Abnormalities of right ventricular (RV) function are associated with poor outcomes in human patients who have right-sided heart disease, such as pulmonary hypertension, as well as in patients with congenital heart disease or left-sided heart disease. Recent studies have shown that RV dysfunction is closely associated with poor outcomes in dogs with myxomatous mitral valve disease, which is the most common cardiac disease of dogs. Therefore, the assessment of RV morphology and function is receiving greater attention in veterinary medicine. Invasive right heart catheterization is the gold-standard technique for assessment of RV function in people and dogs. In particular, catheterization-derived pressure-volume loop indices such as end-systolic elastance (ESE) can be used to assess load-independent cardiac function. However, catheterization techniques have limited applicability in dogs owing to the requirement for general anesthesia, and noninvasive echocardiographic indices have been used for clinical assessment of RV function, such as tricuspid annular plane systolic excursion (TAPSE), RV fractional area change, peak myocardial systolic velocity of the lateral tricuspid annulus, and RV free wall only–assessed RVS and RVSR significantly decreased in the inspiratory phase, compared with the expiratory phase. There were no significant differences in end-systolic elastance or RVGA-assessed RVS or RVSR between respiratory phases. The RVGA-assessed RVSR was significantly associated with stroke volume and end-systolic elastance.

CONCLUSIONS AND CLINICAL RELEVANCE
Specific RV echocardiographic variables were significantly affected by respiration. In contrast, RVS and RVSR determined with RVGA were not affected by respiration and were associated with hemodynamic indicators of RV contractility.

*Investigation of the influence of manual ventilation-controlled respiration on right ventricular pressure-volume loops and echocardiographic variables in healthy anesthetized dogs*

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OBJECTIVE
To evaluate the influence of manual ventilation-controlled respiration on right ventricular (RV) pressure-volume loop–derived and echocardiographic variables in dogs.

ANIMALS
8 healthy, anesthetized Beagles.

PROCEDURES
In a prospective experimental study, pressure-volume catheters were percutaneously inserted into the right ventricle of each dog, and manual ventilation was performed; RV pressure-volume loop (hemodynamic) data and conventional echocardiographic variables were assessed. Two-dimensional speckle tracking echocardiography–derived RV strain (RVS) and RV systolic strain rate (RVSR) were obtained with RV free wall–only analysis (free wall) and RV global analysis (RVGA; interventricular septum). Variables were compared between end-inspiratory and end-expiratory phases of respiration by statistical methods. Multiple regression analysis was used to assess associations between selected hemodynamic and echocardiographic variables.

RESULTS
The RV pressure significantly increased, and RV volume, stroke volume, tricuspid annular plane systolic excursion, RV fractional area change, peak myocardial systolic velocity of the lateral tricuspid annulus, and RV free wall only–assessed RVS and RVSR significantly decreased in the inspiratory phase, compared with the expiratory phase. There were no significant differences in end-systolic elastance or RVGA-assessed RVS or RVSR between respiratory phases. The RVGA-assessed RVSR was significantly associated with stroke volume and end-systolic elastance.

CONCLUSIONS AND CLINICAL RELEVANCE
Specific RV echocardiographic variables were significantly affected by respiration. In contrast, RVS and RVSR determined with RVGA were not affected by respiration and were associated with hemodynamic indicators of RV contractility.
the echocardiographic indices of RV function. An additional study found that the tidal volume in patients undergoing mechanical ventilation can also influence these variables. However, to the authors’ knowledge, no studies that assessed the influence of respiration on echocardiographic assessment of RV function in dogs have been published.

The purpose of the study reported here was to evaluate the influence of positive-pressure ventilation on RV morphology and function in dogs as measured with the gold-standard method of right heart catheterization–derived pressure-volume loop assessment and the more commonly used clinical method of echocardiography. We hypothesized that positive-pressure ventilation would decrease venous return, affecting related RV echocardiographic variables and influencing the clinical evaluation of RV function.

