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STANDARD ARTICLE

Vitamin D metabolism in dogs with and without hypercalciuric calcium oxalate urolithiasis

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Background: There are abnormalities in vitamin D metabolism in people with calcium nephrolithiasis, but limited data are available on vitamin D status in dogs with calcium oxalate (CaOx) urolithiasis.

Objective: To compare serum concentrations of vitamin D metabolites in dogs with and without hypercalciuric CaOx urolithiasis.

Animals: Thirty-eight dogs with (n = 19) and without (n = 19) a history of CaOx urolithiasis and hypercalciuria.

Methods: Retrospective cross-sectional study. Serum 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], and 24,25-dihydroxyvitamin D [24,25$(OH)_2$D] were measured. The ratios of 25(OH)D/24,25$(OH)_2$D and 1,25$(OH)_2$D/25(OH)D were compared between cases and controls.

Results: There were no significant differences between cases and controls when comparing 25(OH)D, 24,25$(OH)_2$D, 1,25$(OH)_2$D, or 1,25$(OH)_2$D/25(OH)D. Cases had higher 25(OH)D/24,25$(OH)_2$D (median = 1.40, range = 0.98-1.58) compared to controls (median = 1.16, range = 0.92-2.75; P = .01). There was overlap in the ranges for 25(OH)D/24,25$(OH)_2$D between cases and controls, but 6 cases (32%) had ratios above the control dog range. There was a moderate positive correlation between the ratio of 25(OH)D/24,25$(OH)_2$D and urinary calcium-to-creatinine ratios (r = 0.40, 95% confidence interval = 0.10-0.64; P = .01).

Conclusions and Clinical Importance: These data suggest that decreased conversion of 25(OH)D to 24,25$(OH)_2$D occurs in a subset of dogs with CaOx urolithiasis. Abnormalities in vitamin D metabolism might contribute to stone risk in dogs.

KEYWORDS

calcitriol, canine, hypercalciuria, stones

1 | INTRODUCTION

Calcium oxalate (CaOx) urolith formation has a multifactorial etiology that is not well understood. In both people and dogs, excess excretion of calcium into the urine is an important risk factor, but the mechanism behind hypercalciuria is poorly understood.[1–5] There are a number of factors including diet and genetics that likely influence the degree of hypercalciuria. Most dogs with CaOx urolithiasis have hypercalciuria and higher blood ionized calcium (iCa) concentration relative to age-, sex-, and breed-matched controls without an obvious cause.[1]

Although some cases of CaOx stones are associated with hyperparathyroidism, parathyroid hormone (PTH) concentrations are normal in most people and dogs with CaOx stones.[2,3,5] Renal leak hypercalciuria occurs in humans secondary to primary renal defects resulting in a decreased ability to reabsorb calcium in the renal tubules.[2,6,7] Renal leak hypercalciuria is considered unlikely to be the primary

Abbreviations: 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; 24,25$(OH)_2$D, 24,25-dihydroxyvitamin D; 24,25$(OH)_3$D, 1,24,25-trihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; CaOx, calcium oxalate; iCa, ionized calcium; PTH, parathyroid hormone; UCa/Cr, urinary calcium-to-creatinine ratio; UMN VMC, University of Minnesota Veterinary Medical Center.

disturbance in dogs with CaOx urolithiasis as it is commonly associated with low to normal blood calcium concentrations, which is not characteristic of the dogs that have been described to date.[1] Bones serve as the major reserve of calcium in humans and dogs. Increased bone resorption is associated with urolithiasis in people.[8–10] In contrast, a marker of bone resorption, serum β-crosslaps, is decreased in dogs with hypercalciuric CaOx urolithiasis, suggesting bone turnover is not the primary mechanism underlying stone risk in dogs.[11]

