**Tissue Doppler assessment of diastolic and systolic alterations of radial and longitudinal left ventricular motions in Golden Retrievers during the preclinical phase of cardiomyopathy associated with muscular dystrophy**

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**Objective**—To quantify radial and longitudinal left ventricular free wall (LVFW) velocities in dogs during the preclinical phase of Golden Retriever muscular dystrophy (GRMD)-associated cardiomyopathy by use of tissue Doppler imaging (TDI).

**Animals**—9 dogs with GRMD and 6 healthy control dogs.

**Procedure**—All dogs (<3 years old) were examined via conventional echocardiography and 2-dimensional color TDI. Myocardial velocities in the LVFW were recorded from right parasternal ventricular short-axis (radial motion) and left apical 4-chamber (longitudinal motion) views. Cardiac assessments via TDI included maximal systolic and early and late diastolic LVFW velocities in the endocardial and epicardial layers (for radial motion) and in the basal and apical segments (for longitudinal motion).

**Results**—No notable ventricular dilatation or alteration of inotropism was detected in dogs with GRMD via conventional echocardiography. Compared with healthy dogs, endocardial velocities were significantly decreased in dogs with GRMD, resulting in marked decreases in radial myocardial velocity gradients during systole and early and late diastole. Similarly, basal and apical velocities were significantly decreased in systole and the former also in early diastole, resulting in significant decreases in the 2 corresponding longitudinal myocardial velocity gradients. The radial epicardial and longitudinal late diastolic velocities were comparable in the 2 groups.

**Conclusion and Clinical Relevance**—Results indicated that GRMD-associated cardiomyopathy in dogs is associated with early marked dysfunction of both radial and longitudinal LVFW motions. These combined regional myocardial abnormalities might be useful criteria for detection of dilated cardiomyopathy at the preclinical stage of the disease in dogs. (Am J Vet Res 2004;65:1335–1341)

_Dilated cardiomyopathy (DCM) is a major cause of illness and death in dogs of various breeds, such as Irish Wolfhounds, Newfoundlands, Boxers, Deerhounds, Great Danes, and Doberman Pinschers._ The diagnosis of overt DCM is made predominantly on the basis of findings of 2-dimensional (2D) and M-mode echocardiographic examinations, including left ventricular dilatation, reduced systolic function, and increased sphericity of the left ventricle. The diagnosis of the preclinical phase of DCM (also called occult DCM) is generally more problematic and remains a challenge for veterinary cardiologists, despite the fact that the preclinical phase (prior to development of clinical signs) may extend over a period of years. Therefore, there is a need to define new imaging indices that will allow early diagnosis of the myocardial dysfunction associated with DCM in dogs.

_Tissue Doppler imaging (TDI) is a recently developed ultrasonographic technique that enables clinicians to quantify regional myocardial function in real time by measurement of myocardial velocities._ Tissue Doppler imaging offers a new noninvasive means by which quantitative in vivo analysis of the 2 intrinsic motions (radial and longitudinal) of the left ventricular free wall (LVFW) during the whole cardiac cycle can be performed. Validation studies of TDI for both radial and longitudinal LVFW movements (ie, evaluation of the within-day and between-day variability of the TDI parameters) have been performed by our group in healthy awake cats and dogs. However, to our knowledge, the clinical relevance and the sensitivity of these new radial and longitudinal TDI indices have not been studied simultaneously in the context of preclinical DCM in dogs.

Various animal models of DCM have been used for research. Golden Retriever muscular dystrophy (GRMD) is an X-linked inherited neuromuscular dis-
order related to a spontaneous mutation in the dystrophin gene, which also affects the myocardium of those dogs. Similar to cardiomyopathy associated with Duchenne dystrophy in humans, GRMD cardiomyopathy is characterized by myocardial lesions that result in progressive heart failure, including muscle necrosis and regeneration and replacement of muscular tissues by fibrosis, fat, and mineralization. The purpose of the study of this report was to quantify radial and longitudinal LVFW velocities in dogs during the preclinical phase of GRMD-associated cardiomyopathy by use of TDI. Our intention was to compare the results with those obtained in healthy control dogs of the same cohort at the same age.

