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Reference intervals and allometric scaling of two-dimensional echocardiographic measurements in 150 healthy cats

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ABSTRACT. The objective of the study was to evaluate the effects of body weight (BW), breed, and sex on two-dimensional (2D) echocardiographic measures, reference ranges, and prediction intervals using allometrically-scaled data of left atrial (LA) and left ventricular (LV) size and LV wall thickness in healthy cats. Study type was retrospective, observational, and clinical cohort. 150 healthy cats were enrolled and 2D echocardiograms analyzed. LA diameter, LV wall thickness, and LV dimension were quantified using three different imaging views. The effect of BW, breed, sex, age, and interaction (BW*sex) on echocardiographic variables was assessed using univariate and multivariate regression and linear mixed model analysis. Standard (using raw data) and allometrically scaled ($Y=a \times M^b$) reference intervals and prediction intervals were determined. BW had a significant ($P<0.05$) independent effect on 2D variables whereas breed, sex, and age did not. There were clinically relevant differences between reference intervals using mean ± 2SD of raw data and mean and 95% prediction interval of allometrically-scaled variables, most prominent in larger (>6 kg) and smaller (<3 kg) cats. A clinically relevant difference between thickness of the interventricular septum (IVS) and dimension of the LV posterior wall (LVPW) was identified. In conclusion, allometric scaling and BW-based 95% prediction intervals should be preferred over conventional 2D echocardiographic reference intervals in cats, in particular in small and large cats. These results are particularly relevant to screening examinations for feline hypertrophic cardiomyopathy.

KEY WORDS: cardiology, cardiovascular system, cat, diagnosis, echocardiography

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The diagnosis of feline hypertrophic cardiomyopathy (HCM) has been challenging due to diagnostic uncertainties. This is most problematic with regard to screening programs but is also relevant to other cat populations. Echocardiography has been the mainstay in the diagnosis of feline heart disease. However, despite decades of collecting data and trying to define phenotypic normalcy including more than 30 individual echocardiographic studies focussing on reference intervals published between 1979 and 2016, much controversy remains as to what defines a normal left ventricle on an echocardiogram in a cat. Rather arbitrary diagnostic cut-offs defining normal diastolic LV (left ventricular) wall thickness have been used (5.0, 5.5, or 6.0 mm) [3, 4, 7, 8, 12–16, 24, 25, 27, 30], although most veterinary cardiologists agree that end-diastolic LV wall thickness in an average-sized cat is around 4 mm [28]. In the majority of studies on echocardiographic reference intervals in cats, M-mode was used for data acquisition [2, 3, 7, 8, 11, 14, 16, 17, 25, 27, 30, 34]. However, HCM is a heterogeneous disease with asymmetrical LV hypertrophy most commonly observed [10, 12] making M-mode a rather poor choice determining maximum LV wall thickness compared to 2D segmental analysis [15]. Moreover, body weight (BW), a seemingly important consideration when linear echocardiographic measures are used for diagnostic purposes [5, 10, 21, 26], has rarely been considered. Applying fixed, BW-independent reference intervals to all cats seems problematic as erroneous (false negative measures in small cats and false positive measures in large cats) will likely be diagnosed [15]. Scaling (or normalization) removes of the effect of body size on echocardiographic measures, and different methods can be applied [5, 16, 21, 22, 29, 32, 35]. Whereas volumetric echocardiographic variables are linearly related to BW, area indices and linear measures have a non-linear (exponential) relationship to BW, with the latter only relating linearly to body length and the former only relating linearly to body surface area [10, 29]. Therefore, while the effect of BW can be removed by simply dividing the volumetric measure by BW (volume is proportional to $BW^{3/3}$; isometric scaling), area indices can only be BW-corrected by dividing the area measure by $BW^{2/3}$(body

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surface area is proportional to $BW^{2/3}$; allometric scaling), and linear indices can only be normalized by dividing the linear measure by $BW^{1/3}$(body length is proportional to $BW^{1/3}$; allometric scaling). However, based on studies in people [26], horses [32], dogs [5], and cats [16, 33], the scaling exponent (b) for one-dimensional measures can relevantly deviate from its theoretical value of 0.33 ($BW^{1/3}$) among species and specific anatomical structures within one species thus requiring study [5, 10, 16, 21, 26, 29, 33].

Therefore, the objective of this study was to explore the independent effects of BW, breed, and sex on linear 2D echocardiographic variables of left atrial (LA) and LV size and wall thickness in a cohort of healthy cats. We hypothesized that conventional echocardiographic reference intervals would clinically relevantly differ from allometrically-scaled reference intervals and that BW but not breed and sex would independently influence LA and LV echocardiographic dimensions.

