Expression of matrix metalloproteinases, their inhibitors, and lysyl oxidase in myocardial samples from dogs with end-stage systemic and cardiac diseases

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Objective—To compare the degree of mRNA expression for matrix metalloproteinases (MMPs), tissue inhibitors (TIMPs), and lysyl oxidase in myocardial samples from dogs with cardiac and systemic diseases and from healthy control dogs.

Sample—Myocardial samples from the atria, ventricles, and septum of 8 control dogs, 6 dogs with systemic diseases, 4 dogs with dilated cardiomyopathy (DCM), and 5 dogs with other cardiac diseases.

Procedures—Degrees of mRNA expression for MMP-1, -2, -3, -9, and -13; TIMP-1, -2, -3, and -4; and lysyl oxidase were measured via quantitative real-time PCR assay. Histologic examination of the hearts was performed to identify pathological changes.

Results—In myocardial samples from control dogs, only TIMP-3 and TIMP-4 mRNA expression was detected, with a significantly higher degree in male versus female dogs. In dogs with systemic and cardiac diseases, all investigated markers were expressed, with a significantly higher degree of mRNA expression than in control dogs. Furthermore, the degree of expression for MMP-2, TIMP-1, and TIMP-2 was significantly higher in dogs with DCM than in dogs with systemic diseases and cardiac diseases other than DCM. Expression was generally greater in atrial than in ventricular tissue for MMP-2, MMP-13, and lysyl oxidase in samples from dogs with atrial fibrillation.

Conclusions and Clinical Relevance—Degrees of myocardial MMP, TIMP, and lysyl oxidase mRNA expression were higher in dogs with cardiac and systemic diseases than in healthy dogs, suggesting that expression of these markers is a nonspecific consequence of end-stage diseases. Selective differences in the expression of some markers may reflect specific pathogenic mechanisms and may play a role in disease progression, morbidity and mortality rates, and treatment response. (Am J Vet Res 2013;74:216–223)
Matrix metalloproteinases and TIMPs are expressed by the major cell types found in the myocardium, cardiac myocytes, fibroblasts, smooth muscle cells, and endothelial cells.2,3 Their expression increases through various mechanisms, including mechanical stretch, neurohormonal activation, and cytokine stimulation.2,3 Therefore, MMPs and TIMPs may not only be important in cardiac diseases but may also be present and play a role in cardiac dysfunction in systemic diseases.17

Cardiac ECM turnover is precisely regulated to maintain the equilibrium between synthesis and degradation. An imbalance results in degradation or production of ECM proteins in the cardiac interstitial space and in cardiac fibrosis.3 Depending on the hemodynamic effect of cardiac diseases, variations in the amounts of MMP and TIMP can occur.2,3 In people with DCM, an increase has been reported in the expression of myocardial MMP-1, MMP-2, TIMP-1, and TIMP-2 as well as a decrease or increase in MMP-9 mRNA expression.3,7,14,15 In dogs with DCM, an increase in MMP-916 and TIMP-117 mRNA expression reportedly occurs, and in dogs with DVD, increases occur in the production of MMP-3 and TIMP-1, -2, and -3 as well as a decrease in MMP-2 production.16–21

Lysyl oxidase, which is an extracellular, matrix-embedded protein, plays an important role in the cross-linking of the collagen fibrils, resulting in the deposition of insoluble collagen fibrils.22 An increase in lysyl oxidase expression, associated with excessive fibrillar collagen cross-linking and fiber deposition, has been identified in humans and other animals with enhanced myocardial stiffness and LV dysfunction and heart failure.7,23 The enzyme also reportedly plays a role in atrial fibrosis and may therefore be involved in the pathogenesis of atrial fibrillation.24

Interestingly, women with cardiac disease have a better outcome than men at similar decreases in systolic function, and cardiac resynchronization treatment might be more effective in women, which suggests different remodeling processes between genders.25,26 In veterinary species, however, the effect of sex on ECM remodeling in naturally occurring cardiac diseases has not yet been assessed.27

An imbalance of MMPs, TIMPs, and lysyl oxidase mRNA and protein expression in dogs with DCM is likely, but few studies16,17 have been conducted to investigate whether this is true. Furthermore, information on constitutive cardiac MMP, TIMP, and lysyl oxidase expression in healthy dogs and whether an increase occurs in dogs with arrhythmia (eg, atrial fibrillation) and systemic diseases is not available, although a constitutive expression with an increase in systemic diseases and cardiac diseases other than DCM is likely to exist.

