

Relation of Vitamin D Status to Congestive Heart Failure and Cardiovascular Events in Dogs

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Background: Vitamin D plays a pivotal role in cardiac function, and there is increasing evidence that vitamin D deficiency is associated with the development of congestive heart failure (CHF) in people.

Hypothesis: Serum vitamin D concentration is lower in dogs with CHF compared with unaffected controls and serum vitamin D concentration is associated with clinical outcome in dogs with CHF.

Animals: Eighty-two client-owned dogs.

Methods: In this cross-sectional study, we examined the association between circulating 25-hydroxyvitamin D [25(OH)D], a measure of vitamin D status, and CHF in dogs. In the prospective cohort study, we examined whether 25(OH)D serum concentration was associated with clinical outcome in dogs with CHF.

Results: Mean 25(OH)D concentration (100 ± 44 nmol/L) in 31 dogs with CHF was significantly lower than that of 51 unaffected dogs (123 ± 42 nmol/L; $P = .023$). The mean calculated vitamin D intake per kg of metabolic body weight in dogs with CHF was no different from that of unaffected dogs (1.37 ± 0.90 μ g/kg metabolic body weight versus 0.98 ± 0.59 μ g/kg body weight, respectively, $P = .097$). There was a significant association of serum 25(OH)D concentration on time to clinical manifestation of CHF or sudden death ($P = .02$).

Conclusion and Clinical Relevance: These findings suggest that low concentrations of 25(OH)D may be a risk factor for CHF in dogs. Low serum 25(OH)D concentration was associated with poor outcome in dogs with CHF. Strategies to improve vitamin D status in some dogs with CHF may prove beneficial without causing toxicity.

Key words: 25-hydroxyvitamin D₃; Canine; Cardiology.

There is extensive evidence that vitamin D influences the cardiovascular system at many sites and has a role in cardiac function in humans and rodents.^{1–4} Cardiac muscle cells possess a vitamin D receptor and a calcitriol-dependent calcium-binding protein.^{5,6} Also, a calcitriol-mediated rapid activation of voltage-dependent calcium channels exists in cardiomyocytes.⁷ Epidemiologic evidence shows that vitamin D deficiency is associated with congestive heart failure (CHF) in dilated cardiomyopathy (DCM), coronary disease, and hypertension in people.^{8–11} Knockout mouse data are even more convincing because vitamin D receptor knockout mice develop typical signs of CHF, such as cardiac hypertrophy, hypertension, and increased concentrations of atrial natriuretic peptide.¹² In large epidemiologic studies in humans, with rare exceptions,¹³ most groups of CHF patients studied have shown insufficient or below reference range limits

Abbreviations:

| | |
|---------|--|
| CHF | congestive heart failure |
| 25(OH)D | 25-hydroxyvitamin D ₃ [25(OH)D] |
| AVD | acquired valve disease |
| DCM | dilated cardiomyopathy |
| BCS | body condition score |

serum concentrations of 25-hydroxyvitamin D [25(OH)D], the best indicator of an individual's vitamin D status.^{14–16} In a study by Zimmerman et al, for example, adults with CHF had 34% lower 25(OH)D concentrations than healthy subjects.¹⁷ In another recent study of 2,312 people, each 10 ng/mL lower 25(OH)D concentration was associated with a 25% greater relative hazard of heart disease.¹⁸ Finally, calcitriol (the principal biologically active form of vitamin D) administration can normalize the impaired contractility of the myocardium that is observed in experimental vitamin D deficiency.^{19,20}

Cardiovascular disease is a common cause of morbidity and mortality in dogs. Acquired valve disease (AVD) and DCM are the major cardiac diseases of dogs, unlike humans who develop CHF because of ischemic disease. AVD is generally degenerative and rare because of an infectious cause; myxomatous degeneration is the most common cardiac disease in the dog. Mitral regurgitation caused by AVD accounts for 75% of cases of heart disease in dogs and the prevalence of AVD markedly increases with age.²¹ Because AVD is characterized by chronic progression, to date, there is little an owner or veterinarian can do to alter the disease process. Evidence suggests that benazepril might be beneficial in dogs with asymptomatic heart disease,²² but 2 trials^{23,24} were not able to demonstrate

