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Risk factors associated with disturbances of calcium homeostasis after initiation of a phosphate-restricted diet in cats with chronic kidney disease

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Abstract

Background: Dietary phosphate restriction improves survival in cats with chronic kidney disease (CKD). However, feeding a phosphate-restricted diet may disrupt calcium homeostasis leading to hypercalcemia in some cats.

Objectives: To identify risk factors associated with increasing plasma total calcium (tCa) concentration after transition to a phosphate-restricted diet and to explore its role in CKD-mineral and bone disorder (CKD-MBD) in cats.

Animals: Seventy-one geriatric (≥9 years) euthyroid client-owned cats with International Renal Interest Society (IRIS) stage 2 to 3 azotemic CKD.

Methods: Retrospective cross-sectional cohort study. Changes in plasma tCa concentration in the first 200 days of diet transition were assessed using linear regression. Binary logistic regressions were performed to identify risk factors for increasing calcium concentration. Changes in clinicopathological variables associated with CKD-MBD over time were explored using linear mixed model and generalized linear mixed model analyses.

Results: Lower baseline plasma potassium (odds ratio [OR] = 1.19 per 0.1 mmol/L decrease; P = .003) and phosphate (OR = 1.15 per 0.1 mmol/L decrease; P = .01) concentrations remained independent risk factors for increasing plasma tCa concentration. Plasma creatinine (β = .069 ± .029 mg/dL; P = .02), symmetric dimethylarginine (β = .64 ± .29 μg/dL; P = .03), phosphate (β = .129 ± .062 mg/dL; P = .04), and ln[FGF23] (β = .103 ± .035 pg/mL; P = .004) concentrations had significantly increased rates of change in cats with increasing plasma tCa concentration over time.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; BCS, body condition score; CI, confidence interval; CKD, chronic kidney disease; CKD-MBD, chronic kidney disease-mineral and bone disorder; EDTA, ethylene-diaminetetraacetic acid; FE, fractional excretion; FGF23, fibroblast growth factor 23; GFR, glomerular filtration rate; GLMM, generalized linear mixed model; HCO_3^-, bicarbonate; HTM, higher-than-median; iCa, ionized calcium; IRIS, International Renal Interest Society; LMM, linear mixed model; ln, natural logarithm; ln[FGF23], log-transformed FGF23; ln[PTH], log-transformed PTH; LTM, lower-than-median; OR, odds ratio; PTH, parathyroid hormone; ROC, receiver operating characteristic; SBP, systolic blood pressure; SDMA, symmetric dimethylarginine; tCa, total calcium; tMg, total magnesium; TT4, total thyroxine; USG, urine specific gravity.

Conclusion and Clinical Importance: Lower plasma potassium or phosphate concentrations or both at the time of transition of cats with CKD to a phosphate-restricted diet are independently associated with increased risk of an increase in plasma tCa concentration. Increasing plasma tCa concentration is associated with progression of CKD.

KEYWORDS

CKD-MBD, feline, FGF23, hypercalcemia, progression, renal diet

1 | INTRODUCTION

Chronic kidney disease (CKD) is a common medical condition, with increased prevalence among geriatric cats.[1] Thirty-one percent of cats >15 years old reportedly have azotemic CKD,[2] yet a more recent study has identified evidence of renal impairment in >80% of cats ≥15 years of age.[3] Hypercalcemia is another condition that also affects older cats, with a median affected age of 9 years.[4] It usually is associated with CKD, neoplasia, and less commonly primary hyperparathyroidism, although in the majority of cats with hypercalcemia it is idiopathic in origin.[4-6] A recent study found that CKD cats had a higher risk of developing increased plasma total calcium (tCa) concentration,[7] with increasing prevalence observed in cats with advancing azotemia.[8] Despite the common comorbidity of CKD and hypercalcemia in geriatric cats, it is unclear whether development of hypercalcemia is attributable to the occurrence of CKD or vice versa.

Chronic kidney disease-mineral and bone disorder (CKD-MBD) is a systemic disorder that involves a complex interplay among mineral and hormonal metabolism, bone remodeling and extraskeletal calcification, that occurs as a result of CKD. Gradual loss of functioning nephrons results in the impairment of excretory capability of phosphate in the kidneys, leading to hyperphosphatemia and development of CKD-MBD.[9] Renal regulation of phosphate homeostasis is a complicated process that involves 2 main phosphaturic hormones: fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH).[10,11] These hormones also play a fundamental role in calcium handling, exhibiting a coordinated interaction between both phosphate and calcium homeostasis.[12]

Fibroblast growth factor 23 is a peptide, consisting of 251 amino acids, primarily produced by osteocytes and osteoblasts in response to phosphate overload. It exerts its phosphaturic activities by interacting with the Klotho-FGF receptor complex and downregulates expression of the principal renal sodium-phosphate cotransporter, NaPi-2a.[13] The FGF23-Klotho signaling also increases the abundance of transient receptor potential vanilloid 5 (TRPV5) in renal distal tubules, resulting in enhanced calcium reabsorption.[14] Parathyroid hormone plays an important role in the maintenance of normal physiological serum calcium concentrations; it is secreted by the parathyroid glands in response to ionized hypocalcemia. Furthermore, PTH is a potent phosphaturic hormone, with a signaling cascade that partially overlaps that of FGF23, and inhibits phosphate reabsorption in the proximal tubules.[11]

Dietary phosphate restriction is the mainstay therapeutic strategy in cats with CKD.[15-17] A previous study showed that ionized hypercalcemia developed in 14.3% of CKD cats within 6 months after transition to a phosphate-restricted diet.[18] With complex regulatory processes and interaction between calcium and phosphate, there is increasing concern about whether dietary phosphate restriction contributes to disturbances of calcium homeostasis in certain azotemic cats. Hence, the objectives of our retrospective study were: (a) to determine the independent risk factors associated with increasing plasma calcium concentration after transition to a phosphate-restricted diet and (b) to assess the change in clinicopathological variables associated with CKD-MBD in relation to dietary phosphate restriction in geriatric CKD cats with different rates of change in plasma calcium concentrations over time.

