \text{sw}$ (=2.77 × sw). The degree of variability was defined as CV <5%, very low variability; 5% to 15%, low variability; 16% to 25%, moderate variability; and >25%, high variability.[47]

TABLE 1 Average heart rate, diastolic, mean arterial, and systolic blood pressure as well as the calculated pulse pressure during echocardiography.

		Rest	Physiological stress		Pharmacological challenge		ANOVA
Variable	Unit	Mean ± SD	Mean ± SD	Difference between means (95% CI)	Mean ± SD	Difference between means (95% CI)	*F* test *P* value
HR	bpm	34 ± 3[a]	46 ± 2[b]	11.4 (5.7 to 17.1)	62 ± 13[c]	27.9 (22.2 to 33.6)	<.001
DAP	mm Hg	71 ± 7[a]	75 ± 7[a]	4.3 (−2.5 to 11.1)	88 ± 14[b]	17.1 (10.3 to 24.0)	<.001
MAP	mm Hg	86 ± 7[a]	93 ± 8[b]	7.4 (0.4 to 14.5)	107 ± 14[c]	20.9 (13.9 to 28.0)	<.001
SAP	mm Hg	114 ± 8[a]	122 ± 12[a]	7.6 (−1.0 to 16.2)	139 ± 13[b]	24.7 (16.1 to 33.2)	<.001
PP	mm Hg	43 ± 5	47 ± 9	3.3 (−2.7 to 9.3)	51 ± 10	7.5 (1.6 to 10.3)	.01

Note: The heart rate was calculated as the mean based on the RR interval preceding the analysed cardiac cycles for echocardiographic measurements. The overall significance level for the general linear model for repeated measures (Univariate Analysis of Variance) was < 0.001 to correct for multiple comparisons. For each variable, mean values with different superscripts indicate a statistically significant difference between examinations after Bonferroni correction for multiple comparisons ($P < .05$). Identical superscripts indicate no statistically significant difference between examinations. Mean difference and its 95% CI are reported.

Abbreviations: ANOVA, analysis of variance; CI, confidence interval; DAP, diastolic arterial blood pressure; HR, heart rate; MAP, mean arterial blood pressure; PP, pulse pressure; SAP, systolic arterial blood pressure; SD, standard deviation.

3 | RESULTS

The study sample included 11 geldings and 9 mares. The mean age of the horses was 14 ± 5 years. Median body condition score was 5/9 (range, 4-6/9).[31] Mean height at the withers was 165 ± 5 cm, mean body length was 193 ± 6 cm, and mean chest circumference was 183 ± 11 cm. Mean estimated BW was 544 ± 71 kg. HR was significantly lower at rest compared with physiological stress and pharmacological challenge (Table 1). Mean arterial BP was significantly higher during physiological stress compared with rest, whereas DAP, SAP as well as pulse pressure (PP) did not significantly increase. Diastolic arterial BP, MAP, and SAP increased significantly between rest and pharmacological challenge (Table 1).

Table 2 summarizes the between-examination measurement variability and the comparison of the echocardiographic variables at rest, physiological stress, and pharmacological challenge. The CV of measurement variability between the 2 scans at rest can be classified as low for most of the echocardiographic variables. Very-low to low-measurement variabilities were seen for 2D and M-mode variables with exception for RV internal diameter at end-systole ($RVIDs_{M\_ch}$) (moderate variability). Higher measurement variabilities were seen for PWD variables and calculated variables in general.

During physiological stress at mildly increased HR below 50 bpm, indices of left atrial contractile function differed significantly compared with rest, with increases in active LA FAC and A_m (Figure 1), whereas LAAa decreased. It should be noted that $R\text{-}LAAd_{end}$ showed a smaller change compared with R-LAAa. LV FAC increased significantly. No significant differences were observed for most other 2D left atrial, left ventricular, aortic and pulmonary dimensions, or calculated variables of cardiac function. No significant changes in left-sided aortic PWD variables were present (Table 2).

Pharmacological challenge with NBBM led to significant changes in 2D, M-mode, PWD, and PW TDI echocardiographic variables compared with rest. Left atrial and diastolic left and right ventricular dimensions decreased. An increase in active left atrial fractional area change, diastolic septal wall thickness, left ventricular relative, and free wall thickness were noted. Aortic and pulmonary dimensions increased, whereas LV SV and EF decreased. In addition, a decrease in PW TDI variable S_m was seen (Table 2). All horses included in this study tolerated the pharmacological challenge test very well, and no pathological dysrhythmias were observed after administration of NBBM. Figure 2 depicts scatter plots indicating individual data points for the echocardiographic variables LAAa, active LA FAC, A_m, and LV internal diameter at end-diastole ($LVIDd_{M\_ch}$) during the different scans.

4 | DISCUSSION

Overall, no clinically relevant differences were found in our study for most 2D, M-mode, and PWD echocardiographic variables in healthy Warmblood horses at slightly increased heart rates during stress (40-45 bpm) compared with rest (30-35 bpm). Small, statistically significant differences were found for some echocardiographic variables at slightly increased physiological heart rate, but the mean difference fell inside the RC value provided for the respective variable, indicating that the measurement difference might be more likely the result of measurement variation than a true response to physiological stress (Table 2). However, physiological stress did result in significant increases in measures of left atrial contractile function (active LA FAC, Am). This might reflect an increased left atrial pump function at physiologically higher HR because of an altered autonomic tone and ventricular filling pressures. The left and right atrium act as a reservoir for venous return during ventricular systole and contribute in an important way to cardiac preload. During early-to-mid ventricular diastole, blood stored in the atria is emptied into the ventricles, whereas active atrial contraction establishes the final ventricular volume during late ventricular diastole.[5,48-50] As HR increases, diastolic ventricular filling time and preload decreases.[50,51] Active left atrial FAC increased significantly in our study because of decreases in left atrial area at atrial

TABLE 2 Echocardiographic measurements at rest, during physiological stress, and during pharmacological challenge.