The purpose of the study reported here was to measure mRNA expression for MMP, TIMP, and lysyl oxidase in the myocardial tissues from these groups of dogs. Other objectives were to determine the enzymes' potential involvement in DCM and other cardiac diseases and to assess whether systemic diseases lead to their imbalance. We hypothesized that differences would exist in the degree of mRNA expression among groups and cardiac regions, with an increase for MMPs and a decrease for TIMPs and lysyl oxidase in dogs with DCM and an increase for TIMPs and lysyl oxidase in atrial samples from dogs with atrial fibrillation.

Materials and Methods

Canine myocardial tissue specimens—The study involved myocardial tissue samples obtained from the cadavers of 8 previously healthy dogs (control samples), 6 dogs with systemic diseases, and 9 dogs with cardiac diseases. Control samples originated from research Beagles from a pharmaceutical company that had been euthanized and examined on site. Myocardial samples immersed in an RNA-stabilizing solution28 had been collected and donated for the study.

The dogs with cardiac and systemic diseases consisted of patients that had undergone a diagnostic workup, and most had been euthanized at the request of the owner because of a poor prognosis. The exception was a dog with recurrent ventricular tachycardia that had developed ventricular fibrillation and died. For dogs with cardiac diseases, the cardiac diagnostic approach included a CBC, serum biochemical analysis, blood pressure measurement, ECG, echocardiography, and thoracic radiography at various points prior to euthanasia.

The heart was removed from each dog within 1 hour after death and grossly examined for any pathological changes. Myocardial samples from the interventricular septum, right atrium and ventricle, and left atrium and ventricle were collected for RNA extraction and stored in RNA stabilizing solution28 at –20°C until analyzed. Hearts were subsequently fixed in neutral-buffered 10% formalin, and samples from the same sites as those for RNA extraction were prepared and routinely embedded in paraffin wax for histologic examination.

Histologic examination of hearts—For the histologic examination, 3- to 5-µm-thick sections of hearts from all diseased dogs were prepared and stained with H&E and Masson trichrome stains. Hearts from healthy dogs were not available for histologic examination. Histopathologic changes identified via light microscopy were recorded, and the most relevant changes (lipo-matosis cordis, cardiac [interstitial, subendocardial, or subepicardial] fibrosis, leukocyte infiltration, muscular hypertrophy of small arteries, and cardiomyocyte necrosis) were scored semiquantitatively. With healthy control hearts used as a reference, changes in hearts from diseased dogs were graded as mild, moderate, or severe.

Quantitative real-time PCR assay—Total RNA was extracted from myocardial samples from healthy dogs,
dogs with systemic disease, and dogs with cardiac disease, and cDNA was synthesized as reported.25

Primers for canine lysyl oxidase were designed with commercial software.6 The forward sequence used for lysyl oxidase was TGCTTGAGGACA-GAGAATG, and the reverse was ACAGGTAGTCTCAGGGGGT. Bioinformatic searches were performed to confirm gene specificity. For the canine housekeeping gene GAPDH, and for MMP-1, -2, published gene sequences18–31 for MMP-1, -2, -3, and -9 and TIMP-1, -2, -3, and -4 were used. Primers were validated with a standard curve of 8 serial dilutions, and primer efficiencies were between 95% and 118%. The PCR assay was performed in accordance with a standard protocol.27,32 Real-time data were analyzed with the aid of detection software.4 Relative degrees of mRNA expression were normalized to GAPDH expression and calculated with the 2-ΔΔCt method.32,33 Results are reported in arbitrary units.

