Calcitonin Response to Naturally Occurring Ionized Hypercalcemia in Cats with Chronic Kidney Disease

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Background: Hypercalcemia is commonly associated with chronic kidney disease (CKD) in cats.

Objectives: To explore the calcitonin response to naturally occurring ionized hypercalcemia in cats with azotemic CKD, and to assess the relationship of plasma calcitonin with ionized calcium, alkaline phosphatase (ALP), and urinary calcium excretion.

Animals: Thirty-three client-owned cats with azotemic CKD and ionized hypercalcemia from first opinion practice.

Methods: Cohort study. Calcitonin was measured with an immunoradiometric assay in heparinized plasma. Simple correlations were assessed with Kendall’s rank correlation, and the within-subject correlations of calcitonin with ionized calcium and other clinicopathological variables were calculated with a bivariate linear mixed effects model.

Results: Calcitonin concentrations above the lower limit of detection (>1.2 pg/mL; range, 1.7–87.2 pg/mL) were observed in 11 of 33 hypercalcemic cats (responders). Blood ionized calcium concentration did not differ significantly between responders (median, 1.59 [1.46, 1.66] mmol/L) and nonresponders (median, 1.48 [1.43, 1.65] mmol/L; P = 0.22). No evidence was found for calcitonin and ionized calcium to correlate between cats (τ_{b} = 0.14; P = 0.31; n = 33), but significant positive correlation was evident within individual responders over time (within-subject correlation coefficient \( r_{within} \), 0.83; 95% confidence interval [CI], 0.63–0.92). Calcitonin correlated negatively over time with plasma ALP \( (r_{within} = -0.55; 95\% CI, -0.79 to -0.16) \). Derangements in hormones involved in calcium homeostasis, such as secondary renal hyperparathyroidism, are common and occur early in cats as part of CKD-mineral and bone disorder.

Conclusions and Clinical Importance: Calcitonin does not appear to have an important role in calcium metabolism in cats with CKD.

Key words: Azotemia; Calcium; Cat; Renal.

Chronic kidney disease (CKD) is the 7th most frequently encountered disorder in cats in general practice in England,1 with a disease prevalence of 30–80% in cats over 10 years old.2,3 and is commonly associated with hypercalcemia.4–6 The kidneys play an important role in calcium regulation,7,8 but the etiology of hypercalcemia in cats with CKD is not completely understood.9 Derangements in hormones involved in calcium homeostasis, such as secondary renal hyperparathyroidism, are common and occur early in cats as part of CKD-mineral and bone disorder.10–12

Although it is traditionally stated that secondary renal hyperparathyroidism could cause ionized hypercalcemia in cats with CKD,9,13 hypercalcemia often appears parathyroid-independent, with secondary suppression of parathyroid hormone (PTH) secretion.14,15

Calcitonin is released by C-cells of the thyroid in response to an increased serum ionized calcium concentration.16,17 It protects against hypercalcemia by inhibition of bone turnover17–19 and renal reabsorption of calcium,20 but does not appear to have an important physiologic role in all species.21 Only 5 of 18 cats with ionized hypercalcemia had measurable plasma calcitonin concentrations,9 and an increase in plasma calcitonin was observed in just 6 of 13 healthy cats in response to acute hypercalcemia induced by calcium chloride infusion.22 The absence of a calcitonin response could cause cats to be susceptible to the development of hypercalcemia. The kidneys are the most important site for calcitonin degradation.23 Calcitonin concentrations are increased in human CKD patients from stage 3 onwards, although this possibly is caused by C-cell hyperplasia rather than reduced metabolism.24,25 Healthy and azotemic normocalcemic cats generally appear to have undetectable plasma calcitonin concentrations.26,27

To date, no longitudinal studies have been published that explore the relationship between plasma calcitonin

Abbreviations:

| Abbreviation | Description |
|--------------|-------------|
| τ_{b}         | Kendall’s rank correlation coefficient tau-b |
| CKD          | chronic kidney disease |
| FE           | fractional excretion |
| HCO_{3}^-     | venous bicarbonate concentration |
| IRIS         | International Renal Interest Society |
| PTH          | parathyroid hormone |
| r_{within}   | within-subject correlation coefficient |
| SBP          | systolic blood pressure |
| USG          | urine specific gravity |

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and ionized calcium and other clinicopathological variables involved in calcium homeostasis in cats with concurrent naturally occurring ionized hypercalcemia and CKD. Therefore, the aims of our study were to explore these relationships.

