Effects of dilated cardiomyopathy on the renin-angiotensin-aldosterone system, atrial natriuretic peptide activity, and thyroid hormone concentrations in dogs

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Objective—To evaluate the effect of dilated cardiomyopathy (DCM) on activity of the renin-angiotensin-aldosterone system (RAAS), the N-terminal fragment of proatrial natriuretic peptide (NT-proANP), and thyroid hormone concentrations in dogs.

Animals—15 dogs with clinical signs of DCM, 15 dogs without clinical signs of DCM, and 15 age-, breed-, and sex-matched control dogs.

Procedure—Physical examinations, thoracic radiography, ECG, and echocardiography were performed on all dogs, and blood and urine samples were collected.

Results—Plasma renin activity (PRA), plasma aldosterone concentration (PAC), urine aldosterone-to-creatinine ratio, and NT-proANP concentrations were significantly increased in dogs with clinical signs of DCM, compared with dogs without clinical signs and control dogs. Thyroid-stimulating hormone and total thyroxine concentrations did not differ significantly among groups; however, free thyroxine (FT_4) concentrations were significantly decreased in dogs with clinical signs of DCM, compared with control dogs and DCM-dogs without clinical signs. Concentrations of PRA, PAC, FT_4, and urine aldosterone-to-creatinine ratio were significantly correlated, whereas plasma concentrations of NT-proANP only correlated with FT_4 concentration.

Conclusion and Clinical Relevance—In dogs with clinical signs of DCM, increased concentrations of components of the RAAS were associated with increased concentrations of NT-proANP. Analysis of the neurohormonal system may aid in identification of clinical stages of DCM for groups of dogs, but the range is too great and there are too many dogs that have neurohormonal concentrations within reference ranges to assess dogs on an individual basis. (*Am J Vet Res* 2001;62:961–967)

Dilated cardiomyopathy (DCM) is one of the most common acquired heart diseases in dogs[1-5]; the disease has been reported in many medium-sized and large-breed dogs.[6] Dilated cardiomyopathy was first characterized in dogs as congestive heart failure in conjunction with dilatation of the cardiac chambers and absence of other clinically important cardiovascular disease.[7] Since the advent of echocardiography, myocardial hypokinesis measured as low **fractional shortening (FS)** and severe left atrial and ventricular dilatation (ie, eccentric hypertrophy) without other detectable cardiac abnormalities have been regarded as diagnostic criteria for DCM.[2,4,8] In an earlier study,[9] the sensitivity of these criteria, in conjunction with clinical and radiographic signs of congestive heart failure, was estimated to be 93%. Dilated cardiomyopathy without clinical signs of congestive heart failure and in the absence of other cardiovascular disease is commonly defined as echocardiographic evidence of left ventricular volume overload hypertrophy and hypokinesis.[10,a,b]

Compensatory mechanisms induced by congestive heart failure involve the **renin-angiotensin-aldosterone system (RAAS)**, the sympathetic nervous system, and alterations in vasopressin activity.[11] Although activation of these hormonal systems may be beneficial in preserving perfusion to vital organs, their effects are considered to be detrimental over long periods, leading to fluid retention and increased peripheral vascular resistance, thus and increasing the workload of the failing heart.[11]

Atrial natriuretic peptide (ANP) inhibits the synthesis of renin and aldosterone, antagonizes the action of angiotensin II, decreases the activity of the sympathetic nervous system, and inhibits the release and action of vasopressin,[12,13] thus counteracting the neurohormonal response to cardiac dysfunction. The major physiologic effects of ANP include natriuresis, diuresis, vasodilatation, and decreased mean arterial blood pressure. The major stimuli for ANP secretion are atrial stretch[14] and atrial tachycardia.[15]

It has been demonstrated that thyroid hormones influence cardiac performance.[16,17] Genes coding for proteins that are essential for myocardial function, such as sodium, K-ATPase, Ca-ATPase, β-adrenergic receptors, and myosin heavy chains, are regulated by **triiodothyronine (T_3)**.[18-21] On a transcriptional level, up-regulation of thyroid hormone β1 and β2 adrenergic receptor mRNA was detected in dogs with heart failure and was attributed to dilatated cardiomyopathy or chronic valvular disease.[22] In dogs with clinical signs of DCM, serum concentrations of **total thyroxine (TT_4)** and T_3 were below reference range values in 17 to 38% of dogs, and results of **thyroid-stimulating hormone (TSH)** stimulation testing did not reveal an increase in prevalence of hypothyroidism.[6,21,23] The importance of these findings in dogs with clinical signs

