Fibroblast Growth Factor 23 in Feline Chronic Kidney Disease

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Background: Fibroblast growth factor 23 (FGF-23) is a phosphaturic hormone involved in the pathogenesis of secondary renal hyperparathyroidism (SRHP) in humans. There are no published studies examining feline FGF-23.

Objectives: Validation of a method for FGF-23 quantification in feline plasma and assessment of the associations among plasma FGF-23, PTH, creatinine, and phosphate concentrations in cats with chronic kidney disease (CKD).

Animals: One hundred nonazotemic and azotemic geriatric (>9 years) client-owned cats.

Methods: Retrospective cross-sectional study: Cats were categorized into 4 groups: control group (plasma creatinine (Cr) ≤ 2.0 mg/dL), stage 2 (Cr 2.1–2.8 mg/dL), stage 3 (Cr 2.9–5.0 mg/dL), stage 4 (Cr >5.0 mg/dL). Stages 2 and 3 were further subdivided based on International Renal Interest Society targets for plasma phosphate concentration (PO4): stage 2a (PO4 ≤ 4.5 mg/dL), stage 2b (PO4 >4.5 mg/dL), stage 3a (PO4 ≤ 5 mg/dL), stage 3b (PO4 >5 mg/dL). Plasma FGF-23 concentrations were measured by a human intact FGF-23 ELISA. Descriptive statistics and linear regression were performed.

Results: The ELISA demonstrated acceptable precision, reproducibility, and specificity. Plasma FGF-23 concentrations increased with increasing plasma creatinine concentrations and were significantly different between all groups (P < 0.006). Plasma FGF-23 concentrations were significantly higher in cats in stage 2b than stage 2a (P = 0.008) and in stage 3b than in stage 3a (P = 0.012). Phosphate, log creatinine, total calcium, log parathyroid hormone, and packed cell volume were all independent predictors of FGF-23.

Conclusions and Clinical Importance: FGF-23 concentrations increase with increasing stage of feline CKD and might be a marker or mediator of feline SRHP.

Key words: Azotemia; Cat; Hyperparathyroidism; Phosphate; PTH.

Chronic kidney disease (CKD) affects 31% of cats over 15 years of age,1 and 84% of cats with azotemic CKD have increased plasma PTH concentrations.2 The traditional understanding of the pathogenesis of secondary renal hyperparathyroidism (SRHP) focuses primarily on the retention of phosphate as glomerular filtration rate (GFR) decreases, and explains phosphate homeostasis by the actions of the principal calcitropic hormones PTH and calcitriol. This has been termed the “trade-off” hypothesis, where the trade-off for regulating plasma phosphate concentration by an increase in PTH is the development of metabolic bone disease.3 However, this hypothesis does not explain a number of aspects of the syndrome of SRHP, particularly the presence of calcitriol deficiency and the increase in PTH concentrations noted in mild CKD in humans.

Fibroblast growth factor 23 (FGF-23) is a novel phosphaturic hormone, initially identified in human patients with a genetic phosphate wasting disorder.4 FGF-23 is also present in healthy human subjects5 and is secreted primarily by osteocytes6 and osteoblasts,7,8 in response to hyperphosphatemia9 and increased plasma calcitriol concentrations.9,10 In the kidney, FGF-23 acts to inhibit calcitriol production by inhibition of the vitamin D synthesis enzyme (25-hydroxyvitamin D-1α-hydroxylase) and to increase phosphaturia by down-regulating sodium-phosphorus type II co-transporters (NaPi-IIa and NaPi-IIc) in the proximal tubules.11 In the parathyroid gland, it acts to decrease PTH production and secretion.12

FGF-23 is a low molecular weight protein, which is cleared by the kidneys. Intact FGF-23 increases with declining renal function in human patients,13–16 and it has been suggested that FGF-23 is primarily a marker of GFR.17 However, plasma FGF-23 concentrations also increase with phosphate loading18 and phosphate has been suggested to be the most significant predictor of plasma FGF-23 concentrations when GFR is markedly reduced.19 Plasma FGF-23 concentrations in humans have been demonstrated to be an independent predictor of progression of CKD16 and are independently associated with mortality rate in patients starting hemodialysis for CKD,20 with a strong concentration-
dependent relationship. Therefore, FGF-23 is now considered to be an important factor in the mechanism of SRHP in humans, but there have been no published studies examining the role of FGF-23 in the cat.

