Effects of experimental cardiac volume loading on left atrial phasic function in healthy dogs

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OBJECTIVE
To elucidate the relationship between acute volume overload and left atrial phasic function in healthy dogs.

ANIMALS
6 healthy Beagles.

PROCEDURES
Dogs were anesthetized. A Swan-Ganz catheter was placed to measure mean pulmonary capillary wedge pressure (PCWP). Cardiac preload was increased by IV infusion with lactated Ringer solution at 150 mL/kg/h for 90 minutes. Transthoracic echocardiography was performed before (baseline) and at 15, 30, 45, 60, 75, and 90 minutes after volume loading began. At each echocardiographic assessment point, apical 4-chamber images were recorded and analyzed to derive time–left atrial area curves. Left atrial total (for reservoir function), passive (for conduit function), and active (for booster-pump function) fractional area changes were calculated from the curves.

RESULTS
Volume overload resulted in a significant increase from baseline in PCWP from 15 to 90 minutes after volume loading began. All fractional area changes at 15 to 90 minutes were significantly increased from baseline. In multiple regression analysis, quadratic regression models were better fitted to the relationships between PCWP and each of the total and active fractional area changes than were linear regression models. A linear regression model was better fitted to the relationship between PCWP and passive fractional area change.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that left atrial phasic function assessed on the basis of left atrial phasic areas was enhanced during experimental cardiac volume loading in healthy dogs. The effect of volume load should be considered when evaluating left atrial phasic function by indices derived from left atrial phasic sizes. (Am J Vet Res 2016;77:952–960)

The left atrium contributes to the maintenance of optimal cardiac performance by modulating the filling of the left ventricle through 5 phasic functions. These consist of a reservoir function (expansion associated with the inflow of blood from the pulmonary veins during ventricular systole), a conduit function (passage of blood from the pulmonary veins to the left ventricle during ventricular diastole), and a booster-pump function (augmentation of left ventricular filling during atrial contraction). In humans with heart diseases such as mitral regurgitation and dilated cardiomyopathy, an association has been identified between left atrial dysfunction and disease severity or cardiovascular events.

Echocardiography has been used for the noninvasive and simple evaluation of left atrial phasic function in humans and other animals, including dogs and cats. Common methods for such evaluations involve calculation of left atrial phasic sizes (diameters, areas, and volumes), pulsed-wave Doppler evaluation of transmitral and pulmonary venous flow, and TDI.

In human medicine, a new technique based on the 2-D speckle tracking method has been developed to calculate left atrial phasic sizes on the basis of the time-left atrial area or volume curve analysis. By this technique, the movement of the left atrial wall throughout the cardiac cycle in 2-D echocardiographic images can be automatically tracked to derive a time-left atrial area or volume curve automatically and precisely. In veterinary medicine, we recently demonstrated that left atrial phasic function can be

ABBREVIATIONS

A wave  Late diastolic transmitral flow wave
A’ wave  Late diastolic wave of myocardial velocity
CI  Confidence interval
E wave  Early diastolic transmitral flow wave
E’ wave  Early diastolic wave of myocardial velocity
EAact  Active emptying area
EApass  Passive emptying area
EAtotal  Total emptying area
LAAmax  Left atrial maximum area at end of ventricular systole
LAAmin  Left atrial minimum area at end of ventricular diastole
LAAp  Left atrial area at onset of the P wave on the ECG
PCWP  Pulmonary capillary wedge pressure
S’ wave  Systolic wave of myocardial velocity
TDI  Tissue Doppler imaging

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evaluated with clinically acceptable repeatability by this technique in healthy dogs.\textsuperscript{11} Furthermore and importantly, we demonstrated that the left atrial reservoir and booster-pump functions assessed by use of this technique were impaired during disease progression and that deterioration of the left atrial booster-pump function was an adverse prognostic factor for dogs with myxomatous mitral valve disease.\textsuperscript{12}

Basic findings for healthy dogs are lacking regarding the effect of volume loading on indices of left atrial phasic function based on time–left atrial area curve analysis. Such findings are necessary to allow future use of these indices to evaluate disease severity for dogs with heart disease. Therefore, the purpose of the study reported here was to elucidate the relationships between cardiac volume loading in healthy dogs and indices of the left atrial phasic function derived through time–left atrial area curve analysis.

