Assessment of left atrial function via strain analysis derived from two-dimensional speckle tracking echocardiography in dogs
(2D スペックルトラッキング心エコー図法による ストレイン解析を用いた犬の左心房機能の評価)

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GENERAL ABBREVIATIONS

A	peak velocity of the late diastolic transmitral flow
A’	peak velocity of the late diastolic mitral annular motion
BW	body weight
CV	coefficient of variation
E	peak velocity of the early diastolic transmitral flow
E’	peak velocity of early diastolic mitral annular motion
E/A	ratio of E to A
E/E’	ratio of E to E’
LA	left atrium (atrial)
LA-FAC	left atrial fractional area change
LA-FVC	left atrial fractional volume change
$LA\text{-}FVC_{active}$	left atrial fractional volume change during atrial contraction
$LA\text{-}FVC_{passive}$	left atrial fractional volume change during early ventricular diastole
$LA\text{-}FVC_{total}$	left atrial fractional volume change during ventricular systole
εA	left atrial longitudinal strain during atrial contraction
εE	left atrial longitudinal strain during early ventricular diastole
εS	left atrial longitudinal strain during ventricular systole
LV	left ventricle

PCWP	pulmonary capillary wedge pressure
S’	peak velocity of the systolic mitral annular motion
S_a	strain before atrial contraction
S_{max}	maximum strain
S_{min}	minimum strain
SR	strain rate
SR_a	second negative peak strain rate during atrial contraction
SR_e	first negative peak strain rate during early ventricular diastole
SR_s	positive peak strain rate during ventricular systole
STE	speckle tracking echocardiography
V_{min}	minimal LA volume
V_p	volume at the frame before the P wave
V_{max}	maximal LA volume
2D	two dimensional

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GENERAL INTRODUCTION

Left atrial function is an important role to modulate LV filling through 3 components and maintain an optimal cardiovascular performance.[1,2] During LV systole and isovolumic relaxation, the LA function serves as a reservoir, receiving blood from the pulmonary vein and storing flow in the form of pressure.[1] The LA function as a conduit, during LV diastole and diastasis, blood is transferred into the LV through the LA via pressure gradient during early diastole and flow passively from pulmonary vein into LV during diastasis.[1] During late LV diastole, the LA serves as a pump function which is modulated by LV compliance, LV end-diastolic pressure and LA intrinsic contractility.[1]

In humans, atrial size and function can be assessed with two-dimensional (2D), three-dimensional echocardiography, cardiac computed tomography and magnetic resonance imaging.[2,23] In dogs, assessment of LA dimensions is most often measured from M-mode and 2D echocardiography,[5-13] and commonly performed by the calculation of fractional area change using 2D echocardiography.[9-13] Although various echocardiographic methods have been developed to analyse atrial morphology and function because of its availability, simple, safety, non-invasive tool, advanced echocardiographic techniques are recently proposed in specific clinical instances. LA function can be indirectly assessed using pulsed wave Doppler evaluation of trans-mitral and pulmonary venous flow.[14-17] However, most of LA functional parameters are dependent on a multitude of factors such as preload, afterload, and angle effect.[1]

The use of many Doppler-based diastolic echocardiographic variables, particularly trans-mitral flow and pulmonary venous flow, in dogs with volume load related disease have been shown to be limited in the prediction of congestive heart failure due to preload dependency.[16,17]

A novel echocardiographic technique on the basis of the 2D speckle tracking method enabled automatic analysis of the time-LA area or volume curve representing LA phasic function in humans[18,19] and dogs.[11,12] The LA fractional area change (LA-FAC) during booster pump function obtained via two-dimensional speckle tracking echocardiography (2D-STE) was lower in dogs with progressively more severe myxomatous mitral valvular heart disease.[5,10] However, a previous study has demonstrated that active FAC is increased during volume loading in normal dogs.[20]

More recently, echocardiographic index focused on cardiac deformation (longitudinal strain) has been developed as an alternative method for measurement of myocardial deformation through Doppler-based[21-23] and speckle tracking-derived strain and strain rate (SR) variable.[3-5,7,10,24,25] Based on several studies focused on the use of Tissue Doppler imaging, this technique is angle dependent and is affected by technical artifacts. The analysis of myocardial deformation with 2D and three dimensional speckle tracking overcame the disadvantages of Tissue Doppler imaging technique and improved the reproducibility of strain and SR measurement.[26,27] The principle of the 2D-STE method relies on tracking movement of the myocardial wall throughout the cardiac cycle.[24,25] The 2D-STE allows evaluation of the atrial myocardial deformation expressed as strain, and the rate of deformation expressed as

SR.[23-25] In clinically normal dogs and dogs with underlying heart disease, evaluation of systolic and diastolic left atrial function by the analysis of speckle tracking based technique, have been shown to be feasible and reproducible.[5-7,12,28,29] The STE measurement of LA longitudinal strain has been considered a promising tool for the early detection of impairment of LA compliance in the patients with asymptomatic chronic mitral regurgitation.[3,4] In addition, εA, a measurement of longitudinal strain during booster pump function obtained from 2D-STE, were lower in congestive heart failure dogs than in those without congestive heart failure.[29]

However, the data on repeatability and the reference intervals for LA longitudinal strain and SR using different echocardiographic machines and offline analysis software applications can show different values.[5,28,29] Furthermore, the εA has been described to be a higher predictive power for congestive heart failure than FAC.[10] The development of pulmonary edema and pulmonary venous pressure, resulting in congestive heart failure is influenced by volume load condition that LA volume is known to be influenced by such condition. However, the extent of load dependency of LA strain is not well known in small animals. The difference in preload dependency may contribute to the superiority of LA strain over FAC.

With the above background, the aim of this thesis was to clarify the feasibility of novel 2D-STE technique to assess LA phasic function via strain imaging analysis in dogs. This thesis was focused on two parts (chapter 1 and chapter 2). In the first chapter, we have confirmed the feasibility of LA strain indices in 6 normal dogs and established the normal reference interval of strain indices obtained via 2D-STE in 120 conscious dogs without cardiac dysfunction. In

the second chapter, we have evaluated the impact of acute volume load change on LA strain, SR side by side with LA volume, in dog models

CHAPTER 1

THE REPEATABILITY AND LEFT ATRIAL STRAIN ANALYSIS OBTAINED VIA SPECKLE TRACKING ECHOCARDIOGRAPHY IN HEALTHY DOGS

1. INTRODUCTION

Left atrial function can provide useful information in dogs with cardiac diseases. Recently, there are several studies focusing on the feasibility of measuring LA longitudinal deformation using 2D-STE technique to estimate LA function in healthy dogs and dogs with progressive mitral valvular heart disease. However, the data on repeatability and the reference intervals for LA longitudinal strain and SR using different echocardiographic machines and offline analysis software applications can show different values. In addition, there are few reports indicated the reference values for conventional 2D echocardiographic parameters as well as other 2D-STE derived variables including LA fractional volume change (LA-FVC) in dogs. The reference intervals for these variables are useful to estimate the LA function in clinical setting. Moreover, several echocardiographic indices of cardiac function are considered to be influenced by non-pathological factors such as the patients' age, gender, and body size in both humans[33] and animals,[8,13,30,31] these variables should be also considered when providing normal values.

Therefore, the goal of chapter 1 aimed to determine the reference intervals of 2D-STE-based strain variables for estimating the LA function in healthy dogs, and the relations of these parameters with 2D-STE-derived LA volumetric indices; in addition, the effect of the patients' age, heart rate and body weight (BW) on STE indices were investigated.

2. MATERIALS AND METHODS

2.1 Animals

Six healthy Beagles (2 males and 4 females, aged 1-4 years, with BW of 8.8-12 kg) from the experimental unit of the faculty of veterinary medicine, Hokkaido University were recruited in the study for evaluation of the repeatability of 2D-STE indices. All dogs were deemed healthy based on routine physical examination including blood examination and standard echocardiography. Each dog underwent echocardiography on three different days at afternoon hours. On a given day, each dog was examined thrice by one of two experienced ultrasonographers (KN, TM); and thereafter, thrice by another ultrasonographer. The order in which the ultrasonographer examined the dog was randomized. In each examination, an apical four-chamber cine-loop containing three consecutive cardiac cycles was recorded for off-line analysis. In each examination, 2D-STE indices were determined from the average of three cardiac cycles.

A sample of 120 privately-owned dogs of varying age, breed, and BW, that visited Hokkaido University Veterinary Teaching Hospital between February 2014 and July 2018, were enrolled in the study to determine the reference intervals of 2D-STE indices. The owner's consent was obtained prior to the animal's recruitment in the study. All dogs were judged with normal cardiac function based on previous medical recordings, absence of any suspected sign of cardiac origin, absence of other abnormal structural cardiac defects, and normal physical and cardiovascular examination including ECG and standard echocardiographic examination

(including M-mode, pulsed-wave, and tissue wave Doppler method).[14,15] Exclusion criteria included the presence of abnormal heart sound, other concurrent cardiac abnormalities, and systemic diseases known to affect the cardiac structure and function; in addition, dogs undergoing treatment with cardio-active medication were excluded. Mild physiological tricuspid and pulmonic regurgitation from colour flow Doppler was defined as physiological tricuspid or pulmonic valve regurgitation under condition of silent to auscultation and normal valve morphology;[14] dogs with mild physiological tricuspid and pulmonic regurgitation were considered as normal and not excluded from this finding. In this study, most of the dogs underwent echocardiogram as preanesthetic evaluation for scheduled magnetic resonance imaging, and the remaining, for purpose of medical health examination. Arterial blood pressure was indirectly measured by means of the oscillometric method (PetMAP graphic; Ramsey Medical Inc., Tampa, FL, USA).[32] Dogs were divided into three groups: Toy breed of BW <5 kg, small breed of BW 5-10 kg, and medium to large breed of BW >10 kg.

2.2 Standard Echocardiography

In all privately-owned dogs, standard echocardiography was fully performed by the same experienced ultrasonographer (KN) using echocardiographic system (Artida; Toshiba Medical System Corp., Tochigi, Japan) with 3-6 MHz sector probe transducer array. All unsedated dogs were manually restrained on a table in the left and right lateral recumbency with simultaneous electrocardiographic recording (ECG trace recording lead II) during the examinations.

The 2D-STE and standard echocardiographic indices were determined from the average of three consecutive cardiac cycles.

The left atrial-to-aortic ratio value was obtained at right-sided parasternal short axis view on the first frame after closure of the aortic valve.[33] With regard to 2D-guided M-mode echocardiographic method, the fractional shortening, left ventricular internal dimension at end diastole and left ventricular internal dimension at end systole, left ventricular free wall and interventricular septal thickness in both systole and diastole were measured at right-sided parasternal short axis 2D view at the level of the chordae tendinae.[34] The left ventricular internal dimension for both the end systole and diastole were normalized on the basis of BW using the following formulae:[35]

normalized left ventricular internal diameter end systole = left ventricular internal diameter end systole (cm)/(BW (kg))$^{0.315}$; normalized left ventricular internal diameter end diastole = left ventricular internal diameter end diastole (cm)/(BW (kg))$^{0.294}$.