MATERIALS AND METHODS

Animals

Medical records of the Veterinary Medical Center at The Ohio State University, Columbus, Ohio from May 2003 until October 2014 were searched for cats with the diagnosis of "Normal cardiovascular findings" that had undergone transthoracic echocardiography. Cats were clinically healthy with a normal physical examination. They were primarily imaged at the request of breeders, due to the presence of a soft heart murmur, peace-of-mind of the client, or as part of a pre-anesthetic exam. Exclusion criteria were presence of heart murmurs >2/6, any disease identified, current administration of medications with a potential effect on the cardiovascular system, dehydration, and poor image quality. If cats had undergone multiple echocardiograms only the last exam meeting inclusion standards was used. Echocardiographic images were inspected and consequently measured. Visual inspection of all cardiac structures including the papillary muscles was the first step to define normality. If in doubt, a maximum end-diastolic LV wall thickness of <6 mm [12] using 2D images and three standard imaging views were required for supportive evidence. Focal segmental wall thickening ≥6 mm or abrupt changes of wall thickness of more than 50% within one segment that was <6 mm was also considered abnormal leading to exclusion. As normal LA dimension in cats in particular cats with low and high BW is poorly defined, the often used diagnostic cut-off measured in the cranial-caudal LA dimension of >16 mm indicating LA enlargement was not used as an independent predictor of disease or abnormality. The majority of healthy cats included were also part of a study on right heart size in cats recently published [35]; however, data from left heart echocardiography have not yet been published.

Echocardiography

Echocardiographic studies and image quantification were done as previously reported [35]. In brief, once cats were identified all echocardiograms were re-analyzed. Images were quantified by one observer (SS) and results reported as the average of three to five measurements. In situations of diagnostic uncertainty, particular variables were not analyzed but were marked as incomplete. All echocardiograms and measurements were subsequently (within three months) reviewed by the principal investigator (KS), evaluated for plausibility and quality, and re-measured as needed. Transthoracic echocardiographic studies were performed using an ultrasound system (Vivid 7 Vantage™ with EchoPAc software version BT06, GE Medical Systems, Waukesha, WI, U.S.A.) with phased-array sector transducers at nominal frequencies of 7 or 10 MHz. Echocardiograms were performed by a board certified cardiologist or resident in training supervised by a cardiologist. Prior sedation with butorphanol (0.15 to 0.20 mg/kg, IM; IVX Animal Health Inc., Miami, FL, U.S.A.) was permitted and not considered a violation of inclusion criteria. Standard echocardiographic imaging views were acquired and 2D, M-mode, spectral Doppler, and pulsed wave tissue Doppler images were recorded as recently reported [31]. A single lead ECG was displayed on the monitor during the entire study. For measurement of the interventricular septum (IVS), the distance from blood-tissue interface to blood-tissue interface was used [20, 37]. Gentle back and forth motion of the image using the trackball of the echocardiographic machine assured identification of false tendons in the region of interest which were avoided. For the LV posterior wall (LVPW), the distance from blood-tissue interface to tissue-pericardial interface was used [17]. The maximum left atrial (Max LAD) cranial-caudal dimension [20, 37] was measured from a right parasternal long-axis 4-chamber view (RPLax, View-1). Maximum end-diastolic dimension of the LV walls (LVPWd and IVSd) and LV dimension (LVDd) were each measured from three imaging views: Right parasternal long-axis (RPLax, View-1), right parasternal short-axis (RPSax, View-2), and left apical 4-chamber (LA4ch, View-3) views [1]. Views and target measurement points as used in all cats are documented in Fig. 1. However, as the location of maximum end-diastolic wall thickness of each segment may occur in a region slightly different from the ones displayed in the figure owing to biological variability, Fig. 1 can only serve as an approximation defining measurement points. Measurement variability was evaluated from repeated analysis of eight randomly selected studies. These studies were measured three times within one week by one observer for determination of intra-observer measurement variability and once by a second observer for evaluation of inter-observer measurement variability. Coefficients of variation (CV) were computed [35].

Data analysis

Analyses were performed with commercially available software (Systat version 13.1, Systat, Chicago, IL, U.S.A. and Graph Pad Prism version 6.07, Graph Pad, San Diego, CA, U.S.A.). Normality of data was evaluated after visual inspection and with the Shapiro-Wilk test. Logarithmic transformation was applied when needed. The Winsorization method was used address outliers [9]. Continuous variables are reported as mean and standard deviation (SD), and categorical data as absolute (number) and frequency (per cent) data.

