

Echocardiographic reference intervals for volumetric measurements of the left ventricle using the Simpson's method of discs in 1331 dogs

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Abstract

Background: Echocardiographic measurements play an important role in detecting cardiac enlargement and assessing cardiac function. In human cardiology, M-mode measurements have been widely replaced by volumetric measurements of the left ventricle (LV) using Simpson's method of disc (SMOD). In veterinary cardiology, more large-scale studies are necessary to generate reference intervals (RIs) for SMOD LV volume measurements.

Objective: To generate body size independent RIs for LV volume measurements in dogs.

Animals: Healthy adult dogs (n = 1331) of variable size and somatotype.

Methods: Prospective study. The SMOD was measured from the right parasternal long axis and the left apical 4-chamber view in clinically healthy dogs. The SMOD measurements were normalized to various allometric scales (kg, kg^{2/3}, or kg^{1/3}). RIs for LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV) using SMOD were estimated as prediction intervals of both a linear and an additive regression model. Additionally, after normalization to body weight, 95% RIs were determined using nonparametric methods with 2.5 and 97.5 percentiles serving as the lower and upper limits. Separate analyses were performed for 120 sighthound breeds and 1211 other breeds.

Results: Echocardiographic LV volumes correlated best with weight in kilograms. The additive model proved to be more flexible and accurate than the other 2 methods to generate RIs. Separate RIs for sighthound and all other breeds are provided.

Abbreviations: 3D, 3-dimensional; A4C view, apical 4-chamber view; ASVCP, American Society for Veterinary Clinical Pathology; BW, body weight in kg; BSA, body surface area; DCM, dilated cardiomyopathy; LV, left ventricle; LVEDV, left ventricular end-diastolic volume; LVEDV-I, left ventricular end-diastolic volume indexed to body weight (kg); LVESV, left ventricular end-systolic volume; LVESV-I, left ventricular end-systolic volume indexed to body weight (kg); PI, prediction intervals; PLAX, parasternal long axis; RI, reference interval; SMOD, Simpson's method of discs; UKC, United Kennel Club.

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Conclusions and Clinical Importance: Body size and breed-independent RIs for LV volume measurements using SMOD were generated prospectively from a large and diverse population of dogs and are available for clinical use.

KEYWORDS

canine, echocardiography, heart dimensions, reference values, volumetric measurements

1 | INTRODUCTION

Echocardiographic measurements of the left ventricle (LV) are important to evaluate the development and progression of heart disease. Mitral valve degeneration and dilated cardiomyopathy (DCM) are the most common acquired heart diseases in dogs, both leading to changes in the diastolic and systolic volumes of the LV as the disease progresses.¹⁻³ Linear measurements have their limitations, and M-mode-derived parameters that calculate cardiac volumes, such as the Teichholz formula, may be inaccurate when converting a 1-dimensional linear measurement to a 3-dimensional (3D) volume.^{4,5} The formula assumes the heart to be elliptical,⁶ but heart shape can differ because of the large variety of dog breeds, and also in the presence of disease.⁷ The Teichholz or Quinones methods of calculating LV volumes and ejection fraction (EF) from LV linear dimensions may result in inaccuracies because of the geometric assumptions required to convert a linear measurement to a 3D volume.^{4,8-10} Accordingly, in human medicine, the American Society of Echocardiography no longer recommends linear-based measurements for calculation of LV volumes and EF, but recommends volumetric measurements using the Simpson's method of discs (SMOD) and 3D echocardiography (if available).¹¹ Using SMOD, heart volume is measured as the summation of parallel cylinders, the diameters of which are derived from endocardial border tracing performed on 1 or 2 orthogonal LV apical views. The underlying principle of this method is the calculation of LV volume from the summation of a stack of elliptical discs. The endocardial borders of the LV are traced, and the length of the ventricle is defined by the distance between the mitral annulus and the apex. The ultrasound system divides the area into equally sized elliptical discs and the volume is calculated by summing them.

The volumes calculated by SMOD show good correlation with measurements by 3D echocardiography and computed tomography-derived volume measurements.^{12,13}

The major advantage of the SMOD over M-mode in the detection of early changes in cardiac chamber sizes in Doberman Pinschers affected by DCM has already been demonstrated.¹⁴ It is therefore considered the best clinical practice to detect the echocardiographic changes related to DCM in Doberman Pinschers.^{1,14} Reference intervals (RIs) for SMOD already have been published for several predisposed breeds,¹⁴⁻¹⁷ and in a study using right parasternal images for SMOD measurements in 122 healthy dogs, including many different breeds.¹⁸

Our hypothesis was that, using a large number of different breeds of different sizes and weights, generally applicable RIs from different

imaging planes could be generated, if the measurements obtained could be related to body weight (BW), size, or other allometric scaling. Our aim was to establish RIs that can be used individually for every dog, independent of BW, breed, and size.

2 | MATERIALS AND METHODS

2.1 | Animals

Our study included dogs that were referred for cardiac evaluation or came for screening examinations to the Cardiology Department of the Ludwig-Maximilian University, Munich, or were recruited for the study between 2004 and 2015 and were classified as echocardiographically healthy. The recruited dogs came from students, faculty and by promoting the study at dog shows and breeding associations, and dogs were prospectively included. The echocardiographic examination had to be complete regarding the measurements, and BW needed to be documented. The examination included at least 1 right parasternal long-axis view (PLAX view) and one left apical 4-chamber view (A4C view) and spectral Doppler measurements of the velocity of aortic and pulmonic flow using continuous or pulsed wave Doppler, as well as color Doppler examinations of all valves. Dogs had to be at least 1 year old because previous echocardiographic studies showed differences in wall thickness and LV diameters in growing dogs as compared with adult individuals.¹⁹

A complete medical history was taken, and a thorough clinical examination was performed before the echocardiographic examination was done.

Exclusion criteria were as follows: (a) pathologic heart murmur, gallop sound, or non-sinus arrhythmia; (b) cardiac abnormalities identified on 2D echocardiography, M-mode, and Doppler echocardiographic examinations, such as congenital or acquired cardiac diseases; (c) current or recent evidence of any systemic illness based on history and physical examination; (d) medications known to affect the cardiovascular system; and (e) uncooperative temperament for echocardiography. Trivial valvular insufficiencies identified on color Doppler were acceptable.