Based on the mitral flow velocity obtained at the left apical four-chambers' view, the following measurements were obtained: Peak velocity of early diastolic trans-mitral flow (E), peak velocity of late trans-mitral flow (A), and the ratio of peak velocity of early diastolic trans-mitral flow to peak velocity of late trans-mitral flow (E/A) using pulsed-wave Doppler. For the mitral valve septal annular velocity derived from tissue Doppler-based imaging, the peak velocity of systolic (S'), early diastolic (E'), and late diastolic mitral annular motion (A') were recorded. The corresponding ratio of peak velocity of early diastolic mitral annular motion to

peak velocity of late diastolic mitral annular motion (E'/A') and the ratio of peak velocity of early diastolic trans-mitral flow to peak velocity of early diastolic mitral annular motion (E/E') were obtained using tissue-wave Doppler imaging. The images used for strain analysis were individually acquired from the left apical four chambers' view with focusing on the LA by adjusting the depth and frame rate. The frame rate of the clips was between 78 and 288 frames per rate, and three consecutive cardiac cycles in sinus rhythm were digitally stored for subsequent off-line analysis.

2.3 Speckle tracking analysis of the left atrium

The obtained echocardiographic cine loops were analysed using 2D speckle tracking software (2D wall motion tracking software; UltraExtend, V3.10; Toshiba Medical Systems Corp., Tochigi, Japan) by one investigator (AD). The LA longitudinal strain and SR were analysed through 2D-STE using the QRS complex as the initiation of calculation. A line was manually traced along the clearly visualized internal edge of the LA wall using the point and click approach; and the epicardial surface of the LA was automatically generated by the software, thus creating an optimal region of interest, with LA myocardial wall thickness divided into six segments with adjustable width to fit the entire LA myocardial wall throughout the cardiac cycle. After tracking, the software generated the longitudinal strain and SR curve for each atrial segment and the average of all six segmental values (global strain and SR), in each dog. Before processing, a cine loop preview was used to confirm the speckle pattern

movement following generation of the LA endocardium; and adequate imaging quality (without dropout speckle pattern) was visually inspected by the operator prior to inclusion in the study. The direction of the LA endocardial and epicardial surface at the junction of the pulmonary veins and LA appendage requires manual adjustment due to artefacts from tracing the region of interest.[28] In addition, the heart rate was recorded at the STE off-line analysis.

The LA longitudinal strain and SR analysis for each phasic function were measured from the global curve at three different timepoints, as reported in our previous study:[29] Minimum strain at negative peak during the ventricular end-diastolic phase, maximum strain at peak during the ventricular systolic phase, and strain before atrial contraction. The LA longitudinal strain for reservoir function was calculated at the time of ventricular systole (εS), that for conduit function at the time of early ventricular diastole (εE), and that for booster-pump function at the time of late ventricular diastole or atrial contraction (εA) corresponding to the cardiac cycle, using the following equations, as reported in the previous study:

$$\varepsilon S = S_{max} - S_{min}, \quad \varepsilon E = S_{max} - S_{a}, \quad \varepsilon A = S_{a} - S_{min}$$

Similarly, positive peak strain rate during ventricular systole (SR_s), first negative peak strain rate during early ventricular diastole (SR_e), and second negative peak strain rate during atrial contraction (SR_a) were identified at the time of ventricular systole at positive peak, early ventricular diastole at first negative peak, and late diastole or atrial contraction at second negative peak, respectively.

For LA-FVC measurement, the software generated automatic construction of LA volume at three frames of the cardiac cycle: Maximal LA volume, the frame before opening of the

mitral valve starts; pre-atrial contraction LA volume, the frame before the P wave on the ECG; and minimal LA volume, the frame at the mitral valve closure. The total, passive, and active LA emptying FVC were calculated based on the following formulae defining each of three LA phasic functions.

LA-FVC during ventricular systole = 100 x (maximal LA volume – minimal LA volume)/maximal LA volume

LA-FVC during early ventricular diastole = 100 x (maximal LA volume – pre-atrial contraction LA volume)/maximal LA volume

LA-FVC during atrial contraction = 100 x (pre-atrial contraction LA volume – minimal LA volume)/pre-atrial contraction LA volume

2.4 Statistical analysis

Statistical analyses were performed using computer software (JMP Pro, 12.2.0; SAS Institute Inc., Cary, NC, USA). In all statistical tests, $p < 0.05$ was considered as significant value. Normal distribution of data was evaluated by means of Shapiro-Wilk test. For evaluation of repeatability of 2D-STE indices, the intra-day, inter-day, and inter-observer coefficients of variance (CVs) were determined using the following linear model:

$$Y_{ijkl} = \mu + observer_i + day_j + dog_k + (observer\ X\ dog)_{ik} + (day\ X\ dog)_{jk} + \varepsilon_{ijkl}$$

where Y_{ijkl} was the value measured for dog k on day j by observer i; μ, the general mean; $observer_i$, the differential effect (considered as fixed) of observer i; dog_k, the differential effect of dog k; $(observer\ X\ dog)_{ik}$, the interaction term between the observer and dog; (day X dog),

the interaction term between the day and dog; and $\varepsilon_{ijkl,}$ the model error. Standard deviation of the intra-day variability was estimated as the residual standard deviation of the model; standard deviation of the inter-day variability, as the standard deviation of the differential effect of day; and standard deviation of the inter-observer variability, as the standard deviation of the different effect of observer. The corresponding CVs were calculated by dividing each standard deviation by the group mean. The degree of repeatability was identified as follows: CV <5%, very high repeatability; 5–15%, high repeatability; 16–25%, moderate repeatability; or >25%, low repeatability.[36]

Descriptive statistics (median, range, reference intervals, 90% confidence intervals of the reference limits) were determined for the 2D-STE indices. The reference intervals were calculated using a robust method with Box-Cox transformation (Reference Value Advisor (Microsoft, Redmond, WA, USA).[37] The 90% confidence intervals of the reference limits were calculated using bootstrapping method.

Since the assumption of normal distribution was not verified for all data, Spearman's correlation analysis was performed to evaluate the relationship between the 2D-STE indices and the standard echocardiographic indices, BW, age, and heart rate. Multiple linear regression analysis with forward stepwise selection based on Akaike's information criterion was used to elucidate the relationship between the echocardiographic indices and BW, age, and heart rate. Assumptions of linearity, normality, homoscedasticity, and independence of the residuals were evaluated by inspection of the standardized residual plots and quantile plot.

3. RESULTS

3.1 Repeatability study

Intra-day, inter-day, and inter-observer CVs of strains, and SRs derived from 2D-STE are summarized in Table 1. On the basis of CVs, the intra-day, inter-day, and inter-observer repeatability of strains and SRs were high to moderate (CVs of <20%).

3.2 Canine characteristics

The signal characteristics in 120 healthy dogs included in the study are presented in Table 3. The mean arterial blood pressure obtained from dogs was 106 ± 11.6 mmHg. Among the various breeds of dogs, Chihuahua was the most frequently presented breed (n=18), followed by Beagle (n=13), Miniature Dachshund (n=11), Toy Poodle (n=9), Miniature Schnauzer (n=8), Yorkshire Terrier (n=8), mixed breed (n=8), Cavalier King Charles Spaniel (n=6), Pomeranian (n=5), Golden Retriever (n=4), Labrador Retriever (n=3), Shiba (n=3), Pug (n=3), Papillon (n=3), Maltese (n=2), Welsh Corgi (n=2), Italian Greyhound (n=2), Bernese Mountain Dog (n=2), American Cocker Spaniel (n=1), Standard Schnauzer (n=1), Border Collie (n=1), Jack Russell (n=1), French Bulldog (n=1), Bichon Frise (n=1), Akita (n=1), Pekingese (n=1), Brussels Griifon (n=1), and Siberian Husky (n=1). The reference values of the longitudinal strain, SR profile, and LA-FVC using 2D-STE are displayed in Table 2. The data of LA longitudinal strain and SR based on the patients' BW separation are summarized in Table 3. Fifty-seven dogs were classified into the Toy breed group, 40 dogs were classified into the

Small breed group, and 23 dogs were classified into the Medium to Large breed group.

3.3 Normal LA longitudinal strain and SR profiles

In all dogs, the mean LA strain curve presented the first positive peak at ventricular systolic phase, and decreased to a plateau at diastolic phase, followed by the second positive peak at preceding period of atrial contraction, and finally, the negative peak at post-atrial contraction. In all dogs, the second positive peak was of less magnitude than the first positive peak (Fig 1A). The SR profiles showed the first positive peak during ventricular systole (SR_s), and two negative peaks at early (SR_e) and late (SR_a) ventricular diastole (Fig 1B). The SR_e curve was more negative than the SR_a with ratio of SR_e:SR_a >1 in 47 dogs; whereas, in 73 dogs, the SR_a was more negative than the SR_e with ratio of SR_e:SR_a<1.

3.4 Correlation of LA longitudinal strain and SR versus 2D doppler echocardiographic parameters of LV function and LA functional indices

The LA phasic function estimated by LA longitudinal strain was significantly correlated with the parameters of LV diastolic function and LA functional indices of conduit function. The εE represented significant moderate positive correlations with the peak velocity of early diastolic transmitral flow (r=0.5560, p<0.001), and the ratio of peak velocity of early diastolic transmitral flow to peak velocity of late transmitral flow (r=0.5515, p<0.001); and moderate negative correlation with the peak velocity of early diastolic mitral annular motion (r= -0.5370,

p<0.001). The εE showed significant weak negative correlation with the peak velocity of late diastolic mitral annular motion (r= -0.2314, p<0.05) and the peak velocity of late transmitral flow (r= -0.2233, p<0.05). The correlation of the εS, εE, and εA was considered strong, both, and moderate with the LA-FVC_{total} (r=0.7628, p<0.001), LA-$FVC_{passive}$ (r=0.7465, p<0.001) and LA-FVC_{active} (r=0.5528, p<0.001), respectively.

3.5 Correlation analysis between LA functional indices and age, body weight and heart rate

The εE, SR_e, and LA-$FVC_{passive}$ were negatively correlated with the age (r=-0.4585, p<0.001, r=-0.4442, p<0.001, and r=-0.4424, p<0.001, respectively); whereas, the εA and LA-FVC_{active} were positively correlated with the age (r=0.2825, p<0.05, and r=0.4564, p<0.001). The εA showed negative correlation with the heart rate (r= -0.2706, p<0.05); whereas, the SR_s and SR_a showed positive correlation with the heart rate (r=0.1967, p<0.05, r=0.3568, p<0.001, respectively). The SR_a and εA showed negative correlation with the BW (r=-0.3709, p<0.001, r=-0.2272, p<0.05, respectively).