The aims of this study were firstly, to validate a method for the quantification of FGF-23 in feline plasma; secondly, to assess the association between plasma FGF-23 concentration and plasma creatinine concentration in a cross-sectional study, including nonazotemic cats and cats with varying severity of azotemic CKD; thirdly, to assess the association between plasma FGF-23 concentration and plasma phosphate concentration in cats with azotemic CKD; and lastly, to identify independent predictors of plasma FGF-23 in cats.

Materials and Methods

Animal Selection

Records from 2 London-based first opinion practices (People's Dispensary for Sick Animals, Bow and the Beaumont Sainsbury Animal Hospital, Camden) between 1 January 2000 and 1 October 2010 were reviewed and cats >9 years of age with or without renal azotemia were identified. Renal azotemia was defined as a plasma creatinine concentration >2.0 mg/dL with a concurrent urine specific gravity (USG) <1.035, or persistent azotemia on 2 consecutive occasions without evidence of a prerenal cause. Azotemic cases already being fed a protein and phosphate restricted diet were excluded. Azotemic and nonazotemic cases were excluded if they had a plasma total thyroxine (TT4) >4.5 mg/dL, stage 4a: PO₄ >5.0 mg/dL, and lastly, to identify independent predictors of plasma FGF-23 in cats.

Blood and Urine Sampling, Sample Processing, and Blood Pressure Measurement

Blood and urine samples were collected with the informed consent of the owner. The Ethics and Welfare Committee of the Royal Veterinary College had approved the study protocol. Systolic blood pressure was measured by the Doppler method as previously described. Blood was obtained via jugular venepuncture and transferred to heparinized and EDTA plasma tubes. Urine samples were collected by cystocentesis. Samples were kept at 4°C before processing, which occurred within 6 hours of collection. Samples were centrifuged at 16 × g for 10 minutes to separate plasma from cellular components. Heparinized plasma was submitted to an external laboratory for biochemical analysis on the day the sample was obtained. Residual heparinized plasma and EDTA plasma were stored at −80°C for future batch analysis of PTH and FGF-23. Urine samples were subjected to measurement of USG by refractometry on the day the samples were obtained.

EDTA plasma samples were used to measure PTH concentrations by a total intact PTH immunoradiometric assay, previously validated for use with feline EDTA plasma samples. The lower limit of detection of this assay has previously been determined to be 5.2 pg/mL; therefore, any samples with a PTH concentration <5.2 pg/mL were given an arbitrary value of 2.6 pg/mL, half the value of the limit of detection. EDTA plasma samples were also used to measure intact plasma FGF-23 concentrations by a sandwich ELISA. When necessary, samples were diluted with the zero standard from the kit as recommended by the manufacturer, to achieve a reading on the standard curve.

FGF-23 Assay Validation

EDTA plasma samples with measured low, medium, and high FGF-23 concentrations were selected. These samples were used to determine precision and reproducibility of the assay by calculation of intra- and interassay coefficients of variation (CV). Dilutional parallelism was assessed by diluting samples from nonazotemic and azotemic cats with the zero standard to achieve a minimum of 3 readings on the standard curve, which were compared with predicted concentrations. Stability of feline FGF-23 was examined by storing samples at 22°C, 4°C, and −20°C and comparing FGF-23 concentrations at baseline, after 24 hours, and after 7 days. The FGF-23 concentration was also measured after 14 days at −20°C. The effect of freeze-thawing samples (that had been previously frozen for storage at −80°C) was assessed by comparing FGF-23 concentrations after each of 4 freeze-thaw cycles. The effect of freezing versus refrigerated storage was also examined by storing EDTA plasma samples within 15 minutes of venepuncture at either 4°C or −20°C for 24 hours before analysis. The lower limit of detection of the assay was assessed by taking 3 standard deviations above the mean from 8 repeated measures of the lowest standard in the kit (0 pg/mL). The upper limit of quantification of the assay was assessed by confirmation of a mean CV <10% for a high sample, as close to the highest standard as possible, measured repeatedly.