**Materials and Methods**

**Animals**

Six healthy Beagles (3 males and 3 females; age, 1 to 3 years; body weight, 8.8 to 11.4 kg) that were part of a research colony at the authors’ laboratory were included in the study. Before the study began, each dog was confirmed as healthy, with no cardiac abnormalities, on the basis of unremarkable results of complete physical, ECG, and standard echocardiographic examinations (including M-mode, pulsed-wave Doppler, and color flow Doppler imaging). All procedures were approved by the Laboratory Animal Experimentation Committee, Graduate School of Veterinary Medicine, Hokkaido University.

**Study protocol**

A 20-gauge over-the-needle catheter was placed in the left and right cephalic veins of each dog to establish a route for IV infusion, and a 24-gauge over-the-needle catheter was placed in the left or right dorsal pedal artery for direct monitoring of arterial blood pressure. Each dog was given atropine sulfate\textsuperscript{a} (0.05 mg/kg, SC), cefazolin sodium hydrate\textsuperscript{b} (20 mg/kg, IV), and heparin sodium\textsuperscript{c} (100 U/kg, IV) and sedated with butorphanol tartrate\textsuperscript{d} (0.2 mg/kg, IV) and midazolam hydrochloride\textsuperscript{e} (0.1 mg/kg, IV). Anesthesia was then induced by administration of propofol\textsuperscript{f} (6 mg/kg, IV). Following endotracheal intubation, anesthesia was maintained with isoflurane\textsuperscript{g} (1.75% to 2.0%) in 100% oxygen.

End-tidal partial pressure of CO\textsubscript{2} was monitored and maintained between 35 and 45 mm Hg by the use of mechanical ventilation, with a tidal volume of 10 to 15 mL/kg and a respiratory rate of 10 to 12 breaths/min. Heart rate and arterial pressure as measured via arterial catheterization were monitored and simultaneously with echocardiographic imaging, an ECG trace (lead II) was recorded by the ECG equipment on the ultrasonographic device, in addition to that on the polygraph instrument. All data were stored digitally and analyzed off-line by 1 observer (TO). The mean of 3 consecutive cardiac cycles was calculated for pressure measurements, and the mean of 4 measurements was calculated for cardiac output.

**Conventional echocardiography**

All echocardiographic examinations were thoracically performed when dogs were in an exploratory phase by use of an ultrasonographic device\textsuperscript{h} equipped with a 3- to 7-MHz sector probe. Simultaneously with echocardiographic imaging, an ECG trace (lead II) was recorded by the ECG equipment on the ultrasonographic device, in addition to that on the polygraph instrument.

**Hemodynamic evaluation**

Mechanical ventilation of dogs was briefly stopped during the procedure to allow measurements of hemodynamic variables. Data were stored digitally. Variables included heart rate, mean arterial blood pressure, mean pulmonary arterial pressure, PCWP, mean right atrial pressure, and cardiac output. Pulmonary arterial and right atrial pressures were measured via the distal and proximal ports of a Swan-Ganz catheter, respectively. The PCWP was determined when the balloon at the end of a Swan-Ganz catheter was inflated to be wedged in a small pulmonary artery. Following pressure recordings, cardiac output was determined by use of the thermodilution technique. Briefly, a 5-mL bolus of cold saline (0.9% NaCl) solution was injected into the right atrium through the proximal port of a Swan-Ganz catheter, and pulmonary arterial blood temperature was recorded by the thermistor at the catheter tip. Cardiac output was calculated from the area under the curve for the resulting blood temperature curve by the cardiac output computer equipped on the polygraph instrument.\textsuperscript{h} The mean of 5 consecutive cardiac cycles was calculated for pressure measurements, and the mean of 4 measurements was calculated for cardiac output.
Pulsed-wave Doppler echocardiography was used to measure the transmural flow velocity with the sample volume positioned at the tip of the mitral valve leaflets from the left apical 4-chamber view. Peak velocities of the E wave and A wave were measured, and the ratio of the peak velocity of the E wave to the peak velocity of the A wave was calculated. When the E and A waves were completely or partially fused, the peak velocities of the E and A waves and the ratio involving them were not determined.

From the left apical 5-chamber view, the aortic Doppler flow profile was obtained with the sample volume placed immediately below the aortic valve. Left ventricular ejection time was measured from the onset to the end of the aortic flow. Left ventricular pre-ejection period was also measured as the interval from the start of the QRS complex to the beginning of aortic flow, and the ratio of the left ventricular pre-ejection time to ejection time was calculated. Values of the pulsed Doppler-derived myocardial performance index were calculated by subtracting the left ventricular ejection time from the interval from the cessation to onset of transmural flow and dividing by left ventricular ejection time.