2.2 | Echocardiographic examination

Dogs were examined in right and left lateral recumbency without sedation. Echocardiographic examination was performed using a

TABLE 1 Distribution of breeds

Breed	Number of animals	Median weight	Minimum weight	Maximum weight
<u>Afghan Hound</u>	5	32.5	30.4	38.6
Airedale Terrier	2	28.6	27.2	30
Akita	1	43	43	43
Alaskan Malamute	3	40	37	53.1
Australian Cattle Dog	1	27.3	27.3	27.3
Australian Shepherd	34	21	12.8	33
Basenji	1	9.8	9.8	9.8
Basset Hound	2	19.6	18.1	21
Bavarian Mountain Scent Dog	6	20	19	28.4
Beagle	13	14	7	25.8
Bearded Collie	5	20	18.4	25
Belgian Shepherd Dog	4	27.2	23	31.5
Berger de Beauce	1	35	35	35
Bernese Mountain Dog	17	43	34	57.3
Bichon Frise	3	4.6	2.2	12
Bloodhound	1	36	36	36
Bolognese	2	4.6	4.5	4.6
Border Collie	12	20.6	16.8	26.3
Border Terrier	2	7.7	7	8.4
<u>Borzoï</u>	16	33.8	24.2	40.7
Boston Terrier	2	8	6	10
Boxer	26	28	20.7	39.5
Briard	4	36.6	29	43
Brittany Spaniel	1	24	24	24
Bull Terrier	3	22	20.6	25
Bulldog	3	46	37.7	46
Cairn Terrier	2	10.2	8.9	11.4
Cavalier King Charles Spaniel	16	8.3	5.9	11.4
Chesapeake Bay Retriever	1	42.8	42.8	42.8
Chihuahua	27	3.1	1.7	5
Chow Chow	1	29	29	29
Collie Rough	5	26.6	22	44.2
Continental Toy Spaniel	2	4.1	2.7	5.4
Coton de Tulear	1	5	5	5
Dachshund	30	8.1	5.4	13.8
Dalmatian	6	33.5	26	40
<u>Deerhound</u>	2	35.2	33.8	36.5
Doberman Pinscher	26	33.8	25	41.2
Dogue de Bordeaux	2	49.1	40	58.2
Dutch Schapendoes	1	19.6	19.6	19.6
Dutch Shepherd Dog	1	25	25	25
English Cocker Spaniel	20	14.6	9.4	17.2
English Pointer	2	26.4	24.8	28
English Springer Spaniel	1	26.8	26.8	26.8
Entlebucher Sennenhund	6	26.3	15.8	31
Eurasian	1	20.4	20.4	20.4

TABLE 1 (Continued)

Breed	Number of animals	Median weight	Minimum weight	Maximum weight
Field Spaniel	1	13	13	13
Fila Brasileiro	1	43	43	43
Flat Coated Retriever	6	34.9	25.4	39
Foxterrier	1	13	13	13
French Bulldog	19	11.7	7.6	15.6
German Hound	3	26	18.8	30.4
German Hunting Terrier	2	8.8	7.8	9.8
German Longhaired Pointer	3	36	25	38
German Pinscher	2	10.9	6.8	15
German Shepherd Dog	36	32.9	23	42
German Shorthaired Pointer	10	30	21.6	37
German Spitz	8	10.2	8.4	12
German Wirehaired Pointer	7	31	27	35
Golden Retriever	65	32	13.6	46.2
Great Dane	37	64.4	37	86
Great Swiss Mountain Dog	1	57	57	57
<u>Greyhound</u>	14	32	24	43.8
Griffon Vendeen	1	25	25	25
Havanese	2	5.7	5.4	5.9
Hovawart	10	39.9	28.2	47
<u>Hungarian Greyhound</u>	7	22	20	33
Hungarian Pointer	1	30.2	30.2	30.2
Ibizan Podenco	2	18.7	16.5	20.9
Irish Red Setter	5	29	23	35
Irish Soft Coated Wheaten Terrier	1	18.7	18.7	18.7
<u>Irish Wolfhound</u>	17	57.4	42	87.5
Italian Corso Dog	1	48	48	48
<u>Italian Sighthound</u>	4	5.6	4.9	8
Jack Russell Terrier	28	7.6	4.8	12
Kerry Blue Terrier	1	15.9	15.9	15.9
Kuvasz	3	49	39.8	56
Labrador Retriever	66	30.8	20	47.6
Lakeland Terrier	1	13	13	13
Leonberger	9	55.6	50	82.8
Lhasa Apso	2	9	8.2	9.8
Maltese	6	6.4	4.8	8.8
Maremma (and the Abruzzes) Sheepdog	3	53	39.6	68
Mastino Napoletano	1	43	43	43
Miniature Pinscher	8	4.8	4	7.5
Miniature Schnauzer	2	7.1	5.6	8.5
Mixed Breed	337	23	4	65.5
Newfoundland	15	60	45	66
Norwegian Buhund	1	14.2	14.2	14.2
Nova Scotia Duck Tolling Retriever	1	20.6	20.6	20.6
Old English Sheepdog	1	39.6	39.6	39.6
Parson Russell Terrier	2	8.9	7.7	10

(Continues)

TABLE 1 (Continued)

Breed	Number of animals	Median weight	Minimum weight	Maximum weight
Pekingese	1	3.6	3.6	3.6
<u>Polish Greyhound</u>	2	33	31.6	34.3
Polish Lowland Sheepdog	4	17.5	16	22
Poodle	12	7.4	5	14.6
Portuguese Podengo	1	6.6	6.6	6.6
Pug	17	8.1	4.7	12.7
Pumi	1	25	25	25
Pyrenean Sheepdog	1	22.2	22.2	22.2
Rhodesian Ridgeback	10	38.6	27	47
Romagna Water Dog	1	17	17	17
Rottweiler	11	44	33	70
Russian-European Laika	1	27.5	27.5	27.5
<u>Saluki</u>	24	22.7	16	30.5
Samoyed	1	22	22	22
Schnauzer	13	9	6	12
Scottish Terrier	1	11	11	11
Shar Pei	2	21.5	21	22
Shetland Sheepdog	3	5.8	5	8.6
Shiba	2	10.3	8.6	11.9
Shih Tzu	6	7	5	8
Siberian Husky	7	25.1	16	30.8
Skye Terrier	1	16	16	16
<u>Sloughi</u>	5	27.7	22.9	32.3
Small Münsterländer	4	26	22	30
<u>Spanish Greyhound</u>	4	25.4	20	28.3
Spanish Water Dog	1	19	19	19
St. Bernhard	4	57.5	46.9	67
Staffordshire Bull Terrier	3	19.4	18.2	26.8
Tatra Shepherd Dog	1	50.5	50.5	50.5
Tibetan Terrier	2	14.1	12.6	15.5
Weimaraner	1	34	34	34
Welsh Terrier	1	7.6	7.6	7.6
West Highland White Terrier	35	9	5.8	15
<u>Whippet</u>	24	13	10.8	17.7
White Swiss Shepherd Dog	3	41.6	27.6	43
Yorkshire Terrier	44	3.4	1.5	9.2

Note: Sorted by alphabet including mean value and weight span of each breed. Only breeds acknowledged by the FCI are listed, any others are summarized under mixed breeds. Sighthound breeds classified after the FCI group 10 are underlined.

Abbreviation: FCI, Fédération Cynologique Internationale.

2.0/3.5 or 4.0/8.0 MHz transducer and simultaneous ECG recording (Vivid 7 and Vivid 7-dimension, General Electric Medical System, Waukesha, Wisconsin). The complete echocardiographic examination was digitally stored and evaluated afterwards offline (EchoPac Clinical Workstation Software, General Electric Medical System, Waukesha, Wisconsin). Imaging and measurements were carried out by an ACVIM diplomate (cardiology, Gerhard Wess) or cardiology residents

under his supervision. Examination followed the guidelines of the Echocardiography Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine.²⁰

First, the heart was subjectively assessed for volume status and systolic function using 2D image loops. Morphology of the valves and any irregularities were noted before measurements. Aortic and pulmonic flow velocity was measured by continuous or pulsed wave

Doppler and had to be <2 m/s and without hemodynamically relevant turbulence on color-flow Doppler. Color Doppler was also used to screen for congenital or acquired cardiac diseases.