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of DCM has been unclear, because severely ill dogs, including dogs with advanced congestive heart failure, often have alterations in thyroid hormone metabolism known as euthyroid sick syndrome.[24]

The purpose of the study reported here was to investigate the effect of naturally occurring DCM on the RAAS, the **N-terminal fragment of proatrial natriuretic peptide (NT-proANP)** activity, and thyroid hormone concentrations in dogs with and without clinical signs of DCM.

Materials and Methods

Dogs—Forty-five dogs of 17 medium-sized and large breeds were included in the study and were allocated to 1 of 3 groups according to the following criteria: group 1 consisted of 15 dogs with clinical signs of DCM (ie, FS of < 25%,[25] as determined by use of M-mode echocardiography), the absence of echocardiographic lesions other than chamber dilatation (eccentric hypertrophy) on **2-dimensional (2D)** echocardiography, and radiographic evidence of left-sided or biventricular cardiac enlargement in association with pulmonary edema or pleural effusion; group 2 consisted of 15 dogs with DCM, using echocardiographic criteria as described, but dogs did not have clinical or radiographic evidence of congestive heart failure; and group 3 consisted of 15 age-, breed-, and sex-matched control dogs (ie, no abnormalities were detected echocardiographically or radiographically). None of the dogs received any treatment or sodium-restricted diet before entering the study.

Echocardiography—M-mode and 2D echocardiography were performed, using a 5-MHz transducer[c] placed on the right precordium, with dogs positioned in right lateral recumbency. Echocardiograms were recorded and analyzed according to the recommendations of the American Society of Echocardiography[26] and the Echocardiographic Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine.[27] Echocardiographic measurements of left atrial and left ventricular dimensions were indexed according to Kittleson and Kienle.[28] Cardiac dimensions were measured in millimeters, and body weight was measured in kilograms. The following calculations were performed to index the cardiac dimensions: **left ventricular end-diastolic diameter (LVEDD)** index = $LVEDD/BW^{0.32}$, **left ventricular end-systolic diameter (LVESD)** index = $LVESD/BW^{0.41}$, **left atrial diameter (LA)** index = $LA/BW^{0.30}$, and **aortic root diameter (Ao)** index = $Ao/BW^{0.35}$. All echocardiographic recordings were reevaluated by a cardiologist (JH) who was unaware of the clinical diagnosis.

Radiography—Thoracic radiography in 2 orthogonal views was performed on all dogs. All radiographic views were evaluated for heart size, evidence of pulmonary congestion and edema, and pleural effusion sequentially[29] but randomly by a radiologist (KH) who was unaware of the clinical diagnosis.

Electrocardiography—Standard 6-lead ECG were recorded and analyzed, using standard ECG criteria for dogs.[30] All ECG recordings were analyzed sequentially but randomly by a cardiologist (JH) who was unaware of the clinical diagnosis.

Blood and urine sample collection—Blood was drawn from the cephalic vein into prechilled silicon-coated evacuated tubes containing different additives in the following order: 10-ml potassium-EDTA tubes containing aprotinin[d] for renin, aldosterone, and NT-proANP assays; 5-ml potassium-EDTA tubes for analysis of BUN, creatinine, and plasma protein concentrations; 5-ml lithium-heparinized tubes for sodium, potassium, and osmolarity analysis; and plain 5 ml tubes for **angiotensin converting enzyme (ACE)** activity assay. A total of 20 ml of blood was collected from each dog; blood was centrifuged at 1500 × g for 15 minutes, after which plasma was separated from blood, placed in polyethylene tubes, and frozen at –20 C. Samples were then stored at –85 C until assays were performed. Urine was collected midstream during micturition and placed into plain silicon-coated vacutainer tubes.[e] A small portion of each urine sample was removed for creatinine analysis; the rest was frozen at –20 C and then stored at –85 C until assays were performed.