### Results

#### Animals—Dogs from which hearts were obtained for the control group included 4 sexually intact males and 4 sexually intact females, with a median age of 3 years. Dogs from which samples were obtained for the cardiac disease group included 4 with DCM, 1 with DVD, and 1 each with arrhythmogenic cardiomyopathy, tricuspid dysplasia, pulmonic stenosis, and aortic stenosis. Their characteristics as well as those of the dogs from which samples were collected for the systemic disease group were summarized (Table 1).

_Dogs with cardiac disease had been treated with furosemide, pimobendan, angiotensin-converting enzyme inhibitor, spironolactone, and hydrochlorothiazide-amiloride. Antiarrhythmic drugs included diltiazem and digoxin for atrial fibrillation and mexiletine and amiodarone for ventricular arrhythmia._

#### Statistical analysis—Data were recorded in spreadsheets, and statistical analysis was performed with a statistical software program.1 After basic descriptive statistics were calculated, the values of several variables were transformed to achieve normal distributions and model assumptions for parametric analysis. Values for MMP-2, MMP-9, and TIMP-1, -2, -3, and -4 were logarithmically transformed. The inverse square root of values was used for lysyl oxidase. For comparison of dog groups, 1-way ANOVA was performed with nonnormalized data to explore the relationships among MMP, TIMP, and lysyl oxidase values of dogs with cardiac diseases. Individual correlations were examined with the Pearson correlation test. Values of P < 0.05 were considered significant for all analyses.

#### Histopathologic findings

<table>
<thead>
<tr>
<th>Group and disease</th>
<th>Breed</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Right atrium</th>
<th>Left atrium</th>
<th>Right ventricle</th>
<th>Interventricular sphincter</th>
<th>LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac disease</td>
<td>Doberman Pinscher</td>
<td>8</td>
<td>F</td>
<td>Marked LC, LI, SepF</td>
<td>LC, LI</td>
<td>LC, LI, marked Inf, AC</td>
<td>LC</td>
<td>LC</td>
</tr>
<tr>
<td>DCM with AF</td>
<td>Great Dane</td>
<td>6</td>
<td>M</td>
<td>Marked LC, LI, SepF, SenF, marked arteriosclerosis</td>
<td>LC, LI, Inf, MH</td>
<td>LC, LI, Inf</td>
<td>LC, LI, Inf</td>
<td>LC</td>
</tr>
<tr>
<td>DCM with AF</td>
<td>Doberman Pinscher</td>
<td>10</td>
<td>M</td>
<td>Marked LC and LI, MI, NI, Inf, MH</td>
<td>LC, LI, Inf</td>
<td>LC, LI, Inf</td>
<td>LC, LI, Inf</td>
<td>LC</td>
</tr>
<tr>
<td>DCM</td>
<td>Bullmastiff</td>
<td>7</td>
<td>M</td>
<td>Marked LC and LI, MI, NI, Inf, MH</td>
<td>LC, LI, Inf, MH</td>
<td>LC, LI</td>
<td>LC</td>
<td>LC</td>
</tr>
<tr>
<td>Tricuspid dysplasia</td>
<td>Labrador Retriever</td>
<td>0.2</td>
<td>M</td>
<td>LC, LI, CN</td>
<td>LC, LI</td>
<td>LC, AC</td>
<td>LC, AC</td>
<td>LC, AC</td>
</tr>
<tr>
<td>Arrhythmogenic cardiomyopathy</td>
<td>Labrador Retriever</td>
<td>8</td>
<td>M</td>
<td>LI, MH</td>
<td>LI</td>
<td>LC</td>
<td>LI</td>
<td>NAD</td>
</tr>
<tr>
<td>Aortic stenosis</td>
<td>Boxer</td>
<td>11</td>
<td>M</td>
<td>LI, MH</td>
<td>LI</td>
<td>LC</td>
<td>LI</td>
<td>MH</td>
</tr>
<tr>
<td>Pulmonic stenosis</td>
<td>Labrador Retriever</td>
<td>9</td>
<td>NF</td>
<td>Marked LI, MH, marked calcification</td>
<td>LI</td>
<td>LI, AC</td>
<td>LI, AC</td>
<td>LI, marked AC</td>
</tr>
<tr>
<td>DVD with AF</td>
<td>German Shepherd Dog</td>
<td>14</td>
<td>NF</td>
<td>LC, LI</td>
<td>LC, LI</td>
<td>Marked LC</td>
<td>LC</td>
<td>LC</td>
</tr>
<tr>
<td>Systemic disease</td>
<td>Lymphoma</td>
<td>7</td>
<td>NF</td>
<td>NAD</td>
<td>NAD</td>
<td>NAD</td>
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<td>NAD</td>
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<tr>
<td>Hypersplenodysplasia</td>
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<td>M</td>
<td>NAD</td>
<td>NAD</td>
<td>NAD</td>
<td>NAD</td>
<td>NAD</td>
</tr>
<tr>
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<td>Cross breed</td>
<td>11</td>
<td>NF</td>
<td>LI</td>
<td>LI</td>
<td>LC</td>
<td>NAD</td>
<td>MH</td>
</tr>
<tr>
<td>Spinal fracture</td>
<td>Labrador Retriever</td>
<td>0.5</td>
<td>M</td>
<td>LI, SenF</td>
<td>LI</td>
<td>LI</td>
<td>NAD</td>
<td>NAD</td>
</tr>
<tr>
<td>Brain tumor</td>
<td>Cocker Spaniel</td>
<td>8</td>
<td>NF</td>
<td>LI</td>
<td>LI</td>
<td>LI</td>
<td>NAD</td>
<td>NAD</td>
</tr>
<tr>
<td>Brain tumor</td>
<td>Boxer</td>
<td>7.5</td>
<td>M</td>
<td>NAD</td>
<td>NAD</td>
<td>NAD</td>
<td>NAD</td>
<td>NAD</td>
</tr>
</tbody>
</table>