2 | METHODS

2.1 | Case selection

Clinical records of 2 first-opinion practices in London (People's Dispensary for Sick Animals in Bow and Beaumont Sainsbury Animal Hospital in Camden) were reviewed between January 1, 2014 and November 1, 2019 and cats ≥9 years old with azotemic CKD were retrospectively identified. Criteria for a diagnosis of azotemic CKD were plasma creatinine concentration ≥2 mg/dL in conjunction with urine specific gravity (USG) <1.035, or plasma creatinine concentration ≥2 mg/dL on 2 consecutive occasions 2 to 4 weeks apart without evidence of prerenal cause. To be enrolled, data on plasma tCa concentration at the time of diagnosis of CKD (baseline visit) and at least 2 follow-up visits within the first 200 days after transition to a phosphate-restricted diet (Feline Veterinary Diet Renal, Royal Canin SAS, Aimargues, France; phosphorus, 0.7-1.1 g/Mcal; calcium : phosphorus ratio, 1.3-1.9) had to be available. Cats were excluded from all analyses if they had been suspected clinically to have hyperthyroidism and their plasma total thyroxine (TT4) concentration was >40 nmol/L, they were undergoing medical treatment for hyperthyroidism, had been diagnosed with diabetes mellitus, were already receiving a phosphate-restricted diet or were being treated with corticosteroids. Cats with a diagnosis of hypercalcemia before

commencement of a phosphate-restricted diet also were excluded, defined as a plasma tCa concentration >11.8 mg/dL or whole blood ionized calcium (iCa) concentration >5.6 mg/dL (1.4 mmol/L). Cats receiving amlodipine besylate for treatment of systemic hypertension were included.

2.2 | Data collection

Collection and storage of blood and urine samples were performed with the informed consent of the owners and approval of the Ethics and Welfare Committee of the Royal Veterinary College (URN20131258 and URN20131258E). Blood samples were obtained by jugular venipuncture and transferred to heparinized and ethylene-diaminetetraacetic acid (EDTA) tubes. Urine samples were collected by cystocentesis. All samples were stored at 4°C after collection, for a maximum of 6 hours before centrifugation and separation. Heparinized plasma was submitted to an external laboratory (IDEXX laboratories, Wetherby, UK) for biochemical analyses on the day of sample collection, including tCa. Ionized calcium concentration measurement was made immediately on untreated whole blood after venipuncture, using a point-of-care blood analyzer (*i-STAT 1*, Abbott Point of Care, Inc, Princeton, New Jersey) that also measured venous pH and bicarbonate (HCO_3^-) concentration, in accordance with the manufacturer's instructions. In-house urinalysis, including measurement of USG by refractometry, dipstick chemical analysis and urine sediment microscopy, were performed on the day urine samples were obtained. A diagnosis of urinary tract infection was confirmed by bacterial culture (Royal Veterinary College Diagnostic Laboratory Services, Hatfield, UK). Residual heparinized and EDTA plasma samples, and urine samples were stored at −80°C for future batch analysis. The EDTA plasma samples were used to measure intact FGF23 and PTH using an ELISA (FGF23 ELISA Kit, Kainos Laboratories, Tokyo, Japan) and a total intact PTH immunoradiometric assay (Total intact PTH immunoradiometric assay-coated bead version, 3KG600, Scantibodies, Santee, California), respectively, both previously validated for use in cats.[19,20] The lower limit of detection for intact PTH with this assay has been determined to be 5.2 pg/mL.[20] Therefore, any samples with PTH concentration <5.2 pg/mL were arbitrarily assigned a concentration of 2.6 pg/mL, half the value of the lower limit of detection.[19]

Systolic blood pressure (SBP) measurements were obtained following a previously described Doppler method.[21] Fundic examination using a retinal camera (ClearView, Optibrand, Fort Collins, Colorado) or by indirect ophthalmoscopy was performed in cats with an average SBP >160 mm Hg. Systemic hypertension was diagnosed in cats with an average SBP >160 mm Hg in conjunction with evidence of ocular pathology consistent with hypertensive damage, or SBP >170 mm Hg on 2 consecutive occasions.

The clinical records were reviewed, with the following historical findings, physical examination findings, and biochemical data extracted: tCa, iCa, plasma creatinine, symmetric dimethylarginine (SDMA), urea, phosphate, potassium, sodium, chloride, total magnesium (tMg), total protein, albumin, TT4, glucose, FGF23 and PTH concentrations, plasma activity of alanine aminotransferase (ALT) and alkaline phosphatase (ALP), PCV, venous blood gases including pH and HCO_3^-, SBP, USG, urine culture result, age, sex, breed, body weight, body condition score (BCS; 9-point scale), muscle condition score (MCS; 4-point scale),[22] lifestyle (indoor vs outdoor), and consumption of dairy products.

2.3 | Statistical analysis

Statistical analyses were performed using commercial software (R 3.6.2 GUI 1.70 El Capitan build, R Foundation for Statistical Computing, Vienna, Austria; IBM SPSS Statistics for MacOS, Version 26, IBM Corp., Armonk, New York). Statistical significance was determined as $P < .05$. Numerical continuous variables were assessed for normality by visual inspection of histogram and using the Shapiro-Wilk test. The assumption of equal variances was tested using Levene's test. Most data were not normally distributed; therefore, for consistency, all numerical data are presented as median [25th, 75th percentile].

2.3.1 | Evaluation of risk factors associated with increasing plasma tCa concentration

Azotemic CKD cats were grouped based on the change in plasma tCa within the first 200 days after transition to a phosphate-restricted diet using linear regression: cats with a tCa regression gradient >0 (uptrend), and those with a tCa regression gradient ≤0 (nonuptrend).

Plasma FGF23 and PTH concentrations were log-transformed (natural logarithm [ln]) for normalization and BCS was categorized into 3 levels ("1-3," "4-6," or "7-9") before analysis. Where clinical biochemical data were not available at the time of transition to a phosphate-restricted diet (baseline visit), measurements from previous visits, within a 2- to 4-week interval, were used for statistical analysis. Comparisons of baseline variables between groups were made by either the independent samples *t* test or Mann-Whitney *U* test for continuous variables with a normal distribution or a skewed distribution, respectively. Proportions with categorical outcomes were compared using either the Chi-squared test or Fisher's exact test.

