Chronic Kidney Disease in Cats and the Risk of Total Hypercalcemia

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Background: Chronic kidney disease (CKD) is a common comorbidity in cats with hypercalcemia, but whether CKD is a risk factor for hypercalcemia is unclear. Hypercalcemia often is diagnosed based on total calcium concentration (tCa), which tends to underestimate the ionized calcium concentration (iCa) in cats.

Objectives: Assessment of the performance of tCa for the diagnosis of ionized hypercalcemia, and exploration of factors influencing the relationship between iCa and tCa. Determination of risk factors for incident total hypercalcemia (ie, the development of hypercalcemia based on tCa during follow-up).

Animals: Records of a cross-section (n = 477) and observational cohort (n = 367) of client-owned cats with and without azotemic CKD from first opinion practice.

Methods: Retrospective cross-sectional and retrospective cohort study. The diagnostic accuracy of tCa as an index test for iCa was evaluated, and risk factors for underestimation were explored by binary logistic and linear regression in a cross-section of cats with and without azotemic CKD. Chronic kidney disease and clinicopathological variables were assessed as predictors of incident total hypercalcemia by both time-invariant and time-dependent Cox regression in a cohort of cats.

Results: Specificity of tCa for identification of ionized hypercalcemia was high (100%), but sensitivity was low. Underestimation was associated with lower venous bicarbonate concentrations. Cats with CKD had increased risk for incident total hypercalcemia (hazard ratio, 4.29; 95% confidence interval, 1.96–9.37; P < .001). Higher tCa predicted incident total hypercalcemia in both azotemic and nonazotemic cats (P < .001).

Conclusions and Clinical Importance: Chronic kidney disease is a risk factor for incident total hypercalcemia, and most cats with increased tCa had concurrent ionized hypercalcemia. Higher baseline tCa predicts incident total hypercalcemia. Prospective studies assessing changes in iCa are warranted.

Key words: Azotemia; Calcium; Feline; Bicarbonate.

Hypercalcemia in cats is associated with chronic kidney disease (CKD), neoplasia, and rarely primary hyperparathyroidism, although hypercalcemia of idiopathic origin is considered the most common etiology. It is unclear whether renal dysfunction is truly a risk factor for the development of hypercalcemia, because CKD is a common comorbidity in older cats, and hypercalcemia could decrease glomerular filtration rate by decreasing medullary tone and renal blood flow. Several mechanisms by which CKD may contribute to hypercalcemia have been proposed, including decreased glomerular filtration, increased tubular reabsorption, and decreased bone storage of calcium. Based on total calcium concentration (tCa), early studies report CKD in 25% of hypercalcemic cats, whereas hypercalcemia was found in 11.5–21% of cats with CKD. The prevalence appears to increase as renal function declines, from 8% in early stage to 32% in end-stage disease. Approximately half of tCa consists of hydrated free calcium ions, and the other half consists of protein-bound calcium and a small but variable portion of ionic complexes, such as calcium phosphate. Hypercalcemia in CKD typically is represented by an increase in tCa, with a normal ionized calcium concentration (iCa). Ionized hypercalcemia has been reported in 10–30% of cats with CKD, even though iCa tends to decrease in end-stage disease.

The ionized calcium fraction is biologically active and therefore superior to tCa in assessing calcium status. The sensitivity and specificity of...

Abbreviations:

- AUROC: area under the receiver operating characteristic curve
- CI: confidence interval
- CKD: chronic kidney disease
- CKD-MBD: chronic kidney disease-mineral and bone disorder
- FGF-23: fibroblast growth factor 23
- HCO₃⁻: venous bicarbonate concentration
- HR: hazard ratio
- iCa: ionized calcium concentration
- IRIS: International Renal Interest Society
- OR: odds ratio
- PTH: parathyroid hormone
- ROC: receiver operating characteristic
- tCa: total calcium concentration
- USG: urine specific gravity
tCa to predict ionized hypercalcemia in cats with CKD are reported to be 30 and 92%, respectively. Hypoalbuminemia, causing a decrease in the protein-bound fraction, could result in underestimation of ionized calcium. However, tCa and albumin concentrations correlate poorly, and the diagnostic accuracy of tCa could not be improved by adjustment for serum albumin concentration in cats. In addition to hypoalbuminemia, metabolic acidosis appears to increase the risk for underestimation of iCa in human CKD, but this has not been reported in cats.

The aims of our study were as follows: first, to assess the performance of plasma tCa in the prediction of ionized hypercalcemia and explore factors influencing the relationship between iCa and tCa, and second, to determine whether azotemic CKD is a risk factor for the development of total hypercalcemia and to explore predictors of incident total hypercalcemia.

Materials and Methods

Case Selection

Cases were retrospectively selected from the records of geriatric cat clinics held at 2 London-based first opinion practices (People's Dispensary for Sick Animals in Bow and Beaumont Sainsbury Animal Hospital in Camden), where apparently healthy cats (≥9 years) had blood samples collected every 6 months for general health screening, and cats with CKD or systemic hypertension were blood sampled every 4 months for disease management. Criteria for a diagnosis of azotemic CKD were plasma creatinine concentration ≥2 mg/dL in conjunction with a urine specific gravity (USG) <1.035, or plasma creatinine concentration ≥2 mg/dL on 2 consecutive occasions 2–4 weeks apart. Records of cats with clinical signs of hyperthyroidism, plasma total thyroxine concentration >40 nmol/L, medical treatment for hyperthyroidism, or diabetes mellitus, or treatment with corticosteroids were excluded from all analyses. Cats receiving amiodipine besylate for treatment of systemic hypertension were included.