		Between-examination measurement variability (at rest)		Rest	Physiological stress	Mean difference between rest and physiological stress (95% CI)	Pharmacological challenge	Mean difference between rest and pharmacological challenge (95% CI)	ANOVA *F* test *P* value
		CV (%)	RC	Mean ± SD	Mean ± SD		Mean ± SD		
2D atrial variables									
R-LADd$_{end}$LAX	cm	4.7	1.2	9.5 ± 0.7	9.2 ± 0.7	−0.2 (−0.5 to 0.0)	9.3 ± 0.7	−0.2 (−0.5 to 0.1)	.1
R-LADd$_{max,end}$LAX	cm	5.2	1.4	10.1 ± 0.9	9.8 ± 0.9	−0.3 (−0.7 to 0.1)	9.8 ± 0.9	0.3 (−0.6 to 0.1)	.08
R-LADs$_{end}$LAX	cm	6.0	1.6	9.5 ± 0.7	9.4 ± 0.7	−0.1 (−0.5 to 0.4)	9.5 ± 0.7	0.0 (−0.4 to 0.5)	.9
R-LADs$_{max,end}$LAX	cm	4.4	1.4	11.5 ± 0.9[a]	11.6 ± 1.0[a]	0.1 (−0.4 to 0.5)	10.8 ± 0.9[b]	−0.7 (−1.2 to −0.3)	<.001
R-LAAd$_{end}$LAX	cm^2	7.9	11.2	51.0 ± 7.9	47.2 ± 6.9	−3.9 (−7.5 to −0.2)	51.4 ± 8.7	0.4 (−3.2 to 4.0)	.01
R-LAAs$_{end}$LAX	cm^2	7.0	15.1	77.1 ± 9.5[a]	79.9 ± 10.2[a]	2.8 (−1.8 to 7.5)	70.1 ± 9.5[b]	−6.9 (−11.6 to −2.3)	<.001
R-LAApLAX	cm^2	7.2	12.8	64.1 ± 10.0	62.5 ± 6.9	−1.6 (−6.8 to 3.6)	66.4 ± 11.6	2.2 (−2.9 to 7.4)	.19
R-LAAaLAX	cm^2	8.3	10.7	46.4 ± 6.2[a]	41.0 ± 6.0[b]	−5.4 (−8.1 to −2.7)	40.8 ± 6.2[b]	−5.6 (−8.3 to −2.9)	<.001
Active LA FAC	%	13.7	10.4	27.3 ± 5.1[a]	34.3 ± 7.5[b]	7.0 (1.2 to 12.8)	37.5 ± 9.1[b]	10.3 (4.5 to 16.0)	<.001
L-LADd$_{end}$LAX	cm	4.5	1.3	10.1 ± 0.8	10.0 ± 1.2	0.0 (−0.4 to 0.4)	9.6 ± 0.6	−0.4 (−0.8 to −0.0)	.01
L-LADd$_{max_end}$LAX	cm	3.0	1.0	11.6 ± 0.8[a]	11.3 ± 1.2[a]	−0.3 (−0.7 to 0.1)	10.9 ± 0.8[b]	−0.7 (−1.1 to −0.3)	<.001
L-LADs$_{end}$LAX	cm	4.5	1.3	10.8 ± 0.9[a]	11.0 ± 0.8[a]	0.2 (−0.2 to 0.5)	10.3 ± 0.7[b]	−0.5 (−0.9 to −0.2)	<.001
L-LADs$_{max_end}$LAX	cm	2.9	1.0	12.4 ± 0.7[a]	12.5 ± 0.9[a]	0.1 (−0.2 to 0.4)	12.0 ± 0.8[b]	−0.5 (−0.7 to −0.2)	<.001
2D ventricular variables									
R-LVAd$_{end}$LAX	cm^2	10.3	39.9	137.9 ± 18.0[a]	143.9 ± 17.6[a]	6.0 (−6.2 to 18.3)	122.1 ± 20.7[b]	−15.8 (−28.0 to −3.5)	<.001
R-LVAs$_{end}$LAX	cm^2	12.0	19.5	57.8 ± 8.8	55.6 ± 9.0	−2.2 (−7.0 to 2.5)	59.5 ± 10.2	1.7 (−3.0 to 6.5)	.13
LV FAC	%	4.5	7.3	58.0 ± 4.2[a]	61.4 ± 4.2[b]	3.4 (0.2 to 6.5)	50.9 ± 6.1[c]	−7.1 (−10.3 to −4.0)	<.001
LVEDV$_{al}$	mL	16.3	461.6	1003 ± 199[a]	1073 ± 205[a]	70 (−69 to 210)	828 ± 249[b]	−175 (−314 to −35)	<.001
LVESV$_{al}$	mL	17.5	123.0	252 ± 62	229 ± 61	−23 (−53 to 8)	258 ± 77	6 (−24 to 36)	.06
LV SV$_{al}$	mL	18.6	390.4	751 ± 167[a]	844 ± 173[a]	93 (−29 to 215)	570 ± 199[b]	−181 (−302 to −59)	<.001
LV EF$_{al}$	%	4.5	9.3	74.6 ± 4.9[a]	78.6 ± 4.3[b]	4.0 (0.4 to 7.6)	68.0 ± 6.7[c]	−6.6 (−10.2 to 3.0)	<.001
LV CO$_{al}$	L/min	19.6	14.2	26.2 ± 5.8[a]	39.6 ± 8.1[b]	13.5 (7.4 to 19.5)	34.6 ± 9.2[b]	8.5 (2.4 to 14.5)	<.001
2D aortic and pulmonary variables									
R-LVOT AoDd$_{end}$	cm	3.6	0.6	6.1 ± 0.4[a]	5.9 ± 0.4[b]	−0.2 (−0.4 to 0.0)	6.6 ± 0.4[c]	0.5 (0.3 to 0.7)	<.001
R-LVOT AoDs$_{peak}$	cm	2.8	0.5	6.6 ± 0.4[a]	6.4 ± 0.4[b]	−0.2 (−0.4 to 0.0)	6.9 ± 0.4[c]	0.3 (0.1 to 0.5)	<.001
R-LVOTs$_{peak}$	cm	5.7	0.9	5.6 ± 0.4	5.4 ± 0.7	−0.2 (−0.6 to 0.2)	5.6 ± 0.5	0.1 (−0.3 to 0.4)	.16

TABLE 2 (Continued)