Gastrointestinal calcium absorption is largely mediated by vitamin D. Vitamin D is absorbed from the diet in an inactive form and converted in the liver to 25-hydroxyvitamin D [25(OH)D]. The 25(OH)D form is then converted to the active form, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], which directly acts on the vitamin D receptor to increase calcium absorption from the diet among other effects. Transcellular and paracellular mechanisms of calcium absorption from the GI tract also exist but are not mediated by vitamin D.[9,10] When circulating 1,25$(OH)_2$D exceeds the body's needs or there is excess 25(OH)D in circulation, both can be converted via CYP24A1 (24-hydroxylase) to the less biologically active metabolites 24,25-dihydroxyvitamin D [24,25$(OH)_2$D] and 1,24,25-trihydroxyvitamin D [1,24,25$(OH)_3$D], respectively, in the liver. There are no commercially available assays for 1,24,25$(OH)_3$D so 24,25$(OH)_2$D is used as the marker of vitamin D inactivation.

There are increased concentrations of 1,25$(OH)_2$D in human patients with calcium kidney stones compared to controls.[5] The stone formers also had a higher ratio of 25(OH)D to the inactive 24,25$(OH)_2$D, and blood calcium was at the upper end of the reference range in the patients. These results suggest that greater activation and decreased relative deactivation of vitamin D might contribute to stone formation.

To the best of our knowledge, only 1 study has reported vitamin D concentrations in dogs with CaOx urolithiasis. It was a small study in Miniature Schnauzers that compared 1,25$(OH)_2$D concentrations in 6 dogs with CaOx stones to 6 without stones.[3] No difference was found between the groups, but other vitamin D metabolites were not measured. Furthermore, most of the controls were younger than the average age of stone formation in dogs and could have been latent stone formers, and urine and blood calcium concentrations were compared to clinical healthy beagle dogs from a previous study.[12]

The objective of this study was to evaluate vitamin D metabolites (25(OH)D, 1,25$(OH)_2$D, and 24,25$(OH)_2$ D) in 20 dogs with a history of hypercalciuric CaOx urolithiasis and in 20 age-, sex-, and breed-matched stone-free control dogs without hypercalciuria. Our hypothesis was that dogs with a history of hypercalciuric CaOx urolithiasis have higher concentrations of 1,25$(OH)_2$D compared to control dogs. In addition, we hypothesized that the ratio of 25(OH)D to 24,25$(OH)_2$D is greater in stone formers.

2 | MATERIALS AND METHODS

2.1 | Study population

This was a retrospective cross-sectional study. The study took place at the University of Minnesota Veterinary Medical Center (UMN VMC). Stored (−80°C) serum samples were available from dogs recruited as cases and controls for genetic and metabolic studies on CaOx urolithiasis between February 2011 and November 2015.[1,11,13] The dogs were recruited from the patient population at the UMN VMC and through outreach to primary care veterinary clinics and breeders in Minnesota, Iowa, and Wisconsin. Miniature Schnauzers, Bichon Frise, and Shih Tzus were selected to represent breeds where idiopathic hypercalciuria has been reported in CaOx stone formers.[1] Cases were defined as dogs with a history of uroliths comprised ≥70% CaOx as determined by stone analysis at the Minnesota Urolith Center (polarized light microscopy and infrared spectroscopy). All cases had hypercalciuria, defined as a fasting urinary calcium-to-creatinine (UCa/Cr, mg/mg) ratio of >0.05 (the upper end of the 95% confidence interval [CI] reported in stone-free control dogs).[1] Controls had no history of urolithiasis and no evidence of urolithiasis on screening abdominal radiographs. All controls had a fasting UCa/Cr < 0.05. All dogs had either iCa concentration evaluated with a blood gas panel on the day of study participation or total serum calcium concentration determined with a biochemistry panel within 30 days of the study participation, and dogs with hypercalcemia were excluded from the study. Medical records were reviewed for medication and health histories. No dog had received glucocorticoids or other drugs reported to alter vitamin D metabolism or urinary calcium excretion (eg, calcitriol, cholecalciferol, calcium carbonate, furosemide, levothyroxine, and theophylline) within the past 30 days. No dog had received hydrochlorothiazide or potassium citrate for greater than 24 hours before sample collection. Dogs were excluded if they had a clinical diagnosis of a disease that alters vitamin D metabolism or urinary calcium excretion (eg, kidney disease, protein-losing enteropathy, hyperparathyroidism, hyperadrenocorticism, hypoadrenocorticism, hypercalcemia of malignancy, osteolytic disease, diabetes mellitus, or granulomatous disease). Stored serum samples were available from 60 dogs (25 cases and 35 controls) that met the criteria above. All samples had been obtained after food was withheld for 12-18 hours. Thirty-eight of the 60 samples (19 cases and 19 controls) were selected for the present study with matching of breed, sex, and age (±1 year) between cases and controls. Samples that did not have appropriate breed, sex, and age matches were excluded from the study. Pedigrees were reviewed, when available, to evaluate for relatedness among study participants. The primary diet fed was obtained from the medical records. Two diets were fed to 3 or more dogs. Both of these diets were therapeutic stone-prevention diets: diet A (Hill's Prescription Diet u/d Canine, Hill's Pet Nutrition Inc, Topeka, Kansas) and diet B (Royal Canin Veterinary Diet Urinary SO, Royal Canin USA, Inc, St. Charles, Missouri).

