


## Standard Article

*J Vet Intern Med* 2017;31:784–790**Fibroblast Growth Factor-23 Concentration in Dogs with Chronic Kidney Disease**L.M. Harjes, V.J. Parker , K. Dembek, G.S. Young, L.H. Giovaninni, M.M. Kogika, D.J. Chew, and R.E. Toribio

**Background:** Chronic kidney disease (CKD) is associated with hyperphosphatemia, decreased vitamin D metabolite concentrations, and hyperparathyroidism. This syndrome is known as CKD-mineral bone disorder (CKD-MBD). Recently, it has been shown that an increase in fibroblast growth factor-23 (FGF-23) concentration is an early biomarker of CKD in people. It is an independent risk factor for both progression of renal disease and survival time in humans and cats with CKD. Information about FGF-23 in healthy dogs and those with CKD is lacking.

**Objectives:** To measure FGF-23 concentration in dogs with different stages of CKD and determine its association with factors involved in CKD-MBD, including serum phosphorus and parathyroid hormone (PTH) concentrations. A secondary aim was to validate an ELISA for measurement of plasma FGF-23 concentration in dogs.

**Animals:** Thirty-two client-owned dogs with naturally occurring CKD and 10 healthy control dogs.

**Methods:** Prospective cross-sectional study. An FGF-23 ELISA was used to measure plasma FGF-23 concentration in dogs and their association with serum creatinine, phosphorus, calcium, and PTH concentrations.

**Results:** Plasma FGF-23 concentrations increased with severity of CKD and were significantly different between IRIS stages 1 and 2 versus stages 3 and 4 ( $P < .0001$ ). Increases in FGF-23 concentrations were more frequent than hyperparathyroidism or hyperphosphatemia in this cohort. Serum creatinine and phosphorus concentrations were the strongest independent predictors of FGF-23 concentration.

**Conclusions and clinical importance:** Plasma FGF-23 concentrations increase in dogs with CKD as disease progresses. Plasma FGF-23 concentrations appear to be useful for further study of the pathophysiology of CKD-MBD in dogs.

**Key words:** Chronic kidney disease-mineral bone disorder; Dog; International Renal Interest Society; Renal secondary hyperparathyroidism.

Chronic kidney disease (CKD) is common in dogs, with a reported prevalence of up to 25% in dogs presented to a veterinary teaching hospital.<sup>1–3</sup> In CKD, hyperphosphatemia occurs in part because of decreased urinary excretion. This contributes to development of renal secondary hyperparathyroidism (RHPT),<sup>4–6</sup> a condition reported in approximately

**Abbreviations:**

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

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76% of dogs with CKD.<sup>4</sup> Chronic kidney disease-mineral and bone disorder (CKD-MBD) is a term that more inclusively accounts for these collective hormonal aberrations and renal osteodystrophy.<sup>7,8</sup> Historically characterized by hyperphosphatemia, as well as decreased 1,25-dihydroxyvitamin D (calcitriol) and increased parathyroid hormone (PTH) concentrations, CKD-MBD recently has been associated with increased fibroblast growth factor-23 (FGF-23) concentrations in people and cats.<sup>9</sup> Increased FGF-23 is associated with disease progression and mortality in humans and cats with CKD.<sup>10,11</sup>

Fibroblast growth factor-23 is a phosphatonin released from osteocytes in response to increased phosphorus, calcitriol, and PTH concentrations.<sup>9</sup> With its coreceptor  $\alpha$ -klotho, FGF-23 decreases phosphorus and calcitriol concentrations by: (1) downregulating sodium-phosphorus cotransporters (NaPi-IIa and IIc) in renal proximal tubules; (2) inhibiting renal  $1\alpha$ -hydroxylase activity; and (3) increasing 24,25-hydroxylase activity.<sup>5,6,9,12,13</sup> Fibroblast growth factor-23 decreases PTH secretion in the early stages of CKD. In advanced stages, FGF-23 leads to decreased calcitriol, indirectly promoting development of hyperparathyroidism, because adequate amounts of calcitriol are needed to

inhibit PTH gene transcription.<sup>9</sup> An additional mechanism for increased PTH concentration is the development of FGF-23 resistance in the parathyroid glands because expression of *klotho* is decreased.<sup>14</sup>

In humans with CKD, FGF-23 concentrations increase early, before disturbances in other markers of mineral metabolism.<sup>10,15</sup> Alterations in serum calcitriol, PTH, and phosphorus concentrations occur during later stages of CKD.<sup>16</sup> However, serum phosphorus concentration is still the variable most routinely tested and targeted therapeutically in dogs with CKD because it is most easily accessible.