		Between-examination measurement variability (at rest)		Rest	Physiological stress	Mean difference between rest and physiological stress (95% CI)	Pharmacological challenge	Mean difference between rest and pharmacological challenge (95% CI)	ANOVA *F* test *P* value
		CV (%)	RC	Mean ± SD	Mean ± SD		Mean ± SD		
R-LVOT $PADd_{end}$	cm	5.9	0.8	4.7 ± 0.5[a]	4.7 ± 0.4[a]	0.1 (−0.2 to 0.3)	5.5 ± 0.3[b]	0.8 (0.6 to 1.1)	<.001
R-LVOT $PADs_{peak}$	cm	4.4	0.6	5.3 ± 0.4[a]	5.3 ± 0.5[a]	0.0 (−0.2 to 0.3)	5.9 ± 0.4[b]	0.7 (−0.5 to 0.9)	<.001
R-LVOT $PADs_{peak}/AoDs_{peak}$	cm	5.6	0.1	0.8 ± 0.1[a]	0.8 ± 0.1[a,b]	0.0 (0.0 to 0.1)	0.9 ± 0.1[b]	0.1 (0.0 to 0.1)	<.001
2D short-axis variables									
R-$Ao_{sx}Ds_{end}$	cm	2.8	0.6	7.4 ± 0.5[a]	7.2 ± 0.5[a]	−0.2 (−0.4 to 0.0)	7.9 ± 0.7[b]	0.4 (0.2 to 0.6)	<.001
R-$LA_{sx}D_{max}$	cm	4.9	1.2	8.7 ± 0.7	8.8 ± 0.6	0.0 (−0.3 tot 0.3)	9.0 ± 0.7	0.2 (−0.1 to 0.5)	.11
R-$LA_{sx}D_{max}/Ao_{sx}Ds_{end}$		5.4	0.2	1.2 ± 0.1	1.2 ± 0.1	0.0 (0.0 to 0.1)	1.1 ± 0.1	0.0 (−0.1 to 0.0)	.01
M-mode variables									
$RVIDd_{M\_ch}$	cm	12.9	1.1	3.4 ± 0.7[a]	3.5 ± 0.6[a]	0.1 (−0.2 to 0.4)	2.7 ± 0.5[b]	−0.6 (−1.0 to −0.3)	<.001
$RVIDs_{M\_ch}$	cm	18.0	1.3	2.7 ± 0.7[a]	2.6 ± 0.5[a]	0.0 (−0.5 to 0.4)	1.9 ± 0.8[b]	−0.8 (−1.2 to −0.4)	<.001
$IVSd_{M\_ch}$	cm	5.7	0.5	3.3 ± 0.4[a]	3.3 ± 0.4[a]	0.0 (−0.2 to 0.2)	3.9 ± 0.4[b]	0.6 (0.4 to 0.8)	<.001
$IVSs_{M\_ch}$	cm	4.5	0.6	4.6 ± 0.3	4.5 ± 0.4	−0.1 (−0.3 to 0.2)	4.6 ± 0.4	0.1 (−0.2 to 0.3)	.52
$LVIDd_{M\_ch}$	cm	3.4	1.0	10.7 ± 0.8[a]	10.8 ± 0.8[a]	0.1 (−0.2 to 0.5)	10.3 ± 0.9[b]	−0.4 (−0.8 to −0.1)	<.001
$LVIDs_{M\_ch}$	cm	7.7	1.4	6.5 ± 0.8	6.4 ± 0.9	−0.1 (−0.7 to 0.4)	6.5 ± 0.9	0.0 (−0.5 to 0.5)	.78
$LVFWd_{M\_ch}$	cm	8.2	0.6	2.5 ± 0.3[a]	2.5 ± 0.2[a]	−0.1 (−0.3 to 0.1)	2.8 ± 0.4[b]	0.3 (0.1 to 0.5)	<.001
$LVFWs_{M\_ch}$	cm	6.4	0.8	4.2 ± 0.5	4.1 ± 0.7	−0.1 (−0.4 to 0.3)	4.1 ± 0.6	−0.1 (−0.5 to 0.2)	.65
LV FS	%	9.7	10.9	39.4 ± 5.0	41.2 ± 6.9	1.8 (−2.7 to 6.3)	37.2 ± 6.7	−2.2 (−6.7 to 2.3)	.01
$RWTd_{M\_ch}$		5.6	0.1	0.6 ± 0.1[a]	0.6 ± 0.1[a]	0.0 (−0.0 to 0.0)	0.7 ± 0.1[b]	0.1 (0.1 to 0.1)	<.001
PWD variables									
L-PWD AoV VTI	cm	14.4	15.5	38.6 ± 5.8[a]	39.7 ± 10.3[a]	1.1 (−4.8 to 7.0)	26.4 ± 6.3[b]	−12.2 (−18.1 to −6.3)	<.001
L-PWD AoV PEP	ms	15.8	42.2	95 ± 16[a]	89 ± 16[a]	−7 (−17 to 4)	146 ± 16[b]	51 (40 to 62)	<.001
L-PWD AoV ET	ms	10.2	154.4	531 ± 51[a]	468 ± 75[b]	−63 (−117 to −9)	379 ± 60[c]	−151 (−205 to −98)	<.001
L-PWD AoV PEP/ET		17.6	0.1	0.2 ± 0.0[a]	0.2 ± 0.0[a]	0.0 (−0.3 to 0.1)	0.4 ± 0.1[b]	0.2 (0.2 to 0.2)	<.001
L-PWD AoV SV	mL	18.2	485.1	948 ± 220[a]	905 ± 306[a]	−43 (−212 to 126)	660 ± 174[b]	−289 (−458 to −120)	<.001
L-PWD AoV CO	L/min	19.5	18.0	32.5 ± 7.9	36.2 ± 12.7	3.7 (−3.8 to 11.2)	38.3 ± 9.9	5.8 (−1.7 to 13.3)	.16

(Continues)

TABLE 2 (Continued)

		Between-examination measurement variability (at rest)		Rest	Physiological stress	Mean difference between rest and physiological stress (95% CI)	Pharmacological challenge	Mean difference between rest and pharmacological challenge (95% CI)	ANOVA *F* test *P* value
		CV (%)	RC	Mean ± SD	Mean ± SD		Mean ± SD		
PW TDI variables									
E_m	cm/s	11.9	8.5	26 ± 6	24 ± 5	−2 (−5 to 2)	21 ± 6	−4 (−8 to −1)	.01
A_m	cm/s	17.1	4.7	10 ± 2[a]	13 ± 3[b]	3 (1 to 6)	10 ± 4[a]	−0 (−3 to 2)	.001
E_m/A_m		18.6	1.4	2.8 ± 0.9	2.0 ± 0.7	−0.8 (−1.4 to −0.1)	2.6 ± 1.3	−0.2 (−0.9 to 0.5)	.01
S_m	cm/s	9.9	3.0	11 ± 2[a]	11 ± 1[a]	0 (−1 to 1)	9 ± 2[b]	−2 (−3 to 0)	.001

Note: Between-examination measurement variability was evaluated based on 2 exams at rest, which were measured by the same blinded observer. From the within-subject variance for repeated measurements, the measurement variability was calculated as the within-subject CV and the RC (95% intermeasurement difference according to BSI recommendations). The degree of variability was defined as CV <5%, very low variability; 5% to 15%, low variability; 16% to 25%, moderate variability; >25%, high variability. The overall significance level for the general linear model for repeated measures (Univariate Analysis of Variance) was ≤.001 to correct for multiple comparisons (51 echocardiographic measurements). For each variable, mean values with different superscripts indicate a statistically significant difference between examinations after Bonferroni correction for multiple comparisons ($P < .05$). Identical superscripts indicate no statistically significant difference between examinations. Mean difference and its 95% CI are reported.