Materials and Methods

Animals

Eight university-owned, healthy adult Beagles (4 males and 4 females; mean ± SD age and body weight, 1.4 ± 0.1 years and 9.9 ± 1.0 kg, respectively) were used in the hypothesis-driven, experimental study. Each dog was determined to be healthy on the basis of results of a complete physical examination, CBC and serum biochemical analysis, thoracic and abdominal radiography, transthoracic and abdominal ultrasonography, and oscillometric method–derived blood pressure measurement.

Study protocol

All procedures followed the Guidelines for Institutional Laboratory Animal Care and Use of Nippon Veterinary and Life Science University in Japan, and the study was approved by the university’s Ethical Committee for Laboratory Animal Use (approval No. 2020S-46).

After a period of food withholding for 12 hours, all dogs were premedicated with butorphanol tartrate (Vetorphale; 0.2 mg/kg, IV) and midazolam hydrochloride (Dormicum; 0.2 mg/kg, IV) and received cefazolin sodium hydrate (Cefamezin α; 20.0 mg/kg, IV). Anesthesia was induced with propofol (4.0 to 6.0 mg/kg to effect, IV) and maintained with 1.5% to 2.0% isoflurane in oxygen. Dogs received an infusion of lactated Ringer solution at a rate of 3.0 mL/kg/h throughout the experiment. Heart rate was calculated from the mean R-R interval across 5 consecutive pressure-volume loops at the end of the inspiratory and expiratory phases of respiration. These included maximal and minimal RVP, and maximal and minimal first derivative of RVP (FDRVP) as well as the following 3 calculated variables: stroke volume, cardiac output, and ESE. Stroke volume and cardiac output were calculated from the maximal RVV, minimal RVV, and heart rate, and ESE was calculated by use of a typical loop with the RV single-beat method as previously described. Heart rate was calculated from the mean R-R intervals obtained from the same cardiac cycle used for hemodynamic measurements.

Echocardiographic measurements

Conventional and Doppler echocardiographic examinations and measurements were performed by 1 observer (YY) using an echocardiographic system (Vivid IQ; GE Healthcare Co) and a 3.5- to 6.9-MHz transducer. Lead II ECGs were recorded simultaneously and displayed on the images. All data, except for the Doppler examination recordings, were stored separately as inspiratory- and expiratory-phase data. Doppler images were stored so that respiratory variation on the variables of interest, spontaneous respiration was restrained and each dog was manually ventilated during the examination. Dogs were positioned in left lateral recumbency, and the right neck region was clipped, aseptically prepared, and draped. A 6F sheath introducer (Fast-Cath hemostasis; St Jude Medical Atrial Fibrillation Division Inc) was inserted into the right jugular vein with a Seldinger retention technique. A 5F pressure-volume catheter (Ventri-Cath 507; Millar Inc) was positioned in the right ventricle by use of fluoroscopic guidance and manually adjusted to obtain accurate RV pressure (RVP)-volume loops. After a 10-minute stabilization period, hemodynamic measurement and echocardiography were performed with manual ventilation so that the airway pressure was 10 to 15 mm Hg at 5-second intervals. When all experimental protocols were completed, the catheter and sheath introducer were removed and atraumatic was performed with direct manual pressure over the catheterization site. All dogs were then allowed to recover completely from anesthesia. Dogs were administered cephalexin (Cefalexin; 20.0 mg/kg, PO, q 12 h) for 3 days and robenacoxib (Onsiro; 2.0 mg/kg, SC, q 24 h as needed) for 3 days. After completing the study protocol, all dogs were transferred for inclusion in another study in our institution.
RV end-diastolic wall thickness (RVEDWT) were measured on the left apical 4-chamber view optimized for the right side of the heart (RV focus view). The RVEDA and RVESA were measured by tracing the endocardial border of the RV inflow region, excluding the papillary muscles, at end-diastole and end-systole, respectively. The RVEDWT was measured as the largest diameter of the RV free wall at end-diastole by use of the B-mode method.