The aim of our study was to measure plasma FGF-23 concentration in dogs with CKD and to determine their association with International Renal Interest Society (IRIS) stages of CKD as well as serum phosphorus and PTH concentrations. The relationship of vitamin D metabolites with components of CKD-MBD is described elsewhere.<sup>17</sup>

## Materials and 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 repeatedly minimally concentrated urine (urine specific gravity [USG] < 1.030) and at least 1 of the following: renal proteinuria, ultrasonographic renal abnormalities, or azotemia in the absence of other diseases likely to cause polyuria or polydipsia. This definition of CKD is derived from the International Renal Interest Society (IRIS) guidelines, with azotemia defined as a serum creatinine concentration  $\geq 1.4$  mg/dL. Ultrasonographic renal findings consistent with CKD included decreased corticomedullary distinction, small, irregular kidneys, or both.<sup>18</sup> Controls consisted of OSU-VMC staff- and student-owned dogs deemed healthy on the basis of history, physical examination findings, and normal results of CBC, serum biochemistry profile, and urinalysis (with USG > 1.030).

Dogs with CKD were classified into 4 stages as defined by IRIS guidelines based on serum creatinine concentrations: stage 1: < 1.4 mg/dL, stage 2: 1.4–2.0 mg/dL, stage 3: 2.1–5.0 mg/dL, and stage 4: > 5.0 mg/dL. They were substage as defined by IRIS guidelines based on urine protein-to-creatinine ratio (UPC) and blood pressure. For UPC, dogs were considered nonproteinuric with a UPC < 0.2, borderline proteinuric with a UPC of 0.2–0.5, and proteinuric with a UPC of > 0.5. Dogs were considered hypertensive when systolic blood pressure was  $\geq 150$  mmHg. Dogs were further divided into normophosphatemic or hyperphosphatemic based on opinions from an expert panel suggesting that maintenance of serum phosphorus concentration 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.<sup>19</sup>

Dogs < 1 year of age, those diagnosed with concurrent diseases, or those receiving medications known to affect PTH concentrations were excluded. Dogs diagnosed with acute kidney injury or suspected of acute exacerbation of CKD were excluded. Ethical approval for this study was obtained from the Institutional Animal Care and Use Committee and The Ohio State University's Clinical Research Advisory Committee. All owners signed a consent form before dogs were enrolled in the study.

### Analytical Procedures

Each CKD dog had a complete physical examination performed and a systolic blood pressure measured by Doppler. Blood was anaerobically collected by jugular venipuncture, with a vacutainer, into red-top and EDTA-containing tubes. The CBC, serum biochemistry profile and serum ionized calcium concentrations were analyzed with commercially available analyzers.<sup>a,b,c</sup> Serum was harvested within 30 minutes of collection. Samples for ionized calcium concentration were handled anaerobically, and as such, pH correction equations were not used to correct for loss of CO<sub>2</sub> from the samples. Urine was collected by cystocentesis for urinalysis and UPC. Additional serum and plasma samples were aliquoted and stored at –80°C for PTH and FGF-23 analysis, respectively. Information regarding medications, diets, and dietary supplements was recorded.

Serum whole PTH concentrations were measured with a human-specific immunoradiometric assay.<sup>d</sup> Plasma FGF-23 concentrations were measured with a human-specific intact FGF-23 ELISA.<sup>e</sup> To achieve a reading on the standard curve, canine samples were diluted with the zero standard supplied with the assay. Precision and reproducibility were assessed by measuring intra- and interassay coefficients of variation (CV) in canine samples with low and high FGF-23 concentrations. Diluting canine samples with the zero standard assessed dilutional parallelism.