Tissue Doppler imaging velocities of myocardial motion were recorded from the left apical 4-chamber view, with the sample volume positioned at the septal mitral annulus. Peak velocities of S’ wave, E’ wave, and A’ wave were measured. The ratio of the peak velocity of the E’ wave to peak velocity of the A’ wave and ratio of the peak velocity of the E wave to the peak velocity of the E’ wave were also calculated. Peak velocities of the E’ and A’ waves, the ratio of the peak velocity of the E’ wave to peak velocity of the A’ wave, and the ratio of the peak velocity of the E wave to the peak velocity of the E’ wave were not measured when the E’ and A’ waves were completely or partially fused.

In addition, TDI-derived myocardial performance index values were determined by adding the isovolumic contraction time to the isovolumic relaxation time and dividing by the duration of the S’ wave. Isovolumic contraction time was defined as the interval from the end of the A’ wave to the onset of the S’ wave. Isovolumic relaxation time was defined as the interval from the end of the S’ wave to the onset of the E’ wave. Duration of the S’ wave was defined as the interval from the onset to the end of the S’ wave.

**2-D speckle tracking echocardiography of the left atrium**

An apical 4-chamber view was obtained by second harmonic grayscale imaging, with the frequency, depth, and sector width adjusted for frame-rate optimization (117 to 154 frames/s). In accordance with previous studies, echocardiographic images were analyzed by use of off-line software. A frame corresponding to the time of the peak R wave on the ECG trace was selected as indicating end of ventricular diastole, and the endocardium of the left atrium was manually traced in that frame. The area of the left atrium was then automatically calculated by the software in each subsequent frame throughout the cardiac cycle to derive a time–left atrial area curve (Figure 1).

Tracking quality was assessed visually. If the tracking quality was unsatisfactory (ie, the blood-tissue border was not tracked), manual tracing of the endocardium was repeated. The LAAtot, LAAmax, and LAAmin were determined. Variables used as indicators of the left atrial phasic function were calculated as follows (Figure 1):

\[
E_{Atot} = \text{LAA}_{\text{max}} - \text{LAA}_{\text{min}}
\]
\[
E_{\text{Apass}} = \text{LA}_{\text{max}} - \text{LA}_{\text{Ap}}
\]
\[
E_{\text{Aact}} = \text{LA}_{\text{Ap}} - \text{LAA}_{\text{min}}
\]

Total fractional area change = 100X EA\text{Atot}/\text{LAA}_{\text{max}}

Passive fractional area change = 100X EA\text{Apass}/LA\text{max}

Active fractional area change = 100X EA\text{Aact}/LA\text{Ap}

Total emptying area and fractional area change were calculated as indicators of the reservoir function, whereas EA\text{Apass} and passive fractional area change were determined as indicators of the conduit function. Active emptying area and fractional area change were calculated as indicators of booster-pump function. From a time–left atrial area curve without the diastasis portion, only the LAAmax, LAmin, and EA\text{Atot} and total fractional area change were calculated.

**Statistical analysis**

Commercially available statistical software was used for statistical analyses. For all analyses,
were performed by obtaining least squares means between the baseline and each assessment point was assessed with the effect of time on values of the measured variables. The effect and dog identity as a random effect. The effect was developed, with time (baseline and 15, 30, 45, 60, 75, and 90 minutes) as a categorical fixed effect and applying the Bonferroni correction to account for multiple comparisons.

