Left ventricular remodeling is a complex process that is mediated in part by activation of the RAAS. In humans with mild to moderate and severe heart failure, blockade of the mineralocorticoid (aldosterone) receptor has been shown to improve survival times. Associations among serum N-terminal procollagen type III concentration, urinary aldosterone-to-creatinine ratio, and ventricular remodeling in dogs with myxomatous mitral valve disease

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Objective—To assess relationships among serum N-terminal procollagen type III concentration, urinary aldosterone-to-creatinine concentration ratio (UAC), and clinical variables in dogs with myxomatous mitral valve disease (MMVD) and healthy dogs.

Animals—162 dogs with MMVD and 24 healthy control dogs of comparable age and body weight.

Procedures—Blood and urine samples were collected from each dog. Dogs with MMVD underwent echocardiography and ECG. Ventricular diameter measurements were normalized for body weight. Serum N-terminal procollagen type III and urinary aldosterone concentrations were measured via radioimmunoassay. Each dog was examined on 1 to 3 occasions. Examinations were repeated at approximately 6-month intervals.

Results—Serum N-terminal procollagen type III concentration decreased with increasing severity of MMVD and was negatively associated with age and left ventricular end-diastolic and end-systolic diameters. The UAC increased with prior percentage change in left ventricular end-diastolic diameter per month, subsequent percentage change in left ventricular end-systolic diameter per month, and treatment with diuretics and was negatively associated with age. Both UAC and serum N-terminal procollagen type III concentration were higher in Cavalier King Charles Spaniels than in other breeds when other measured variables were controlled for.

Conclusions and Clinical Relevance—In dogs with MMVD, echocardiographic indicators of left ventricular remodeling appeared to be associated with a decrease in serum concentration of a marker of collagen type III turnover and an increase in urinary aldosterone concentration. (Am J Vet Res 2012;73:1765–1774)
This benefit is suggested to be attributable to a decrease in aldosterone-mediated interstitial fibrosis. In an experimental model of atrial fibrillation in dogs, treatment with spironolactone, which is a mineralocorticoid receptor antagonist, inhibited the development of atrial dilatation and fibrosis. In dogs with MMVD, survival time (from diagnosis to death or euthanasia due to cardiac disease) is negatively correlated with the myocardial fibrosis score at postmortem examination, and in dogs with heart failure, spironolactone administration appears to reduce the risk of death. Plasma aldosterone concentration is reportedly higher in the 6 months prior to cardiac decompensation than at the time of decompensation. Measurement of UAC has been suggested to estimate 24-hour aldosterone production and therefore may be more reflective of RAAS activation than measurement of the more labile plasma aldosterone concentration.

Circulating PIIINP is a marker of collagen type III turnover and hence ECM turnover. In humans, higher circulating PIIINP concentrations have been associated with more advanced cardiac disease and are considered a marker of myocardial fibrosis. Mineralocorticoid receptor blockade is most beneficial in humans with receptor antagonist, inhibited the development of atrial fibrillation. Measurement of UAC has been suggested to estimate 24-hour aldosterone production and therefore may be more reflective of RAAS activation than measurement of the more labile plasma aldosterone concentration.

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Materials and Methods

Animals—All dogs enrolled in the study were privately owned, and owner consent was obtained prior to enrollment. For inclusion in the control group, dogs were required to be healthy on the basis of their history and results of physical examination. Relevant mitral regurgitation was deemed to be absent on the basis of unremarkable auscultatory findings.

For inclusion in the MMVD group, dogs were required to have an echocardiographic diagnosis of MMVD, as made on the basis of characteristic abnormalities of the valve leaflets (thickening or prolapse) and evidence of regurgitant flow across the valve detected with Doppler techniques. Dogs with any other cardiac disease or major organ-related or systemic disease were excluded; however, dogs receiving medication for heart failure were included. The study protocol was approved by the Royal Veterinary College ethics and welfare committee.

Sample collection—Blood samples were collected from all dogs via jugular venipuncture into serum gel tubes. Owners collected urine samples from their dogs during natural voiding into sterile urine specimen containers on the morning of the examination (n = 161) or the previous evening (25). Urine samples collected the previous evening were stored at 2° to 4°C until centrifugation. Blood samples were stored at 2° to 4°C for up to 6 hours; urine samples were stored at 2° to 4°C for up to 18 hours. All samples were centrifuged for 15 minutes at 1,000 X g, and the serum and urine supernatants were stored at –80°C for subsequent analysis.