Abbreviations: A_m, peak radial wall motion velocity during late diastole; ANOVA, analysis of variance; BSI, British Standards Institution; CI, confidence interval; CV, coefficient of variation; E_m, peak radial wall motion velocity during early diastole; E_m/A_m, ratio of radial wall motion velocity during early and late diastole; $IVSd_{M\_ch}$, interventricular septal thickness at end-diastole; $IVSs_{M\_ch}$, interventricular septal thickness at end-systole; L, left-sided; LA FAC, left atrial fractional area change; L-$LADd_{end}$LAX, LA diameter at end-diastole left-sided LA diameter at end-diastole; L-$LADd_{max_end}$LAX, maximal LA diameter at end-diastole; L-$LADs_{end}$LAX, LA diameter at end-systole left-sided LA diameter at end-systole; L-$LADs_{max_end}$LAX, maximal LA diameter at end-systole; L-PWD AoV CO, LV cardiac output determined by PW Doppler; L-PWD AoV ET, LV ejection time determined by PW Doppler; L-PWD AoV PEP/ET, ratio of the LV pre-ejection period versus ejection time determined by PW Doppler; L-PWD AoV PEP, LV pre-ejection period determined by PW Doppler; L-PWD AoV SV, LV stroke volume determined by PW Doppler; L-PWD AoV Vel_{max}, LV maximal outflow velocity determined by PW Doppler; L-PWD AoV VTI, LV velocity time integral determined by PW Doppler; LV CO_{al}, cardiac output determined by area-length method; LV EF_{al}, LV ejection fraction determined by area-length method; LV FAC, fractional area change of LV area; LV FS, LV fractional shortening; LV SV_{al}, LV stroke volume determined by area-length method; $LVEDV_{al}$, LV end-diastolic volume determined by area-length method; $LVESV_{al}$, LV end-systolic volume determined by area-length method; $LVFWd_{M\_ch}$, LV free wall thickness at end-diastole; $LVFWs_{M\_ch}$, LV free wall thickness at end-systole; $LVIDd_{M\_ch}$, LV internal diameter at end-diastole; $LVIDs_{M\_ch}$, LV internal diameter at end-systole; PWD, pulsed wave Doppler echocardiography; R, right-sided; R-$Ao_{sx}Ds_{end}$, short axis diameter of the Ao at end-systole; RC, repeatability coefficient; R-LAAaLAX, LA area at maximal atrial contraction; R-$LAAd_{end}$LAX, LA area at end-diastole; R-LAApLAX, LA area at the onset of the P wave; R-$LAAs_{end}$LAX, LA area at end-systole; R-$LADd_{end}$LAX, LA diameter at end-diastole; R-$LADd_{max,end}$LAX, maximal LA diameter at end-diastole; R-$LADs_{end}$LAX, LA diameter at end-systole; R-$LADs_{max,end}$LAX, maximal LA diameter at end-systole; R-$LA_{sx}D_{max}$, short axis diameter of the LA at end-systole; R-$LA_{sx}D_{max}$/$Ao_{sx}Ds_{end}$, ratio of the short axis diameter of the LA and Ao at end-systole; R-$LVAd_{end}$LAX, LV area at end-diastole; R-LVOT $AoDd_{end}$, Ao diameter at sinotubular junction at end-diastole; R-LVOT $AoDs_{peak}$, Ao diameter at sinotubular junction at peak-systole; R-LVOT $PADd_{end}$, PA diameter parallel to sinotubular junction at end-diastole; R-LVOT $PADs_{peak}$, PA diameter parallel to sinotubular junction at peak-systole; R-LVOT $PADs_{peak}$/$AoDs_{peak}$, ratio of the pulmonary artery to aortic diameter parallel to sinotubular junction at peak-systole; R-$LVOTs_{peak}$, left ventricular outflow tract diameter at peak-systole; $RVIDd_{M\_ch}$, RV internal diameter at end-diastole; $RVIDs_{M\_ch}$, RV internal diameter at end-systole; $RWTd_{M\_ch}$, LV relative wall thickness at end-diastole; SD, standard deviation; S_m, peak radial wall motion velocity during peak-systole; TDI, tissue Doppler imaging.

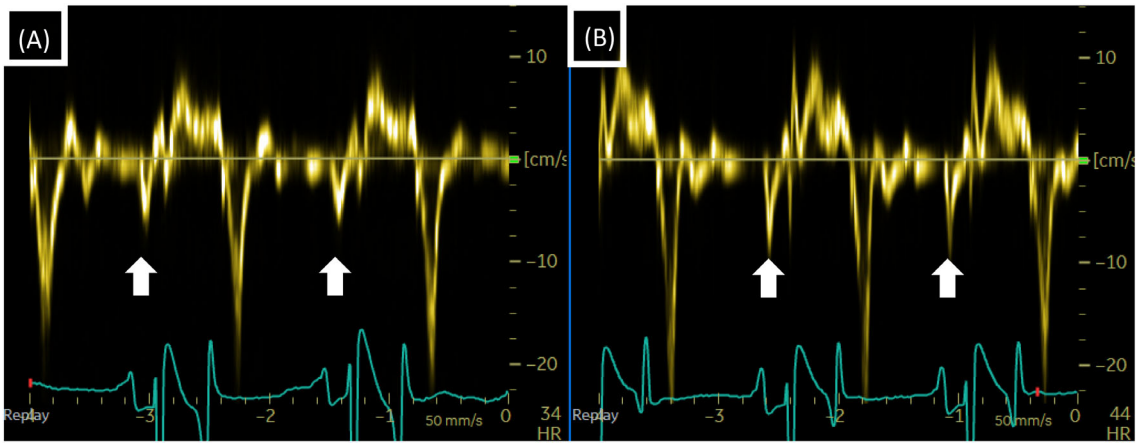

FIGURE 1 Pulsed wave tissue Doppler recording of the left ventricular free wall myocardial velocity from a right short axis view of the left ventricle at chordal level at 2 different physiological heart rates in a healthy Warmblood mare of 17 years old. The peak radial wall motion velocity during late diastole (A_m) indicated by arrows is higher at a heart rate of 44 beats per minute (bpm) (B, physiological stress scan) compared with a heart rate of 34 bpm (A, rest scan).

contraction. This finding of an increased active LA FAC demonstrates an enhanced booster pump function of the LA at slightly increased physiological HR in horses.[40,48,50] Significant increases in PW TDI A_m further support an increased atrial pump function at slightly increased heart rates. TDI has been suggested to be a promising tool for a more sensitive, quantitative, global, or focal assessment of myocardial function in horses compared with other echocardiographic techniques.[39] LA active pump function and LV compliance are the major determinants of the A_m velocity, whereas the peak early-to-late diastolic filling velocity ratio (E_m/A_m ratio) reflects the relative contribution of atrial pump function to ventricular filling.[5,8,39,46,48] An excellent correlation between A_m velocity and atrial function as well as LA ejection fraction, force, and kinetic energy has been demonstrated in a large number of studies in human medicine.[50] In healthy human individuals and cats, an age- and HR-related increase in tissue Doppler A_m velocities have been described.[50,52,53] These findings emphasize the importance of HR while evaluating atrial contractile function by PW TDI echocardiography.[50] This increased left atrial pump function at physiologically higher HR should be considered during the echocardiographic assessment of horses under hospital conditions as this situation should be considered as stressful event. It should be noticed that active LA FAC was calculated from area measurements before atrial contraction (LAAp) and at maximal atrial contraction (LAAa), as previously reported.[40-42] The left atrial area at atrial contraction refers to the truly smallest area of the LA during cardiac cycle, and atrial contraction acts as additional filling of the LV before atrioventricular valve closure. Left atrial FAC calculated in this way was shown to be useful to assess the risk of atrial fibrillation recurrence postconversion.[41,42] Left atrial FAC has also been calculated using different area measurements, namely the area before atrial contraction (named LAAa in those studies, instead of LAAp) and the area at mitral valve closure (named LAAmin in those studies).[5,54] The latter corresponds to the $LAAd_{end}$ of our study, which represents the atrial area after atrial contraction, and is influenced by the PQ interval. During physiological stress, LAAa showed a larger decrease (from 46.4 ± 6.2 cm^2 to 41.0 ± 6.0 cm^2, $P < .001$) compared with $LAAd_{end}$ (from 51.0 ± 7.9 cm^2 to 47.2 ± 6.9 cm^2, $P = .03$). During pharmacological challenge, $LAAd_{end}$ showed no significant decrease, whereas LAAa was significantly decreased ($P < .001$). These results suggest that the measurement during true atrial contraction more correctly reflects atrial booster pump function.