2.2 | Vitamin D assays

Samples had been stored frozen (−80°C) for 1-5 years before the vitamin D assays; vitamin D metabolites are reported to be highly stable at this temperature.[14–16] The serum samples were submitted to a Vitamin D External Quality Assessment Scheme-certified laboratory for analysis (Heartland Assays, Inc, Ames, Iowa). Three vitamin D metabolites were measured: 1,25$(OH)_2$D was measured using radioimmunoassay and 24,25$(OH)_2$D and 25(OH)D were measured using liquid chromatography-mass spectrometry.

2.3 | Statistical analysis

Data distribution and normality were determined by inspection of Q-Q plots and the Shapiro-Wilk test. A Student's *t* test was used to compare normally distributed data, including the mean age, sample storage time, iCa concentration, blood urea nitrogen (BUN), creatinine, 1,25(OH)$_2$D, and 25(OH)D between case and control groups. Wilcoxon rank-sum tests were used for case-control group comparisons of data that did not follow a normal distribution, including UCa/Cr, 24,25(OH)$_2$D, and the ratios of 25(OH)D/24,25(OH)$_2$D and 1,25(OH)$_2$D/25(OH)D. A multivariable regression was also performed to determine the effects of sex (male versus female), age (years), breed (Miniature Schnauzer, Bichon Frise, or Shih Tzu), diet (diet A, diet B, or "other," which encompassed all remaining diets for which numbers were too few for individual analysis), and stone status (case versus control) on the log(10)-transformed ratio of 25(OH)D/24,25(OH)$_2$D (log25(OH)D/24,25(OH)$_2$D). The significance of each model predictor was determined using Type II tests (ANCOVA). For ease of interpretation, estimates of the regression coefficient and 95% CI were back-transformed to their original scale for reporting and therefore represent estimates for the multiplicative (factor) change in 25(OH)D/24,25(OH)$_2$D rather than the additive change. A Pearson correlation was used to test the relationship between the log25(OH)D/24,25(OH)$_2$D and log (10)-transformed UCa/Cr (logUCa/Cr) and determine the 95% CI. All analyses were performed using R software for statistical computing (R, version 3.3.1. www.r-project.org) and a *P* value <.05 was considered significant.