Laboratory analyses—Serum PIIINP concentration was measured with a commercially available RIA based on highly purified human PIIINP that had been validated for use with canine serum and bronchoalveolar lavage fluid. Urinary aldosterone concentration was measured following mild acid hydrolysis and extraction into ethyl acetate. Each urine sample (0.25 mL) was incubated with 25 mL of 3.2N hydrochloric acid in the dark for 24 hours at room temperature (mean temperature, 22°C). Ethyl acetate (2.5 mL) was then added to each sample followed by mixing at 30 revolutions/min for 60 minutes in a rotary mixer at room temperature. The samples were centrifuged at 600 X g for 5 minutes at 4°C, and 0.3 mL of the solvent phase was evaporated to dryness under a gentle stream of nitrogen at 37°C. The residues were reconstituted in assay zero standard (0.5 mL) and aldosterone concentration was measured via a commercially available RIA validated for use with canine urine. All in-house assays were performed precisely in accordance with the manufacturers’ instructions. Urinary creatinine concentrations were measured at a commercial laboratory.

Samples from multiple dogs were pooled to provide serum with low, medium, and high PIIINP concentrations and urine with low, medium, and high aldosterone concentrations. Intra-assay variability was determined by measuring the sample pools 6 times within 1 RIA. Interassay variability was determined by measuring the sample pools in 6 RIAs. For measurements of aldosterone concentration, the extraction procedure was performed separately for each sample replicate for both intra- and interassay variability determinations. The results are reported as coefficients of variation, calculated as the SD as a percentage of the mean. Urinary aldosterone and creatinine values were used to calculate the UAC in grams per mole.

Echocardiography—All echocardiographic examinations were performed with dogs unsedated by a board-certified cardiologist. Dogs were positioned in right lateral recumbency and then left lateral recumbency on an ultrasonography examination table. The echocardiographic examination was performed with an ultrasonographic unit equipped with 2- to 4-MHz and 3- to 7-MHz phased-array transducers and ECG monitoring. Standard imaging planes were digitally stored. Assessment of mitral valve structures was performed from the right parasternal long-axis view and the left apical 4-chamber view. Measurements of left atrial and aortic root diameters were made from the 2-D right parasternal short-axis view at the level of the aortic valve leaflets. Measurements were made at the onset of the P wave and were used to calculate the LA:Ao. The LVEDD,
left ventricular end systolic diameter, and LVFWd were measured from the right parasternal short-axis M-mode at the level of the papillary muscles and used to calculate the LVEDD:LFWFd ratio. The LVEDDN was determined by dividing by body weight (kg)^0.294. The LVESDN was determined by dividing by body weight (kg)^0.315.

Dogs with MMVD were classified according to their echocardiographic measurements and clinical status, as recommended in the ACVIM consensus statement. Categories assigned were no cardiomegaly (LVEDDN ≤ 1.8 cm/[kg^0.294] and LA:Ao ≤ 1.5; B1), cardiomegaly (LVEDDN > 1.8 cm/[kg^0.294] or LA:Ao > 1.5) but no current or previous clinical signs of congestive heart failure (B2), and cardiomegaly (LVEDDN > 1.8 cm/[kg^0.294] or LA:Ao > 1.5) plus current or previous clinical signs of congestive heart failure requiring medical management (C).

Data for dogs in class B1 that received treatment for congestive heart failure were excluded from between-group statistical comparisons because we believed congestive heart failure in the absence of cardiomegaly to be unlikely and any confounding effects of treatment on UAC and serum PIIINP concentration would be inappropriately increased by including these dogs in the analysis. These dogs were not excluded from linear regression analyses because the confounding effects of treatment were reduced by their inclusion as covariates in the multivariable models when appropriate.

**ECG**—All dogs with MMVD underwent ECG. Heart rate was measured from a 60-second ECG trace.

**Measurement of MMVD progression**—All procedures (blood and urine sample collection and assays, echocardiography, and ECG) were repeated for dogs with MMVD at approximately 6-month intervals unless the dogs died or were lost to follow-up. Each dog was examined on a maximum of 3 occasions, and to assess echocardiographic progression, a baseline visit was defined for comparison of findings with those of the previous or subsequent visit. For dogs examined 3 times, the second visit was designated as the baseline visit. For dogs examined only twice, either the first or second visit was randomly designated the baseline visit on the basis of a coin toss.

