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Echocardiographic Assessment of Left Atrial Size and Function in Warmblood Horses: Reference Intervals, Allometric Scaling, and Agreement of Different Echocardiographic Variables

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Background: Echocardiographic assessment of left atrial (LA) size and function in horses is not standardized.

Objectives: The aim of this study was to establish reference intervals for echocardiographic indices of LA size and function in Warmblood horses and to provide proof of concept for allometric scaling of variables and for the clinical use of area-based indices.

Animals: Thirty-one healthy Warmblood horses and 91 Warmblood horses with a primary diagnosis of mitral regurgitation (MR) or aortic regurgitation (AR).

Methods: Retrospective study. Echocardiographic indices of LA size and function were measured and scaled to body weight (BWT). Reference intervals were calculated, the influence of BWT, age, and valvular regurgitation on LA size and function was investigated and agreement between different measurements of LA size was assessed.

Results: Allometric scaling of variables of LA size allowed for correction of differences in BWT. Indices of LA size documented LA enlargement with moderate and severe MR and AR, whereas most indices of LA mechanical function were not significantly altered by valvular regurgitation. Different indices of LA size were in fair to good agreement but still lead to discordant conclusions with regard to assessment of LA enlargement in individual horses.

Conclusions and Clinical Importance: Allometric scaling of echocardiographic variables of LA size is advised to correct for differences in BWT among Warmblood horses. Assessment of LA dimensions should be based on an integrative approach combining subjective evaluation and assessment of multiple measurements, including area-based variables. The clinical relevance of indices of LA mechanical function remains unclear when used in horses with mitral or aortic regurgitation.

Key words: Equine; Heart; Imaging; Valvular regurgitation.

Assessment of left atrial (LA) dimensions constitutes a central part of every echocardiographic examination. It provides important information on the hemodynamic effects and severity of a variety of heart diseases and allows for monitoring of disease progression over time. In horses, assessment of LA size has traditionally been limited to subjective evaluation and measurement of the LA diameter in a left-parasternal long-axis view.[1,2] However, using linear dimensions as the sole measure of LA size might be misleading, as it neglects the fact that the LA can enlarge in multiple directions, thereby changing its three-dimensional geometry.[3] In addition, the exact timing of measurements within the cardiac cycle is often undefined in clinical practice and measurements are usually not corrected for differences in body size, although LA size is known to be related

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Abbreviations:

2D	two-dimensional
2DE	two-dimensional echocardiography
AC	area change
AR	aortic regurgitation
A_m	late-diastolic left ventricular radial wall motion velocity
ANOVA	analysis of variance
AAD	aortic annular diameter
AoD	aortic sinus diameter
AoA	aortic area
BWT	body weight
CI	confidence interval
d	diastolic
ECG	electrocardiogram
E_m	early-diastolic left ventricular radial wall motion velocity
FAC	fractional area change
FS	fractional shortening
HR	heart rate
IVS	interventricular septal thickness
LA	left atrium or left atrial
LAD	left atrial diameter
LAA	left atrial area
llx	left-parasternal long-axis view
LV	left ventricle or left ventricular
LVFW	left-ventricular free wall
LVID	left-ventricular internal diameter
M-mode	motion mode
MR	mitral regurgitation
MWT	mean wall thickness
NSR	normal sinus rhythm
PAD	pulmonary artery diameter
PR	pulmonic regurgitation
RI	reservoir index

RWT	relative wall thickness
SD	standard deviation
s	systolic
sx	short axis
TR	tricuspid regurgitation
WB	Warmblood horse

to body weight.[4–7] Finally, LA mechanical function might have a prognostic implication in a variety of diseases,[8–11] but is rarely considered during routine echocardiographic examinations in horses.

Methods and reliability of a variety of conventional (linear) and novel (area-based) indices could allow a more comprehensive assessment of LA size and mechanical function in horses.[12,13] However, their clinical use is not standardized across centers and the use of novel indices is poorly established. Reference intervals are lacking and the influence of age and body weight is unknown. Although two-dimensional (2D) (area) measurements of LA dimensions might be more sensitive for detection of mild LA enlargement compared to one-dimensional (linear) indices, this has not been proven in horses.

The goals of this study were to (1) assess the influence of age and body weight (BWT) on LA size and function in Warmblood horses, (2) support the concept for allometric scaling of variables, (3) establish reference intervals for echocardiographic indices of LA size and mechanical function in Warmblood horses, and (4) provide prove of concept for the clinical use of area-based indices of LA size in this species. The effect of various degrees of mitral (MR) and aortic regurgitation (AR) on indices of LA size and mechanical function was described and agreement between conventional linear measurements of LA size and novel area-based indices of LA size was assessed in a population of healthy horses and horses with valvular regurgitation.

Materials and Methods

Study Population

The study population was chosen retrospectively and included Warmblood horses (WB) that had undergone a standardized echocardiographic examination at the University of Zurich Equine Hospital between June 2007 and January 2014. Enrollment criteria were the following: BWT >300 kg; age >2 years; no sedation prior or during the examination; normal sinus rhythm; absence of cardiovascular disease (healthy group) or presence of mitral or aortic regurgitation as a primary diagnosis (diseased group); and the availability of a complete, standardized echocardiogram of good quality, with an electrocardiogram (ECG) recorded simultaneously and performed by a single experienced operator (CCS).

One hundred and twenty-two Warmblood horses fulfilled the inclusion criteria. Thirty-one horses (12 female, 19 male castrated; 6–23 (12 ± 4) years; 450–707 (574 ± 58) kg [range (mean ± standard deviation, SD)]) were considered healthy based on medical history, physical examination, electrocardiography and transthoracic echocardiography. The remaining 91 horses (30 female, 5 male, 56 male castrated; 3–28 (14 ± 6) years; 430–720 (577 ± 60) kg) had a primary diagnosis of MR or AR, diagnosed by auscultation and confirmed and graded by echocardiography. Grading of the severity of valvular regurgitation was based on the duration of the regurgitant signal, high-velocity jet area and flow disturbance, and the number of imaging planes in which the high-velocity jet could be observed in the receiving chamber.[14] The horses were grouped according to the primarily affected valve, as judged by the clinician performing the echocardiogram (CCS). The group "trivial-mild MR" (n = 27) contained horses with trivial MR (n = 2); trivial MR plus trivial pulmonic regurgitation (PR, n = 2); mild MR (n = 14); mild MR plus trivial to mild AR (n = 6); and mild MR plus trivial to mild PR and/or tricuspid regurgitation (TR, n = 3). The group "moderate MR" (n = 25) contained horses with moderate MR (n = 19); moderate MR plus mild AR (n = 3); and moderate MR plus trivial to moderate TR (n = 3). The group "severe MR" (n = 3) contained horses with severe MR (n = 2); and severe MR plus mild AR, TR and PR (n = 1). The group "trivial-mild AR" (n = 9) contained horses with trivial AR (n = 1); trivial AR plus trivial PR and TR (n = 1); mild AR (n = 5); and mild AR plus mild PR (n = 2). The group "moderate AR" (n = 13) contained horses with moderate AR (n = 11); and moderate AR plus mild MR (n = 1) or moderate PR (n = 1). The group "severe AR" (n = 14) contained horses with severe AR (n = 13); and severe AR plus mild PR (n = 1). None of the horses were in congestive heart failure.