Collection and storage of blood samples was performed with owner consent and approval of the Ethics and Welfare Committee of the Royal Veterinary College. Blood was obtained by jugular venipuncture, collected in a heparinized tube, and stored on ice for a maximum of 6 hours until centrifugation and separation. Heparinized plasma was sent to an external laboratory for routine bioanalyses. Cats receiving amlodipine besylate for treatment of systemic hypertension and/or in agreement based on reference ranges, the amount of underestimation of iCa status as the event of interest. Only ionized hypercalcemic cats were used in this analysis, classified as either underestimated if the standard score of tCa was lower than the matched total calcium status for which the laboratory reference interval (8.20–11.80 mg/dL) was used.

Evaluation of Risk of Incident Total Hypercalcemia in Cats with and Without Renal Azotemia

For the retrospective cohort, the records between January 1, 2000 and September 1, 2014 were reviewed to identify nonazotemic cats and cats with a diagnosis of azotemic CKD. Data extraction was performed on July 27, 2015. For nonazotemic cats, the first visit to the geriatric cat clinic was designated as baseline, whereas for CKD cases the date of diagnosis of azotemic CKD was used. All records were included from baseline to the visit when total hypercalcemia was noted (ie, incident total hypercalcemia; tCa >11.80 mg/dL). Animals that remained normocalcemic based on iCa had to have follow-up of at least 330 days to be included. Cases with a diagnosis of total hypercalcemia before or at baseline were excluded. Cats were classified according to renal azotemia and development of total hypercalcemia, resulting in the following 4 groups: cats with a diagnosis of CKD that remained normocalcemic during follow-up (CKD-NCa group); cats with a diagnosis of CKD that developed total hypercalcemia (CKD-HCa group); nonazotemic cats that remained normocalcemic (H-NCa group); and nonazotemic cats that developed total hypercalcemia (H-HCa group).

Statistical Analysis

All statistical analyses were performed using commercial software. Statistical significance was set as P < .05. Continuous clinical data are presented as mean (range) or median [25th and 75th percentiles]. Normality was assessed by visual inspection of histograms, and skewed variables were log-transformed for normalization. Correlations were assessed by Pearson’s correlation (r).

Evaluation of Diagnostic Accuracy of tCa as a Marker of Ionized Hypercalcemia

Simple linear regression was used to assess the association between tCa and iCa. Sensitivity and specificity were calculated for tCa as an index test of true ionized hypercalcemia. Measures of accuracy are presented as percentage (95% confidence interval [CI]). Receiver operating characteristic (ROC) curves were plotted to assess overall diagnostic accuracy of tCa for detecting ionized hypercalcemia. Age, sex, weight, creatinine, phosphate, albumin, cholesterol, sodium, potassium, chloride, HCO₃⁻, pH, and PCV were assessed as risk factors by binary logistic regression with underestimation of iCa status as the event of interest. Only ionized hypercalcemic cats were used in this analysis, classified as either underestimated (total normocalcemia with ionized hypercalcemia) or in agreement (both total and ionized hypercalcemia). Only variables from the univariable analysis with a type I error <10% were entered into multivariable logistic regression analysis. The final models were derived by backward elimination. Results are reported as odds ratio (OR) (95% CI).

In addition to dichotomization of cases as either underestimated or in agreement based on reference ranges, the amount of underestimation was quantified by standardizing tCa and iCa using the formula: z-score = (measured value – reference range mean)/standard deviation of the reference range. Cats were classified as underestimated if the standard score of tCa was lower than the
Evaluation of Risk of Incident Total Hypercalcemia in Cats with and Without Renal Azotemia

Baseline variables of the 4 groups were compared using 1-way ANOVA with Bonferroni posthoc comparison or independent samples t-test and proportions by Fisher’s exact test. To assess whether azotemic CKD is a risk factor for incident total hypercalcemia, Kaplan–Meier curves of the nonazotemic and CKD group were compared using log-rank test, and a hazard ratio (HR) was calculated with univariable Cox proportional hazard analysis. Incident total hypercalcemia was the event of interest, and cats that remained normocalcemic were censored when lost to follow-up. Cats with CKD had blood samples collected more frequently (every 4 months) because of their condition than did nonazotemic cats (every 6 months). A subanalysis that only included visits at 6-month intervals, ignoring other visits, was carried out to evaluate the above-mentioned potential bias.

Baseline predictors of incident total hypercalcemia for the azotemic and nonazotemic groups were explored with time-invariant Cox regression, and repeatedly measured predictors were assessed using time-dependent Cox regression. No missing data imputation was performed. Age, weight, USG, iCa, plasma creatinine, phosphate, tCa, FGF-23, chloride, sodium, and potassium concentrations were entered as continuous variables, and hair length, sex, and plasma albumin and PTH concentrations based on terciles, as categorical variables. Variables with a type I error <10% were assessed for multivariable analysis. The final time-invariant and time-dependent models were derived by backward elimination. Results are reported as regression coefficient (β) (95% CI). Receiver operating characteristic curves (ROC) were constructed to explore cutoff values of baseline predictors of incident total hypercalcemia.

Results

Evaluation of Diagnostic Accuracy of tCa as a Marker of Ionized Hypercalcemia

A total of 590 independent samples were identified with both tCa and iCa measured, of which 113 were excluded because of a diagnosis of hyperthyroidism (n = 85), or undetermined renal function (n = 28), allowing 477 cats for analysis, of which 168 were nonazotemic and 309 had CKD. Domestic shorthair was the most common breed (n = 368), followed by domestic longhair (n = 51), and purebreds or crosses of the following breeds: Burmese (n = 15), Persian (n = 15), Siamese (n = 8), Birman (n = 4), 3 each of British blue, British shorthair, Chinchilla, and Russian blue, 2 Maine Coons, 1 Abyssinian, and 1 Tiffany. In the CKD group, 222 cats had International Renal Interest Society (IRIS) (www.iris-kidney.com) stage 2; 66, IRIS stage 3; and 21, IRIS stage 4 CKD. Clinicopathologic variables are summarized in Table 1. Based on iCa, 58 cats were hypercalcemic, 31 hypocalcemic, and 388 normocalcemic. In ionized hypercalcemic cats, mean iCa was 5.91 (range, 5.52–7.40) mg/dL and mean tCa 11.20 (range, 9.48–14.84) mg/dL. No concurrent diseases other than CKD, hypertension, or bacterial urinary tract infections were diagnosed.