Analysis of NT-proANP, aldosterone, plasma renin activity, and ACE activity—The N-terminal proANP concentration was measured by use of direct **radioimmunoassay (RIA)**, as described.[31] The antibodies were raised in rabbits against the 79 to 98 sequence of human proANP, which is identical to the corresponding sequence in dogs except for 1 amino acid (number 95). The bound and free fractions were separated by double antibody precipitation in the presence of PEG 6000.[f] Serially diluted plasma samples from dogs and human NT-proANP standards were diluted in parallel, thus validating quantification of canine NT-proANP, using the assay. However, although the relative changes observed in NT-proANP concentrations were accurate, the absolute values obtained may not have been, because the assay is heterologous for canine samples. The limit of detection for the method was 62 pmol/L. Recovery ranged from 91 to 108%. The intra- and interassay **coefficients of variation (CV)** for this RIA were 4 to 10% and 9%, respectively.

Renin activity and aldosterone concentration were determined, using commercially available RIA.[g,h] Both these RIA had been validated for canine samples.[32,33] Recovery ranged from 71 to 98% for the aldosterone assay. The intra- and interassay CV for the aldosterone RIA were 8% and 10%, respectively. For the **plasma renin activity (PRA)** assay, the intra-assay CV was 8% and interassay CV was < 10% within the working range of 0.087 to 4.465 ng/ml/h.

The ACE activity in serum was determined by use of a direct radioenzymatic test,[i] as described.[34] The inter- and intra-assay CV for this assay in our laboratory were 8.2% (n = 11) and 2.9%, respectively, for low ACE-activity samples (< 8.5 ACE units) and < 1% in the higher reference range (> 15 ACE units).

Analysis of TSH, free thyroxine, and TT_4 concentrations—Serum concentrations of TSH, **free thyroxine (FT_4)**, and TT_4 were measured, using a commercially available assay system.[j] The TSH and TT_4 assays are solid-phase chemiluminescent enzyme immunometric assays intended for canine samples. Thus, these methods have been validated for use in canine samples by the manufacturer. The FT_4 assay is based on the same principles as those for TSH and TT_4. The FT_4 assay is intended for human samples, although its use for canine samples has been validated in our laboratory. The inter- and intra-assay CV were as follows: TSH, 5.2 to 13.8% and 3.9 to 10.8%, respectively; FT_4, 9.0 to 10.9% and 5.3 to 8.1%, respectively; and TT_4, 6.3 to 8.2% and 3.8 to 5.0%, respectively. The reference ranges established for these variables in our laboratory were: TSH, < 40 mU/L; FT_4, 5 to 25 pmol/L; and TT_4, 18 to 38 nmol/L.

Blood and urine biochemical analyses—Blood urea nitrogen concentration was determined by use of an enzymatic test,[k] as described.[35] Urine and plasma creatinine concentrations were measured by use of a commercially available kit[l] based on the method by Jaffe modified by Fabiny and Ertinghausen.[36] Plasma protein concentration was measured by use of refractometry.[m] Urine and plasma sodium and potassium concentrations were measured by use of ion-selec-

tive electrodes.[n] Osmolarity was determined in plasma and urine by measuring freezing point depression.[o]

Statistical methods—All statistical calculations were performed by use of a computerized statistical program.[p] Statistical methods used, in normally distributed data sets, were 1-way ANOVA and the Tukey-Kramer test, which was applied for multiple comparisons. Equal variances between groups were tested by use of the F-test (variance ratio test). Logarithmic transformation was performed to correct for non-normality on plasma concentrations of NT-proANP, aldosterone, urinary aldosterone- to-creatinine ratio, and urinary- to-plasma creatinine ratio. For PRA and plasma sodium concentrations, the different distributions between groups could not be corrected by logarithmic transformation; therefore, nonparametric methods (eg, the Wilcoxon rank sum test) were applied. For continuous variables, the Spearman rank correlation was used as a nonparametric measure of association of echocardiographic, hormonal, and biochemical variables. Differences between groups with categorical data were determined by use of the χ^2 test. The Fisher exact test was used when $n < 5$. Values were considered significant when $P < 0.05$. Data are reported as mean ± SD.