To investigate the relationship between PCWP and each of the indices of left atrial phasic function, multiple regression analysis was performed with each of the left atrial function parameters as the dependent variable. In model 1, the PCWP and dummy coding of the enrolled dogs were included as covariates (linear regression model). In model 2, the linear and applying the Bonferroni correction to account for multiple comparisons.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean PAP (mm Hg)</td>
<td>9.5 (7.4–11.6)</td>
<td>12.7 (10.5–14.8)†</td>
<td>15.0 (12.9–17.1)†</td>
<td>16.3 (14.2–18.5)†</td>
<td>17.3 (15.2–19.5)†</td>
<td>17.5 (15.3–19.6)†</td>
<td>17.5 (15.4–19.6)†</td>
</tr>
<tr>
<td>Mean PCWP (mm Hg)</td>
<td>3.3 (1.3–5.4)</td>
<td>8.0 (6.0–10.0)†</td>
<td>10.2 (8.1–12.2)†</td>
<td>11.3 (9.3–13.4)†</td>
<td>12.7 (10.6–14.7)†</td>
<td>13.2 (11.1–15.2)†</td>
<td>14.0 (12.0–16.0)†</td>
</tr>
<tr>
<td>Mean RAP (mm Hg)</td>
<td>0.3 (0.9–1.6)</td>
<td>5.3 (4.1–6.6)†</td>
<td>6.3 (5.1–7.6)†</td>
<td>7.2 (5.9–8.4)†</td>
<td>7.3 (6.1–8.6)†</td>
<td>8.2 (6.9–9.4)†</td>
<td>8.3 (7.1–9.6)†</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>56 (49–62)</td>
<td>54 (48–61)</td>
<td>57 (50–63)</td>
<td>58 (52–65)</td>
<td>60 (53–66)</td>
<td>60 (54–67)</td>
<td>59 (53–66)</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>2.1 (1.9–2.4)</td>
<td>2.5 (2.3–2.8)†</td>
<td>2.8 (2.6–3.1)†</td>
<td>2.9 (2.7–3.2)†</td>
<td>3.0 (2.7–3.2)†</td>
<td>3.1 (2.9–3.4)†</td>
<td>3.1 (2.8–3.3)†</td>
</tr>
</tbody>
</table>

Table 1—Least squares means (95% CIs) obtained from linear mixed model analysis of hemodynamic data obtained before (baseline) and at various times during experimental cardiac volume overloading in 6 healthy Beagles.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>E wave (m/s)§</td>
<td>0.64 (0.58–0.70)</td>
<td>0.81 (0.74–0.87)†</td>
<td>0.80 (0.74–0.86)</td>
<td>0.80 (0.73–0.87)</td>
<td>0.85 (0.77–0.93)†</td>
<td>0.83 (0.75–0.91)†</td>
<td>0.78 (0.70–0.86)†</td>
</tr>
<tr>
<td>A wave (m/s)§</td>
<td>0.34 (0.28–0.40)</td>
<td>0.45 (0.39–0.51)§</td>
<td>0.47 (0.41–0.53)§</td>
<td>0.49 (0.42–0.55)§</td>
<td>0.47 (0.39–0.54)§</td>
<td>0.50 (0.43–0.57)§</td>
<td>0.51 (0.44–0.58)§</td>
</tr>
<tr>
<td>E wave/A wave§</td>
<td>2.02 (1.69–2.35)</td>
<td>1.83 (1.51–2.16)</td>
<td>1.74 (1.42–2.07)</td>
<td>1.69 (1.31–2.06)</td>
<td>1.84 (1.42–2.27)</td>
<td>1.65 (1.23–2.08)</td>
<td>1.54 (1.16–1.91)</td>
</tr>
<tr>
<td>PEP (ms)</td>
<td>69 (63–74)</td>
<td>57 (51–62)†</td>
<td>52 (47–58)†</td>
<td>52 (47–58)†</td>
<td>52 (47–58)†</td>
<td>53 (48–59)†</td>
<td>53 (47–59)†</td>
</tr>
<tr>
<td>PEP:ET</td>
<td>0.38 (0.35–0.41)</td>
<td>0.25 (0.22–0.28)</td>
<td>0.22 (0.19–0.25)</td>
<td>0.21 (0.18–0.24)</td>
<td>0.21 (0.18–0.24)</td>
<td>0.21 (0.18–0.24)</td>
<td>0.20 (0.17–0.23)</td>
</tr>
<tr>
<td>PD-MPI</td>
<td>0.49 (0.40–0.57)</td>
<td>0.33 (0.27–0.43)§</td>
<td>0.29 (0.21–0.38)§</td>
<td>0.29 (0.21–0.38)§</td>
<td>0.32 (0.23–0.40)§</td>
<td>0.30 (0.21–0.38)§</td>
<td>0.32 (0.23–0.40)§</td>
</tr>
</tbody>
</table>

Table 2—Least squares means (95% CIs) obtained from linear mixed model analysis of conventional echocardiographic data obtained before (baseline) and at various times during experimental cardiac volume overloading in 6 healthy Beagles.