### Statistical Analysis

Results are presented as median and range. Due to positive skewness and nonconstant variance, concentrations were log-transformed before parametric modeling. Fibroblast growth factor-23 (FGF-23) and PTH were compared by IRIS stage by a linear model with heterogeneous variance. The Tukey-Kramer adjustment was used to control for multiplicity. Pearson's correlation coefficients were used to assess the relationships between variables. A multivariable model for FGF-23 was created by utilizing a backwards variable selection method. All analyses were conducted by commercially available statistical analysis programs.<sup>f,g</sup>

## Results

The FGF-23 assay was performed with canine plasma, with all samples run in duplicate. The lowest and highest standards supplied with the ELISA were 0 and 800 pg/mL, respectively. Sample dilution (up to 1:128) was required to measure FGF-23 concentrations in all CKD dogs. Samples outside the standard curve were serially diluted before inclusion in the assay. The intra-assay coefficients of variation (CV) for samples measuring 223, 274, 3789, and 7242 (n = 4) pg/mL FGF-23 were 4.8, 2.2, 1.2, and 7.2%, respectively. The interassay CVs for samples measuring 240, 289 (n = 6), and 360 (n = 4) pg/mL FGF-23 were 6.7, 3.1, and 6.1%, respectively.

Thirty-two dogs met the inclusion criteria for enrollment in the CKD group. Median age was 10.5 years (range, 3.1–15.7 years). The most common breeds were mixed breed (n = 14) and Labrador retriever (n = 2) and 1 each of the following breeds: Australian Cattle Dog, Boxer, Doberman Pinscher, Fox Terrier, German Shepherd Dog, Golden Retriever, Greyhound, Jack Russell Terrier, Miniature Schnauzer, Pekingese, Pomeranian, Shih Tzu, Vizsla, Weimaraner, Welsh Terrier, and Whippet. Twelve castrated males, 1 intact male

and 19 spayed female dogs were included. In accordance with the IRIS staging system, dogs were classified as stage 1 (n = 7), stage 2 (n = 9), stage 3 (n = 10), or stage 4 (n = 6).

Ten healthy dogs were enrolled in the control group. The median age was 4.3 years (range, 1.4–10.3 years) and represented breeds included mixed breed (n = 4), Pit Bull Terrier (n = 3), German Shepherd Dog (n = 2) and Rottweiler (n = 1). Six dogs were castrated males and 4 were spayed females.

Not all CKD dogs were enrolled at the time of diagnosis of CKD, and for ethical considerations, treatment was not withheld before enrollment. These patients were receiving a variety of medications including enalapril (n = 8), antibiotics (n = 5), 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 prevention (n = 3), phenylpropanolamine (n = 1), diethylstilbestrol (n = 2), nonsteroidal anti-inflammatory drugs (n = 2), ectoparasite prevention (n = 2), mirtazapine (n = 1), sevelamer (n = 1), levothyroxine (n = 1), trazodone (n = 1), aspirin (n = 1), and cyclosporine (n = 1). The CKD dogs were fed a variety of diets, with 14 (44%) eating a veterinary therapeutic renal diet, 2 (6%) a home-cooked diet and 16 (50%) other commercial dog diets. All control dogs were fed commercial dog diets and 8/10 were receiving heartworm and ectoparasite preventatives.

Details regarding IRIS stage and substages of each enrolled dog are outlined in Table S1. Median serum creatinine concentration for CKD dogs was 2.3 mg/dL (range, 0.6–12.9 mg/dL; Table 1). Median serum phosphorus concentration for CKD dogs was 4.5 mg/dL (range, 1.6–14.4 mg/dL). According to previously defined optimal serum phosphorus concentrations based on IRIS stage,<sup>19</sup> 41% (13/32) of dogs with CKD had hyperphosphatemia. Dogs with IRIS stage 3 CKD were subdivided into normophosphatemic (stage 3a; n = 5) and hyperphosphatemic (stage 3b; n = 5) groups. Median serum phosphorus concentration in stage 3a was 3.4 mg/dL (range, 2.7–4.8 mg/dL) and stage 3b dogs was 6.2 mg/dL (range, 5.6–6.6 mg/dL). Dogs in IRIS

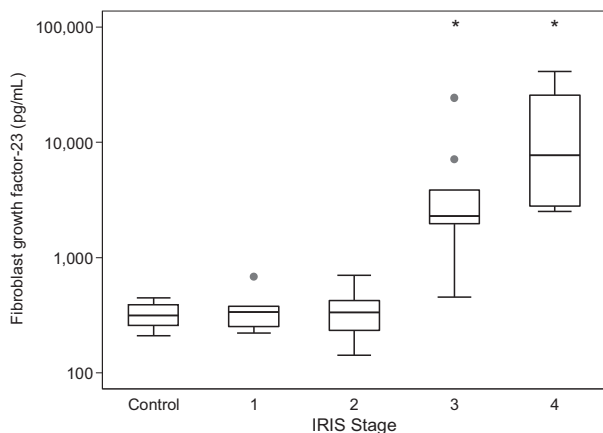
stages 2 and 4 were not subdivided because most dogs with stage 2 CKD were normophosphatemic (7/9) and most dogs with stage 4 CKD were hyperphosphatemic (5/6).