Results

Significant differences were not detected between DCM dogs with clinical signs, DCM dogs without clinical signs, and control dogs regarding breed, age, sex, or body weight. Twenty-six (58%) dogs were male. Ages ranged from 2.4 to 11.9 years (mean, 7.0 ± 2.6 years). Body weights ranged from 12 to 70 kg (mean, 35.8 ± 14 kg).

Echocardiographic findings—For indexed echocardiographic variables, significant increases in LVEDD and LVESD were detected in both groups of dogs with DCM, compared with control dogs (**Table 1**). Fractional shortening was significantly decreased in both groups of dogs with DCM, compared with control dogs. Dogs with clinical signs of DCM had a significant increase in size of the left atrium and a significant decrease in size of the aorta, compared with dogs without clinical signs of DCM and control dogs.

Electrocardiographic findings—Heart rate was significantly increased in DCM dogs with clinical signs, compared with DCM dogs without clinical signs and control dogs (Table 1). Results of ECG included normal sinus rhythm in 21 dogs (1 dog with clinical signs of DCM; 5 with no clinical signs; 15 controls), sinus tachycardia in 11 dogs (10 with clinical signs; 1 with no clinical signs), atrial fibrillation in 9 dogs (4 with clinical signs; 5 with no clinical signs), ventricular premature depolarizations in 3 DCM dogs with no clinical signs, and sinus bradycardia in 1 DCM dog with no clinical signs.

Plasma renin activity, plasma aldosterone concentration (PAC), NT-proANP concentration, urinary aldosterone-to-creatinine ratio, and ACE activity—Plasma renin activity, PAC, NT-proANP concentration, and urinary aldosterone-to-creatinine ratio were significantly increased in DCM dogs with clinical signs, compared with DCM dogs with no clinical signs and control dogs. (**Table 2**; **Fig 1**) The variation of NT-proANP concentrations were greater in DCM dogs without clinical signs than in control dogs, but not significantly so. The NT-proANP concentrations were particularly high in dogs with atrial fibrillation. Indeed, results of the Spearman rank correlation test indicated that NT-proANP concentrations were increased with increasing heart rate, left atrial index ($\rho = 0.56$ and 0.65, respectively; both $P < 0.01$), and indexed echocardiographic dimensions of the left ventricle (LVEDD index and LVESD index, $P = 0.55$ and 0.66, respectively; both $P < 0.01$), and with decreasing FS (**Fig 2**). The PRA, PAC, and urinary aldosterone-to-creatinine ratio were highly covariate as indicated by results of the Spearman rank correlation test. However, a poor correlation was found between these variables and heart rate and

Table 1—Mean (± SD) age, sex, body weight, heart rate, and indexed echocardiographic variables for 15 clinically normal dogs, 15 dogs without clinical signs of dilated cardiomyopathy (DCM), and 15 dogs with clinical signs of DCM. Heart diameters were indexed as described.[28]

Variable	Normal dogs	DCM dogs without clinical signs	DCM dogs with clinical signs
Age (y)	6.5 ± 2.6[a]	7.4 ± 2.5[a]	7.2 ± 2.8[a]
Sex (M/F)	7/8[a]	9/6[a]	10/5[a]
Body weight (kg)	33.6 ± 14.1[a]	39.2 ± 14.9[a]	34.8 ± 13.2[a]
Heart rate (beats/min)	95 ± 16[a]	126 ± 48[a]	183 ± 49[b]
LVEDD index	14.6 ± 1.1[a]	18.2 ± 2.2[b]	21.8 ± 2.4[c]
LVESD index	7.3 ± 1.1[a]	11.1 ± 2.0[b]	13.2 ± 2.5[c]
FS (%)	32.0 ± 4.4[a]	16.1 ± 4.1[b]	13.0 ± 5.7[b]
LA index	9.2 ± 0.9[a]	11.2 ± 2.0[a]	15.3 ± 3.2[b]
Ao index	7.6 ± 0.8[a]	7.8 ± 1.1[a]	6.4 ± 0.9[b]
LA/Ao	1.02 ± 0.08[a]	1.23 ± 0.31[a]	2.06 ± 0.71[b]

Values with different superscript letters indicate significant ($P < 0.05$) differences between groups.

