

Standard Article

J Vet Intern Med 2017;31:791–798

Association of Vitamin D Metabolites with Parathyroid Hormone, Fibroblast Growth Factor-23, Calcium, and Phosphorus in Dogs with Various Stages of Chronic Kidney Disease

V.J. Parker, L.M. Harjes, K. Dembek, G.S. Young, D.J. Chew, and R.E. Toribio

Background: Hypovitaminosis D is associated with progression of renal disease, development of renal secondary hyperparathyroidism (RHPT), chronic kidney disease-mineral bone disorder (CKD-MBD), and increased mortality in people with CKD. Despite what is known regarding vitamin D dysregulation in humans with CKD, little is known about vitamin D metabolism in dogs with CKD.

Objectives: The purpose of our study was to further elucidate vitamin D status in dogs with different stages of CKD and to relate it to factors that affect the development of CKD-MBD, including parathyroid hormone (PTH), fibroblast growth factor-23 (FGF-23), calcium, and phosphorus concentrations.

Methods: Thirty-seven dogs with naturally occurring CKD were compared to 10 healthy dogs. Serum 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [1,25(OH)₂D], and 24,25-dihydroxyvitamin D [24,25(OH)₂D], and PTH and FGF-23 concentrations were measured. Their association with serum calcium and phosphorus concentrations and IRIS stage was determined.

Results: Compared to healthy dogs, all vitamin D metabolite concentrations were significantly lower in dogs with International Renal Interest Society (IRIS) stages 3 and 4 CKD (*r* [creatinine]: −0.49 to −0.60; *P* < .05) but not different in dogs with stages 1 and 2 CKD. All vitamin D metabolites were negatively correlated with PTH, FGF-23, and phosphorus concentrations (*r*: −0.39 to −0.64; *P* < .01).

Conclusions and Clinical Importance: CKD in dogs is associated with decreases in all vitamin D metabolites evaluated suggesting that multiple mechanisms, in addition to decreased renal mass, affect their metabolism. This information could have prognostic and therapeutic implications.

Key words: calcitriol; diet; international renal interest society; renal secondary hyperparathyroidism.

Chronic kidney disease (CKD) in dogs is a condition characterized by progressive loss of function, with a reported prevalence of up to 25% of dogs.^{1–3} Major consequences of CKD include development of renal secondary hyperparathyroidism (RHPT) and CKD-mineral bone disorder (CKD-MBD). In 1 study, by the International Renal Interest Society (IRIS) CKD staging system, the overall prevalence of RHPT in dogs was 76%, increasing from 36% for stage 2 to 100% for stage 4 dogs.⁴

The development of RHPT is influenced by complex interactions of ionized calcium, phosphorus, vitamin D metabolites, parathyroid hormone (PTH), and fibroblast growth factor-23 (FGF-23). There is limited data regarding vitamin D status in dogs with CKD. Both 25-hydroxyvitamin D [25(OH)D] and

Abbreviations:

1,25(OH) ₂ D	1,25-dihydroxyvitamin D
24,25(OH) ₂ D	24,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
CKD	chronic kidney disease
CKD-MBD	chronic kidney disease-mineral bone disorder
FGF-23	fibroblast growth factor-23
IRIS	International Renal Interest Society
PTH	parathyroid hormone
RHPT	renal secondary hyperparathyroidism
UPC	urine protein:creatinine ratio

1,25-dihydroxyvitamin D [1,25(OH)₂D; calcitriol] concentrations have been shown to be lower in dogs with CKD as compared to healthy dogs,^{4–6} but only 1 study correlated these with IRIS stage. In that study, calcitriol concentrations were inversely associated with IRIS stage and PTH concentrations.⁴ To our knowledge, no study has correlated 25(OH)D to IRIS stage, and no information on 24,25-dihydroxyvitamin D [24,25(OH)₂D] concentrations is available in dogs with clinical kidney disease.

Fibroblast growth factor-23 is secreted by osteocytes in response to net phosphate balance (i.e., dietary load, serum concentration), 1,25(OH)₂D, and PTH.^{7,8} It promotes renal phosphorus excretion (i.e., acts as a phosphatonin) by suppressing 1 α -hydroxylase activity (therefore decreasing 1,25(OH)₂D synthesis) and renal sodium-phosphorus co-transporters.^{7,9–11} By increasing 24-hydroxylase activity, calcitriol concentrations are further decreased.¹¹ Fibroblast growth factor-23 directly decreases PTH secretion in early stages of CKD, but in more advanced stages

From the Veterinary Clinical Sciences, The Ohio State University, Columbus, OH (Parker, Harjes, Dembek, Chew, Toribio); Center for Biostatistics, The Ohio State University, Columbus, OH (Young).

Corresponding author: V.J. Parker, DVM, DACVIM, DACVN, The Ohio State University College of Veterinary Medicine, 601 Vernon L. Tharp St., Columbus, OH 43210; e-mail: parker.888@osu.edu.

Submitted May 16, 2016; Revised October 28, 2016; Accepted December 6, 2016.

Copyright © 2017 The Authors. *Journal of Veterinary Internal Medicine* published by Wiley Periodicals, Inc. on behalf of the American College of Veterinary Internal Medicine.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

DOI: 10.1111/jvim.14653

of CKD, FGF-23 leads to decreased 1,25(OH)₂D concentration that indirectly promotes development of RHPT, because adequate amounts of 1,25(OH)₂D are needed to inhibit PTH gene transcription.¹⁰ An additional mechanism for increased PTH is the development of FGF-23 resistance in the parathyroid glands because expression of its co-receptor klotho is decreased during CKD progression.¹² Increased FGF-23 is associated with progression of CKD, development of RHPT, and higher mortality rates in people.^{13–15}

Both PTH and FGF-23 can impact calcium and phosphorus concentrations. Both hormones tend to decrease circulating phosphorus whereas they have divergent effects on calcium. Whereas PTH tends to mobilize calcium, in part due to generation of calcitriol, FGF-23 minimizes calcium mobilization due to decreased calcitriol production. Calcium and phosphorus concentrations have been shown to be variably affected in CKD dogs with total calcium × phosphorus product (CPP) associated with IRIS stage and mortality.^{4,16,17}

Hypovitaminosis D and increased serum PTH and FGF-23 concentrations are associated with CKD progression, development of RHPT, and increased mortality in people with CKD.^{18–20} Despite the clear role of vitamin D dysregulation in humans with CKD, information on vitamin D metabolites in dogs with different stages of renal disease is lacking. The primary goal of our study was to measure vitamin D metabolites, PTH, and FGF-23 concentrations in dogs with CKD and to determine their association with IRIS stages of CKD. We hypothesize that: (1) dogs with CKD will have lower vitamin D metabolite and higher PTH and FGF-23 concentrations than healthy dogs; and (2) these aberrations will be proportional to IRIS stage. A secondary goal of our study was to assess the relationship of calcium and phosphorus concentrations to IRIS stage. Lastly, we wanted to determine whether there was an association between vitamin D metabolites, specifically 25(OH)D, and dietary vitamin D (i.e., cholecalciferol) intake.