Echocardiography

All echocardiographic examinations and measurements were performed by a single operator (CCS) according to a standardized protocol. During the examination, all horses were standing in a quiet room and restrained by an experienced handler. All horses were unsedated during the examination. Transthoracic echocardiography[a] was performed with a phased array transducer[b] at a frequency of 1.9/4.0 MHz (octave harmonics). A single-lead base-apex electrocardiogram was recorded simultaneously. Recordings were stored as still frames or cine-loops in digital raw format for offline analysis.[c] Three representative non-consecutive cardiac cycles were measured and averaged for each variable. Cycles immediately following a sinus pause, second-degree atrioventricular block or ectopic beat were precluded from analysis. The heart rate (HR) of each measured cycle was calculated based on the RR interval (ms) preceding the analyzed cycle (HR = 60,000/RR). All measurements were performed at the time of examination of the horses, strictly adhering to a predetermined measurement protocol that was used throughout the duration of data collection.

Routine transthoracic two-dimensional (2DE), motion mode (M-mode), tissue Doppler and color Doppler echocardiography were performed to assess cardiac structures, valvular competence, great vessel dimensions, chamber dimensions, and left ventricular (LV) systolic and diastolic function by use of standard right-parasternal long-axis and short-axis views.[1,2,9,12,13,15,16] The main attention was then directed to the assessment of LA size and mechanical function using the methods previously described.[9,12,13] The variables and indices used in this study are listed in detail in Appendix 1 and the measurements are shown in the supporting information (Figure S1).

The measurements of great vessel and chamber dimensions were corrected for differences in BWT according to the principles of allometric scaling.[6,17] Specifically, the measurements of LA and LV dimensions were normalized to a BWT of 500 kg using the following equations: diameter (500) = measured diameter / $BWT^{1/3} \times 500^{1/3}$; area (500) = measured area / $BWT^{2/3} \times 500^{2/3}$. In addition, linear indices were indexed to aortic annular diameter (AAD) and area measurements were indexed to AAD^2 and aortic short-axis area, respectively, as an alternative method to correct for differences in body size.[13]

Data Analysis and Statistics

Data collection, graphical presentation, data analysis, and statistics were performed using commercially available computer software.[d,e,f,g]

The relationship of echocardiographic variables obtained in healthy Warmblood horses to age and BWT was assessed using linear regression analyses. For dimensional variables (ie, variables of great vessel and chamber size), both raw data and weight-corrected data were included in linear regression analyses in order to assess the effect of weight correction.

The reference intervals for the measured and calculated variables were calculated based on the data of 31 healthy Warmblood horses using a dedicated software package.[f] For dimensional variables, only the weight-corrected measurements were used. Distribution of the data was checked using raw data box-and-whisker plots, histograms, and normal probability plots. For symmetrically distributed data, standard methods were used to calculate the lower and upper limit of the reference interval on untransformed data. For $PA_{sx}D/AoD$, $LA_{sx}A_{max}/Ao_{sx}A$ and $LAD_{max}/LVID_d$, normal distribution could not be assumed; therefore, the reference interval was calculated using standard methods based on Box-Cox transformed data. The 90% confidence intervals (CI) of the limits of the reference intervals were determined using a bootstrap method.

Echocardiographic indices obtained in healthy horses were compared to those obtained in horses with trivial-mild MR, moderate MR, and severe MR, and to those obtained in horses with trivial-mild AR, moderate AR, and severe AR, using a one-way analysis of variance (ANOVA) with Dunnett's posthoc test.[e] Homogeneity of variances was assessed by graphical display of the data and validity of the normality assumption was confirmed by assessment of normal probability plots of the residuals. Summary statistics were calculated for each group and expressed as mean ± SD.

The number of horses in which different methods of measurement obtained during a single examination revealed discordant results concerning left atrial enlargement (ie, one variable indicated normal LA size and another variable indicated LA enlargement) was expressed as proportion and percentage for a variety of combinations. The relationship between different indices of LA size was assessed using linear regression analyses. Agreement of different indices for detection of reduced, normal, and increased LA size (as judged based on the calculated reference intervals) in all horses and in horses with valvular regurgitation, respectively, was quantified using weighted kappa (κ_w) statistics.[g] Thereby, $\kappa_w > 0.75$ indicated excellent agreement, κ_w ranging from 0.40 to 0.75 indicated fair to good agreement, and $\kappa_w < 0.40$ indicated poor agreement.[18] Finally, Bland–Altman analyses were performed to calculate mean bias and 95% limits of agreement for comparison between linear measurements of LA size and between area measurements of LA size.[19,20]

The level of significance for all statistical analyses was $P = .05$.

Results

Linear regression analyses indicated that before correction of dimensional variables for differences in BWT, LAD_{max} ($P = .010$, $r^2 = 0.21$), LAA_{max} ($P < .001$, $r^2 = 0.59$), LAA_a ($P < .001$, $r^2 = 0.34$), LAA_{min} ($P < .001$, $r^2 = 0.41$), $LA_{sx}A_{max}$ ($P < .001$, $r^2 = 0.43$), $LAD_{llx\text{-}max}$ ($P = .0012$, $r^2 = 0.31$), AoD ($P = .021$, $r^2 = 0.17$), AAD ($P = .0026$, $r^2 = 0.27$), $Ao_{sx}A$ ($P = .0034$, $r^2 = 0.26$), $PA_{sx}D$ ($P < .001$, $r^2 = 0.37$), and $LVFW_s$ ($P = .035$, $r^2 = 0.14$) were positively related to BWT in healthy Warmblood horses. After allometric scaling to a standard BWT of 500 kg, none of these variables remained significantly related to BWT. The IVS_s (500) was the only scaled measurement that was (inversely) related to BWT ($P = .008$, $r^2 = 0.22$). With exception of passive LA FAC ($P = .050$, $r^2 = 0.13$) and IVS_d (500) ($P = .036$, $r^2 = 0.14$), which both increased with higher age, none of the echocardiographic variables was significantly related to age.

The reference intervals for echocardiographic variables of LA size and mechanical function and for basic variables of great vessel size and LV size and function are summarized in Table 1.