**Materials and Methods**

**Animals**—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 Allort, France. Nine young adult dogs with GRMD (age, 8 to 76 months; weight, 12 to 23 kg) were chosen from the GRMD cohort of the National Veterinary School of Allort on the basis of the following cardiovascular criteria: identification of the GRMD mutation via DNA analysis, absence of clinical or radiographic signs of congestive heart failure, and normal ECG findings. Six healthy Golden Retriever dogs with a normal genotype (age, 17 to 24 months; weight, 23 to 38 kg) of similar age and weight were also used as controls.

**Conventional echocardiography**—Standard transthoracic echocardiography with continuous ECG monitoring was performed by 1 trained observer (VC) by use of an ultrasound unit equipped with a 2.2- to 3.5-MHz phased-array transducer. All the conventional ultrasonographic examinations were performed in awake dogs that were gently restrained in a standing position. Hair was clipped from the area overlying the right fourth to sixth intercostal spaces. For each variable, a mean value was calculated from 3 measurements (on the same frame) obtained from 3 consecutive cardiac cycles. Ventricular measurements were taken from the right parasternal location (short-axis view) by use of 2D-guided, M-mode quantitation, according to the recommendations of the American Society of Echocardiography. Left ventricular end-diastolic and end-systolic diameter were measured. The left ventricular shortening fraction (SF; %) was then calculated. Measurements of the aorta and left atrium were performed by use of a 2D method and involved a short-axis right-sided parasternal view obtained at the level of the aortic valve, where the commissures of the cusps are observable during diastole. The internal short-axis diameter of the aorta (Ao) was measured along the commissure between the noncoronary and left coronary aortic valve cusps. The diameter of the left atrium (LA) was measured in the same frame along a line extending from and parallel to the commissure between the noncoronary and left coronary aortic valve cusps. The LA:Ao ratio was then calculated.

**Color TDI examination**—Two-dimensional color TDI examinations were performed with continuous ECG monitoring by the trained observer (VC) who completed the conventional echocardiographic examinations; the same echocardiograph and transducer were used for both types of echocardiographic examination. In each examination, the gray-scale receive gain was set to optimize the clarity of the endocardial and epicardial boundaries of the LVFW. 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 later reviewed by use of a stand-alone off-line measuring system by the person who had performed the conventional echocardiographic examinations. A 2 X 2-mm sampling was used, and a tissue velocity profile was displayed in each sample location. Variables assessed via TDI included maximal systolic and early diastolic and late diastolic LVFW velocities. For each velocity, a mean value was calculated from 2 measurements (on the same frame) obtained during 2 consecutive cardiac cycles. In our experience, this TDI technique has good repeatability and reproducibility for most variables.

**Quantification of radial left ventricular motion**

Velocities of the LVFW resulting from the radial left ventricular motion were measured in the right parasternal short-axis view between the 2 papillary muscles. The angle of the beam was carefully aligned to be perpendicular to the LVFW. Measurements were made in an endocardial and epicardial segment of the LVFW; simultaneous endocaridal and epicardial velocity profiles were obtained during the off-line analysis. Radial myocardial velocities were determined in systole and early and late diastole. Radial myocardial velocity gradients (MVGs; cm/s), defined as the difference between endocardial and epicardial velocities, were then calculated.

**Quantification of longitudinal left ventricular motion**

Velocities of the LVFW resulting from the longitudinal left ventricular motion were measured in a standard left apical 4-chamber view. The angle of the beam was carefully aligned to be parallel to the LVFW. Measurements were made of 2 myocardial segments of the internal midportion of the LVFW (the basal and apical segments); simultaneous basal and apical velocity profiles were obtained during the off-line analysis. Longitudinal myocardial velocities were determined in systole and early and late diastole. Longitudinal MVGs, defined as the difference between basal and apical velocities, were then calculated.