Determination of a 95% Reference Interval for FGF-23 in Geriatric Cats

EDTA samples from healthy, nonazotemic geriatric cats, age matched to the cats in the cross-sectional study, which remained nonazotemic for at least 12 months postsampling, were used to establish a reference interval for plasma FGF-23. The results were assessed for outliers by the Dixon method and the reference interval was calculated by taking nonparametric 2.5th–97.5th percentiles.

Statistical Analysis

Statistical analyses were performed by a computerized statistical software package. Results are reported as median [25th, 75th percentiles] and statistical significance was determined as P < .05.
Variables were assessed for normality by visual inspection of histograms. Comparisons between groups were made by the Kruskal–Wallis test and Mann–Whitney U-test. Bonferroni corrections were applied where appropriate and significance then determined as \( P < .008 \). Related samples were compared by the Friedman test and the Wilcoxon Signed Rank test.

The correlations between plasma FGF-23 and PTH concentrations and plasma creatinine concentration were assessed using Spearman’s correlation coefficient. The assumptions for multivariable linear regression analyses were performed and variables significant at the 5% level were included in a forward multivariable linear regression analysis to identify independent predictors of FGF-23.

**Results**

**Validation of the ELISA**

The intra-assay CVs for samples measuring 52, 221, 324, 499, 552 (\( n = 5 \)), and 780 (\( n = 4 \)) pg/mL FGF-23 were 6.3%, 8.4%, 1.4%, 2.6%, 1.9%, and 1.7%, respectively. The interassay CVs for samples measuring 110, 179, 253 (\( n = 5 \)), 459, 522, 782 (\( n = 4 \)) pg/mL FGF-23 were 8.1%, 10.1%, 4.7%, 5.3%, 4.4%, and 1.5%, respectively. The FGF-23 dilutional parallelism (\( n = 13 \)) indicated a mean recovery (\( \pm \)standard deviation) of 103.6% (\( \pm \)13.6%). There was no significant change in the percentage recovery of FGF-23 for samples (\( n = 4 \)) stored for up to 14 days at \(-20^\circ C\) (\( P = .33 \)) or up to 7 days at \(22^\circ C\) (\( P = .37 \)). Overall, there was a significant increase in the mean \( \pm \)standard deviation percentage recovery of FGF-23 after 24 hours (109 \( \pm \)6%) and after 7 days (113 \( \pm \)5%) for samples (\( n = 4 \)) stored at \(4^\circ C\) (\( P = .039 \)), but the changes were not significant when each time point was compared during posthoc analysis (\( P \geq .068 \)).

There was no significant change in FGF-23 concentrations when samples (\( n = 3 \)) were subjected to 4 freeze-thaw cycles (\( P = .24 \)). There was no significant difference between samples (\( n = 4 \)) that were kept at \(4^\circ C\) for 24 hours when compared with paired samples kept at \(-20^\circ C\) for 24 hours (\( P = 1.0 \)).

The lowest and highest standards supplied with the ELISA were 0 and 800 pg/mL, respectively. The lower limit of detection was 10 pg/mL. A sample with a mean concentration of 780 pg/mL was measured 4 times with a CV of 1.7%.

**FGF-23 Cross-Sectional Study**

EDTA plasma samples were available for FGF-23 measurement in 100 cats. Of these, 55 were neutered males and 45 were neutered females. In the control group (\( n = 44 \)), 12-month follow-up information was available for 34 cats. The remaining 10 cats had no follow-up information, but had plasma creatinine concentrations <1.6 mg/dL. TT4 measurements were available for 69 cases and the remaining cases had no clinical evidence of hyperthyroidism. PTH measurements were available for 79 cats.