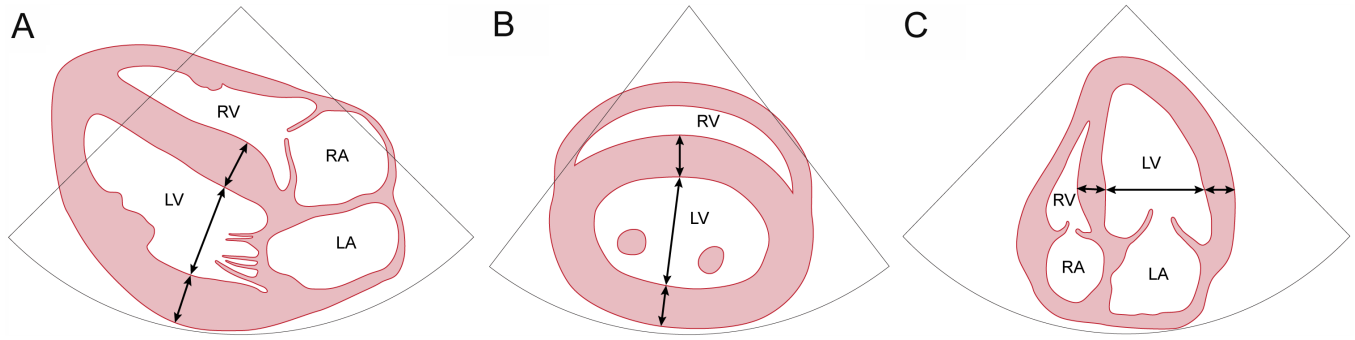

Fig. 1. Artistically rendered images of the three echocardiographic imaging views used for measurements of the interventricular septum and the left ventricular posterior wall. Panel A, right parasternal long-axis view (View-1). Panel B, right parasternal short-axis view (View-2). Panel C, left apical 4-chamber view (View-3). LA, left atrium. RA, right atrium. LV, left ventricle, RV, right ventricle.

Reference intervals were defined by both the conventional method using the mean ± 2 SD [23] and the method of allometric scaling considering the effects of BW on linear echocardiographic measures [5, 21]. For both, mean + 2 SD was considered the upper reference limit (URL), and mean −2 SD the lower reference limit (LRL). Univariate linear regression, multivariate analysis (ANCOVA [GLM] model), and linear mixed model regression were done to evaluate the effects of BW, age, sex, and breed on outcome variables. To explore the potential effect of co-linearity between BW and sex, the interaction term (BW*sex) was added to the general linear model (full model: Y=BW + age + sex + breed + sex*BW). Allometric scaling was used to specifically address the impact of BW on echocardiographic measurements. In brief, the constants and exponents from the logarithmic form of the allometric scaling equation [log (*Y*)=log (*a*) + *b* ×log (BW)] were derived, where *a* is the proportionality constant, *b* is the slope (scaling exponent), and *Y* is the echocardiographic variable and BW is body weight in kg. The 95% prediction interval (PI) was then determined using the following formula:

$$Yc \pm tSx,\, y \sqrt{1 + \frac{1}{n} + \frac{(xi - X)^2}{\sum (xi - X)^2}}$$

Yc is the calculated value of Y for a given value of *Xi* (BW in this case), *t* is the student's *t*-statistics for n-2 degrees of freedom, n is sample size, *Sx, y* is the standard error of the estimate (root mean square error), *X* is the mean, and *xi* is the individual value of *x* [5, 33].

For all analyses, a *P* value of ≤0.05 was considered significant.

RESULTS

A total of 168 cats were identified of which 150 met inclusion criteria. Reasons for exclusion were incomplete echocardiographic data and poor image quality (n=15), a heart murmur >2/6 (n=2), and current administration of medications with potential cardiovascular effects (n=1). There were 82 (55%) females and 68 (45%) males. Mean age was 3.76 years (SD, 3.66) and median age was two years (range three months to 16 years). Seventy nine (52%) cats were <2 years old, 37 (25%) cats were between 2 and 6 years old, and 34 (23%) cats were above six years old. Mean BW was 4.37 kg (SD, 1.28). The BW of 24 (16%) cats was <3 kg, 112 (75%) cats were between 3 and 6 kg, and 14 (9%) cats were above 6 kg. Cats were from 11 different breeds: 57 (38%) domestic cats (Domestic short hair, Domestic medium hair, and Domestic long hair cats), 51 (34%) Bengal cats, 14 Maine Coon cats, 12 Norwegian Forrest cats, six Turkish Angora, four Persian, two each Himalayan, Sphinx, and Ragdoll cats. Ten (7%) cats were sedated. On the day of study, two cats were on a heartworm prevention drug. The remaining 148 cats were not on any medication. None of the cats had visual evidence of LV wall thickening or LA enlargement in any imaging view. However, 26 (17%) had a maximum LA dimension of >16 mm, and six (4%) cats had at least one LV wall segment measured >6 mm. Cats with a maximum LA dimension of >16 mm were 12 Maine Coon cats, 7 Bengal cats, and 7 domestic cats, 16 male and 10 female, and with a higher BW (5.10 ± 1.10 vs. 4.48 ± 1.29 kg, *P*=0.02). Two cats had minimal mitral valve regurgitation whereas 13 (9%) cats had trivial tricuspid regurgitation. One cat had evidence of systolic chordal anterior motion and five cats had evidence of mild dynamic RV outflow tract obstruction (Vmax <2.2 m/s). Mean ± SD heart rate was 184 ± 31 beats/min. Mean ± SD LV shortening fraction was 50% ± 10 (min−max, 30−78).