Materials & Methods

Case Selection Criteria

Client-owned dogs diagnosed with CKD were prospectively recruited from the patient population referred to The Ohio State University Veterinary Medical Center (OSU-VMC) between January 2014 and July 2015. A diagnosis of CKD was made based on the presence of at least 2 episodes, over at least 3 months, of minimally concentrated urine (urine specific gravity [USG] < 1.030) with or without azotemia in the absence of other diseases likely to cause polyuria or polydipsia. Additional factors used to determine eligibility included the presence of renal proteinuria, ultrasonographic changes consistent with CKD (e.g., loss of corticomedullary distinction) or both. Dogs were not consistently enrolled at the time of diagnosis, nor were all dogs enrolled in fasted states because of the manner in which dogs were presented to the teaching hospital. Based on serum creatinine concentrations, dogs were assigned to 1 of 4 CKD IRIS stages: <1.4 mg/dL—stage 1; 1.4–2.0 mg/dL—stage 2; 2.1–5.0 mg/dL—stage 3; and >5.0 mg/dL—stage 4, respectively.²¹

Dogs <1 year of age, those diagnosed primary hyperparathyroidism or primary hypoparathyroidism, protein-losing

enteropathy, or neoplasia were excluded. Dogs receiving corticosteroids and dogs diagnosed with acute kidney injury or suspected acute exacerbation of CKD were excluded.

Dogs enrolled as controls, recruited specifically for this study, were deemed healthy based on normal history, physical examination, CBC, serum biochemistry profile, and urinalysis with a USG >1.030. The study was approved by The Ohio State University's Institutional Animal Care and Use Committee and the Clinical Research Advisory Committee, and all owners signed a consent form before dogs were enrolled in the study.

Study Design

After determining eligibility, each CKD dog had a complete physical examination performed, including body weight, body condition score (BCS), and muscle condition score (MCS). A Doppler systolic blood pressure was measured. Blood was collected by jugular venipuncture, and urine was collected by cystocentesis for CBC, serum biochemistry profile, serum ionized calcium concentration, urinalysis, urine culture, and urine protein:creatinine (UPC) ratio. Additional serum was stored at –80°C for analysis of vitamin D metabolite and PTH concentrations, and EDTA plasma was stored at –80°C for FGF-23 analysis. Information regarding medications, diets, and dietary supplements was recorded, and nutrient profiles of the diets the dogs were eating were obtained from the manufacturers.

Vitamin D Analysis

Serum 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)₂D] were measured by radioimmunoassay, and 24,25(OH)₂D by liquid chromatography-mass spectrometry by a Vitamin D External Quality Assessment Scheme (DEQUAS)-certified laboratory.^a

PTH and FGF-23 Analysis

Serum whole PTH concentrations were measured with an immunoradiometric assay utilizing a polyclonal 1-84 PTH antibody.^b Interassay coefficient of variation is reported to be 10%, intra-assay coefficient of variation is reported to be 3%, and functional sensitivity is reported to be 0.3 pmol/L for this assay. Plasma FGF-23 concentrations were measured using a human-specific ELISA,^c previously validated for measurement in dogs.

Data Analysis

Concentrations were log-transformed before analysis to improve their normality and reduce heteroscedasticity. Results are presented as medians and ranges. Pearson correlations were used to assess the relationship between continuous variables. Analysis of variance was used to compare outcomes by IRIS stage, utilizing a Tukey-Kramer adjustment for multiple comparisons. Multivariable linear regression also was used to explore the relationship between vitamin D metabolites and the other relevant biomarkers, taking care to not include predictors that were collinear. Diagnostic plots of the residuals were used to assess model assumptions. Statistical analysis was performed using a commercial statistical software package.^d *P*-values ≤.05 were considered statistically significant.

Results

Thirty-seven dogs with IRIS stage 1–4 CKD and 10 control dogs were enrolled. Median age for CKD dogs was 10.2 years (range, 3.1–15.7 years). Median age for control dogs was 4.3 years (range, 1.4–10.3 years).

Breeds represented among CKD dogs were mixed breed (n = 15), Labrador Retriever (n = 4), and Golden Retriever (n = 3). There were 2 Cocker Spaniels, 2 Shetland Sheepdogs, and 1 each of the following breeds: Australian Cattle Dog, Boxer, Doberman, Fox Terrier, Greyhound, German Shepherd, Jack Russell Terrier, Miniature Schnauzer, Pekingese, Pomeranian, Shih Tzu, Vizsla, Weimaraner, Welsh Terrier, and Whippet. Sixteen male (15 castrated) and 21 female (20 spayed) dogs were included. Control dogs included mixed breed (n = 4), American Pit Bull Terrier (n = 3), German Shepherd (n = 2), and Rottweiler (n = 1). Six dogs were castrated males, and 4 were spayed females.

Median body weight of CKD dogs was 20.0 kg (range, 3.6–58.7 kg). Using the 9-point scoring system, median BCS was 6 (range, 2–8). Three dogs were under-conditioned (BCS < 4), 15 dogs had an ideal BCS (4–5), and 19 dogs were overconditioned (BCS > 5). Body condition score was negatively correlated with serum creatinine concentration ($r = -0.45$; $P = .002$). The MCS was assessed to be normal in 26 dogs. Muscle loss was noted to be mild in 8 dogs, moderate in 1 dog, and severe in 2 dogs. Muscle condition did not correlate with IRIS stage. Median body weight of control dogs was 26.2 kg (range, 13.5–47.0 kg). Median BCS was 6 (range, 4.5–8). All control dogs had normal MCS.

