

Assessment of repeatability, reproducibility, and effect of anesthesia on determination of radial and longitudinal left ventricular velocities via tissue Doppler imaging in dogs

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Objective—To determine left ventricular free wall (LVFW) motions and assess their intra- and interday variability via tissue Doppler imaging (TDI) in healthy awake and anesthetized dogs.

Animals—6 healthy adult Beagles.

Procedure—In the first part of the study, 72 TDI examinations (36 radial and 36 longitudinal) were performed by the same observer on 4 days during a 2-week period in all dogs. In the second part, 3 dogs were anesthetized with isoflurane and vecuronium. Two measurements of each TDI parameter were made on 2 consecutive cardiac cycles when ventilation was transiently stopped. The TDI parameters included maximal systolic, early, and late diastolic LVFW velocities.

Results—The LVFW velocities were significantly higher in the endocardial than in the epicardial layers and also significantly higher in the basal than in the mid-segments in systole, late diastole, and early diastole. The intraday coefficients of variation (CVs) for systole were 16.4% and 22%, and the interday CV values were 11.2% and 16.4% in the endocardial and epicardial layers, respectively. Isoflurane anesthesia significantly improved the intraday CV but induced a decrease in LVFW velocities, except late diastolic in endocardial layers and early diastolic in epicardial layers.

Conclusions and Clinical Relevance—Left ventricular motion can be adequately quantified in dogs and can provide new noninvasive indices of myocardial function. General anesthesia improved repeatability of the procedure but cannot be recommended because it induces a decrease in myocardial velocities. (*Am J Vet Res* 2004;65:909–915)

Quantitative measurement of regional left ventricular contraction may provide relevant information, especially for understanding pathophysiology and therapeutic purposes. **Tissue Doppler imaging (TDI)** is a recently introduced technique in veterinary medicine.¹ It enables online acquisition of myocardial wall velocities,² including noninvasive evaluation of all regional myocardial movements, as revealed in human patients³ and more recently in canine models of cardiomyopathy.^{a,b} Longitudinal motion has already been assessed in dogs but only indirectly by use of the Simpson rule^{4,5} or the mitral annular motion analysis on M-mode.⁶ However, the velocities of different segments of the left ventricular myocardium during longitudinal motion have never been studied. Radial motion was recently assessed with TDI but was restricted to dogs with myocardial diseases only.^{a,b} Moreover, assessment of repeatability and reproducibility of TDI is a major prerequisite before recommending its use in longitudinal studies in a large cohort of dogs with spontaneous myocardial disease to test its sensitivity and specificity. Validation studies of TDI have already been performed in vitro by use of tissue-mimicking phantoms⁷ and during the last decade in humans for radial^{3,8} and longitudinal myocardial movements.^{9,10}

Another relevant issue is the validation of TDI in anesthetized dogs because anesthesia may be required in some stressed dogs. Animals may also have to be anesthetized under experimental conditions for assessment of effects of drugs, cardiac surgical procedures, and pathophysiologic conditions (eg, hemorrhagic shock) of myocardial function. Therefore, TDI may offer an interesting noninvasive and quantitative alternative for such in vivo evaluation of myocardial motion. In fact, 1 of the prerequisites for recommending the use of TDI in such experimental settings is to assess the repeatability of the technique in anesthetized animals and evaluate the potential impact of anesthesia on left ventricular free wall (LVFW) velocities. The effect of anesthesia with isoflurane combined with vecuronium-induced neuromuscular blocking was therefore assessed in the study reported here.

The purpose of the study reported here was to provide a description of radial and longitudinal left ventricular contraction in clinically normal awake dogs by use of TDI, determine the within-day (repeatability) and between-day (reproducibility) variabilities of the technique, and evaluate the influence of anesthesia on the TDI results.

Recent advances in human cardiology indicate that investigations of left ventricular wall motion are essential for the diagnosis of heart dysfunction.