Results of linear regression analysis of logarithmically transformed echocardiographic variables including the proportionality constants (*a*) and allometric scaling exponents (*b*) are reported in Table 1. In Table 2, echocardiographic reference values after allometric transformation of data are reported. Conventional reference intervals (mean ± 2SD) and BW-adjusted intervals for all variables using allometric scaling [5, 33] and the allometric equation $Y=a \times M^b$ are summarized in Table 3. IVSd of raw data and allometrically scaled data, grouped according to BW, was consistently thicker than LVPWd in all imaging views (Tables 2 and 3; *P*<0.05). More specifically, the difference between the IVSd and LVPWd averaged approximately 0.5 mm in View-1, 0.4 mm in

Table 1. Results of linear regression analysis of logarithmically transformed echocardiographic variables and body weight including the proportionality constants (*a*) and allometric scaling exponents (*b*) from 150 healthy control cats

Echo variable	Imaging view	Log (*a*)	*a*	Prediction interval for *a*		*b*	SE of *Y* est	R^2	*P*
				LRL (2.5%)	URL (97.5%)				
Max LAD	1	1.05	11.23	9.24	13.65	0.177	0.043	0.205	<0.001
IVSd	1	0.57	3.71	2.80	4.93	0.158	0.062	0.090	<0.001
	2	0.57	3.73	2.77	5.01	0.177	0.065	0.101	<0.001
	3	0.55	3.55	2.60	4.87	0.165	0.069	0.071	0.005
LVDd	1	1.03	10.70	8.22	13.93	0.202	0.058	0.157	<0.001
	2	1.00	9.96	7.74	12.82	0.247	0.055	0.234	<0.001
	3	1.09	12.21	9.28	16.07	0.090	0.060	0.029	0.085
LVPWd	1	0.44	2.76	2.17	3.51	0.278	0.053	0.295	<0.001
	2	0.51	3.21	2.59	3.97	0.212	0.047	0.241	<0.001
	3	0.44	2.77	2.17	3.53	0.289	0.053	0.276	<0.001

For linear regression analysis, the logarithmic form of the allometric equation log (*Y*)=log (*a*) + *b* ×log (BW) was used. *Y* represents the echocardiographic variable and BW represents body weight. Regression yields the constant *b*, which represents the slope of the regression line and the constant *a*, which is the antilog ($\log^{-1}$) of the y-intercept of the regression line. Rewritten, the equation can be documented as the allometric equation $Y=a \times M^b$. The prediction intervals (PI, 95% confidence intervals) for *a* were calculated from the formula $PI=\log^{-1}[\log (a) \pm t \times Sx,y]$, and the significance (*P* value) of each individual regression is given. SE of *Y* est, standard error of the *Y* estimate. t, critical value based on the Student's *t*-statistics for n-2 degrees of freedom and an alpha of 0.05. S*x, y* is the standard error of the echocardiographic estimate (*Y*) obtained from the regression model (root mean square error). Imaging view 1, right parasternal long-axis view. Imaging view 2, right parasternal short-axis view. Imaging view 3, left apical 4-chamber view. Max LAD, maximum cranial-caudal dimension of the left atrium. IVS, maximum thickness of the interventricular septum. LVD, maximum internal dimension of the left ventricle. LVPW, maximum thickness of the left ventricular posterior wall. d, measured at end-diastole. All variables were determined from two-dimensional images.

Table 2. Echocardiographic reference values (mean ± SD and 95% reference interval) of left atrial and left ventricular dimensions after allometric transformation of variables in 150 healthy control cats

Variable	Imaging view	Reference interval
Max LAD (mm)	1	14.49 ± 0.72 (13.03–15.95)
IVSd (mm)	1	4.66 ± 0.21 (4.24–5.08)
	2	4.80 ± 0.24 (4.32–5.28)
	3	4.51 ± 0.21 (4.08–4.92)
LVDd (mm)	1	14.33 ± 0.82 (12.68–15.97)
	2	14.23 ± 1.00 (12.24–16.22)
	3	13.90 ± 0.35 (13.19–14.61)
LVPWd (mm)	1	4.12 ± 0.32 (3.47–4.77)
	2	4.36 ± 0.26 (3.83–4.88)
	3	4.21 ± 0.34 (3.52–4.90)

For allometric scaling, the equation $Y=a \times M^b$ was used. *See* Table 1 for remainder of key.