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a significant effect of enalapril on time to develop CHF. DCM is characterized by a slowly progressive presymptomatic phase during which ventricular premature contractions occur along with progressive left ventricular dysfunction and, usually, progressively more severe ventricular tachyarrhythmias.^{25–27} Outcome of dogs with DCM is usually either sudden death or CHF.²⁵ Recently, there are data to support the early use of pimobendan in Doberman Pinchers as it prolongs the time to the onset of clinical signs and extends survival.²⁸ Whether or not pimobendan is effective in other breeds with DCM is not known. Therefore, interventions to delay the onset of disease would be desirable and investigation into the utility of vitamin D may be worthwhile.

To date, no study has investigated the association between vitamin D status and CHF in dogs. In people, serum 25(OH)D concentrations reflect cumulative exposure to sunlight (ie, ultraviolet light) and dietary vitamin D.²⁹ In dogs, serum vitamin D concentration is not influenced by ultraviolet light exposure, because dogs do not convert cutaneous 7-dehydrocholesterol into vitamin D₃ as do many other species.³⁰ This difference in environmental exposure leaves dietary intake as the primary means of obtaining vitamin D₂ or vitamin D₃ for conversion into 25(OH)D and thereby eliminating a potentially confounding variable during investigation.

Herein, we report a cross-sectional study to examine the correlation between circulating 25(OH)D concentration and CHF in dogs. Also, we report results of a prospective cohort study to determine the association of serum 25(OH)D concentration status on incident CV outcomes in dogs with CHF.

Materials and Methods

Study Groups

Recruitment for these studies occurred between January 2009 and June 2010. Only dogs that had not been fed for at least 8 hours were included. Client-owned dogs with CHF were included as affected cases. Dogs were considered to have CHF if they had radiographic evidence of cardiogenic pulmonary edema within 3 months of study entry (Class C). Clinical signs of decompensated CHF had to either be present at the time of entering the study or have been resolved by cardiac medications that were still being administered. Only dogs with CHF attributable to AVD or DCM were included; structural disease was documented by echocardiography. Healthy, client-owned dogs served as unaffected controls and were enrolled on the basis of a normal medical history and physical examination performed by a board-certified cardiologist. All healthy dogs were auscultated by a cardiologist to confirm the absence of any auscultatory cardiac abnormalities (eg, murmurs, arrhythmias) or pulmonary abnormalities (eg, crackles, wheezes). Because dogs with CHF secondary to AVD and DCM are generally middle-to-older aged, only dogs >5 years of age were included as unaffected healthy controls to minimize the chance of age as a confounding variable. For both affected cases and unaffected controls, dogs with known hepatic insufficiency, renal insufficiency, diabetes mellitus, or clinically relevant systemic or infectious diseases were excluded. All dogs' owners provided written informed consent to participation

in the study, which was approved by the Institutional Animal Care and Use Committee of Cornell University.

Study Variables

Fasted blood and urine samples were collected from each dog. Blood samples were protected from light, allowed to clot, and then centrifuged at $3800 \times g$ for 10 minutes. An aliquot of serum was immediately stored at -70°C until analysis of 25(OH)D was performed. Circulating concentrations of 25(OH)D were measured with a commercially available radioimmunoassay kit.^a Acetonitrile extraction of samples and assay procedures were performed according to the manufacturer's recommendations. Calibrators of 25(OH) D were included for the standard curve. The manufacturer reported the following percent cross-reactivity with related compounds: vitamin D₂ or D₃ (0.8%), 25-hydroxyvitamin D₂ or D₃ (100%), 1,25-dihydroxyvitamin D₂ or D₃ (11%), 24,25-dihydroxyvitamin D₂ or D₃ (100%), 25,26-dihydroxyvitamin D₂ or D₃ (100%). However, the dihydroxyvitamin D metabolites are in pmol/L concentrations in the circulation, and cross-reactivity would be negligible in quantification of 25(OH)D.