3.6 Multiple linear regression analysis between LA functional indices and age, body weight ad heart rate

In multiple linear regression analysis, the relationships were significant, but generally weak. Only age was significantly associated with all the parameters for conduit function

[standardized partial regression coefficient (β)=-0.46, r^2=0.21, p<0.001 for εE, r^2=0.19, β=-0.46, p<0.001 for SR_e, and r^2=0.19, β=-0.45, p<0.001 for $LA\text{-}FVC_{passive}$]. The age, BW, and heart rate were significantly related to the εA (age, r^2=0.08, β=0.25, p=0.0025; BW, r^2=0.05, β=-0.25, p=0.0039; heart rate, r^2=0.07, β=-0.31, p=0.0004). The heart rate and BW were significantly related to the SR_a (heart rate, r^2=0.12, β=0.31, p=0.0003; BW, r^2=0.14, β=-0.31, p=0.0003). The age and heart rate were significantly associated with the $LA\text{-}FVC_{active}$ (age, r^2=0.21, β=0.46, p<0.001; heart rate, r^2=0.04, β=-0.21, p=0.01).

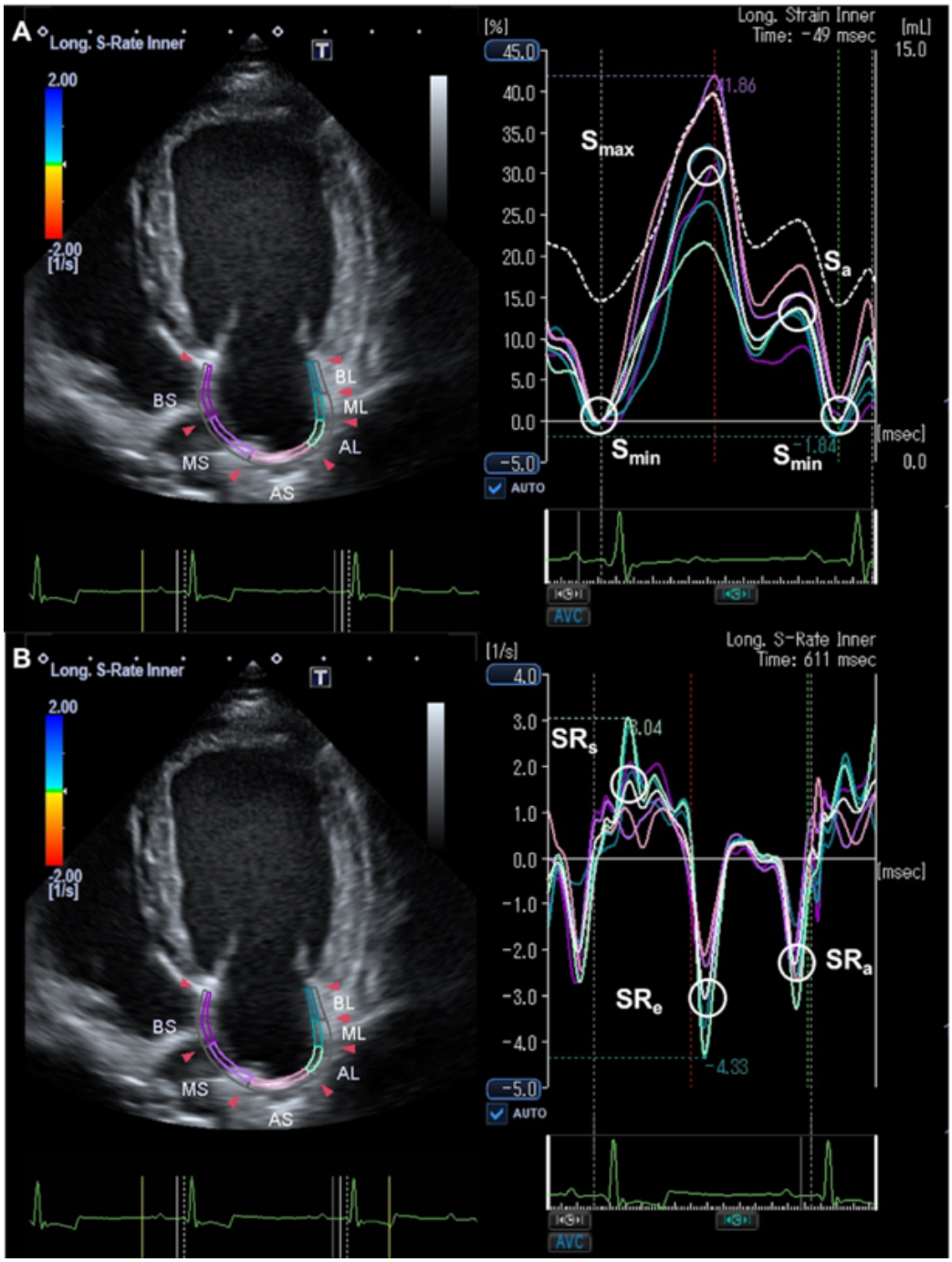

Figure 1. Longitudinal strain and strain rate images obtained from two-dimensional speckle tracking echocardiography. LA longitudinal strain and SR curves of normal dogs obtained on the apical 4-chamber view. Strain and SR curves color-coded refer to the myocardial segment of LA. White lines represent the averaged global strain and SR. (A) S_{min}, S_{max}, S_a were obtained

from average global curves to calculate LA longitudinal strain during each LA phasic function; (B) SR_s was calculated by averaging first peak longitudinal strain rate values in all six segments of the LA. SR_e and SR_a were calculated by averaging first and second negative peak longitudinal strain rate values in all six segments of the LA, respectively.

SR, strain rate; S_{min}, minimum strain; S_{max}, maximum strain; S_a, strain before atrial contraction; SR_s, positive peak strain rate during ventricular systole; SR_e, first negative peak strain rate during early ventricular diastole; SR_a, second negative peak strain rate during atrial contraction

Table 1. Variability of strain and strain rate analysis obtained by 2D-STE from normal beagle dogs (n=6)

Strain and SR parameters	CV (%)		
	Intraday	**Interday**	**Inter observer**
εS	7.1	14.4	2.6
εE	9.9	13.3	5.5
εA	9.5	18.4	3.6
SR_s	9.3	19.0	18.6
SR_e	8.8	12.1	17.4
SR_a	10.7	15.8	8.3

CV, coefficient of variation; εS /SR_s, left atrial longitudinal strain/strain rate during ventricular systole; εE/ SR_e, left atrial longitudinal strain/strain rate during early ventricular diastole; εA/SR_a, left atrial longitudinal strain/strain rate during atrial contraction

Table 2. Descriptive statistics for the LA function indices in conscious dogs (n=120)

Parameters	Median	Min-max	Lower RI	90% CI	Upper RI	90% CI
εS	25.37	16.36- 41.56	17.61	16.79-18.42	37.33	35.47-39.35
εE	11.06	4.35-22.69	4.25	3.81-4.80	20.93	19.49-22.35
εA	14.17	4.77-27.48	8.00	7.27-8.77	22.48	21.17-23.85
SR_s	1.87	1.15-3.46	1.23	1.18-1.29	2.88	2.72-3.02
SR_e	-2.32	-1.07 to -4.39	-1.13	-1.11 to -1.24	-4.25	-3.96 to -4.55
SR_a	-2.71	-1.17 to -4.65	-1.42	-1.25 to -1.58	-4.09	-3.91 to -4.28
LA-FVC_{total} (%)	52.44	35.93-67.13	40.84	39.30-42.38	65.19	63.49-66.82
LA-$FVC_{passive}$ (%)	32.75	13.98-48.74	17.64	15.61-19.63	47.13	45.36-48.87
LA-FVC_{active} (%)	28.99	15.08-53.17	17.36	16.07-18.87	45.86	43.39-48.40

εS /SR_s, LA-FVC_{total}, left atrial longitudinal strain/strain rate, fractional volume change during ventricular systole; εE/SR_e, LA-$FVC_{passive}$, left atrial longitudinal strain/strain rate, fractional volume change during early ventricular diastole; εA/SR_a, LA-FVC_{active}, left atrial longitudinal strain/strain rate, fractional volume change during atrial contraction;

Min-max, minimum-maximum; RI, reference interval calculated using a robust method with a Box-Cox transformation; CI, confidence interval

Table 3. Body weight separated normal values of LA strain and SR in 120 dogs

Profiles	Toy breed < 5 kg (n=57)	Small breed 5-10 kg (n=40)	Medium to large breed >10 kg (n=23)
Characteristics			
Age (years)	6 (1-16)	6 (1-13)	7 (1-12)
Sex (male/female)	28/29	21/19	10/13
Body weight (kg)	3.4 (1.2-4.8)	6.9 (5.1-10)	20.9 (10.4-60)
Heart rate (bpm)	118 (58-180)	106 (53-152)	107 (63-147)
Mean arterial blood pressure (mmHg)	106 (89-129)	101 (80-126)	110 (89-126)
Echocardiographic values			
left atrial-to-aortic ratio	1.3 (1.1-1.6)	1.4 (1.0-1.6)	1.3 (1.1-1.5)
nLVIDd	1.3 (1.1-1.9)	1.4 (1.1-1.9)	1.4 (1.2-1.8)
nLVIDs	0.7 (0.4-1.1)	0.8 (0.6-1.3)	0.8 (0.5-1.2)
FS (%)	44.7(28.1-62.2)	41.9 (27-58.8)	35.6 (28.7-50.9)
E (m/s)	60.7 (42-93.2)	72.2 (38.2-95.2)	72.7 (52-112.6)
A (m/s)	59.3 (25.7-96.2)	58.8 (31.3-100.3)	57.8 (39.9-87.9)
E:A	1.1 (0.6-1.9)	1.3 (0.6-2.2)	1.2 (0.9-1.9)
E’ (m/s)	6.5 (3.0-12.2)	7.8 (4.2-12.0)	8.5 (5.3-12.6)
A’ (m/s)	7.6 (5.2-12.1)	8.0 (3.7-12.9)	7.9 (5.1-11.2)
S’ (m/s)	7.6 (5-11.9)	8.8 (5.7-12.9)	10.6 (7.4-17.1)
E:E’	9.2 (5.7-15.4)	8.8 (5.5-13.7)	8.9 (5.9-12.8)