M-mode measurements were obtained from the PLAX or LV short-axis view, and dogs classified as having normal cardiac measurements according to breed-specific RIs were included.^{14-17,21-45} If no breed-specific RIs were available, previously published RIs were used as supportive information about normal cardiac size, but these investigators based the assessment of normality on the interpretation of their results at their own discretion, and by systematically excluding all possible cardiac abnormalities.⁴⁶ To avoid that the new reference values would be established based on a 95% prediction interval (PI), the study population was completed by another 5% of dogs that were subjectively healthy and had just slight deviations above or below the previously published PIs.⁴⁶

For the SMOD measurements, echo loops with at least 3 consecutive cardiac cycles were chosen, and the LV had to be completely displayed. The LV volume determined by SMOD was measured either directly or offline afterwards in the PLAX view and in the A4C view,

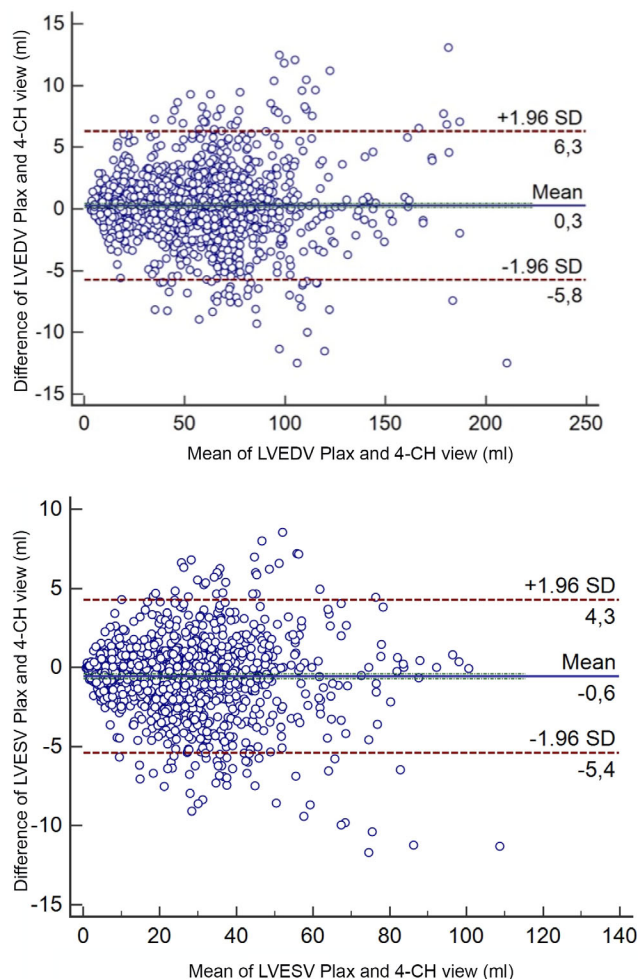


FIGURE 1 Bland-Altman graphs showing good correlation between LVEDV and LVESV obtained from the right parasternal long-axis view and left apical 4-chamber view. LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume

with the aorta not being visible in either view, by tracing the endocardial border on each selected image. The frame used to measure the LV end-diastolic volume (LVEDV) was selected from the frames around the onset of the QRS complex, when the mitral valve was closed and the volume at its largest (which might not have been exactly at onset of the QRS complex) and the frame used to measure the LV end systolic volume (LVESV) was selected as the last frame

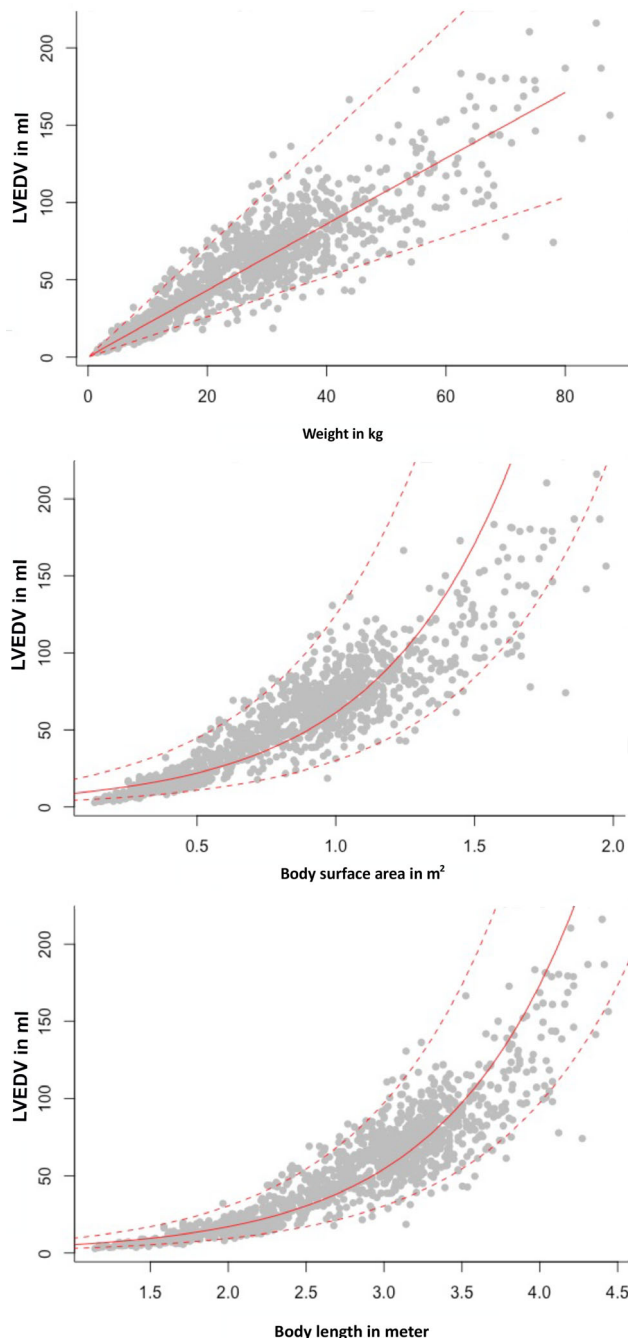


FIGURE 2 Linear regression model of LVEDV with the scaling parameters such as body weight in kilograms, body surface area in square meters ($\text{kg}^{2/3}$), and body length in meters ($\text{kg}^{1/3}$). LVEDV, left ventricular end-diastolic volume