Binary logistic regression was performed to explore baseline risk factors associated with "uptrend" calcium status in CKD cats after the initiation of a phosphate-restricted diet. Age, body weight, tCa, iCa, creatinine, SDMA, urea, phosphate, potassium, sodium, chloride, tMg, total protein, albumin, glucose, ln[FGF23], ln[PTH], ALT, ALP, PCV, venous pH, HCO_3^-, and USG were entered as continuous variables, whereas sex, breed, BCS, MCS, lifestyle, consumption of dairy products, and hypertension status were entered as categorical variables, for univariable analyses. Variables associated with an "uptrend" calcium status with $P < .1$ were entered into a multivariable model. The final model was derived by manual backward elimination. The Hosmer-Lemeshow test was used to assess the goodness-of-fit of the model, and presence of colinearity among the significant risk factors ($P < .05$) was evaluated by variance inflation factor. Receiver

operating characteristic (ROC) curve analysis was performed to assess the performance of the final multivariable model for prediction. Linear relationship between continuous predictors and the logit was assessed by categorizing the variables into quartiles in the logistic regression analysis and evaluating the increasing or decreasing trend of the coefficients. Interaction among significant risk factors also was examined; continuous variables were treated as categorical data by dichotomization based on their respective median values (termed high or low), followed by multivariable binary logistic regression. Results are reported as odds ratio (OR; 95% confidence interval [CI]).

2.3.2 | Evaluation of changes in CKD-MBD parameters in relation to dietary phosphate restriction

Changes in continuous and ordinal clinicopathological variables over time were assessed using linear mixed model (LMM) and generalized linear mixed model (GLMM) with logistic link function, respectively. Longitudinal data from all available visits during the first 200 days while cats were fed a phosphate-restricted diet were included for the following variables: body weight, BCS, MCS, tCa, iCa, creatinine, SDMA, urea, phosphate, potassium, sodium, chloride, tMg, total protein, albumin, glucose, ln[FGF23], ln[PTH], ALT, ALP, PCV, venous pH, and HCO_3^-. Group ("uptrend" vs "nonuptrend"), time (in months [30.4 days]), and the interaction between group and time were treated as fixed factors in the model. The case number of each individual cat and time nested within cats were included as 2 uncorrelated random effects. Residuals were assumed to be independent in the model. For BCS, MCS, and body weight, only the case number of each individual cat was included as random effect because of a model convergence issue. No missing data imputation was performed. Results are reported as coefficient (β) ± SE, with statistical significance set at $P < .05$.

2.3.3 | Evaluation of fractional excretion of analytes

Urinary fractional excretion (FE) of calcium, phosphate, potassium, sodium, and chloride were calculated where urine samples were available at the baseline visit or 2 to 4 weeks before baseline (termed prediet visit), as well as the first follow-up visit >30 days after transition to a phosphate-restricted diet (termed postdiet visit). Where measurements had not already been performed, stored heparinized plasma and urine samples were sent to an external laboratory (IDEXX GmbH, Ludwigsburg, Germany) for biochemical analyses to calculate the FE of analytes. The FE of an individual analyte was calculated relative to urinary clearance of creatinine, according to the formula[23]:

$$FE_{analyte}(\%) = \frac{Urinary_{analyte} \times Plasma_{creatinine}}{Plasma_{analyte} \times Urinary_{creatinine}} \times 100$$

Variables at pre- and postdiet visits were compared between groups using either the independent samples *t* test or Mann-Whitney *U* tests. Change in FE of analytes was determined by either the paired *t* test or Wilcoxon signed ranked test, as appropriate.

3 | RESULTS

3.1 | Evaluation of risk factors associated with "uptrend" plasma calcium concentrations

A total of 208 geriatric cats diagnosed with CKD were identified between January 1, 2014 and November 1, 2019, of which 64 were excluded because of suspected or documented hyperthyroidism (n = 61), diabetes mellitus (n = 1), or administration of corticosteroids (n = 2). Of the remaining 144 cats, an additional 73 were excluded from all analyses because of the absence of phosphate-diet transition (n = 6), lack of baseline tCa measurement (n = 5), or insufficient follow-up visits with measurements of tCa available (n = 62). In total, 71 CKD cats (International Renal Interest Society [IRIS] stage 2, n = 54; IRIS stage 3, n = 17) were enrolled in the study, with 40 cats having an "uptrend" calcium status and 31 having a "nonuptrend" status. The most common breed was domestic shorthair (n = 59), followed by domestic longhair (n = 5), Birman (n = 2), Burmese (n = 2), Siamese (n = 2), and British shorthair (n = 1). Seventy-six percent (n = 54) of cats had 3 visits with tCa measurements available within the first 200 days, whereas 23% (n = 16) and 1% (n = 1) of cats had 4 and 5 visits, respectively (Figure S1A). Baseline clinicopathological variables are presented in Table 1. Baseline plasma potassium, phosphate, and sodium concentrations were significantly lower in "uptrend" cats. No significant difference in other clinicopathological variables between groups was found.

Univariable binary logistic regression analyses identified that lower plasma phosphate, potassium and sodium concentrations and hypertension status were associated with increasing plasma tCa concentration after the initiation of a phosphate-restricted diet at the 10% level (Table 2). In the final multivariable model, baseline plasma potassium (OR = 1.19 per 0.1 mmol/L decrease; $P = .003$) and phosphate concentration (OR = 1.15 per 0.1 mmol/L decrease; $P = .01$) remained independent risk factors for "uptrend" calcium status (Table 2). The area under the ROC curve was 0.76 (95% CI = 0.64-0.87; Figure 1). The median plasma potassium and phosphate concentrations of the study population were 3.84 mEq/L and 3.90 mg/dL, respectively. At baseline, 44.4% (n = 16) of cats with "uptrend" calcium status had both lower-than-median (LTM) potassium (≤3.84 mEq/L) and LTM phosphate (≤3.90 mg/dL) concentrations, whereas only 16.7% (n = 5) of "nonuptrend" cats had concomitant LTM potassium and LTM phosphate. In contrast, half of "nonuptrend" cats in our study population (n = 15) had higher-than-median (HTM) potassium (>3.84 mEq/L) and HTM phosphate (>3.90 mg/dL) concentrations. The CKD cats with a combination of LTM baseline potassium and phosphate concentrations had >9 times the odds of developing increased calcium concentrations compared with cats that had HTM

TABLE 1 Descriptive statistics for cats enrolled in this retrospective cohort study, dichotomized based on the changes in plasma total calcium concentration after transition to a phosphate-restricted diet