View-2, and 0.3 mm in View-3 with IVSd >LVPWd. Considering raw data only; 75, 73 and 65% of cats had a thicker IVSd compared to LVPWd in Views-1, -2 and -3, respectively.

Univariate analysis identified an effect of BW on all continuous echocardiographic variables in all imaging views (for Max LAD: r=0.44, P<0.001; for IVSd: r between 0.27 and 0.29, all P<0.005; for LVPWd: r between 0.26 and 0.56, all P<0.002; and for LVDd: r between 0.21 and 0.40, all P<0.01). Age had an effect on IVSd measured in View-1 (P=0.037). Sex had an effect on all variables (all P<0.05), except LVDd in View-1 (P=0.67). Breed had an effect on LVDd in all three imaging views (all P<0.018) with LVDd in Bengal cats being larger than in Domestic cats, using raw data. There was no difference of LV wall thickness and Max LAD between Bengal cats and Domestic cats (P>0.20) for all imaging views.

Multivariate analysis including BW, age, sex, breed, and the interaction term BW*sex in the model revealed a consistent effect of BW on all variables (all P<0.05). No effect (all P>0.10) of breed, age, and sex (beyond BW) was observed in the model. Looking specifically at cats which had IVSd >LVPWd using multivariate regression, an effect of increased BW in View-1 (P=0.033) was observed. Prediction intervals using allometrically-scaled data and BW groups are presented in Table 4. For all variables, BW-based values and prediction intervals increased with increasing BW. Results of regression analysis using logarithmically transformed echocardiographic variables including the proportionality constants (*a*) and allometric scaling exponents (*b*) found in Domestic cats and Bengals are reported in Table 5. There were no differences between Bengal and Domestic cats for any variable in any BW group with almost identical BW-based values (all P>0.60).

Intra-observer measurement variability for all variables in all imaging views was between 1.7 and 6.5% with an average of 3.9% for IVSd and 4.2% for LVPWd. Inter-observer measurement variability was between 3.4% and 10.9% with an average of 8.5% for IVSd and 10.9% for LVPWd.

DISCUSSION

The results of this study in 150 healthy cats demonstrate that (1) BW has a significant and clinically relevant effect on linear 2D echocardiographic measures of LA and LV size, (2) allometric scaling eliminates the effect of BW on those measurements, (3) the IVSd measures thicker that the LVPWd in the majority of cats rejecting the use of a fixed diagnostic wall-thickness cut-off, and (4)

Table 3. Echocardiographic reference intervals (mean ± 2SD) and intervals of allometrically scaled data [95% lower bound RI and 95% upper bound RI] of left atrial and left ventricular dimensions in 150 healthy control cats