Profiles	Toy breed < 5 kg (n=57)	Small breed 5-10 kg (n=40)	Medium to large breed >10 kg (n=23)
2D-STE derived parameters			
LA-FVC_{total} (%)	52.4 (35.9-66.8)	52.9 (42.3-63.4)	51.0 (41.9-67.1)
LA-$FVC_{passive}$ (%)	30.9 (13.9-47.7)	35.4 (16.9-47.9)	30.7 (15.7-48.7)
LA-FVC_{active} (%)	29.4 (15.1-51.2)	28.1 (15.6-40.2)	29.2 (16.3-53.2)
εS	25.1 (16.4-37.9)	26.1 (19.7-41.6)	24.4 (17.6-34.8)
εE	9.5 (4.7-19.5)	12.6 (4.3-22.1)	11.2 (5.3-22.7)
εA	14.9 (7.5-27.5)	13.8 (8.6-19.0)	12.6 (4.8-23.2)
SR_s	1.9 (1.2-2.9)	1.9 (1.2-2.9)	1.9 (1.2-3.5)
SR_e	-2.4 (-1.1 to -4.1)	-2.5 (-1.1 to -4.4)	-2.2 (-1.3 to -4.1)
SR_a	-2.9 (-1.6 to -4.7)	-2.6 (-1.5 to -3.9)	-2.1 (-1.2 to -4.0)

nLVIDd, normalized LVIDd; nLVIDs, normalized LVIDs; FS, fractional shortening; E, peak velocity of early diastolic transmitral flow; A, peak velocity of late transmitral flow; E:A, ratio of E to A; E', peak velocity of early diastolic mitral annular motion as determined by pulse wave Doppler; A', peak velocity of diastolic mitral annular motion as determined by pulse wave Doppler; S', peak velocity of systolic mitral annular motion as determined by pulse wave Doppler; E:E', ratio of E to E';

εS /SR_s, LA-FVC_{total}, left atrial longitudinal strain/strain rate, fractional volume change during ventricular systole; εE/SR_e, LA-$FVC_{passive}$, left atrial longitudinal strain/strain rate, fractional volume change during early ventricular diastole; εA/SR_a, LA-FVC_{active}, left atrial longitudinal strain/strain rate, fractional volume change during atrial contraction;

data are expressed as median (range)

4. DISCUSSION

Results of this study indicated that the assessment of the LA phasic function through the 2D-STE variables enabled LA longitudinal strain and SR analysis to estimate the three phasic functions of the LA including the reservoir, conduit, and booster-pump function in healthy dogs. Intra-observer variability within-day for LA longitudinal strain was considered as a repeatable parameter with CV of <20%.[38,39] However, intra-observer variability between-day and inter-observer variability of some SR revealed relatively higher value than those in the strain variables. The higher variability of some SR variables may be software-dependent due to automatic generation of the curves after tracing the region of interest.[28] Therefore, the SR should be interpreted under careful consideration of the reliability. Moreover, the CVs relative to the LA longitudinal strain and SR obtained in this study were slightly higher than those obtained in a previous study showing CVs <16% for all tested variables,[5] which could be explained by the difference in the software and canine population in each study. The good repeatability of the STE might support the finding of lack of angle dependency which is an advantage over conventional techniques.[1,2] Based on a previous report in humans,[40] the LA strain parameter through offline analysis using a different software was validated with high feasibility and good agreement. In veterinary medicine, the repeatability and reproducibility of measurements of the LA function assessed with STE in healthy dogs are also clinically relevant.[5,12]

In the study, among the strain variables, strain corresponding to the reservoir phase showed the strongest relationship with that corresponding to the conduit phase. However, other representative indices such as the pulmonary venous systolic flow, left ventricular end-diastolic pressure, left ventricular end-diastolic volume, left ventricular ejection fraction, or isovolumetric contraction time may be required to understand more detailed insight of the LA reservoir function and left ventricular hemodynamic measurement.[40,41] Likewise, STE derived LA strain parameters have shown to be correlated with the Doppler-based parameters used to describe LV diastolic function and the corresponding FVC. The relationships of LA conduit function and Doppler-based parameters investigated in this results can be explained by the utility of mitral inflow as indirect parameters of the LV diastolic and LA functions based on occurrence initial filling of the LA, and consequent relaxation of the left ventricle, resulting in high peak velocity of early diastolic trans-mitral flow as the blood enters the left ventricle, and consequently, a high passive LA emptying fraction.[21,25,40]

In addition, the results indicated that LA function analysed by speckle tracking was age dependent. All LA functional indices for conduit function were inversely related with increasing age. This may be explained by the effect of ageing process on impaired early left ventricular diastolic filling.[25] In contrast, the changes in strain corresponding to the booster pump phasic parameters were positively influenced by age increases, which is most likely to be a compensatory mechanism for impaired left ventricular performance.[25,40,42] Moreover, the strain and SR parameters corresponding to booster-pump function were relatively higher than

those corresponding to conduit function; whereas, the LA-FVC at conduit phase was relatively higher than that at booster pump phase. Thus, the 2D-STE derived LA strain parameters showed potential for use as sensitive indicators of the subclinical change in atrial function with aging; further studies are needed to determine maintenance of the changes in volumetric indices with ageing despite relative decrease in the values in LA strain. Additionally, a higher number of dogs with more negative SR_a curve than the SR_e was indicated in this study, which extends an earlier observation regarding the adverse effect of ageing on the LA conduit function.[28,30] Most dogs used in this study were in the older age group and represented the inversion of ratio of E to A on the pulse wave Doppler interrogation of the mitral valve, without changes in the LA size and heart rate.[21,28] This trend may be considered as an important influencing factor on the age-associated change in cardiac function. However, to determine the effect of ageing on LA function, a study including a large number of dogs of different aged-groups (young, middle, and older group) for subsequent analysis to detect the changes of LA function parameters, is required.[25,30,43-45]

This study provides evidence of minor effect of BW on atrial SR, particularly booster-pump function, in contrast to the absence of association between LA strain, SR and BW in a previous report.[28] The STE values in right ventricular strain in larger breed dogs was also reported as lower than that in smaller breed dogs.[46] Therefore, a possible effect of BW on the STE variables cannot be excluded. The effect value of atrial SR_a might be underestimated due to the wide variation of SR. In this current study, strain and SR parameters alone for booster-

pump function were significantly associated with the BW and heart rate. These findings support the consideration of the effect of BW and heart rate on SR in interpretation of cardiac diseases.[25,31]

Currently, normal values of LA longitudinal strain and SR in a large number of healthy dogs are lacking. This study reported the central tendencies for estimating the mean of LA longitudinal strain and SR at each LA phasic function, including data by BW. In addition to tracking algorithm, the image quality, ultrasound system, and software used for this analysis could increase the differences in interpretation of reported mean values atrial strain, SR and those in other studies.[5,28] The image of LA strain and SR obtained in this study was generated by Artida and Ultra-Extend (Toshiba Medical System), compared with MyLab and XStrain™ (Esaote), iE33 and QLAB quantification software (Phillips) used in the previous studies. In humans, the longitudinal strain values of LV using Artida were reported to be lower than those using Esaote.[47,48] There are no available reference intervals of LA longitudinal strain and SR obtained from the software used in this study. Thus, this is the first report to provide the reference intervals of atrial deformation parameters through different software in healthy dogs as compared to previous literature. Therefore, to provide suitable normal values for wider use in dogs with disease, investigators should additionally consider variations due to different methodology as well as STE software for LA, instead of LV software.[23]

This study included a larger number of samples as compared to other echocardiographic studies focused on normal LA deformation in animals,[5,8,28] and provided better sample size for

the recommended number of reference intervals in at least 120 reference subjects.[49] However, this study limits to control the respiratory cycle or stress factor in conscious dogs, which can lead to larger variability in the analysis. Additionally, the current study lacked long-term follow-up of the dogs used to identify the reference intervals; therefore, it was not possible to examine the effect of the subclinical myocardial disease-free status of included dogs on the LA function. Finally, the results in this study did not present in the form of body-size dependent allometric equations for LA longitudinal and SR variables, as a previous report did,[31] due to the small effect of body size on the strain and SR variables.

In conclusion, the current study demonstrated that the LA phasic function indices of strain and SR were a feasible, reproducible measurement tool to assess the LA function in awake dogs without cardiac disease. However, the age, heart rate, and BW have shown to have potential effect in the interpretation of these parameters in clinical setting. The reference intervals of LA strain and SR enable future studies to investigate parameters to predict the cardiovascular outcome. Next, the possible effect of preload condition on LA function will be investigated in the chapter 2.

5. SUMMARY

In this chapter, the intra-observer within-day variations of strain variables showed adequate repeatability to assess the LA function in awake dogs without cardiac disease (CV<20%). The mean values of strain were 25.37 for the LA reservoir function, 11.06 for the LA conduit function, and 14.17 for the LA booster-pump function; and the strain was significantly correlated with the corresponding LA fractional volume change. The LA strain conduit function showed moderate correlation with the parameters through LV diastolic Doppler (the peak velocity of early diastolic trans-mitral flow, peak velocity of early diastolic mitral annular motion and ratio of peak velocity of early diastolic trans-mitral flow to peak velocity of late trans-mitral flow). In multiple regression analysis, only age was significantly related to the strain/SR and volumetric change indices, indicating conduit function.

Left atrial speckle tracking echocardiographic analysis provided useful information to assess the LA function in healthy dogs. However, the influencing factors on strain and SR variables including the age, body weight, and heart rate should be considered in interpretation of these parameters in clinical setting.

CHAPTER 2

EFFECT OF VOLUME LOADING ON LEFT ATRIAL STRAIN VALUES DERIVED FROM TWO-DIMENSIONAL SPECKLE TRACKING ECHOCARDIOGRAPHY IN DOG MODELS

1. INTRODUCTION

Notably, LA booster pump dysfunction indicated by strain imaging using 2D-STE was shown to be the best predictor of heart failure complications in dogs and had a higher predictive power for evaluating congestive heart failure over the LA-FAC for booster pump function.[10] Volume load dependency of echocardiographic indices is of clinical concern when using the indices in heart diseases associated with volume overload: LA dysfunction can be masked by the enhancing effect of the volume loading on LA function indices.[50] A previous experimental study using healthy beagles have shown that the volumetric LA function indices (i.e., LA fractional area changes) determined with 2D-STE are volume load-dependent and enhanced by cardiac volume loading.[20] On the other hand, the degree of volume load dependency on LA function indices derived from strain imaging using 2D-STE remains unclear in dogs.

Therefore, the aim of this chapter was to elucidate the effect of clinically relevant changes of acute volume loading on strain and SR parameters derived using the 2D-STE method in dog models. The results of the present study could describe the degree of volume load dependency on LA myocardial deformation for further therapeutic strategy and prognostic information of the LA.