Gross pathological and histologic evaluation—
No abnormalities were grossly evident in the hearts from the healthy dogs. Findings of gross pathological evaluation confirmed the clinical diagnosis of all dogs with heart disease. It was determined during histologic examination that all dogs with DCM had features consistent with the fibrous fatty type described in the literature (ie, lipomatosis cordis; interstitial, subendocardial, and subepicardial fibrosis; leukocyte infiltration; and focal cardiomyocyte necrosis); muscular hypertrophy of small arteries was also common. In hearts from dogs with other cardiac diseases, changes were primarily restricted to mild lipomatosis cords and lymphocytic infiltration as well as mild muscular hypertrophy of small arteries (Table 1).

The hearts from dogs with systemic disease had no evidence of gross pathological change. The histologic examination did not yield any evidence of disease-related pathological changes, such as neoplastic cell infiltration in hearts from dogs with malignant tumors. Histopathologic changes were primarily restricted to mild myocardial lymphocytic infiltration in both atria of 3 dogs and mild lipomatosis cords in the right ventricle of 2 dogs (Table 1).

PCR assay—In the control myocardial samples, only mRNA for TIMP-3 (mean ± SD expression, 2.26 ± 1.16) and TIMP-4 (2.0 ± 1.43) was detected, with significantly (P < 0.001) expression in males (2.88 ± 0.30 and 3.3 ± 0.36, respectively) than in females (0.29 ± 0.32 and 0.64 ± 0.53, respectively). In myocardial samples from dogs with cardiac and systemic diseases, the mRNA for all MMPs and TIMPs was expressed and the degree of expression for TIMP-3 and TIMP-4 was significantly (P < 0.001) higher than that in control dogs (Table 2). For atrial samples from dogs with car-