	Nonuptrend (n = 31)		Uptrend (n = 40)		
Variables (reference interval)	**Median [25th, 75th percentile]**	**n**	**Median [25th, 75th percentile]**	**n**	***P* value**
Age (y)	15.8 [14.5, 17.1]	31	15.7 [14.0, 17.9]	40	.92
Dairy product (yes, n [%])	13 [57]	23	19 [58]	33	.94
BCS ("1-3," "4-6," "7-9," n [%])	14 [45], 14[45], 3 [10]	31	16 [41], 20[51], 3 [8]	39	.94
MCS ("0," "1," "2," "3," n [%])	2 [6], 26 [84], 3 [10], 0 [0]	31	0 [0], 34 [87], 5 [13], 0[0]	39	.39
Weight (kg)	3.7 [3.2, 4.2]	30	3.6 [3.1, 4.4]	38	.83
Sex (female neutered, n [%])	14 [45]	31	24 [60]	40	.21
Albumin (2.5-4.5 g/dL)	3.1 [2.8, 3.2]	31	3.1 [2.9, 3.3]	40	.46
ALP (≤60 U/L)	28 [22, 35]	31	33 [22, 46]	40	.34
ALT (5-60 U/L)	50 [42, 69]	31	56 [51, 70]	40	.26
Chloride (100-124 mEq/L)	118 [115, 120]	30	117 [114119]	36	.33
Creatinine (0.23-2 mg/dL)	2.60 [2.31, 2.95]	31	2.41 [2.13, 2.71]	40	.18
FGF23[a] (56-700 pg/mL)	562 [312, 1093]	21	599 [273, 862]	26	.86
Glucose (54-117 mg/dL)	105 [95, 121]	28	115 [99, 137]	37	.27
Venous HCO_3^- (17-24 mEq/L)	20.9 [19.1, 22.7]	25	20.2 [19.0, 22.2]	34	.66
Hypertension (controlled) (n [%])	4 [13]	31	12 [30]	40	.09
iCa (4.76-5.48 mg/dL)	5.23 [5.13, 5.32]	26	5.29 [5.13, 5.41]	33	.25
PCV (30%-45%)	35 [27, 39]	31	35 [31, 38]	40	.53
Venous pH (7.21-7.44)	7.35 [7.33, 7.38]	25	7.35 [7.31, 7.39]	34	.93
Phosphate (2.79-6.81 mg/dL)	4.30 [3.81, 4.77]	31	3.70 [3.37, 4.54]	40	**.03**
Potassium (3.5-5.5 mEq/L)	4.08 [3.82, 4.29]	30	3.72 [3.46, 4.01]	36	**.009**
PTH[a] (2.6-17.6 pg/mL)	19.5 [15.8, 47.6]	26	22.3 [13.0, 40.5]	32	.72
SBP (<160 mm Hg)	128 [119, 147]	30	133 [119, 149]	39	.36
SDMA (1-14 μg/dL)	19 [17, 22]	20	17 [14, 21]	25	.19
Sodium (145-157 mEq/L)	152 [150, 155]	30	150 [148, 153]	36	**.04**
tCa (8.2-11.8 mg/dL)	9.94 [9.76, 10.34]	31	9.88 [9.45, 10.18]	40	.39
tMg (1.73-2.57 mg/dL)	2.09 [1.96, 2.35]	11	2.19 [1.95, 2.29]	17	.85
Total protein (6.0-8.0 g/dL)	7.8 [7.3, 8.1]	31	8.0 [7.6, 8.5]	40	.16
Urea (7.0-27.7 mmol/L)	56.9 [47.3, 67.1]	22	51.0 [41.7, 63.7]	29	.17
USG (≥1.035)	1.017 [1.014, 1.019]	15	1.015 [1.014, 1.017]	21	.54

Note: Significant difference between groups ($P < .05$) are highlighted in bold.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; BCS, body condition score; FGF23, fibroblast growth factor 23; HCO_3^-, bicarbonate; iCa, ionized calcium; MCS, muscle condition score; n, number of cats; PTH, parathyroid hormone; SBP, systolic blood pressure; SDMA, symmetric dimethylarginine; tCa, total calcium; tMg, total magnesium; USG, urine specific gravity.
[a]Baseline FGF23 and PTH were log-transformed for comparison using Mann-Whitney *U* test.

TABLE 2 Univariable and multivariable backward binary logistic regression to identify risk factors at baseline associated with "uptrend" calcium status in CKD cats after transition to a phosphate-restricted diet

	Univariable analysis			Multivariable analysis		
Variables	**OR (95% CI)**	**n**	***P* value**	**OR (95% CI)**	**n**	***P* value**
Hypertension status	2.89 (0.88-11.37)	71	.08			
Phosphate (mg/dL)	1.10 (1.00-1.25)	71	.06	1.15 (1.03-1.32)	66	.01
Potassium (mEq/L)	1.16 (1.04-1.33)	66	.007	1.19 (1.06-1.38)	66	.003
Sodium (mEq/L)	1.01 (1.00-1.03)	66	.07			

Note: Hypertension status was treated as a categorical variable in the model.
Abbreviations: CI, confidence interval; CKD, chronic kidney disease; OR, odds ratio.

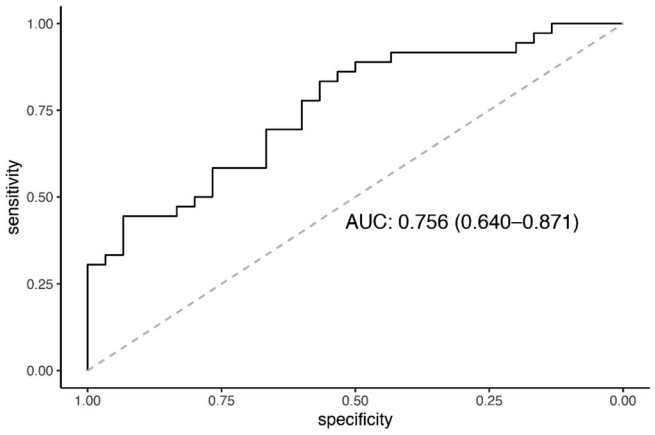

FIGURE 1 Receiver operating characteristic curve illustrating the performance of the final multivariable model in predicting an "uptrend" calcium status after transition to a phosphate-restricted diet in CKD cats. AUC, area under curve; CKD, chronic kidney disease

baseline potassium and phosphate concentrations (OR = 9.6; 95% CI = 2.5-44.3; P = .002).