When the first visit was designated the baseline visit, subsequent percentage changes in LVEDDN and LVESDN per month were calculated. When the second visit was designated the baseline visit, prior percentage changes were calculated.

Prior percentage changes in LVEDDN and LVESDN per month were calculated by comparing the measurements from the baseline and previous examinations as follows:

\[
\text{Prior percentage change per month} = \left(\frac{|V_1 - V_2|/V_1\times 100}{T_{2-3}}\right)
\]

where \(V\) is the measurement at visit \(n\) and \(T\) is the time between visits in months. Similarly, subsequent percentage changes in LVEDDN and LVESDN were calculated by comparing the measurements from the baseline and subsequent examinations as follows:

Subsequent percentage change per month = \(\left(\frac{|V_3 - V_2|/V_2\times 100}{T_{1-2}}\right)\)

**Statistical analysis**—Statistical analyses were performed with commercially available software. Values of \(P < 0.05\) was considered significant. Data were assessed graphically for normality. Values for UAC, body weight, and LA:Ao were not normally distributed and were consequently logarithmically transformed prior to further analysis. The logarithmically transformed variables were normally distributed.

Comparisons of continuous variables were made for measurements obtained from healthy control dogs and dogs in the MMVD group at the baseline visit via the independent sample \(t\) test or 1-way ANOVA with Tukey post hoc comparisons, as appropriate. Proportions were compared via the \(\chi^2\) test. Correlations were assessed via calculation of Pearson (\(r\)) correlation coefficients. Univariate linear regression analyses were performed to assess associations in dogs with MMVD among UAC and serum PIIINP concentration and clinical characteristics (age, breed [CKCS vs non-CKCS], body weight, heart rate obtained from the ECG, echocardiographic measurements [LA:Ao, LVEDD:LFWFd ratio, LVEDDN, LVESDN, prior percentage change in LVEDDN and LVESDN per month, and subsequent percentage change in LVEDDN and LVESDN per month] and treatment with ACE inhibitors [yes vs no], pimobendan [yes vs no], or diuretics [yes vs no]).

The CKCS breed was chosen as the comparator breed because this was the most common breed in the study. Cavalier King Charles Spaniels are particularly prone to MMVD, and another study revealed differences in the natural history of the disease in this breed. In the univariate analyses, the assumption of linearity was assessed graphically by plotting the independent variables against the dependent variable (PIIINP concentration or UAC). The distributions of residuals were graphically tested for normality by plotting the predicted values against the residuals.

Variables associated with PIIINP concentration and UAC with values of \(P < 0.20\) in the univariate analysis were entered into multivariable linear regression analyses. Separate models were constructed for the outcomes UAC and serum PIIINP concentration. When treatment with furosemide (yes vs no) was not significant in the final model, the modeling process for UAC was repeated, forcing treatment with furosemide (yes vs no) into the final models. Multivariable models for UAC and serum PIIINP concentration were repeated, excluding dogs receiving medication for cardiac disease.

Not all dogs had been examined on > 1 occasion, so data for prior percentage change in LVEDDN and LVESDN per month and subsequent percentage change in LVEDDN and LVESDN per month were unavailable for dogs with only 1 evaluation. For a dog to have data for both prior percentage change in LVEDDN and LVESDN per month and subsequent percentage change in LVEDDN and LVESDN per month, it had to have been examined on at least 3 occasions. Therefore, separate multivariable models including and excluding these variables were constructed. In the multivariable regression models, analyses were performed in a backward stepwise manner. All variables were initially in-
cluded, and the variable with the highest $P$ value was removed until all remaining variables had a value of $P < 0.05$, which was considered significant.

The distributions of residuals in the multivariable analyses were graphically tested for normality by plotting the predicted values against the residuals. First-order interactions were assessed between variables that were significant in the multivariable analyses by including the product term of each pair of variables in the model. When a product term was found to be significant, it was retained in the final multivariable model.