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Atria</th>
<th>P value*</th>
<th>Ventricles</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1*</td>
<td>0.35 ± 0.29</td>
<td>0.59 ± 0.27</td>
<td>0.002</td>
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</tr>
<tr>
<td>MMP-2</td>
<td>2.67 ± 0.66</td>
<td>2.73 ± 0.93</td>
<td>0.006</td>
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</tr>
<tr>
<td>MMP-3*</td>
<td>0.56 ± 0.34</td>
<td>0.42 ± 0.40</td>
<td>0.57</td>
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</tr>
<tr>
<td>MMP-9</td>
<td>0.68 ± 0.72</td>
<td>0.78 ± 0.66</td>
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<tr>
<td>MMP-13*</td>
<td>0.46 ± 0.27</td>
<td>0.52 ± 0.32</td>
<td>0.42</td>
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<tr>
<td>TIMP-1</td>
<td>2.98 ± 0.59</td>
<td>2.85 ± 0.49</td>
<td>0.34</td>
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<tr>
<td>TIMP-2</td>
<td>3.99 ± 0.34</td>
<td>3.80 ± 0.25</td>
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<tr>
<td>TIMP-3</td>
<td>4.29 ± 0.41</td>
<td>4.03 ± 0.41</td>
<td>0.032</td>
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<tr>
<td>TIMP-4</td>
<td>3.40 ± 0.32</td>
<td>3.25 ± 0.57</td>
<td>0.10</td>
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<tr>
<td>Lysyl oxidase</td>
<td>48.30 ± 26.00</td>
<td>38.12 ± 16.79</td>
<td>0.077</td>
<td></td>
</tr>
</tbody>
</table>

*The inverse square root was applied to achieve a normal data distribution. This resulted in smaller numbers meaning a greater degree of mRNA expression.

Values of P < 0.05 were considered significant.

Table 2—Mean ± SD degrees of MMP TIMP and lysyl oxidase expression (mRNA transcription) in atrial tissue (2 samples/dog) and ventricular tissue (2 samples/dog) from hearts of dogs with systemic (n = 6) and cardiac (10) diseases.

Systemic disease

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Atria</th>
<th>P value*</th>
<th>Ventricles</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1*</td>
<td>0.35 ± 0.30</td>
<td>0.51 ± 0.32</td>
<td>0.002</td>
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<tr>
<td>MMP-2</td>
<td>2.67 ± 0.88</td>
<td>2.86 ± 0.52</td>
<td>0.002</td>
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</tr>
<tr>
<td>MMP-3*</td>
<td>0.39 ± 0.40</td>
<td>0.42 ± 0.57</td>
<td>0.57</td>
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</tr>
<tr>
<td>MMP-9</td>
<td>0.77 ± 0.68</td>
<td>0.61 ± 0.77</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>MMP-13*</td>
<td>0.52 ± 0.32</td>
<td>0.47 ± 0.30</td>
<td>0.48</td>
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</tr>
<tr>
<td>TIMP-1</td>
<td>2.67 ± 0.37</td>
<td>3.0 ± 0.64</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>TIMP-2</td>
<td>3.95 ± 0.20</td>
<td>3.95 ± 0.36</td>
<td>0.024</td>
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</tr>
<tr>
<td>TIMP-3</td>
<td>4.22 ± 0.31</td>
<td>4.01 ± 0.37</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>TIMP-4</td>
<td>3.33 ± 0.26</td>
<td>3.23 ± 0.42</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Lysyl oxidase</td>
<td>36.78 ± 16.8</td>
<td>42.6 ± 21.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3—Mean ± SD degrees of MMP TIMP and lysyl oxidase expression (mRNA transcription) in atrial tissue (2 samples/dog) and ventricular tissue (2 samples/dog) from hearts of dogs with cardiac disease or various cardiac diseases.

Values of P < 0.05 were considered significant.

Table 4—Mean ± SD degrees of MMP TIMP and lysyl oxidase expression (mRNA transcription) in atrial and ventricular tissue from the dogs in Table 3, with (n = 4; 8 samples) or without (6; 10 samples) atrial fibrillation.
diac and systemic diseases, comparison of the degree of mRNA expression for MMP, TIMP, and lysyl oxidase in atrial versus ventricular tissue revealed significantly greater values for MMP-1 (P = 0.002), MMP-2 (P = 0.006), TIMP-2 (P = 0.014), and TIMP-3 (P = 0.032).

Myocardial tissue from dogs with CHF had significantly higher MMP-2 (P = 0.010) and TIMP-1 (P = 0.024) mRNA expression than did tissue from dogs without CHF or dogs with systemic diseases (Table 3). Furthermore, expression of MMP-2 (P = 0.019), TIMP-1 (P < 0.001), and TIMP-2 (P = 0.028) was significantly greater in myocardial tissue from dogs with DCM than in tissue from dogs with systemic diseases and other cardiac diseases.