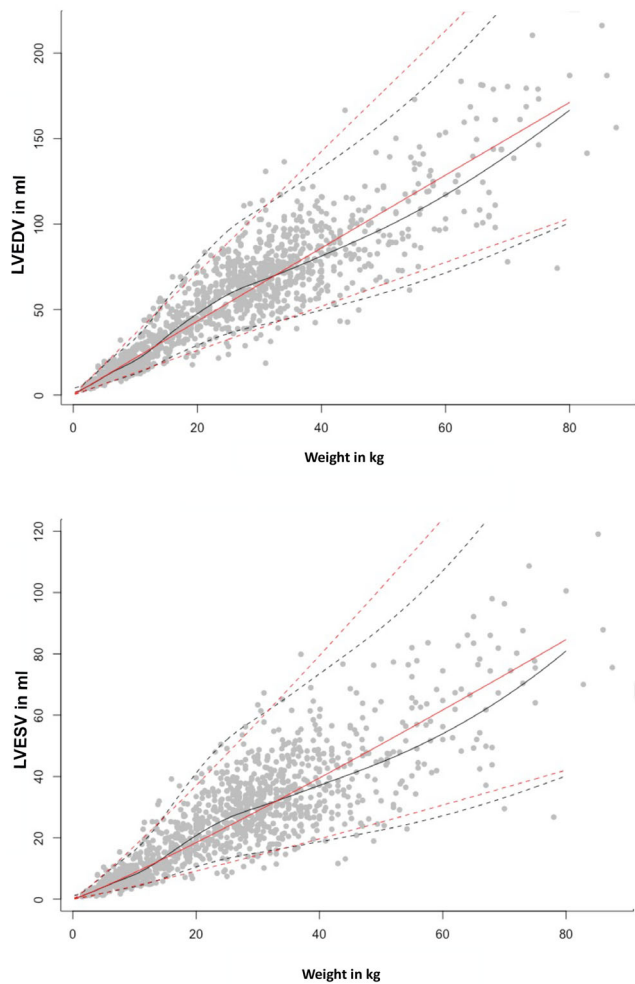


FIGURE 3 Comparison between the linear regression (red lines) and the additive model (black lines) for LVEDV and LVESV. Upper and lower prediction intervals are marked with dotted lines, the median is displayed as a continuous line. Gray dots are representing the study population. LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume

before mitral valve opening, typically after the end of the T wave, where the volume was at its smallest.¹ In the chosen frame, endocardial borders were traced starting from the anterior valve leaflet to the posterior valve leaflet and a line was drawn across the mitral annulus to mark the area of the LV. The maximum length of the LV was defined by the distance between the mitral annulus and the apex. A mathematical algorithm divided this area into equal discs, and LV volume was calculated by the following formula:

$$V = \frac{\pi}{4} \sum_{i=1}^n a_i b_i \frac{L}{n},$$

where n corresponds to the number of discs, L stands for the LV length, and a and b correlate with the diameter of the discs.⁶

The value used to generate reference values was established using the mean of 3 consecutive measurements.

TABLE 2 Prediction intervals (PI) for non-sighthound breeds, estimated as 95% PIs for left ventricular end-diastolic and end-systolic volumes in milliliters, measured by Simpson method of discs

Body weight	Diastole Lower PI	Upper PI	Systole Lower PI	Upper PI
1	1.4	4.7	0.4	1.5
2	2.8	7.2	0.9	2.3
3	4.0	10.1	1.4	3.5
4	5.4	13.3	1.9	4.8
5	6.8	16.8	2.5	6.2
6	8.1	20.1	3.1	7.6
7	9.3	23.1	3.6	9.0
8	10.4	25.8	4.1	10.1
9	11.4	28.2	4.4	11.0
10	12.4	30.8	4.8	11.8
11	13.6	33.8	5.2	13.0
12	15.0	37.3	5.8	14.4
13	16.6	41.2	6.5	16.2
14	18.4	45.5	7.3	18.2
15	20.2	50.1	8.2	20.3
16	22.0	54.6	9.1	22.5
17	23.8	59.0	9.9	24.7
18	25.6	63.4	10.8	26.9
19	27.3	67.6	11.7	29.1
20	29.0	71.8	12.5	31.2
22.5	32.9	81.4	14.4	35.8
25	36.2	89.6	16.0	39.7
27.5	38.8	96.1	17.3	42.9
30	41.1	101.6	18.4	45.6
32.5	43.3	107.1	19.5	48.2
35	45.5	112.6	20.5	50.8
37.5	47.8	118.3	21.5	53.3
40	50.1	124.0	22.5	56.0
42.5	52.4	129.9	23.6	58.7
45	54.8	135.8	24.8	61.5
47.5	57.3	142.0	26.0	64.5
50	59.9	148.5	27.2	67.8
52.5	62.7	155.4	28.6	71.3
55	65.6	162.8	30.1	75.0
57.5	68.7	170.7	31.6	79.0
60	72.1	179.0	33.3	83.3
62.5	75.6	187.9	35.1	87.8
65	79.3	197.4	37.0	92.7
67.5	83.3	207.4	39.0	98.0
70	87.4	218.1	41.1	103.6
72.5	91.7	229.4	43.2	109.7
75	96.1	241.6	45.4	116.3
77.5	100.6	254.6	47.6	123.6
80	105.2	268.6	49.8	131.6

Note: Assorted by weight in kilograms.

TABLE 3 Prediction intervals (PI) for sighthound breeds, estimated as 95% PIs for left ventricular end-diastolic and end-systolic volumes in milliliter, measured by SMOD

Body weight	Diastole Lower PI	Upper PI	Systole Lower PI	Upper PI
1	2.4	12.9	0.9	5.1
2	3.4	14.7	1.3	5.9
3	4.9	16.9	1.9	6.9
4	6.7	19.7	2.7	8.3
5	9.1	23.7	3.8	10.1
6	11.8	29.0	5.0	12.5
7	14.7	35.3	6.3	15.4
8	17.5	41.6	7.6	18.3
9	20.1	47.3	8.9	21.0
10	22.4	52.5	10.0	23.5
12.5	28.3	65.4	12.8	29.8
15	33.8	78.0	15.5	35.9
17.5	38.8	89.5	18.0	41.6
20	43.3	99.9	20.2	46.8
22.5	47.4	109.1	22.3	51.5
25	50.9	117.2	24.2	55.7
27.5	54.1	124.3	25.8	59.5
30	57.2	131.3	27.5	63.1
32.5	60.6	139.2	29.2	67.1
35	64.3	147.7	30.9	71.2
37.5	68.1	156.5	32.7	75.4
40	71.6	165.0	34.4	79.4
42.5	74.7	172.4	35.8	82.9
45	77.4	178.8	37.1	85.9
47.5	79.8	184.5	38.2	88.6
50	82.1	189.9	39.3	91.1
52.5	84.4	195.4	40.3	93.8
55	86.7	201.0	41.4	96.5
57.5	89.0	206.7	42.6	99.3
60	91.4	212.6	43.7	102.3
62.5	93.7	218.7	44.9	105.4
65	96.1	224.9	46.0	108.6
67.5	98.4	231.4	47.2	112.0
70	100.7	238.2	48.3	115.6
72.5	102.9	245.3	49.5	119.4
75	105.1	252.8	50.6	123.5
77.5	107.1	260.7	51.7	127.9
80	109.0	269.2	52.7	132.6

Note: Assorted by weight in kilograms.

Abbreviation: SMOD, Simpson method of disc.

2.3 | Statistical analysis

Statistical analysis was performed using commercially available software (PASW Statistics, Version 25.0.0, IBM Corporation, Armonk, New York;

MedCalc Version 15.6, Ostend, Belgium) and the open-source software R (R Core Team, Vienna, Austria). Following the Guidelines for the Determination of Reference Intervals in Veterinary Species by the American Society of Veterinary Clinical Pathology (ASVCP),⁴⁷ all variables were evaluated for possible outliers by Horn's algorithm using Tukey's interquartile ranges⁴⁸ and, if necessary, excluded. Respective measurements (ie, statistical outliers) only were considered for removal if an obvious measurement error was thought to have occurred.