The distribution of FGF-23 was determined to be non-Gaussian from visual inspection; therefore, nonparametric tests were used. Plasma FGF-23 concentrations increased with increasing creatinine concentrations and measurements were significantly different between groups (control group: 146 [111, 264], \( n = 44 \), stage 2: 354 [239, 473], \( n = 20 \), stage 3: 1282 [428, 7658], \( n = 22 \), stage 4: 33478 [4241, 86204] pg/mL, \( n = 14 \); \( P < .001 \)) (Fig 1). Posthoc testing with Bonferroni correction found that there was a significant difference between all groups (all \( P \leq .002 \)). Plasma PTH concentrations were significantly different between groups (control group: 7.5 [2.6, 11.9], \( n = 41 \), stage 2: 6.9 [2.6, 19.3], \( n = 14 \), stage 3: 15.3 [2.6, 36.4], \( n = 14 \), stage 4: 20.2 [8.8, 115.0] pg/mL, \( n = 10 \); \( P = .013 \)) (Fig 2). Posthoc testing with Bonferroni correction only found a significant difference between the control group and stage 4 (\( P = .003 \)).

FGF-23 concentrations were significantly higher in cats with higher plasma phosphate concentrations in both stage 2 (stage 2a: 329 [237, 370], \( n = 15 \) versus stage 2b: 770 [376, 1912] pg/mL, \( n = 5 \); \( P = .008 \)).
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Fig 2. Box and whisker plot illustrating the plasma parathyroid hormone (PTH) concentrations in nonazotemic and azotemic geriatric cats. Cats were divided into 4 groups: control group; plasma creatinine concentration $ \leq 2.0$ mg/dL (n = 41), stage 2; plasma creatinine concentration $2.1$–$2.8$ mg/dL (n = 14), stage 3; plasma creatinine concentration $2.9$–$5.0$ mg/dL (n = 14), and stage 4: plasma creatinine concentration $>5.0$ mg/dL (n = 10). 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 dotted line represents the maximum value in the control group, which compromised healthy, nonazotemic cats. The Kruskal–Wallis test found that PTH concentrations were different between the groups (P = .013). Mann–Whitney U-tests with Bonferroni correction (significance therefore P < .008) found that PTH concentrations were only significantly different between the control group and stage 4 (P = .003).

Fig 3. Box and whisker plot illustrating the plasma fibroblast growth factor (FGF)-23 concentrations for cats in stage 2 (plasma creatinine concentration $2.0$–$2.8$ mg/dL [n = 20]) according to plasma phosphate concentration. Stage 2a: plasma phosphate concentration $\leq 4.5$ mg/dL (n = 15) and stage 2b: plasma phosphate concentration $>4.5$ mg/dL (n = 5). 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 scale for FGF-23 is logarithmic. The Mann–Whitney U-test found that FGF-23 concentrations were significantly different between the groups (P = .008).

Fig 4. Box and whisker plot illustrating the plasma fibroblast growth factor (FGF)-23 concentrations for cats in stage 3 (plasma creatinine concentration $2.9$–$5.0$ mg/dL [n = 22]) according to plasma phosphate concentration. Stage 3a: plasma phosphate concentration $\leq 5.0$ mg/dL (n = 9) and stage 3b: plasma phosphate concentration $>5.0$ mg/dL (n = 13). 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 scale for FGF-23 is logarithmic. The Mann–Whitney U-test found that FGF-23 concentrations were significantly different between the groups (P = .012).

Plasma FGF-23 concentration was strongly correlated ($r_s = 0.81$, P < .001) and plasma PTH concentration was moderately correlated ($r_s = 0.4$, P < .001) with plasma creatinine concentration. Before linear regression being performed, FGF-23 was log-transformed because of its non-Gaussian distribution. Variables with...
nonnormally distributed residuals were also log-transformed, including creatinine and PTH, to yield the variables log creatinine and log PTH. Log creatinine (P < .001), phosphate (P < .001), age (P = .001), total calcium (P < .001), PCV (P < .001), and log PTH (P < .001) were found to be predictors of logFGF-23 in the univariable analyses. SBP (P = .20), ALP (P = .62), ALT (P = .84), and sex (P = .60) were not significant predictors of log FGF-23 in the univariable analyses. Log creatinine, phosphate, total calcium, PCV, and log PTH were found to be independent predictors of FGF-23 in the multivariable analysis (Table 1).