Pharmacological challenge with NBBM, a parasympatholytic agent with anticholinergic and antimuscarinic properties, caused similar echocardiographic effects as classical pharmacological stress testing, with decreased left ventricular dimensions and stroke volume and increased relative ventricular wall and interventricular septum thicknesses.[18,20,23,27] Cardiovascular effects of NBBM on HR and BP were visible within 2 minutes after IV administration of NBBM with a gradual reduction to baseline which occurred beyond image acquisition, similar to the findings of Morton et al,[28] who showed significant peak effects on HR and BP within 2 minutes after IV administration of NBB. Peak HR elevation occurred in the study of Morton et al 5 minutes post administration of NBB and persisted 25 minutes for DAP, MAP, and SAP and 50 minutes for HR with a gradual return to baseline.[28] Geimer et al documented a significant increase in HR and CO for at least 16 minutes and a short decrease in SV after administration of NBBM.[55] Pharmacological challenge in our study with NBBM led to significant decreases for most atrial and ventricular dimensions, volumes, and areas. Decreased left ventricular end-diastolic dimensions ($LVIDd_{M\_ch}$ and LV end-diastolic volume [$LVED-V_{al}$]) are considered as a surrogate for diastolic ventricular filling and a noninvasive measurement of cardiac preload (Table 2).[51,56] Although LA FAC was increased, LV A_m and E_m/A_m ratio were not altered. Therefore, it appears that the effects of an increased heart rate and a decreased diastolic LV filling time outweigh the effects of an enhanced left atrial booster pump function, leading to a decreased LV preload and filling.[51] Aortic dimensions increased significantly during pharmacological challenge in our study, as described in human patients with hypertension, probably because of increases in BP.[57-60] Left ventricular PEP increased,

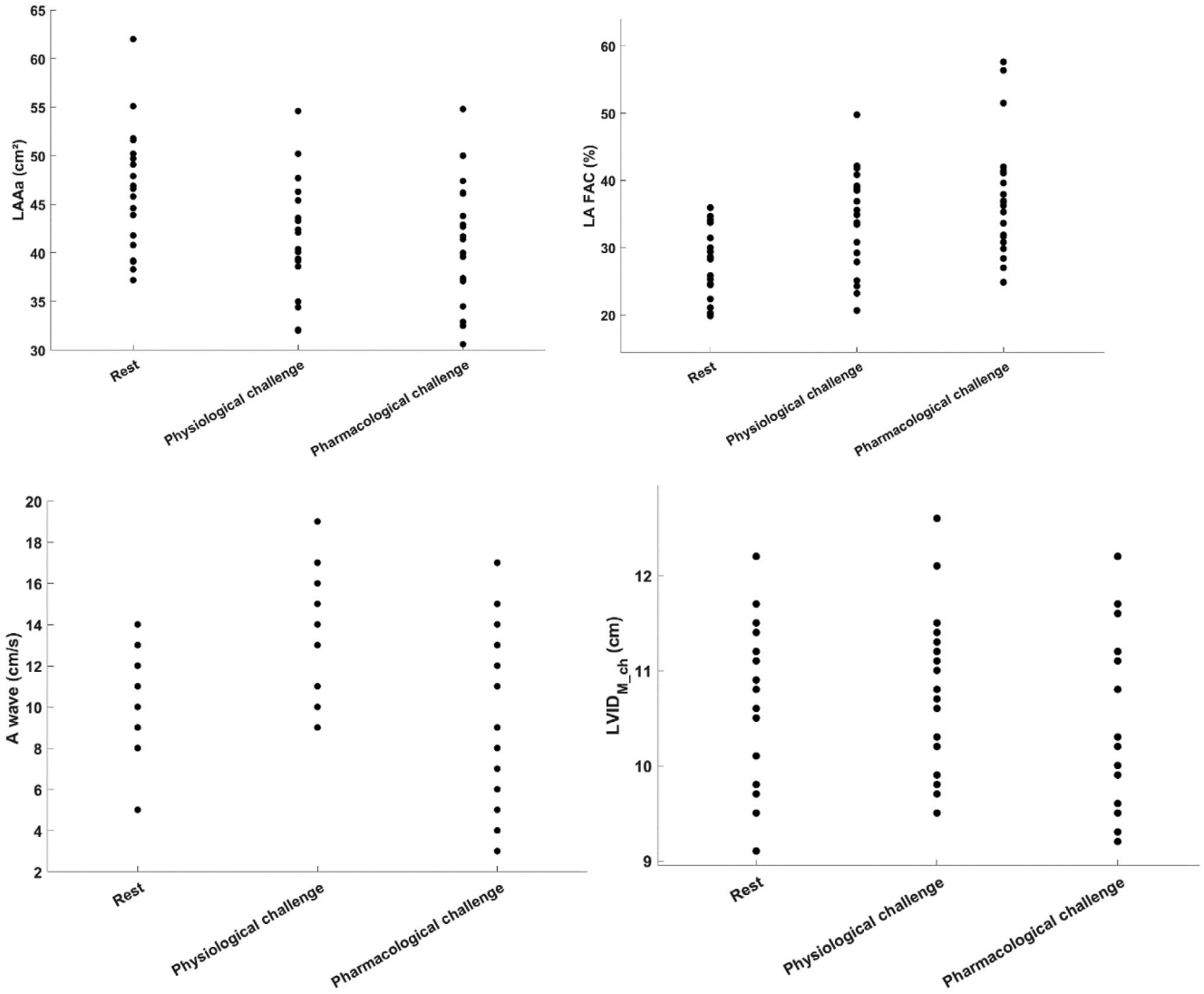

FIGURE 2 Scatter plots indicating individual data points for the echocardiographic variables left atrial area at maximal atrial contraction (LAAa), active left atrial fractional area change (LA FAC), peak radial wall motion velocity during late diastole (A_m), and left ventricular internal diameter at end-diastole ($LVIDd_{M\_ch}$) during the different scans.

which might be related to alterations in heart rate, conduction velocity, left ventricular end-diastolic pressure, left ventricular end-diastolic volume, afterload, and the inotropic state of the myocardium. The latter has been reported to be the dominant factor.[61] Left ventricular PEP is influenced by the sympathetic activity of beta-1 adrenoreceptors and should therefore shorten under stimulation.[62] The unexpected prolongation of LVPEP might be explained by increases in BP, which leads to a prolonged time to overcome the aortic pressure.[63,64] Increases in pulmonary dimensions after pharmacological challenge might also be caused by an increase in mean pulmonary arterial pressure, although this was not measured directly. These findings should be considered when examining horses with substantially increased heart rates or BP.

Repeatability of echocardiographic measurements in horses has been evaluated in a number of studies.[1,5,6] Overall, 2D and M-mode measurements appeared to be more repeatable compared with other echocardiographic techniques.[1,5,65] A clinically significant difference in echocardiographic variables should be larger than the 95% inter-measurement difference according to British Standards Institution (RC).[47] Statistically significant differences were identified for a number of variables after induction of physiological or pharmacological stress in our study, but many of these results do not represent clinically relevant differences because of the inherent measurement variabilities of the variables. However, mild increases in heart rate from 30 - 35 to 40 - 45 bpm may limit the effects of physiological stress in our study as heart rates might raise substantially during an echocardiographic exam under physiological stress. In addition, our study sample included healthy warmblood horses without an audible cardiac murmur or substantial valvular regurgitation. In cases with valvular regurgitation, small differences in HR and BP might contribute to significant changes in echocardiographic variables as the degree of valvular regurgitation is heavily influenced by variations in heart rate and BP.[66,67] Further research is needed to investigate the influence of

small changes in HR and BP on echocardiographic dimensions in horses with valvular disease.[25] Echocardiographic assessment of horses under hospital conditions might result in higher HR and BP alterations which mimic those obtained in our study during the pharmacological challenge. This could result in clinically significant differences in echocardiographic measurements.