The sensitivity of the assay, defined as the concentration of 25(OH)D at 90% specific binding on the standard curve, was 7 nmol/L. The highest calibrator on the standard curve was 250 nmol/L. The upper limit of detection is not precisely defined, but experience from dilution of clinical samples suggests that it is in the vicinity of 800 nmol/L. Extracted aliquots of a canine serum pool (165 nmol/L) were diluted 1 : 2, 1 : 3, 1 : 4, and 1 : 5 with acetonitrile. Assay of these diluted samples yielded respective recovery rates of 107, 113, 114, and 118%. When 25(OH) D₃ was added to aliquots of a canine serum sample (47 nmol/L) to achieve increases of 15, 25, 50, or 125 nmol/L, 98, 101, 90, and 94% of added 25-hydroxyvitamin D were measured in the assay. For intra-assay repeatability (12 replicates), the % coefficients of variation for serum pools of 30, 109, and 183 nmol/L were 5.0, 4.6, and 4.2%, respectively. For interassay repeatability (13 assays), the % coefficients of variation serum pools of 32 and 115 nmol/L were 15.3 and 10.3%, respectively.

Serum chemistry and urinalysis were performed on the remaining aliquots of serum and urine to ensure adequate organ function. At enrollment, all dogs' owners completed a questionnaire that included questions about dietary intake during the 3 months before enrollment. The following information about each case and control was recorded: body condition score based on a modified body condition scoring system³¹ (BCS; on a scale of 1–5, where briefly, a BCS of 1 was consistent with emaciation; BCS of 2 was thin; BCS of 3 was optimal; BCS of 4 was overweight; BCS of 5 was obese), body weight, sex, age, and micrograms of vitamin D intake per kg body weight calculated from the dietary questionnaire. Information regarding the total vitamin D (cholecalciferol and ergocalciferol) content of canine foods, treats, and supplements was acquired by contacting manufacturers when not reported on the label. Total daily dietary vitamin D intake was calculated by adding the food, supplement, and treat quantities based on known portion consumption per day. The total intake of vitamin D was divided by the kilograms of body weight and the kilograms of metabolic body weight of the individual dog to provide a microgram per kg body weight or microgram per kg of metabolic body weight measure. If, on the basis of the questionnaire, table foods comprised $\geq 25\%$ of the metabolizable energy (ME) of the diet, dogs' owners were contacted to get a more complete diet history including types and frequency of foods fed. If the dog was fed $< 25\%$ ME in table foods, but still received meat, fish, dairy, or animal product oils, those owners were also contacted for a more complete diet history. NutritionistPro™ (<http://www.nutritionistpro.com/dietanalysis.php>) was used to

assist with nutrient analysis of table foods. If the nutrient content was not available or could not be calculated, the case or control was excluded from the dietary intake analysis portion of the study (see below).

Statistical Analysis

Cross-Sectional Study. In the cross-sectional study, 25(OH)D concentration was analyzed as a continuous variable. The relationship between CHF cases and controls and 25(OH)D concentration was evaluated using nonpaired Student's *t*-tests based on normally distributed data (Shapiro–Wilk test). A logistical multivariable regression analysis was done, factoring in age (dichotomized at the median and continuous) and BCS (dichotomized at the median, and BCS as <4 and ≥4) in the multivariable model. Differences in vitamin D intake were examined between CHF cases and controls using a Wilcoxon ranked sum test based on a lack of normality in affected dogs.

Prospective Cohort Study. In the prospective cohort study, the effect of 25(OH)D concentration on time to another CV event was evaluated by use of Cox regression and Kaplan–Meier methods. Incident CV events were analyzed by 2 methods. First, an incident cardiovascular event was defined as either another documented episode of CHF or sudden death. Event-free interval (EFI) was defined as number of days from the first episode of CHF to either a second clinical manifestation of CHF or sudden death. Dogs without an incident cardiovascular event and dogs with incomplete data with respect to EFI were censored at the last date at which cardiac status was adequately assessed. In the second incident cardiovascular event analysis, an event was more broadly defined (“any event”). In addition to another documented episode of CHF or sudden death, adjustment of cardiac medications for suspected, but not confirmed, CHF was also included as “any event”. EFI and censoring were similar (as described above). The Kaplan–Meier product-limit method was used to estimate EFI curves for 25(OH)D (dichotomized at the median among dogs with CHF, <111 nmol/L versus ≥111 nmol/L) and the log-rank test for censored data was used to compare EFI curves. To evaluate risk of experiencing a cardiovascular event, hazard ratio was determined by use of a Cox proportional hazard model. All statistical analyses were performed by a software package,^b 2-sided values of $P \leq .05$ were considered significant.