**Statistical analyses**—All data are expressed as mean ± SEM. Age, weight, heart rate, and conventional echocardiographic variables were compared between the control and GRMD groups by use of a Student unpaired t test. The radial and longitudinal velocities between the different localizations (endocardial vs subepicardial and basal vs apical, respectively) in each of the 2 groups (control dogs vs dogs with GRMD) were compared by use of a 2-way ANOVA and followed, if necessary, by a Student post-hoc t test with Bonferroni corrections. Values of P < 0.05 were considered significant.

**Results**

**Animals**—Age and heart rate did not differ significantly between dogs with GRMD and control dogs. As expected, mean ± SEM weight was lower (P < 0.01) in dogs with GRMD (17 ± 1 kg), compared with the weight of control dogs (29 ± 2 kg; Table 1).

**Conventional echocardiographic measurements**—With regard to left ventricular dimensions and values of SF and LA:Ao ratio, no significant difference was found between the control dogs and dogs with GRMD (Table 1). However, 3 of 9 dogs with GRMD already had an increased systolic left ventricular diameter (39, 49, and 51 mm, respectively; reference range, 18 to 35 mm); in 2 of those dogs, this was associated with an increased
TDI results—After a short isovolumic contraction phase, all radial and longitudinal LVFW velocity profiles included 1 positive systolic wave and after a short isovolumic relaxation phase, 2 negative diastolic waves (1 each in early and late diastole). Overall, 60 LVFW segments (15 endocardial, 15 epicardial, 15 basal, and 15 apical segments) were assessed in the 15 study dogs.

Radial motion
As expected, the systolic LVFW velocity was significantly higher in the endocardial layers than in the epicardial layers in control dogs (P < 0.001) as well as in dogs with GRMD (P < 0.01; Table 2). However, compared with values in control dogs, systolic endocardial velocities were significantly (P < 0.001) lower in dogs with GRMD, which resulted in markedly lower systolic radial MVG (Table 3; Figures 1–3). As expected, the early and late diastolic LVFW velocities were comparable in the 2 groups. Longitudinal motion
As expected, the systolic LVFW velocity was significantly (P < 0.01) higher in the basal segments than in the apical segments in control dogs as well as in dogs with GRMD (Table 4; Figure 4). However, compared with values in control dogs, systolic LVFW velocities in dogs with GRMD were also higher (P < 0.05 and < 0.01, respectively) in the endocardial layers than in the epicardial layers in dogs with GRMD. However, compared with control dogs, endocardial velocities in dogs with GRMD were significantly lower in early (P < 0.001) and late (P < 0.05) diastole, which resulted in markedly lower diastolic MVG. All radial systolic and diastolic epicardial velocities were comparable in the 2 groups.

Table 1—Mean ± SEM weight, age, heart rate, and echocardiographic variables of 6 healthy control dogs and 9 dogs with Golden Retriever muscular dystrophy (GRMD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control dogs</th>
<th>Dogs with GRMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>29 ± 2</td>
<td>17 ± 1*</td>
</tr>
<tr>
<td>Age (mo)</td>
<td>22 ± 2</td>
<td>23 ± 7</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>122 ± 11</td>
<td>120 ± 4</td>
</tr>
<tr>
<td>LVDD (mm)</td>
<td>49 ± 2</td>
<td>44 ± 4</td>
</tr>
<tr>
<td>LVSD (mm)</td>
<td>30 ± 1</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>SF (%)</td>
<td>39 ± 1</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>L:Ao ratio</td>
<td>0.8 ± 0.0</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

*Value significantly (P < 0.01) different from the value of the control group.
LVDD = Left ventricular diastolic diameter. LVSD = Left ventricular systolic diameter. SF = Shortening fraction. L:Ao = Ratio of the diameter of the left atrium to the diameter of the aorta diameter.