Our study has several limitations. An a priori power analysis was conducted. The sample size was sufficient to demonstrate a 5% to 10% difference in cardiac chamber dimensions, depending on the variability of the different measurements. Therefore, differences of <5% could have been missed. However, these differences were deemed to be clinically insignificant. Increases in HR caused by physiological stress could in some horses only be achieved by forward or backward walking of the horse during the echocardiographic examination. This might have influenced image quality compared with the examinations at rest or during pharmacological challenge. In our experiment, BP was measured noninvasively and correlations with invasive measurement techniques have not been performed. A percentage of error (15%-28%) has been reported between noninvasively and invasively BP measurements for standing horses.[68] However, aim of our study was to estimate and differentiate trends in BP among scans and not to assess BP most precisely or accurately. For this purpose, the used technique and type of device have been validated in horses.[69] The order in which image acquisition was performed during the pharmacological challenge could have had an influence on the results as images acquired shortly after drug administration may be more prone to drug effects than those recorded later on. Therefore, image acquisition was performed in random order. Furthermore, the physiological stress scan was performed at only mildly increased HR. In a clinical setting, HR might rise substantially during an echocardiographic exam and exceed 45 bpm, which could lead to significant differences compared with rest. This situation could be more comparable to the pharmacological challenge, although BP might not rise as substantially. Hydration state of the horses was not assessed during this crossover study, although hydration deficits or hypovolemia could have affected echocardiographic variables during the different scans.[70] However, none of the horses showed clinical signs of dehydration during clinical examinations before the scans and hydration deficits were assumed to be unlikely. A few reported echocardiographic variables are prone to error or limitations. Assessment of RV echocardiographic variables is influenced by respiration as well as apical trabeculation. Therefore, RV measurements appear generally to be less reliable. Decloedt et al. (2015) demonstrated a low variability for most 2D and M-mode echocardiographic variables (CV < 15% for $RVIDd_{M\_ch}$ and $RVIDs_{M\_ch}$).[20] Our study showed low to moderate CV's for RV M-mode variables. Volumetric variables are calculated variables based on geometric assumptions and subsequently prone to error.[51] PW Doppler–derived LV SV and CO are calculated estimates based on aortic dimensions and outflow velocity profiles, which are highly angle dependent. Inadequate alignment, variation in cursor placement, and inaccuracies in the determination of the flow area predispose to error. PW Doppler echocardiographic variables are therefore generally regarded to be less reliable and accurate.[39]

In conclusion, physiological stress with mildly increased HR (40-45 bpm) resulted in increases in ventricular peak radial wall motion velocity during atrial contraction, reflecting an increased left atrial pump function. Most other left atrial, left ventricular, aortic and pulmonary 2D, M-mode, or Ao PWD measurements did not differ significantly with mildly increased HR (40-45 bpm) in healthy horses. This finding emphasizes an important influence of HR on measures of atrial contractile function. Pharmacological challenge with NBBM led to decreased left atrial and diastolic ventricular dimensions and increased aortic and pulmonary dimensions.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Study (EC 2020/100) was performed after approval and following the guidelines of the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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REFERENCES

1. Buhl R, Ersbøll AK, Eriksen L, Koch J. Sources and magnitude of variation of echocardiographic measurements in normal standardbred horses. *Vet Radiol Ultrasound*. 2004;45(6):505-512. doi:10.1111/j.1740-8261.2004.04086.x
2. Menzies-Gow NJ. Effects of sedation with acepromazine on echocardiographic measurements in eight healthy thoroughbred horses. *Vet Rec*. 2008;163(1):21-25. doi:10.1136/vr.163.1.21
3. Reef VB, Bonagura J, Buhl R, et al. Recommendations for management of equine athletes with cardiovascular abnormalities. *J Vet Intern Med*. 2014;28(3):749-761. doi:10.1111/jvim.12340
4. Schwarzwald CC. Disorders of the cardiovascular system. In: Reed S, Bayly W, Sellon D, eds. *Equine Internal Medicine*. Elsevier, St. Louis, Missouri; 2018:387-541.
5. Schwarzwald CC, Schober KE, Bonagura JD. Methods and reliability of echocardiographic assessment of left atrial size and mechanical function in horses. *Am J Vet Res*. 2007;68(7):735-747. doi:10.2460/ajvr.68.7.735