2. MATERIAL AND METHOD

2.1 Animals

Six laboratory beagles (aged 1-3 years, with body weight of 8.8 to 11.4 kg), which were part of an experimental unit at Hokkaido University, were enrolled in this study. All dogs were healthy and had no abnormalities of cardiac function on the basis of routine physical examination, including blood examination, electrocardiogram, and standard echocardiography (including M-mode, pulsed-wave Doppler, and color flow Doppler–based imaging). All procedures were reviewed and approved by the laboratory animal experimentation committee of the Graduate School of Veterinary Medicine, Hokkaido University (approval No. 15-0087).

2.2 Procedure

The protocol used in this study was the same as in a previous report.[20] An intravenous infusion route was established in each dog on the left and right cephalic veins with a 20-gauge over-the-needle catheter, and a 24-gauge over-the-needle catheter was placed in the left or right dorsal pedal artery to directly monitor arterial blood pressure. Each dog was administered atropine sulfate (Mitsubishi Tanabe Pharma Corp., Osaka, Japan) 0.05 mg/kg, subcutaneously, cefazolin sodium hydrate (Astellas Pharma Inc., Tokyo, Japan) 20 mg/kg intravenously (IV), and heparin sodium (Ajinomoto Pharmaceuticals Co., Ltd., Tokyo, Japan) 100 units/kg IV, and sedated with butorphanol tartate (Meiji Seika Pharma Co., Ltd., Tokyo, Japan) 0.2 mg/kg IV

and midazolam hydrochloride (Astellas Pharma Inc., Tokyo, Japan) 0.1 mg/kg IV. Then, anesthesia was induced with administration of propofol (Mylan Inc., Canonsburg, PA, U.S.A.) 6 mg/kg IV. Thereafter, each dog was endotracheally intubated, and anesthesia was maintained with isoflurane (DS Pharma Animal Health Co., Ltd., Osaka, Japan) 1.75% to 2.0% in 100% oxygen. End-tidal partial pressure of carbon dioxide was continuously monitored and maintained between 35 and 45 mmHg with mechanical ventilation, with a tidal volume of 10 to 15 m*l*/kg and a respiratory rate of 10 to 12 breaths/min. Heart rate and arterial pressure measured with arterial catheterization were continuously recorded with a commercial polygraph instrument (Nihon Kohden Co., Ltd., Tokyo, Japan).

A 6F, 12-cm introducer sheath (St. Jude Medical Inc., Minnetonka, MN, U.S.A.) was percutaneously inserted into the right external jugular vein using the Seldinger technique in each dog which was positioned in in the position of left lateral recumbency. A 5F, 75-cm Swan-Ganz catheter (Edwards Lifesciences Corp., Irvine, CA, U.S.A.) was advanced into the pulmonary artery with fluoroscopy guidance. The catheter was connected to polygraph equipment for acquisition of hemodynamic data.

Following a stabilization period of about 10 min, baseline recordings of hemodynamic and echocardiographic indices were performed. Thereafter, cardiac preload was increased by IV infusion of warmed lactated Ringer solution (Terumo Corp., Tokyo, Japan) at 150 ml/kg/hr for 90 min.[20] This dose was modified from the dose used in previous studies.[51,52] After the fluid infusion began, hemodynamic and echocardiographic evaluations were performed every 15

min. The hemodynamic data were obtained before echocardiography at each time point assessment. Following the final echocardiographic examination, each dog was administered furosemide (Sanofi K K, Tokyo, Japan) 4 to 6 mg/kg IV and allowed to recovery from anesthesia.

2.3 Hemodynamic assessment

All hemodynamic data including heart rate, mean arterial blood pressure, mean pulmonary arterial pressure, pulmonary capillary wedge pressure (PCWP), mean right atrial pressure, and cardiac output were recorded by a polygraph instrument and digitally stored. Mechanical ventilation was briefly stopped during the recordings of hemodynamic indices. The distal and proximal ports of a Swan-Ganz catheter were used to measure pulmonary arterial and right atrial pressures, respectively. The PCWP was determined when the balloon at the end of the Swan-Ganz catheter was inflated to be wedged in a small pulmonary artery. After pressure recordings, cardiac output was determined using the thermodilution method with the injection of a 5-ml bolus of cold saline (0.9% NaCl) into the right atrium through the proximal port of a Swan-Ganz catheter. Stroke volume was calculated by dividing cardiac output by heart rate. For pressure measurements, the mean of five consecutive cardiac cycles was calculated, and the average of four measurements was calculated for cardiac output.

2.4 Standard echocardiographic methods

Echocardiography was performed by the same experienced investigator (KN) using a Toshiba Artida^{TM} echocardiographic system (Toshiba Medical System Corp., Tochigi, Japan) with a 3- to 7-MHz sector probe transducer array. All echocardiographic indices were recorded when dogs were in an expiratory phase. An electrocardiogram trace (lead II) was recorded simultaneously with echocardiographic imaging by the electrocardiogram equipment on the ultrasonographic device, in addition to that on the polygraph instrument. The mean of 3 consecutive cardiac cycles was calculated for all echocardiographic indices, including those determined by 2D-STE.

Pulsed-wave Doppler echocardiography was performed to measure the trans-mitral flow velocity from the left apical four-chamber view as described in chapter 1. The sample gate for trans-mitral flow was placed at the tip of the mitral valve leaflets when they were opened.[61] The following indices were measured: peak velocity of the early diastolic mitral flow (E), peak velocity of the late diastolic mitral flow (A), and the ratio of E to A. These indices were not determined when those waves were completely or partially fused.

The aortic Doppler flow profile was obtained with the sample gate positioned immediately below the aortic valve from the left apical five-chamber view. Left ventricular ejection time was measured as the interval from the onset to the end of the aortic flow. Left ventricular pre-ejection period was measured as the interval from the start of the QRS complex to the beginning of aortic flow. The ratio of the LV ejection time to LV pre-ejection period was calculated.

Myocardial motion velocities derived from tissue Doppler imaging were recorded with the sample gate placed at the septal mitral annulus from the left apical four-chamber view.[61] The peak velocity of the systolic mitral annular motion (S′ wave), peak velocity of the early diastolic mitral annular motion (E′ wave), and peak velocity of the late diastolic mitral annular motion (A′ wave) were measured, the ratio of E to A and the ratio of E to E' were calculated. These indices other than the peak velocity of the S' wave were not determined when the E' and A' waves were completely or partially fused. Additionally, from the tissue Doppler imaging velocities of myocardial motion at the septal mitral annulus, the isovolumic relaxation time and the isovolumic contraction time was measured: the isovolumic relaxation time was correspondence to the interval from the end of the S′ wave to the beginning of the E′ wave, while the isovolumic contraction time was correspondence to the interval from the end of the A′ wave to the beginning of the S′ wave.

2.5 2D-STE of the LA

From the left apical four-chamber view, the image used for strain imaging using 2D-STE of LA was acquired with the frequency, depth, and sector width adjusted for optimization of frame rate (between 151 and 229 frames per rate). The image of three consecutive cardiac cycles was digitally stored at each assessment point for later offline analysis. The assessment of LA strain and SR derived from 2D-STE was described in chapter 1. The software also automatically generated a LA volume curve which was calculated by the monoplane area-

length method of LA and LA-FVC were calculated as detailed in chapter 1.

2.6 Statistical analysis

Statistical analyses were performed on JMP Pro 12.2.0 software (SAS Institute, Cary, NC, U.S.A.). Normal distribution of the data was confirmed by a Shapiro-Wilk test. A linear mixed model was developed with time (baseline, 15, 30, 45, 60, 75, and 90 min) as a categorical fixed effect and dog identity as a random effect. The *F* test was performed to assess the effect of time on the values of the measured variables. Pairwise comparisons between the baseline and each time point were performed by obtaining the least squares means and using the Bonferroni correction to account for multiple comparisons. The relationship between PCWP and each of the indices of LA functional strain/SR were investigated using multiple regression analysis. In model 1, the PCWP and dummy coding of the enrolled dogs were included as covariates (linear regression model). In model 2, the quadratic terms of PCWP and dummy coding of the enrolled dogs were entered as covariates (quadratic regression model). For each LA function parameter, model 2 was accepted if the effect of the quadratic term of the PCWP was significant, and a log-likelihood ratio χ^2 test revealed that model 2 had a fit superior to that of model 1. After constructing each model, assumptions of linearity, normality, homoscedasticity, and independence of the residuals were evaluated by inspection of the standardized residual plots and quantile plots.

3. RESULTS

3.1 Change in hemodynamic variables

The changes in hemodynamic variables before (baseline) and at each assessment point after IV infusion of fluid are summarized in Table 4. Mean PCWP and cardiac output were significantly greater than at baseline from 15 to 90 min after acute volume loading began. Heart rate and mean arterial blood pressure were not significantly changed from baseline at 15 to 90 min for all 6 dogs, whereas stroke volume was significantly changed from baseline at 15 to 90 min.

3.2 Change in echocardiographic parameters

The changes in echocardiographic parameters before (baseline) and at each assessment point after acute volume loading began are summarized in Table 5. Peak velocities of E, A, E′, and A′ wave were determined without fusion of the E and A wave and the fusion of the E′ wave and A′ wave for all dogs. Acute volume loading induced a significant increase from baseline at 15 to 90 min in peak velocity of the E wave, E′ wave, and at 30 min in the A′ wave. LV ejection time was significantly increased and the ratio of LV pre-ejection period to ejection time was significantly decreased from baseline at 15 to 90 min. The isovolumic relaxation time and isovolumic contraction time did not show a significant change after volume infusion. For LA strain and SR variables (Table 6), acute volume loading caused a significantly increased

LA strain from baseline, corresponding to reservoir function and conduit function from baseline at 15 to 90 min and at 45 to 90 min in εA (Fig. 2.). Acute volume loading caused a significant increase from baseline in all phasic functions of SR at 15 min. The LA volumes and LA-FVCs at each assessment point after acute volume loading are shown in Table 7. The V_{min}, V_{preA}, and V_{max} were obtained from speckle tracking analysis. The V_{preA} was significantly increased from baseline at 60 to 90 min, at 15 to 90 min in V_{max}, and at 75 to 90 min in V_{min}.

A quadratic multiple regression model (model 2) provided to be a better fit model when compared with the linear regression model (model 1) for relationships between PCWP and all phasic functions of LA function indices, including LA strains, SRs, and LA-FVCs (Table 8; Fig. 3.)