Intraobserver measurement variability was quantified by calculating coefficients of variation (CV) based on 2 measurements of 20 patients on 2 different days, chosen by an independent statistician. The observer was blinded to the previous measurements. Comparisons between measurements from the right PLAX view and the left A4C view were conducted using a Wilcoxon test. Limits of agreement were analyzed and graphically displayed using a Bland-Altman plot.⁴⁹ Theoretically, volumetric measurements should relate best with BW, but the relationships with body length ($\text{kg}^{1/3}$) and body surface area (BSA; $\text{kg}^{2/3}$) also were calculated as reported in previous studies.⁴⁶

The LV volumetric RIs were estimated using a regression model on the logarithmized LV volume. The logarithmic transformation was performed because the original distribution of the data was substantially skewed. Values were transformed back for calculation of the RIs. To determine if BW linearly influences the response parameter, the model was estimated both as a linear regression model and as an additive model with a potentially nonlinear effect. The nonlinear effect was estimated using a P-spline basis.⁵⁰ The models were of the form:

$$\log(y_i) = \beta_0 + f(\text{weight}_i) + \varepsilon_i,$$

$$\varepsilon_i \stackrel{iid}{\sim} N(0, \sigma^2),$$

where y_i refers to the LV volume of dog i , β_0 in the formula mentioned earlier corresponds to the intercept (the intersection at the ordinate), f corresponds to the linear or smooth effect of weight that is to be estimated, and ε_i refers to the unexplained error for dog i , where the errors are assumed independent and identically distributed (iid). The additive model was estimated using the function *gam* from the R package *mgcv*.⁵⁰ Reference intervals are estimated as 95% PIs, which were calculated as previously described.⁵⁰

For the linear model where $f(\text{weight}_i) = \beta_1 * \text{weight}_i$, 95% PIs for a specific value weight_0 are estimated as follows:

$$(\hat{\beta}_0 + \hat{\beta}_1 * \text{weight}_0) \pm t_{n-2}(0.975) * \hat{\sigma}_{\hat{y}_0},$$

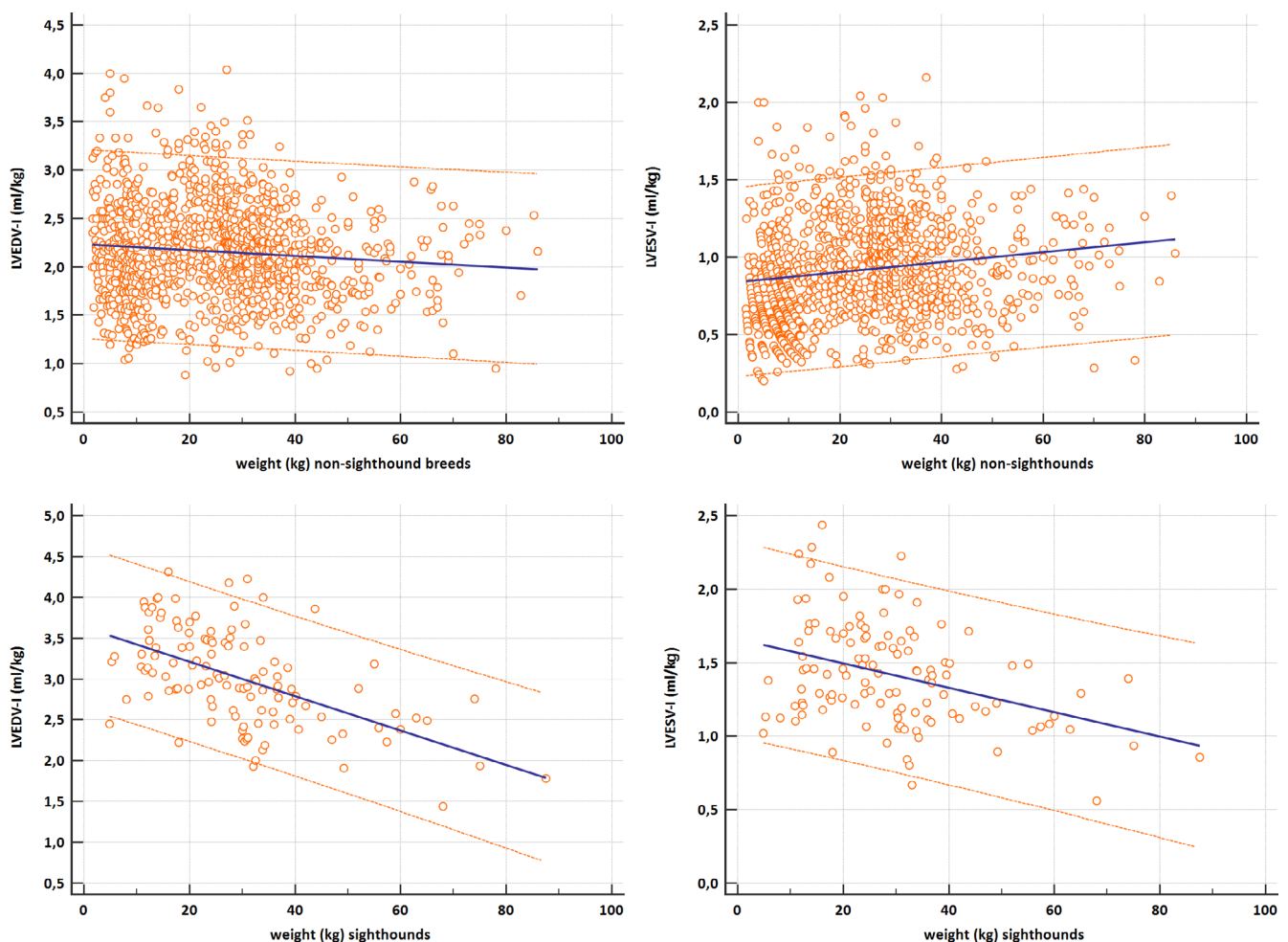
where $\hat{\beta}_0$ and $\hat{\beta}_1$ are the estimated regression coefficients, $t_{n-2}(0.975)$ is the 97.5% quantile of the t -distribution, and $\hat{\sigma}_{\hat{y}_0}$ is the estimated SD of the predicted value as previously described.⁵⁰ Prediction intervals for the nonlinear model were estimated in a corresponding manner.

Comparison between the linear and additive version of the regression model was performed using a likelihood-ratio test.⁵⁰ In all analyses, a P value $< .05$ was considered significant.