Atrial tissue from dogs with atrial fibrillation had a significantly greater degree of mRNA expression for MMP-2 (P = 0.024), MMP-13 (P = 0.046), and lysyl oxidase (P = 0.005) and significantly greater ventricular TIMP-2 (P = 0.025) values than did atrial and ventricular tissue, respectively, from dogs with systemic diseases (Table 4). In myocardial tissue from dogs with cardiac diseases, lower mRNA expression for MMP-9 (P = 0.002) and TIMP-1 (P = 0.024) was evident in atrial samples and lower expression for TIMP-1 (P = 0.045) was found in ventricular tissues from dogs with versus without atrial fibrillation.

Cluster analysis and correlations—The cluster and Pearson correlation analyses identified 3 clusters in dogs with cardiac diseases. Several significant (P < 0.001) positive correlations were detected in the degree of mRNA expression among the MMPs, TIMPs, and lysyl oxidase (Table 5).

**Discussion**

The purpose of the present study was to assess the degree of mRNA expression for MMPs, TIMPs, and lysyl oxidase in myocardial tissue. These enzymes are known to be of relevance in tissue remodeling and would therefore be expected to contribute to the pathogenesis of DCM and to possibly be involved in other cardiac diseases or end-stage systemic diseases.\(^\text{16,17}\) To obtain information on the basal degree of mRNA expression in both sexes and to avoid the influence of any potential age-related pathological and functional changes, the hearts of young, healthy Beagles were also examined in the study. Only the mRNA for TIMP-3 and TIMP-4 was expressed in these healthy dogs and at a significantly higher degree in male versus female Beagles. A study\(^\text{16}\) of the constitutive transcription of genes for MMPs and TIMPs in canine hearts was performed previously; investigators focused on the mitral valve leaflets and detected MMP-2, MMP-9, and TIMP-3 mRNA expression. The reason for this difference between study findings may be that heart valves are mainly composed of interstitial cells, glycosaminoglycans, proteoglycans, collagen, and elastin fibers,\(^\text{34–36}\) whereas myocardial tissue was the subject of interest in our study. Also, potential sex differences were not assessed in the other study.\(^\text{16}\) In humans, however, hormonal influences on the degree of MMP and TIMP expression exist.\(^\text{37–39}\)

The most abundant TIMP in murine myocardium is TIMP-4, which is believed to be heart specific, although its role is not completely understood.\(^\text{10,18}\) In a different study\(^\text{14}\) involving dogs, we found greater tumor necrosis factor-α and transforming growth factor-β1 and -β3 mRNA expression in myocardial samples from healthy male versus healthy female dogs. These results suggest a generally higher fibrogenic potential in (young) male dogs than in female dogs. We were not able to include older control dogs in the present study to assess whether the degrees and patterns of myocardial mRNA expression change with age. However, a study\(^\text{36}\) of MMP and TIMP expression in LV samples from young (18 months) and old (> 8 years) healthy sheep did not reveal age-related changes in mRNA expression, apart from a higher degree for MMP-2 with age.

Our findings supported those of previous studies\(^\text{16–18,20,21,34}\) and suggested that transcription of genes for all examined enzymes takes place in the myocardium of dogs with cardiac diseases. Interestingly, however, myocardial samples from dogs with severe systemic diseases and without remarkable pathological cardiac changes also had evidence of mRNA expression for all

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**Table 5—Correlations among the degrees of mRNA transcription for MMPs, TIMPs, and lysyl oxidase in myocardial samples from dogs with cardiac diseases (n = 9; 5 samples/dog).**