Table 2 summarizes the comparison of echocardiographic variables of LA size and mechanical function and basic variables of great vessel dimensions and LV size and function in healthy horses and horses with various degrees of MR and AR. Several indices of LA size, including LAD_{max} (500), LAD_{max}/AAD, LAA_{max} (500), LAA_{max}/AAD^2, $LA_{sx}A_{max}$ (500), and $LAD_{llx\text{-}max}$ (500) were significantly higher in horses with moderate and severe valvular regurgitation. Conversely, with the exception of passive LA FAC in horses with severe AR, the indices of LA mechanical function (ie, active LA FAC, LA RI, active:total LA AC, and A_m) were not significantly altered in horses with mitral and aortic regurgitation.

Table 3 lists the proportions (percentages) of horses in which different methods of measurement obtained during a single echocardiographic examination revealed discordant results concerning left atrial enlargement. Finally, agreement of different echocardiographic variables used for assessment of LA size is summarized in Figure 1.

Discussion

This study provides support for allometric scaling of echocardiographic variables of LA size and defines reference intervals for a multitude of echocardiographic indices of LA size and function in Warmblood horses. It further provides proof of concept for the use of area-based indices for assessment of LA dimensions in horses.

The results of this study confirm that measurements of LA dimensions are significantly related to BWT. This is in agreement with other studies in horses, demonstrating that cardiac dimensions are affected by body size.[5,6,21–23] Therefore, appropriate correction for differences in BWT is necessary to compare echocardiographic measurements between individuals.[17] Different variants of allometric scaling have been described for dogs,[6,17,24] horses,[6] and foals,[25] overall suggesting that the theoretical assumptions that cardiac volumes are linearly related to BWT, cross-sectional areas are linearly related to $BWT^{2/3}$ (proportional to body surface area), and linear dimensions are linearly related to $BWT^{1/3}$ (proportional to body length) are clinically applicable to correct echocardiographic measurements for differences in BWT. However, some of the approaches are not very practical for daily clinical use. Therefore, we chose an allometric scaling approach that corrects echocardiographic variables to a standard body weight of 500 kg and allows intuitive interpretation of

Table 1. Reference values of healthy Warmblood horses.

Variable	Unit	n	Mean	SD	Lower Limit of Reference Interval (90% CI)	Upper Limit of Reference Interval (90% CI)
Left atrium						
LAD_{max} (500)	cm	31	11.9	0.7	10.5 (10.2–10.8)	13.2 (12.9–13.5)
LAD_{max}/AAD		31	1.9	0.1	1.6 (1.5–1.6)	2.1 (2.1–2.2)
LAA_{max} (500)	cm^2	31	92.8	5.0	82.3 (79.9–84.8)	103.2 (100.5–105.8)
LAA_{max}/AAD^2		30	2.3	0.3	1.7 (1.6–1.9)	2.8 (2.6–2.9)
LAA_a (500)	cm^2	31	71.1	5.6	59.4 (56.7–62.2)	82.8 (79.8–85.7)
LAA_a/AAD^2		31	1.7	0.2	1.3 (1.2–1.4)	2.2 (2.1–2.3)
LAA_{min} (500)	cm^2	29	57.9	3.9	49.9 (48.0–51.9)	66.0 (63.9–68.0)
LAA_{min}/AAD^2		31	1.4	0.2	1.1 (0.9–1.1)	1.8 (1.7–1.9)
Active LA FAC	%	31	20	7	6 (3–9)	33 (30–37)
Passive LA FAC	%	31	23	5	13 (11–16)	33 (31–36)
LA RI	%	30	62	11	39 (34–45)	86 (80–91)
Active:total LA AC		31	0.39	0.12	0.13 (0.08–0.20)	0.65 (0.58–0.71)
$LA_{sx}A_{max}$ (500)	cm^2	31	108.8	12.2	83.5 (77.7–89.6)	134.1 (127.7–140.3)
$LA_{sx}A_{max}/Ao_{sx}A$		31	2.5	0.3	2.0 (1.9–2.1)	3.2 (2.9–3.4)
$LAD_{llx\text{-}max}$ (500)	cm	31	12.9	0.5	11.8 (11.6–12.1)	14.0 (13.7–14.3)
$LAD_{llx\text{-}max}/AAD$		31	2.0	0.1	1.7 (1.7–1.8)	2.3 (2.2–2.4)
Great vessels						
PAD (500)	cm	30	6.5	0.4	5.6 (5.4–5.9)	7.4 (7.2–7.6)
AoD (500)	cm	31	7.6	0.5	6.5 (6.3–6.8)	8.7 (8.4–9.0)
PAD/AoD		31	0.86	0.07	0.71 (0.68–0.75)	1.00 (0.96–1.04)
$PA_{sx}D$ (500)	cm	31	5.0	0.3	4.3 (4.2–4.5)	5.6 (5.4–5.8)
$PA_{sx}D/AoD$		31	0.66	0.06	0.55 (0.53–0.57)	0.79 (0.75–0.84)
AAD (500)	cm	31	6.4	0.4	5.6 (5.4–5.8)	7.2 (7.0–7.4)
$Ao_{sx}A$ (500)	cm^2	31	44.8	5.5	33.5 (30.9–36.2)	56.2 (53.3–59.0)
Left ventricle						
IVS_d (500)	cm	31	3.0	0.3	2.3 (2.2–2.5)	3.7 (3.6–3.9)
$LVFW_d$ (500)	cm	30	2.5	0.3	1.9 (1.8–2.1)	3.1 (3.0–3.3)
$LVID_d$ (500)	cm	31	11.1	0.9	9.3 (8.9–9.7)	12.9 (12.4–13.3)
$LVID_d/AAD$		31	1.7	0.2	1.4 (1.3–1.5)	2.1 (2.0–2.2)
$LAD_{max}/LVID_d$		31	1.1	0.1	0.9 (0.9–1.0)	1.3 (1.2–1.5)
IVS_s (500)	cm	31	4.4	0.4	3.5 (3.3–3.7)	5.2 (5.0–5.5)
$LVFW_s$ (500)	cm	31	4.4	0.4	3.7 (3.5–3.8)	5.1 (5.0–5.3)
$LVID_s$ (500)	cm	31	6.7	0.8	5.0 (4.6–5.4)	8.4 (7.9–8.8)
$LVID_s/AAD$		31	1.1	0.2	0.7 (0.7–0.8)	1.4 (1.3–1.4)
RWT_d	cm	31	0.51	0.05	0.40 (0.37–0.42)	0.62 (0.59–0.65)
MWT_d (500)	cm	31	2.8	0.2	2.4 (2.3–2.5)	3.2 (3.1–3.3)
LV FS	%	31	40	5	30 (28–32)	50 (47–52)
E_m	cm/s	28	33	4	24 (22–26)	41 (39–43)
A_m	cm/s	27	11	2	7 (6–8)	14 (13–15)
E_m/A_m		30	3.1	0.8	1.4 (1.0–1.8)	4.7 (4.3–5.1)

n, number of horses; SD, standard deviation; CI, confidence interval.
For detailed explanation of echocardiographic indices see Appendix 1.