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Materials and Methods

Dogs—The procedures used in this experiment were carried out in accordance with the *Guide For the Care and Use of Laboratory Animals* and approved by the Animal Use and Care Committee of the National Veterinary School of Alfort. Six healthy Beagles (sexually intact females; mean \pm SD age, 2.5 ± 1.2 years old; mean \pm SD body weight, 12.9 ± 1.6 kg) were used. A complete clinical examination was performed before inclusion in the study, and all cardiovascular variables (ECG, indirect arterial blood pressure, 2-dimensional and M-mode echocardiography, and color and pulsed-wave Doppler examination) were within reference ranges. M-mode echocardiography parameters included right and left ventricular diameter, LVFW and interventricular septal thickness, and left ventricular shortening fraction. Measurements of the aorta and left atrial diameter were performed with 2-dimensional mode. Pulmonary, aortic, mitral, and tricuspid valve competency was assessed by use of color and pulsed-wave Doppler imaging.

Color TDI examination—Two-dimensional color TDI examinations were performed with continuous ECG monitoring by use of an ultrasound machine^c equipped with a 5.5- to 7.5-MHz phased-array transducer. In each examination, the gray scale receive gain was set to optimize the clarity of the LVFW endocardial and epicardial boundaries. Segmental myocardial motion was measured off-line from color Doppler images of the LVFW. Real-time color Doppler was superimposed on the gray scale with a frame rate ≥ 100 frames/s. The Doppler receive gain was adjusted to maintain optimal coloring of the myocardium, and Doppler velocity range was set as low as possible to avoid occurrence of aliasing. Digital images were obtained, stored, and reviewed later by use of a stand-alone, off-line measuring system.^d Nine-by-nine pixel sampling (2×2 mm) was used, and a tissue velocity profile was displayed in each sample location.

Quantification of radial left ventricular motion—Measurement of LVFW velocities resulting from the radial left ventricular motion was obtained by use of the right parasternal ventricular short-axis view between the 2 papillary muscles (Figure 1). The angle of interrogation of the beam was carefully aligned to be perpendicular to the LVFW. Measurements were made in an endocardial and epicardial segment of the LVFW of each dog. Simultaneous endocardial and epicardial velocity profiles were obtained during the off-line analysis.

Quantification of longitudinal left ventricular motion—Measurement of LVFW velocities resulting from the longitudinal left ventricular motion was obtained by use of the standard left apical 4-chamber view (Figure 2). The angle of the beam was carefully aligned to be parallel to the left ventricular caudal wall. Measurements were made of 2 myocardial segments of the internal midportion of the left ventricular caudal wall: the basal (or annular) segment and midsegment (at the level of the maximal diastolic opening of the LVFW mitral valve leaflet). Simultaneous basal and mid-velocity profiles were obtained during the off-line analysis.

Assessment of TDI inter- and intraday variability—All TDI examinations were randomized and blindly performed. In each, the observer (same person as the echocardiographer) measured the velocity with the calipers but could not see the screen values. Another person was responsible for data collection. Two protocols were performed, including protocol 1 in awake dogs and protocol 2 in anesthetized dogs.

Protocol 1—To determine the intraday and interday variability of the TDI technique, 72 TDI examinations (36 radial and 36 longitudinal) were performed by the same

observer on 4 days during a 2-week period on the 6 clinically normal dogs. For a given dog, the radial examination was first made from the right side of the thorax and then the longitudinal examination was made from the left side of the thorax. On a given day, 3 dogs were examined at 3 nonconsecutive times. Each velocity was measured twice on 2 consecutive cardiac cycles by use of the same frame, and mean values were used for determination of the intraday and interday variabilities and comparison of the different segmental LVFW movements (in the endocardial, epicardial, basal, and mid-segments).

Protocol 2—In this protocol, to determine the influence of anesthesia on the beat-to-beat variability of each TDI parameter (absolute difference between 2 beats or intra-examination variability), 3 of the 6 clinically normal dogs (mean \pm SD body weight, 12.7 ± 2.2 kg) were included. In each TDI exam, 2 measurements of each TDI parameter were made on 2 consecutive cardiac cycles. The mean of the 2 beat-to-beat TDI measurements was not determined as in protocol 1; they were used as 2 distinct values for statistical analysis (ie, to determine the variability between 2 beats [intra-examination variability] and compare the different segmental LVFW movements).