For indicators of RV function, TAPSE, RVFAC, RVS, and RV myocardial performance index (RVMPI) were measured. All RV functional indices were obtained from the RV focus view. The TAPSE was measured as the total displacement of the lateral tricuspid annulus from end-diastole to end-systole by use of the B-mode method. The RVFAC was calculated as a percentage with the following formula:

\[
\frac{(RVEDA - RVESA)}{RVEDA} \times 100\%
\]

The RVs' and RVMPI were obtained from the tissue Doppler imaging–derived lateral tricuspid annular motion wave. The RVMPI was measured according to the following formula:

\[
\frac{(b - a)}{a}
\]

where \(a\) represents the duration of the systolic tricuspid annular motion wave and \(b\) represents the interval from the end of the late diastolic tricuspid annular motion wave to the onset of the early diastolic tricuspid annular motion wave.

All 2D-STE analyses were performed by the same investigator using the same ultrasound equipment and offline workstation as those used for standard echocardiography. The RV strain (RVS) and RV systolic strain rate (RVSR) were obtained from the RV focus view by use of left ventricular 4-chamber algorithms. The region of interest for 2D-STE was defined by manually tracing the RV endocardial border. The RV free wall–only analysis (RVFWA; 3 segments of the RV free wall) was performed by tracing the endocardial border from the level of the lateral tricuspid annulus to the RV apex. In addition, RV global analysis (RVGA; 3 segments each of the interventricular septum and RV free wall) was performed by tracing the endocardial border from the lateral tricuspid annulus to the septal tricuspid annulus (including the interventricular septum) via the RV apex. Manual adjustments were made to include and track the entire myocardial thickness throughout the cardiac cycle when necessary. When the automated software could not track the myocardial regions, the regions of interest were retraced and recalculated. The RVS was defined as the absolute value of the negative peak obtained from the strain wave. The RVSR was defined as the absolute value of the negative peak obtained from the strain rate curve during the ejection phase as identified from the ECG (Figure 1).

**Statistical analysis**

All statistical analyses were performed with commercial statistical software (EZR software version 1.41; Saitama Medical Center, Jichi Medical University). The normality of data was evaluated with the Shapiro-Wilk test. Continuous variables were reported as the mean ± SD. To evaluate respiratory variation, all continuous variables were compared between inspiratory and expiratory phases by use of the paired \(t\) test for normally distributed data and the Wilcoxon signed-rank test for non-normally distributed data. Additionally, multiple regression analysis with a backward stepwise approach was performed to assess the relationships between hemodynamic variables, especially stroke volume, maximal FDRVP, ESE, and echocardiographic indices. Variables that had values of \(P < 0.20\) in single regression analysis and were adjusted for confounding factors were included in the multiple regression analyses. Values of \(P < 0.05\) were considered significant.

**Results**

In total, 83 measurements (50 hemodynamic and 33 echocardiographic) were obtained from the study sample of 8 dogs. The end-tidal partial pressure of carbon dioxide, tissue oxygen saturation, and blood pressure measurements were within the expected ranges for healthy dogs throughout the experimental procedures. Dogs were anesthetized for 42 ± 6 minutes and received a total volume of 20.7 ± 1.6 mL of lactated Ringer solution during the procedures.

**Hemodynamic measurements**

The hemodynamic measurements obtained from RVP-volume loops in each respiratory phase were summarized (Table 1). The RVP-volume loops shifted to the upper left side in the inspiratory phase (Figure 2). In accordance with this, maximal and minimal RVP increased significantly, and maximal and minimal RVV, stroke volume, and cardiac output decreased significantly, compared with the values for the expiratory phase. However, heart rate, maximal and minimal FDRVP, and ESE did not differ significantly between respiratory phases.