*Value differs significantly (P < 0.05) from corresponding baseline value. †Value differs significantly (P < 0.01) from corresponding baseline value. PAP = Pulmonary arterial blood pressure. RAP = Right atrial blood pressure. MAP = Mean arterial blood pressure as measured via arterial catheterization.

Values of P < 0.05 were considered significant. Normal distribution of the data was confirmed with the Shapiro-Wilk test. A linear mixed model was developed, with time (baseline and 15, 30, 45, 60, 75, and 90 minutes) as a categorical fixed effect and dog identity as a random effect. The effect of time on values of the measured variables was assessed with the F test. Pairwise comparisons between the baseline and each assessment point were performed by obtaining least squares means and applying the Bonferroni correction to account for multiple comparisons.
Table 3—Least squares means (95% CIs) obtained from linear mixed model analysis of left atrial phasic function data determined via 2-D speckle tracking echocardiography before (baseline) and at various times during experimental cardiac volume overloading in 6 healthy Beagles.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAAmax (cm²)</td>
<td>5.34 (4.65–6.02)</td>
<td>7.06 (6.37–7.75)*</td>
<td>7.76 (7.07–8.45)*</td>
<td>7.88 (7.19–8.57)*</td>
<td>7.92 (7.23–8.61)*</td>
<td>8.14 (7.45–8.83)*</td>
<td>8.13 (7.44–8.81)*</td>
</tr>
<tr>
<td>LAAp (cm²)†</td>
<td>3.91 (3.51–4.32)</td>
<td>4.70 (4.29–5.10)</td>
<td>5.03 (4.62–5.43)*</td>
<td>4.97 (4.56–5.38)*</td>
<td>4.74 (4.31–5.16)*</td>
<td>4.84 (4.42–5.27)*</td>
<td>4.92 (4.48–5.36)*</td>
</tr>
<tr>
<td>LAAmin (cm²)</td>
<td>3.34 (2.89–3.80)</td>
<td>3.70 (3.24–4.16)</td>
<td>3.88 (3.43–4.34)</td>
<td>3.94 (3.48–4.39)</td>
<td>3.89 (3.43–4.35)</td>
<td>4.01 (3.55–4.47)*</td>
<td>4.00 (3.54–4.46)*</td>
</tr>
<tr>
<td>Total</td>
<td>2.00 (1.62–2.38)</td>
<td>3.36 (2.98–3.74)</td>
<td>3.87 (3.49–4.25)</td>
<td>3.95 (3.57–4.33)</td>
<td>4.03 (3.65–4.41)</td>
<td>4.13 (3.75–4.51)*</td>
<td>4.13 (3.75–4.51)*</td>
</tr>
<tr>
<td>Passive†</td>
<td>1.43 (1.08–1.77)</td>
<td>2.37 (2.02–2.71)</td>
<td>2.73 (2.39–3.07)*</td>
<td>2.74 (2.37–3.10)*</td>
<td>3.00 (2.61–3.40)*</td>
<td>3.49 (3.10–3.89)*</td>
<td>3.16 (2.73–3.59)*</td>
</tr>
<tr>
<td>Active†</td>
<td>0.57 (0.34–0.79)</td>
<td>0.99 (0.77–1.22)</td>
<td>1.14 (0.92–1.37)</td>
<td>1.09 (0.86–1.32)</td>
<td>1.02 (0.77–1.27)</td>
<td>1.03 (0.79–1.28)*</td>
<td>1.06 (0.80–1.33)*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fractional area change (%)</th>
<th>Baseline</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>37.3 (34.1–40.5)</td>
<td>47.5 (44.3–50.7)*</td>
<td>50.0 (46.7–53.2)*</td>
<td>50.0 (46.8–53.2)*</td>
<td>51.0 (47.8–54.2)*</td>
<td>51.0 (47.8–54.2)*</td>
<td>51.0 (47.8–54.2)*</td>
</tr>
<tr>
<td>Passive†</td>
<td>26.6 (23.6–29.5)</td>
<td>33.4 (30.5–36.3)*</td>
<td>35.3 (32.4–38.2)*</td>
<td>35.4 (32.3–38.5)</td>
<td>38.9 (35.6–42.3)*</td>
<td>39.3 (36.0–42.7)*</td>
<td>38.9 (35.2–42.6)*</td>
</tr>
<tr>
<td>Active†</td>
<td>14.6 (10.3–18.9)</td>
<td>21.1 (16.8–25.4)</td>
<td>22.6 (18.3–26.9)*</td>
<td>22.0 (17.6–26.5)*</td>
<td>21.7 (17.0–26.3)*</td>
<td>21.4 (16.8–26.0)*</td>
<td>21.7 (16.8–26.5)*</td>
</tr>
</tbody>
</table>

*Value differs significantly (P < 0.01) from corresponding baseline value. †Data represent all 6 dogs at baseline and 15 and 30 minutes, 5 dogs at 45 minutes, 4 dogs at 60 and 75 minutes, and 3 dogs at 90 minutes.