Radial and longitudinal TDI exams were first performed on awake dogs on 1 day at 2 nonconsecutive times. On the same day, identical radial and longitudinal TDI exams were performed twice at nonconsecutive times under general anesthesia. Induction of anesthesia was achieved with diazepam^f (0.2 mg/kg, IV), followed by a bolus of thiopental^l (10 mg/kg,

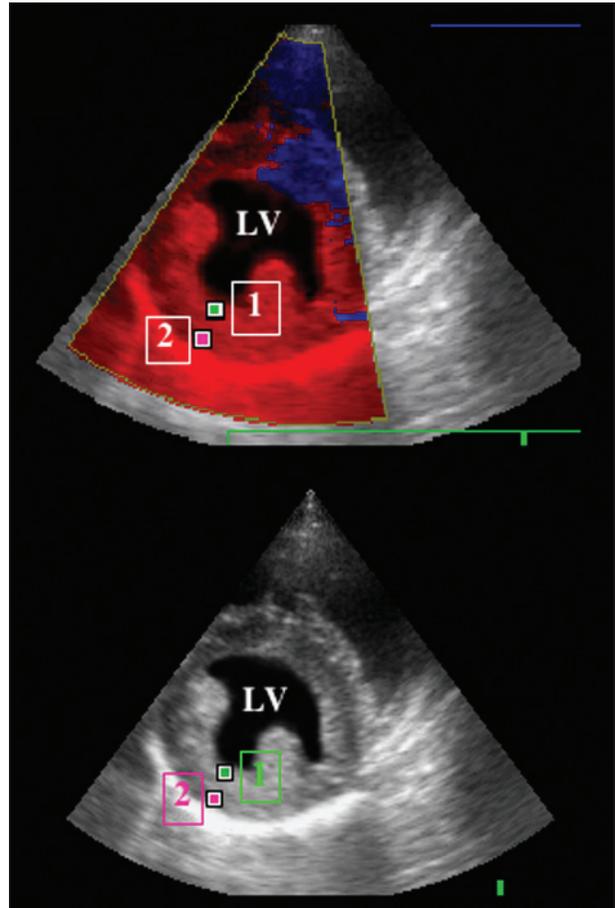


Figure 1—Two-dimensional color tissue Doppler imaging (TDI) view of the heart of a dog (right parasternal short-axis view). Notice location of the endocardial segment (1) and the epicardial segment (2) used for the left ventricular free wall (LVFW) radial motion analysis. LV = Left ventricle.

IV). Anesthesia was maintained with isoflurane.⁸ End-tidal PCO₂ was monitored and maintained between 35 and 45 mm Hg. Vecuronium bromide^b was injected IV at a dose of 80 µg/kg after 10 minutes of spontaneous breathing, and intermittent positive-pressure ventilation was initiated. All TDI measurements were performed after artificial ventilation had been temporarily stopped. The duration of the respiratory arrest induced to perform the TDI measurements was < 30 seconds. The duration of artificial ventilation was 20 ± 10 minutes (mean ± SD). Spontaneous breathing resumed after 30 ± 5 minutes following injection of vecuronium bromide.

Statistical analyses—Data are expressed as mean ± SD. Statistical analysis was performed with software.ⁱ Endocardial and epicardial LVFW velocities and basal and midvelocities were compared in awake (protocol 1) and anesthetized (protocol 2) dogs by use of the Student *t* test. The same test was used to compare protodiastolic and telediastolic velocities in the same dogs. For the intraday and interday variability study of protocol 1, the following linear model was used for each TDI parameter:

$$Y_{ijk} = \mu + \text{day}_i + \text{dog}_j + (\text{day} \times \text{dog})_{ij} + \epsilon_{ijk}$$

where Y_{ijk} is the *k*th value measured for dog *j* on day *i*, μ is the general mean, day_i is the differential effect of day *i*, dog_j

is the differential effect of dog *j*, $(\text{day} \times \text{dog})_{ij}$ is the interaction term between day and dog, and ϵ_{ijk} is the model error. The SD of repeatability was estimated as the residual SD of the model and the SD of reproducibility as the SD of the differential effect of day. The corresponding coefficients of variation (CVs) were determined by dividing each SD by the mean. For protocol 2, to determine the intra-examination TDI variabilities, beat-to-beat variances of awake and anesthetized dogs were compared by use of the Fisher *F* test. For all comparisons, values of $P < 0.05$ were considered significant.