The association between tCa and iCa was moderate (r = 0.57), venous bicarbonate; iCa, ionized calcium; tCa, total calcium; USG, urine specific gravity.

Table 1. Clinicopathologic variables for cats included in the cross-sectional study grouped according to renal status.

| Variable         | CKD (n = 309) | Nonazotemic (n = 168) |
|------------------|---------------|-----------------------|
| (Reference Interval) | Median [25th, 75th Percentile] | Median [25th, 75th Percentile] |
| Age (years)      | 14.4 [11.4, 16.6] | 12.3 [11.0, 14.6] |
| Weight (kg)      | 3.90 [3.20, 4.54] | 4.25 [3.43, 5.04] |
| % Male           | 55.5 (95% CI, 50.6) |
| Albumin (2.5–4.5 g/dL) | 3.2 [3.0, 3.3] | 3.2 [3.0, 3.4] |
| Chloride (100–124 mEq/L) | 119 [116, 122] | 120 [118, 122] |
| Creatinine (0.23–2.00 mg/dL) | 2.48 [2.17, 2.91] | 1.53 [1.38, 1.73] |
| HCO₃⁻ (17–24 mEq/L) | 20 [18, 22] | 20 [19, 22] |
| iCa (4.76–5.48 mg/dL) | 5.16 [5.00, 5.36] | 5.12 [4.96, 5.27] |
| PCV (30–45%)      | 34 [30, 38] | 37 [33, 40] |
| Venous pH (7.21–7.44) | 7.36 [7.31, 7.39] | 7.36 [7.32, 7.39] |
| Phosphate (2.79–6.81 mg/dL) | 3.93 [3.41, 5.05] | 3.60 [3.14, 4.11] |
| Potassium (3.5–5.5 mEq/L) | 3.7 [3.5, 4.0] | 3.7 [3.4, 3.9] |
| Sodium (145–157 mEq/L) | 151 [150, 153] | 151 [150, 152] |
| tCa (8.2–11.8 mg/dL) | 10.2 [9.7, 10.5] | 9.7 [9.4, 10.2] |
| Total protein (6.0–8.0 g/dL) | 7.6 [7.3, 8.0] | 7.7 [7.3, 8.1] |
| USG (≥1.035) | 1.018 [1.015, 1.021] | 1.043 [1.030, 1.050] |

CKD, chronic kidney disease; n, number of cats; HCO₃⁻, venous bicarbonate; iCa, ionized calcium; tCa, total calcium; USG, urine specific gravity.
With binary logistic regression analysis, lower HCO₃⁻ was identified as a significant risk factor for underestimation of calcium status if the azotemic and nonazotemic ionized hypercalcemic cats were combined for analysis (OR, 0.74; 95% CI, 0.55–0.93; \(P = 0.007\)), but not when the CKD group was analyzed separately (\(n = 47; P = 0.062\)).

The standardized tCa was lower than the standardized iCa in 171 cats with CKD and 99 nonazotemic cats. Linear regression analysis was performed to assess what variables influenced plasma tCa in these underestimated cases. Venous HCO₃⁻, and plasma creatinine and albumin concentrations were associated with plasma tCa at the 10% level in both groups (Table 2). In the multivariable models adjusted for iCa, HCO₃⁻, creatinine, and albumin concentrations remained significant in the CKD group, and albumin and HCO₃⁻ concentrations in the nonazotemic group.

**Evaluation of Risk of Incident Total Hypercalcemia in Cats with and Without Renal Azotemia**

For the observational cohort, 2,198 cats were identified, of which 1,818 were excluded from analysis because of insufficient follow-up (\(n = 566\)), suspect or documented hyperthyroidism during follow-up (\(n = 1251\)), and corticosteroid administration (\(n = 1\)). Thereafter, 13 cats were excluded for total hypercalcemia at baseline. In total, 367 cats were included: 176 nonazotemic cats, of which 10 developed total hypercalcemia, and 191 cats with a diagnosis of azotemic CKD, of which 60 developed total hypercalcemia. Most common breeds were domestic shorthair (\(n = 270\)) followed by domestic longhair (\(n = 41\)), purebreds or cross-breeds of Burmese (\(n = 15\)), Persian (\(n = 14\)), Siamese (\(n = 6\)), British shorthair (\(n = 5\)), British blue (\(n = 3\)), 2 each of Russian blue, Tiffany, Bengal, and Exotic shorthair, and 1 each of Abyssinian, Maine Coon, American shorthair, Devon rex, and Ocicat. Of the 191 cats with

**Table 2.** Univariable and multivariable linear regression models to identify predictors of plasma total calcium concentration in azotemic and nonazotemic cats with underestimated ionized calcium concentration.

|                      | Univariable Results |             |             | Multivariable Results |             |             |
|----------------------|---------------------|-------------|-------------|-----------------------|-------------|-------------|
|                      | \(\beta\) (95% CI)  | \(P\)       | \(\beta\) (95% CI) | \(P\)       |
| **CKD cats (\(n = 171\))** |                     |             |             |                       |             |             |
| Ionized calcium      | 1.89 (1.65–2.13)    | <.001       | 1.74 (1.50–1.97) | <.001       |
| (mg/dL)              |                     |             |             |                       |             |             |
| HCO₃⁻ (mEq/L)        | 0.08 (0.03–0.13)    | .002        | 0.06 (0.03–0.09) | <.001       |
| Creatinine (mg/dL)   | 0.30 (0.09–0.51)    | .005        | 0.23 (0.09–0.38) | .001        |
| Albumin (g/dL)       | 0.62 (0.15–1.09)    | .010        | 0.48 (0.17–0.78) | .002        |
| **Nonazotemic cats (\(n = 99\))** |                     |             |             |                       |             |             |
| Ionized calcium      | 2.03 (1.71–2.35)    | <.001       | 1.92 (1.66–2.19) | <.001       |
| (mg/dL)              |                     |             |             |                       |             |             |
| Albumin (g/dL)       | 0.87 (0.49–1.26)    | <.001       | 0.68 (0.47–0.89) | <.001       |
| HCO₃⁻ (mEq/L)        | 0.05 (0.00–0.11)    | .065        | 0.04 (0.02–0.07) | .002        |
| Creatinine (mg/dL)   | 0.52 (0.02–1.02)    | .042        |                     |             |