3 | RESULTS

Thirty-eight dogs were included in the study. The cases and controls were breed and sex matched. Each group included 10 Miniature Schnauzers (5 male neutered and 5 female spayed), 5 Shih Tzus (3 male neutered and 2 female spayed), and 4 Bichon Frise (3 male neutered and 1 female spayed). Thirty-four of the dogs had participated in previously published studies on urinary metabolites[1,13] and 29 had participated in a study on a serum marker of bone turnover[11] in dogs with and without CaOx urolithiasis. Serum sample storage time before vitamin D metabolite analysis was not significantly different between groups (*P* = .55) with an average of 3.5 ± 0.9 years for cases and 3.3 ± 1.4 years for controls. The mean age of the cases (9.7 ± 2.0 years) at the time of sample collection was not significantly different from the mean age of the controls (10.4 ± 2.0 years, *P* = .29). In the case group, the mean age of first stone formation was 8.3 ± 2.0 years (range 4-13 years). Twelve of the 19 cases (63%) were recurrent stone formers with between 2 and 4 episodes of CaOx urolithiasis. Twelve cases were documented to have uroliths on the date of study participation. Three cases were fed diet A, and 4 cases were fed diet B. The remaining 31 dogs were fed 27 different diets (classified as "other"). Three cases were being treated with potassium citrate and 1 was also receiving hydrochlorothiazide for CaOx urolith prevention; as per inclusion criteria, these medications were discontinued a minimum of 24 hours before study participation and sample collection. Four generation pedigrees were available for 12 of the 38 dogs. Three Miniature Schnauzers, 2 cases and 1 control, were closely related (1 1st degree relationship and 2 3rd degree relationships), and 2 control Bichon Frise were closely related (1st degree). The 2 related control Bichon Frise also lived in the same household. No other relationships were identified.

TABLE 1 Calcium (blood and urine) and vitamin D metabolite analysis of the study population. For normally distributed data, mean is reported with ±SD; data that did not follow a normal distribution are reported as median (range)

Variable	Controls (n = 19)	Cases (n = 19)	*P* value
iCa, mg/dL	5.2 ± 0.2	5.5 ± 0.2	**<.001**
UCa/Cr, mg/mg	0.022 (0.005-0.049)	0.121 (0.059-0.292)	**<.001**
25(OH)D, ng/mL	33 ± 13	40 ± 15	.15
1,25(OH)$_2$D, pg/mL	165 ± 71	199 ± 83	.18
24,25(OH)$_2$D, ng/mL	26 (11-40)	27 (4-40)	.97
25(OH)D/24,25(OH)$_2$D, ng/ng	1.16 (0.98-1.58)	1.40 (0.92-2.75)	**.01**
1,25(OH)$_2$D/25(OH)D, pg/ng	5.3 ± 2.3	5.2 ± 1.8	.94

P values in bold denote significance (*P* < .05).

Fasting urine calcium-to-creatinine ratios were measured in all dogs. Data are presented in Table 1. Hypercalciuria was a selection criterion for the cases, and as expected, they had a significantly higher UCa/Cr than the controls (Table 1, *P* < .001). Blood iCa was measured in 34 dogs (18 cases and 16 controls) on the day of study participation. Blood iCa was also significantly higher in the cases as compared to the controls (*P* < .001), although all measured iCa concentrations were within the laboratory reference range. The other 6 dogs did not have iCa measured but had serum total calcium concentrations determined within 30 days of study participation that were within the laboratory reference range. BUN and creatinine were available for all dogs and were not significantly different between cases (BUN = 13 ± 8 mg/dL, creatinine = 0.9 ± 0.3) and controls (BUN = 13 ± 6 mg/dL, creatinine = 0.8 ± 0.2; *P* = .86 and .63, respectively).

Serum vitamin D metabolite data are presented in Table 1. The mean concentrations of 25(OH)D and 1,25(OH)$_2$D were not statistically different between cases and controls (*P* = .15 and .18, respectively). There was also no significant difference in 24,25(OH)$_2$D concentrations or 1,25(OH)2D/25(OH)D between cases and controls (*P* = .97 and .94, respectively).

When comparing ratios of vitamin D metabolites, the median 25(OH)D/24,25(OH)$_2$D was significantly higher in cases versus controls (Table 1, *P* = .01). The ranges overlapped for the 2 groups, but 6 of the 19 cases (32%) had ratios above the highest ratio observed in the control group (Figure 1); these 6 highest ratios were present in 4 Miniature Schnauzers and 2 Shih Tzus. There was a moderate positive correlation (*r* = 0.40, 95% CI = 0.10-0.64; *P* = .01) between log25(OH)D/24,25(OH)$_2$ and logUCa/Cr in the population as a whole. Further analysis revealed differences in this relationship between the groups. Within the control group, there was a strong positive correlation between 25(OH)D/24,25(OH)$_2$ and logUCa/Cr (*r* = 0.56, 95% CI = 0.15-0.81; *P* = .01, Figure 2); in contrast, the correlation within the case group did not reach statistical significance (*r* = −0.29, 95% CI = −0.66-0.19; *P* = .23).