The reference interval (n = 79) was derived from the FGF-23 measurements of the 34 cats from the control group that had 12-month follow-up data and an additional 45 nonazotemic cats included in a separate study, which remained nonazotemic and did not develop concurrent disease for at least a further 12 months. There was no significant difference in age between the cats from which samples were taken to generate the reference interval (13 [10, 15] years; n = 79) and the azotemic cats in the cross-sectional study (13 [11, 15] years; n = 56) (P = .31). In univariable analyses, age was not a significant predictor of logFGF-23 (P = .26) in the nonazotemic reference interval cats. The 95% reference interval for geriatric feline plasma FGF-23 was determined to be 56–700 pg/mL.

**Discussion**

To our knowledge, this is the 1st study to examine FGF-23 in feline CKD. The results of this study demonstrate that FGF-23 increases as stage of CKD increases. FGF-23 is higher in azotemic cats with hyperphosphatemia, when compared with cats with normophosphatemia, even within the same IRIS stage. In addition, phosphate is an independent predictor of plasma FGF-23 concentration.

**Table 1.** Multivariable forward linear regression model to identify predictors of plasma logFGF-23, adjusted R² model = 0.781, P value <.001.

<table>
<thead>
<tr>
<th>Unstandardized Coefficients</th>
<th>B</th>
<th>SE</th>
<th>Sig.</th>
<th>95% CI for B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log (Creatinine [mg/dL])</td>
<td>1.089</td>
<td>0.313</td>
<td>0.001</td>
<td>0.466–1.712</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>-0.024</td>
<td>0.008</td>
<td>0.004</td>
<td>-0.040 to -0.008</td>
</tr>
<tr>
<td>Log (PTH [pg/ml])</td>
<td>0.234</td>
<td>0.108</td>
<td>0.033</td>
<td>0.019–0.448</td>
</tr>
<tr>
<td>Total Calcium (mg/dL)</td>
<td>0.193</td>
<td>0.071</td>
<td>0.008</td>
<td>0.052–0.333</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>0.065</td>
<td>0.021</td>
<td>0.003</td>
<td>0.023–0.108</td>
</tr>
<tr>
<td>Constant</td>
<td>0.672</td>
<td>0.705</td>
<td>0.34</td>
<td>-0.733 to 2.077</td>
</tr>
</tbody>
</table>

B, coefficient; CI, confidence interval; PCV, packed cell volume; PTH, parathyroid hormone; SE, standard error; Sig., significance.

The human intact FGF-23 ELISA used in this study demonstrates acceptable precision, reproducibility, and specificity for feline FGF-23. A recent publication has suggested that there is rapid loss of human intact FGF-23 within 2 hours of sampling unless a protease inhibitor is added to the sample immediately after venepuncture. It is impossible in the clinical setting to analyze plasma samples for FGF-23 immediately; the samples must initially be centrifuged to separate the packed cells from the plasma and subsequently the samples require batch analysis. Therefore, it was important to establish the stability of FGF-23 in feline plasma before cold storage and after defrosting. The results of this study demonstrate that in previously frozen EDTA plasma samples, feline FGF-23 is a stable molecule for up to 7 days even when stored at room temperature. In addition, there is no significant difference in feline FGF-23 concentrations between samples, which have never been frozen and stored at room temperature for 24 hours, with samples placed into -20°C storage within 15 minutes of venepuncture for 24 hours. This suggests that feline FGF-23 is not subject to protease degradation and the use of a protease inhibitor with feline samples is unnecessary.