Median (range) plasma FGF-23 concentrations in all CKD dogs were higher (582, 142–41,265 pg/mL) than those of healthy dogs (315, 211–449 pg/mL; Fig 1). Fibroblast growth factor-23 concentrations were significantly lower in control dogs as compared to those with IRIS stages 3 and 4 ( $P < .0001$ ). Using the upper FGF-23 concentration from healthy dogs as the cutoff to define high FGF-23 concentration (449 pg/mL), 59% (19/32) of CKD dogs had increased FGF-23 concentrations. Fibroblast growth factor-23 concentrations increased with IRIS stage (stage 1: 338 [221–684]; stage 2: 336 [142–704]; stage 3: 2,302 [445–24,409]; and stage 4: 7,733 [2,520–41,265]) pg/mL. Plasma FGF-23 concentrations were significantly increased in dogs with IRIS stages 3 and 4 as compared to stages 1 and 2 ( $P < .0001$ ). Plasma FGF-23 concentrations were positively correlated with serum creatinine ( $r = 0.87$ ;  $P < .0001$ ; Fig 2) and serum phosphorus ( $r = 0.68$ ,  $P < .0001$ ) concentrations (Fig 3). The estimated fold difference between FGF-23 concentrations in stage 3a versus 3b was 2.53 (95% confidence interval [CI]: 0.49, 13.06), but there was not a statistically significant difference between these 2 groups ( $P = .23$ ; Fig 4). There was no correlation between FGF-23 and either total ( $P = .24$ ) or ionized calcium ( $P = .27$ ).

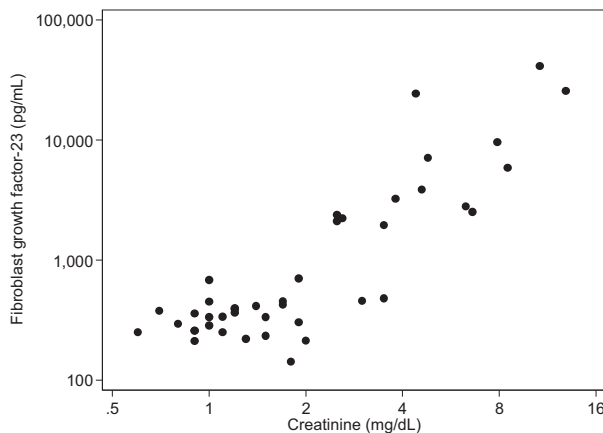
Serum PTH concentration in all CKD dogs was 2.8 (0.9–229) pmol/L as compared to healthy dog concentrations of 1.1 (0.7–7.8) pmol/L (Fig 5). Plasma FGF-23 concentrations were positively correlated with serum PTH concentrations in CKD dogs ( $r = 0.74$ ,  $P < .0001$ ; Fig 6). Serum PTH concentrations increased with IRIS stage (stage 1: 1.6 [0.9–5.2]; stage 2: 1.7 [1.2–3.4]; stage 3: 4.5 [1.6–14.2]; stage 4: 32.2 [5.1–229] pmol/L). Serum PTH concentrations were significantly higher in stages 3 and 4 than in all other stages and PTH concentrations were significantly higher in stage 4 versus stage 3 dogs ( $P < .05$ ). Eight CKD dogs had PTH concentrations above the upper limit of the laboratory's reference range of 5.8 pmol/L. Of these dogs, 3 had stage 3 and 5 had stage 4 CKD.

**Table 1.** Comparison of creatinine, phosphorus, fibroblast growth factor-23 (FGF-23), parathyroid hormone (PTH) and calcium concentrations in control and chronic kidney disease (CKD) dogs. Results are presented as median and range.