weight-corrected variables.[26,27] The results of this study show that allometric scaling of echocardiographic measurements of LA size in Warmblood horses is effective and eliminates the significant relationship of LA dimensions to body weight. It is important to notice that allometric scaling might not be applicable for use across different equine breeds, particularly when including small breeds and ponies. Whereas further studies are needed to investigate the use of allometric scaling across different breeds, it seems unlikely that it will completely replace the need for breed-specific reference intervals. Another limitation that needs to be considered is the potential impact of body condition. In this study, the horses' height and their body condition score were not considered for allometric scaling. Theoretically, the use of the ideal body weight as opposed to the actual body weight might result in even better correction for differences in BWT. However, the ideal body weight could only be estimated by approximation, which would be an additional source of error.

Except for passive LA FAC and IVS_d, none of the variables of this study is significantly affected by age. In people, advanced age is associated with depressed left atrial passive emptying function and increased left atrial volume, contributing to an increase in atrial ejection force and active atrial stroke volume. This might

Table 2. Echocardiographic variables obtained in healthy Warmblood horses and in Warmblood horses with valvular regurgitation. Significant differences between groups are marked in bold.

Variable	Unit	Healthy (mean ± SD)	Mitral regurgitation (mean ± SD) (*P* value posthoc test)				Aortic regurgitation (mean ± SD) (*P* value posthoc test)			
			P value *F*-test	Trivial-mild	Moderate	Severe	*P* value *F*-test	Trivial-mild	Moderate	Severe
n		31		27	25	3		9	13	14
Age	y	12 ± 4	.79	12 ± 5	12 ± 6	9 ± 6	**< .001**	14 ± 5 **.27**	17 ± 6 **.0014**	20 ± 4 **< .001**
BWT	kg	574 ± 58	.71	590 ± 70	574 ± 57	559 ± 50	.81	583 ± 42	560 ± 69	575 ± 47
HR	min^{-1}	39 ± 6	.17	41 ± 12	38 ± 6	50 ± 20	.40	41 ± 4	39 ± 7	42 ± 6
Left atrium										
LAD_{max} (500)	cm	12 ± 0.65	**< .001**	12 ± 0.94 .97	13 ± 1.1 **< .001**	15 ± 2.2 **< .001**	**.038**	12 ± 0.73 .92	13 ± 0.84 **.015**	12 ± 0.98 .11
LAD_{max}/AAD		1.9 ± 0.14	**< .001**	1.9 ± 0.21 >.99	2.0 ± 0.20 **.046**	2.4 ± 0.62 **< .001**	.75	1.9 ± 0.17	1.9 ± 0.18	1.9 ± 0.19
LAA_{max} (500)	cm^2	93 ± 5.0	**< .001**	93 ± 10 .99	106 ± 16 **.0012**	152 ± 46 **< .001**	.077	93 ± 9.9	101 ± 11	95 ± 15
LAA_{max}/AAD^2		2.3 ± 0.27	**< .001**	2.2 ± 0.40 .99	2.5 ± 0.50 .17	4.1 ± 2.1 **< .001**	.98	2.3 ± 0.38	2.3 ± 0.37	2.2 ± 0.52
Active LA FAC	%	20 ± 6.7	.41	20 ± 7.5	19 ± 11	12 ± 14	.57	17 ± 8.6	21 ± 5.6	19 ± 7.0
Passive LA FAC	%	23 ± 4.8	.054	20 ± 6.3	20 ± 5.7	26 ± 7.6	**.025**	23 ± 7.2 .96	21 ± 3.9 .30	18 ± 7.3 **.0096**
LA RI	%	64 ± 14	.63	58 ± 17	59 ± 24	57 ± 25	.086	58 ± 15	61 ± 12	52 ± 16
Active:total LA AC		0.39 ± 0.12	.33	0.44 ± 0.15	0.40 ± 0.23	0.23 ± 0.26	.21	0.38 ± 0.18	0.45 ± 0.10	0.47 ± 0.16
$LA_{sx}A_{max}$ (500)	cm^2	109 ± 12	**< .001**	110 ± 14 .98	120 ± 15 **.014**	162 ± 36 **< .001**	**.0027**	113 ± 11 .76	123 ± 17 **.0031**	121 ± 11 **.015**
$LA_{sx}A_{max}/Ao_{sx}A$		2.4 ± 0.28	**< .001**	2.4 ± 0.41 .97	2.6 ± 0.36 .22	3.7 ± 1.6 **< .001**	.94	2.4 ± 0.26	2.4 ± 0.42	2.4 ± 0.36
$LAD_{llx\text{-}max}$(500)	cm	13 ± 0.52	**< .001**	13 ± 0.90 .64	14 ± 1.1 **.0041**	15 ± 2.3 **< .001**	**.026**	13 ± 0.64 .99	13 ± 0.81 .35	14 ± 2.2 **.011**
$LAD_{llx\text{-}max}$/AAD		2.0 ± 0.14	**.0055**	2.0 ± 0.22 .99	2.1 ± 0.20 .21	2.4 ± 0.64 **.0033**	.47	2.04 ± 0.19	2.01 ± 0.14	2.1 ± 0.28
Great vessels										
PAD (500)	cm	6.5 ± 0.47	.062	6.5 ± 0.48	6.5 ± 0.47	7.3 ± 0.87	**< .001**	6.8 ± 0.29 .26	6.9 ± 0.42 .076	7.5 ± 0.78 **< .001**
AoD (500)	cm	7.6 ± 0.53	.40	7.6 ± 0.55	7.7 ± 0.49	8.2 ± 0.80	.10	7.6 ± 0.64	8.02 ± 0.45	7.8 ± 0.44
PAD/AoD		0.86 ± 0.070	.61	0.87 ± 0.069	0.86 ± 0.064	0.91 ± 0.20	**< .001**	0.90 ± 0.047 .31	0.86 ± 0.064 .99	0.96 ± 0.086 **< .001**

(continued)

Table 2 (Continued)