LVEDD = Left ventricular end-diastolic diameter. LVSED = Left ventricular end-systolic diameter. FS = Fractional shortening. LA = Left atrium. Ao = Aortic root diameter. LA/Ao = Left atrium-to-aortic root ratio.

Table 2—Means (± SD) of various neurohormonal and biochemical variables in serum and urine from 15 clinically normal dogs, 15 dogs without clinical signs of DCM, and 15 dogs with clinical signs of DCM.

Variable	Normal Dogs	DCM dogs without clinical signs	DCM dogs with clinical signs
Plasma			
ACE (ACE units)	20.4 ± 5.1[a]	20.3 ± 4.3[a]	19.4 ± 7.8[a]
TSH (mU/L)	25.6 ± 7.7[a]	22.2 ± 3.9[a]	30.3 ± 13.7[a]
TT_4 (nmol/L)	20.1 ± 9.1[a]	16.0 ± 7.9[a]	18.2 ± 8.2[a]
Na^+ (mmol/L)	146.4 ± 1.6[a]	149.1 ± 4.8[a]	148.0 ± 2.3[a]
K^+ (mmol/L)	4.6 ± 0.59[a]	4.3 ± 0.41[a]	4.4 ± 0.45[a]
K^+/Na^+	0.15 ± 0.37[a]	0.25 ± 0.45[a]	0.18 ± 0.37[a]
Osmolarity (mOsm/L)	307 ± 3[a]	306 ± 6[a]	308 ± 6[a]
Protein (g/L)	6.9 ± 0.3[a]	7.0 ± 0.6[a]	5.8 ± 0.6[b]
BUN (mmol/L)	6.3 ± 1.8[a]	2.3 ± 2.3[a]	6.4 ± 3.6[a]
Creatinine (mmol/L)	96 ± 17[a]	92 ± 23[a]	103 ± 35[a]
Urine			
Specific gravity	1.03 ± 0.01[a]	1.02 ± 0.01[a]	1.02 ± 0.01[a]
Crea (mmol/L)	17,523 ± 11,358[a]	10,725 ± 7,011[a]	9,262 ± 4,757[a]
U-Crea/P-Crea	181 ± 116[a]	112 ± 65[a]	92 ± 52[b]
Na^+/Crea (mmol/mmol)	6.3 ± 7.7[a]	6.7 ± 6.3[a]	7.4 ± 6.3[a]
K^+/Crea (mmol/mmol)	6.5 ± 3.8[a]	7.8 ± 5.0[a]	11.0 ± 6.6[a]
Osmolarity (mOsm/L)	945 ± 606[a]	709 ± 412[a]	971 ± 478[a]
Aldo/Crea (pmol/mmol)	309.5 ± 262.8[a]	776.0 ± 702.5[b]	1,501.5 ± 1,748.9[b]

Values with different superscript letters indicate significant ($P < 0.05$) differences among groups.

ACE = Plasma angiotensin-converting enzyme. TSH = Plasma thyroid-stimulating hormone. TT_4 = Plasma total thyroxine. Crea = Creatinine. U-Crea/P-Crea = Urine creatinine-to-plasma creatinine ratio. Aldo = Aldosterone.

echocardiographic dimensions of the left atrium and the left ventricle. The ACE activity was comparable in the 3 groups of dogs, and the only variable that correlated significantly with ACE activity was the urinary-to-plasma creatinine ratio (ρ = -0.43; $P < 0.01$).

Concentrations of TSH, FT_4, and TT_4—Thyroid stimulating hormone and TT_4 concentration did not differ significantly among groups. However, FT_4 concentrations were significantly decreased in DCM dogs with clinical signs, compared with dogs without clinical signs and control dogs (Table 2; Fig 1d). The decreased FT_4 concentration in the DCM dogs with clinical signs was significantly correlated with PRA, PAC, and urinary aldosterone-to-creatinine ratio, whereas TSH concentration did not appear to be correlated with any of the other variables.