The LAAmax, LAAmin, EAtotal, and total fractional area change were calculated from time–left atrial area curves without the diastasis portion.

Results

Hemodynamic and echocardiographic data for the 6 healthy anesthetized Beagles, including indices of left atrial phasic function at baseline (before volume loading began) and during volume loading, were summarized (Tables 1–3). Significant (P < 0.05) time effects were detected for all but 6 hemodynamic and echocardiographic variables (mean arterial blood pressure, ratio of the peak velocity of the E wave to the peak velocity of the A wave, peak velocity of the S’ wave, ratio of the peak velocity of the E’ wave to the peak velocity of the A’ wave, ratio of the peak velocity of the E wave to the peak velocity of the E’ wave, and isovolumic relaxation time).

Mean PCWP was significantly greater than at baseline from 15 to 90 minutes after volume loading began and reached approximately 10 mm Hg greater than at baseline after approximately 45 to 60 minutes (Table 1). Heart rate was significantly greater than at baseline 60 minutes after volume loading began. Furthermore, volume loading induced a significant increase from baseline in mean pulmonary arterial pressure, right atrial pressure, and cardiac output from 15 to 90 minutes.

Peak velocities of E and A waves and ratio of the peak velocity of the E wave to the peak velocity of the A wave were determined without fusion of the E and A waves for all dogs at baseline and at 15 and 30 minutes, for 4 dogs at 45 and 90 minutes, and for 3 dogs at 60 and 75 minutes after volume loading began. Significant increases from baseline were identified in peak velocities of the E and A waves and left ventricular ejection time (Table 2). Also, compared with baseline values, there were significant decreases from 15 to 90 minutes in the left ventricular pre-ejection period, ratio of left ventricular ejection time to pre-ejection period, and pulsed Doppler-derived myocardial performance index value.

Peak velocities of the E’ and A’ waves and the ratio of the peak velocity of the E’ wave to peak velocity of the A’ wave were determined without fusion of the E’ and A’ waves for all dogs at baseline and at 15 and 30 minutes, for 5 dogs at 45 minutes, for 3 dogs at 60 minutes, and for 4 dogs at 75 and 90 minutes after volume loading began. Also, the ratio of the peak velocity of the E wave to peak velocity of the E’ wave was obtained for all dogs at baseline and at 15 and 30 minutes, for 4 dogs at 45 and 90 minutes, and for 3 dogs at 60 and 75 minutes. Volume loading induced significant increases from baseline in peak velocities of the E’ and A’ waves and the duration of the S’ wave at 15 to 90 minutes (Table 2). Furthermore, the TDI-derived myocardial performance index value was significantly decreased from baseline from 15 to 90 minutes. Isovolumic contraction time was significantly decreased from baseline from 45 to 90 minutes after volume loading began.

The LAAP, EAPass, EAact, and passive and active fractional area changes were measured from time–left atrial area curves with the diastasis portion for all dogs at baseline and at 15 and 30 minutes, for 5 dogs at 45 minutes, for 4 dogs at 60 and 75 minutes, and for 3 dogs at 90 minutes after volume loading.
began (Figure 2). Volume loading caused significant increases from baseline in LAAmax, LAAp, EAtotal, EApass, and EAact and also caused total, passive, and active fractional area changes from 15 to 90 minutes (Table 3). The LAAmin was significantly increased from baseline from 30 to 90 minutes.

A quadratic multiple regression modeling approach (model 2) provided the model of better fit (Table 4; Figure 3).