Variable	Unit	Healthy (mean ± SD)	Mitral regurgitation (mean ± SD) (*P* value posthoc test)				Aortic regurgitation (mean ± SD) (*P* value posthoc test)			
			P value *F*-test	Trivial-mild	Moderate	Severe	*P* value *F*-test	Trivial-mild	Moderate	Severe
$PA_{sx}D$ (500)	cm	5.0 ± 0.31	**.0014**	5.0 ± 0.53 .86	5.2 ± 0.43 .32	6.2 ± 1.6 **< .001**	**.01**	5.1 ± 0.42 .61	5.3 ± 0.28 .06	5.4 ± 0.80 **.007**
$PA_{sx}D/AoD$		0.66 ± 0.059	.12	0.67 ± 0.077	0.67 ± 0.059	0.77 ± 0.26	.42	0.68 ± 0.082	0.67 ± 0.062	0.70 ± 0.11
AAD (500)	cm	6.4 ± 0.39	.79	6.5 ± 0.50	6.5 ± 0.37	6.3 ± 0.71	.13	6.4 ± 0.57	6.7 ± 0.36	6.7 ± 0.76
$Ao_{sx}A$ (500)	cm^2	45 ± 5.5	.73	46 ± 6.1	46 ± 5.7	47 ± 11	**< .001**	47 ± 5.1 .61	52 ± 6.8 **< .001**	51 ± 5.5 **.005**
Left ventricle										
IVS_d (500)	cm	3.0 ± 0.34	**.036**	2.9 ± 0.29 .46	2.9 ± 0.29 .067	2.6 ± 0.46 .055	.73	3.0 ± 0.32	2.9 ± 0.32	3.0 ± 0.52
$LVFW_d$ (500)	cm	2.5 ± 0.32	.67	2.5 ± 0.26	2.5 ± 0.29	2.4 ± 0.51	.11	2.5 ± 0.30	2.8 ± 0.20	2.6 ± 0.39
$LVID_d$ (500)	cm	11 ± 1	**< .001**	11 ± 0.98 .83	12 ± 0.84 **< .001**	14 ± 2.9 **< .001**	**< .001**	11 ± 0.82 .93	13 ± 0.98 **< .001**	14 ± 2.1 **< .001**
$LVID_d/AAD$		1.7 ± 0.18	**< .001**	1.7 ± 0.20 .99	1.9 ± 0.15 **.028**	2.3 ± 0.70 **< .001**	**< .001**	1.8 ± 0.12 .96	1.9 ± 0.17 .10	2.1 ± 0.36 **< .001**
$LAD_{max}/LVID_d$		1.1 ± 0.10	.90	1.1 ± 0.077	1.1 ± 0.087	1.1 ± 0.077	**< .001**	1.1 ± 0.070 .99	1.0 ± 0.064 .10	0.93 ± 0.12 **< .001**
IVS_s (500)	cm	4.4 ± 0.41	**.0090**	4.1 ± 0.36 **.0055**	4.1 ± 0.45 **.049**	4.0 ± 0.35 .21	.35	4.3 ± 0.39	4.3 ± 0.43	4.6 ± 0.67
$LVFW_s$ (500)	cm	4.4 ± 0.36	**.0060**	4.2 ± 0.27 **.037**	4.2 ± 0.48 **.039**	3.8 ± 0.55 **.017**	.91	4.3 ± 0.45	4.4 ± 0.41	4.3 ± 0.62
$LVID_s$ (500)	cm	6.7 ± 0.81	**< .001**	6.9 ± 0.84 .61	7.5 ± 0.87 **< .001**	8.5 ± 1.4 **.0020**	**< .001**	6.5 ± 0.71 .98	7.8 ± 1.01 **.0083**	8.3 ± 1.8 **< .001**
$LVID_s/AAD$		1.0 ± 0.15	**.001**	1.1 ± 0.15 .92	1.2 ± 0.16 **.014**	1.4 ± 0.32 **.0036**	**.001**	1.0 ± 0.11 .97	1.2 ± 0.13 .083	1.3 ± 0.26 **.0013**
RWT_d	cm	0.51 ± 0.053	**< .001**	0.48 ± 0.060 .32	0.44 ± 0.044 **< .001**	0.37 ± 0.15 **< .001**	**.0031**	0.48 ± 0.072 .74	0.45 ± 0.048 .067	0.42 ± 0.12 **.0013**
MWT_d (500)	cm	2.8 ± 0.19	.053	2.7 ± 0.20	2.7 ± 0.21	2.5 ± 0.44	.70	2.7 ± 0.28	2.8 ± 0.21	2.8 ± 0.41
LV FS	%	40 ± 4.8	.61	39 ± 4.2	38 ± 6.4	40 ± 5.3	.39	42 ± 5.0	38 ± 5.3	40 ± 5.6
E_m	cm/s	33 ± 4.9	.16	31 ± 6.0	34 ± 4.0	35 ± 7.8	**.032**	29 ± 4.7 .13	32 ± 4.7 .68	29 ± 6.9 **.021**
A_m	cm/s	11 ± 2.7	.67	12 ± 3.9	12 ± 3.1	11 ± 3.2	.38	13 ± 3.6	13 ± 3.2	12 ± 4.7
E_m/A_m		3.1 ± 0.80	.53	2.8 ± 1.3	3.0 ± 0.84	3.7 ± 2.01	.32	2.4 ± 0.69	2.7 ± 0.94	2.8 ± 1.6

n, number of horses; BWT, body weight; HR, heart rate.
For detailed explanation of echocardiographic indices see Appendix 1.

Table 3. Proportion (percentage) of horses in which different methods of measurement obtained during a single examination revealed discordant results concerning left atrial enlargement.