Blood and urine biochemical analyses—Plasma protein concentration and urine-to-plasma creatinine ratio were significantly decreased in DCM dogs with clinical signs, compared with DCM dogs without clinical signs and control dogs (Table 2). Plasma protein concentration was significantly correlated with heart rate (ρ = –0.50; $P < 0.001$), indexed echocardiographic dimensions of the left ventricle (LVEDD index, LVESD index, and FS, ρ = –0.49, –0.63, and 0.48 respectively; $P < 0.001$) and left atrium (LA/Ao, ρ = –0.53; $P < 0.001$), PRA (ρ = –0.46; $P < 0.001$), PAC (ρ = –0.41; $P < 0.01$), and plasma NT-proANP and FT_4 concentrations (ρ = –0.59 and 0.56, respectively; both $P < 0.001$). Likewise, the urine-to-plasma creatinine ratio correlated significantly with PRA, PAC, NT-proANP concentration (ρ = –0.34, –0.29, and –0.33, respectively), and urinary aldosterone-to-creatinine ratio (ρ = –0.49; $P < 0.001$). The potassium to creatinine ratio was significantly correlated to the urinary aldosterone-to-creatinine ratio (ρ = –0.40; $P < 0.01$). Significant correlations were not detected between plasma sodium concentration, plasma potassium concentration, plasma sodium-to-potassium ratio, plasma or urine osmolarity, urine potassium-to-creatinine ratio, or urine sodium-to-creatinine ratio. Significant

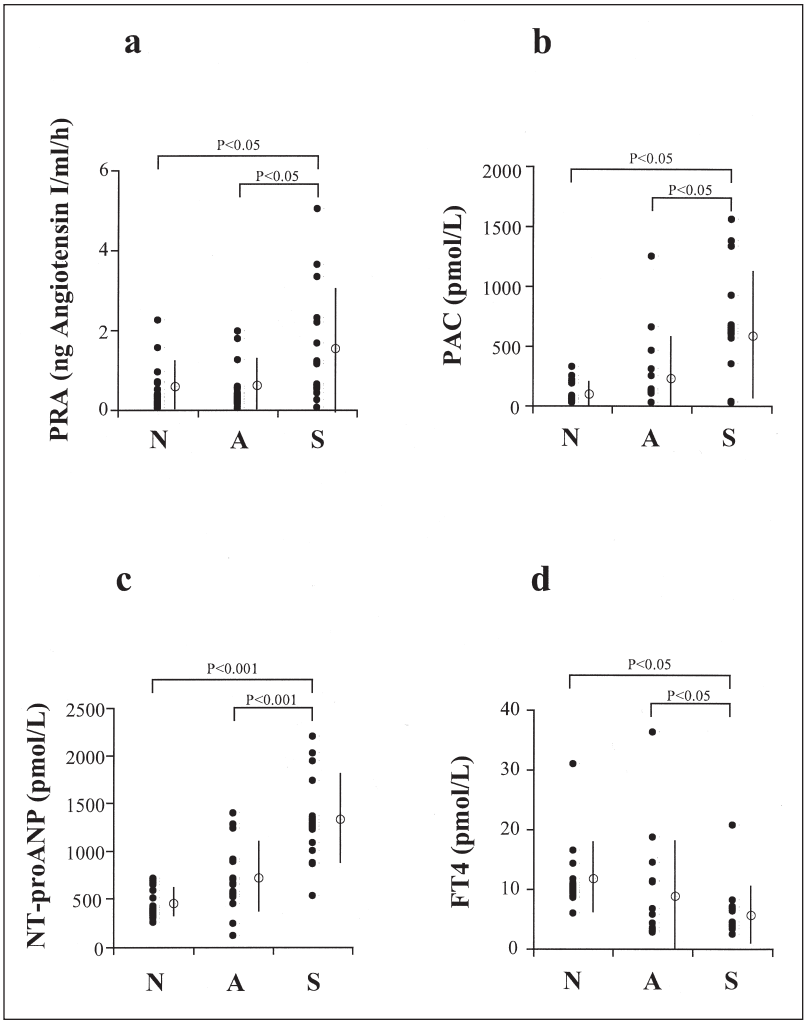

Figure 1—Plasma renin activity (PRA; panel a), plasma aldosterone concentration (PAC; panel b), N-terminal proatrial natriuretic peptide concentration (NT-proANP; panel c), and free thyroxine concentrations (FT_4; panel d) in 15 control dogs (N), 15 dogs without clinical signs of DCM (A), and 15 dogs with clinical signs of DCM (S). Vertical lines with an open circle indicate mean ± SD.