<table>
<thead>
<tr>
<th>MMPs</th>
<th>MMP-2</th>
<th>MMP-3</th>
<th>MMP-9</th>
<th>MMP-12</th>
<th>TIMP-1</th>
<th>TIMP-2</th>
<th>TIMP-3</th>
<th>TIMP-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>0.118</td>
<td>0.998</td>
<td>0.963</td>
<td>0.891</td>
<td>0.253</td>
<td>0.325</td>
<td>0.666</td>
<td>0.416</td>
</tr>
<tr>
<td>P</td>
<td>0.415</td>
<td>0.001</td>
<td>0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lysyl oxidase</td>
<td>0.118</td>
<td>0.076</td>
<td>0.067</td>
<td>0.063</td>
<td>0.064</td>
<td>0.272</td>
<td>0.666</td>
<td>0.416</td>
</tr>
<tr>
<td>Value</td>
<td>0.415</td>
<td>0.001</td>
<td>0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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<td>= Not applicable.</td>
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<td></td>
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</tbody>
</table>

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examined enzymes, even at degrees similar to those in tissues from dogs with cardiac diseases. Therefore, it is possible that generalized cardiac remodeling occurs not only in cardiac diseases but also in end-stage systemic diseases, potentially causing myocardial dysfunction.4,5 However, histologic examination of the hearts did not reveal any cardiac changes that would indicate relevant cardiac remodeling. Accordingly, a more acute functional change may be involved, particularly given that we also observed an increase in inflammatory cytokine mRNA expression in the myocardium of the dogs in that study.31

An increase in the local cytokine production in addition to systemic activation of the inflammatory cascade with an increase in the amount of circulating catecholamines and an activation of the renin-angiotensin-aldosterone-system might result in myocardial imbalance of MMP and TIMP mRNA expression and subsequent impairment of cardiac myocyte function and cardiac remodeling.46–48 Most dogs with systemic diseases of the present study were oncology patients and euthanized because of malignant, final-stage tumors, and an activation of the inflammatory system was likely present. Echocardiography was not performed to identify myocardial dysfunction immediately prior to euthanasia; however, the apparent proinflammatory state of the myocardium in dogs with systemic diseases is of interest and requires additional investigation because this state might influence morbidity, mortality rate, and long-term survival rates.

In all diseased dogs of the present study, atrial tissue samples had a significantly higher degree of mRNA expression for MMP-1, MMP-2, TIMP-2, and TIMP-3 than did ventricular tissues. In dogs with experimentally induced tachycardia leading to CHF, atria have greater cardiomyocyte degeneration, MMP activity, leukocyte infiltration, and fibrosis than do ventricles.47

Myocardial tissues from dogs with certain cardiac diseases had an increase in MMP and TIMP mRNA expression that was particular to those diseases. In tissues from dogs with clinically diagnosed CHF, MMP-2 and TIMP-1 mRNA expression was significantly higher than in tissues from dogs with systemic diseases and cardiac disease without CHF. Dogs with DCM had greater MMP-2, TIMP-1, and TIMP-2 expression than did dogs with systemic diseases or other cardiac diseases. These findings are partly similar to those in humans with cardiomyopathy and CHF, in which expression of MMP-2, -3, and -9 as well as TIMP-1 and -2 is significantly greater than that in humans without cardiac disease.17

In the mitral valves of dogs with DVD, mRNA expression of MMP-1, MMP-3, and TIMP-2, -3, and -4, but interestingly neither MMP-2 nor MMP-9, has been detected.31 An increase was evident for all MMPs and TIMPs investigated in the study reported here when results for hearts from dogs with cardiac disease were compared with those from control dogs. Considering that the histopathologic changes in hearts from dogs with DCM were more severe than in hearts from dogs with other cardiac diseases, it is possible that the severity rather than the type of clinical cardiac disease is responsible for the observed increase in MMP and TIMP mRNA expression.

The roles of MMP-2 and MMP-9, both of which are gelatinases, in cardiac disease have been extensively researched. Both are induced by myocardial stretch and hypoxia and through neurohormonal and immune activation.3,7,8 Matrix metalloproteinases induce a reduction of fibrillar collagen cross-link formation and development of cardiac dysfunction.3,7,8,46–49 Therefore, the observed increase in the degree of MMP-2 expression in dogs with DCM and CHF was not surprising. An increase in the expression of TIMP-1, which induces fibrosis and remodeling,46 predicts the presence of heart failure in people with LV hypertrophy.31 In addition to profibrotic TIMP-1, TIMP-2 induces fibroblast proliferation and myofibroblast development, which contributes to fibrosis.3 In the study reported here, all DCM-affected hearts had some degree of the characteristic interstitial, subendocardial, or subepicardial fibrosis described for the condition.33 The increase in fibrosis could have been the consequence of an increase in MMP-2, TIMP-1, and TIMP-2 production in the myocardium. The same pathogenesis is also suspected in end-stage human DCM, in which TIMP-1 and TIMP-2 production is profoundly increased.32