Variables indicate normal LA dimensions	Variables indicate LA enlargement	All horses n = 122		Horses with valvular regurgitation n = 91	
LAD_{max} (500)	$LAD_{llx\text{-}max}$ (500)	9/95	9.5%	9/64	14%
	LAA_{max} (500)	8/97	8.2%	8/66	12%
	$LA_{sx}A_{max}$ (500)	3/97	3.1%	3/66	4.5%
$LAD_{llx\text{-}max}$ (500)	LAD_{max} (500)	6/91	6.6%	6/61	9.8%
	LAA_{max} (500)	11/91	12%	11/61	18%
	$LA_{sx}A_{max}$ (500)	3/91	3.3%	3/61	4.9%
LAA_{max} (500)	LAD_{max} (500)	2/83	2.4%	2/52	3.8%
	$LAD_{llx\text{-}max}$ (500)	6/81	7.4%	6/50	12%
	$LA_{sx}A_{max}$ (500)	4/83	4.8%	4/52	7.7%
$LA_{sx}A_{max}$ (500)	LAD_{max} (500)	13/108	12%	13/78	17%
	$LAD_{llx\text{-}max}$ (500)	17/106	16%	17/76	22%
	LAA_{max} (500)	20/108	19%	20/78	26%
LAD_{max} (500) and $LAD_{llx\text{-}max}$ (500)	LAA_{max} (500)	7/85	8.2%	7/55	13%
	$LA_{sx}A_{max}$ (500)	2/85	2.4%	2/55	3.6%
LAA_{max} (500) and $LA_{sx}A_{max}$ (500)	LAD_{max} (500)	1/78	1.3%	1/48	2.1%
	$LAD_{llx\text{-}max}$ (500)	6/78	7.7%	6/48	13%
LAD_{max} (500)	LAD_{max}/AAD	9/97	9.3%	7/66	11%
LAD_{max}/AAD	LAD_{max} (500)	10/94	11%	10/65	15%
$LAD_{llx\text{-}max}$ (500)	$LAD_{llx\text{-}max}/AAD$	1/91	1.1%	1/61	1.6%
$LAD_{llx\text{-}max}/AAD$	$LAD_{llx\text{-}max}$ (500)	15/106	14%	15/75	20%
LAA_{max} (500)	LAA_{max}/AAD^2	4/83	4.8%	3/52	5.8%
LAA_{max}/AAD^2	LAA_{max} (500)	16/98	16%	16/68	24%
$LA_{sx}A_{max}$ (500)	$LA_{sx}A_{max}/Ao_{sx}A$	2/108	1.9%	2/78	2.6%
$LA_{sx}A_{max}/Ao_{sx}A$	$LA_{sx}A_{max}$ (500)	9/109	8.3%	9/78	12%

For detailed explanation of echocardiographic indices see Appendix 1.

represent a compensatory mechanism to increase the atrial contribution of ventricular filling to overcome the normal age-related decrease in LV relaxation.[28–30] The results of this study suggest that these mechanisms might not hold true for normally aging healthy Warmblood horses. It is possible that the range of different ages available in the study population was not wide enough to be able to detect age-related changes in LA and LV size and function. The reference intervals provided in this study (Table 1) should therefore be used with caution for Warmblood horses younger than 6 and older than 19 years, as 30 of the 31 healthy horses included in this study were between 6 and 19 years of age. Also, training status and athletic condition might influence cardiac size and mechanical function of different age groups, with young adults and middle aged-horses being more likely to be in athletic condition compared to adolescent and older horses. The data available for this study did not allow assessing the influence of training and athletic condition on cardiac size and mechanical function, since the training status and athletic condition was not objectively assessed and recorded.

Progressive mitral and aortic regurgitation are associated with LA and LV volume overload, with the degree of chamber enlargement depending on the severity of valvular regurgitation.[31–38] Therefore, even in the absence of a gold standard for assessment of LA size and function, comparison of echocardiographic variables between healthy horses and horses with different severities of valvular regurgitation allows evaluation of the variables' relative clinical value to detect disease-related alterations. Specifically, the results of this study indicate that on a population level all echocardiographic indices of LA size, scaled to a standard BWT of 500 kg, are able to identify significant LA enlargement in horses with moderate and severe mitral regurgitation (Table 2). Left atrial dilation is less consistent in horses with AR and is usually only present in advanced stages of disease.[38] Accordingly, in the groups of horses with moderate and severe AR, LA enlargement is not consistently detected using weight-corrected indices of LA size.

In addition to the allometric scaling of variables to a standard body weight of 500 kg, a second method was applied to correct for different body size by indexing LA dimensions to aortic size.[6,13,24] This was done under the assumption that aortic dimensions are directly related to BWT (which is confirmed by the results of this study) and can serve as an internal reference for body size in lack of an accurate body weight. Because the fibrous aortic annulus is likely to be less affected by alterations in stroke volume, blood pressure, and wall stretch than the more elastic aortic sinus or sino-tubular junction, the aortic annular diameter (AAD), and the short-axis area of the aorta ($Ao_{sx}A$) measured at the level of the valve cusps (close to the aortic annulus), but not the aortic sinus diameter (AoD), were used for indexing. Indeed, the regression analyses revealed that the coefficient of determination is