**Results**

**Animals**—The control group consisted of 24 healthy dogs (14 neutered females, 3 sexually intact males, and 7 neutered males), with ages ranging from 3 to 13 years (mean ± SD, 8.9 ± 3.0 years) and body weights ranging from 4.2 to 26.9 kg (median, 11.2 kg [interquartile range, 7.1 to 13.8 kg]). There were 5 (21%) CKCSs and various other purebred and crossbred dogs. None of the dogs was treated with ACE inhibitors.

The MMVD group comprised 162 dogs (10 sexually intact females, 59 spayed females, 25 sexually intact males, and 68 neutered males), with ages ranging from 3 to 18 years (mean ± SD, 10.1 ± 2.9 years) and body weights ranging from 1.9 to 48.7 kg (median, 10.6 kg [interquartile range, 8.4 to 14.2 kg]). There were 68 (42%) CKCSs and various other purebred and crossbred dogs. Thirty-nine (24%) were undergoing treatment with ACE inhibitors, 24 (15%) were receiving diuretics, and 24 (15%) were receiving pimobendan. Seventy-two dogs with MMVD were evaluated 3 times during the study period, and 44 dogs were evaluated twice (13 had the first visit designated as the baseline visit, and 31 had the second visit designated as such). The remaining 46 dogs were examined on only 1 occasion.

Cardiopulmonary measurements at the baseline evaluation of dogs with MMVD were summarized (Table 1). At the baseline visit, there was no significant ($P = 0.804$) difference in body weight between the control and MMVD groups; control dogs were younger but not significantly ($P = 0.059$) so. Distributions of male and female dogs were similar ($P = 0.220$) between the control and MMVD groups.

**Assay variation**—The intra-assay coefficients of variation for low (5.9 µg/L), medium (10.2 µg/L), and high (15.7 µg/L) serum concentrations of PIIINP were 2.3%, 1.8%, and 1.5%, respectively. The interassay coefficients of variation for low, medium, and high serum concentrations of PIIINP were 8.2%, 3.3%, and 8.5%, respectively. The intra-assay coefficients of variation for low (168.3 pg/mL), medium (992.0 pg/mL), and high

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of dogs</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA:Ao</td>
<td>159</td>
<td>1.29 (0.83 to 2.75)</td>
</tr>
<tr>
<td>LVEDD:LVPWh ratio</td>
<td>158</td>
<td>3.92 (2.17 to 6.62)</td>
</tr>
<tr>
<td>LVEDDN (cm/[kg^{0.294}])</td>
<td>158</td>
<td>1.71 (1.14 to 2.58)</td>
</tr>
<tr>
<td>Prior change in LVEDDN per month (%)</td>
<td>85</td>
<td>0.47 (–2.30 to 4.45)</td>
</tr>
<tr>
<td>Subsequent change in LVEDDN in per month (%)</td>
<td>103</td>
<td>0.48 (–3.05 to 4.73)</td>
</tr>
<tr>
<td>LVESDN (cm/[kg^{0.315}])</td>
<td>158</td>
<td>1.01 (0.63 to 1.84)</td>
</tr>
<tr>
<td>Prior change in LVESDN per month (%)</td>
<td>85</td>
<td>0.22 (–6.10 to 3.98)</td>
</tr>
<tr>
<td>Subsequent change in LVESDN per month (%)</td>
<td>103</td>
<td>0.53 (–2.49 to 4.62)</td>
</tr>
<tr>
<td>HR measured from the ECG (beats/min)</td>
<td>151</td>
<td>128 (61 to 204)</td>
</tr>
</tbody>
</table>

![Figure 1](11-09-0314r.indd)
Dogs in the B1 group receiving treatment for congestive heart failure were included in subsequent analyses. There were no significant differences in UAC (P = 0.903) or serum PIIINP concentration (P = 0.163) between sexes. No correlation was detected between UAC and serum PIIINP concentration when all dogs (P = 0.391) or only dogs with MMVD (P = 0.483) were included in the analysis. In the healthy control group, there was no evidence for a difference in UAC (P = 0.211) or serum PIIINP concentration (P = 0.801) between CKCSs and other breeds.