Table 2—Mean ± SEM peak radial velocities in the endocardial and epicardial layers of the left ventricular free wall (LVFW) measured during systole and early and late diastole in 6 control dogs and 9 dogs with GRMD.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Cardiac region</th>
<th>Control dogs</th>
<th>Dogs with GRMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systole</td>
<td>Endocardial layer</td>
<td>7.7 ± 0.47</td>
<td>4.7 ± 0.26*</td>
</tr>
<tr>
<td></td>
<td>Epicardial layer</td>
<td>4.6 ± 0.26*</td>
<td>4.0 ± 0.23†</td>
</tr>
<tr>
<td>Early diastole</td>
<td>Endocardial layer</td>
<td>9.0 ± 0.61</td>
<td>4.2 ± 0.51*</td>
</tr>
<tr>
<td></td>
<td>Epicardial layer</td>
<td>5.0 ± 0.55†</td>
<td>3.6 ± 0.48†</td>
</tr>
<tr>
<td>Late diastole</td>
<td>Endocardial layer</td>
<td>6.5 ± 0.32</td>
<td>3.8 ± 0.72*</td>
</tr>
<tr>
<td></td>
<td>Epicardial layer</td>
<td>3.2 ± 0.87†</td>
<td>3.0 ± 0.69†</td>
</tr>
</tbody>
</table>

*Value significantly (P < 0.01) different from the value for that layer in control dogs. †Value significantly (P < 0.05) different from the value of the endocardial layer in the same group.

Figure 1—Results of 2-dimensional (2D) color tissue Doppler imaging (TDI) evaluation of the radial motion of the left ventricular free wall (LVFW) recorded from the right parasternal short-axis view (A) in a healthy control dog and simultaneous recording of the velocities in the endocardial and epicardial segments (B). Notice that in panel B, the endocardial layers (yellow curve) are moving more rapidly than the epicardial layers (green curve) in systole and also in diastole, thus defining marked systolic and early and late diastolic myocardial velocity gradients (MVGs). LV = Left ventricle. S = Peak velocity of the LVFW during systole. A = Peak velocity of the LVFW during late diastole. IVC = Isovolumic contraction phase. IVR = Isovolumic relaxation phase. E = Peak velocity of the LVFW during early diastole.
pared with the control dogs, systolic basal and apical velocities were significantly ($P < 0.01$) lower in dogs with GRMD, which resulted in markedly ($P < 0.01$) low systolic MVG. As expected, the early and late diastolic LVFW velocities were higher in the basal segments than in the apical segments in control dogs ($P < 0.001$); the early and late diastolic LVFW velocities were also higher ($P < 0.05$ and $< 0.01$, respectively) in dogs with GRMD.

Table 4—Mean ± SEM peak longitudinal velocities in the basal and apical segments of the LVFW measured during systole and early and late diastole in 6 control dogs and 9 dogs with GRMD.

<table>
<thead>
<tr>
<th>Group</th>
<th>Phase</th>
<th>Cardiac Segment</th>
<th>Control dogs</th>
<th>Dogs with GRMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td></td>
<td>11.3 ± 1.82</td>
<td>4.7 ± 0.50*</td>
</tr>
<tr>
<td></td>
<td>Apical</td>
<td></td>
<td>2.4 ± 0.411</td>
<td>0.9 ± 0.25†*</td>
</tr>
<tr>
<td>Early diastole</td>
<td>Basal</td>
<td></td>
<td>9.1 ± 0.34</td>
<td>5.3 ± 1.00*</td>
</tr>
<tr>
<td></td>
<td>Apical</td>
<td></td>
<td>2.2 ± 0.471</td>
<td>1.9 ± 0.81†</td>
</tr>
<tr>
<td>Late diastole</td>
<td>Basal</td>
<td></td>
<td>7.3 ± 0.69</td>
<td>5.4 ± 0.83</td>
</tr>
<tr>
<td></td>
<td>Apical</td>
<td></td>
<td>1.1 ± 0.191</td>
<td>1.0 ± 0.67†</td>
</tr>
</tbody>
</table>

*Value significantly ($P < 0.05$) different from value for the same segment during the same cardiac phase in control dogs. †Value significantly ($P < 0.05$) different from value of the basal segment in the same group.