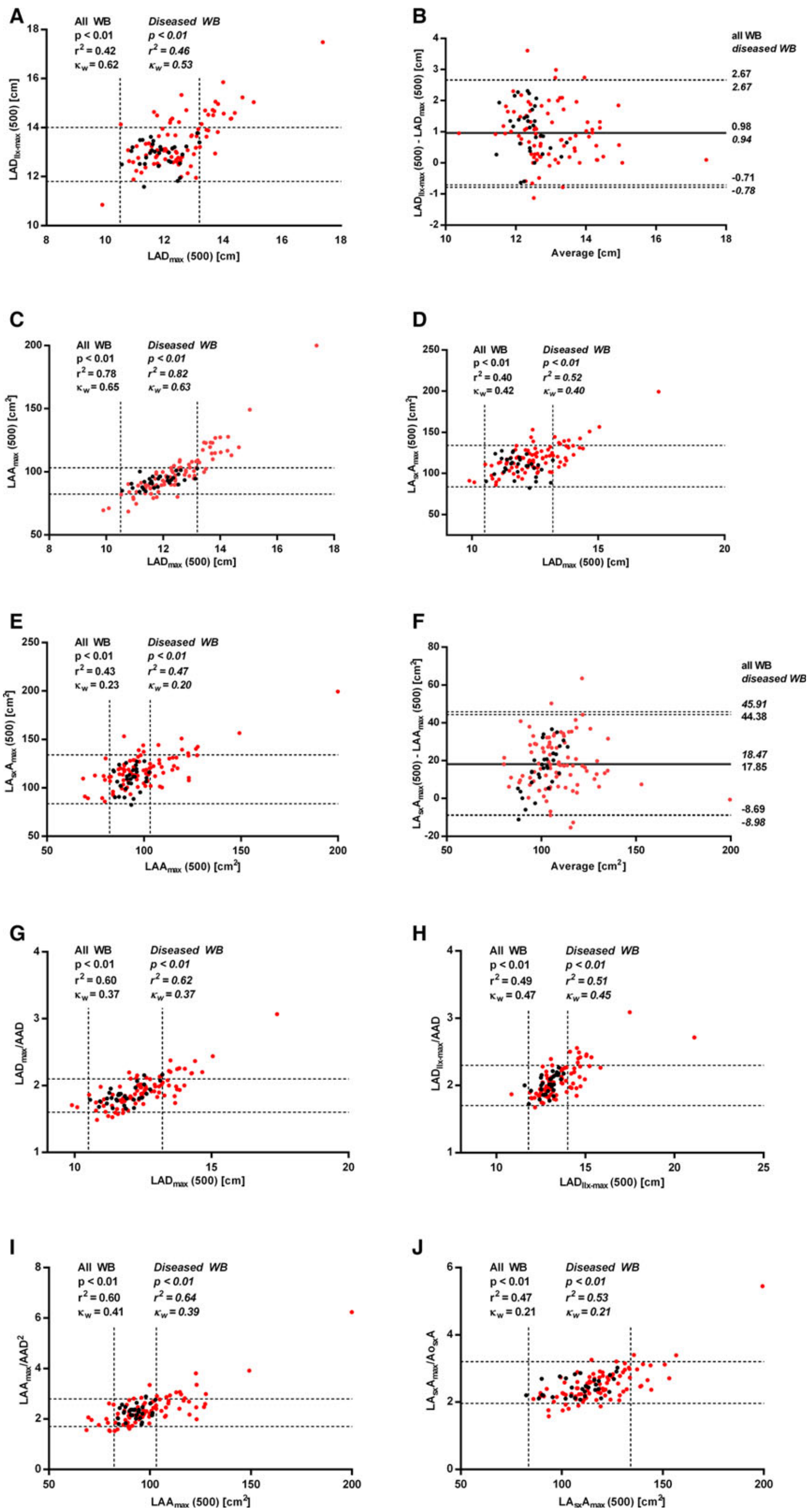

Fig 1. Method agreement for different variables of left atrial size. The black dots indicate the subpopulation of healthy horses and the red dots represent the subpopulation of horses with valvular regurgitation. **A, C-E, G-J:** Linear regression analyses and Kappa statistics. The dotted lines illustrate the reference intervals of the respective indices. **B, F:** Bland–Altman analyses. The solid lines represent the mean bias, the dotted lines indicate the 95% limits of agreement. Numeric results are reported separately for analyses including all (ie, healthy and diseased) Warmblood horses and for analyses including diseased Warmblood horses only. WB, Warmblood; *P*, *P* value of linear regression statistics; r^2, coefficient of determination; κ_w, weighted kappa. For detailed explanation of echocardiographic indices see Appendix 1.

higher for AAD (r^2 = 0.27) and $Ao_{sx}A$ (r^2 = 0.26) than for AoD (r^2 = 0.17), suggesting that AAD and $Ao_{sx}A$ show a stronger relation to BWT and are less influenced by other factors than AoD. However, indexing of dimensional variables to aortic size might not be valid for horses with aortic valve disease, because dilatation of the aortic root is expected in horses with moderate to severe aortic regurgitation.[36] Although there is no significant enlargement of AoD (500) (P = .10) and AAD (500) (P = .13) in horses with AR, $Ao_{sx}A$ (500) indicates aortic root enlargement in horses with moderate and severe AR. Furthermore, the results summarized in Table 2 indicate that LA dimensions indexed to AAD might not be as sensitive to detect LA enlargement as LA dimensions normalized to a standard BWT. This is in agreement with a study in dogs, which revealed that allometrically scaled 2DE measurements of LA size correlate well with measurements obtained by real-time three-dimensional echocardiography, whereas corresponding indexed measurements do not.[24] Therefore, normalization of measurements of LA size to a BWT of 500 kg appears preferable and provides a clinically applicable and intuitive method for weight correction of echocardiographic variables of LA size in horses.

Echocardiographic indices of LA mechanical function have previously been described in horses.[13] They are sufficiently reliable for routine clinical use, allow documenting LA mechanical dysfunction after conversion of atrial fibrillation to sinus rhythm[9,39,40] and could have prognostic implications in horses recovering from atrial fibrillation.[8] However, the use of these indices in horses with MR and AR has not been described. The effects of chronic mitral regurgitation on LA function have been examined in dogs using LA pressure–dimension relationships.[31] The left atrial contribution to LV filling can be augmented as a result of activation of the Frank–Starling mechanism by LA dilation. The LA becomes more compliant and its reservoir function is enhanced, attenuating increases in LA pressure while simultaneously maintaining adequate LV filling volume. However, chronic LA volume overload and chamber dilation might eventually result in reduced LA emptying fraction and LA mechanical dysfunction.[31,41] Hence, both increased and decreased active LA function might be observed in horses with valvular regurgitation and LA volume overload, depending on the stage of disease. In this study, E_m and passive LA FAC are significantly decreased in horses with severe AR, consistent with reduced LA reservoir and conduit function. This likely results from impaired diastolic empting of the LA related to increased LV diastolic pressures and interference of mitral inflow with the aortic regurgitation jet. However, none of the other echocardiographic indices of LA function shows significant alterations with valvular regurgitation on a population level. Therefore, the results of this study are inconclusive with regard to the clinical value of echocardiographic indices of LA mechanical function in horses with MR and AR. The data suggest that the functional response of the LA to valvular regurgitation can be variable in individual horses. However, the population size of this study does not allow more detailed subgroup analyses and comprehensive investigation of the influence of different stages or causes of MR and AR on LA mechanical function. One could argue that assessment of active LA contraction by 2DE should be based on measurement of LA area at the time of maximum atrial contraction (determined subjectively),[8,39,40] since at the time of MV closure (which occurs some time after maximum atrial contraction) the LA dimensions have again slightly increased because of pulmonary venous return and beginning LA relaxation. However, measurement of LA dimensions at the time of maximum LA contraction can be difficult in horses with less vigorous or complete lack of active atrial contraction, such as horses with atrial stunning. The standard measurement protocol used in this study included only LA dimensions at the time of MV closure. Certainly, indices of LA function based on measurements of LA area at the time of maximum LA contraction would be different to those reported in this study, but it is currently unclear whether the difference would be clinically relevant. The data available in this study do not allow investigating this difference.

With the lack of a gold standard, this study does not allow quantifying accuracy of the respective variables or proving the superiority of area-based measurements of LA size over unidimensional variables. However, the results indicate that agreement of different indices for detection of abnormal LA size is fair to good for the majority of weight-corrected variables of LA size, with the exception of poor agreement between LAA_{max} (500) and $LA_{sx}A_{max}$ (500). On average, the weight-corrected LA diameter measures approximately 1 cm larger in a left-parasternal compared to a right-parasternal long-axis view (Fig 1B) and the weight-corrected LA area measures approximately 18 cm^2 larger in a right-parasternal short-axis compared to a long-axis view (Fig 1F). Agreement between LA dimensions scaled to a BWT of 500 kg and corresponding dimensions indexed to AAD is fair to good for $LAD_{llx\text{-}max}$ and LAA_{max} (when considering all horses) but poor for LAD_{max}, $LA_{sx}A_{max}$, and LAA_{max} (when considering diseased horses only) (Fig 1G–J). Even for variables with fair to good agreement, the use of different variables may lead to discordant conclusions with regard to the presence of LA enlargement in individual horses (Table 3 and Fig 1). This can likely be explained by inherent measurement variability and by the fact that variables represent different uniplanar or biplanar dimensions of an asymmetrical three-dimensional structure that can enlarge in a multidirectional fashion.[42] Although on a theoretical basis the use of area-based variables might be preferable, the results of this study do not unconditionally support this assumption. Our results however strongly suggest that in addition to subjective assessment of LA size and function, a variety of different variables, including conventional linear measurements and novel area-based measurements, should be jointly considered for diagnosing and documenting LA dilation in horses.