**Echocardiographic measurements**

Comparisons of echocardiographic measurements between respiratory phases were provided (Table 2). For RV size, RVEDA significantly decreased, RVEDWT significantly increased, and RVESA did not change significantly in the inspiratory phase, compared with the respective expiratory phase values. Among the echocardiographic indices for RV function, TAPSE, RVFAC, and RVS' were significantly decreased and RVMPI was significantly increased in the inspiratory phase.

The 2D-STE examinations were recorded at 116 ± 3.2 frames/s. All myocardial segments were included in the statistical analyses. The RVS and RVSR determined with RVFWA decreased significantly in the inspiratory phase, whereas the RVS and RVSR deter-
mined with RVGA did not differ significantly between respiratory phases.

Regression analysis

In the single regression analyses, variables with values of \( P < 0.20 \) for association with stroke volume included RVs' (\( P = 0.046 \)), RVFAC (\( P = 0.110 \)), and RVSR determined with the RVGA method (\( P = 0.185 \)). For association with maximal FDRVP, variables that met this criterion included RVEDA (\( P = 0.068 \)), RVESA (\( P = 0.032 \)), RVS determined with the RVFWA (\( P = 0.020 \)) and RVFWA' (\( P = 0.045 \)), RVEDA determined with RVSA (\( P = 0.048 \)).

Table 1—Comparison of mean ± SD right ventricular (RV) hemodynamic measurements obtained at the end of the inspiratory and expiratory phases of respiration for 8 healthy anesthetized Beagles in a study to evaluate the influence of manual ventilation-controlled respiration on RV pressure (RVP)-volume loop–derived and echocardiographic variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Inspiration</th>
<th>Expiration</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>100.8 ± 15.4</td>
<td>100.8 ± 14.6</td>
<td>0.871</td>
</tr>
<tr>
<td>RVP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal</td>
<td>24.6 ± 3.6</td>
<td>20.7 ± 3.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Minimal</td>
<td>8.6 ± 1.4</td>
<td>5.2 ± 1.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RVV (mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal</td>
<td>52.4 ± 9.1</td>
<td>55.7 ± 9.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Minimal</td>
<td>47.2 ± 8.1</td>
<td>49.2 ± 8.4</td>
<td>0.005</td>
</tr>
<tr>
<td>SV (mL)</td>
<td>5.2 ± 1.3</td>
<td>6.5 ± 1.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>0.53 ± 0.2</td>
<td>0.66 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FDRVP (mm Hg/s)</td>
<td>308.3 ± 45.3</td>
<td>299.6 ± 40.4</td>
<td>0.682</td>
</tr>
<tr>
<td>Maximal</td>
<td>–256.1 ± 52.1</td>
<td>–253.6 ± 91.9</td>
<td>0.926</td>
</tr>
<tr>
<td>Minimal</td>
<td>3.6 ± 1.3</td>
<td>3.6 ± 1.0</td>
<td>0.962</td>
</tr>
</tbody>
</table>

CO = Cardiac output. ESE = End-systolic elastance. FDRVP = Minimal first derivative of RVP. RVV = RV volume. SV = Stroke volume.

Values of \( P < 0.05 \) (paired t test or Wilcoxon signed rank test) were considered significant.
0.061) or RVGA (P = 0.024) method, and RVSR determined with the RVFWA (P = 0.106) or RVGA (P = 0.012) method. For association with ESE, these included RVs' (P = 0.186) and RVSR determined with the RVGA method (P = 0.046). After adjustment for confounding factors in multiple regression analysis, only RVSR determined with the RVGA method was significantly associated with stroke volume (P = 0.048; multiple adjusted r² = 0.523) and ESE (P = 0.047; multiple adjusted r² = 0.296), and RVESA and RV determined with the RVGA method were significantly associated with maximal FDRVP (P = 0.043 and P = 0.029, respectively; multiple adjusted r² = 0.522).