CKD, chronic kidney disease; \(\beta\), regression coefficient; 95% CI, 95% confidence interval; HCO₃⁻, venous bicarbonate; \(P\), significance.
CKD, 143 had IRIS stage 2, 46 IRIS stage 3, and 2 IRIS stage 4 CKD. Forty-six of the cats that developed total hypercalcemia were part of the study population used for the cross-sectional analysis, with 3 visits being identical. Baseline values of clinicopathologic variables can be found in Table 3. Baseline iCa was higher in both nonazotemic and azotemic cats that developed total hypercalcemia during follow-up, and iCa was higher in the CKD-HCa group. Baseline plasma intact PTH concentration was lower in cats that developed total hypercalcemia. Plasma creatinine concentration was 2.44 [2.23, 3.07] mg/dL, and a >25% increase compared to baseline was observed in 8 cats. Parathyroid hormone concentration was <2.6 pg/mL in 7 and 8.0 pg/mL in 1 cat, and decreased compared to baseline in 5 cats (baseline median, 21.5 [9.0, 47.8] pg/mL). Five cats were treated PO with aluminum hydroxide. Concurrent findings with total hypercalcemia were abdominal masses (n = 2), calcium oxalate crystalluria (n = 2), and ureterolithiasis (n = 1).

The incidence rate of total hypercalcemia was 0.03 per patient-year for the nonazotemic group and 0.18 per patient-year for the CKD group. Risk of total hypercalcemia within the first year from baseline was 2.3% for nonazotemic cats and 23.4% for CKD cats. The Kaplan-Meier curves (Fig 2A) were significantly different between the 2 groups (P < .001), and cats with azotemic CKD were at significantly higher risk of developing total hypercalcemia (HR, 6.66; 95% CI, 3.41–13.02; P < .001). When adjusted for frequency of sampling, the study population included 174 nonazotemic cats, of which 8 developed total hypercalcemia, and 172 CKD cases, of which 30 cats developed hypercalcemia. The incidence rate of total hypercalcemia was 0.02 per patient-year for the nonazotemic group and

### Table 3. Clinicopathological variables for cats included in the retrospective cohort study grouped according to renal status and development of total hypercalcemia.

| Variable (Reference Interval) | H-NCa (n = 166) | H-HCa (n = 10) | CKD-NCa (n = 131) | CKD-HCa (n = 60) |
|-----------------------------|----------------|---------------|-------------------|-----------------|
| Age (years)                 | Median [25th, 75th Percentile] | Median [25th, 75th Percentile] | Median [25th, 75th Percentile] | Median [25th, 75th Percentile] |
| Weight (kg)                 | 4.43 [3.53, 5.29] | 4.29 [3.71, 5.76] | 3.94 [3.39, 4.71] | 3.41 [3.21, 4.94] |
| % Male                      | 49 | 166 | 52 | 131 |
| % Longhair                  | 18 | 166 | 15 | 131 |
| Albumin (2.5-4.5 g/dL)      | 3.2 [3.1, 3.4] | 3.4 [3.2, 3.5] | 3.1 [3.0, 3.3] | 3.1 [2.9, 3.4] |
| Calcidiol (65-170 nmol/L)   | 0 | 166 | 0 | 131 |
| Calcitriol (90-342 pmol/L)  | 0 | 166 | 418.0 [366.0, 447.0] | 15 |
| Chloride (100-124 mEq/L)    | 119 [117, 120] | 118 [117, 120] | 118 [116, 121] | 118 [116, 120] |
| Creatinine 1.47 [1.34, 1.70] | 166 | 1.59 [1.34, 2.04] | 2.37 [2.13, 2.68] | 2.42 [2.19, 2.90] |
| FGF-23 (56-700 pg/mL)       | 164 [108, 243] | 261 [221, 604] | 491 [283, 1035] | 518 [342, 1701] |
| iCa (4.76-5.48 mg/dL)       | 5.08 [5.00, 5.24] | 5.16 [5.02, 5.32] | 5.16 [5.02, 5.32] | 5.34 [5.20, 5.44] |
| PCV (30-45%)                | 38 [34, 40] | 39 [36, 42] | 35 [31, 38] | 39 [30, 39] |
| Venous pH (7.21-7.44)       | 7.35 [7.32, 7.38] | 7.35 [-] | 7.36 [7.31, 7.39] | 7.35 [7.31, 7.37] |
| Phosphate 3.84 [3.35, 4.37] | 166 | 3.53 [3.18, 4.46] | 4.03 [3.50, 4.84] | 4.12 [3.43, 4.95] |