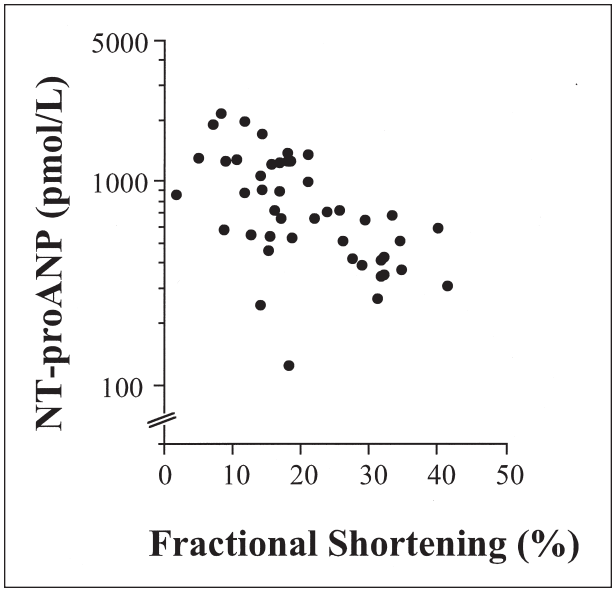

Figure 2—Relationship between the fractional shortening (FS) and plasma concentration of NT-proANP in 45 dogs (ρ =–0.60; $P < 0.001$).

differences were not detected in BUN, plasma creatinine concentration, or urine creatinine concentration among the 3 groups.

Discussion

Results of our study revealed that PRA, PAC, and urinary aldosterone to creatinine ratio were significantly increased in DCM dogs with clinical signs but not in DCM dogs without clinical signs, compared with control dogs, which is in agreement with results of previous investigations in dogs with DCM.[10] The significant correlations between PRA, PAC, and urine aldosterone to creatinine ratio were expected. Renin release is stimulated by a decrease in extracellular fluid volume and systemic blood pressure or increased sympathetic output.[31,32] Aldosterone concentration increases in response to increased release of adrenocorticotropic hormone, release of renin (via angiotensin II), and to hyperkalemia or hyponatremia.[33] Congestive heart failure has previously been associated with increased RAAS activity in relation to clinical staging of the disease (New York Heart Association class I to IV) in dogs with various cardiac diseases[34] and humans with DCM.[35] A more recent investigation in dogs with early decompensated mitral regurgitation attributable to chronic valvular disease indicated there was no change in circulating RAAS activity,[36] possibly attributable to the fact that these dogs were less severely affected with congestive heart failure, compared with the dogs in our study.

The NT-proANP concentration was significantly increased in DCM dogs with clinical signs, compared with DCM dogs without clinical signs and control dogs in our study. An increase in NT-proANP concentration, which is released on an equimolar basis with the C-terminal active hormone[37] and has prolonged stability in vitro, is associated with substantially decreased survival time in humans with congestive heart failure.[38] Because the major stimuli for ANP secretion are atrial stretch[14] and tachycardia,[15] and left atrial diameter and heart rate were significantly increased in DCM dogs with clinical signs, the increase in NT-proANP concentration was expected. Indeed, results of our study revealed that NT-proANP concentration was highly correlated with heart rate, echocardiographic dimensions of the left atrium and left ventricle, and FS, which is in agreement with previous studies in dogs with decompensated chronic valvular disease in which increased NT-proANP activity was associated with enlargement of the left atrium and ventricle.[36,39] Expression of ANP appears to be positively correlated to the degree of ventricular impairment and dilatation in humans with DCM.[40] Plasma concentrations of ANP were found to be 3 to 4 times higher in human patients with DCM than in those with left-sided valvular heart disease.[41] A disturbed peripheral metabolism of ANP and a resistance to its biological effects has been demonstrated in humans with symptomatic DCM as well as in those with asymptomatic DCM, who have circulating concentrations of ANP and atrial pressure and volume within reference ranges.[42]