IRIS Stages and Substages

According to the IRIS CKD staging system (Table 1), dogs were classified as stage 1 (n = 10), stage 2 (n = 9), stage 3 (n = 12), or stage 4 (n = 6). Median serum creatinine concentration was 2.0 mg/dL (range, 0.5–12.9 mg/dL). Median urine specific gravity was 1.013 (range, 1.003–1.028). Median urine protein:creatinine ratio (UPC) was 0.6 (range, 0.1–9.6). Based on IRIS substages, dogs were classified as nonproteinuric (n = 11), borderline proteinuric (n = 7), or proteinuric (n = 19). Three dogs had bacterial growth of *Escherichia coli* in their urine. One dog had light growth (600 colony-forming units [cfu] per mL), 1 had 30,000 cfu/mL, and 1 had >100,000 cfu/mL. Their UPC values were 1.2, 2.4, and 0.7, respectively. After removing these dogs from UPC calculations, the median UPC was 0.5. Median systolic blood pressure was 148 mm Hg (range, 115–240 mm Hg). Each dog received a blood pressure substage: minimal risk (n = 18), low risk (n = 5), moderate risk (n = 7), or high risk (n = 6). One dog did not have its blood pressure measured. None of the control dogs was proteinuric. One dog had an increased systolic blood pressure of 180 mm Hg. No specific underlying etiology was determined to account for this dog's hypertension.

Calcium and Phosphorus

Median serum total calcium concentration was 10.8 mg/dL (range, 8.1–13.0 mg/dL). It was within reference range (9.3–11.6 mg/dL) for 31 CKD dogs, low in 1 dog, and high in 5 dogs. Median serum ionized calcium concentration was 5.31 mg/dL (range, 4.03–6.02 mg/dL). Serum ionized calcium concentrations

Table 1. Laboratory parameters of chronic kidney disease (CKD) and healthy control dogs.

Laboratory parameter	Control dogs (n = 10)	All CKD dogs (n = 37)	Stage 1 CKD (n = 10)	Stage 2 CKD (n = 9)	Stage 3 CKD (n = 12)	Stage 4 CKD (n = 6)
BUN	19 (14–23)	44 (8–164)	13 (8–25)	32 (20–75)	59 (28–96)	117 (63–164)
Creatinine (mg/dL)	1.0 (0.8–1.2)	2.0 (0.5–12.9)	1.0 (0.5–1.3)	1.7 (1.4–2.0)	3.3 (2.1–4.8)	8.2 (6.3–12.9)
Phosphorus (mg/dL)	4.0 (2.7–5.6)	4.3 (1.6–14.4)	3.7 (1.7–6.0)	3.7 (1.6–7.3)	4.9 (2.7–6.6)	8.2 (4.5–14.4)
Total calcium (mg/dL)	10.2 (9.4–10.8)	10.8 (8.1–13.0)	10.6 (10.2–11.1)	10.8 (10.3–13.0)	11.1 (9.4–12.2)	10.6 (8.1–11.8)
Ionized calcium (mg/dL)	–	5.31 (4.03–6.02)	5.36 (4.72–5.54)	5.17 (5.00–5.36)	5.17 (4.88–5.58)	5.30 (4.03–6.02)
Ca × P product (mg ² /dL ²)	39.3 (27.0–60.5)	44.9 (17.3–133.9)	39.5 (18.2–64.2)	39.1 (17.3–94.9)	54.5 (29.7–75.6)	95.0 (44.6–133.9)
PTH (pmol/L)	1.1 (0.7–7.8)	2.5 (0.8–229.0)	1.2 (0.8–5.2)	1.7 (1.2–3.4)	3.6 (1.6–14.2)	23.2 (5.1–229)
FGF-23 (pg/mL)	315 (211–449)	467 (142–41,265)	338 (n = 9) (221–684)	336 (142–704)	2,302 (n = 10) (455–24,409)	7,733 (2,520–41,265)
25(OH)D (ng/mL)	75.1 (50.4–97.9)	48.2 (3.5–95.8)	53.7 (32.6–87.3)	55.7 (34.5–93.5)	42.7 (3.5–95.8)	25.0 (5.7–52.5)
1,25(OH) ₂ D (pg/mL)	209.6 (168.9–428.0)	120.8 (19.0–286.0)	157.6 (94.8–202.4)	143.2 (96.4–286.0)	104.8 (29.2–228.7)	64.7 (19.0–91.1)
24,25(OH) ₂ D (ng/mL)	38.7 (24.0–89.5)	18.9 (0.3–48.5)	24.75 (11.2–48.5)	30.3 (14.8–46.8)	10.25 (0.3–42.1)	7.0 (2.0–16.4)
USG	1.043 (1.031–1.053)	1.013 (1.003–1.028)	1.013 (1.003–1.028)	1.019 (1.009–1.027)	1.013 (1.009–1.020)	1.010 (1.008–1.018)
UPC	0.1 (0.1–0.1)	0.6 (0.1–9.6)	0.3 (0.1–3.5)	0.2 (0.1–1.7)	1.1 (0.1–9.6)	1.5 (0.7–4.7)
BP (mm Hg)	137 (100–180)	148 (115–240)	140 (115–240)	145 (130–210)	150 (115–185)	158 (130–200)

were within reference range (4.9–5.8 mg/dL) in 32 CKD dogs, low in 4 dogs and high in 1 dog.

Median serum phosphorus concentration in CKD dogs was 4.3 mg/dL (range, 1.6–14.4 mg/dL). A recent expert panel suggested that maintenance of serum phosphate concentrations within the following ranges is optimal management for dogs with CKD: 2.5–4.5 mg/dL for dogs with stages 1 and 2 CKD, 2.5–5.0 mg/dL for stage 3, and 2.5–6.0 mg/dL for stage 4.⁶ Based on these recommendations, 3 of 10 stage 1 CKD dogs were hypophosphatemic and 7 of 10 were normophosphatemic. Of stage 2 CKD dogs, 1 of 9 was hypophosphatemic, 6 of 9 were normophosphatemic, and 2 of 9 were hyperphosphatemic. Of stage 3 CKD dogs, 7 of 12 were normophosphatemic, and 5 of 12 were hyperphosphatemic. Of stage 4 CKD dogs, 1 of 6 was normophosphatemic and 5 of 6 were hyperphosphatemic.