Results

Cross-Sectional Study

Thirty-one dogs with CHF (20 AVD and 11 DCM) and 51 unaffected control dogs were enrolled (Table 1). Mean serum 25(OH)D concentrations were approximately 20% lower in CHF dogs when compared with controls; $P = .023$; Fig 1). 25(OH)D concentrations were not statistically different between the DCM and AVD dogs ($P = .75$). The multivariable analysis showed that body condition score was not different between groups ($P = .60$) and sex was not different between groups ($P = .08$). Age was different between groups ($P < .05$). However, in the multivariable regression analysis, 25(OH)D remained different between controls and cases when controlling for dichotomized age ($P = .02$) as well as continuous age ($P = .037$). Univariate linear regression of all dogs across both populations confirmed that serum 25(OH)D and age were not significant ($P = .321$). The mean vitamin D intake per kg body weight, calculated on

Table 1. Characteristics of dogs in the case-control cross-sectional study to evaluate serum 25-hydroxyvitamin D₃ [25(OH)D] as a risk factor for congestive heart failure

| | Dogs with Congestive Heart Failure (n = 31) | Unaffected Control Dogs (n = 51) |
|--------------------------------------|---|----------------------------------|
| Age (years, mean + SD) | 9.6 + 2.9 | 7.6 + 2.8 |
| Weight (kg, mean) | 28.3 + 20.4 | 32.4 + 6.7 |
| Body Condition Score ^a | | |
| <4 (No. of each BCS) | 25 (8 = 2; 15 = 3) | 38 (2 = 2, 36 = 3) |
| ≥4 | 6 (6 = 4) | 13 (13 = 4) |
| Sex | | |
| Male (No. of intact) | 19 (3) | 19 (3) |
| Female | 12 (1) | 32 (3) |
| Serum 25(OH)D (nmol/L, mean ± SD) | 100 ± 42 | 123 ± 44 |
| AVD (n = 20) | 101 (±37) | NA |
| DCM (n = 11) | 98 (±55) | NA |
| Concurrent drug therapy ^b | | |
| Digitalis | 2 | 0 |
| Beta-blocker | 2 | 0 |
| Vasodilators | 14 | 0 |
| Diuretics | | |
| Loop | 18 | 0 |
| Aldosterone antagonists | 2 | 0 |
| Pimobendan | 10 | 0 |
| Calcium channel blockers | 3 | 0 |

AVD, acquired valve disease; DCM, dilated cardiomyopathy; NA, not applicable.

^aBody condition score: 1 was consistent with emaciation, 2 was thin, 3 was optimal, 4 was fat, 5 was obese.

^bSeveral patients were receiving more than 1 medication.

the basis of dietary questionnaire, in dogs with CHF was significantly higher than that of unaffected dogs (0.67 ± 0.49 µg/kg body weight versus 0.42 ± 0.27 µg/kg body weight, respectively, $P = .07$); whereas when adjusted for metabolic body weights attributable to the size differences in the affected group, there was no significant difference either (1.37 ± 0.90 µg/kg body weight versus 0.98 ± 0.59 µg/kg body weight, respectively, $P = .097$; Fig 2).