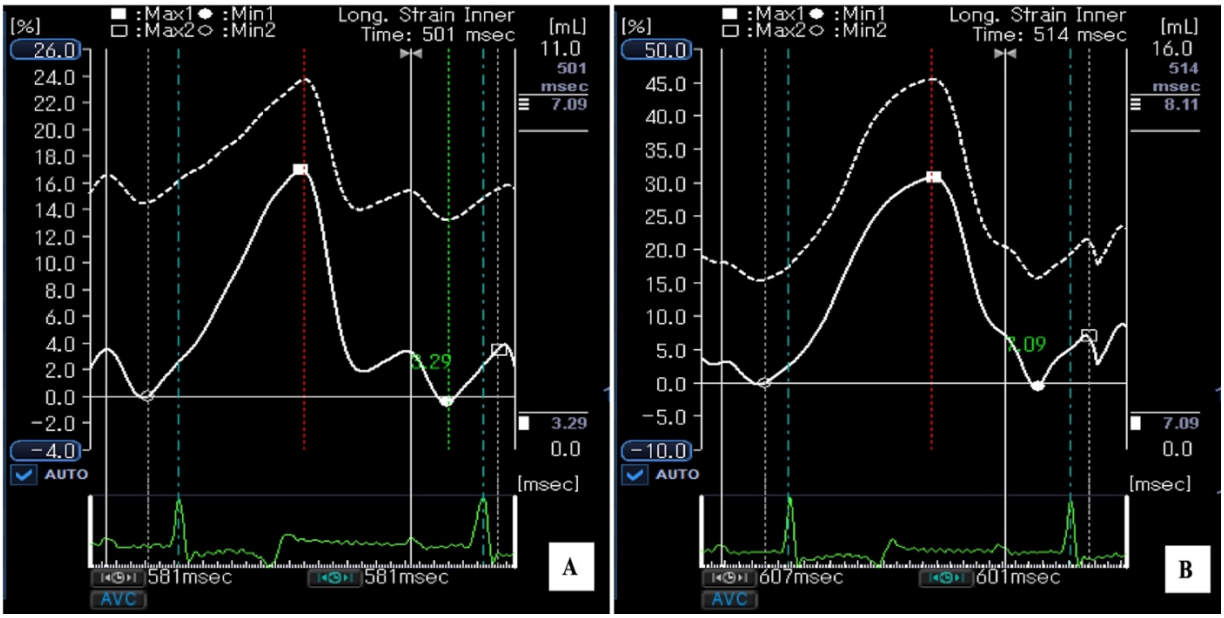

Figure 2. Representative software generated a strain curve for a single cardiac cycle of a healthy beagle at baseline (A) and at 90 minutes after cardiac volume loading (B).

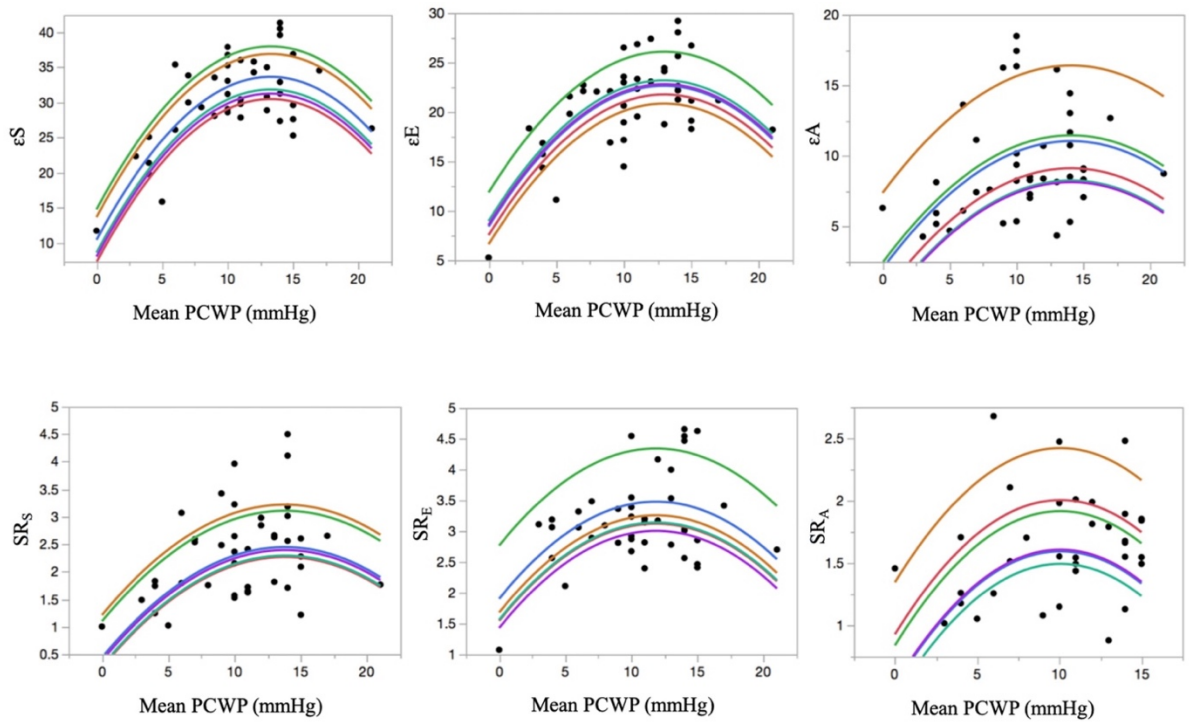

Figure 3. Relationships between pulmonary capillary wedge pressure (PCWP) in 6 healthy beagles and the following indices of left atrial (LA) phasic function. Quadratic regression lines better fit the relationships between PCWP and LA deformation indices, indicating three phases of LA function.

Table 4. Least square mean (95% CI) obtained from linear mixed model for hemodynamic data before (baseline) and each time point during experimental cardiac volume loading in 6 healthy beagles

Variables	Baseline	15 min	30 min	45 min	60 min	75 min	90 min
MBP	55 (48-63)	55 (48-62)	57 (49-64)	58 (51-65)	60 (53-67)	60 (53-67)	59 (52-67)
Heart rate (beats/min)	110 (103-117)	101 (94-108)*	109 (102-116)	110 (103-117)	113 (106-120)	114 (107-121)	109 (102-116)
PAPm (mmHg)	9.5 (7.1-11.9)	12.7 (10.2-15.1)*	15 (12.6-17.4)*	16.3 (13.9-18.8)*	17.3 (14.9-19.8)*	17.8 (15.4-20.3)*	17.5 (15.1-19.9)*
PCWPm (mmHg)	3.3 (1.1-5.6)	8 (5.8-10.2)*	10.3 (8.1-12.6)*	12 (9.8-14.2)*	12.7 (10.4-14.9)*	13.3 (11.1-15.6)*	14 (11.8-16.2)*
RAPm (mmHg)	0.3 (-1.1-1.7)	5.5 (4.1-6.9)*	6.5 (5.1-7.9)*	7.2 (5.8-8.6)*	7.5 (6.1-8.9)*	8.2 (6.8-9.6)*	8.3 (6.9-9.7)*
Cardiac output (L/min)	2.1 (1.8-2.4)	2.6 (2.3-2.8)*	2.8 (2.5-3.1)*	2.9 (2.7-3.2)*	3 (2.7-3.3)*	3.1 (2.8-3.4)*	3.1(2.8-3.4)*
Stroke volume (mL)	19 (17-22)	25 (23-28)*	26 (24-28)*	27 (24-29)*	27 (24-29)*	28 (25-30)*	28 (26-31)*

*Value differs significantly ($P < 0.05$) from corresponding baseline value.

PAP = Pulmonary arterial blood pressure. RAP = Right atrial blood pressure. PCWP = Pulmonary capillary wedge pressure. MAP = Mean arterial blood pressure as measured via arterial catheterization.

Table 5. Least square mean (95% CI) obtained from linear mixed model for conventional echocardiographic parameters before (baseline) and each time point during experimental cardiac volume loading in 6 healthy beagles

Variables	Baseline	15 min	30 min	45 min	60 min	75 min	90 min
E wave (m/s)	0.71 (0.65-0.77)	0.84 (0.78-0.89)	0.85 (0.79-0.91)*	0.83 (0.77-0.89)	0.87 (0.81-0.92)*	0.91 (0.85-0.97)*	0.85 (0.79-0.91)*
A wave (m/s)	0.43 (0.33-0.53)	0.48 (0.38-0.58)	0.55 (0.45-0.66)	0.55 (0.45-0.65)	0.53 (0.42-0.63)	0.56 (0.45-0.66)	0.49 (0.38-0.59)
Ratio of E and A	1.72 (1.33-2.10)	1.81 (1.42-2.19)	1.58 (1.19-1.97)	1.66 (1.27-2.05)	1.69 (1.29-2.07)	1.77 (1.39-2.16)	1.86 (1.47-2.25)
PEP (ms)	73.67 (66.17-81.16)	66.17 (58.67-73.66)*	65.67 (58.17-73.16)*	62.33 (54.84-69.83)*	59.17 (51.67-66.66)*	60.50 (53.01-67.99)*	61.83 (54.34-69.33)*
ET (ms)	185.00 (169.76-200.24)	234.83 (219.59-250.07)*	238.67 (223.43-253.90)*	243.00 (227.76-258.24)*	254.67 (239.43-269.90)*	253.33 (238.09-268.57)*	260.83 (245.59-276.07)*
Ratio of PEP and ET	0.40 (0.36-0.44)	0.28 (0.25-0.32)*	0.27 (0.24-0.31)*	0.26 (0.22-0.29)*	0.23 (0.19-0.27)*	0.24 (0.20-0.28)*	0.24 (0.20-0.27)*
E' wave (cm/s)	6.47 (4.95-7.98)	9.04 (7.52-10.55)*	9.75 (8.23-11.27)*	9.17 (7.66-10.69)*	9.88 (8.36-11.39)*	9.29 (7.78-10.82)*	8.72 (7.20-10.24)*
A' wave (cm/s)	3.54 (2.35-4.73)	4.80 (3.61-5.99)	5.18 (3.99-6.36)*	5.56 (4.37-6.75)*	6.53 (5.34-7.71)*	5.17 (3.98-6.36)*	5.29 (4.09-6.48)*
S' wave (cm/s)	5.44 (4.59-6.29)	5.42 (4.57-6.27)	5.56 (4.71-6.41)	5.57 (4.72-6.42)	5.96 (5.11-6.81)	5.75 (4.90-6.59)	5.41 (4.56-6.26)
Ratio of E and E'	11.38 (9.68-13.09)	9.39 (7.68-11.09)	8.98 (7.28-10.69)	9.16 (7.45-10.86)	9.26 (7.55-10.96)	10.17 (8.46-11.88)	9.89 (8.18-11.59)
IVCT	54.50 (44.76-64.24)	49.33 (39.59-59.07)	52.67 (42.93-62.41)	47.17 (37.43-56.91)	46.17 (36.43-55.91)	46.00 (36.26-55.74)	48.33 (38.59-58.07)
IVRT	54.17 (46.65-61.68)	56.50 (48.98-64.02)	58.83 (51.32-66.35)	55.67 (48.15-63.18)	59.50 (51.98-67.02)	59.33 (51.82-66.85)	55.67 (48.15-63.18)

*Value differs significantly ($P < 0.05$) from corresponding baseline value.