Serum CPP were determined. Median CPP for CKD dogs was 39.3 (range, 27.0–60.5 mg²/dL²). Nine dogs had CPP > 70 mg²/dL². Of those dogs, stage 2 (n = 1), stage 3 (n = 3), and stage 4 (n = 5) CKD were represented. Serum CPP was negatively correlated with all vitamin D metabolites (Table 2; $P < .001$). Serum CPP was positively correlated with creatinine, PTH, and FGF-23 ($P < .001$).

Vitamin D Metabolites

All vitamin D metabolites were lower in CKD compared to healthy control dogs (Table 1), reaching statistical significance with IRIS stages 3 and 4 (all adjusted P -values <.05). Vitamin D metabolite concentrations were not significantly different between controls and dogs with stages 1 and 2 CKD (Figs 1-3). Serum creatinine concentration was negatively correlated with all vitamin D metabolites. Pearson correlation coefficients between vitamin D metabolites and creatinine, FGF-23, PTH, calcium, and phosphorus are listed in Table 2.

Serum 25(OH)D concentration was negatively correlated with phosphorus ($r = -0.55$; $P < .001$), PTH ($r = -0.42$; $P = .003$), FGF-23 ($r = -0.39$; $P = .009$), and CPP ($r = -0.43$; $P = .002$). A doubling of serum creatinine concentration was associated with a 14% decrease in 25(OH)D ($P = .005$) (Fig 4). Positive correlations were found between 25(OH)D and total calcium ($r = 0.33$; $P = .02$) and ionized calcium ($r = 0.33$;

Table 2. Pearson correlations (r) between vitamin D metabolites and other parameters.

Variable	25(OH)D	1,25(OH) ₂ D	24,25(OH) ₂ D
Creatinine	-0.49 [^]	-0.60 [#]	-0.60 [#]
Phosphorus	-0.55 [#]	-0.61 [#]	-0.55 [#]
PTH	-0.42 [^]	-0.50 [^]	-0.48 [^]
FGF-23	-0.39 [^]	-0.64 [#]	-0.55 [#]
Total calcium	0.33 [*]		
Ionized calcium	0.33 [*]		
Calcium × phosphorus product (CPP)	-0.43 [^]	-0.49 [#]	-0.49 [#]

* $P < .05$; [^] $P < .01$; [#] $P < 0.001$.

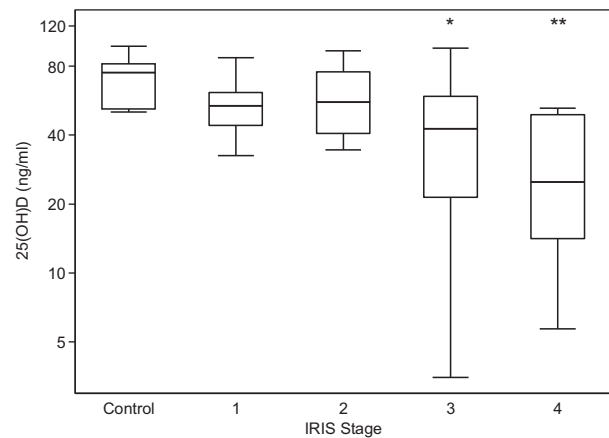


Fig 1. Serum 25(OH)D concentrations based on International Renal Interest Society stage (1–4) and healthy control dogs. The boxes represent the 25th and 75th percentiles, and the central lines in the boxes represent the median values. The whiskers represent the range of concentrations. The single asterisk represents significantly different from control dogs ($P < .05$). The double asterisk represents significantly different from control dogs ($P < .01$).

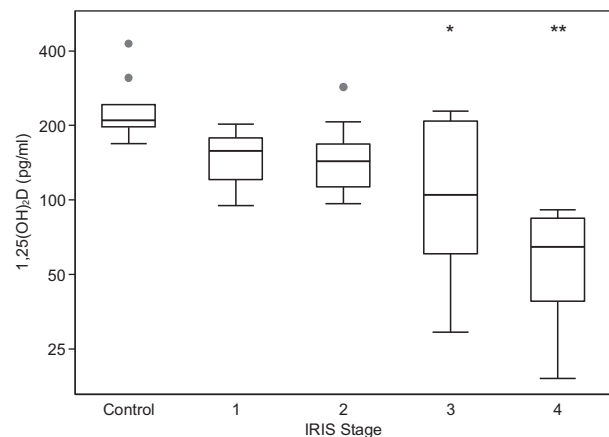


Fig 2. Serum 1,25(OH)₂D concentrations based on International Renal Interest Society stage (1–4) and healthy control dogs. The boxes represent the 25th and 75th percentiles, and the central lines in the boxes represent the median values. The whiskers represent the range of concentrations. Dots represent outliers. The single asterisk represents significantly different from control ($P < .01$). The double asterisk represents significantly different from control and IRIS stages 1 and 2 dogs. ($P < .01$).

$P = .047$). Serum 1,25(OH)₂D concentration was negatively correlated with FGF-23 ($r = -0.64$; $P < .001$), phosphorus ($r = -0.61$; $P < .001$), and PTH ($r = -0.50$; $P < .001$). Serum 24,25(OH)₂D concentration was negatively correlated FGF-23 ($r = -0.55$; $P < .001$), phosphorus ($r = -0.55$; $P < .001$), and PTH ($r = -0.48$; $P < .001$).