Results

Left ventricular radial motion in awake dogs (protocol 1)—After a short isovolumic contraction phase, all radial velocity profiles included 1 positive systolic wave (S), and after a short isovolumic relaxation phase, all radial velocity profiles included 2 diastolic negative waves (E and A), respectively, in proto- and telediastole (Figure 3). Regional synchrony was observed between the endocardial and epicardial layers. Systolic LVFW velocities were significantly ($P < 0.001$) higher in the endocardial (6.0 ± 1.01 cm/s) than in the epicardial layers (3.4 ± 0.77 cm/s). Early and late diastolic LVFW velocities were higher ($P < 0.001$) in the endocardial (8.1 ± 1.82 cm/s and 3.7 ± 0.99 cm/s) than in the epicardial layers (3.5 ± 0.78 cm/s and 1.7 ± 0.65 cm/s). They were also higher in protodiastole than in telediastole ($P < 0.001$). The intraday CV values were 16.4% and 2% for the S wave, 15.1% and 17.6% for the E wave, and 26.6% and 36% for the A wave in the endocardial and epicardial layers, respectively. The interday CV values were 11.2% and 16.4% for the S wave and 44.4% and 69.2% for the A wave in the endocardial and epicardial layers, respectively. The interday CV value was 25% for the E wave in the endocardial layer. A significant interaction between dog and day was observed for

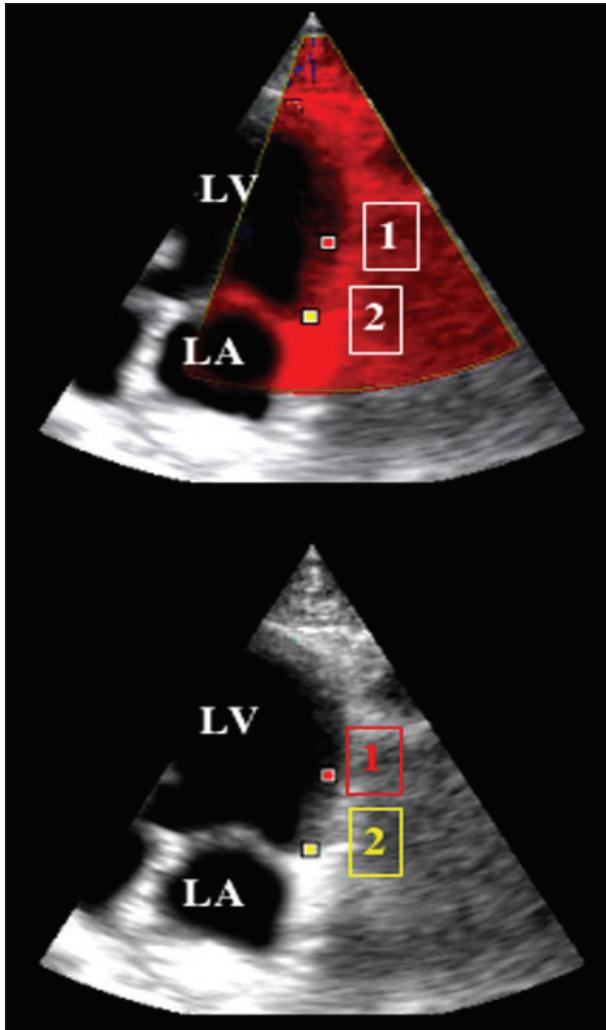


Figure 2—Two-dimensional color TDI view of the heart of a dog (left apical 4-chamber view). Notice location of the basal segment (1) and the midsegment (2) used for LFW longitudinal motion analysis. LA = Left atrium.