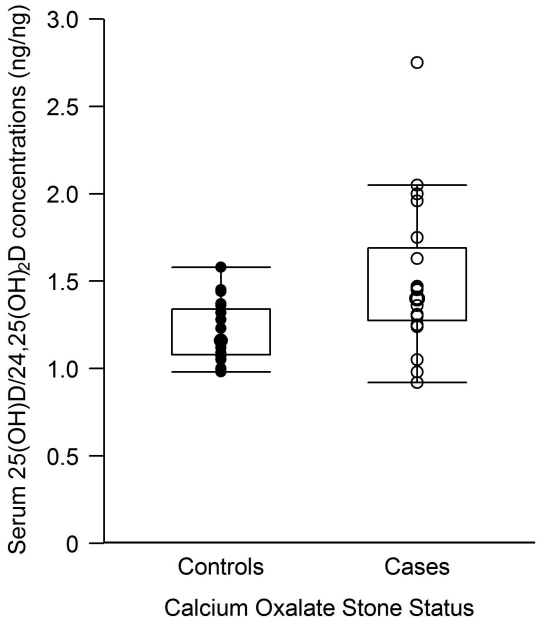

FIGURE 1 Box and whisker plots of serum 25(OH)D/24,25(OH)$_2$D concentrations (ng/ng) in cases versus controls (P = .01). The boxes represent the 25th and 75th percentiles. The whiskers represent range of ratios. The dots represent each individual within the specified group (open circles indicate cases and closed circles indicate controls)

A multivariable regression analysis was performed to evaluate for the effects of sex, breed, age, and diet on 25(OH)D/24,25(OH)$_2$D (Table 2). Calcium oxalate stone status was the strongest predictor of 25(OH)D/24,25(OH)$_2$D (estimate of 1.33-fold change, 95% CI = 1.14-1.55; P < .001). There was also a significant negative association between diet B and 25(OH)D/24,25(OH)$_2$D (estimate of 0.75-fold change, 95% CI = 0.56-0.99; P = .04).

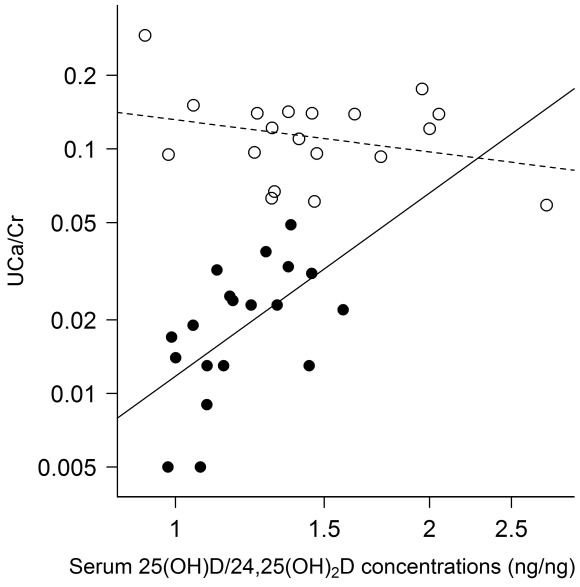

FIGURE 2 Relationship between serum 25(OH)D/24,25(OH)$_2$ concentrations (ng/ng) and UCa/Cr in cases (open circles, dotted line; r = −0.29, P = .23) and controls (closed circles, solid line; r = 0.56, P = .01). Data are plotted on a base 10 logarithmic scale

TABLE 2 Multivariable regression model for the effects of age, sex, breed, diet, and calcium oxalate (CaOx) stone status on the ratio of serum 25(OH)D/24,25(OH)$_2$D concentrations