PTH and FGF-23

Median PTH concentration in CKD dogs was 2.5 pmol/L (range, 0.8–229 pmol/L). Serum PTH was significantly higher in IRIS stage 3 ($P = .0065$) and

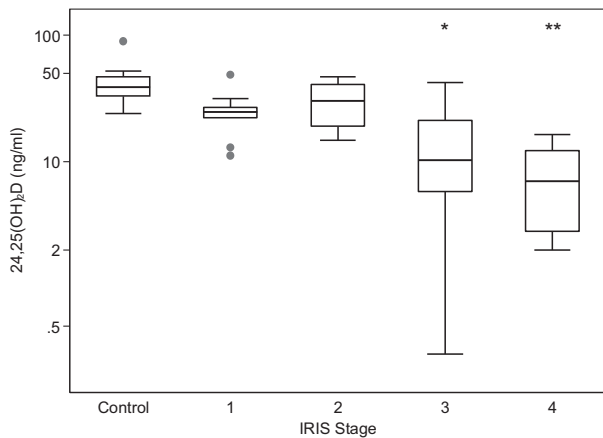


Fig 3. Serum 24,25(OH)₂D concentrations based on International Renal Interest Society stage (1–4) and healthy control dogs. The boxes represent the 25th and 75th percentiles, and the central lines in the boxes represent the median values. The whiskers represent the range of concentrations. Dots represent outliers. The single asterisk represents significantly different from control and IRIS stages 1 and 2 dogs. ($P < .05$). The double asterisk represents significantly different from control and IRIS stages 1 and 2 dogs. ($P < .02$).

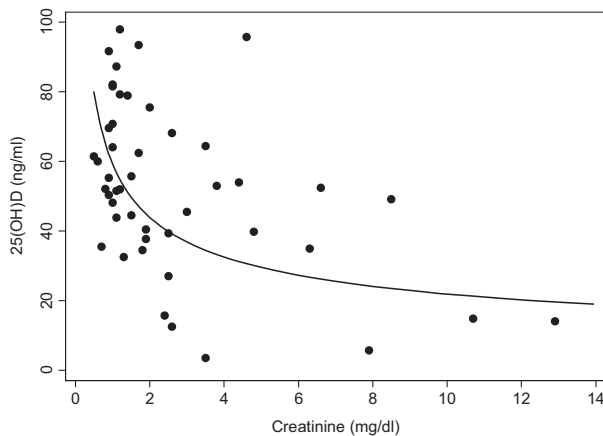


Fig 4. Relationship between serum 25-hydroxyvitamin D and creatinine concentrations.

stage 4 ($P < .001$) CKD dogs compared to healthy control dogs. Eight dogs had PTH concentrations above the upper limit of the laboratory's reference range of 5.8 pmol/L. Of these 8 dogs, 3 were classified as stage 3 CKD and 5 were classified as stage 4 CKD. These dogs had a median serum phosphorus concentration of 8.4 mg/dL (range, 2.7–14.4 mg/dL), notably higher than observed in dogs with lower stages of CKD. Median PTH in control dogs was 1.1 pmol/L (range, 0.7–7.8 pmol/L). One control dog had a serum PTH concentration above the upper limit of the laboratory's reference range. There was no identifiable disease present to explain it. There was no additional serum available to recheck this result from the time of enrollment, but approximately 18 months later, the dog had a normal serum PTH concentration of 1.2 pmol/L. Using this repeated result, median PTH concentration for

control dogs was 1.1 pmol/L (range, 0.7–1.8 pmol/L). We hypothesize that this dog's blood was collected early in the morning and was affected by diurnal variation in serum PTH concentration in dogs.²²

Median FGF-23 concentration in 34 CKD dogs was 467 pg/mL (range, 142–41,265 pg/mL).^f As compared to control dogs, FGF-23 concentrations were significantly higher in CKD dogs with IRIS stages 3 and 4 disease ($P < .001$), but not between controls and earlier stages. Compared to the upper range of 449 pg/mL in the control dogs, 19 of 34 CKD dogs had high FGF-23 concentrations with a median of 2,520 pg/mL (range, 454–41,265 pg/mL). All IRIS stages were represented: stage 1 ($n = 1$), stage 2 ($n = 2$), stage 3 ($n = 10$), and stage 4 ($n = 6$). Three CKD dogs, IRIS stage 1 ($n = 1$) and stage 3 ($n = 2$), did not have their FGF-23 concentrations measured.

Medications and Diet

Dogs with CKD were receiving a variety of medications, including enalapril ($n = 9$), antibiotics ($n = 6$), aluminum hydroxide ($n = 5$), famotidine ($n = 5$), tramadol ($n = 5$), gabapentin ($n = 4$), omeprazole ($n = 4$), amlodipine ($n = 3$), ondansetron ($n = 3$), SC fluids ($n = 3$), diphenhydramine ($n = 3$), heartworm preventative ($n = 3$), mirtazapine ($n = 2$), phenylpropanolamine ($n = 2$), diethylstilbestrol (DES; $n = 2$), nonsteroidal anti-inflammatory drug (NSAID; $n = 2$), flea/tick preventative ($n = 2$), sevelamer ($n = 1$), maropitant ($n = 1$), soloxine ($n = 1$), trazodone ($n = 1$), aspirin ($n = 1$), and cyclosporine ($n = 1$). Eight control dogs were receiving heartworm and flea/tick preventatives.

At the time of enrollment, 17 dogs were reported to be eating ≥ 1 veterinary therapeutic renal diets and 18 dogs were eating a variety of other commercial diets. Two dogs were eating home-prepared diets. Most dogs were eating a predominantly dry kibble diet ($n = 24$). Ten dogs ate a combination of dry and canned diets, and 1 dog ate canned food exclusively. Almost all dogs ($n = 34$) were reported to receive a variety of treats and foods intended for human consumption. The specific amount of ingested cholecalciferol could not be determined given the variability in daily intake and the lack of many owners' abilities to provide exact amounts fed.

To assess for a relationship between dietary cholecalciferol intake and serum 25(OH)D concentrations, dietary intake was defined by the IU/100 kcal of cholecalciferol from the dog's primary diet source. The amount of dietary cholecalciferol ingested was unavailable for 6 dogs, 4 that were eating commercial diets and 2 that were eating variable home-prepared diets. There was no relationship between cholecalciferol intake and serum 25(OH)D ($r = 0.19$, $P = .25$).

Most CKD dogs ($n = 20$) were not receiving any dietary supplements. Supplements that were administered included fish oil ($n = 10$), joint health supplements ($n = 7$), multivitamin ($n = 2$), cranberry supplement ($n = 2$), symbiotic ($n = 2$), probiotic ($n = 1$), S-Adenosylmethionine/silybin ($n = 1$), niacinamide ($n = 1$), calcium carbonate ($n = 1$), calcitriol ($n = 1$), and vitamin

E (n = 1). Four control dogs were receiving glucosamine supplements.