6. Schwarzwald CC, Schober KE, Bonagura JD. Methods and reliability of tissue Doppler imaging for assessment of left ventricular radial wall motion in horses. *J Vet Intern Med*. 2009;23(3):643-652. doi:10.1111/j.1939-1676.2009.0287.x
7. Reef VB. Heart murmurs in horses: determining their significance with echocardiography. *Equine Vet J Suppl*. 1995;19:71-80. doi:10.1111/j.2042-3306.1995.tb04992.x
8. Ven S, Decloedt A, van der Vekens N, de Clercq D, van Loon G. Assessing aortic regurgitation severity from 2D, M-mode and pulsed wave Doppler echocardiographic measurements in horses. *Vet J*. 2016;210:34-38. doi:10.1016/j.tvjl.2016.01.011
9. Keen JA. Examination of horses with cardiac disease. *Vet Clin North Am Equine Pract*. 2019;35(1):23-42. doi:10.1016/j.cveq.2018.12.006
10. Patteson MW. Chapter 4: Diagnostic aids in equine cardiology. In: Patteson MW, ed. *Equine Cardiology*. Blackwell Science Ltd., Oxford; 1996:87.
11. Gasthuys F, De Moor A, Parmentier D. Haemodynamic changes during sedation in ponies. *Vet Res Commun*. 1990;14(4):309-327. doi:10.1007/BF00350713
12. Buhl R, Ersbøll AK, Larsen NH, Eriksen L, Koch J. The effects of detomidine, romifidine or acepromazine on echocardiographic measurements and cardiac function in normal horses. *Vet Anaesth Analg*. 2007;34(1):1-8. doi:10.1111/j.1467-2995.2005.00269.x
13. Yamashita K, Tsubakishita S, Futaok S, et al. Cardiovascular effects of medetomidine, detomidine and xylazine in horses. *J Vet Med Sci*. 2000;62(10):1025-1032. doi:10.1292/jvms.62.1025
14. Mama KR, Grimsrud K, Snell T, Stanley S. Plasma concentrations, behavioural and physiological effects following intravenous and intramuscular detomidine in horses. *Equine Vet J*. 2009;41(8):772-777. doi:10.2746/042516409x421624
15. Gehlen H, Kroker K, Deegen E, Stadler P. Einfluss von Detomidin auf echokardiographische Funktionsparameter und kardiale Hämodynamik bei Pferden mit und ohne Herzgeräusch. *Schweiz Arch Tierheilkd*. 2004;146(3):119-126.
16. Frye MA, Bright JM, Dargatz DA, et al. A comparison of dobutamine infusion to exercise as a cardiac stress test in healthy horses. *J Vet Intern Med*. 2003;17(1):58-64. doi:10.1892/0891-6640(2003)0172.3.co;2
17. Durando MM, Slack J, Reef VB, Birks EK. Right ventricular pressure dynamics and stress echocardiography in pharmacological and exercise stress testing. *Equine Vet J Suppl*. 2006;36:183-192. doi:10.1111/j.2042-3306.2006.tb05537.x
18. Gehlen H, Marnette S, Rohn K, Stadler P. Stress echocardiography in warmblood horses: comparison of dobutamine/atropine with treadmill exercise as cardiac stressors. *J Vet Intern Med*. 2006;20(3):562-568. doi:10.1892/0891-6640(2006)20[562:seiwhc]2.0.co;2
19. Sandersen CF, Amory H. Stress echocardiography in horses—a review. *Pferdeheilkunde*. 2006;22(5):609-617.
20. Decloedt A, De Clercq D, Ven Sofie S, et al. Echocardiographic measurements of right heart size and function in healthy horses. *Equine Vet J*. 2017;49(1):58-64. doi:10.1111/evj.12554
21. Meier M, Bettschart-Wolfensberger R, Schwarzwald CC, Portier K, Gysler A, Ringer SK. Effects of dobutamine on cardiovascular function and oxygen delivery in standing horses. *J Vet Pharmacol Ther*. 2020;43(5):470-476. doi:10.1111/jvp.12869
22. Sandersen CF, Detilleux J, Delguste C, Pierard L, van Loon G, Amory H. Atropine reduces dobutamine-induced side effects in ponies undergoing a pharmacological stress protocol. *Equine Vet J*. 2005;37(2):128-132. doi:10.2746/0425164054223868
23. Sandersen CF, Detilleux J, de Moffarts B, van Loon G, Amory H. Effect of atropine-dobutamine stress test on left ventricular echocardiographic parameters in untrained warmblood horses. *J Vet Intern Med*. 2006;20(3):575-580. doi:10.1892/0891-6640(2006)20[575:eoasto]2.0.co;2
24. Gehlen H, Marnette S, Stadler P. Stress echocardiography in warmblood horses: active stress induction by treadmill and longing exercise. *Pferdeheilkunde*. 2005;21(4):303-310.
25. Gehlen H, Becker J, Deegen E, Stadler P. Veränderung echokardiographischer Funktionsparameter unter Dobutaminwirkung bei Warmblutpferden mit und ohne Herzgeräusch. *Vet Med*. 2004;91:103-111.
26. Gehlen H, Marnette S, Stadler P. The influence of adrenaline on echocardiographic parameters of left ventricular function in the horse. *Equine Comp Exerc Physiol*. 2005;2(2):89-96.
27. Sandersen C, Detilleux J, Art T, Amory H. Exercise and pharmacological stress echocardiography in healthy horses. *Equine Vet J Suppl*. 2006;36:159-162. doi:10.1111/j.2042-3306.2006.tb05533.x
28. Morton AJ, Varney CR, Ekiri AB, Grosche A. Cardiovascular effects of N-butylscopolammonium bromide and xylazine in horses. *Equine Vet J Suppl*. 2011;39:117-122. doi:10.1111/j.2042-3306.2011.00400.x
29. de Lagarde M, Rodrigues N, Chevigny M, Beauchamp G, Albrecht B, Lavoie JP. N-butylscopolammonium bromide causes fewer side effects than atropine when assessing bronchoconstriction reversibility in horses with heaves. *Equine Vet J*. 2014;46(4):474-478. doi:10.1111/evj.12229
30. Tapio HA, Raekallio MR, Mykkänen A, et al. Effects of MK-467 hydrochloride and hyoscine butylbromide on cardiorespiratory and gastrointestinal changes induced by detomidine hydrochloride in horses. *Am J Vet Res*. 2018;79(4):376-387. doi:10.2460/ajvr.79.4.376
31. Henneke DR, Potter GD, Kreider JL, Yeates BF. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J*. 1983;15(4):371-372. doi:10.1111/j.2042-3306.1983.tb01826.x
32. Carroll CL, Huntington PJ. Body condition scoring and weight estimation of horses. *Equine Vet J*. 1988;20(1):41-45. doi:10.1111/j.2042-3306.1988.tb01451.x
33. Wagner EL, Tyler PJ. A comparison of weight estimation methods in adult horses. *J Equine Vet*. 2011;31(12):706-710.
34. Vernemmen I, Vera L, Van Steenkiste G, van Loon G, Decloedt A. Reference values for 2-dimensional and M-mode echocardiography in Friesian and Warmblood horses. *J Vet Intern Med*. 2020;34(6):2701-2709. doi:10.1111/jvim.15938
35. Verheyen T, Decloedt A, De Clercq D, Deprez P, Sys S, van Loon G. Electrocardiography in horses, part 1: how to make a good recording. *Vlaams Diergeneeskd Tijdschr*. 2010;79(5):331-336.
36. Vera L, Van Steenkiste G, Decloedt A, Chiers K, van Loon G. Age-related differences in blood pressure, ultrasound-derived arterial diameters and arterial wall stiffness parameters in horses. *Equine Vet J*. 2020;52(6):868-875. doi:10.1111/evj.13263
37. Vera L, De Clercq D, Van Steenkiste G, Decloedt A, Chiers K, van Loon G. Differences in ultrasound-derived arterial wall stiffness parameters and noninvasive blood pressure between Friesian horses and Warmblood horses. *J Vet Intern Med*. 2020;34(2):893-901. doi:10.1111/jvim.15705
38. Ven S, Decloedt A, De Clercq D, Vera L, Rademakers F, van Loon G. Detection of subclinical left ventricular dysfunction by tissue Doppler imaging in horses with aortic regurgitation. *Equine Vet J*. 2018;50(5):587-593. doi:10.1111/evj.12805
39. Schwarzwald CC. Equine echocardiography. *Vet Clin North Am Equine Pract*. 2019;35(1):43-64. doi:10.1016/j.cveq.2018.12.008
40. De Clercq D, van Loon G, Tavernier R, Duchateau L, Deprez P. Atrial and ventricular electrical and contractile remodeling and reverse remodeling owing to short-term pacing-induced atrial fibrillation in horses. *J Vet Intern Med*. 2008;22(6):1353-1359. doi:10.1111/j.1939-1676.2008.0202.x
41. Decloedt A, Schwarzwald CC, De Clercq D, et al. Risk factors for recurrence of atrial fibrillation in horses after cardioversion to sinus rhythm. *J Vet Intern Med*. 2015;29(3):946-953. doi:10.1111/jvim.12606