Linear regression analyses—In the univariate analyses in dogs with MMVD, age, breed (CKCS vs non-CKCS), LA:Ao, LVEDDN, prior percentage change in LVEDDN per month, LVEDSN, prior percentage change in LVEDSN per month, heart rate, treatment with ACE inhibitors (yes vs no), and ACVIM heart disease class were correlated with PIIINP concentration (P < 0.20) and were submitted for consideration in the multivariable models (Table 2; Figure 2). The LVEDDN and LVEDSN were strongly correlated (r = 0.758; P < 0.001), so separate models were constructed excluding each of these variables in turn. The model with the highest adjusted R² value was reported in each situation. In the multivariable model excluding prior percentage change data, age (P = 0.007) and LVEDDN (P = 0.003) were negatively associated with PIIINP concentration and breed (CKCS vs non-CKCS) was positively associated with PIIINP concentration (Table 3; P = 0.007; adjusted R² = 0.159).

No significant interactions were identified among pairs of variables. Neither prior percentage change in LVEDDN nor prior percentage change in LVEDSN was significant in a multivariable model including these variables (adjusted R² = 0.267).

The multivariable models were reconstructed to include data on only the 111 dogs remaining after those receiving medication for cardiac disease were excluded (Table 4). In the multivariable model excluding percentage change data, age (P = 0.015) and LVEDDDN (P = 0.039) were negatively associated with PIIINP concentration and heart rate (P = 0.021) was positively associ-

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of dogs</th>
<th>P value</th>
<th>β</th>
<th>95% CI for β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>162</td>
<td>&lt; 0.001</td>
<td>−0.39</td>
<td>−0.60 to −0.19</td>
</tr>
<tr>
<td>Logarithm of body weight</td>
<td>162</td>
<td>0.015</td>
<td>1.53</td>
<td>0.31 to 2.76</td>
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<tr>
<td>CKCS (yes vs no)</td>
<td>162</td>
<td>0.024</td>
<td>−7.56</td>
<td>14.10 to −1.02</td>
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<tr>
<td>LVEDDDN-LVFWd ratio</td>
<td>158</td>
<td>0.533</td>
<td>−0.22</td>
<td>−0.92 to 0.48</td>
</tr>
<tr>
<td>LVEDDN</td>
<td>158</td>
<td>0.031</td>
<td>−2.33</td>
<td>−4.45 to −0.21</td>
</tr>
<tr>
<td>Prior change in LVEDDN per month (%)</td>
<td>85</td>
<td>0.185</td>
<td>−0.75</td>
<td>−1.13 to 0.22</td>
</tr>
<tr>
<td>Subsequent change in LVEDDN per month (%)</td>
<td>103</td>
<td>0.723</td>
<td>0.10</td>
<td>−0.44 to 0.63</td>
</tr>
<tr>
<td>LVEDSDN</td>
<td>158</td>
<td>0.092</td>
<td>−3.07</td>
<td>−6.65 to 0.51</td>
</tr>
<tr>
<td>Prior change in LVEDSN per month (%)</td>
<td>85</td>
<td>0.070</td>
<td>−0.34</td>
<td>−0.71 to 0.03</td>
</tr>
<tr>
<td>Subsequent change in LVESDN per month (%)</td>
<td>103</td>
<td>0.639</td>
<td>−0.11</td>
<td>−0.55 to 0.34</td>
</tr>
<tr>
<td>Heart rate measured from the ECG</td>
<td>151</td>
<td>0.036</td>
<td>0.02</td>
<td>0.002 to 0.047</td>
</tr>
<tr>
<td>Treatment with ACE inhibitors (yes vs no)</td>
<td>162</td>
<td>0.075</td>
<td>−1.30</td>
<td>−2.72 to 0.13</td>
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<tr>
<td>Treatment with diuretics (yes vs no)</td>
<td>162</td>
<td>0.422</td>
<td>−0.71</td>
<td>−2.44 to 1.03</td>
</tr>
<tr>
<td>Treatment with pimobendan (yes vs no)</td>
<td>162</td>
<td>0.500</td>
<td>−0.59</td>
<td>−2.32 to 1.14</td>
</tr>
<tr>
<td>ACVIM class</td>
<td>162</td>
<td>0.018</td>
<td>−1.07</td>
<td>−1.95 to −0.19</td>
</tr>
</tbody>
</table>

β = Regression coefficient, representing the slope of the relationship between the variables. Values of P < 0.05 were considered significant.