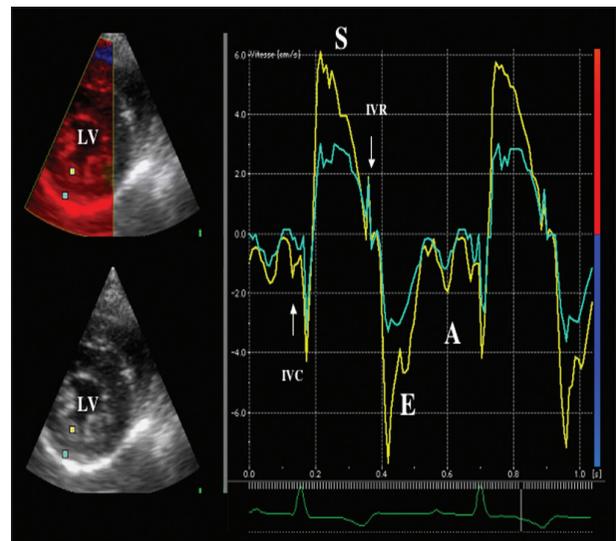


Figure 3—Two-dimensional color TDI view of the heart of a dog (right parasternal short-axis view) and simultaneous velocity recording of LVFW segmental radial motion. The simultaneous recording of the velocities in the 2 segments (endocardial and epicardial) reveals that the endocardial layers (yellow curve) are moving more rapidly than the epicardial layers (green curve) in systole and diastole. IVC = Isovolumic contraction phase. S, E, and A = Peak velocities of the LVFW during systole and early and late diastole, respectively. IVR = Isovolumic relaxation phase.

Table 1—Comparison of radial left ventricular velocities for intra-examination variability (SD and coefficients of variation [CVs]) in awake and anesthetized dogs with blocked ventilation.

Velocity	SD (cm/s) in awake dogs	SD (cm/s) in anesthetized dogs	P value	CVs (%) in awake dogs	CVs (%) in anesthetized dogs
S, endocardium	0.443	0.187	0.027	7.6	5.2
S, epicardium	0.190	0.055	0.004	6.2	2.8
E, endocardium	0.834	0.145	< 0.001	8.9	2.5
E, epicardium	0.716	0.148	< 0.001	21.8	5.0
A, endocardium	0.429	0.084	< 0.001	13.4	4.1
A, epicardium	0.444	0.045	< 0.001	32.9	8.3

S = Maximal systolic radial velocity. E = Maximal protodiastolic radial velocity. A = Maximal telediastolic radial velocity.

Table 2—Comparison of longitudinal left ventricular velocities for intra-examination variability (SD and CV) in awake and anesthetized dogs with blocked ventilation.

Velocity	SD (cm/s) in awake dogs	SD (cm/s) in anesthetized dogs	P value	CVs (%) in awake dogs	CVs (%) in anesthetized dogs
S, basal segment	0.251	0.110	0.0318	4.9	3.9
S, midsegment	0.574	0.148	0.0022	19.4	9.2
E, basal segment	0.367	0.170	0.0416	4.2	4.4
E, midsegment	0.565	0.253	0.0357	7.4	8.1
A, basal segment	0.663	0.230	0.0105	13.6	8.5
A, midsegment	0.958	0.217	0.0010	31.0	12.9

See Table 1 for key.

the E wave in the epicardium, so the SD and CV values of reproducibility could not be estimated.

Left ventricular longitudinal motion in awake dogs (protocol 1)—All longitudinal velocity profiles included 1 positive systolic wave (S) and 2 diastolic negative waves (E and A in proto- and telediastole, respectively). Regional synchrony was observed between the basal and midmotions. Systolic LVFW velocities were significantly ($P < 0.001$) higher in the basal (7.9 ± 2.26 cm/s) than in the midsegments (5.0 ± 2.04 cm/s) of the LVFW. Diastolic LVFW velocities were significantly ($P < 0.001$) higher in protodiastole (10.0 ± 1.67 cm/s and 9.6 ± 1.98 cm/s in the basal and midsegment, respectively) than in telediastole (5.7 ± 1.87 cm/s and 4.2 ± 1.84 cm/s in the basal and midsegment, respectively). Diastolic LVFW velocities were also significantly ($P < 0.05$ for the E wave; $P < 0.001$ for the A wave) higher in the basal than in the midsegments. The intraday CV values were 22.7% and 38% for the S wave, 12.5% and 18.7% for the E wave, and 28.3% and 43.3% for the A wave in the basal and mid-LVFW segments, respectively. The interday CV values were 27% and 22.7% for the S wave, 19.0% and 24.6% for the E wave, and 17.8% and 50.9% for the A wave in the basal and mid-LVFW segments, respectively.