TABLE 4 Proposed reference intervals for left ventricular volume measurements normalized to body weight using the Simpson method of discs and ejection fraction in 1211 non-sighthound breeds and 120 sighthound breeds

Variable	Non-sighthound dogs Median	95% reference interval 2.5 centile (90% CI) to 97.5 centile (90% CI)	Sighthound breeds Median	95% reference interval 2.5 centile (90% CI) to 97.5 centile (90% CI)
LVEDV-I (mL/kg) PLAX view	2.15	1.25 (1.20, 1.29) to 3.27 (3.18, 3.33)	3.00	1.92 (1.44, 2.13) to 4.17 (3.98, 4.31)
LVEDV-I (mL/kg) 4-CH view	2.14	1.25 (1.17, 1.30) to 3.21 (3.12, 3.40)	3.04	2.02 (1.88, 2.16) to 4.11 (3.97, 4.25)
LVESV-I (mL/kg) PLAX view	0.88	0.30 (0.27, 0.32) to 1.54 (1.51, 1.56)	1.40	0.72 (0.63, 0.81) to 2.11 (2.01, 2.20)
LVESV-I (mL/kg) 4-CH view	0.95	0.35 (0.32, 0.37) to 1.57 (1.54, 1.59)	1.38	0.82 (0.74, 0.91) to 2.11 (2.02, 2.19)
EF PLAX (%)	58	40.5 (39.8, 41.2) to 75.3 (74.6, 76.1)	53	41.3 (39.8, 42.9) to 65.1 (63.5, 66.7)
EF 4-CH view (%)	56	39.6 (38.9, 40.4) to 71.9 (71.2, 72.7)	52	37.8 (35.9, 39.7) to 65.9 (64.1, 67.9)

Abbreviations: CI, confidence interval; LVEDV-I, left ventricular end-diastolic volume indexed to body weight (kg); LVESV-I left ventricular end-systolic volume indexed to body weight (kg); and PLAX, parasternal long axis.

**FIGURE 4** Scatter diagram with a regression line and 95% prediction interval for the regression curve for LVEDV-I (mL/kg) and LVESV-I (mL/kg) plotted against body weight (BW; kg). The upper graphs are for non-sighthound breeds (n = 1211) and the lower graphs for sighthound breed (n = 120). LVEDV-I, left ventricular end-diastolic volume normalized to BW in kilograms; LVESV-I, left ventricular end-systolic volume normalized to BW in kilograms

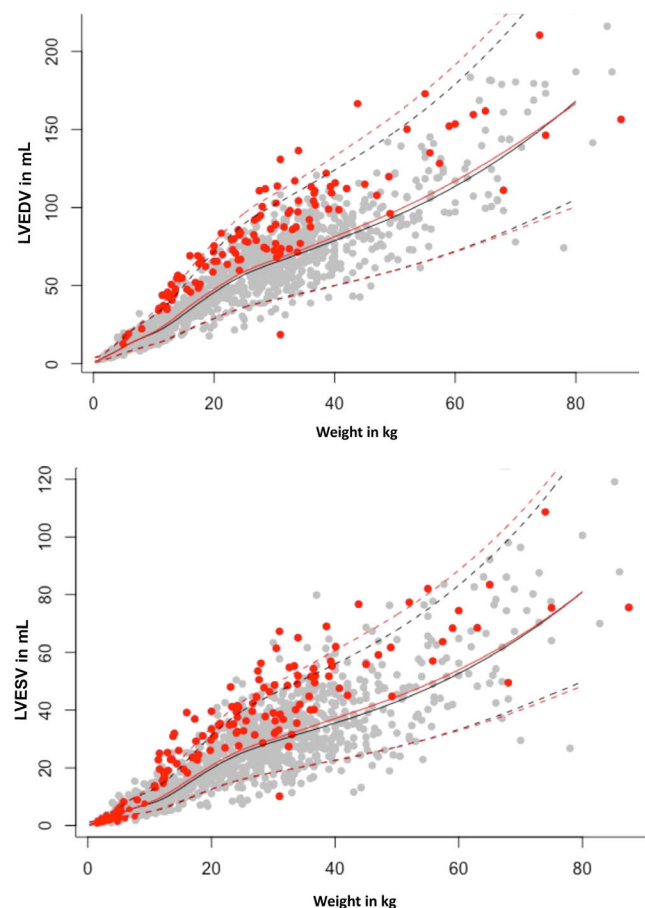


FIGURE 5 Comparison between the diastolic (above) and systolic (below) prediction intervals (PIs) including and excluding sighthound breeds. Prediction intervals including sighthound breeds (red dots) are represented in red lines. Gray dots represent all other dogs, PI without sighthound breeds is displayed in black lines. Dotted lines stand for upper and lower PI, the median is displayed as a continuous line. LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume

Additionally, LVEDV and LVESV measurements first were indexed to BW in kilograms (LVEDV-I, LVESV-I) and then RI values were calculated using the nonparametric percentile method as recommended by the Guidelines for the Determination of Reference Intervals in Veterinary Species by the ASVCP⁴⁷ and the Clinical and Laboratory Standards Institute (CLSI) guidelines for establishing RIs.⁵¹ The 2.5th and 97.5th percentiles were defined as the lower and upper reference limits, respectively. As recommended, 90% confidence intervals (CIs) around these limits also were determined.^{47,51}

3 | RESULTS

A total of 1331 dogs were enrolled in the study. The population consisted of 128 different breeds and many mixed breeds (Table 1). Six-hundred sixty-seven dogs were female (338 spayed) and 654 were male (226 neutered). Age varied between 1 and 16.9 years with a median of 6.16 years. Median weight was 24.7 kg (range,

1.5–87.5 kg). As stated in Section 2, we wanted to avoid including only dogs in the 95% RI of a previous study⁴⁶ or breed-specific RIs, which also were a population of 1263 healthy dogs (based on the reference values previously published). Therefore, 68 dogs (34 dogs each from the 2.5% above and below the reference values) were added to the 1263 dogs to build the study population of 1331 dogs (100%).

After the first analysis, the study population was separated into 2 groups. Group 1 included only dogs belonging to sighthound breeds (120 animals), and group 2 included all other dogs (1211 animals).

Intraobserver measurement variability was good with 3.46% and 8.62% for PLAX view in diastole and systole, and 4.11% and 4.87% for left A4C view in diastole and systole, respectively.

Volumes obtained from PLAX and left A4C views were significantly different (LVEDV, $P = .004$; LVESV, $P < .0001$). However, the mean difference was only 0.2 mL (LVEDV) and 1.2 mL (LVESV). Bland-Altman analysis showed no systematic underestimation or overestimation with a median of 0.3 (LVEDV) and -0.6 (LVESV) and limits of agreement of -5.8 mL to 6.3 mL in diastole and -5.4 mL to 4.3 mL in systole (Figure 1). For further analyses, mean volumes from right parasternal and left apical views were calculated (LVEDV biplane, LVESV biplane) and used to create the RIs. The linear regressions of LVEDV with the scaling parameters such as BW in kilograms, BSA ($\text{kg}^{2/3}$) in square meters, and body length in meters ($\text{kg}^{1/3}$) are displayed in Figure 2; LVESV showed comparable results. The graphical display comparing the linear regression model using the additive model with their PIs in Figure 3 shows that the additive model fits better to the available data, with a slight curvilinear curve, than the linear regression model (Figure 3). The likelihood ratio test showed that the goodness of fit was significantly different ($P < .001$) between both models, confirming that the relationship is nonlinear. Results of the weight-based RI for LVEDV and LVESV obtained by SMOD using the RIs of the additive model are shown in Table 2 for all non-sighthound breeds and Table 3 for sighthound breeds. The RIs for LVEDV-I and LVESV-I, calculated after normalizing the LV volumes to BW, are provided separately for non-sighthound breeds and sighthound breeds, as well as EF for both the right PLAX and the left A4C views in Table 4. The EF exhibited a weak negative correlation with BW (-0.39 ; $P < .001$). In Figure 4, normalized LVEDV-I and LVESV-I values are plotted in a scatter diagram with a regression line and 95% PI for the regression curve against the BW.