**Discussion**

The goal of the present study was to obtain basic findings for healthy dogs of changes in indices of left atrial phasic function assessed by means of time–left atrial area curve analysis in response to cardiac volume loading. This was necessary to elucidate potential relationships between these indices and changes in volume load caused by disease progression in dogs with heart disease. Findings indicated that left atrial phasic function determined in this manner was enhanced in healthy dogs during volume loading.
addition, the results suggested that the relationships between volume loading and reservoir and booster-pump functions were quadratic rather than linear, whereas conduit function enhanced linearly with volume loading. These data represent the first to be reported on the responses of left atrial phasic function evaluated by means of 2-D speckle tracking echocardiography attributable to volume loading in dogs.

Evidence is accumulating to indicate that the eventual change in left atrial function is determined by various cardiovascular factors as well as by left atrial intrinsic properties. The reservoir function is modulated by left atrial intrinsic relaxation, left atrial chamber compliance, left atrial booster-pump function, left ventricular systolic function associated with the descent of the left ventricular base, and right ventricular output.\(^1,2\) The conduit function is determined by left ventricular relaxation and the early diastolic pressure gradient between the left ventricle and atrium.\(^2\) The booster-pump function is modulated by left atrial intrinsic contractility, left atrial preload (ie, left atrial volume before active contraction), and left atrial afterload determined by left ventricular end-diastolic pressure dependent on left ventricular volume and chamber compliance, left atrial radius, and left atrial wall thickness.\(^2\) Therefore, evaluation of left atrial phasic function in dogs with heart disease would facilitate detection of the deterioration of the aforementioned cardiovascular factors associated with disease progression or assessment of their improvement during cardiovascular treatment.

Results of previous studies\(^2,19,20\) suggest that volume overload could initially enhance and then impair left atrial phasic function. During early phases of volume loading, the booster-pump function is augmented by the Frank-Starling mechanism associated with an increased left atrial preload, as supported by the increase in LAAp identified in the present study. Also, the increase in volume load enhances the reservoir function by augmenting the left atrial booster-pump function and left ventricular systolic function, as supported by decreases in the ratio of the left ventricular pre-ejection time to ejection time and the left ventricular isovolumic contraction time in the present study, and by increasing cardiac output in accordance with the Frank-Starling mechanism.\(^2,19,20\) Furthermore, volume loading augments the conduit function by increasing the early diastolic pressure gradient between the left ventricle and atrium,\(^2\) as suggested by increases in the peak velocities of \(E\) and \(E’\) waves in the present and previous studies.\(^13,14\)

On the contrary, during later phases of volume loading, the increase in left atrial afterload, as suggested by increases in the LAAp and PCWP in the present study, could impair the booster-pump function (ie, afterload mismatch). Also, a previous experimental study\(^21\) involving healthy dogs revealed...
that volume loading decreases the degree of left atrial compliance, which could suppress the reservoir function. Furthermore, the impairment of booster-pump function and left ventricular systolic function caused by the increase in left ventricular afterload (the increased left ventricular radius) could impair reservoir function. Besides, left ventricular relaxation is impaired during volume loading, causing the impairment of conduit function, although this effect would be surpassed by the effect of the increased early diastolic pressure gradient between the left ventricle and atrium in healthy dogs receiving volume loading. Taken together, these findings indicated that the enhancing effect of volume loading on the 3 phasic functions could have surpassed its suppressive effect. In addition, during later phases of volume loading, the enhancing effect on the reservoir and booster-pump functions could have been offset by its suppressive effect.

The findings reported here have several clinical implications. First, the data indicated that detection of left atrial dysfunction on the basis of indices of left atrial phasic areas might be precluded by the enhancing effect of volume loading on left atrial phasic function. This could be of concern for dogs with myxomatous mitral valve disease, particularly disease of milder severity in which the left atrial myocardial impairment and the decrement in left atrial wall compliance related to volume loading are still mild and the afterload mismatch does not occur in the left atrium. Second, considering the quadratic relationships identified between volume loading and variables reflecting reservoir and booster-pump functions, it is possible that these variables rather than those reflecting conduit function would be more useful for evaluation of disease severity. In humans with asymptomatic mitral valve regurgitation, all of the left atrial reservoir, conduit, and booster-pump functions evaluated on the basis of left atrial phasic volumes are enhanced. On the other hand, in humans with severe mitral valve regurgitation requiring mitral surgery, the left atrial reservoir and booster-pump functions are deteriorated, whereas the conduit function is not. In addition, in dogs with myxomatous mitral valve disease, we have demonstrated that impairment of the left atrial reservoir and booster-pump functions is associated with shorter survival times.