Laboratory Variable and Reference Range	Control (n = 10)	All CKD Dogs (n = 32)	Stage 1 (n = 7)	Stage 2 (n = 9)	Stage 3 (n = 10)	Stage 4 (n = 6)
Creatinine (0.6–1.6 mg/dL)	1.0 (0.8–1.2)	2.3 (0.6–12.9)	1.0 (0.6–1.3)	1.7 (1.4–2.0)	3.5 (2.5–4.6)	8.2 (6.3–12.9)
Phosphorus (3.2–8.1 mg/dL)	4.0 (2.7–5.6)	4.5 (1.6–14.4)	4.0 (2.0–6.0)	3.7 (1.6–7.3)	5.6 (2.7–6.6)	8.2 (4.5–14.4)
FGF-23 (pg/mL)	315 (211–449)	582 (142–41,265)	338 (221–684)	336 (142–704)	2,302 (455–24,409)	7,733 (2,520–41,265)
PTH (0.5–5.8 pmol/L)	1.1 (0.7–7.8)	2.8 (0.9–229.0)	1.6 (0.9–5.2)	1.7 (1.2–3.4)	4.5 (1.6–14.2)	32.2 (5.1–229.0)
Total calcium (9.3–11.6 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)	11.3 (9.4–12.2)	10.6 (8.1–11.8)
Ionized calcium (4.9–5.8 mg/dL)	–	5.2 (4.0–6.0)	5.4 (4.7–5.5)	5.2 (5.0–5.4)	5.1 (4.9–5.6)	5.3 (4.0–6.0)



**Fig 1.** Box and whisker plot illustrating the plasma fibroblast growth factor-23 (FGF-23) 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 scale for FGF-23 is logarithmic. Asterisks represent statistically increased FGF-23 concentration in dogs with IRIS stages 3 and 4 as compared to control dogs and those with stages 1 and 2 ( $P < .0001$ ).



**Fig 2.** Scatterplot illustrating plasma FGF-23 concentrations by serum creatinine concentrations. The scales for both axes are logarithmic. Plasma FGF-23 concentrations were positively correlated with creatinine concentrations ( $r = 0.87$ ,  $P < .0001$ ).

One control dog had a PTH concentration above the upper limit of the laboratory's reference range (7.8 pmol/L). There was no additional serum available to recheck this result from the time of enrollment, but 18 months later the dog had a normal serum PTH concentration of 1.2 pmol/L. Removing this patient from the population did not significantly alter PTH results. Using this repeated result, median PTH concentration for control dogs was 1.1 pmol/L (range, 0.7–1.8 pmol/L). Comparing PTH concentrations of the CKD dogs to the upper range of the control dogs (i.e., with a cutoff of 1.8 pmol/L), 22/32 (69%) of CKD dogs had hyperparathyroidism with a median PTH of 5.0 pmol/L (range, 2.1–229 pmol/L). These included dogs with all

stages of CKD: stage 1 ( $n = 3$ ); stage 2 ( $n = 4$ ), stage 3 ( $n = 9$ ), stage 4 ( $n = 6$ ).

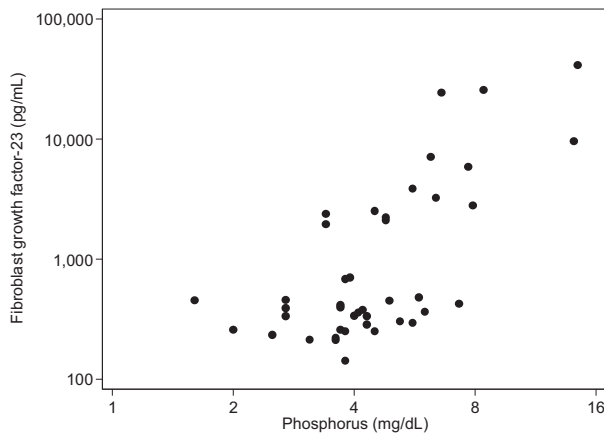
Serum creatinine, phosphorus, calcium and PTH concentrations were assessed in the multivariable model to predict FGF-23 concentrations. Of these, serum creatinine ( $P < .0001$ ) and phosphorus ( $P = .01$ ) concentrations were retained in the final model ( $r^2 = 0.79$ ). From this model, a doubling of serum creatinine concentration was associated with a 150% (95% CI: 97%, 218%) increase in FGF-23 and a doubling of serum phosphorus concentration with a 78% (95% CI: 15%, 176%) increase in FGF-23 (Table 2).