Discussion

In our study, we found CKD in dogs to be associated with a decrease in several vitamin D metabolites. Our results are consistent with previous studies that found 25(OH)D and 1,25(OH)₂D concentrations to be decreased in advanced IRIS stages.^{4,5} Vitamin D metabolites were negatively correlated with PTH and FGF-23 concentrations, suggesting that these hormones interact in the development of RHPT.

The development of hypovitaminosis D is likely multifactorial. Decreased concentrations of 25(OH)D may relate to decreased nutritional intake, proteinuria, increased inflammatory cytokines, and increased FGF-23. Dietary intake of parent vitamin D compounds (i.e., cholecalciferol [vitamin D₃], ergocalciferol [vitamin D₂]) does affect 25(OH)D status in people and dogs.^{23–26} Dietary cholecalciferol was quite variable in the diets CKD dogs were consuming, ranging from 10 to 91 International Units (IU) per 100 kilocalories (kcal). The veterinary therapeutic renal diets provided 25–91 IU cholecalciferol per 100 kcal. At the time of study enrollment, The Association of American Feed Control Officials (AAFCO) recommended that adult maintenance diets for dogs provide a minimum of 12.5 and a maximum of 75 IU vitamin D₃ per 100 kcal. The control dogs were eating diets with 25–110 IU cholecalciferol per 100 kcal.

In our study, there was no well-defined relationship between dietary cholecalciferol intake, as defined by the IU/100 kcal of cholecalciferol from the dog's primary diet source, and 25(OH)D concentrations. The CKD dogs were eating a wide variety of diets, treats, and foods intended for human consumption, and did not consistently eat the same amounts on a daily basis. It has been shown that 82 healthy dogs eating the same diet can have quite variable serum 25(OH)D concentrations (personal communication, Dr. Rondo Middleton). It would have been ideal to determine what, if any, effect appetite and specific caloric intake had on serum 25(OH)D concentration.

One CKD dog was receiving calcium carbonate and calcitriol supplements, but this dog was eating an unbalanced home-cooked diet that was deficient in many essential nutrients, and it is unlikely that the calcium and calcitriol supplements were contributing any relevant quantities to affect systemic status. One CKD dog was receiving a glucosamine-chondroitin supplement that contained 500 IU cholecalciferol per tablet.⁸ Based on the dog's estimated daily caloric intake, this added <0.5 IU/100 kcal, so it was unlikely to have made a relevant impact on systemic 25(OH)D status. Both dogs receiving multivitamin supplements were eating veterinary therapeutic renal diets, and it is unknown why they were receiving additional vitamin and mineral supplementation.

Another factor that could account for low 25(OH)D is that cholecalciferol may not be absorbed well in dogs

with CKD. One study proposed that there may be a breed-associated effect related to intestinal absorption of cholecalciferol.²⁵ None of the dogs had known intestinal disease. Yet another possibility is that cholecalciferol is not as readily transformed in the liver to 25(OH)D in animals with CKD. Decreased transformation of cholecalciferol to 25(OH)D has been demonstrated in rats with induced kidney disease as a result of decreased hepatic cytochrome P450 isoforms that affect 25-hydroxylase activity.²⁷

Proteinuria may influence 25(OH)D concentrations in dogs.^{6,h} In people, it has been suggested that 25(OH)D metabolites may decrease as a result of urinary loss when bound to vitamin D binding protein.²⁸ Decreased megalin expression in renal tubules could contribute to this loss of 25(OH)D into urine,²⁹ but a recent report in people failed to show a difference in serum 25(OH)D and 1,25(OH)₂D concentrations despite decreased urinary loss of vitamin D binding protein.³⁰ Because vitamin D metabolism and synthesis are different in dogs, this area requires additional investigation. Additionally, chronic inflammation, a hallmark of CKD, may contribute to decreased 25(OH)D concentrations. The relationship between hypovitaminosis D and inflammatory markers (e.g., interleukin-6, C-reactive protein) has been established in people.^{31,32}

Lastly, it has been postulated that increased FGF-23 concentrations may contribute to decreased 25(OH)D by upregulation of 24-hydroxylase, the enzyme responsible for converting both 25(OH)D and 1,25(OH)₂D to 24,25(OH)₂D.³³ This idea recently was refuted in a study that prospectively monitored vitamin D metabolites and FGF-23 in people with CKD receiving cholecalciferol supplementation because 24,25(OH)₂D concentrations did not increase.³⁴ Our study also failed to support this hypothesis because serum 24,25(OH)₂D concentrations decreased with CKD progression despite increasing FGF-23 concentrations. It is likely that 24,25(OH)₂D decreased as a result of lesser availability of its 25(OH)D substrate. Additionally, decreased serum 1,25(OH)₂D concentrations should preferentially shunt metabolism of 25(OH)D toward 1,25(OH)₂D.³⁵

Serum 24,25(OH)₂D concentrations have been reported infrequently in the veterinary literature. Healthy control dogs in our study had similar concentrations as were observed in control and stage-stop racing sled dogs in another study.³⁶ Both studies highlight the fact that dogs typically consume a substantially higher amount of dietary vitamin D than do humans, ultimately resulting in substantially higher vitamin D metabolite concentrations. This variability is reflected in what is considered "normal" or "sufficient" serum 25(OH)D concentrations, which is controversial in both species.^{25,37,38}

Decreased 1,25(OH)₂D has been documented previously in dogs with CKD. Its deficiency also is likely a multifactorial process. Several consequences of CKD affect 1 α -hydroxylase activity, the enzyme responsible for converting 25(OH)D to 1,25(OH)₂D. Conventional knowledge of CKD states that decreased renal mass will decrease 1 α -hydroxylase activity. However, both PTH and FGF-23 also influence this enzyme. Increased PTH

concentrations (1) stimulate 1α -hydroxylase activity to ultimately increase calcitriol synthesis and subsequently intestinal calcium and phosphorus absorption, and (2) decrease 24-hydroxylase activity. Conversely, FGF-23, which is in part stimulated by $1,25(\text{OH})_2\text{D}$, downregulates 1α -hydroxylase and upregulates 24-hydroxylase.