The 1st published reference interval for FGF-23 in healthy adult humans was 8.2–54.3 pg/mL, and similar values for healthy adult humans have been demonstrated again since. In contrast, the reference interval for FGF-23 in geriatric cats is 56–700 pg/mL. The reason for this markedly elevated and much broader reference interval in cats is currently unknown; at present, a purified source of feline FGF-23 to use as a standard in the human intact FGF-23 ELISA is not available and therefore it is not possible to confirm that the results for feline samples are comparable to results from human samples. However, the demonstration of dilutional parallelism and biological validity of feline FGF-23 with this assay suggests that it is measuring the feline equivalent of human FGF-23. It is always difficult to establish a true control population from which to derive a reference interval and it is possible that some of the cats used in this study may have had subclinical CKD. Urine specific gravity measurements that were available in this population ranged from 1.014 to 1.085, but all of these cases remained nonazotemic for at least 12 months, which is a robust method for trying to establish a group of geriatric cats without CKD in the absence of available GFR measurements. It is possible that in a younger population of cats, the reference range would be lower and narrower, but this would not be as good a control population for the geriatric cats in this study. Another factor that may have contributed to the breadth of the reference range may have been the variety of diets that the cats were being fed on at the time of sampling, because no attempt was made to control for diet in this study. It is possible that FGF-23 may be consistently higher in cats, as a feline evolutionary response to eating a diet high in phosphate, or perhaps may be a result of the apparent lack of an FGF-23 protease in the cat resulting in a longer half life for feline FGF-23.
Feline FGF-23 increases as the stage of CKD increases. This is consistent with the well-documented finding that FGF-23 in human CKD patients increases as GFR decreases. The relationship between feline FGF-23 and GFR has been explored in a separate study. The cats in this study were grouped according to a modified IRIS staging system, to allow for comparison between healthy, nonazotemic cats with no evidence of CKD and cats diagnosed with CKD in 3 different stages of the disease. FGF-23 was significantly higher in azotemic cats in comparison to healthy, nonazotemic cats where CKD was not suspected clinically. In addition, FGF-23 was significantly higher in stage 3 compared with stage 2, and in stage 4 compared with stage 3. FGF-23 reached extremely increased concentrations of up to 111,600 pg/mL in 2 of the stage 4 (end-stage CKD) cats. These high concentrations are similar to absolute values published for human end-stage dialysis CKD patients. In contrast, plasma PTH concentrations were only statistically significantly increased in the cats in stage 4, with end-stage CKD, when compared with the nonazotemic cats. There was a tendency for PTH to increase with increasing stage of CKD; therefore, it is possible a significant increase may be apparent with a larger study population. However, comparisons of Figures 1 and 2 illustrate that there is a much greater overlap between the groups for plasma PTH concentrations than for plasma FGF-23 concentrations.

Feline FGF-23 is significantly higher in azotemic cats with hyperphosphatemia, when compared with normophosphatemic cats in the same IRIS stage. In addition, phosphate is associated with logFGF-23, after adjustment for other variables. It has been demonstrated in rodents that FGF-23 concentrations increase in response to an increase in plasma phosphate concentration. FGF-23 then acts to inhibit phosphate reabsorption in the kidney, an action previously thought to be caused primarily by PTH. In this study, PTH concentrations were higher in the hyperphosphatemic cats in stage 3, but were not significantly higher in the hyperphosphatemic cats in stage 2. However, in stage 2, there was a tendency for PTH to be higher in the hyperphosphatemic cats and it is likely that this did not reach significance because of the small number of cats with a PTH measurement in stage 2b. This suggests that both plasma FGF-23 and PTH rise in azotemic CKD cats in response to hyperphosphatemia; longitudinal studies are now required to identify whether the increases in FGF-23 and PTH occur concurrently or if one hormone is seen to increase first. GFR has been shown to be the most significant determinant of FGF-23 increases in human CKD, which makes biological sense as FGF-23 is a low molecular weight protein, which will be freely filtered by the glomerulus. However, in this study, it was confirmed that plasma creatinine concentrations were not significantly different between the normo- and hyperphosphatemic cats in stages 2 and 3; therefore, the significant increase in FGF-23 in the hyperphosphatemic cats is unlikely to be simply attributable to a difference in GFR. GFR is not the only determinant of FGF-23 concentration because once the failing kidneys lose their ability to regulate phosphate homeostasis, the subsequent development of hyperphosphatemia stimulates further FGF-23 secretion, a hypothesis supported by the results of this study.