The effect of sighthound breeds on the calculation of RIs for LVEDV and LVESV using the additive model is presented in Figure 5. To illustrate the distribution of various breeds within the RI, the data points of Great Danes, Newfoundlands, West Highland White Terriers, and French Bulldogs are shown in Figure 6.

4 | DISCUSSION

Veterinary cardiologists face the problem of dealing with many different breeds and therefore a wide range of different body and BWs. To diagnose cardiac enlargement or, less commonly, to evaluate if a heart is volume underloaded, it is necessary to compare the obtained

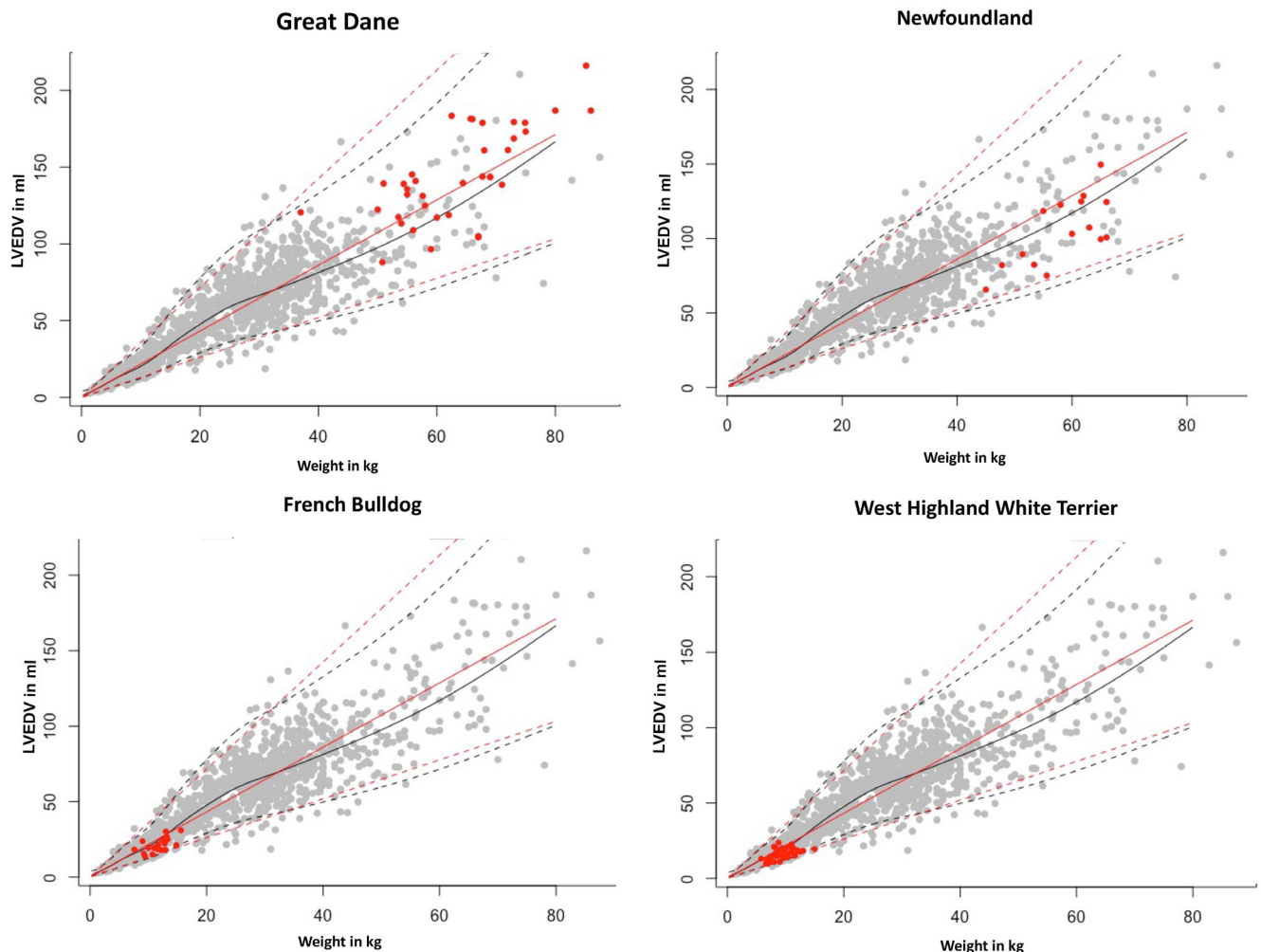


FIGURE 6 Comparison between the prediction interval (PI) for all breeds without sighthounds and selected individual breeds (red dots). The PI is displayed in black lines. LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume

measurements to RIs. The optimal situation would be to have breed-specific RIs, generated from a large healthy population. Because such RIs are not available for many breeds, and there are many mixed breed dogs among patients, RIs generated from a large sample size of dogs of different sizes and breeds are an alternative, but those RIs must be related to a scale basis. If allometric scaling is used to normalize cardiac dimensions to BW, cross-sectional areas should be proportional to BSA (proportional to $BW^{2/3}$), linear dimensions (eg, M-mode) should relate to body length (commonly calculated as $BW^{1/3}$) and volumes theoretically should relate linearly to BW.⁴⁶ Recent studies found better relationships between volumes and BSA, and thus reference values for humans as well as Doberman Pinschers, Boxers, Salukis, and Whippets have been published in mL/m^2 , but the relationship of volumes to BW was almost identical.^{15,16,52} In our study, the volumetric measurements using SMOD related best with BW (Figure 2), and therefore the reference values in our study are presented based on BW (kg). The use of a table with different BWs also makes use in clinical practice much easier, because no normalization to BSA (ie, no calculations) must be done. All studies that found better

correlation of volume to BSA than to BW in kg used single breeds, which might explain why previous studies did not find a better relationship to BW, because they were dealing with a small range of different weights.

Measurements of LV volume are essential in assessing the progression of left-sided heart disease. As discussed earlier, linear methods as well as volumetric methods based on linear measurements carry the risk of too many assumptions.^{4,9} The SMOD is not only the echocardiographic standard method used in human medicine to evaluate EF and LV volume, but also has been recommended as the preferred method for the diagnosis of DCM in the Doberman Pinscher.^{1,14} The superiority of SMOD over the Teichholz method also already has been shown in previous studies.¹⁴

Our study included a wide variety of different breeds and had a large number of enrolled dogs. Not every dog presented at the clinic was enrolled into the study group. Because the clinic has a special interest in the Doberman, Boxer, Whippet, and Saluki breeds, and therefore more dogs of these breeds are presented, the number of dogs enrolled of those particular breeds was decreased to a median

size of $n = 24$ for Whippets and Salukis, and $n = 26$ for Dobermans and Boxers to avoid overrepresentation. Dogs selected for analysis from the overrepresented breeds were selected randomly from the database.