3.2 | Evaluation of changes in CKD-MBD parameters in relation to dietary phosphate restriction

Changes in clinicopathological variables over time, after initiation of a phosphate-restricted diet in CKD cats, were assessed using LMM and GLMM analyses (Tables 3 and S1 and Figure S1). The unit of time was expressed as month (30.4 days). Changes in plasma iCa concentration were similar to those of tCa and significantly increased over time in cats with "uptrend" tCa status (β = .052 ± .013 mg/dL; P < .001) but the rate did not change significantly for the "nonuptrend" cats (β = −.008 ± .014 mg/dL; P = .55; Figure 2A,B). Both plasma creatinine and SDMA concentrations were significantly increased over time in the "uptrend" group (creatinine, β = .069 ± .029 mg/dL; P = .02; SDMA, β = .64 ± .29 μg/dL; P = .03) but the rate of both creatinine and SDMA did not change over time in the "nonuptrend" group (creatinine, β = −.043 ± .032 mg/dL; P = .19; SDMA, β = .08 ± .29 μg/dL; P = .8; Figure 2C,D). Plasma phosphate concentration significantly increased over time in cats with "uptrend" calcium status (β = .129 ± .062 mg/dL; P = .04) but the rate did not change significantly in the "nonuptrend" group (β = −.086 ± .068 mg/dL; P = .21; Figure 2E). Log-transformed PTH (ln[PTH]) concentration significantly decreased over time in both "uptrend" (β = −.155 ± .036 pg/mL; P < .001) and "nonuptrend" cats (β = −.135 ± .040 pg/mL; P = .002), but the rate of change did not differ significantly between groups (Figure 2F). Log-transformed FGF23 (ln[FGF23]) significantly increased over time (β = .103 ± .035 pg/mL; P = .004) in the "uptrend" group but the rate did not change significantly in cats with "nonuptrend" calcium status (β = −.064 ± .039 pg/mL; P = .11; Figure 2G).

TABLE 3 Linear mixed model and generalized linear mixed model analyses examining the change in clinicopathological variables over time (first 200 days after the transition to a phosphate-restricted diet) in CKD cats (n = 71)

Variables	Group	Time	Group*Time
BCS[a] ("1-3," "4-6," "7-9")	.87	**.008**	.17
MCS[a] ("0," "1," "2," "3")	.25	.14	.15
Body weight[a] (kg)	.99	**.006**	.29
Albumin (g/dL)	.41	**.006**	**.04**
ALP (U/L)	.39	.33	.85
ALT (U/L)	.67	.84	.22
Chloride (mEq/L)	.18	.86	.4
Creatinine (mg/dL)	.33	.54	**.01**
ln[FGF23] (pg/mL)	.78	.46	**.002**
Glucose (mg/dL)	.21	.26	.27
Venous HCO_3^- (mEq/L)	.57	.64	.13
iCa (mg/dL)	.71	**.02**	**.002**
PCV (%)	.12	**.009**	.91
Venous pH	.59	.62	.28
Phosphate (mg/dL)	**.03**	.64	**.02**
Potassium (mEq/L)	**.002**	.58	.17
ln[PTH] (pg/mL)	.95	**<.001**	.71
SDMA (μg/dL)	.17	.18	.08
SBP (mm Hg)	.75	.49	.2
Sodium (mEq/L)	**.03**	.07	.11
tCa (mg/dL)	.54	**.002**	**<.001**
tMg (mg/dL)	.61	.32	.35
Total protein (g/dL)	.24	.09	.48
Urea (mmol/L)	.55	**.01**	.81

Note: Summary of P values for all variables included in the model. Group represents cats in "uptrend" or "nonuptrend" group based on the trend of plasma total calcium concentration. Outcome variables showing significant change over time and between groups (P < .05) are highlighted in bold. The unit used for time was month (30.4 days). A significant difference in the group column indicates a significant difference between the two groups at baseline for a given parameter (the start of the regression line at time 0). A significant difference in Group*Time indicates that the outcome variable differs significantly between groups ("uptrend" vs "nonuptrend") over time. If Group*Time was not significant, a significant difference in Time indicates the overall rate of change of the outcome variable differs significantly from baseline over time, regardless of the trend of plasma calcium.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; BCS, body condition score; CKD, chronic kidney disease; HCO_3^-, bicarbonate; iCa, ionized calcium; ln[FGF23], log-transformed fibroblast growth factor 23; ln[PTH], log-transformed parathyroid hormone; MCS, muscle condition score; SBP, systolic blood pressure; SDMA, symmetric dimethylarginine; tCa, total calcium; tMg, total magnesium.
[a]Only the case number of each individual cat was included as random effect in the model.

3.3 | Evaluation of FE of analytes

Thirty paired urine samples ("uptrend," n = 17; "nonuptrend," n = 13) were available for determination of FE of analytes.