## Discussion

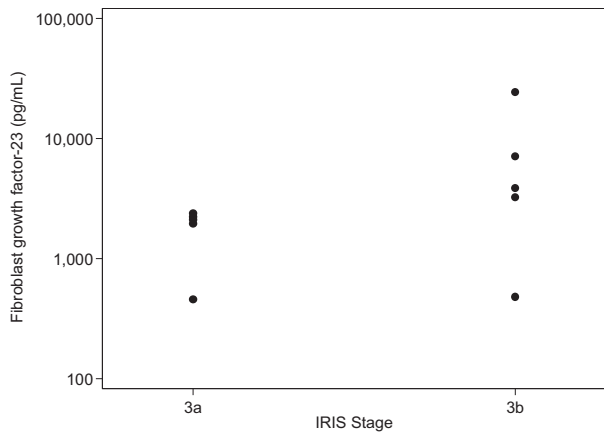
Our study showed that CKD in dogs is associated with increases in plasma FGF-23 concentration and that concentrations increase with the degree of severity as assessed by the IRIS staging system. The positive association of FGF-23 concentrations with serum creatinine, phosphorus, and PTH concentrations supports a possible role of FGF-23 in the pathogenesis of renal disease in dogs.

Under physiological conditions, FGF-23 suppresses PTH and calcitriol secretion to decrease serum phosphorus concentrations. Fibroblast growth factor-23 activity requires the cofactor klotho.<sup>20</sup> Klotho expression is decreased in CKD patients and results in an early decrease in serum klotho concentration.<sup>21</sup> This early decrease in circulating klotho downregulates the klotho-FGF receptor complexes in the parathyroid gland so that PTH synthesis is no longer adequately suppressed. Renal secondary hyperparathyroidism occurs when PTH synthesis and secretion become excessive as a result of parathyroid gland hyperplasia and hypertrophy.<sup>22</sup> Finding increased PTH concentrations despite increased FGF-23 concentrations reported in this study suggests that FGF-23 resistance is involved in RHPT in dogs. It is possible that PTH concentrations would have been even higher without the documented increased FGF-23 concentrations. We attempted to measure serum, plasma, and urine klotho concentrations in this CKD cohort and healthy dogs, but we were not able to detect measurable concentrations of klotho in any dog with a commercially available  $\alpha$ -klotho ELISA kit.<sup>h</sup>

Although no significant differences in FGF-23 concentrations were found between hyperphosphatemic dogs with stage 3b CKD versus normophosphatemic dogs with stage 3a CKD, the trend for higher FGF-23 in stage 3b CKD dogs suggests that a larger number of dogs in these stages will provide further insight on the progression of CKD and how it affects FGF-23 and the development of hyperparathyroidism. There was a tendency for FGF-23 concentrations to be higher in stage 3b dogs as compared with stage 3a dogs, with a fairly large estimated fold difference among subgroups, but there were too few cases and too much variability to detect a significant difference. A larger cohort would be needed to determine whether there is in fact a significant increase in FGF-23 concentrations in hyperphosphatemic dogs within the same IRIS stage.

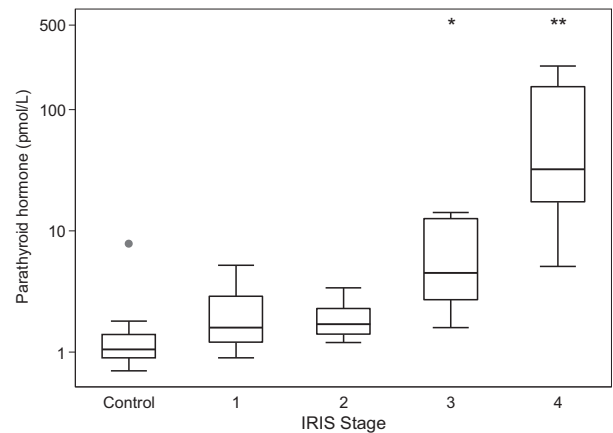


**Fig 3.** Scatterplot illustrating plasma FGF-23 concentrations by serum phosphate concentrations. The scales for both axes are logarithmic. Plasma FGF-23 concentrations were positively correlated with phosphate concentrations ( $r = 0.68$ ,  $P < .0001$ ).