Discussion

Our results for hemodynamic measurements with the gold-standard method of pressure-volume loop assessment demonstrated that positive-pressure ventilation shifted the RVP-volume loops to the upper left side (ie, decreased the RVV and increased the RVP in the inspiratory phase); however, intrinsic RV contractility assessed by pressure-volume loop–derived ESE was not affected by the respiratory phase. The conventional RV echocardiographic indices of RVEDA, RVEDWT, TAPSE, FAC, RVs', and RVMPI, as well as 2D-STE–derived RVS and RVSR determined by means of RVFWA, were significantly influenced by respiratory phase. On the other hand, RVS and RVSR determined by means of RVGA did not differ significantly between the inspiratory and expiratory phases; in addition, RVSR determined by means of RVGA was significantly associated with ESE, regardless of the respiratory phase.

In the study reported here, hemodynamic indices obtained from RVP-volume loops had significant fluctuations in accordance with the respiratory phase. Higher intrathoracic pressure in the inspiratory phase (ie, positive-pressure ventilation) may have induced the decreased venous return and lowered the RVV that followed. This has been reported in previous studies.28-30 Certain RV echocardiographic indices, such as RVEDA, RVEDWT, TAPSE, RVFAC, RVs', and RVMPI, were also significantly affected by respiratory fluctuations in our study. In a previous report,25 volume overload affected the RV echocardiographic indices in healthy anesthetized dogs. Our results suggested that temporarily decreased venous return might also affect these RV functional indices. Furthermore, the increase in RVP during the inspiratory phase might also impact these echocardiographic indices. Although the variables in the present study were evaluated in healthy dogs, increased RVP might become especially evident in dogs with RV remod-

Figure 2—Representative example of an RV pressure (RVP)-volume loop from 1 of 8 healthy anesthetized Beagles in a study to evaluate the influence of manual ventilation-controlled respiration on RVP-volume loop–derived and echocardiographic variables. The black and gray lines represent RV pressure-volume loops in the inspiratory and expiratory phases, respectively.

Table 2—Comparison of mean ± SD echocardiographic measurements obtained at the end of the inspiratory and expiratory phases of respiration for the 8 Beagles in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Inspiration</th>
<th>Expiration</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVEDA (cm²)</td>
<td>4.6 ± 0.8</td>
<td>5.3 ± 0.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RVESA (cm²)</td>
<td>3.2 ± 0.7</td>
<td>3.2 ± 0.5</td>
<td>0.470</td>
</tr>
<tr>
<td>RVEDWT (mm)</td>
<td>4.7 ± 0.3</td>
<td>4.4 ± 0.4</td>
<td>0.011</td>
</tr>
<tr>
<td>TAPSE (mm)</td>
<td>5.6 ± 0.9</td>
<td>7.7 ± 0.9</td>
<td>0.012</td>
</tr>
<tr>
<td>RVFAC (%)</td>
<td>30.8 ± 2.4</td>
<td>39.3 ± 4.0</td>
<td>0.012</td>
</tr>
<tr>
<td>RVs' (cm/s)</td>
<td>5.0 ± 1.4</td>
<td>6.6 ± 1.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RVMPI</td>
<td>0.57 ± 0.2</td>
<td>0.35 ± 0.1</td>
<td>0.003</td>
</tr>
<tr>
<td>RVS (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVFWA</td>
<td>17.5 ± 3.1</td>
<td>19.7 ± 2.2</td>
<td>0.032</td>
</tr>
<tr>
<td>RVGA</td>
<td>16.6 ± 2.5</td>
<td>17.6 ± 1.9</td>
<td>0.071</td>
</tr>
<tr>
<td>RVSR (s⁻¹)</td>
<td>1.7 ± 0.3</td>
<td>1.9 ± 0.4</td>
<td>0.028</td>
</tr>
<tr>
<td>RVFWA</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>0.465</td>
</tr>
</tbody>
</table>

RVEDA = RV end-diastolic area. RVEDWT = RV end-diastolic wall thickness. RVESA = RV end-systolic area. RVFAC = RV fractional area change. RVFWA = RV free wall–only analysis. RVGA = RV global analysis. RVMPI = RV myocardial performance index. RVS = RV strain. RVs' = Peak systolic myocardial velocity of the lateral tricuspid annulus. RVSR = RV systolic strain rate. TAPSE = Tricuspid annular plane systolic excursion.