The present study had several limitations. First, our data could not be extrapolated to dogs with greater PCWPs. In a study of healthy dogs given an IV infusion of dextran solution, indices of left atrial reservoir and booster-pump functions calculated from left atrial diameters as measured with a sonomicrometer were higher than baseline values at mean left atrial pressures < 15 mm Hg and then decreased to baseline values at pressures > 15 mm Hg. Second, our study lacked the use of invasive cardiovascular procedures for measurement of left heart function. Left atrial pressure-volume loop analysis, which is the reference standard for evaluation of left atrial phasic function, may be needed to identify changes in left atrial intrinsic properties. In addition, our assumptions regarding left ventricular properties were made solely on the basis of echocardiographic variables.

Third, our study lacked examination of dogs after furosemide was administered; therefore, it remains unknown whether index values based on left atrial phasic areas would be decreased by the unloading achieved with cardiovascular medications, such as diuretics, venodilators, and inodilators, contrary to volume loading. When values of these indices are decreased by unloading, assessment of hemodynamic improvement attributable to cardiovascular medications on the basis of indices representing left atrial phasic areas can be obscured because the decrease in the volume load might mask the augmentation of left atrial phasic function. However, this presumption, based on findings in healthy dogs, may not be applicable to dogs with clinical changes in left atrial intrinsic properties and chronic and severe volume overload. Indeed, inodilators can enhance the left atrial reservoir and booster-pump functions despite a decrease in left atrial volume load in humans with heart failure.

Fourth, a complete autonomic nerve block was not used in the present study. Acute volume loading by fluid infusion, particularly in dogs, can cause the Bainbridge reflex, by which withdrawal of vagal tone and an associated increase in the heart rate can occur. This might have led to a state of sympathetic dominance, augmenting left atrial function, or the increase in heart rate might have prevented some of left atrial volume loading.

Fifth, sample sizes were small and indices of conduit and booster-pump functions were not determined at all echocardiographic assessment points for each dog, as also occurred in a previous study, in which variables representing transmitral flow in dogs receiving volume loading could not be measured at all assessment points. The failure to determine indices of conduit and booster-pump functions was possibly attributable to the decrease in the interval from the onset of left atrial passive emptying to the end of the active emptying associated with the prolonged left ventricular ejection time (Figure 2) and the increase in the heart rate caused by volume loading.

Sixth, the effects of anesthesia on cardiac function could not be eliminated. Isoflurane can cause vasodilation (arteriolar dilation and venodilation) and impairment of myocardial contractility and lusitropy in the left ventricle and atrium. Therefore, the enhancing effect of volume loading on left atrial function in the dogs of the present study might have been blunted by the use of isoflurane.

Results of the present study indicated that in healthy dogs, left atrial phasic function determined on the basis of left atrial phasic areas obtained by time–left atrial area curve analysis was enhanced during volume loading. The effect of volume load should
be considered when evaluating left atrial phasic function by indices derived from left atrial phasic sizes in dogs with heart disease.

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Footnotes

a. Atropine sulfate injection, Mitsubishi Tanabe Pharma Corp, Osaka, Japan.
b. Cefamezin α, Astellas Pharma Inc, Tokyo, Japan.
c. Heparin sodium injection, Ajinomoto Pharmaceuticals Co Ltd, Tokyo, Japan.
d. Veturphale, Meiji Seika Pharma Co Ltd, Tokyo, Japan.
e. Dormicium injection, Astellas Pharma Inc, Tokyo, Japan.
g. Isoflu, DS Pharma Animal Health Co Ltd, Osaka, Japan.
h. RMC-4000, Nikoh Koden Co, Tokyo, Japan.
i. FAST-CATH hemostasis introducers, St. Jude Medical Inc, Minnetonka, Minn.
k. Solulact, Terumo Corp, Tokyo, Japan.
l. Lasix Injection, Sanofi K K, Tokyo, Japan.
m. HI VISION Preirus, Hitachi Aloka Medical Ltd, Tokyo, Japan.
n. EUP-852, Hitachi Aloka Medical Ltd, Tokyo, Japan.
o. Left Atrial Tracking, Hitachi Aloka Medical Ltd, Tokyo, Japan.
p. JMP Pro, version 10.0, SAS Institute Inc, Cary, NC.
q. IBM SPSS Statistics, version 21, IBM Corp, Chicago, Ill.

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