In heart failure, retention and excretion of sodium and potassium is balanced by activation of the RAAS and simultaneous regulation of ANP activity. Although the activity of these hormones was significantly different in DCM dogs with clinical signs, compared with dogs with no clinical signs and control dogs, differences in plasma sodium or potassium concentrations were not detected between groups. However, plasma sodium and potassium concentrations may not be representative of total body content of these ions. Plasma sodium concentration indicates the amount of sodium relative to the amount of water in the extracellular fluid but provides no direct information regarding total body sodium content.[43] Because intracellular potassium accounts for 95 to 98% of total body potassium and because potassium distribution is influenced by many factors, plasma potassium concentration may not represent total body potassium content.[44] Thus, the lack of differences in plasma sodium and potassium concentrations between the 3 groups of dogs may be attributable to the balance among the RAAS and ANP activity or to factors influencing the distribution of these ions in the different fluid compartments.

The heart is a primary target organ for thyroid hormone action. There is a strong correlation between increasing concentrations of T_3 and positive inotropism[45,46] and chronotropism.[47,48] Subclinical thyroid disorders have been described in humans with DCM.[49] It has been suggested that hypothyroidism may play a role in the development of DCM in dogs; however, this has not been convincingly documented. In DCM dogs with clinical signs, T_4 and T_3 concentrations were below reference range values in 10 of 58 dogs in 1 study,[6] and T_4 concentrations before TSH stimulation testing were below reference range values in 5 of 13 dogs in another study.[21] A more recent investigation failed to identify any relationship between hypothyroidism and DCM in 79 Doberman Pinschers.[23] Determination of basal thyroid hormone and TSH concentrations for evaluation of thyroid function can be misleading, because many factors such as age, breed, environmental and body tem-

perature, diurnal rhythm, hourly fluctuations, obesity, and malnutrition may influence test results. Serum concentrations of thyroid hormones often decrease in dogs as a result of concurrent illness (sick euthyroid syndrome).[50] In our study, TSH and TT_4 concentrations did not differ significantly between groups. However, FT_4 concentrations were significantly decreased in DCM dogs with clinical signs, compared with dogs with no clinical signs and control dogs. Measuring FT_4 concentration, although it is only 1/1000 of the TT_4 concentration and thus more difficult to measure accurately, is sometimes considered to be more accurate than measuring TT_4 concentration for the detection of hypothyroid states. In general, a TSH stimulation test is needed to verify the diagnosis of hypothyroidism if results of baseline TT_4, FT_4, and TSH concentrations are discordant.[51] Low FT_4 concentrations in DCM dogs with clinical signs may be attributable to increased protein binding of T_4 or increased conversion to the biologically active hormone T_3 at the tissue level.

The decrease in plasma protein concentration in DCM dogs with clinical signs most likely reflects the increased plasma volume that is observed with congestive heart failure. Expansion of plasma volume develops secondary to increased activity of the RAAS but is counteracted by the diuretic action of ANP. Renal excretory function was assessed in the dogs of this study by measuring plasma urea and creatinine concentrations and the urine-to-plasma creatinine ratio. Because neither glomerular membrane disease nor hepatic or gastrointestinal disease causing loss of protein were investigated in these dogs, other causes of decreased plasma protein concentration cannot be completely ruled out.

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[e]Becton & Dickinson, Meylex Cedex, France.

[f]Art 81260, Fluka, Buchs, Switzerland.

[g]Coat-A-Count Aldosterone, Diagnostic Products Co, Los Angeles, Calif.

[h]GammaCoat Plasma Renin Activity (PRA) method, Incstar, Stillwater, Mass.

[i]Angiotensin-converting-enzyme (ACE) direct-radioenzymatic assay (REA), Code RK-ACD, Bühlmann Laboratories AG, Allschwil, Switzerland.

[j]Diagnostic Products Co, Los Angeles, Calif.

[k]Unimate 5 Urea/BUN, Art. 073685, Roche, Basel, Switzerland.

[l]Unimate 7 Crea, Art 0736678, Roche, Basel, Switzerland.

[m]AO Instrument Co, Buffalo, NY.

[n]Model E2A electrolyte analyzer, Beckman Instruments, Stockholm, Sweden.

[o]Osmometer VAO 1, Herman Roebling Messtechnik, Berlin, Germany.

[p]JMP, version 3.2, SAS Institute Inc, Cary, NC.

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