Both total and ionized calcium concentrations were positively correlated with $25(\text{OH})\text{D}$ concentrations. This is not surprising because $25(\text{OH})\text{D}$ is metabolized to $1,25(\text{OH})_2\text{D}$, which then positively influences intestinal calcium absorption and subsequently serum calcium concentrations. Similar to what has been documented previously,^{17,39} serum total calcium concentration was not a good predictor of serum ionized calcium status in the CKD dogs, thus highlighting the importance of evaluating serum ionized calcium for CKD dogs. Serum CPP increased with IRIS stage. One limitation to reporting CPP is that it represents a momentary snapshot in time. It is probably more useful to monitor and report trends in CPP than individual time-points.

Lastly, BCS was negatively correlated with IRIS stage (i.e., serum creatinine concentration). The finding of poor condition with later stage CKD is likely multifactorial, resulting from decreased caloric intake and increased catabolic inflammatory cytokines. In a retrospective study, dogs with poor BCS (i.e., $\leq 3/9$) had shorter survival than dogs with a BCS $\geq 4/9$.⁴⁰ There was no association between BCS and vitamin D metabolites. It remains to be determined whether vitamin D metabolites are associated with survival in CKD dogs.

Limitations of this study included a relatively small number of dogs represented in each IRIS stage, especially stage 4. Study enrollment was not consistently performed on fasted patients. Some dogs may have been classified incorrectly based on the IRIS staging system, but we consider this unlikely. Other diseases that can cause polyuria and polydipsia could have been incorrectly classified as having IRIS stage 1 CKD. Many dogs did have abdominal ultrasound examinations performed, but dogs were not required to have this test performed nor were they required to have hyperadrenocorticism excluded. Dogs with neoplasia were excluded because there may be aberrations in calcium, vitamin D, and PTH metabolism in various types of cancers.^{38,41,42} Another limitation is that control dogs were not age-matched, but there was no difference in serum $25(\text{OH})\text{D}$ concentration by age in 1 study of 320 dogs.²⁵

Three dogs had documented *E. coli* lower urinary tract infections, with mixed patterns of growth, ranging from mild (<600 cfu/mL) to heavy ($>100,000$ cfu/mL) quantitative growth. Infection is reported to affect UPC concentrations, although it is interesting to note that the dog with the heaviest growth had the lowest UPC (0.7) among the 3 dogs. None of the dogs exhibited overt clinical signs of pyelonephritis, but this possibility cannot be excluded. Although it is possible that these dogs may have been misclassified by IRIS stage, we consider this to be unlikely.

In summary, our study shows that vitamin D metabolites are decreased in CKD dogs and related to CKD severity. An inverse relationship exists between

vitamin D metabolites and serum PTH, FGF-23, and phosphorus concentrations, supporting the complex relationship these hormones play in the development of RHPT. Additional studies are needed to determine whether targeted regulation of vitamin D metabolite status in dogs with CKD is warranted and the best way to increase vitamin D concentrations and decrease PTH and FGF-23 concentrations while avoiding toxicity (e.g., hypercalcemia). Historically, emphasis has been placed on supplementation with calcitriol,⁴³ but other potential options include nutritional vitamin D (e.g., cholecalciferol, ergocalciferol) or even $25(\text{OH})\text{D}$.⁴⁴

Footnotes

- ^a Heartland Assays, Inc., Ames, IA
^b Michigan State University Diagnostic Center for Population and Animal Health (MSU-DCPAH), East Lansing, MI
^c Kainos FGF-23 ELISA, Japan
^d SAS v9.4, SAS Institute, Cary, NC
^e Vetoquinol proceedings
^f Harjes LM, et al. JVIM 2016; In review
^g Simply Right Glucosamine Chondroitin + Vitamin D-3
^h Parker VJ, et al. Association between hypovitaminosis D and proteinuria. Research report, ACVIM 2016
-

Acknowledgments

The authors thank the Clinical Trials Office (CTO) at the College of Veterinary Medicine for coordinating sample collection, storage, and handling.

This study was supported by a Canine Funds grant from the College of Veterinary Medicine at The Ohio State University.

The project described was supported by Award Number Grant UL1TR001070 from the National Center for Advancing Translational Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Advancing Translational Sciences or the National Institutes of Health.

Presented in part at the 2016 American College of Veterinary Internal Medicine Forum, Denver, CO.

Conflict of Interest Declaration: Drs. Parker and Chew have received honoraria to travel and speak at ACVIM Forums.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

- Lund EM, Armstrong PJ, Kirk CA, et al. Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. *J Am Vet Med Assoc* 1999;214:1336–1341.
- Bartlett PC, Van Buren JW, Neterer M, et al. Disease surveillance and referral bias in the veterinary medical database. *Prev Vet Med* 2010;94:264–271.