The multivariable model showed that logCreatinine, phosphate, logPTH, total calcium, and PCV were all independent predictors of logFGF-23, together explaining 79% of the variability in logFGF-23 concentration. The influences of PTH on FGF-23 have been examined in previous studies in humans and rodents. The PTH receptor is present in osteocytes and osteoblasts, the cells responsible for FGF-23 production. An activating mutation of the PTH receptor can result in increased plasma concentrations of FGF-23 in human patients with Jansen’s metaphyseal chondroplasia, a condition characterized by hypophosphatemia, hypercalcemia, and low or undetectable PTH concentrations. This suggests that PTH is able to directly stimulate FGF-23 secretion and FGF-23 directly inhibits PTH production and secretion, thereby forming a feedback loop.

The relationship between calcium and FGF-23 has not been well characterized. Calcitriol is a known stimulant of FGF-23 secretion, and in another feedback loop, FGF-23 also inhibits calcitriol production in the kidney via inhibition of the 1α-hydroxylase enzyme, which converts calcidiol into biologically active calcitriol. Because calcitriol acts to increase plasma calcium concentrations, the relationship between calcium and FGF-23 may be dependent on calcitriol, which unfortunately was not measured in this study because of insufficient available samples. This is the main limitation in this study as it is difficult to fully interpret relationships of the other factors involved in SRHP without measurements for calcitriol. In this study, PCV was found to be independently negatively associated with logFGF-23. This is a novel finding and the reason for this remains currently unclear.

The interactions among FGF-23, PTH, phosphate, calcium, calcitriol, and renal function are complex. Because of the retrospective nature of this study, there were additional limitations. Firstly, creatinine was used as a marker of GFR. Creatinine is not a sensitive marker of GFR until azotemia develops; therefore, ideally GFR would have been measured directly. Secondly, we were unable to obtain measurements for ionized calcium, the biologically active form of calcium, and have therefore used measurements of total calcium in the multivariable linear regression model. It has been shown that measurement of plasma total calcium is a poor predictor of plasma ionized calcium in cats, especially in cases of CKD. Despite these limitations, the linear regression model in this study was able to explain 78% of the variability in logFGF23, suggesting that we have identified many of the factors influencing plasma FGF-23 concentration in this multivariable model.

In conclusion, feline FGF-23 increases as the stage of CKD increases, and is further elevated in azotemic patients with hyperphosphatemia. Independent predictors
of feline FGF-23 include logCreatinine, phosphate, logPTH, total calcium, and PCV. This is the 1st study investigating FGF-23 in feline azotemic CKD. Further work is now required to elucidate the complex relationships between FGF-23 and the other factors involved in feline SRHP and to establish if FGF-23 is a mediator of feline SRHP or simply a marker of CKD in cats.

Footnotes

\( ^{a} \) Idexx Laboratories, Wetherby, UK
\( ^{b} \) Refractometer, Model RHC-200ATC, Burton's Medical Equipment Limited, Kent, UK
\( ^{c} \) Total intact PTH immunoradiometric assay – coated bead version. Part number 3KG600, S Santibodies, Santee, CA
\( ^{d} \) Kainos Laboratories, Tokyo, Japan
\( ^{e} \) PASW Statistics 18, SPSS Inc, Chicago, IL

Acknowledgments

Conflict of Interest: The Renal Research Clinic at the Royal Veterinary College acknowledges support from Royal Canin for its research on feline hyperphosphatemia and chronic kidney disease. R. Geddes is in receipt of an Everts Luff Trust Research Training Fellowship.

References