| Variable                     | Median [25th, 75th Percentile] | Median [25th, 75th Percentile] | Median [25th, 75th Percentile] | Median [25th, 75th Percentile] |
|-----------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Potassium (3.5-5.5 mEq/L)   | 3.9 [3.7, 4.3] | 4.0 [3.8, 4.2] | 4.0 [3.7, 4.3] | 4.0 [3.8, 4.3] |
| PTH (2.6-17.6 pg/mL)        | 7.0 [2.6, 12.0] | 4.6 [2.6, 7.1] | 15.0 [8.7, 27.7] | 7.9 [2.6, 22.0] |
| SBP (<160 mmHg)             | 136 [122, 152] | 134 [121, 159] | 142 [126, 158] | 131 [123, 156] |
| Sodium (145-157 mEq/L)      | 152.3 [150.9, 153.8] | 151.9 [150.5, 153.9] | 152.5 [150.8, 154.6] | 152.9 [150.0, 154.6] |
| iCa (8.2-11.8 mg/dL)        | 7.8 [7.5, 8.1] | 7.5 [7.1, 8.1] | 7.8 [7.4, 8.1] | 7.7 [7.4, 8.1] |
| Total protein (6.0-8.0 g/dL) | 7.6 [7.3, 8.1] | 7.5 [7.1, 8.1] | 7.8 [7.4, 8.1] | 7.7 [7.4, 8.1] |
| Urea (7.0-27.7 mg/dL)       | 29.1 [24.9, 32.8] | 27.8 [24.3, 33.8] | 47.3 [39.9, 55.7] | 45.4 [37.7, 55.6] |
| USG (≥0.035)                | 1.044 [1.031, 1.056] | 1.060 [1.041, 1.070] | 1.020 [1.016, 1.022] | 1.020 [1.016, 1.024] |
| Follow-up (days)            | 732 [516, 1023] | 386 [166, 542] | 679 [490, 1023] | 137 [63, 403] |

| H-NCa, apparently healthy-normocalcemic; H-HCa, apparently healthy-developed hypercalcemia; CKD-NCa, CKD-normocalcemic; CKD-HCa, CKD-developed hypercalcemia; n, number of cats; FGF-23, fibroblast growth factor 23; PTH, parathyroid hormone; SBP, systolic blood pressure; USG, urine specific gravity; P, significance.
| Rows bearing a different superscript letter are significantly different from one another. |
Results of our retrospective study show that cats with azotemic CKD were more likely to develop total hypercalcemia than nonazotemic cats. Higher baseline tCa
predicted incident total hypercalcemia in both azotemic and nonazotemic animals. At any time, higher plasma tCa and creatinine concentrations were associated with incident total hypercalcemia in cats with azotemic CKD. The majority of cats with total hypercalcemia had concomitant ionized hypercalcemia. Conversely, underestimation of iCa by tCa was common and associated with lower venous HCO₃⁻, and plasma albumin and creatinine concentrations.

Although the biologically active ionized calcium fraction most accurately reflects true calcium status, tCa usually is relied on in practice. Total calcium concentration only shows moderate association with iCa in cats. The correlation of plasma iCa to whole blood iCa found in this study ($r = 0.63$) is similar to the previously reported correlation for cats with CKD ($r = 0.68$). This association was found to be higher in the general population ($r = 0.87$), but here the association in nonazotemic cats ($r = 0.62$) was similar to that in cats with renal azotemia. Differences in age and sample handling could have accounted for this observation. Age was not a selection criterion in the previous study, whereas nonazotemic cats in our study were ≥9 years. Both analytes were measured in serum in the previous study, whereas in our study iCa was measured in whole blood directly after sampling, and tCa was measured in heparinized plasma.

In contrast to the concept that hypercalcemia in cats with CKD generally represents an increase in tCa only, the majority of cats with hypercalcemia based on iCa in our study had a coexistent increase in iCa. Specificity for tCa to detect ionized hypercalcemia was close to 100%, which is in agreement with high specificity values reported previously for cats and humans with CKD, but dissimilar to the situation in dogs with CKD, in which tCa tends to overestimate iCa.

A large proportion of ionized hypercalcemic cats had a normal tCa in the cross-sectional study. The ROC curve analysis showed that the possibility of ionized hypercalcemia should be considered if tCa is within the upper half of the reference interval. Low sensitivity of tCa to detect ionized hypercalcemia has been reported previously in humans and cats with CKD, and was associated with lower total calcium and albumin concentrations in human CKD patients. Our study identified lower HCO₃⁻ as a risk factor for failure of tCa to detect ionized hypercalcemia in cats. Noncorrected tCa previously was shown to be influenced by HCO₃⁻ and albumin concentrations in human CKD patients with underestimated iCa. In agreement with this finding, HCO₃⁻, albumin, and creatinine were predictors of tCa in cats with CKD. These associations were independent of iCa, and lower concentrations of these analytes could therefore increase the risk for underestimation of iCa.

Carbonate concentration, reflecting acid-base status, affects plasma calcium concentration by influencing bone storage, protein binding, and tubular reabsorption of calcium. Metabolic acidosis stimulates osteoclastic bone resorption but decreases the protein-bound calcium fraction as calcium and hydrogen ions compete for albumin binding sites. Free and complexed calcium concentrations could possibly increase as a result, but these ultrafilterable fractions are under control of renal regulation. Indeed, metabolic acidosis was associated with increased urinary calcium excretion in dogs, whereas iCa did not appear affected. However, if renal calcium excretion were to be impaired, ionized hypercalcemia could occur in conjunction with total hypercalcemia. This association between creatinine and tCa supports a role for metabolic acidosis, because its prevalence increases with a decrease in kidney function. Conversely, a higher HCO₃⁻ is expected to increase tCa by stimulation of tubular calcium reabsorption and increased protein binding, as reflected by the iCa-independent association between HCO₃⁻ and tCa found in the cross-sectional analysis.

Fig 3. Receiver operating characteristic (ROC) curves illustrating the predictive ability of different baseline plasma tCa for development of total hypercalcemia in (A) chronic kidney disease (CKD) and (B) nonazotemic cases. A large proportion of ionized hypercalcemic cats had a normal tCa in the cross-sectional study.
A lower albumin concentration would decrease the protein-bound calcium fraction and was found to increase risk of underestimation. Historically, correction formulas were developed in an attempt to adjust tCa for albumin binding in humans and dogs, but not for cats because of the particularly weak association between calcium and albumin in this species. Correction formulas currently are considered obsolete due to poor performance.