Table 2—Results of univariate linear regression analysis of the relationships between serum PIIINP concentration and other variables in dogs with MMVD.

Figure 2—Box-and-whisker plots of serum PIIINP concentration in the dogs of the MMVD group that were CKCSs (n = 67) or any other breed (95). See Figure 1 for key.

(7,378.4 pg/mL) urine concentrations of aldosterone were 1.7%, 3.4%, and 2.4%, respectively. The interassay coefficients of variation for low, medium, and high urine concentrations of aldosterone were 7.4%, 2.1%, and 4.6%, respectively. The lower limit of quantification was 14.2 pg/mL.

Groupwise comparisons—When dogs in the B1 group receiving treatment for congestive heart failure (n = 24) were excluded from the analyses, for the remaining 162 dogs (148 dogs in the MMVD group plus 24 healthy control dogs), an overall significant (P = 0.004) difference in serum PIIINP concentration was evident among groups (control, B1, B2, and C). Dogs in the B1 category (n = 75) had a higher mean concentration of serum PIIINP (12.2 ± 4.5 µg/L) than those in the B2 (33; P = 0.009) and C (30; P = 0.049) categories (9.7 ± 2.7 µg/L and 10.1 ± 3.3 µg/L, respectively; Figure 1). No significant differences were detected in serum PIIINP concentrations between any other group pairs. There were no significant (P = 0.820) differences in UAC among control, B1, B2, and C groups.
ated with PIIINP concentration (adjusted $R^2 = 0.161$). No significant interactions were identified. In the multivariable model including percentage change data, age ($P = 0.006$) and prior percentage change in LVEDDN per month ($P = 0.012$) were negatively associated with PIIINP concentration (adjusted $R^2 = 0.221$). No significant interaction was identified.

In the univariate analyses involving dogs with MMVD, age, breed (CKCS vs non-CKCS), LVEDD:LVFWd ratio, LVEDDN, prior percentage change in LVEDDN per month, LVESDN, subsequent percentage change in LVESDN per month, treatment with diuretics (yes vs no), and treatment with pimobendan (yes vs no) were correlated with the logarithm of UAC at a value of $P < 0.20$ and were taken further for consideration in multivariable modeling (Table 5; Figure 3). The LVEDDN and LVESDN ($r = 0.758; P < 0.001$) and LVEDDN and LVEDD:LVFWd ratio ($r = 0.807; P < 0.001$) were strongly correlated, so separate models were constructed excluding each of these variables in turn. The model with the highest adjusted $R^2$ value is reported in each situation.

In the multivariable model excluding prior percentage change data, breed (CKCS vs non-CKCS; $P = 0.007$) and treatment with diuretics (yes vs no; $P = 0.005$) were positively associated with UAC and age was negatively associated with UAC ($P = 0.002$; Table 6) (adjusted $R^2 = 0.292$). No significant interactions were identified. In the multivariable model including prior and subsequent percentage change data, breed (CKCS vs non-CKCS; $P = 0.018$), prior percentage change in LVEDDN per month ($P = 0.036$), and subsequent percentage change in LVEDDN per month ($P = 0.023$) were positively associated with UAC, and age was negatively associated with UAC ($P = 0.028$). The adjusted $R^2$ for the model was 0.257.

The multivariable models were repeated for 111 dogs remaining after excluding dogs receiving medicatio...
tion for cardiac disease (Table 8). In the multivariable model excluding percentage change data, breed (CKCS vs non-CKCS) was positively associated with UAC ($P < 0.001$; adjusted $R^2 = 0.201$).
model including percentage change data, breed (CKCS vs non-CKCS; P < 0.001) and subsequent percentage change in LVESDN per month (P = 0.024) were positively associated with UAC (adjusted R² = 0.288). No significant interaction was identified.

**Discussion**

The present study was the first to demonstrate an independent relationship between UAC and prior percentage change in LVEDDD and subsequent percentage change in LVESDN over time in dogs with MMVD. It also revealed a negative relationship between serum PIIINP concentration and echocardiographic measurements of left ventricular diameter.