Variable (mm)	Imaging view	Conventional method All cats (n=150)	Allometric scaling method Cats grouped according to body weight (kg)[a)] ≤3 (n=24)	>3 to ≤4 (n=49)	>4 to ≤5 (n=38)	>5 to ≤6 (n=25)	>6 to ≤7 (n=9)	>7 (n=5)
Max LAD	1	14.45 ± 3.22 (11.23–17.77)	13.37 ± 0.60 (12.76–13.98)	14.09 ± 0.40 (13.69–14.49)	14.65 ± 0.28 (14.37–14.93)	15.20 ± 0.28 (14.92–15.48)	15.64 ± 0.22 (15.42–15.85)	16.12 ± 0.16 (15.96–16.28)
IVSd	1	4.69 ± 1.16 (3.53–5.84)	4.34 ± 0.18 (4.16–4.52)	4.55 ± 0.12 (4.43–4.67)	4.71 ± 0.10 (4.61–4.81)	4.87 ± 0.10 (4.77–4.97)	4.99 ± 0.08 (4.91–5.07)	5.13 ± 0.08 (5.05–5.21)
	2	4.85 ± 1.42 (3.43–6.27)	4.44 ± 0.20 (4.24–4.64)	4.67 ± 0.14 (4.53–4.81)	4.86 ± 0.10 (4.76–4.96)	5.04 ± 0.10 (4.94–5.14)	5.19 ± 0.10 (5.09–5.29)	5.35 ± 0.08 (5.27–5.43)
	3	4.58 ± 1.49 (3.09–6.08)	4.18 ± 0.18 (4.00–4.38)	4.39 ± 0.12 (4.27–4.51)	4.55 ± 0.10 (4.45–4.65)	4.71 ± 0.10 (4.61–4.81)	4.84 ± 0.08 (4.76–4.92)	4.98 ± 0.06 (4.92–5.04)
LVDd	1	14.42 ± 4.16 (10.26–18.58)	13.07 ± 0.68 (12.39–13.75)	13.87 ± 0.46 (13.41–14.33)	14.50 ± 0.32 (14.18–14.82)	15.13 ± 0.32 (14.81–15.45)	15.63 ± 0.24 (15.39–15.87)	16.18 ± 0.18 (16.00–16.36)
	2	14.32 ± 4.24 (10.08–18.56)	12.72 ± 0.80 (11.92–13.52)	13.68 ± 0.54 (13.14–14.22)	14.41 ± 0.38 (14.03–14.79)	15.21 ± 0.58 (14.63–15.79)	15.82 ± 0.30 (15.52–16.12)	16.51 ± 0.22 (16.29–16.73)
	3	14.11 ± 3.86 (10.25–17.97)	13.34 ± 0.32 (13.02–13.66)	13.71 ± 0.20 (13.51–13.91)	13.99 ± 0.14 (13.85–14.13)	14.25 ± 0.14 (14.13–14.39)	14.46 ± 0.10 (14.36–14.56)	14.68 ± 0.08 (14.60–14.76)
LVPWd	1	4.18 ± 1.16 (3.02–5.34)	3.63 ± 0.26 (3.37–3.89)	3.94 ± 0.18 (3.76–4.12)	4.19 ± 0.14 (4.05–4.33)	4.44 ± 0.14 (4.30–4.58)	4.64 ± 0.10 (4.54–4.74)	4.80 ± 0.14 (4.66–4.94)
	2	4.39 ± 1.08 (3.31–5.47)	3.96 ± 0.22 (3.74–4.18)	4.21 ± 0.16 (4.05–4.37)	4.41 ± 0.12 (4.29–4.53)	4.62 ± 0.12 (4.50–4.74)	4.78 ± 0.10 (4.68–4.88)	4.95 ± 0.10 (4.85–5.05)
	3	4.31 ± 1.20 (3.11–5.51)	3.69 ± 0.28 (3.41–3.97)	4.02 ± 0.20 (3.82–4.22)	4.28 ± 0.14 (4.14–4.42)	4.55 ± 0.14 (4.41–4.69)	4.76 ± 0.12 (4.64–4.88)	5.01 ± 0.10 (4.91–5.11)

n, number of observations. For allometric scaling, the equation $Y=a \times M^b$ was used. *See* Table 1 for remainder of key. a) values in parentheses underneath the reference intervals represent the 95% upper and 95% lower bounds.

Table 4. Body weight-based means and 95% prediction intervals of left atrial and left ventricular dimensions and left ventricular wall thickness measurements derived from allometric scaling parameters in 150 healthy control cats

Variable (mm)	Imaging view	Body weight (kg) 2	3	4	5	6	7	8
Max LAD	1	12.7 (10.29–15.52)	13.64 (11.21–16.61)	14.36 (11.80–17.46)	14.93 (12.27–18.17)	15.42 (12.66–18.78)	15.84 (13.00–19.32)	16.23 (13.29–19.81)
IVSd	1	4.14 (3.10–5.54)	4.42 (3.32–5.87)	4.62 (4.48–6.14)	4.79 (3.60–6.36)	4.93 (3.70–6.55)	5.05 (3.79–6.72)	5.16 (3.86–6.88)
	2	4.21 (3.11–5.71)	4.53 (3.36–6.10)	4.76 (3.54–6.41)	4.95 (3.67–6.67)	5.11 (3.79–6.90)	5.25 (3.89–7.10)	5.38 (3.97–7.28)
	3	3.99 (2.87–5.54)	4.26 (3.10–5.86)	4.47 (3.25–6.13)	4.63 (3.37–6.36)	4.77 (3.47–6.57)	4.9 (3.55–6.76)	5.01 (3.62–6.93)
LVDd	1	12.31 (9.40–16.40)	13.37 (10.25–17.44)	14.17 (10.88–18.46)	14.83 (11.38–19.32)	15.38 (11.79–20.07)	15.87 (12.15–20.73)	16.3 (12.45–21.33)
	2	11.83 (9.13–15.33)	13.08 (10.14–16.86)	14.04 (10.90–18.08)	14.83 (11.51–19.11)	15.52 (12.04–20.01)	16.12 (12.48–20.82)	16.66 (12.88–21.55)
	3	13.01 (9.75–17.34)	13.49 (10.20–17.83)	13.84 (10.49–18.25)	12.12 (10.70–18.63)	14.36 (10.86–18.97)	14.56 (10.99–19.28)	14.73 (11.10–19.57)
LVPWd	1	3.34 (2.61–4.29)	3.75 (2.94–4.78)	4.06 (3.19–5.17)	4.32 (3.39–5.51)	4.54 (3.56–5.79)	4.74 (3.71–6.05)	4.92 (3.85–6.29)
	2	3.72 (2.99–4.62)	4.05 (3.27–5.02)	4.31 (3.48–5.33)	4.52 (3.65–5.59)	4.69 (3.79–5.82)	4.85 (3.91–6.02)	4.99 (4.02–6.20)
	3	3.39 (2.63–4.37)	3.81 (2.97–4.88)	4.14 (3.24–5.29)	4.42 (3.45–5.64)	4.65 (3.64–5.96)	4.87 (3.79–6.24)	5.06 (3.93–6.50)