Prospective Cohort Study

The 31 dogs with CHF were included in the EFI analysis. Twenty-five dogs experienced a cardiovascular event; the first cardiovascular event was a documented second episode of complications attributable to CHF in 11 dogs, adjustment of cardiac medications (upward titration, primarily diuretics) attributable to suspected CHF by the attending clinician in 6 dogs, and sudden death in 8 dogs. Six dogs were censored in the EFI analysis because they were still alive and without evidence of a cardiovascular event at the time of data analysis. Median follow-up time for these dogs

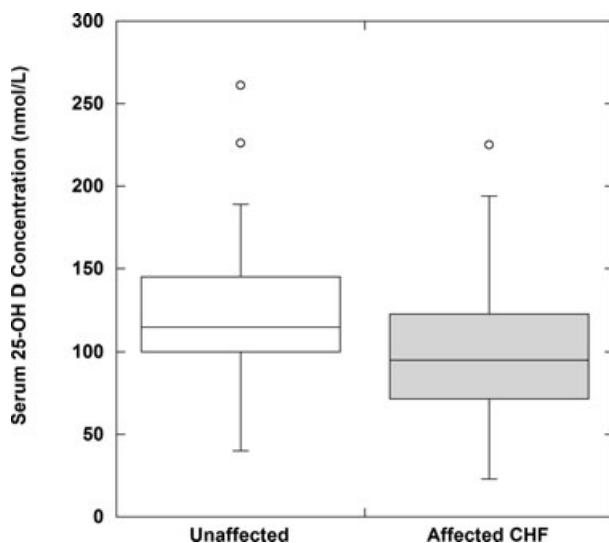


Fig 1. Box and whisker plot showing serum 25-hydroxyvitamin D [25(OH)D] concentrations in 31 dogs with congestive heart failure (CHF) and 51 unaffected control dogs. Values are means (black horizontal lines) with 75th and 25th interquartile ranges represented (boxes) and standard deviations represented by vertical lines. Outliers greater than 2 standard deviations from the mean are depicted (small circles). Mean 25(OH)D concentration in dogs with CHF was significantly lower than that of unaffected dogs ($P = .023$).

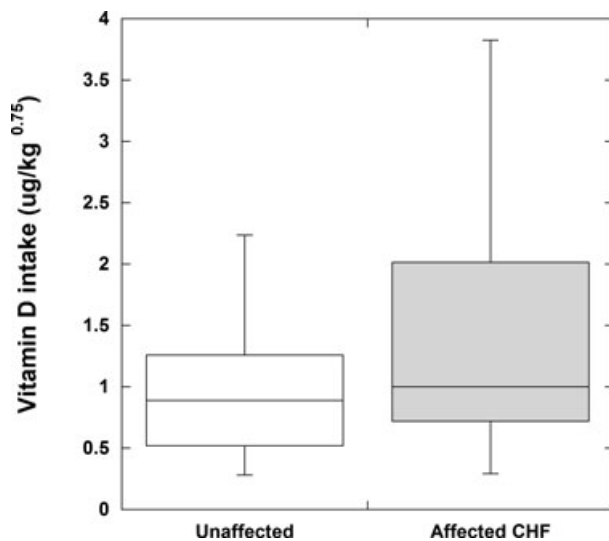


Fig 2. Box and whisker plot showing calculated vitamin D intake per kg of metabolic body weight in 20 of 31 dogs with congestive heart failure (CHF) and 40 of 51 unaffected control dogs. Values are medians (black horizontal lines) with 75th and 25th interquartile ranges represented (boxes) and the range is represented by vertical lines. The median vitamin D intake per kg metabolic body weight in dogs with CHF was no different from unaffected dogs ($P = .097$).

was 116 days (range 17–315 days). Analysis of the censored EFI curve indicated that the overall median EFI was 154 days (95% confidence interval, 97–211 days). There was a significant effect of 25(OH)D and time to

another episode of complication attributable to CHF or sudden death ($P = .019$; Cox regression) and 25(OH)D and “any event” ($P = .003$; Cox regression). Median EFI for the 20 dogs with AVD was 151 days (range 28–493 days). Median EFI for 11 dogs with DCM was 227 days (range 10–520 days). Serum 25(OH)D was a risk factor significantly associated with EFI when cardiovascular event was broadly defined as “any event”; median overall EFI time was significantly lower in 11 dogs with serum 25(OH)D <111 nmol/L (151 days; 95% confidence interval, 78–224 days) compared with 20 dogs with serum 25(OH)D \geq 111 nmol/L (171 days; 95% confidence interval, 33–309 days; log-rank $P = .036$; Fig 3). Dogs with serum 25(OH)D <111 nmol/L had a 2.6 times greater hazard of having cardiovascular event than dogs with serum 25(OH)D \geq 111 nmol/L (95% confidence interval, 1.03–6.67; $P = .03$). Serum 25(OH)D was not associated with EFI when event was defined only as a second episode of complications attributable to CHF or sudden death ($P = .12$).