Figure 2—Results of 2D TDI evaluation of the radial motion of the LVFW recorded from the right parasternal short-axis view in a dog with Golden Retriever muscular dystrophy (GRMD). Notice that the endocardial (yellow curve) and epicardial (green curve) velocity profiles are nearly superimposed during the whole cardiac cycle, indicating the lack of systolic and diastolic MVGs. See Figure 1 for key.

Figure 3—Plots of systolic (A) and early (B) and late (C) diastolic radial velocities in the endocardial (left panels) and in the epicardial (right panels) layers of 9 dogs with (GRMD) and 6 healthy control dogs. *Data points for the 3 Golden Retrievers with muscular dystrophy that had abnormal conventional echocardiography are highlighted.
the basal segments than in the apical segments in dogs with GRMD. However, compared with control dogs, basal velocities in dogs with GRMD were significantly (P < 0.05) lower in early diastole, which resulted in lower longitudinal MVG (P < 0.05; Table 3). No significant differences in variables measured during late diastole were detected between groups.

**Discussion**

The main goal of our TDI study was to evaluate the systolic and diastolic radial and longitudinal LVFW motion in dogs in the preclinical stage of GRMD-associated DCM. Overall, 60 LVFW segments (15 endocardial, 15 epicardial, 15 basal, and 15 apical segments) were analyzed in 9 dogs with GRMD and 6 healthy age-matched control dogs of the same breed. Our results indicated that, compared with findings in the control dogs, both radial and longitudinal left ventricular motions were significantly impaired in dogs with GRMD during systole as well as during diastole, despite the absence of clinical and radiographic signs of heart disease and lack of abnormal findings via ECG examination in these dogs.

Histologic, echocardiographic, and radionuclide investigations in dogs have revealed cardiomyopathic changes associated with GRMD. These myocardial lesions result in progressive myocardial dysfunction, followed by left ventricular and left atrial dilation. Congestive heart failure generally develops at a later stage of the disease, often after several months or years. Therefore, the progressive cardiac dysfunction associated with GRMD provides an opportunity to study the preclinical stage of DCM and assess early markers of myocardial alteration such as TDI measurements. To determine the accuracy of TDI indices for early detection of GRMD-associated DCM, we evaluated young adult dogs with GRMD that had little or no risk of cardiac arrhythmias or congestive heart failure. Left atrial dilatation was detected in none of the dogs with GRMD; moreover, myocardial function, as determined by conventional echocardiographic assessments, was normal for most dogs (6/9 dogs).

Several studies have revealed that TDI can be used to establish a preclinical diagnosis of familial hypertrophic cardiomyopathy in both human patients and in rabbits, prior to and independently of the development of left ventricular hypertrophy. Also, TDI has been proven to be more sensitive than conventional echocardiography for detection of moderate myocardial alterations in different clinical settings, such as ischemia and heart transplant disorders.

In a preliminary study, our group analyzed systolic radial LVFW motion in puppies with GRMD and compared those findings with data from healthy puppies. Despite normal left ventricular dimensions and shortening fraction, puppies with GRMD had lower systolic endocardial velocity and systolic radial MVG than control puppies. However, the early and late diastolic radial velocities were not measured, and the longitudinal LVFW motion was not assessed. In the present study, both radial and longitudinal LVFW motions during the whole cardiac cycle were analyzed, thus providing complete TDI information on the heart movement of dogs with GRMD.