Left ventricular radial motion in anesthetized dogs (protocol 2)—As in the awake dogs of protocol 1, all radial velocity profiles obtained in the anesthetized dogs when ventilation was blocked included 1 positive systolic wave (S) and 2 diastolic negative waves (E and A). Synchrony was good between the 2 LVFW segments, but LVFW velocities were higher in the endocardial than in the epicardial layers. Left ventricular free wall velocities

were lower in the anesthetized dogs when ventilation was blocked than in the same dogs before anesthesia, both in the endocardial and epicardial layers, respectively: 3.6 ± 0.49 cm/s and 1.9 ± 0.43 cm/s for the S wave (vs 5.9 ± 0.62 cm/s and 3.1 ± 0.43 cm/s [$P < 0.001$]); 5.7 ± 2.06 cm/s and 2.9 ± 1.59 cm/s for the E wave (vs 9.4 ± 1.32 cm/s and 3.3 ± 0.30 cm/s [$P < 0.01$ and $P > 0.05$]); and 2.1 ± 1.33 cm/s and 0.6 ± 0.62 cm/s for the A wave (vs 3.2 ± 1.09 cm/s and 1.4 ± 0.41 cm/s [$P > 0.05$ and $P < 0.05$]). A significant improvement of intra-examination CVs was observed for all radial TDI parameters (Table 1), particularly in diastole ($P < 0.001$); all CVs were $< 10\%$ when ventilation was blocked, with a range of 2.8% to 8.3% (vs 6.2% to 32.9% in awake dogs).

TDI for left ventricular longitudinal motion in anesthetized dogs (protocol 2)—As in the awake dogs of protocol 1, all longitudinal velocity profiles obtained in the anesthetized dogs when ventilation was blocked included 1 positive systolic wave (S) and 2 diastolic negative waves (E and A), with good synchrony and higher velocities in the basal than in the middle of the lateral segments. As for radial motion, LVFW velocities were lower in anesthetized dogs when ventilation was blocked, compared with the same dogs before anesthesia, both in the basal and midsegments, respectively: 2.8 ± 0.70 cm/s and 1.6 ± 0.61 cm/s for the S wave (vs 5.1 ± 0.71 cm/s and 3.0 ± 0.52 cm/s [$P < 0.001$ and $P < 0.01$]); 3.9 ± 0.83 cm/s and 3.1 ± 0.74 cm/s for the E wave (vs 8.7 ± 2.17 cm/s and 7.7 ± 2.56 cm/s [$P < 0.001$ and $P < 0.01$]); and 2.7 ± 0.38 cm/s and 1.7 ± 0.63 cm/s for the A wave (vs 5.1 ± 0.71 cm/s and 3.0 ± 0.52 cm/s [$P < 0.001$ and $P < 0.05$]). A significant ($P < 0.01$) improvement of intra-examination CVs was observed for all longitudinal TDI parameters, particularly in telediastole (Table 2). All CVs were $< 13\%$

when ventilation was blocked, with a range of 3.9% to 12.9% (vs 4.2% to 31% in awake dogs).

Discussion

The main goal of this study was to document LVFW motion during the cardiac cycle in healthy dogs. Overall, 144 LVFW segments (36 endocardial, 36 epicardial, 36 basal, and 36 midsegments) were analyzed in the first protocol. Our results indicated that radial and longitudinal left ventricular motions may be quantified in different LVFW segments by use of TDI in awake dogs, with a relatively limited intraday and interday variability.

Although no study under blinded and well-controlled conditions has yet been published on the subject, it is generally accepted among cardiologists that 3 to 5 cardiac cycles are used when assessing echocardiography or Doppler echocardiography. However, this could not be done with our echocardiograph because of technical reasons; with the software we used, only 2 cycles could be analyzed simultaneously. This represents the main limitation of our TDI study.