Degrees of mRNA expression for MMP-2 and -13 and lysyl oxidase in atrial tissues and for TIMP-2 in ventricular tissues were significantly higher when the source was dogs with atrial fibrillation rather than those with systemic disease. Similarly, greater MMP-2, TIMP-2, and lysyl oxidase expression has been detected in atria of people with atrial fibrillation and is hypothesized to contribute to an increase in fibrosis and structural remodeling.2,4

Interestingly, in the study reported here, the amount of MMP-9 and TIMP-1 expression was lower in atrial tissue from dogs with atrial fibrillation than in tissues from dogs with cardiac disease but without atrial fibrillation. The combination of a decrease in TIMP-1 expression and increase in MMP-2 and MMP-13 expression suggested the existence of ECM degradation. Such a phenomenon could lead to atrial dilatation, which is a consistent feature of atrial fibrillation. An increase in the degree of lysyl oxidase expression and decrease in MMP-9 expression might counterbalance the latter and induce ECM deposition, thereby increasing the amount of fibrosis, which could be a protective mechanism to avoid progressive atrial dilatation.2,4,23,46,54 Histologic examination of most hearts from dogs with atrial fibrillation in our study revealed atrial fibrosis and leukocyte infiltration consistent with a chronic inflammatory process. Similar changes were also observed in atrial tissue from the DCM-affected dog without atrial fibrillation, which suggested that the dog might have developed atrial fibrillation had it lived longer; however, this supposition remains speculative. Regardless, the results of the present study suggested that atrial remodeling occurs in dogs with DCM, which might contribute to the development of atrial dysfunction and arrhythmia.2,4

Limitations in the present study were mainly attributable to the small number of hearts used, composition of the control group, and inhomogeneous diseased groups. Healthy control dogs had not undergone specific cardiac assessment, which would have left minor functional or structural cardiac abnormalities unnoticed. Hearts from healthy dogs were grossly unaltered but were unavailable for histologic examination. Ac-
cordingly, the observed apparent constitutive mRNA expression of TIMP-3 and TIMP-4 in those dogs should be interpreted with caution. The dogs from which the control hearts originated were also significantly (P = 0.02) younger than the dogs in the diseased groups, which did not allow examination of potential age-related changes in constitutive transcription. That said, a study involving sheep did not reveal any age-related differences in the expression of TIMPs.

The dogs with cardiac disease from which hearts were obtained had DCM, DVD, arrhythmic cardiomyopathy, or congenital cardiac disease, and some also had CHE. However, subgroup formation (DCM and cardiac diseases other than DCM) with meaningful statistical evaluation was possible and provided interesting results. Most diseased dogs were end-stage patients, many of which had undergone long-term and different treatments. This may have had an impact on the degree of myocardial MMP, TIMP, and lysyl oxidase expression.9,10,11,12

A methodological limitation of the present study was that gene transcription and not protein expression was measured. With regard to MMPs and TIMPs, protein expression is not necessarily reflected by mRNA expression.96 Matrix metalloproteinases are secreted as proenzymes that require cleavage of the propeptide domain before they become activated.12 Nonetheless, changes in the degree of mRNA expression indicate functional changes. Findings of the present study suggested that the myocardium of healthy young adult dogs is in a proinflammatory state because of the increase in mRNA expression of TIMP-3 and TIMP-4. Sex differences were identified for systemic diseases other than DCM with meaningful statistical evaluation was possible and provided interesting results. Most diseased dogs were end-stage patients, many of which had undergone long-term and different treatments. This may have had an impact on the degree of myocardial MMP, TIMP, and lysyl oxidase expression.9,10,11,12

References