Variable	Estimate of the coefficient[a]	95% CI		P value
		Lower	Upper	
CaOx case	1.33	1.14	1.55	**<.001**
Age (year)	1.03	1.00	1.06	.08
Sex (male)	0.94	0.82	1.08	.36
Breed				.78
Miniature Schnauzer	Referent			NA
Bichon Frise	0.96	0.78	1.18	.69
Shih Tzu	1.03	0.87	1.22	.73
Diet				.10
Other	Referent			NA
Diet A	0.99	0.74	1.33	.95
Diet B	0.75	0.56	0.99	**.04**

P values in bold denote significance (<.05).
[a]The regression was performed with log-transformed 25(OH)D/24,25(OH)$_2$D as the outcome. For ease of interpretation, the regression coefficients and 95% confidence interval (CI) have been back-transformed to the original scale and represent estimates for the factor change in 25(OH)D/24,25(OH)$_2$D per status change (categorical) or 1-unit increase (continuous) in the variable.

4 | DISCUSSION

In this study, we found that dogs with hypercalciuric CaOx urolithiasis had a greater ratio of 25(OH)D/24,25(OH)$_2$D compared to matched stone-free controls. Examination of the data showed that there was considerable overlap in this ratio between groups and that the difference was driven by a subset of stone forming dogs (6/19, 32%) with an increased ratio. This suggests that decreased 24-hydroxylation of 25(OH)D to 24,25(OH)$_2$D might contribute to CaOx stone risk in some but not all dogs.

There are alterations in vitamin D metabolism because of a deficiency in the CYP24A1 gene in humans. This gene encodes 24-hydroxylase enzyme that converts 25(OH)D and 1,25(OH)$_2$D to 24,25(OH)$_2$D and 1, 24,25(OH)$_3$D.[17] Loss of function of this gene leads to a relative increase in vitamin D metabolites with more vitamin D receptor activity as compared to vitamin D metabolites with less receptor activity. This results in higher than normal blood calcium and compensatory hypercalciuria. Approximately, a third of the cases in our population showed similar ratio changes. However, because there is no data on ratios expected in dogs with CYP24A1 deficiency, we cannot determine if the degree of the change is consistent with this hereditary disorder. Given the lack of previous data, we also cannot be certain that the 25(OH)D/24,25(OH)$_2$ difference represents disease within our case population or rather a protective mechanism in our control group. Of note, the ratios observed in cases varied not only across but also within breeds, suggesting that the pathophysiology of stone formation might differ even between individuals of the same breed.

Multiple metabolites of vitamin D were measured in the present study to completely evaluate the vitamin D status of the included dogs. Although the only significant difference between cases and controls was the ratio 25(OH)D/24,25(OH)$_2$D, the median 25(OH)D and 1,25(OH)$_2$D were higher in cases versus control dogs. This study included a small number of dogs, and the interindividual variability was great. Furthers studies

with larger sample sizes are needed to confirm whether there are differences between 25(OH)D and 1,25$(OH)_2$D in cases and controls.

There was a significant correlation between 25(OH)D/24,25$(OH)_2$D and UCa/Cr in control dogs but not in cases. All cases were selected to be hypercalciuric, which could eliminate the ability to detect a correlation. However, although all had UCa/Cr above the threshold of 0.05, there was variation within the groups; the UCa/Cr in cases ranged from just above the highest value in controls to approximately 6 times it. This lack of correlation in the cases further demonstrates the multifactorial nature of hypercalciuria.