See Table 1 for key.
eling, pulmonary vascular disease, and pericardial disease. Notably, TAPSE, which is widely used for RV systolic functional indications, has been shown to deviate from the reference range in the inspiratory phase, although it was within the reference range in the expiratory phase. Therefore, our results supported that respiratory variation should be taken into account when evaluating RV function by use of these conventional echocardiographic indices.

The use of 2D-STE with an RV focus view revealed that RVS and RVSR measured by means of RVFWA significantly decreased in the inspiratory phase; these variables may be influenced by respiration-induced low-volume load and high-pressure load as well as conventional RV echocardiographic indicators. Previous studies have demonstrated that an acute change in volume load might affect RV free wall myocardial function in healthy anesthetized dogs and healthy people. Myocardial function in the RV free wall might be sensitive to respiration-related load fluctuation. On the other hand, RVS and RVSR determined with RVGA did not differ significantly between respiratory phases in our study, and RVSR measured with this method was significantly associated with pressure-volume loop–derived ESE and stroke volume. Experimental studies have found that the interventricular septum plays an important role in the cardiac output of the RV. The RVS and RVSR measured by means of RVGA, which reflect myocardial function in not only the RV free wall but also in the interventricular septum, were less sensitive to respiration-related load fluctuation and have the potential to be robust indicators of RV function. Also, the RVSR determined with RVGA may reflect intrinsic RV contractility regardless of the respiratory phase. However, myocardial function in the interventricular septum might be affected by left ventricular function. Further studies that include the assessment of left ventricular function are warranted.

The present study had several limitations. First, we compared the influence of respiration on RV morphology and function in anesthetized dogs undergoing positive-pressure ventilation. Our results may have been different if the assessment was performed on conscious dogs with spontaneous respiration induced by negative pressure. The respiratory cycle was controlled by manual ventilation, which might have caused some errors in measures associated with respiratory condition, volume and pressure loading conditions, and cardiovascular function. Studies that evaluate the effects of pressure- or volume-controlled ventilation and spontaneous respiration on RV morphology and function are warranted in the future. Furthermore, dogs were kept in left lateral recumbency during the study period, which might have impacted heart-lung interactions. Some drugs used to maintain anesthesia, especially isoflurane, might have affected RV afterload and contractility. It should also be considered that the sample size in our study might have had insufficient power to detect the differences between respiratory phases for some variables. Our study included only healthy dogs of one breed (Beagles), and results of similar analyses may differ in dogs of other breeds or in those that have heart diseases affecting RV function. Finally, we evaluated only longitudinal RVS and RVSR, and RV circumferential function would also contribute to RV systolic function.

The present study, certain conventional echocardiographic variables for RV assessment including RVEDA, RVEDWT, TAPSE, RVFAc, RVS, and RVMPI and 2D-STE–derived RVS and RVSR measured with RVFWA were significantly influenced by respiration because of the fluctuations in venous return. Conversely, 2D-STE–derived RVS and RVSR determined by use of RVGA did not change significantly between the inspiratory and expiratory phases, and the latter was significantly associated with intrinsic RV contractility (ESE), regardless of the respiratory phase. These results suggested that respiration should be taken into account when evaluating RV function by use of conventional echocardiographic variables and that determination of RVS and RVSR with RVGA may be a useful method to assess RV contractility regardless of the respiratory phase.

Acknowledgments

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