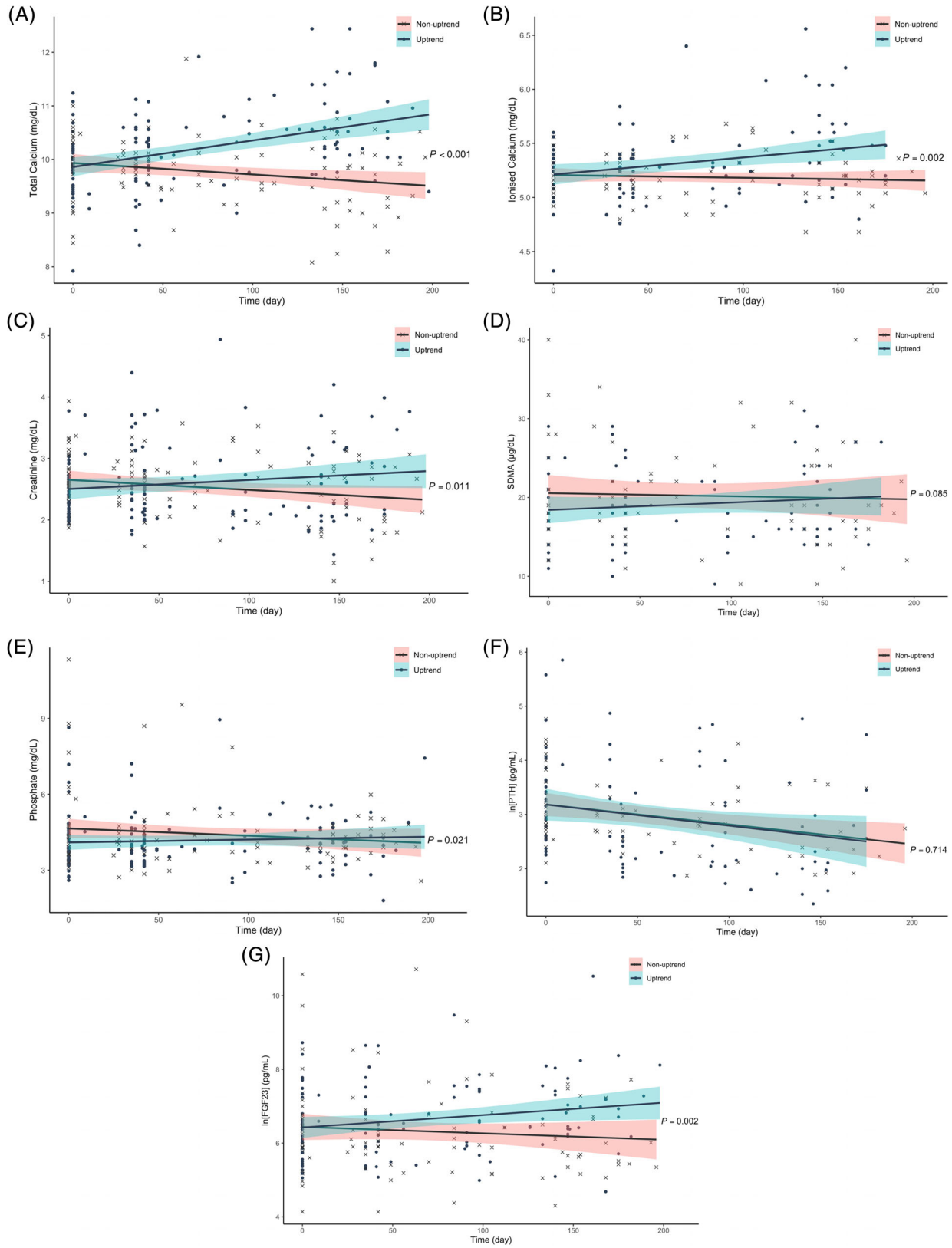

FIGURE 2 Scatter plots illustrating the linear change of, A, total calcium; B, ionized calcium; C, creatinine; D, symmetric dimethylarginine (SDMA); E, phosphate; F, log-transformed PTH (ln[PTH]); and, G, log-transformed fibroblast growth factor 23 (ln[FGF23]) in CKD cats grouped according to the trend of plasma total calcium concentration ("uptrend" vs "nonuptrend") during the first 200 days after transition to a phosphate-restricted diet. The solid lines represent the regression lines and the shaded areas represent 95% confidence interval (95% CI) for the fitted linear regression. Outliers have been omitted from the graph for creatinine, SDMA, and phosphate. The *P* values shown are the interactions between groups and the rate of change for these analyses within groups are presented in Table S1. CKD, chronic kidney disease; PTH, parathyroid hormone

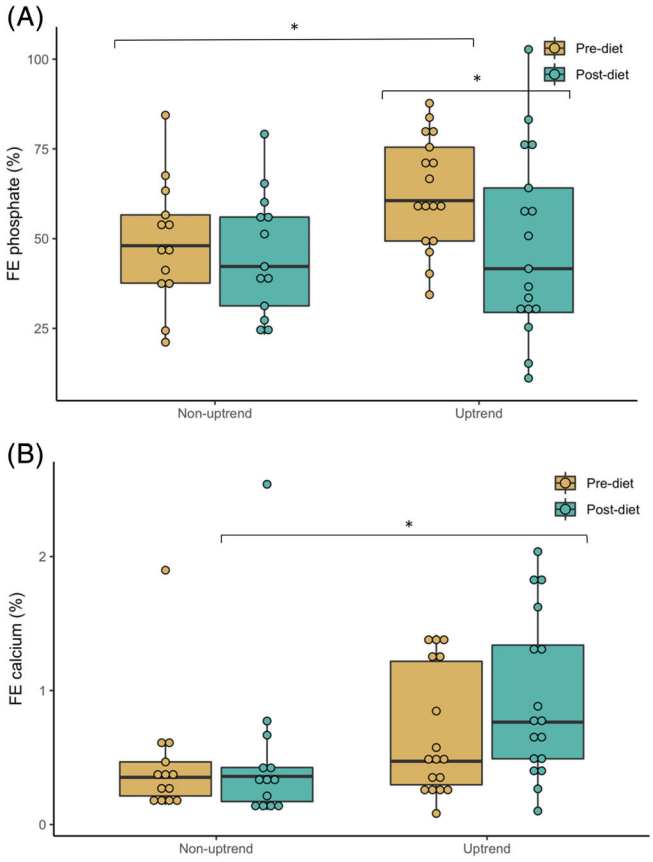

FIGURE 3 Boxplots illustration the fractional excretion of, A, phosphate and, B, calcium between groups of cats according to the trend of plasma total calcium concentration after transition to a phosphate-restricted diet ("nonuptrend" [n = 13] vs "uptrend" [n = 17]) at baseline visit (termed prediet) and postdiet visit (termed postdiet). Asterisk indicates significant difference ($P < .05$) was observed, either between groups or between visits

The time interval between pre- and postdiet visits both were a median of 91 days ("uptrend" range, 35-259 vs "nonuptrend" range, 35-175), with no significant difference in the time period observed ($P = .84$). Cats with "uptrend" plasma calcium status had significantly higher FE of phosphate at prediet visits than did cats in the "nonuptrend" group ("uptrend," 60.6% [49.3, 75.5] vs "nonuptrend," 48.0% [37.6, 56.6]; $P = .03$), but no difference was detected at the postdiet visits between groups ($P = .76$; Figure 3A). The FE of phosphate decreased significantly between visits in the "uptrend" group ($P = .02$) but no significant change between visits was detected in the "nonuptrend" group ($P = .46$). The FE of calcium did not differ significantly at prediet visits between the groups ($P = .2$). Cats with "uptrend" calcium status had significantly higher FE of calcium when compared to the "nonuptrend" cats at postdiet visits ($P = .02$; Figure 3B). No significant difference in FE of potassium, sodium, and chloride between groups at either prediet or postdiet visits, as well as between visits for both groups, was observed (data not shown).