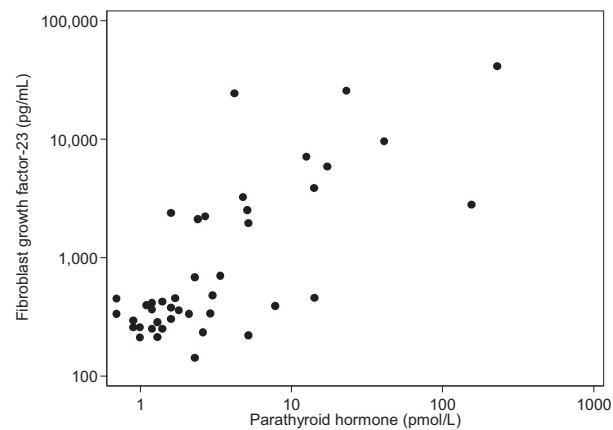


**Fig 4.** Scatterplot of plasma FGF-23 in IRIS stage 3 normophosphatemic (3a) versus hyperphosphatemic (3b). The scale for FGF-23 is logarithmic. The estimated fold difference between FGF-23 concentrations in stage 3a versus 3b was 2.53 (95% confidence interval [CI]: 0.49, 13.06), but there was no statistically significant difference between these 2 groups ( $P = .23$ ).

Depending upon which cutoff for increased PTH concentration was used, the prevalence of RHPT in our study was either 25 or 69%. There have been conflicting results about the prevalence of hyperparathyroidism in dogs and cats with CKD. In a previous study, hyperparathyroidism was documented in 76% of dogs with CKD, including animals in IRIS stage 1.<sup>4</sup> In cats, onset of RHPT, defined by increased PTH concentration, has been documented even before the onset of azotemia.<sup>23</sup> It has also been reported that PTH concentrations do not increase significantly in cats until end-stage disease is present.<sup>24,25</sup> The discrepancies in these results may be a reflection of different laboratory reference ranges among studies or a reflection of patient variability we have yet to understand. Additionally, it has been shown that there is significant diurnal PTH variability in healthy dogs, with a distinct peak at 7:00 am.<sup>26</sup>



**Fig 5.** Box and whisker plot illustrating serum parathyroid hormone (PTH) concentrations based on International Renal Interest Society (IRIS) 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 scale for PTH is logarithmic. The single asterisk represents a significantly increased PTH in dogs with IRIS stages 3 as compared to control dogs and those with IRIS stages 1 and 2 ( $P < .05$ ) CKD. The double asterisk represents significantly increased PTH in dogs with IRIS stage 4 than all other groups ( $P < .05$ ).



**Fig 6.** Scatterplot illustrating plasma FGF-23 concentrations by serum PTH concentrations. The scale for FGF-23 is logarithmic. Plasma FGF-23 concentrations were positively correlated with serum PTH concentrations ( $r = 0.74$ ,  $P < .0001$ ).

**Table 2.** Multivariable linear regression model to identify predictors of plasma fibroblast growth factor-23.  $R^2$  model = 0.79 ( $P < .0001$ ).

Variable	Parameter Estimate	Standard Error	t Value	P-value
Intercept	1.98	0.18	10.84	<.0001
Log <sub>10</sub> Creatinine (mg/dL)	1.32	0.17	7.70	<.0001
Log <sub>10</sub> phosphorus (mg/dL)	0.84	0.31	2.68	.01

Longitudinal studies would be beneficial in determining the onset of hyperparathyroidism because most studies, including ours, only evaluated at a single time point. It also should be clearly documented at what time of day the samples are collected.

The multivariable regression analysis showed that serum creatinine and phosphorus concentrations were independent predictors of FGF-23. These factors explained 79% of the variability in logFGF-23 concentration. Interestingly, PTH and calcium were not significant predictors in the presence of creatinine and phosphorus. This model suggests we have identified the main variables influencing FGF-23 concentration in dogs.

The human intact FGF-23 double antibody sandwich ELISA used in our study can measure canine FGF-23 concentrations over a wide range and could be valuable to assess disease status and progression in the clinical setting because FGF-23 increased during progressive stages of CKD and concentrations were similar to those reported in cats with CKD, suggesting that the assay is measuring canine FGF-23.<sup>24</sup> More rigorous standardization of this assay is warranted before clinical use because CVs were from samples at all extremes of the standard curve instead of high on the standard curve (600–800 pg/mL).