Discussion

The main finding of this cross-sectional study is that serum 25(OH)D concentrations were significantly lower in dogs with CHF compared with unaffected controls. This result suggests that low serum vitamin D concentration might be a risk factor for development of CHF in dogs. Similar associations between low circulating 25(OH)D and CHF have been found in

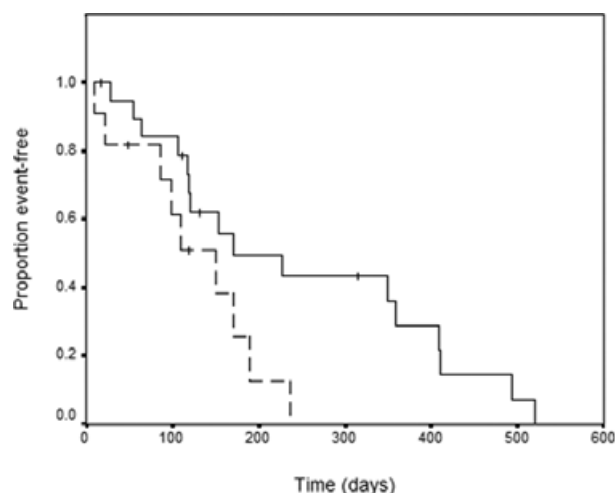


Fig 3. Kaplan-Meier curves depicting serum 25-hydroxyvitamin D₃ [25(OH)D] and incident cardiovascular events in dogs with congestive heart failure (CHF). Incident CV events were defined as second clinical manifestation of CHF, adjustment of cardiac medications attributable to suspected CHF, or sudden death. Eleven dogs had serum 25(OH)D <111 nmol/L (broken line) and 20 dogs had serum 25(OH)D > 111 nmol/L (solid line). Median time to another cardiovascular event for dogs with serum 25(OH)D <111 nmol/L (151 days; 95% confidence interval, 78–224 days) was significantly ($P = .036$, log-rank) shorter than the median time to another CV event for dogs with serum 25(OH)D \geq 111 nmol/L (171 days; 95% confidence interval, 33–309 days). Vertical marks represent censored data.

people.³² However, the relationship can be potentially confounded because people with low 25(OH)D are generally older, are heavier, and have comorbidities that might lead to a greater estimated cardiovascular risk than individuals with higher 25(OH)D concentration.³³ In this study, we adjusted for similar confounders (eg, age, BCS). Because BCS is an approximate measure of body fat or body mass index³¹ and 25(OH)D can be sequestered by adipose tissue, it is important to take BCS into consideration. The relatively normal body conditions of both populations, however, likely precluded any influence of body condition on serum 25(OH)D concentrations.³⁴ Also, it is possible that other factors such as breed, sex, or undiagnosed diseases might have impacted results.^{35–38} Finally, we cannot definitively exclude that concurrent medications, especially diuretics, given to dogs with CHF (Table 1) did not contribute to the low serum 25(OH)D concentrations by increased metabolism or excretion. However, one would expect excessive diuresis to lower serum calcium concentration and activate parathyroid hormone, which enhances the absorption of calcium in the intestine by increasing the production of activated vitamin D.³⁹ Despite the complex interactions of diuretics on calcium, vitamin D, and parathyroid hormone, our results are similar to what is found in humans with cardiovascular disease.^{4,14–16}