3. Guidi G, Rossini C, Cinelli C, et al. Canine chronic kidney disease: Retrospective study of a 10-year period of clinical activity. In: Pugliese A, Gaiti A, Boiti C, eds. *Veterinary Science: Current aspects in Biology, Animal Pathology, Clinic and Food Hygiene*. New York: Springer; 2012:115–118.
4. Cortadellas O, Fernandez del Palacio MJ, Talavera J, et al. Calcium and phosphorus homeostasis in dogs with spontaneous chronic kidney disease at different stages of severity. *J Vet Intern Med* 2010;24:73–79.
5. Gerber B, Hassig M, Reush CE. Serum concentrations of 1,25-dihydroxycholecalciferol and 25-hydroxycholecalciferol in clinically normal dogs and dogs with acute and chronic renal failure. *Am J Vet Res* 2003;64:1161–1166.
6. Galler A, Tran JL, Krammer-Lukas S, et al. Blood vitamin levels in dogs with chronic kidney disease. *Vet J* 2012;192:226–231.
7. Wolf M. Update on fibroblast growth factor 23 in chronic kidney disease. *Kidney Int* 2012;82:737–747.
8. Saito H, Maeda A, Ohtomo S, et al. Circulating FGF-23 is regulated by 1 α ,25-dihydroxyvitamin D₃ and phosphorus in vivo. *J Biol Chem* 2005;280:2543–2549.
9. Quarles LD. Skeletal secretion of FGF-23 regulates phosphate and vitamin D metabolism. *Nat Rev Endocrinol* 2012;8:276–286.
10. Silver J, Naveh-Manly T. FGF-23 and secondary hyperparathyroidism in chronic kidney disease. *Nat Rev Nephrol* 2013;9:641–649.
11. Shimada T, Hasegawa H, Yamazaki Y, et al. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J Bone Miner Res* 2004;19:429–435.
12. Hu MC, Kuro-o M, Moe OW. Klotho and chronic kidney disease. *Contrib Nephrol* 2013;180:47–63.
13. Shaikh A, Berndt T, Kumar R. Regulation of phosphate homeostasis by the phosphatonins and other novel mediators. *Pediatr Nephrol* 2008;23:1203–1210.
14. Kuro-o M. Phosphate and klotho. *Kidney Int* 2011;29:S20–S23.
15. Juppner H. Phosphate and FGF-23. *Kidney Int* 2011;79:S24–S27.
16. Lippi I, Guidi G, Marchetti V, et al. Prognostic role of the product of serum calcium and phosphorus concentrations in dogs with chronic kidney disease: 31 cases. *J Am Vet Med Assoc* 2014;245:1135–1140.
17. Kogika MM, Lustoza MD, Notomi MK, et al. Serum ionized calcium in dogs with chronic renal failure and metabolic acidosis. *Vet Clin Path* 2006;35:441–445.
18. Ravani P, Malberti F, Tripepi G, et al. Vitamin D levels and patient outcome in chronic kidney disease. *Kidney Int* 2009;75:88–95.
19. Isakova T, Xie H, Yang W, et al. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. *J Am Med Assoc* 2011;305:2432–2439.
20. Jean G, Terrat JC, Vanel T, et al. High levels of serum fibroblast growth factor (FGF)-23 are associated with increased mortality in long haemodialysis patients. *Nephrol Dial Transpl* 2009;24:2792–2796.
21. International Renal Interest Society IRIS staging of CKD. Available at <http://iris-kidney.com/pdf/staging-of-ckd.pdf>. Accessed August 1, 2015.
22. Lopez I, Aguilera-Tejero E, Estepa JC, et al. Diurnal variations in the plasma concentration of parathyroid hormone in dogs. *Vet Rec* 2005;157:344–347.
23. Tran JL, Horvath C, Krammer S, et al. Blood vitamin concentrations in privately owned dogs fed non-standardized commercial diets and after intake of diets with specified vitamin concentrations. *J Anim Phys Anim Nutr* 2007;91:40–47.
24. Kim SM, Choi HJ, Lee JP, et al. Prevalence of vitamin D deficiency and effects of supplementation with cholecalciferol in patients with chronic kidney disease. *J Renal Nutr* 2014;24:20–25.
25. Sharp CR, Selting KA, Ringold R. The effect of diet on serum 25-hydroxyvitamin D concentrations in dogs. *BMC Res Notes* 2015;8:442–447.
26. Krassilnikova M, Ostrow K, Bader A, et al. Low dietary intake of vitamin D and vitamin D deficiency in hemodialysis patients. *J Nephrol Thera* 2014;4:166–170.
27. Michaud J, Naud J, Ouimet D, et al. Reduced hepatic synthesis of calcidiol in uremia. *J Am Soc Nephrol* 2010;21:1488–1497.
28. Denburg MR, Bhan I. Vitamin D-binding protein in health and chronic kidney disease. *Semin Dialysis* 2015;28:636–644.
29. Dusso A, Gonzalez EA, Martin KJ. Vitamin D in chronic kidney disease. *Best Pract Res Cl En* 2011;25:647–655.
30. Doorenbos CR, de Cuba MM, Vogt L, et al. Antiproteinuric treatment reduces urinary loss of vitamin D-binding protein but does not affect vitamin D status in patients with chronic kidney disease. *J Steroid Biochem* 2012;128:56–61.
31. Bucharles S, Barberato SH, Stingham AE, et al. Hypovitaminosis D is associated with systemic inflammation and concentric myocardial geometric pattern in hemodialysis patients with low iPTH levels. *Nephron Clin Pract* 2011;118:384–391.
32. Ansemant T, Mahy S, Piroth C, et al. Severe hypovitaminosis D correlates with increased inflammatory markers in HIV infected patients. *BMC Infect Dis* 2013;13:7.
33. Petkovich M, Jones G. CYP24A1 and kidney disease. *Curr Opin Nephrol* 2011;20:337–344.
34. Stubbs JR, Zhang S, Friedman PA, et al. Decreased conversion of 25-hydroxyvitamin D₃ to 24,25-dihydroxyvitamin D₃ following cholecalciferol therapy in patients with CKD. *Clin J Am Soc Nephrol* 2014;9:1965–1973.
35. Hazewinkel HA, Tryfonidou MA. Vitamin D₃ metabolism in dogs. *Mol Cell Endocrinol* 2002;197:23–33.
36. Spoo JW, Downey RL, Griffiths C, et al. Plasma vitamin D metabolites and C-reactive protein in stage-stop racing endurance sled dogs. *J Vet Intern Med* 2015;29:519–525.
37. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266–281.
38. Selting KA, Sharp CR, Ringold R, et al. Serum 25-hydroxyvitamin D concentrations in dogs - correlation with health and cancer risk. *Vet Comp Onc* 2016;14:295–305.
39. Schenck PA, Chew DJ. Prediction of serum ionized calcium concentration by use of serum total calcium concentration in dogs. *Am J Vet Res* 2005;66:1330–1336.
40. Parker VJ, Freeman LM. Association between body condition and survival in dogs with acquired chronic kidney disease. *J Vet Intern Med* 2011;25:1306–1311.
41. Wakshlag JJ, Rassnick KM, Malone EK, et al. Cross-sectional study to investigate the association between vitamin D status and cutaneous mast cell tumours in Labrador retrievers. *Brit J Nutr* 2011;106:S60–S63.
42. Weidner N, Verbrugge A. Current knowledge of vitamin D in dogs. *Crit Rev Food Sci Nutr* 2016. doi:10.1080/10408398.2016.1171202.
43. de Brito Galvao JF, Nagode LA, Schenck PA, et al. Calcitriol, calcidiol, parathyroid hormone, and fibroblast growth factor-23 interactions in chronic kidney disease. *J Vet Emerg Crit Care* 2013;23:134–162.
44. Petkovich M, Melnick J, White J, et al. Modified-release oral calcifediol corrects vitamin D insufficiency with minimal CYP24A1 upregulation. *J Steroid Biochem* 2015;148:283–289.