We did not find a significant association between 25(OH)D/24,25$(OH)_2$D and breed, sex, or age. There are breed differences in vitamin D metabolites in growing dogs. Large-breed puppies are reported to have evidence for decreased 24-hydroxylation, with increased 1,25$(OH)_2$D, decreased 24,25$(OH)_2$D, or a combination of both relative to small-breed dogs. This is hypothesized because of higher growth hormone and insulin-like growth factor I in larger breed dogs, as these hormones inhibit CYP24A1.[18,19] However, it is unknown if breed differences in vitamin D metabolites are present in adult dogs. Greyhounds have no significant difference in 25(OH)D concentrations compared to mixed breed dogs, but other metabolites concentrations are unknown.[20] There is no effect of sex on 25(OH)D concentrations in Greyhounds.[20] In contrast, 25(OH)D concentrations are greater in females compared to males in young adult research colony dogs (1-5 years of age), but this difference is not present in geriatric research colony dogs (9-14 years).[21] There are no significant differences in 1,25$(OH)_2$D or 24,25$(OH)_2$D concentrations between sexes in research colony dogs.[21] Lower 25(OH)D is reported geriatric research colony dogs (9-14 years) as compared to younger dogs (1-5 years), but there are no significant differences in 1,25$(OH)_2$D or 24,25$(OH)_2$D.[21] Calcium deficiency has been reported in elderly human populations which is thought to be secondary to several mechanisms including decreasing production of 1,25$(OH)_2$D by aging kidneys and decreasing sensitivity of the GI tract to 1,25$(OH)_2$D, as well as other molecular mechanisms.[22] It has also been proposed that there is upregulation of CYP24A activity with age.[23] These changes would be expected to decrease 25(OH)D/24,25$(OH)_2$D. The absence of a significant relationship between 25(OH)D/24,25$(OH)_2$D and breed, sex, and age in our study population could be because of a true lack of effect of these variables on the ratio in dogs but might also be because of the limited sample size, the population studied (eg, all dogs were spayed/neutered), in the case of age, and the narrow range evaluated (ie, most study dogs were ≥9 years of age).

There was no statistically significant difference between individual vitamin D metabolites or the ratio of 1,25$(OH)_2$D to 25(OH)D between cases and controls. In people, 1,25$(OH)_2$D was reported to be higher in a cohort with calcium kidney stones compared to controls.[5] The lack of a significant difference in 1,25$(OH)_2$D between the cases and controls in this study could be a factor of limited sample size. It is also likely that our case population included mechanisms of hypercalciuria other than altered vitamin D metabolism. This variation could mask disturbances present in small subsets of dogs within the case population. There is significant variation in 25(OH)D between dogs being fed a variety of home cooked and commercially available.[24] Diet fed to each dog in this study was recorded, but, with the exception of urinary stone prevention diets, the numbers of each diet type were too low to draw any conclusions regarding impact on serum vitamin D metabolites in this study. There was an association between a lower ratio of 25(OH)D/24,25$(OH)_2$D and being fed a specific stone-prevention diet (diet B), although this association was not present in a second stone-prevention diet (diet A). Diet B has a lower vitamin D_3 content as compared to diet A (26.4 IU/100 kcal versus 45.5 IU/100 kcal, respectively, for dry formulations). No data exists to further elucidate how these specific diets might impact vitamin D metabolism.

There are a few limitations to this study. Only 3 breeds were included, and there were small numbers of dogs in each breed. Blood phosphorus and hormones involved in vitamin D regulation were not measured in present study including PTH, and fibroblast growth factor 23. The control dogs were screened for stones using abdominal radiographs which are less sensitive than other modalities including contrast radiography or ultrasound for detecting small uroliths.[25] However, the use of UCa/Cr to exclude controls with hypercalciuria reduces the possibility that latent stone formers were included in the control population.

In conclusion, these data suggest that decreased conversion of 25(OH)D to the inactive 24,25$(OH)_2$D occurs in a subset of dogs with hypercalciuric CaOx urolithiasis. Confirmation and characterization of these abnormalities could lead to development novel therapeutic targets in some dogs. Measurement of 25(OH)D/24,25$(OH)_2$D and 1,25$(OH)_2$D/24,25$(OH)_2$D ratios in dogs could be useful in assessing vitamin D status in other canine diseases. Although a commercially available assay for 1,24,25$(OH)_3$D is not currently available, measurement of this metabolite and the ratio of 1,25$(OH)_2$D to 1,24,25$(OH)_3$D could also provide further insight into vitamin D metabolism in the future.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

University of Minnesota protocol 1509-33019A. Stored samples were used for this study; they had been previously collected with approval of the protocol above.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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