Dogs, unlike humans, cannot synthesize vitamin D from cholesterol precursors and ultraviolet irradiation at the level of the skin, making oral intake of either cholecalciferol or ergocalciferol a dietary requirement.³⁰ On the basis of the dietary questionnaire used in this study, dogs with CHF did not show any difference in vitamin D intake when compared with unaffected controls, but there was more variation in intake in the CHF group. The variation in intake in the CHF is interesting, but must be examined with caution. First, foods fed to dogs in this study were not subjected to quantitative analysis and actual vitamin D content could not be determined. In many instances, although pet food companies were contacted by telephone, they frequently did not have specific analytical results, but instead provided the amounts of vitamin D that were included in the vitamin and mineral premix added to their food. Second, food frequency questionnaires are typically used in epidemiologic studies in humans to assess dietary intake in relation to development of disease.⁴⁰ However, food questionnaires should be validated and compared with a gold standard diet analysis technique for the specific population under study.^{40,41} The diet questionnaire used in this study has not been validated and it is possible that vitamin D intake was overestimated in dogs with CHF or underestimated in the unaffected controls, and therefore must be considered a very crude estimation of vitamin D intake. Third, we did assess that relative number of dogs being fed >25% of their food as table food and over 80% of both groups were fed all or 75% of their intake as commercial dog food according to our questionnaire. Lastly, on the basis of the diet questionnaires, there were only 2 dogs in the CHF

group and 4 dogs in the control group being provided vitamin or mineral supplements or fish oil that might contain supplemental vitamin D. Hence, differences in table food intake and supplements are unlikely to be contributing factors to the lower serum 25(OH)D concentrations in CHF dogs. Furthermore, as presented in Figure 2, vitamin D intake could not be calculated for all cases or control dogs, so it is possible that missing data might have influenced results.

In the prospective cohort study, among the dogs with CHF, low circulating 25(OH)D concentration was associated with poor outcome. This is similar to people in end-stage CHF in whom low vitamin D metabolites also have been shown to be associated with poor clinical outcome.⁴² For example, in the study by Zimmerman et al, survival rates were only 26% for patients with vitamin D metabolite concentrations in the lowest tertile, compared with 40% and 61% for those in the intermediate and highest tertiles, respectively. Also, in that study, people with CHF and vitamin D metabolites in the lowest tertile were 2.4 times more likely to die of CHF compared with those with concentrations in the highest tertile.⁴²

Significance in the prospective cohort study was found only when the endpoint was another clinical episode of CHF, sudden death, or adjustment of cardiac medications for suspected CHF. When the latter group of dogs was excluded, results were no longer significant, suggesting that our population with clinical manifestation and sudden death was likely underpowered. We had arbitrarily used the median 25(OH)D concentration (111 nmol/L) to compare the dogs. A more biologically relevant 25(OH)D concentration might be present in dogs with respect to what concentration represents deficiency, insufficiency, or adequacy. Serum parathyroid hormone activity, for example, has been established as a marker in human populations in whom increases in parathyroid hormone serve as an indicator of 25(OH)D insufficiency.⁴³ In people, the inverse relationship between parathyroid hormone and vitamin D concentrations is used to define both vitamin D inadequacy and target serum concentrations of vitamin D necessitating supplementation.⁴⁴ Efforts to define a similar relationship between parathyroid hormone and vitamin D in dogs should be considered.

Overall, findings of the study reported here suggest that low concentrations of 25(OH)D may be a risk factor for CHF in dogs. The demonstration of association, as reported here, does not prove causality. These results are supported by extensive evidence that vitamin D has cardioprotective actions by suppressing the renin-angiotensin-aldosterone system, decreasing myocardial hypertrophy, inhibiting proinflammatory cytokines, and improving endothelial dysfunction and atherosclerosis.⁴⁵ Additional studies are needed to determine if routine screening of all dogs with CHF for inadequate vitamin D concentrations followed by vitamin D supplementation will delay onset of clinical signs or improve survival, while also examining these patients for signs of potential vitamin D toxicity.

Footnotes

- ^a 25-Hydroxyvitamin D ¹²⁵I RIA Kit, DiaSorin, Stillwater, MN
^b SPSS, statistical analytical software, SPSS Inc, Chicago, IL
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Conflict of Interest Declaration: Authors disclose no conflict of interest.

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