4 | DISCUSSION

Results of our retrospective study indicate that lower plasma potassium and phosphate concentrations were independent risk factors for increasing plasma tCa concentration after initiation of a phosphate-restricted diet in CKD cats. Cats with increasing plasma tCa concentrations were found to have higher urinary excretion of calcium after 5 to 37 weeks of dietary phosphate restriction when compared to the cats showing no increase in plasma tCa concentrations. We also identified that plasma creatinine, SDMA, phosphate, FGF23, and iCa concentrations increased over time in cats with "uptrend" calcium status after transition to a phosphate-restricted diet.

Plasma creatinine and SDMA concentrations indirectly reflect glomerular filtration rate (GFR) and increases in creatinine and SDMA are associated with decreased kidney function.[24,25] We found that these GFR surrogate biomarkers significantly increased in the cats that developed an "uptrend" calcium status after dietary phosphate restriction during our study period, suggesting an association between increasing plasma calcium concentration and progression of kidney disease. To our knowledge, an association between changes in plasma calcium concentration and progression of CKD has not been reported previously in cats. We found that the rate of change in plasma creatinine and SDMA concentrations over time was significantly different between the "uptrend" and "nonuptrend" groups. Strikingly, cats with an "uptrend" calcium status had average 2.7% and 3.6% increments in plasma creatinine and SDMA concentrations per month, respectively. A significantly higher proportion of cats (25%; n = 10) in the "uptrend" group developed sustained progression of CKD (defined as ≥25% increase in plasma creatinine concentration) compared to those in the "nonuptrend" group (6.5%; n = 2) over the 200-day period after transition to a phosphate-restricted diet ($P = .04$). It is well documented in human CKD patients that positive calcium balance is independently associated with increased risk for vascular calcification and cardiovascular morbidity, and higher mortality.[26,27] However, perturbations in calcium homeostasis have not been proven to contribute to disease progression, notwithstanding the deleterious impact of hypercalcemia on kidney function as evident by the decrease in GFR.[28,29]

Apart from progressive azotemia, cats with "uptrend" calcium status also experienced increasing plasma phosphate concentrations, with an average 3.2% increment per month during the study period. Increased phosphate concentrations are more commonly detected in advanced stages of CKD and a reverse relationship between serum phosphate concentration and GFR is well established in human patients.[30,31] Moreover, hyperphosphatemia is associated with progression of azotemia and shorter survival times in CKD cats.[32] We identified a cohort of CKD cats with a combination of increasing tCa, iCa, creatinine, SDMA, phosphate, and FGF23 concentrations after transition to a phosphate-restricted diet, suggesting a reciprocal interplay among calcium, phosphate and GFR that might reflect the detrimental effects of calcium on CKD progression. A causal relationship, however, cannot be proven by a retrospective observational study

and the reason for the observed associations requires prospective interventional investigation.

An increase in plasma tCa concentration, in principle, results from either a decrease in urinary excretion, an increase in intestinal absorption or an increase in bone resorption, or some combination of these.[33] It can also be attributed to an increase in circulating complexes of calcium with anions. One possible mechanism suggested to account for the development of hypercalcemia is impaired calcium excretory function in CKD. Urinary FE measurements therefore were performed to assess the renal handling of various electrolytes, including calcium.[23] We found a higher urinary excretion of calcium in cats with "uptrend" calcium status after transition to a phosphate-restricted diet, suggesting that enhanced renal calcium excretion acts as a compensatory mechanism, although the extent of compensation was inadequate to prevent plasma calcium concentration from increasing.

As in human patients, dietary phosphate restriction is a well-recognized strategy to improve survival in cats with CKD.[15-17] Hyperparathyroidism and increased FGF23 concentrations have been associated with advancing kidney disease and reported to be negative prognostic indicators.[8,19,34,35] Feeding azotemic cats a phosphate-restricted diet decreases plasma PTH and FGF23 concentrations.[18,36] In the present study, we also identified decreasing plasma concentrations of PTH with comparable rate of decrease over time in both groups. Interestingly, although FGF23 concentrations decreased, albeit nonsignificantly, in the "nonuptrend" group after decreased dietary phosphate intake, it increased significantly over time, with an average 1.1% increment in ln[FGF23] per month in cats with "uptrend" calcium status. This finding is in contrast with a previous study,[36] but such a finding could be attributed to the increasing plasma tCa concentrations in this cohort of cats. Increasing evidence has suggested that FGF23 is regulated, at least in part, by calcium.[37-39] Dietary calcium supplementation has been shown to stimulate FGF23 mRNA expression in bone, leading to increased circulating FGF23 concentrations.[39] Increases in serum FGF23 concentrations after intraperitoneal injections of calcium gluconate also have been observed in wild-type mice, as well as in mice with targeted inactivation of PTH and the calcium-sensing receptor.[38] These findings provide further evidence to support the positive correlation found between FGF23 and plasma tCa concentration in our study. Furthermore, FGF23 inhibits vitamin D activation and PTH secretion,[40,41] contributing to a net decrease in extracellular calcium concentration and thus maintains a negative feedback circuit. Fibroblast growth factor 23 also suppresses expression of α-Klotho, which is involved in renal reabsorption of calcium.[42] Nevertheless, the incremental increase in FGF23 detected in the "uptrend" cats may have been confounded by the concurrent positive trend of phosphate in these cats. Considering the close relationship between FGF23 and phosphate metabolism,[19] we cannot exclude the possibility that increasing FGF23 could be a response to the increased phosphate. In addition, an increase in plasma FGF23 concentration could be a consequence of decreased renal excretion, which is supported by the progressive azotemia observed in our cats with increasing plasma calcium concentrations. Interestingly, cats with lower plasma phosphate concentration at the time of transition to a phosphate-restricted diet were found to have increased risk of developing an "uptrend" calcium status. The mechanism underlying this finding remains to be determined, but it is possible that hormonal regulation in these cats was adapted to maintain lower plasma phosphate concentrations, as evidenced by the increased urinary clearance of phosphate, and they could be more sensitive to dietary phosphate modification.