The finding that creatinine was independently associated with tCa in CKD cats could be indicative not only of a role for metabolic acidosis, but also for increased availability of calcium-binding anions. In dogs with CKD, tCa is mainly influenced by increases in the complexed calcium fraction, which underlies the tendency of tCa to overestimate the prevalence of ionized hypercalcemia in that species. Data on the plasma concentrations of most calcium-binding anions were not available for our study, except for phosphate, which showed no association with risk of underestimation ($P = .802$).

Results from our retrospective cohort study show that cats with CKD are at risk for the development of total hypercalcemia. Sixty of 191 cats (31.4%) developed total hypercalcemia at a median of 175 [63, 403] days after diagnosis of azotemic CKD. Total hypercalcemia previously was reported with a prevalence of 10–32% of cats with CKD and incident hypercalcemia, with both tCa and iCa increased, in 2 of 15 cats with idiopathic hypercalcemia.16,17 Data on the plasma concentrations of most calcium-binding anions were not available for our study, except for phosphate, which showed no association with risk of underestimation ($P = .802$).

The 3 most common causes of hypercalcemia in human CKD patients are neoplasia, hyperparathyroidism, and milk-alkali syndrome. In a previous publication on total hypercalcemia in cats, 6 of 33 azotemic cats had concurrent neoplastic disease, and 9 had concurrent hyperparathyroidism. In our study, 7 of 20 cats with concurrent neoplasia had hyperparathyroidism, and 1 cat had hyperparathyroidism and calcium oxalate crystalluria were identified in 2 cases each. Ionized hypercalcemia in cats with CKD is thought to occur secondary to severe renal hyperparathyroidism, or as a result of administration of vitamin D metabolites (eg, calcitriol). Hypervitaminosis D appears to be an unlikely cause of total hypercalcemia in our study because none of the cats were known to have been exposed to or had been treated with vitamin D analogues or metabolites. Furthermore, plasma phosphate concentrations were generally within the reference interval. Severe hyperparathyroidism cannot be ruled out. Plasma PTH concentrations were not available in most cats at the hypercalcemic visit. In cats with total hypercalcemia that had PTH measured, concentrations were low and generally had decreased compared to baseline. In a previous study, plasma PTH concentrations of cats that developed hypercalcemia after diagnosis of CKD decreased to below the lowest limit of detection of the assay.18 Additionally, a diagnosis of hyperparathyroidism was shown to increase with severity of CKD previously, from 8% in early stage kidney disease to 32% in end-stage CKD. As discussed above, a decline in kidney function could contribute to an increase in calcium-containing organic complexes, as has been shown in dogs with CKD. Metabolic acidosis was found with increasing frequency in cats as renal function declines. In rodent models, metabolic acidosis has been documented to stimulate osteoclastic bone resorption, releasing calcium carbonate into the circulation. Increased bone resorption and decreased bone mineral density are known consequences of CKD in cats. Interestingly, a similar finding has been shown that hypochloremia develops before manifestation of metabolic acidosis in cats with CKD, possibly in an attempt to maintain $\mathrm{HCO}_3^-$ concentrations within normal limits. In our study, lower baseline plasma chloride concentrations appeared associated with incident total hypercalcemia with a type I error <10% in univariable analysis ($P = .089$). Therefore, an increased
requirement for skeletal buffering of acid could be related to total hypercalcemia in cats with CKD. Further work is warranted to test this hypothesis.

By contrast, milk-alkali syndrome is a form of hypercalcemia in CKD associated with metabolic alkalosis, often caused by ingestion of calcium carbonate. Alkali ingestion, tubular reabsorption of HCO₃⁻, and suppression of PTH contribute to metabolic alkalosis, which in turn leads to increased tubular calcium reabsorption. Cats in the retrospective cohort were not treated with calcium carbonate, and classic milk-alkali syndrome is unlikely to have caused total hypercalcemia. However, HCO₃⁻ was positively associated with tCa in the cross-sectional analysis, and it could be hypothesized that higher tCa could in part be due to bicarbonate-induced increases in tubular calcium reabsorption.

Our study had a number of limitations because of its retrospective design. Cats ate a wide variety of commercial cat foods at baseline, and all CKD cats were offered a phosphate-restricted renal diet for management of their disease, but precise data on what proportion of the renal diet was fed and other dietary contributions often were unavailable. Data on dietary calcium and vitamin D concentrations were not available. Therefore, no conclusions could be drawn on any possible dietary interaction.

Although no diagnosis other than azotemic CKD was made in the majority of primary care cases presented, concurrent conditions including occult neoplasia and hyperparathyroidism, cannot be ruled out because diagnostic tests other than biochemistry and urinalysis were not consistently performed. Lack of blood gas data prohibited evaluation of the effect of acid-base disturbances on development of total hypercalcemia. Classification was based purely on tCa. Because of low sensitivity of tCa to detect ionized hypercalcemia, ionized hypercalcemic cats likely were misclassified in the normocalcemic groups. Although our study focused on total hypercalcemia, factors affecting tCa could be expected to influence iCa, and differences between cats that remained normocalcemic and cats that developed total hypercalcemia could have been missed due to misclassification.