During the echocardiographic examination, the angle of the transducer was optimized to avoid underestimation of the LV volume. In the right PLAX view, the less experienced examiner tends to position the probe too close to the sternum, or not cranial enough, so the LV is not displayed in its full size. Also, the left apical view can be displayed incorrectly when the probe is positioned too far cranial, or too far away from the sternum (ie, the LV might be foreshortened). Imaging was done only by diplomates or by residents under supervision, so that possible mistakes could be immediately corrected. Overlapping of the lungs also should be avoided, otherwise the endocardial borders cannot be traced exactly. To predict exact LV volumes, 2 orthogonal views are recommended in human medicine.⁹ We recommend measuring SMOD using both the right PLAX and the left A4C view and using the larger volume, because overestimation is not possible as long as the aorta is not visualized (ie, the view is a true 4-chamber view).

Linear regression models, as used in previous studies, did not provide satisfactory results in our study.⁵³ Problems detected were negative RIs in the low weight range, when using nonlogarithmized numbers, and an unrealistically wide range in the sector of heavy weight when logarithmizing the measurements to avoid the first problem. The linear model therefore was limited and did not provide enough flexibility. To solve this problem, another model independent of linearity or any other function was used. This additive model was based on dividing the codomain into smaller intervals bordering at the intersections. The effect of weight on the command variable was estimated based on a polynomial function for each interval. The more intervals applied, the more flexible is the global function of the estimated effect. The effect of weight was almost linear but had a curved deviance in the middle sector. To obtain the most exact RIs, more intersections were set up in this sector. The resulting RIs are therefore adapted to the given data in the best possible way. This method to calculate RI therefore has the advantage of adjusting and fitting the RIs better in certain weight classes, which might occur because of body size and breed differences. Estimating RIs based only on the 2.5th and 97.5th percentiles of weight-normalized measurements, as recommended by CLSI⁵¹ and used recently in a veterinary study estimating RIs for SMOD and other parameters,¹⁸ would lead to much simpler RIs (appropriate for all BWs), compared to our model-based intervals. We also performed those calculations (Table 4). This approach however would implicitly make 2 statistical assumptions about the normalized values that do not hold for our data. Both points are visualized in Figure 4, which plots the normalized LVEDV-I and LVESV-I values against BW, for both the non-sighthound dogs and the sighthound breeds. The figure additionally includes a linear regression line from BW on the normalized values and uncertainty intervals of constant size across BW to make the following interpretations easier. First, deriving 1 RI from these normalized values is only reasonable if the normalized values have a comparable range of observed measurements over all BWs. This however is not the case because the

normalized LVEDV-I values get smaller for higher BWs (Figure 4). Second, 1 RI would assume a comparable variation of the normalized values over all BWs. This also is not the case, because the variation is higher for small BW and substantially smaller for higher BWs (Figure 4). Because of these 2 drawbacks, it would not be statistically sound to use this more simplistic method based on the normalized values, because the resulting RIs would not sufficiently describe the variation in the data, but we included them for illustrative and comparative purposes.

The calculated RIs in non-sighthound breeds appear not to be very different among different BWs and might be used clinically as recommended in a previous study,¹⁸ whereas the use in sighthounds is questionable, and RIs calculated from our additive model are more accurate because the model adjusts to the observed variations (Table 3). The normalized RIs (mL/kg) in Table 4 are slightly larger than those of a previous study using 122 normal dogs with an LVEDV-I upper RI of 3.27 mL/kg compared to 2.99 mL/kg¹⁸ and an LVESV-I upper normal RI of 1.54 mL/kg compared to 1.35 mL/kg,¹⁸ with smaller CIs in our study, which might be explained by the much larger population used in our study. The recently published study also used only right parasternal images for SMOD measurements.¹⁸

Earlier studies, which published RIs for specific breeds, found that sighthound breeds, categorized into group 10 by the Fédération Cynologique Internationale following guidelines of the United Kennel Club (UKC), tended to have larger hearts than other breeds. Reference values for M-mode as well as SMOD have been published showing larger RI than those of comparable breeds (height and weight).^{15,36,54,55} The effect of sighthound breeds on the calculation of RIs for LVEDV and LVESV in our study using the additive model (Figure 5) shows that the RIs clearly were smaller when the sighthound breeds were excluded. This observation confirms that sighthound breeds should be excluded or handled separately when RIs are calculated for a general canine population. The “athletic heart” is described in human medicine as a cardiac change in athletes⁵⁶ where athletes had larger LV volumes than the inactive control group.⁵⁷ It is not known if the larger LV volumes in sighthound breeds represent a training effect or are related to genetic background.⁵⁸ To avoid the problem of missing pathologic changes based on an RI that is too wide, subgroups have been established in our study, dividing the study population into sighthounds and other breeds. This approach is recommended by the guidelines of the Quality Assurance and Laboratory Standards Committee if the reference values will have clinical relevance and the literature has already documented differences among the subgroups. This subdivision is only allowed if at least 40 individuals can be assigned to each group.⁴⁷

The reference values of sighthound breeds contain 120 individuals, the group of all other breeds with sighthound breeds excluded consists of 1211 individuals. Thus, the larger volumes of sighthound breeds had no effect on the overall RI. Even in the group of all other breeds, differences among breeds can be noted, showing tendencies to affect RI. French Bulldogs, Newfoundland Dogs, West Highland White Terriers, and Great Danes especially showed strong bias. These differences can be explained by the influence of body shape on the

size and shape of the heart. Deep-chested breeds such as sighthound breeds as well as Great Danes have larger heart volumes than do flat-chested such as French Bulldogs and West Highland White Terriers. Those differences may have their origin in the primary use of the dogs: sighthound breeds and Great Danes were used for hunting, whereas high athletic performance is not expected from a companion dog such as the French Bulldog. Breed-specific RIs still are important for selected breeds, but the numbers of individuals in our study were not high enough to establish such RIs.

Our study had several limitations. The health status was only evaluated by physical examination and echocardiography, and asymptomatic systemic diseases that may have an influence on the echocardiographic findings were not ruled out by additional diagnostic testing. Including dogs deemed to be normal and healthy was based on as many variables as possible (primarily physical examination, history, echocardiography, experience, and subjective impression), but also based on previously published breed-specific RIs and if not available based on previously published PIs that might have biased the results.⁴⁶ Also, potential arrhythmias, such as arrhythmogenic cardiomyopathy, were not further evaluated by Holter examinations.

The examinations were done by >1 cardiologist, but interobserver measurement variability for SMOD measurements has been shown to be low in previous studies. This factor might also be a strength, because the results of the study potentially can be used by other cardiologists.

The RI > 40 kg includes fewer individuals than the lower weight ranges and thus is not so robust. Additional studies including heavier dogs (>40 kg) should be performed, as well as determining breed-specific RIs. Nevertheless, it is still possible to evaluate dogs of higher weight based on the RIs of our study, but individual and breed-specific variances should be considered when interpreting the results.

It has not yet been proven that cardiac dimensions change with age as observed in humans, but age-related variations must be considered.

5 | CONCLUSIONS

Our study provides echocardiographic RIs for SMOD, independent of breed and size and might enable veterinary cardiologist to use this method for all dogs, using the PLAX or A4C view or both. Additional studies must demonstrate the superiority of the SMOD over M-mode in detecting earlier chamber enlargement as is already proven in Doberman Pinschers.¹⁴

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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

The study is within German laws for animal welfare.

HUMAN ETHICS APPROVAL DECLARATION

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

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