The UAC was found to be negatively associated with age and positively associated with diuretic treatment, as expected. The relationships in the multivariable regression analyses between UAC and prior percentage change in LVEDDD per month and subsequent percentage change in LVESDN per month, but not with absolute measurements of LVEDDD or LVESDN, suggested that aldosterone concentration may increase around times of active ventricular remodeling rather than continuously throughout the course of the disease. This potentially intermittent nature of aldosterone release may explain the lack of correlation between UAC and serum PIIINP concentration and the lack of an increase in aldosterone with ACVIM heart disease class. It may also explain the apparently conflicting findings in studies in which plasma aldosterone concentration was measured in dogs with MMVD.

Aldosterone is a recognized mediator of ventricular fibrosis. It is interesting to speculate whether a causal relationship exists between the high concentration of circulating aldosterone suggested by increasing UAC and the deteriorating ventricular systolic function suggested by an increase in LVESDN over time.

As serum PIIINP concentration decreased in dogs with MMVD, left ventricular diastolic diameter increased. This negative association was independent of the reported age-related decrease in PIIINP concentration. Given that PIIINP concentration reflects collagen type III turnover, a decrease in turnover might be consistent with the decrease in myocardial collagen found in dogs with experimentally induced mitral regurgitation. However, given that PIIINP concentration reflects both production and degradation of collagen type III, any such relationship remains speculative.

We expected to find a relationship between increasing concentrations of fibrosis markers and increasing severity of MMVD, but this was not reflected in serum PIIINP concentrations. The lack of effect on PIIINP concentration may have been attributable to the balance in MMVD favoring a decrease in ECM turnover, despite an increase in fibrosis seen in some studies. Alternatively, turnover of collagen type III might not be an important component of fibrosis development in MMVD or fibrosis might not have developed in the study dogs. Although PIIINP concentration is strongly correlated with fibrosis in humans, whether such a relationship exists in dogs is not known. Additional studies are required to investigate the relationship between serum PIIINP concentration and fibrosis in dogs.

In dogs with MMVD but not healthy control dogs, PIIINP concentration and UAC were higher in CKCSs than non-CKCS breeds, despite statistically controlling for the influence of other variables known to affect concentrations of these analytes, such as age, heart size measurements, and treatment. This finding suggested that there might be breed-specific differences in collagen type III turnover and RAAS activation and hence differences in the pathophysiologic response to mitral regurgitation. No significant difference in UAC or serum PIIINP concentration was found between healthy control dogs and dogs with MMVD, which suggests that neither of these markers is suitable for use as a diagnostic test in dogs with clinical MMVD.

The UAC increases with furosemide administration in dogs and may decrease with ACE inhibitor administration. However, in the medium to long term, the effects of treatment with ACE inhibitors on UAC are diminished by the potential for angiotensin I to be converted to angiotensin II by enzymes other than ACE, such as chymase, a phenomenon known as aldosterone breakthrough. Short-term administration of pimobendan has no effect on UAC in healthy dogs, although the effects of long-term administration or administration to dogs with naturally occurring heart disease remain unclear. Serum C-terminal propeptide of procollagen type I concentrations do not change on administration of furosemide in human patients. In hypertensive humans treated with imidapril, serum PIIINP concentration decreases over time with regression of left ventricular hypertrophy.

Any effects of treatment with furosemide, ACE inhibitors, or pimobendan on serum PIIINP concentration in dogs with MMVD remain to be elucidated. Therefore, measurements of UAC and serum PIIINP concentration might be confounded by the effects of treatment in studies that include dogs receiving medications for cardiac disease. Attempts were made to control for these effects in the present study by the inclusion of the following variables in the linear regression models: treatment with furosemide (yes vs no), treatment with ACE inhibitors (yes vs no), and treatment with pimobendan (yes vs no). Although these variables did not take into account any effect of dose on UAC or serum PIIINP concentration, no evidence for dose-dependent relationships was indicated by linear regression analysis (data not shown). As expected, treatment with furosemide was positively associated with UAC.

Treatment with furosemide was not significant in the multivariable model including percentage change over time data; however, to contribute data to this model, dogs with MMVD had to be examined on 3 occasions. Dogs with 3 evaluations were likely to be less severely affected and therefore less likely to be receiving furosemide, which might explain why treatment with furosemide was not significant in this model.