A wave = Peak velocity of the A wave. A′ wave = Peak velocity of the A′ wave. ET = Left ventricular ejection time. E wave = Peak velocity of the E wave. E′ wave = Peak velocity of E′ wave. IVCT = Isovolumic contraction time. IVRT = Isovolumic relaxation time. PEP = Left ventricular pre-ejection period. S′ wave = Peak velocity of S′ wave.

Table 6. Least square mean (95% CI) obtained from linear mixed model for LA strain and strain rate before (baseline) and each time point during experimental cardiac volume loading in 6 healthy beagles

Variables	Baseline	15 min	30 min	45 min	60 min	75 min	90 min
εS	19.4 (15.5-23.3)	31.1 (27-35.2)*	32.2 (28.3-36)*	32.5 (28.6-36.4)*	33.9 (30-37.8)*	33.1 (29.3-37)*	30.3 (26.4-34.1)*
εE	13.6 (10.3-16.9)	22.3 (18.9-25.7)*	23.9 (20.7-27.3)*	21.8 (18.5-25.1)*	21.5 (18.2-24.7)*	22.6 (19.3-25.8)*	20.7 (17.4-24)*
εA	5.8 (2.6-8.9)	8.8 (5.5-12)	8.3 (5.1-11.4)	10.4 (7.2-13.5)*	12.1 (8.9-15.3)*	10.4 (7.3-13.6)*	9.7 (6.6-12.9)*
SR_S	1.4 (0.8-2)	2.3 (1.7-2.9)*	2.3 (1.7-2.9)*	2.5 (1.9-3.1)*	3.1 (2.5-3.7)*	2.6 (1.9-3.2)*	2.2 (1.5-2.8)*
SR_E	2.5 (1.9-3.2)	3.5 (2.8-4.1)*	3.3 (2.7-3.9)*	3.2 (2.6-3.8)*	3.4 (2.8-4)*	3.3 (2.7-3.9)*	3.1 (2.5-3.7)*
SR_A	1.3 (0.9-1.7)	1.9 (1.5-2.2)*	1.7 (1.4-2.1)*	1.9 (1.5-2.3)*	1.8 (1.4-2.2)*	1.7 (1.3-2.1)*	1.5 (1-1.9)

*Value differs significantly ($P < 0.05$) from corresponding baseline value.

εS/ SR_S, εE/ SR_E, εA/ SR_A represent strain/strain rate during reservoir, conduit and booster pump function, respectively.

Table 7. Least square mean (95% CI) obtained from linear mixed model for LA phasic function variables derived from 2D-STE before (baseline) and each time point during experimental cardiac volume loading in 6 healthy beagles

Variables	Baseline	15 min	30 min	45 min	60 min	75 min	90 min
Vmin	6.12 (4.47-7.77)	6.91 (5.24-8.58)	7.93 (6.29-9.58)	6.66 (5.01-8.30)	7.44 (5.79-9.09)	8.52 (6.87-10.17)*	8.76 (7.11-10.41)*
VpreA	7.31 (4.58-10.04)	8.94 (6.17-11.71)	10.33 (7.60-13.06)	9.33 (6.59-12.06)	10.89 (8.16-13.62)*	11.79 (9.07-14.53)*	11.75 (9.02-14.48)*
Vmax	11.05 (7.03-15.08)	16.63 (12.54-20.71)*	19.19 (15.17-23.22)*	16.59 (12.57-20.62)*	18.83 (14.81-22.86)*	21.19 (17.17-25.22)*	20.51 (16.48-24.53)*
LA-FVC_{total}	43.55 (38.67-48.43)	58.06 (52.89-63.22)*	58.74 (53.86-63.62)*	59.91 (55.03-64.78)*	60.13 (55.25-65.01)*	59.23 (54.36-64.11)*	57.41 (52.53-62.29)*
LA-$FVC_{passive}$	32.30 (26.13-38.48)	45.76 (39.35-52.18)*	46.66 (40.49-52.84)*	43.89 (37.72-50.07)*	42.69 (36.52-48.87)*	44.91 (38.74-51.09)*	43.31 (37.13-49.48)*
LA-FVC_{active}	16.47 (10.23-22.72)	22.91 (16.42-29.40)	22.64 (16.39-28.89)	28.33 (22.09-34.58)*	29.73 (23.49-35.97)*	25.51 (19.26-31.75)*	24.21 (17.97-30.46)*

*Value differs significantly ($P < 0.05$) from corresponding baseline value.

LA-FVC_{total}, LA-$FVC_{passive}$, LA-FVC_{active} represent left atrial fractional volume change during reservoir, conduit and booster pump function, respectively.

Table 8. Maximum likelihood estimates (95% CIs), adjusted coefficients of determination (R^2), and results of log-likelihood ratio χ^2 tests for multiple linear (1) and quadratic (2) regression models of the association between variables of left atrial phasic function and PCWP in 6 healthy Beagles.

Variables	Model 1		Model 2			Log-likelihood ratio χ^2 test	
	PCWP	R^2	PCWP	$PCWP^2$	R^2	Accepted	P-value
εS	0.8 (0.4-1.2)	0.55	0.7 (0.5-0.9)	-0.13 (-0.2 to -0.1)	0.79	Q	<.0001
εE	0.5 (0.3-0.8)	0.50	0.4 (0.2-0.7)	-0.08 (-0.1 to -0.04)	0.68	Q	<.0001
εA	0.4 (0.2-0.6)	0.64	0.3 (0.1-0.5)	-0.05 (-0.1 to -0.02)	0.72	Q	0.0017
SR_S	0.08 (0.03- 0.14)	0.44	0.07 (0.02-0.1)	-0.01(-0.02 to -0.002)	0.53	Q	0.0067
SR_E	0.05 (0.004 -0.1)	0.55	0.03 (-0.01-0.1)	-0.01 (-0.02 to -0.005)	0.68	Q	<.0001
SR_A	0.03 (-0.003-0.1)	0.42	0.001 (-0.04-0.04)	-0.01 (-0.02 to -0.003)	0.58	Q	0.0067
$LA\text{-}FVC_{Total}$	1.2 (0.7-1.6)	0.53	0.9 (0.6-1.3)	-0.15 (-0.2 to -0.1)	0.77	Q	<.0001
$LA\text{-}FVC_{Passive}$	0.9 (0.4-1.4)	0.51	0.7 (0.3-1.1)	-0.1 (-0.2 to -0.04)	0.62	Q	0.0017
$LA\text{-}FVC_{Active}$	0.8 (0.4-1.3)	0.59	0.7 (0.3-1.1)	-0.1 (-0.2 to -0.03)	0.67	Q	0.0067

L = Linear regression model (model 1). $PCWP^2$ = Quadratic term of PCWP. Q = Quadratic regression model (model 2).

4. DISCUSSION

The major finding of this chapter indicated that the LA phasic function assessed by strain imaging via 2D-STE was enhanced during experimental cardiac volume overload as LA phasic function assessed by LA-FVCs did. According to the previous literatures,[1,20] the volumetric LA function indices including LA-FACs and LA-FVCs are known to have a limitation of volume load dependency. In heart diseases with volume overload, LA dysfunction evaluated on the basis of these indices can be masked by the enhancing effect of the volume loading.

Regarding the change in LA phasic function, the reservoir function is modulated by LA intrinsic relaxation, LA chamber stiffness, and the property of LV contraction.[50,54] For LA conduit function, it is related to the early diastolic pressure between LA and LV, and relaxation of the LV.[50] The booster pump of LA function is determined by intrinsic LA contractility, LA preload (i.e., LA volume before atrial contraction), and LV end-diastolic filling pressure and compliance.[50] The results of this study showed that the LA function at booster pump function was enhanced in response to an increasing cardiac preload, as supported by increased volume before atrial contraction (V_{preA}) identified in the study.[50] On the other hand, the change in LA function during the early atrial contraction phase follows Starling's law. Furthermore, the results could be described the enhancement of reservoir function after the increased volume load by stimulating the LA booster pump function and LV systolic function, as determined by the reduction in the ratio of the LV pre-ejection period and ejection time and by increasing the

cardiac output according to the Frank starling mechanism in this study.[1,20,55,56] Additionally, the enhancement of conduit function was mainly caused by increased LA pressure during the early diastolic period, as suggested by increased peak velocities of E wave in this study. Mitral valve E velocity, a Doppler-derived measurement of not only the filling pressure gradient between LA and LV but also LV relaxation, has been applied to estimate LA pressure.[57] In the study reported here, the ratio of peak velocities of the E to velocity of the E′ wave and the ratio of the velocity of the E to the velocity of the A wave were not changed significantly. This could be possibly explained by the effect of preload on that ratio was suggested as a minimal preload dependency.[58] Moreover, the change in conduit function was thought to be surpassed by the effect of increased reservoir phase. It is known that the LA reservoir and conduit function is correlated with maintaining LV performance, whether the LA is initially filled with blood or volume and consequent relaxation of LV, resulting in increased blood flow entering the LV. However, the relationship between the LA reservoir and conduit is likely to vary depending on atrial pressure, volume, and neural control, as described in a previous investigation.[59] As mentioned in chapter 1, to provide a better understanding more insights of the relationship between reservoir and conduit function, it is required the measurement from other representative hemodynamic indices.

This was the first report to elucidate the relationship between acute volume loading and the three phasic functions of LA obtained via strain imaging with 2D-STE in dogs. The relationships between PCWP and LA function indices derived from strain imaging with 2D-

STE were quadratic: the LA function indices were initially enhanced and then started to be impaired when the severity of cardiac volume load was occurred. This finding is similar to a previous study where the changes in LA diameter representing reservoir and booster pump functions were initially enhanced and then later impaired in healthy dogs given cardiac volume loading with IV infusion of dextran.[55] During later phases of volume load, results of previous studies suggested that LA afterload, as suggested by an increase in PCWP in this study, could suppress LA booster pump and reservoir functions.[20,55] Indeed, those LA functional parameters during reservoir and booster pump function in this study were not decreased from baseline in the later phase. This observation might imply that the enhancing effect of the volume load of those functions could have been offset by its suppressive effect.[20] In addition, it is possible that the degree of the elevation of PCWP was relatively mild (mean PCWP of about 15 mmHg), such that the results of dogs with higher PCWP could not be extrapolated. In general, a mean PCWP greater than 15 to 25 mmHg can be associated with left-sided heart failure.[64]

Currently, there have been conflicting reports between LA function indices determined by strain imaging with 2D-STE in humans. A previous study enrolling infants with patent ductus arteriosus demonstrated that cardiac volume overload secondary to this disease was associated with the impairment of reservoir and booster pump functions evaluated with LA strains and SRs.[61] On the contrary, another previous study including healthy humans showed that the acute decrease in cardiac volume load caused by a tilt maneuver was associated with the impairment of reservoir, conduit, and booster pump functions assessed on the basis of LA

strains.[62] The discrepancies between the present study and mentioned previous studies might have resulted from the difference in the duration of the change in cardiac volume load, the difference in the degree of the change in cardiac volume load, or the difference in the direction of the change in cardiac volume load.