This study provides the first description of radial and longitudinal LVFW motion in healthy dogs. To our knowledge, longitudinal LVFW contraction has never been studied in dogs before. Results of our study clearly indicate that the canine heart has 2 types of intrinsic myocardial motion, namely radial or circumferential motion and longitudinal motion. These 2 motions were recorded by use of 2 views; with the right parasternal ventricular short axis view, radial motion was well quantified, but as the longitudinal motion vector formed an angle of nearly 90° with the Doppler signal, longitudinal LVFW motion could not be analyzed. The opposite occurred with the left apical 4-chamber view; the longitudinal motion was well quantified, whereas the circumferential LVFW motion could not be analyzed. In the human left ventricle, these 2 left ventricular motions suggest involvement of different myocardial fibers.⁹ Radial contraction is attributable to circumferential fibers in the midportion of the LVFW wall, whereas longitudinal motion results from longitudinal fibers located in the subendocardium. These fibers may be altered separately and to various degrees in some pathologic situations. This explains why the use of TDI to distinguish these 2 LVFW motions may be clinically relevant.⁹

Our results indicated that all time-velocity plots had good regional synchrony during the whole cardiac cycle for both radial and longitudinal motions. The loss of this synchrony may be an abnormal motion pattern, but this must be confirmed by further studies, including studies in large populations of healthy and diseased dogs.

In the study reported here, all radial and longitudinal velocity profiles included, after a short isovolumic contraction phase, 1 positive systolic wave (S), and after a short isovolumic relaxation phase, 2 diastolic negative waves (E and A, in early and late diastole, respectively, with E systematically greater than A for each examination). However, LVFW segments move with various velocities; during the whole cardiac cycle, the endocar-

dial layers move more rapidly than the epicardial layers, defining an intramyocardial radial velocity gradient. Similarly, the LVFW velocities decrease substantially from the base to the midsegment of the left lateral wall, defining an intramyocardial longitudinal velocity gradient. In humans, similar systolic gradients and early and late diastolic transmural gradients have been described³ and are reliable indicators of regional left ventricular function, which is even more sensitive than isolated velocity values.^{10,11} Concerning the radial contraction, we have already demonstrated the accuracy of TDI for detecting early myocardial dysfunction in a dog model of dilated cardiomyopathy; despite normal left ventricular dimensions and shortening, TDI identified the diseased puppies early because of a decrease in the endocardial-epicardial gradient.^{4a} This intramyocardial gradient may be a useful early marker of subclinical cardiomyopathy in dogs. Further studies are required to determine its diagnostic value in other canine myocardial diseases. Nevertheless, it should be emphasized that further studies in a larger number of animals are required to provide adequate reference ranges. However, 1 of the prerequisites for such studies is to assess the repeatability and reproducibility of the measurements.

As for the validation of any technique, our results are only valid for our conditions (site, observer, dogs, and materials). In such conditions, intraday and interday variability was correct for most TDI parameters, except in late diastole (A wave) in which the highest CV values were observed. Consequently, use of radial and longitudinal A velocities cannot be recommended because of their poor repeatability and reproducibility. Our results may be compared with those obtained by our group in awake cats.¹² In that study, the CV could not be calculated for the A wave because E and A waves were summated in 47% and 64% of TDI radial and longitudinal examinations, respectively. This EA fusion, attributable to high heart rate (> 220 beats/min), was not observed in our study. Despite this discrepancy, as in dogs, repeatability and reproducibility of TDI were adequate for longitudinal and radial left ventricular motions, and the lowest within-day and between-day CV values were also observed in the endocardial layers and basal segment. The values were lower than ours in dogs; the within-day CV was 8.2% and 6.5% in the endocardial layers and 8.3% and 8.5% in the basal segment for the S and E waves, respectively. Conversely, all CV values obtained in awake dogs were > 10%. Presently, no benchmark has been defined for longitudinal and radial left ventricular motions. It is difficult to define what an adequate repeatability and reproducibility should be. Nevertheless, the lowest within-day and between-day CV values were from 3.1% to 13.8% for echocardiographic variables in dogs,¹³ which means that the repeatability and reproducibility of TDI measurements in our study were similar to those of routine echocardiography.

The second protocol was conducted to study the influence of anesthesia on radial and longitudinal left ventricular velocities as well as the beat-to-beat (intra-examination) variability. A limitation of our study was that intraday and interday variabilities of TDI measurements were not assessed because the dogs were awake.