Treatment with neither ACE inhibitors nor pimobendan was significant in either multivariable model for UAC, and no treatment was significant in either multivariable model for PIIINP concentration. Those
findings yield no evidence of important confounding effects of these treatments on the models.

To further investigate any potential effects of treatment on UAC and serum PIIINP concentration, the multivariable models were repeated, excluding dogs receiving medication for cardiac disease. Although some variables that were significant in the final multivariable model that included all dogs were not significant in the final multivariable model that included dogs receiving medication for cardiac disease, the regression coefficients for the variables that were significant in both multivariable models were similar. This suggests that the changes in the final models were most likely to be the result of the decrease in study power created by the elimination of data for 32% of the dogs. Additionally, it should be noted that the dogs excluded from these analyses were typically those with the most severe disease; therefore, their exclusion resulted in a decrease in the range of cardiac size over which the analyes were measured.

The low adjusted R² values for the multivariable regression models in the present study may have reflected the fact that neither PIIINP concentration nor UAC is a specific marker of cardiac disease. For example, in humans, serum PIIINP concentration increases with hepatic disease, systemic sclerosis, and neoplasia. The excess amount of circulating aldosterone may be associated with primary hyperaldosteronism, coartation of the aorta, renin-secreting tumors, disor- orders associated with local circulating volume, hepatic cirrhosis, or nephrotic syndrome. Plasma aldosterone concentration also increases in dogs fed a low-sodium diet. In addition, circulating aldosterone concentration may not be directly related to cardiac tissue aldosterone concentration. In our sample of middle-aged and old- er dogs, it was likely that other systemic processes influenced UACs and serum PIIINP concentrations, decreasing the strength of associations with cardiac variables.

The dogs in the multivariable analysis for which percentage change over time data were available needed to have been examined on at least 3 occasions, which might have biased findings toward less severely affected dogs, although death was not the only reason animals were lost to follow-up. The adjusted R² value for the UAC model including the percentage change over time data was higher than that for the model excluding these data, suggesting that the model including percentage change more fully explained the relationships between the variables. However, the adjusted R² value < 0.5, suggesting that important factors influencing UAC were not included in the model.

The present study had a number of limitations. Only 1 marker of ECM turnover was measured, and serum procollagen type I concentration may have a different relationship with left ventricular echocardiographic measurements than serum PIIINP concentration. Histologic evaluation was not performed to investigate the relationship between UAC and serum PIIINP concentration and fibrosis; therefore, any putative relationship remains speculative.

Sodium-restricted diets are associated with increases in plasma aldosterone concentration in dogs. Although none of the dogs in the study reported here were fed such a diet, dietary sodium intake was not controlled and therefore may have influenced urinary aldosterone measurements. Intraobserver variability in the types of echocardiographic measurements made is reportedly approximately 10%, and this variability might have decreased the ability to detect significant associations between echocardiographic variables and PIIINP concentration or UAC.

Dogs were receiving various treatments, although the inclusion of these treatments in the multivariable model limited any potential for confounding of the results given these variables. The healthy control dogs were generally younger than the dogs with MMVD; therefore, an additional study to replicate the results of the present study would be ideal.

The healthy control dogs did not undergo echocardiography, so it was not possible to entirely rule out the presence of any cardiac disease in these dogs. However, given the types of heart disease to which dogs of this age and size are susceptible, a lack of abnormal physical examination findings made the presence of cardiac disease unlikely. Data from this group were included only for groupwise comparisons of PIIINP concentration and UAC and not in the construction of the multivariable models. Therefore, their inclusion did not affect the conclusions of the present study.

Finally, the primary veterinarians retained responsibility for all treatment decisions. Not all dogs with clinical signs of congestive heart failure underwent radiography prior to the initiation of treatment, meaning that diagnosis may have been inaccurate in dogs included in the C category of heart disease. Such inaccuracy would have had no effect on the major conclusions of the study because class was not a factor in the final multivariable models.

Regardless of the aforementioned limitations, serum PIIINP concentration and therefore, we believe, collagen type III turnover decreased with increasing measurements of left ventricular size in dogs with naturally occurring MMVD. Urinary aldosterone concentration increased with increasing rates of change in left ventricular measurements. Findings also suggested that there might be breed-specific differences in the pathophysiologic response to mitral regurgitation in dogs.

References

dosterone blocker, in patients with left ventricular dysfunctional

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