In conclusion, this study defines reference intervals for echocardiographic indices of LA size and function in Warmblood horses and suggests that novel area-based measurements and indices are in fair to good agreement with conventional unidimensional indices of LA size and function. Allometric scaling appears to be an effective and practical method to correct for differences in body size in a population of Warmblood horses. Weight-corrected variables might be preferred to aortic indexing for assessing LA size, particularly in horses with moderate to severe AR. Most of the echocardiographic LA indices are able to identify LA enlargement in horses with mitral and aortic regurgitation. However, various echocardiographic indices can result in different conclusions with regard to identification of LA enlargement, suggesting that assessment of LA dimensions should be based on an integrative approach of subjective evaluation and joint assessment of a combination of multiple measurements and indices. The clinical relevance of echocardiographic assessment of LA mechanical function in horses with mitral or aortic regurgitation remains unclear and needs to be further investigated.

Conflict of Interest Declaration. Dr Colin Schwarzwald is an associate editor of the Journal of Veterinary Internal Medicine. He was not involved in the review of this article.

Off-label Antimicrobial Declaration. Authors declare no off-label use of antimicrobials.

Footnotes

[a] GE Vivid 7 Ultrasound system, GE Healthcare, Glattbrugg, Switzerland

[b] M4S phased array transducer, GE Healthcare, Glattbrugg, Switzerland

[c] EchoPAC, GE Healthcare, Glattbrugg, Switzerland

[d] Microsoft Excel 2010, Microsoft Corporation, Santa Rosa, CA

[e] GraphPad Prism v5.02 for Windows, GraphPad Software, La Jolla, CA

[f] Reference Value Advisor v2.1, National Veterinary School, Toulouse, France

[g] GraphPad QuickCalcs, Online Calculator, www.graphpad.com/quickcalcs, GraphPad Software, La Jolla, CA

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Appendix 1: Echocardiographic variables (see also supporting information Figure S1).[12,13,15,16]

Left atrium (LA)	
2DE, right-parasternal long-axis four chamber view, optimized to image the LA	
LAD_{max} (cm)	Internal left atrial diameter measured at the widest distance parallel to the mitral valve annulus during maximum atrial filling (at end-systole, one frame before mitral valve opening)
LAD_{max}/AAD	LAD_{max}-to-AAD ratio
LAA_{max} (cm^2)	Internal left atrial area measured during maximum atrial filling (at end-systole, one frame before mitral valve opening)
LAA_{max}/AAD^2	LAA_{max}-to-AAD^2 ratio
LAA_a (cm^2)	Internal left atrial area measured at the onset of active atrial contraction (at the onset of the electrocardiographic P wave)
LAA_a/AAD^2	LAA_a-to-AAD^2 ratio
LAA_{min} (cm^2)	Internal left atrial area measured during minimum atrial filling (at closure of the mitral valve)
LAA_{min}/AAD^2	LAA_{min}-to-AAD^2 ratio
Calculated variables of LA mechanical function	
Active LA FAC (%)	Active fractional area change of the LA [active LA FAC = $(LAA_a - LAA_{min}) / LAA_a \times 100$]
Passive LA FAC (%)	Passive fractional area change of the LA [passive LA FAC = $(LAA_{max} - LAA_a) / LAA_{max} \times 100$]
LA RI (%)	LA reservoir index [LA RI = $(LAA_{max} - LAA_{min}) / LAA_{min} \times 100$]
Active:total LA AC	Ratio of active-to-total LA area change [active:total LA AC = $(LAA_a - LAA_{min})/(LAA_{max} - LAA_{min})$]
2DE, right-parasternal short-axis view of the aorta and the LA, optimized to image the LA and the LA appendage	
$LA_{sx}A_{max}$ (cm^2)	Internal area of the left atrium during maximum atrial filling (at time of aortic valve closure)
$Ao_{sx}A$ (cm^2)	Internal area of the aorta at time of aortic valve closure
$LA_{sx}A_{max}/Ao_{sx}A$	$LA_{sx}A_{max}$-to-$Ao_{sx}A$ ratio
2DE, left-parasternal long-axis view, optimized to image the LA	
$LAD_{llx\text{-}max}$ (cm)	Left atrial diameter measured at the widest distance during maximum atrial filling (at end-systole, one frame before mitral valve opening)
$LAD_{llx\text{-}max}/AAD$	$LAD_{llx\text{-}max}$-to-AAD ratio
Great vessels	
2DE, right-parasternal long-axis right ventricular outflow tract (RVOT) view	
PAD (cm)	Pulmonary artery sinus diameter measured at end-diastole

(continued)

Appendix 1 (Continued)

2DE, right-parasternal long-axis left ventricular outflow tract (LVOT) view	
AoD (cm)	Aortic sinus diameter measured at end-diastole
$PA_{sx}D$ (cm)	Cross-sectional pulmonary artery diameter at end-diastole
AAD (cm)	Aortic annular diameter at peak systole
PAD/AoD	PAD-to-AoD ratio
$PA_{sx}D$/AoD	$PA_{sx}D$-to-AoD ratio
Left ventricle (LV)	
M-mode, right-parasternal short-axis view at the chordal level	
IVS_d, IVS_s (cm)	Interventricular septal thickness at end-diastole and at peak systole
$LVID_d$, $LVID_s$ (cm)	Left ventricular internal diameter at end-diastole and at peak systole
$LVFW_d$, $LVFW_s$ (cm)	Left ventricular free wall thickness at end-diastole and at peak systole
$LVID_d$/AAD	$LVID_d$-to-AAD ratio
$LVID_s$/AAD	$LVID_s$-to-AAD ratio
$LAD_{max}/LVID_d$	LAD_{max}-to-$LVID_d$ ratio
LV FS (%)	Left ventricular fractional shortening [LV FS = $(LVID_d–LVID_s) / LVID_d \times 100$]
Pulsed-wave tissue Doppler imaging, right-parasternal short-axis view at the chordal level, cursor placed on LV free wall	
E_m (cm/s)	Early-diastolic peak radial LV wall motion velocity
A_m (cm/s)	Late-diastolic peak radial LV wall motion velocity
E_m/A_m	E_m-to-A_m ratio

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Overview on image planes and echocardiographic measurements.