Our study had a few limitations. Glomerular filtration rate (GFR) was not measured, making it is possible that some dogs were incorrectly classified as having CKD. Dogs were enrolled before the symmetric dimethylarginine (SDMA) test was routinely being performed on dogs. The majority of our dogs, however, had concurrent systemic hypertension, renal proteinuria, or ultrasonographic changes consistent with CKD. Within IRIS stage 1, 2/7 dogs were enrolled solely based on repeated isosthenuria over at least 3 months. Within IRIS stage 2, 3/9 dogs did not have proteinuria nor was an abdominal ultrasound examination performed for further documentation of CKD. In contrast to reports in humans and cats, stage 1 dogs did not have a statistically higher FGF-23 concentration than healthy control dogs. This may support an inappropriate diagnosis of CKD in the current study or may be a reflection of the small study population negatively impacting the ability to derive statistically significant differences among groups.

It would have been ideal to include dogs before initiating treatment, but because of the ethical implications of withholding treatment, many CKD dogs were already receiving medications or veterinary therapeutic diets at the time of enrollment. Many of these therapies are known to affect phosphorus homeostasis, including veterinary therapeutic renal diets (n = 14) and intestinal phosphorus binders (n = 6). Cats eating phosphorus-restricted diets can have lower plasma FGF-23 concentrations.<sup>27</sup> Given that only 1 time point was collected, it was impossible to determine if these treatments lowered FGF-23 concentrations in individual dogs. Additionally, venipuncture was not consistently performed on fasted patients, which may affect serum phosphorus concentrations. Despite this variability, there was no significant difference in FGF-23 concentrations based on diet or phosphorus binders within IRIS stage.

One control dog initially had an increased PTH concentration of 7.8 pmol/L. This was a young dog without documented hypercalcemia. Thus, primary hyperparathyroidism was unlikely. The dog was eating a complete and balanced diet. Thus, nutritional secondary hyperparathyroidism was unlikely. The serum PTH concentration was repeated approximately 18 months after initial enrollment due to lack of available serum from the first time point and serum PTH concentration was normal at 1.2 pmol/L. We hypothesize that because this dog's blood initially was collected early in the morning that the PTH concentration was influenced by diurnal variation.<sup>26</sup> For reference, blood collected for re-evaluation was collected at 11:00 am. It is unlikely that diurnal variation influenced any of the CKD dogs because these dogs typically were presented to the hospital for late morning or early afternoon appointments and their blood was collected mid-day. This finding highlights the variability in canine PTH measurements.

In conclusion, plasma FGF-23 concentrations increased as IRIS stage advanced in dogs with CKD. Our results indicate that increases in FGF-23 concentrations occur early in CKD in dogs, and therefore it can be used as a biomarker of disease progression as in other species. Whether increased FGF-23 in dogs with CKD merely reflects decreased GFR, with increases in total body phosphorus burden, or contributes to progression of CKD or mortality in dogs with CKD has yet to be established. It remains to be determined if FGF-23 concentrations affect survival and progression of CKD in dogs or if there may be a benefit to use FGF-23 as a therapeutic target.

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## Footnotes

- <sup>a</sup> Advia 2120i Hematology Analyzer, Siemens Medical Solutions, Malvern, PA  
<sup>b</sup> Roche c501 Automatic Analyzer, Roche Diagnostics, Indianapolis, IN  
<sup>c</sup> Nova CCX Electrolyte Analyzer, Nova Biomedical Corporation, Waltham, MA  
<sup>d</sup> Michigan State University Diagnostic Center for Population and Animal Health; East Lansing, MI  
<sup>e</sup> Kainos FGF-23 ELISA, Japan  
<sup>f</sup> SPSS Statistics, version 23 (SAS Institute, Cary, NC)  
<sup>g</sup> Stata v13.1 (StataCorp, College Station, TX)  
<sup>h</sup> IBL-America, Minneapolis, MN
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*Conflict of Interest Declaration:* Authors declare no conflict of interest.

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

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## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Table S1.** Characteristics of dogs classified as having chronic kidney disease (CKD).