See Table 1 for key.

Table 5. Results of linear regression analysis of logarithmically transformed echocardiographic variables and body weight including the proportionality constants (*a*) and allometric scaling exponents (*b*) from 51 healthy Bengal cats and 57 healthy domestic cats

Echo variable	Imaging view	Breed	*a*	*b*
Max LAD	1	Bengal	11.38	0.179
		Domestic	11.67	0.135
IVDd	1	Bengal	3.65	0.166
		Domestic	4.06	0.107
	2	Bengal	3.78	0.187
		Domestic	3.65	0.193
	3	Bengal	3.82	0.141
		Domestic	3.11	0.248
LVDd	1	Bengal	11.31	0.211
		Domestic	10.34	0.177
	2	Bengal	10.98	0.217
		Domestic	10.28	0.181
	3	Bengal	12.67	0.105
		Domestic	11.82	0.082
LVPWd	1	Bengal	2.65	0.303
		Domestic	2.89	0.247
	2	Bengal	3.15	0.231
		Domestic	3.07	0.243
	3	Bengal	2.66	0.324
		Domestic	2.64	0.307

For linear regression analysis, the logarithmic form of the allometric equation log (*Y*)=log (*a*) + *b* × log (BW) was used. *Y* represents the echocardiographic variable and BW represents body weight. Regression yields the constant *b*, which represents the slope of the regression line and the constant *a*, which is the antilog ($\log^{-1}$) of the y-intercept of the regression line. Rewritten, the equation can be documented as the allometric equation $Y=a \times M^b$. *See* Table 1 for remainder of key.

there is no independent effect of breed and sex on 2D echocardiographic variables. The use of BW-based 95% prediction intervals is recommended when evaluating individual cats, in particular during echocardiographic screening examinations.

To the authors knowledge, only two prior echocardiographic studies in healthy cats have specifically addressed the effect of BW on left heart echocardiographic reference values and applying the principles of allometric scaling to determine 95% prediction intervals [16, 33]. The association between BW and linear echocardiographic variables is well known [29] and has been reported in numerous feline studies [2, 7, 8, 11, 14, 17, 25, 30]. However, due to the relatively small BW of cats ranging from approximately 2 to 10 kg, the impact of BW on echocardiographic measures has often been ignored and considered clinically irrelevant [6, 28]. Rather low coefficients of determination (R^2) between BW and linear echocardiographic measurements of <0.25 have been reported in most studies [3, 7, 14, 16, 25] indicating that factors other than BW contribute to the variability of echocardiographic variables.

Removal of the effect of body size on echocardiographic dimensions is the goal of scaling (or normalization) and facilitates both correct comparisons within and among cohorts, as well as accurate construction of reference standards of normality [5, 29]. Although LA size has routinely been normalized using aortic root dimension (LA:Ao ratio) in cats, scaling of LV wall thickness and LV dimension have largely been ignored. Using different imaging views and 2D echocardiography, we could demonstrate that allometric (non-linear) scaling in cats is feasible with scaling exponents relevantly deviating from values in people [26], foals [32], and dogs [5]. Similar results were recently published in studies of healthy purebred cats using both 2D imaging and M-mode for data acquisition [33] or M-mode only [16]. The latter study [16] included a very large cohort of cats permitting definition of true reference intervals for the population. However, study design in both studies varied markedly, making a direct comparison challenging. In contrast to our study, only purebread cats were studied [16, 33] which do not represent the standard feline population most veterinarians examine in veterinary practice. The age of the cats was lower [16], only M-mode (as opposed to 2D) was used [16] with differences between M-mode measures and 2D measures to be expected [6], inclusion criteria regarding LV wall thickness were different ('normality' was arbitrarily defined *a-priori* by diastolic LV wall thickness <5 mm) [16], studies were performed and measurements generated relatively uncontrolled by a diverse group of specialized and non-specialized examiners in different countries (versus a single center study with all images re-measured in a standardized fashion), and measurement technique was different (leading edge-to-leading edge [16] versus leading edge-to-trailing edge). In addition to a much smaller number of cats included, those differences may explain the higher predicted dimensions and wider prediction intervals found in our study.