As reported in clinically normal Beagles, all radial and longitudinal velocity profiles obtained in dogs with GRMD and in healthy control dogs in our study included 1 positive systolic wave that occurred after a short isovolumic contraction phase and 2 diastolic negative waves (1 each detected in early and late diastole) that occurred after a short isovolumic relaxation phase. In the 2 groups of dogs, LVFW segments moved with various velocities; during the whole cardiac cycle, the endocardial layers moved more rapidly than the epicardial layers, defining 3 radial MVGs (ie, systolic and early and late diastolic MVGs). The LVFW velocities also decreased significantly from base to apex, defining 3 longitudinal MVGs. Similar radial and longitudinal transmural gradients have been described in humans and cats.

Compared with control dogs, dogs with GRMD had marked impairment of nearly all radial and longitudinal TDI indices. This impairment was much more pronounced during systole and early diastole for both motions. The 3 radial MVGs were significantly lower in dogs with GRMD than they were in control dogs. These marked MVG alterations in dogs with GRMD were related to significantly lower endocardial velocities, whereas epicardial velocities were comparable in the 2 groups. Systolic and early diastolic longitudinal MVGs were also significantly lower because of lower basal velocities. This basal alteration may be related to the location of the myocardial lesions in GRMD-associated cardiomyopathy, which develop predominantly in the posterobasal aspect of the LVFW initially. Further studies in very young dogs with GRMD are required to assess which TDI indices are the most sensitive markers of the GRMD-associated cardiomyopathy.

In a previous study, our group determined that longitudinal and radial TDI indices have a significantly smaller variability when measurements are performed in anesthetized dogs during transient respiratory arrest, compared with measurements obtained in awake dogs.
These results suggested that breathing may contribute to the variability in TDI indices in awake dogs. One limitation of the study of this report is that, for technical (software) reasons, only 2 consecutive cardiac cycles could be analyzed simultaneously. Calculation of a mean value from > 2 measurements for each LVFW velocity would have been preferable to minimize the effects of changes in cardiac positioning related to the breathing movements and the position of the heart within the thorax. When assessing conventional or Doppler echocardiography, 3 to 5 cardiac cycles are generally evaluated to obtain mean measurements for each variable, although to our knowledge, no study under blinded and well-controlled conditions has yet confirmed scientifically the need of this practice. Another limitation of our study is that the dogs with GRMD included 3 dogs with echocardiographic evidence of DCM, although these dogs had an absence of clinical and radiographic signs of heart disease and a lack of abnormal findings via ECG examination. Evaluation of a more homogeneous group, including only dogs with GRMD in the occult phase of the disease, would have been preferable. The last limitation of our study is that although TDI measurements were performed during off-line analysis by an observer who was unaware of the genotype results, conventional echocardiography could not be performed under masked conditions; all dogs with GRMD had signs of skeletal muscle dystrophy, including stiff gait and truncal and temporal amyotrophy of various degrees. These clinical signs of muscular dystrophy in these dogs are similar to those detected in humans with Duchenne dystrophy; the signs are associated with the lack of dystrophin that results in severe early fiber necrosis and regeneration and progressive replacement of muscular tissues by fibrosis, fat, and mineralization with secondary muscular atrophy and joint ankylosis. These skeletal lesions develop early, even during the neonatal period in some muscles such as the deltoid, trapezius, extensor carpi radialis, and sartorius muscles. Conversely, histologic lesions in the heart are detected in the later stages of the disease, and morphologic changes are generally not detectable before 3 months of age. The results of the present study have indicated that systolic and diastolic radial and longitudinal LVFW velocities are significantly impaired in dogs in the preclinical phase of the GRMD-associated cardiomyopathy. These data have suggested that TDI may be a useful tool for early detection of DCM, at a time when findings of cardiovascular clinical examination, thoracic radiography, and ECG examination are still within reference limits and left ventricular dimensions and function are still preserved in the affected dog. Whether our results are applicable for early screening of other forms of spontaneous DCM in dogs remains to be elucidated; longitudinal prospective studies would also be needed to determine the prognostic value of these new TDI indices.

References
21. Thomas WP, Gaber CE, Jacobs GJ, et al. Recommendations for standards in transl thoracic two-dimensional echocardiography in...