42. Decloedt A, Verheyen T, Van Der Vekens N, Sys S, De Clercq D, van Loon G. Long-term follow-up of atrial function after cardioversion of atrial fibrillation in horses. *Vet J*. 2013;197(3):583-588. doi:10.1016/j.tvjl.2013.05.032
43. Vandecasteele T, Cornillie P, van Steenkiste G, et al. Echocardiographic identification of atrial-related structures and vessels in horses validated by computed tomography of casted hearts. *Equine Vet J*. 2019;51(1):90-96. doi:10.1111/evj.12969
44. Berthoud D, Schwarzwald CC. Echocardiographic assessment of left ventricular size and systolic function in Warmblood horses using linear measurements, area-based indices, and volume estimates: a retrospective database analysis. *J Vet Intern Med*. 2021;35(1):504-520. doi:10.1111/jvim.15968
45. Koenig TR, Mitchell KJ, Schwarzwald CC. Echocardiographic assessment of left ventricular function in healthy horses and in horses with heart disease using pulsed-wave tissue Doppler imaging. *J Vet Intern Med*. 2017;31(2):556-567. doi:10.1111/jvim.14641
46. Decloedt A, Verheyen T, Sys S, De Clercq D, van Loon G. Evaluation of tissue Doppler imaging for regional quantification of radial left ventricular wall motion in healthy horses. *Am J Vet Res*. 2013;74(1):53-61. doi:10.2460/ajvr.74.1.53
47. Bland M. Clinical measurement. In: Bland M, ed. *An Introduction to Medical Statistics*. 4th ed. Oxford: Oxford University Press; 2015:313-346.
48. Piotrowski G, Goch A, Wlazłowski R, Gawor Z, Goch JH. Noninvasive methods of atrial function evaluation in heart diseases. *Med Sci Monit*. 2000;6(4):827-839.
49. Pagel PS, Kehl F, Gare M, Hettrick DA, Kersten JR, Warltier DC. Mechanical function of the left atrium: new insights based on analysis of pressure-volume relations and Doppler echocardiography. *Anesthesiology*. 2003;98(4):975-994. doi:10.1097/00000542-200304000-00027
50. Blume GG, Mcleod CJ, Barnes ME, et al. Left atrial function: physiology, assessment, and clinical implications. *Eur J Echocardiogr*. 2011;12(6):421-430. doi:10.1093/ejechocard/jeq175
51. Schefer KD, Bitschnau C, Weishaupt MA, Schwarzwald CC. Quantitative analysis of stress echocardiograms in healthy horses with 2-dimensional (2D) echocardiography, anatomical M-mode, tissue Doppler imaging, and 2D speckle tracking. *J Vet Intern Med*. 2010;24(4):918-931. doi:10.1111/j.1939-1676.2010.0542.x
52. Yu CM, Sanderson JE. Right and left ventricular diastolic function in patients with and without heart failure: effect of age, sex, heart rate, and respiration on Doppler-derived measurements. *Am Heart J*. 1997;134(3):426-434. doi:10.1016/s0002-8703(97)70077-2
53. Disatian S, Bright JM, Boon J. Association of age and heart rate with pulsed-wave Doppler measurements in healthy, nonsedated cats. *J Vet Intern Med*. 2008;22(2):351-356. doi:10.1111/j.1939-1676.2008.0066.x
54. Huesler IM, Mitchell KJ, Schwarzwald CC. Echocardiographic assessment of left atrial size and function in Warmblood horses: reference intervals, allometric scaling, and agreement of different echocardiographic variables. *J Vet Intern Med*. 2016;30(4):1241-1252. doi:10.1111/jvim.14368
55. Geimer TR, Ekström PM, Ludders JW, Erichsen DF, Gleed RD. Haemodynamic effects of hyoscine-N-butylbromide in ponies. *J Vet Pharmacol Ther*. 1995;18(1):13-16. doi:10.1111/j.1365-2885.1995.tb00544.x
56. Cnota JF, Mays WA, Knecht SK, et al. Cardiovascular physiology during supine cycle ergometry and dobutamine stress. *Med Sci Sports Exerc*. 2003;35(9):1503-1510. doi:10.1249/01.MSS.0000084436.15808.52
57. Covella M, Milan A, Totaro S, et al. Echocardiographic aortic root dilatation in hypertensive patients: a systematic review and meta-analysis. *J Hypertens*. 2014;32(10):1928-1935. doi:10.1097/HJH.0000000000000286
58. Vizzardi E, Maffessanti F, Lorusso R, et al. Ascending aortic dimensions in hypertensive subjects: reference values for two-dimensional echocardiography. *J Am Soc Echocardiogr*. 2016;29(9):827-837. doi:10.1016/j.echo.2016.03.016
59. Farasat SM, Morrell CH, Scuteri A, et al. Do hypertensive individuals have enlarged aortic root diameters? Insights from studying the various subtypes of hypertension. *Am J Hypertens*. 2008;21(5):558-563. doi:10.1038/ajh.2008.10
60. Vasan RS, Larson MG, Levy D. Determinants of echocardiographic aortic root size. The Framingham Heart Study. *Circulation*. 1995;91(3):734-740. doi:10.1161/01.cir.91.3.734
61. Mertens HM, Mannebach H, Trieb G, Gleichmann U. Influence of heart rate on systolic time intervals: effects of atrial pacing versus dynamic exercise. *Clin Cardiol*. 1981;4(1):22-27. doi:10.1002/clc.4960040106
62. Bonagura JD, Fuentes VL. Echocardiography. In: Mattoon JS, Nyland TG, eds. *Small Animal Diagnostic Ultrasound*. Elsevier, St. Louis, Missouri; 2015:217-331.
63. Lanfranchi PA, Pépin JL, Somers VK. Cardiovascular physiology. In: Kryger M, Roth T, Dement WC, eds. *Principles and Practice of Sleep Medicine*. Elsevier, St. Louis, Missouri; 2017:142-154.
64. Finnegan E, Davidson S, Harford M, et al. Pulse arrival time as a surrogate of blood pressure. *Sci Rep*. 2021;11(1):22767.
65. Slack J, Durandot MM, Belcher CN, et al. Intraoperator, intraobserver and interoperator variability of echocardiographic measurements in healthy foals. *Equine Vet J Suppl*. 2012;41:69-75. doi:10.1111/j.2042-3306.2011.00503.x
66. Robinson S, Ring L, Augustine DX, et al. The assessment of mitral valve disease: a guideline from the British Society of Echocardiography. *Echo Res Pract*. 2021;8(1):G87-G136. doi:10.1530/ERP-20-0034
67. Grayburn PA, Weissman NJ, Zamorano JL. Quantitation of mitral regurgitation. *Circulation*. 2012;126(16):2005-2017. doi:10.1161/CIRCULATIONAHA.112.12159
68. Olsen E, Pedersen TL, Robinson R, Haubro AP. Accuracy and precision of oscillometric blood pressure in standing conscious horses. *J Vet Emerg Crit Care*. 2016;26(1):85-92. doi:10.1111/vec.12411
69. Heliczer N, Lorello O, Casoni D, Navas de Solis C. Accuracy and precision of noninvasive blood pressure in normo-, hyper-, and hypotensive standing and anesthetized adult horses. *J Vet Intern Med*. 2016;30(3):866-872. doi:10.1111/jvim.13928
70. Underwood C, Norton JL, Nolen-Walston RD, Dallap-Schaer BL, Boston R, Slack J. Echocardiographic changes in heart size in hypohydrated horses. *J Vet Intern Med*. 2011;25(3):563-569. doi:10.1111/j.1939-1676.2010.0612.x

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