Sex was a significant covariate of LV wall thickness in a recent report on healthy Bengal cats [33]. Although its effect was significant and independent of BW, it was rather weak and likely clinically not relevant. An effect of sex on all 2D measures was also observed in our study, using univariate analysis and with similar findings reported elsewhere [3, 8, 25]. However, multivariate

modeling using a general linear model did not confirm such findings. Non-significance of the interaction term (BW*sex) in the regression model (Y=age + sex + breed + BW + sex*BW) demonstrated that the relationship between (Y) and other variables in the model was not different for males and females. Collinearity between BW and sex related to sexual dimorphism is the most likely reason for female/male differences observed using non-adjusted values [17]. As allometric scaling corrects for the effect of BW, sex does not need to be taken into consideration if allometric data and 95% prediction intervals are used.

Variations in echocardiographic variables amongst breeds have been reported and breed-specific reference intervals published [2, 3, 8, 13, 14, 17, 25, 33]. However, it is still unclear whether the breed effect is independent related to true physiological differences or is rather due to the association between breed and body size [2]. Our results indicate that breed is not an independent covariate beyond BW, considering Domestic cats and Bengals for comparison. Despite considerable differences of all 2D variables quantified using univariate analysis and also identified in other studies [2, 3], those differences disappeared after adjusting for BW, age, and sex. Therefore, we conclude that an independent effect of breed on 2D LA and LV echocardiographic variables is likely less relevant than previously assumed. However, studies involving more breeds with a larger number of observations are needed to substantiate this statement.

A rather surprising finding in this study was the difference in mean wall thickness between the IVSd and the LVPWd. IVSd was measured thicker than LVPWd in approximately 75% of cats, leading to a mean difference between segments of 0.3 to 0.5 mm, depending on imaging view. This difference is clinically important. Using a fixed cut-off for diagnostic decision-making would potentially lead to misclassification of cats. However, cardiologists do not commonly consider a differential cut-off when evaluating cats via echocardiography. Although not specifically discussed, the finding of septal thickness being larger than free wall thickness has been documented prior in cats, with differences of 0.2 to 0.7 mm between IVSd and LVPWd in favor of IVSd [2, 3, 14, 24, 33]. No difference was found in other studies, though [8, 13, 17, 25]. Potential reasons for the discrepancy between IVSd and LVPWd could include true differences, systematic measurement or recording error, imaging artifact in the near field, contamination of the region of interest with papillary muscles or chordae tendineae of the right ventricle, and inclusion of false tendons often found in the LV outflow tract in normal cats [15, 18, 19, 38]. However, the presence of false tendons seems rather problematic when M-mode echocardiography is used but is less likely with 2D measurements. We tried to identify and avoid false tendons or insertions thereof sometimes visible as focal bumps or punctual hyperechogenicity although measurement error cannot be excluded with certainty. Increased age can lead to focal dorsal septal thickening in cats, but multivariate analysis did not identify age as an independent variable contributing to the difference between IVSd and LVPWd.

This study has limitations. The number of cats was relatively low, a relevant problem in particular when partitioning cats into BW groups for determination of prediction intervals. A low number of observations affect decision intervals and make them clinically less useful. No definitive test was applied to determine whether or not the cats were free of myocardial disease or had occult HCM. Cats were relatively young with a limited age range covered. Inclusion and exclusion criteria of any study determine the sample population and thus influence the results in terms of their general applicability. Thus, selection bias is unavoidable and may have occurred in this study. We may have rejected normal cats, eliminated equivoval ("diagnostic grey-zone") cats, and included cats that had occult HCM. Therefore, extrapolation of our results to the entire cat population in order to separate 'normal' cats from 'abnormal' cats may be inaccurate. Moreover, physiological variants independent of BW and breed including the anatomy of the papillary muscles, the shape of the LV, and distribution and insertion of false tendons all not specifically considered in this study may have led to misclassification. Image quality was suboptimal in some cats, owing to the retrospective use of previously recorded data. Echocardiographic images were acquired by different observers with different levels of experience and expertise. Body condition score [22] and heart rate [36] were not considered, but are known to influence 2D echocardiographic variables in cats.

In conclusion, allometric scaling and BW-based 95% prediction intervals should be preferred over conventional 2D echocardiographic reference intervals in cats, in particular in small and large cats. The effect of sex and breed on echocardiographic reference intervals is small and thus can likely be ignored. The IVS and LVPW require different reference ranges and prediction intervals for diagnostic evaluation. These results are particularly relevant to screening examinations for feline hypertrophic cardiomyopathy.

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