Our study highlights the importance of hypercalcemia in cats with CKD because total hypercalcemia developed in 60 of 191 cats (31.4%). The supposition that hypercalcemia in cats with CKD represents total hypercalcemia only, with the biologically active ionized calcium fraction adequately regulated and within its reference interval, should be questioned. Supported by the high specificity reported here and previously, most cats with total hypercalcemia are suspected to have ionized hypercalcemia. Sensitivity of tCa to detect ionized hypercalcemia is low, and therefore the necessity for accurate assessment of calcium status in cats. In human medicine, increased serum calcium concentrations as part of chronic kidney disease-mineral and bone disorder (CKD-MBD) have been associated with vascular calcification, myocardial infarction, cardiovascular morbidity, and increased mortality. Similarly, in cats, previous case reports have identified calcification of the aorta, gastric wall, paws, and kidneys associated with a high calcium-phosphorus product, although the full extent of soft tissue mineralization as a component of CKD-MBD in cats remains to be fully elucidated. Higher baseline tCa did not predict all-cause mortality in cats with CKD, but underdiagnosis of ionized hypercalcemia may have impacted these results and therefore the full effect of hypercalcemia on prognosis in cats with CKD remains to be determined. Prospective studies that longitudinally assess iCa throughout the course of CKD are warranted to further understand both the biochemical and clinical consequences of calcium derangements in cats with CKD.

**Footnotes**

a Idexx laboratories, Wetherby, UK
b i-STAT 1 point-of-care analyzer, Abbott Point of Care Inc., Princeton, NJ
c FGF-23 ELISA Kit, Kainos Laboratories, Tokyo, Japan
d Total intact PTH immunoradiometric assay—coated bead version, 3KGr600, Scantibodies, Santee, CA
e Michigan State University Diagnostic Center for Population and Animal Health, Lansing, MI
f R 1386 3.1.5, R Foundation for Statistical Computing, Vienna, Austria, and GraphPad Prism 6, GraphPad Software, La Jolla, CA
gh Schenck PA, Chew DJ, Refsal K, Nachreiner R, Rick M. Calcium metabolic hormones in feline idiopathic hypercalcemia. *J Vet Intern Med* 2004; 18: 442 (abstract)
hi Geddes RF, Forcada Y, Catchpole B, Elliott J, Syme HM. Eight novel polymorphisms identified in the feline calcium sensing receptor in cats with varying plasma ionised calcium concentrations. *J Vet Intern Med* 2013; 27: 687 (abstract)