However, this study lacked the use of left heart catherization, which is a gold standard for measurement of the mechanical function of the LA. In the study, a Swan-Ganz catheter and right heart catheterization was used for measuring hemodynamic variables. Although the LA pressure-volume loop analysis may be needed to measure LA intrinsic properties,[50] the invasive nature of this method and the expertise required to obtain appropriate data limit the application of this approach. In addition, the measurement of LV properties was made only by echocardiographic parameters. Regarding the changes of LA phasic function during unloading effect, it remains unknown whether the LA deformation indices would be decreased after the volume unloading effect since the LA function indices after examination of diuretic administration was not be measured. Besides, the number of dogs enrolled in the study was relatively small, such that a significant change in some echocardiographic data could not be determined. The indices of conduit and booster pump function in some images could not clearly be determined, which might possibly be related to the observed increase in the heart rate caused by acute volume loading.[63] The enhancing effect of acute volume loading on LA phasic function in this study might have been affected by the use of general anesthesia. As described in a previous literature,[64] isoflurane alters the active and passive mechanical properties of the

LA. This agent can depress LA myocardial contractility, delay relaxation, and also enhance reservoir function.[64] Furthermore, this was a clinically normal animal study of the acute volume load effect intervention on the LA strain imaging that the possibility of chronic adaptations in awake clinical dogs could not be excluded. Such chronic adaptations may lead to different response to changes in loading condition.

In conclusion, the LA phasic functions assessed by strain imaging with 2D-STE are affected by changes in acute volume loading condition and correlated with invasive measurement of PCWP in clinically normal dogs. Therefore, the diagnosis on the basis of LA phasic function obtained by strain and SR analysis obtained from 2D-STE should be considered with caution. Strain variables obtained from 2D-STE may serve as a sensitive indicator and provide additional information of the dogs with acute volume loading-stated heart diseases.

5. SUMMARY

In this chapter showed that acute volume loading significantly increased from baseline during LA strain and SR as assessed by the speckle tracking–based technique during reservoir and conduit function at 15 to 90 min after volume load began, and strain indices representing booster pump function were enhanced at 45 to 90 min. In addition, acute volume loading resulted in a significantly greater PCWP after fluid infusion. On multiple regression analysis, quadratic regression analysis was a better fit for the relationship between PCWP and all LA functional indices. These findings indicated that LA function analyzed by strain and SR was enhanced during cardiac acute volume loading in healthy dogs. The change in strain and SR during acute volume loading should be interpreted with caution during the diagnosis of heart diseases related to volume overload.

GENERAL CONCLUSION

The goal of this study was to show feasibility of the assessment of LA function via LA strain and SR imaging based on 2D-STE in dogs. The findings of this present study indicate that this technique is clinically acceptable for using in dogs. The normal reference interval of LA strain and SR was established in a larger number of clinically normal dogs. The reference intervals of LA strain and SR enable future studies to investigate parameters to predict the cardiovascular outcome. Furthermore, this study has obtained the basic findings in healthy dogs about the relationships between LA strain and SR indices and other STE derived LA functional indices as well as pathological effects such as age, body weight and heart rate including the hemodynamic changes from acute volume loading effect observed in experimental dog models. Such information will further allow for the evaluation of the interactions of LA strain and the hemodynamic changes observed in dogs with volume stated heart disease.

In chapter 1, the feasibility, repeatability and reproducibility of LA functional indices via strain imaging was determined in healthy dogs. The intra-observer within-day variations of strain variables showed adequate repeatability and this technique was considered to be clinical acceptability. These findings demonstrate that strain imaging based on 2D-STE can be applied for the assessment of LA function in dogs. With regard to interpreting these parameters by different day and observer, data in the study indicated relatively high intra-observer between-day and inter-observer variation, particular in data of SR. Therefore, SR parameters based on

2D-STE should be considered with caution when apply this parameter in clinical setting. Moreover, the reported normal values of LA strain in this study have shown to be correlated with the Doppler-based parameters used to describe left ventricular diastolic function and FVC estimated LA function, which highlights the potential clinical applicability of LA strain analysis. The LA strain parameters related to the corresponding FVC were indicated to have a possible interaction of the LA myocardial deformation and volumetric change, especially under conditions of cardiovascular disease states. Furthermore, the influencing factors on strain and SR variables including the age, body weight, and heart rate should be considered in interpretation of these parameters in clinical setting as well. Although the reference intervals for LA strain and SR using different system are needed for wider use in dogs with disease, investigators should also consider variations due to different methodology as well as specific STE software for LA. All of the dogs included in the repeatability study were healthy; therefore, caution has to be urged in interpreting these data to groups of diseased dogs, especially those with high heart rate. Besides, the accuracy of interpretation using LA strain analysis depends on the image quality and experience of tracking ability of the investigator that should be also taken into account in the analysis.

In chapter 2, the effect of clinically relevant changes of acute volume loading on strain and SR parameters derived using the 2D-STE method was elucidated in dog models. LA strain and SR as assessed by 2D-STE representing 3 phasic functions of the LA were affected by acute volume loading. The acute load of volume in this study resulted in a greater LA pressure

indirectly measured by PCWP, consequently resulted in an increased LA phasic function. The change in increased LA phasic function can described by determinants of cardiovascular factors, in the same way for LV. However, in this study lacked the use of a gold standard method which would strengthen the results for measuring hemodynamic changes of the mechanical function of the LA. Besides, the difference in the degree, duration or direction of the change in volume load, might be the causes of different results obtained among studies. Further studies are also needed to elucidate the possibility of different response due to chronic adaptation of loading condition in awake clinical dogs with heart disease. Therefore, the diagnosis on the basis of LA phasic function obtained by strain and SR analysis obtained from 2D-STE should be considered with caution especially in dogs with volume loaded heart disease.

In order to clarify the use of LA function assessed by strain and SR with 2D-STE in dogs with heart disease, further studies are necessary to follow-up the effect of subclinical myocardial disease on LA strain and SR in dogs prior to apply the usefulness of these parameters in other heart diseases, such as cardiomyopathies or congenital heart diseases. Also, it is needed to investigate the relationship between goal standard measurement of LA derived hemodynamic variables and the LA phasic function in dogs with clinical condition, especially dogs with heart failure model. In addition, it would be useful if the changes in the LA strain and SR is evaluated in dogs during the use of cardiovascular medications.

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JAPANESE SUMMARY (要旨)

Assessment of left atrial function via strain analysis derived from two-dimensional speckle tracking echocardiography in dogs

（2D スペックルトラッキング心エコー図法によるストレイン解析を用いた犬の左心房機能の評価）

従来、心エコー図検査による心疾患の重症度評価には、左心房の大きさが用いられてきたが近年は左心房機能の評価に注目が集まっている。心エコー図検査による左心房機能の評価は以前から試みられており、代表的なものとしてパルスドプラ法を用いた左心室流入血流波形解析、左心房容積変化率を用いる評価方法あるいは組織ドプラ法による僧帽弁輪部移動速度測定などがあげられる。しかし、これらの方法は再現性の低さや、角度依存性を有するという点あるいは容量負荷に強く影響を受けるといった問題を抱えており、広く臨床応用されるには至っていない。以上のように左心房機能は注目されてきた領域ではあるものの、それを簡便かつ正確に測定する方法がなかった。

これを打開したのが心エコー図検査における新たな画像解析技術、スペックルトラッキング法によるストレインイメージング法である。本法は心筋局所のエコー輝

度の動きを追跡することで心筋の歪みを捉えることができる新しい方法である。この方法によって左心房心筋局所の歪み（ストレイン）を角度非依存性に高い再現性で評価することが可能となった。医学領域においては、左心房ストレインイメージング法が虚血性心疾患、僧帽弁逆流、肥大型心筋症などにおける心疾患関連事象発生の予測因子として有用であることが報告されている。

獣医学領域においても左心房機能評価の有用性は検討されてきており、容積変化率による左心房機能指標の犬種、年齢による差異の検討が正常犬において報告されている。また、過去に私たちのグループは左心房断面積変化率から求めた左心房機能指標が慢性僧帽弁疾患犬における有力な予後指標であることを報告している。一方で、スペックルトラッキング法を用いた左心房ストレインイメージング法に関する報告は少ない。臨床応用の前には検査の再現性および正常値の設定が必須であるが、これらのデータは十分ではない。また、心エコー図検査に用いる多くのパラメータは年齢や体重の影響を受けることが知られているが、左心房機能指標においては不明である。さらに、現在広く用いられている方法の多くは、容量負荷の影響を受けやすいことが問題となっている。犬で最も多い僧帽弁逆流においては左心房に容量負荷が生じているが、その影響を臨床例で検討するのは困難である。

そこで、本研究では犬の左心房ストレインイメージング法の有用性を評価することを目的とし、下記の 3 点について検討を行った。

1. 左心房ストレインイメージング法の再現性の検討

2. 左心房ストレインイメージング法の正常値の設定

3. 容量負荷が左心房ストレインイメージング法に与える影響の検討

はじめに、左心房ストレインイメージング法が臨床応用可能な検査であるかどうかを検討するために、北海道大学大学院獣医学研究院実験動物施設で飼育されている健康なビーグル犬6頭を用いて再現性の評価を行った。2人の検者がそれぞれの犬に対し、1日に3回を3日間、合計18回の検査を行い、日内変動、日差変動および検者間変動の変動係数を算出した。その結果、左心房ストレインイメージング法は、日内変動、日差変動および検者間変動のいずれも臨床的に許容可能とされる20%以下であることが示された。

続いて、北海道大学附属動物獣医療センターに来院し、健康診断を目的に心エコー図検査を実施した家庭飼育犬 120 頭を対象として左心房機能指標と年齢、体重、性別との関連性を検討した。その結果、年齢と左心房機能指標には相関関係が認められ、年齢ごとに正常値を設定する必要性が確認された。

最後に、実験的な容量負荷モデル犬において左心房機能を評価し、容量負荷が左心房機能へ与える影響を評価した。健常な実験犬に対して急速静脈内輸液による容量負荷を誘発し、心臓カテーテル法により得られた血行動態指標とストレインイメージングにより得られた左心房機能指標を比較した。その結果、ストレインイメージングによって得られた左心房機能指標は全て急性の容量負荷によって影響を受け、左房圧との正の相関が認められた。

以上のように、本研究は犬におけるストレインイメージングによって得られた左心房機能の指標が臨床応用に耐えうる再現性を有することを示した。さらに、これらの指標が年齢に応じて変動すること、さらには容量負荷の影響を受けることを明らかとした。本研究の成果は、臨床例において本法を適応し左心房機能を評価する上で重要な知見となる。

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