Repeated measurements at 2 times on the same day would have been relevant, but it would have been difficult to interpret any change in the measurements because the repeatability of the technique and effect of prolonged anesthesia would have been confounded in such a protocol. Interday variability was not assessed here because experimental protocols involving anesthetized animals with induction of acute myocardial changes are generally performed on the same day. In view of the results in the first part of this study, it is obvious that when repeated measurements are required over several days in the same dog, performance of the examination without anesthesia is the best approach.

Isoflurane was selected here because it is probably the most widely used volatile agent for canine anesthesia in cardiology. Moreover, use of neuromuscular blocking agents is very common for many experimental settings that require assessment of heart function, such as open-thorax surgery, heart transplantation, and induction of myocardial infarction. Anesthesia significantly decreased the LVFW velocities for radial motions. The mean decrease that was significant was 39% for endocardial and epicardial S waves and the endocardial E wave. The maximal decrease (57%) was observed for epicardial A. Similarly for longitudinal motions, a mean decrease from 43% to 47% was observed for all LVFW velocities except for E, which gave the greatest decrease (55% and 60% in basal and midsegments, respectively). The underlying cause of such an alteration, compared with values observed in the nonanesthetized dogs, is difficult to identify. Isoflurane is known to depress canine myocardial contractility,¹⁴⁻¹⁶ mainly by decreasing Ca⁺⁺ handling in excitation-contraction coupling.¹⁷ Therefore, the decrease in LVFW velocities could be explained by the fact that isoflurane directly decreases contractility. Vecuronium causes a positive inotropic response¹⁸; nevertheless, it may have indirect effects on myocardial contractility because it also has parasympatholytic effects in dogs.¹⁹ Ventilation with positive end-expiratory pressure on cardiac function does not affect left ventricular contractility in closed-thorax anesthetized dogs.²⁰

Another relevant result was that the intra-examination variability was better under anesthetized conditions. This may result from the fact that perfect immobility of the animal improved repeatability of TDI measurements. Another explanation is that motion of the heart attributable to the respiratory cycle could interfere with determination of myocardial velocities. All systolic and diastolic myocardial movements are a combination of the intrinsic myocardial motion and the complex heart displacement within the thorax.⁸ This heart movement is the result of its relatively loose attachments within the thorax and the respiratory movements. Effects of respiration on conventional echocardiographic and Doppler parameters have already been demonstrated in human patients. They sometimes result in large variations that may lead to inaccurate assessment of cardiac dysfunction.^{21,22} Stopping ventilation with vecuronium under general anesthesia may explain the improvement of the intra-examination variability for all parameters, particularly in late diastole. These results are in accordance with conventional echo-Doppler studies that reveal that a

period of breath-holding in human patients significantly reduces the variability of M-mode parameters²³ and blood flow velocities.²⁴ The fact that all TDI parameters had a significantly smaller variability when measurements were performed under anesthesia during transient respiratory arrest (< 30 seconds) than under awake conditions suggests that breathing may explain TDI variability in awake dogs. In a human TDI study,²⁵ consecutive beats are mostly recorded during apnea. In docile animals, temporarily stopping the ventilation during the TDI recording by manually blocking the nostrils could be a simple way to limit this negative breathing influence and may improve the repeatability and reproducibility of the technique. Further TDI studies with simultaneous recording of respiration movements are required to compare variability of TDI parameters between 2 beats at end-expiration and end-inspiration to determine the optimal moment for TDI recording during breathing.

^aChetboul V, Escriou C, Blot S, et al. Early detection of regional myocardial function alterations in a dog model of dilated cardiomyopathy by Tissue Doppler imaging study (abstr). *Circulation* 2001;104(suppl 17):351.

^bChetboul V, Escriou C, Thibaud JL, et al. Antenatal detection of Duchenne's cardiomyopathy by tissue doppler (abstr). *Circulation* 2002;106(suppl 19):397.

^cGeneral Electric medical system, GE Vingmed Ultrasound, Waukesha, Wis

^dEcho Pac 5.4 software for System 5, Waukesha, Wis.

^eValium, Roche, Neuilly-sur-Seine, France.

^fNesdonal, Merial, Lyon, France.

^gForene, Abbot France 10, Rungis, France.

^hNorcuron Organon Teknika, Paris, France.

ⁱSystat, version 10.0, SPSS Inc, Chicago, Ill.

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