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

1. Barber PJ, Elliott J. Feline chronic renal failure: calcium homeostasis in 80 cases diagnosed between 1992 and 1995. J Small Anim Pract 1998;39:108–116.
2. Savary KC, Price GS, Vaden SL. Hypercalcemia in cats: a retrospective study of 71 cases (1991–1997). J Vet Intern Med 2000;14:184–189.
3. DiBartola SP, Rutgers HC, Zack PM, et al. Clinico-pathologic findings associated with chronic renal disease in cats: 74 cases (1973–1984). J Am Vet Med Assoc 1987;190:1196–1202.
4. Middkiff AM, Chew DJ, Randolph JF, et al. Idiopathic hypercalcemia in cats. J Vet Intern Med 2000;14:619–626.
5. Lulich JP, Osborne CA, O’Brien TD, et al. Feline renal-failure - questions, answers, questions. Compend Cont Educ Vet 1992;14:127.
6. Marino CL, Lascelles BD, Vaden SL, et al. Prevalence and classification of chronic kidney disease in cats randomly selected from four age groups and in cats recruited for degenerative joint disease studies. J Feline Med Surg 2014;16:465–472.
7. Levi M, Peterson L, Berl T. Mechanism of concentrating defect in hypercalcemia. Role of polydipsia and prostaglandins. Kidney Int 1983;23:489–497.
8. Levi M, Ellis MA, Berl T. Control of renal hemodynamics and glomerular filtration rate in chronic hypercalcemia. Role of prostaglandins, renin-angiotensin system, and calcium. J Clin Invest 1983;71:1624–1632.
9. Peacock M. Calcium metabolism in health and disease. Clin J Am Soc Nephrol 2010;5(Suppl 1):S23–S30.
10. Schenck PA, Chew DJ. Prediction of serum ionized calcium concentration by serum total calcium measurement in cats. Can J Vet Res 2010;74:207–213.
11. Schenck PA, Chew DJ. Calcium: total or ionized? Vet Clin North Am Small Anim Pract 2008;38:497–502, ix.
12. Schenck PA, Chew DJ. Hypercalcemia: a quick reference. Vet Clin North Am Small Anim Pract 2008;38:449–453, viii.
13. Schenck PA. Electrolyte disorders: Ca-P and Mg. In: Ettenger SJ, Feldman EC. Textbook of Veterinary Internal Medicine: Diseases of the Dog and the Cat. St. Louis, MO, USA: Saunders Elsevier; 2010:308.
14. Finch NC. Hypercalcemia in cats: The complexities of calcium regulation and associated clinical challenges. J Feline Med Surg 2016;18:387–399.
15. Gauci C, Moranne O, Fouqueray B, et al. Pitfalls of measuring total blood calcium in patients with CKD. J Am Soc Nephrol 2008;19:1592–1598.
16. Flanders JA, Scarlett JM, Blue JT, et al. Adjustment of total serum calcium concentration for binding to albumin and protein in cats: 291 cases (1986–1987). J Am Vet Med Assoc 1989;194:1609–1611.
17. Biennze D, Jacobs RM, Lumsden JH. Relationship of serum total calcium to serum albumin in dogs, cats, horses and cattle. Can Vet J 1993;34:360–364.
18. Malatesha G, Singh NK, Bharaja A, et al. Comparison of arterial and venous pH, bicarbonate, PCO2 and PO2 in initial emergency department assessment. Emerg Med J 2007;24:569–571.
19. Kelly AM, McAlpine R, Kyle E. Agreement between bicarbonate measured on arterial and venous blood gases. Emerg Med Aust 2004;16:407–409.
20. Geddes RF, Finch NC, Elliott J, et al. Fibroblast growth factor 23 in feline chronic kidney disease. J Vet Intern Med 2013;27:234–241.
21. Williams TL, Elliott J, Syme HM. Calcium and phosphate homeostasis in hyperthyroid cats: associations with development of azotaemia and survival time. J Small Anim Pract 2012;53:561–571.
22. Geddes RF. Calcium-Phosphate Homeostasis in Feline Chronic Kidney Disease. London: Royal Veterinary College, University of London; 2014. PhD Thesis.
23. Must A, Anderson SE. Body mass index in children and adolescents: considerations for population-based applications. Int J Obes 2006;30:590–594.
24. Abd H. Z-scores. Encyclopedia of Measurement and Statistics. Thousand Oaks, CA: Sage; 2007.
25. Schenck PA, Chew DJ. Prediction of serum ionized calcium concentration by use of serum total calcium concentration in dogs. Am J Vet Res 2005;66:1330–1336.
26. Bushinsky DA. Stimulated osteoclastic and suppressed osteoblastic activity in metabolic but not respiratory acidosis. Am J Physiol 1995;268:C80–C88.
27. Lemann J Jr, Litzow JR, Lennon EJ. The effects of chronic acid loads in normal man: further evidence for the participation of bone mineral in the defense against chronic metabolic acidosis. J Clin Invest 1966;45:1608–1614.
28. Burnell JM. Changes in bone sodium and carbonate in metabolic acidosis and alkalosis in the dog. J Clin Invest 1971;50:327–331.
29. Fogh-Andersen N. Albumin/calcium association at different pH, as determined by potentiometry. Clin Chem 1973;23:2122–2126.
30. Morone CC, Wong NL, Sutton RA, et al. Effects of metabolic alkalosis on calcium excretion in the conscious dog. J Lab Clin Med 1983;101:264–273.
31. Costanzo LS. Regulation of calcium and phosphate homeostasis. Adv Physiol Educ 1998;20:S206–S216.
32. Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. Clin J Am Soc Nephrol 2015;10:1257–1272.
33. Sutton RA, Wong NL, Dirks JH. Effects of metabolic acidosis and alkalosis on sodium and calcium transport in the dog kidney. Kidney Int 1979;15:520–533.
34. Kogika MM, Lustoza MD, Notomi MK, et al. Serum ionized calcium in dogs with chronic renal failure and metabolic acidosis. Vet Clin Pathol 2006;35:441–445.
35. Elliott J, Syme HM, Reubens E, et al. Assessment of acid-base status of cats with naturally occurring chronic renal failure. J Small Anim Pract 2003;44:65–70.
36. Berry EM, Gupta MM, Turner SJ, et al. Variation in plasma calcium with induced changes in plasma specific gravity, total protein, and albumin. Br Med J 1973;4:640–643.
37. Payne RB, Little AJ, Williams RB, et al. Interpretation of serum calcium in patients with abnormal serum proteins. Br Med J 1973;4:643–646.
38. Meuten DJ, Chew DJ, Capen CC, et al. Relationship of serum total calcium to albumin and total protein in dogs. J Am Vet Med Assoc 1982;180:63–67.
39. Schenck PA, Chew DJ. Determination of calcium fractionation in dogs with chronic renal failure. Am J Vet Res 2002;64:1181–1184.
40. Barber PJ, Rawlings JM, Markwell PJ, et al. Effect of dietary phosphate restriction on renal secondary hyperparathyroidism in the cat. J Small Anim Pract 1999;40:62–70.
41. Picolos MK, Lavis VR, Orlander PR. Milk-alkali syndrome is a major cause of hypercalcemia among non-end-stage renal disease (non-ESRD) inpatients. Clin Endocrinol 2005;63:566–576.
42. Polzin DJ. Chronic kidney disease. In: Ettenger SJ, Feldman EC, eds. Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat, 7th ed. St. Louis, MO, USA: Saunders; 2010:1990–2021.
43. Schenck PA, Chew DJ, Nagode LA, et al. Disorders of calcium: hypercalcemia and hypocalcemia. In: DiBartola SP. Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice, 4th edn. St. Louis, MO, USA: Saunders; 2012:120–194.
44. Block GA, Wheeler DC, Persky MS, et al. Effects of phosphate binders in moderate CKD. J Am Soc Nephrol 2012;23:1407–1415.
45. Pieper AK, Haffner D, Hoppe B, et al. A randomized crossover trial comparing sevelamer with calcium acetate in children with CKD. Am J Kidney Dis 2006;47:625–635.
46. Rafferty K, Heaney RP. Nutrient effects on the calcium economy: emphasizing the potassium controversy. J Nutr 2008;138:166S–171S.
47. Gal A, Ridge TK, Graves TK. Cloning and sequencing of the calcium-sensing receptor from the feline parathyroid gland. Domest Anim Endocrinol 2010;38:57–61.
48. Cole DE, Vieth R, Trang HM, et al. Association between total serum calcium and the A986S polymorphism of the calcium-sensing receptor gene. Mol Genet Metab 2001;72:168–174.
49. He Y, Han L, Li W, et al. Effects of the calcium-sensing receptor A986S polymorphism on serum calcium and parathyroid hormone levels in healthy individuals: a meta-analysis. Gene 2012;491:110–115.
50. Andress DL. Adynamic bone in patients with chronic kidney disease. Kidney Int 2008;73:1345–1354.
51. Kurz P, Monierfaugere MC, Bognar B, et al. Evidence for abnormal calcium homeostasis in patients with adynamic bone-disease. Kidney Int 1994;46:855–861.
52. Piraino B, Chen T, Puschet JB. Elevated bone aluminum and suppressed parathyroid hormone levels in hypercalcemic dialysis patients. Am J Nephrol 2009;30:114–121.
53. Bertazzolo W, Toscani L, Calcaterra S, et al. Clinicopathological findings in five cats with paw calcification. J Feline Med Surg 2003;5:11–17.
54. Geddes RF, Elliott J, Syme HM. Relationship between plasma fibroblast growth factor-23 concentration and survival time in cats with chronic kidney disease. J Vet Intern Med 2015;29:1494–1501.