Lower baseline plasma potassium concentration also was identified as an independent risk factor for development of increasing plasma calcium concentrations in CKD cats after dietary phosphate restriction. A potential explanation could be involvement of the renin-angiotensin-aldosterone system (RAAS). The RAAS is a key player in the maintenance of sodium and potassium balance and regulation of arterial pressure in the body. Renal potassium handling is predominantly regulated by aldosterone with a negative feedback circuit.[43] Therefore, we postulate that upregulation of aldosterone may contribute to lower baseline potassium concentrations in the "uptrend" cats. A relationship between hyperaldosteronism and systemic hypertension has been well documented in humans,[44] and more recently, in CKD cats.[45] Azotemic cats with systemic hypertension have higher serum aldosterone concentrations, as well as increased aldosterone-to-renin ratio, than do normotensive azotemic cats.[45] Hypertensive cats also have lower plasma potassium concentrations than do normotensive cats.[21,46] Although no difference in baseline SBP between the 2 groups was detected in our study, this most likely is explained by our clinic protocol in which systemic hypertension is controlled (<160 mm Hg) before transition of CKD cats to a phosphate-restricted diet. However, 30% of this cohort were observed to have controlled hypertension (using amlodipine besylate) at baseline, which is more prevalent, albeit not statistically significantly different, than the 13% detected in the "nonuptrend" group (P = .09). In human patients with CKD, both hyperaldosteronemia and hypokalemia are implicated in progression of renal disease and increased mortality.[47-51] Assessment of the RAAS system before and after transition to a clinical renal diet is warranted in future studies to examine this possibility further.

Plasma albumin concentrations were found to decrease by an average of 0.9% per month in cats with "nonuptrend" calcium status. Lower plasma albumin concentration recently has been shown to underestimate iCa using tCa in CKD cats,[7] and it can decrease the protein-bound fraction of calcium. Therefore, the decreasing albumin concentration may have an effect on the tCa measurements.[52] It should be acknowledged, however, that this should not affect the iCa concentrations,[53] and therefore, the parallel trends of iCa and tCa observed in both groups should support our classification of calcium trend. Moreover, the fact that tCa was increased by an average of 1.7% per month in the "uptrend" group indicates that changes in tCa in the "nonuptrend" group could not be explained simply by decreased plasma albumin concentration.

In addition to plasma albumin concentration, BCS and body weight also decreased in cats with "nonuptrend" calcium, but not in the "uptrend" cats. One explanation for these changes is that these

cats were in a catabolic state with increased rate of protein catabolism after transition to a renal diet because this diet is also restricted in protein, and protein is an important source of phosphorus.[54] It is possible that decreased albumin concentration, BCS and body weight may reflect cachexia and affect measurement of plasma creatinine concentration[55,56]; however, cachexia should not affect the concentration of SDMA, supporting the lack of evidence of progressive kidney disease in these cats. The mechanisms behind the changes in plasma albumin concentration, BCS and body weight after transition to a phosphate-restricted diet in these cats remain unclear. The median age of our study population was >15 years and therefore, the possibility that these changes are attributable to concurrent nonrenal illnesses or part of the natural aging process cannot be excluded. It is unknown whether the magnitude of changes in these variables is of biological or clinical relevance, and no untreated control animals were available for comparison. Additional studies are required to understand the potential reasons behind these changes and whether or not they are of clinical relevance.

Our study had several limitations, mostly attributable to its retrospective observational nature. Classification of cohort cases was based on the regression gradient of plasma tCa concentrations from at least 3 visits. However, plasma tCa concentration may not be an accurate assessment of calcium status in cats, and measurements of the biologically active plasma iCa concentration were not available for all cats at all visits. Of the 71 cats enrolled, 42 cats had iCa measurements from the baseline visit and at least 2 follow-up visits ("iCa uptrend," n = 12; "iCa nonuptrend," n = 30). Subanalysis using LMM was performed on these cats based on iCa. The results showed divergent patterns in creatinine, SDMA, phosphate, and ln[FGF23] concentrations over time between these 2 groups, with increasing concentrations of these variables in the "iCa uptrend" group. Although these subanalysis results were not significant, most likely as a consequence of the small number of cases in the "iCa uptrend" group, they are in concordance with our main findings. Although all cats enrolled were transitioned to the same phosphate-restricted diet, the actual amount of renal diet being consumed was unknown. Cats also were fed a variety of commercial diets before transition to the phosphate-restricted diet, and this information was highly variable and challenging to characterize. Further study using detailed dietary history will be required to investigate whether the different calcium responses of cats fed a phosphate-restricted diet is attributable to prior dietary adaption to a particular formulation of diet. In addition, hypercalcemia has been detected in cats at first diagnosis of CKD or in those not transitioned to a phosphate-restricted diet. Based on the retrospective design of our study, a causal relationship between dietary phosphate restriction and increase in plasma tCa concentration cannot be definitively concluded. Another limitation of our study is that FE of electrolytes is commonly subject to a large amount of variation, both intra- and interindividually, under physiological conditions.[23] Renal clearance of electrolytes was assessed using spot urine samples in our study. Although such an approach has the benefit of being practical in clinical settings, it may not accurately reflect longer term FE of electrolytes in cats and results therefore should be interpreted with caution.[57]

In conclusion, we identified that lower plasma potassium and phosphate concentrations at the time of transition to a phosphate-restricted diet are independent risk factors for increasing plasma tCa concentration in cats with azotemic CKD. Prospective randomized controlled trials using longitudinal measurements of plasma iCa concentration are warranted to better determine the effects of dietary phosphate restriction on calcium homeostasis and its associated clinical implications in cats with CKD. Lastly, little is known about the involvement of potassium and aldosterone in the regulation of calcium balance in cats with CKD, and further investigation is necessary to explore the complex interplay between potassium and calcium, and whether low plasma potassium concentration is a modifiable risk factor.

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CONFLICT OF INTEREST DECLARATION

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OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

This study was part of a larger observational cohort for which approval of the Ethics and Welfare Committee of the Royal Veterinary College had been granted.

HUMAN ETHICS APPROVAL DECLARATION

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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