**Methods**

**Case Selection**

Cats diagnosed with azotemic CKD and concurrent ionized hypercalcemia between January 2002 and March 2014 were retrospectively identified from the records of 2 first opinion practices in central London (Beaumont Sainsbury Animal Hospital in Camden and People’s Dispensary for Sick Animals in Bow). Azotemic CKD had been diagnosed based on a plasma creatinine concentration $\geq 1.37$ mmol/L in conjunction with a urine specific gravity (USG) $< 1.035$, or a plasma creatinine concentration $> 2$ mg/dL on 2 consecutive visits 2–4 weeks apart. A blood ionized calcium concentration $> 1.37$ mmol/L was classified as hypercalcemia based on a previously derived reference interval in healthy age-matched cats. Cats were included in this study if data on blood ionized calcium concentration were available for at least 2 visits within a 12-month period with blood ionized calcium concentration exceeding 1.37 mmol/L on at least one of these visits, and if stored heparinized plasma samples obtained on those visits were available for measurement of plasma calcitonin concentrations. Cats with medical treatment for hyperthyroidism, clinical signs of hyperthyroidism, plasma total thyroxine concentration $> 40$ nmol/L, previous bilateral thyroidectomy, diabetes mellitus, or receiving glucocorticosteroids or diuretics were excluded from analysis. Cats treated for systemic hypertension with amlopidine besylate were eligible to be included in the study. For all cats with azotemic CKD it was advised they be fed a renal diet and, if necessary, phosphate binders had been added to the treatment. Cats receiving these forms of PO phosphate restriction were eligible for inclusion in this study.

Cats were reexamined approximately every 8 weeks for management of CKD. Clinic visits consisted of full history and physical examination, systolic blood pressure (SBP) measurement, and blood and urine sample collection. Collection and storage of blood and urine samples had been performed with owner consent and approval of the Ethics and Welfare Committee of the Royal Veterinary College. Blood samples were obtained via jugular venipuncture and urine samples via cystocentesis. After ionized calcium concentration and blood gases had been measured directly following venipuncture, ionized calcium concentration and bone-specific ALP is more reliable, but total ALP can be used to assess bone turnover, although it has not been explicitly validated for this purpose in cats. Cats with concurrent abnormal changes in plasma alanine transaminase activity were excluded from this analysis to minimize the impact of liver-specific ALP.

**Comparison of Responders versus Nonresponders**

Clinical data obtained at each cat’s visit with the highest whole blood ionized calcium concentration were used for this part of the study. Comparisons between groups were made either with independent 2-sample $t$-tests for continuous variables with a normal distribution, or with Mann–Whitney $U$-tests for skewed variables or if group size was $< 5$. Proportions were compared with Fisher’s exact test. The hypercalcemic responders were compared to nonresponders primarily to assess group differences in blood ionized calcium concentration, plasma total alkaline phosphatase (ALP) activity, and FE of calcium, and second to identify any difference in other clinicopathological variables that could be related to a calcitonin response. Plasma total ALP was used as a marker of bone formation. Total ALP activity in serum of mature, healthy cats is mainly comprised of liver-specific ALP, followed by bone-specific ALP, a marker of osteoblastic bone formation. Bone-specific ALP is more reliable, but total ALP can be used to assess bone turnover, although it has not been explicitly validated for this purpose in cats. Cats with concurrent abnormal changes in plasma alanine transaminase activity were excluded from this analysis to minimize the impact of liver-specific ALP.

Given that multiple cats had their plasma calcitonin concentration below the lower limit of detection, the relationship between plasma calcitonin concentration and other clinicopathological variables was assessed by calculating the Kendall’s rank correlation coefficient $\tau$-b, which is a preferred nonparametric measure of correlation for analysis of data with ties or small sample size.

**Longitudinal Analysis of Responders**

To characterize the relationship between plasma calcitonin and blood ionized calcium concentration within individual cats over time, data
from available follow-up samples of responders were used to calculate the within-subject correlation of plasma calcitonin with ionized calcium using a bivariate linear mixed effects model. Specifically, unstructured (co)variance types were used between random effects of plasma calcitonin and random effects of ionized calcium as well as between the residuals of these 2 variables. By the same method, the within-subject correlations in responders were calculated between plasma calcitonin concentration and plasma total ALP activity, FE calcium, and other clinicopathological variables involved in calcium homeostasis (plasma total calcium, phosphate, PTH, calcitriol, and calcidiol concentrations, venous pH and bicarbonate concentration [HCO\textsubscript{3}\textsuperscript{−}], and FE phosphate). Plasma calcitonin, PTH and calcitriol were log-transformed before analysis (logarithmus naturalis). No missing data imputation was performed. The assumptions of normality and of linear relationship between variables were checked by visual inspection of histograms of the residuals and scatter plots of the residuals against the fitted values. Results are presented as within-subject correlation coefficient ($r_{\text{within}}$; 95% confidence intervals [95% CI]). Correlations were considered statistically significant if the 95% CI did not include zero.

**Results**

Between January 2002 and March 2014, 68 individual cats with azotemic CKD were diagnosed with ionized hypercalcemia during the course of their CKD. Thirty-five cats were excluded from this study because of concurrent hyperthyroidism (n = 7), previous bilateral thyroidectomy (n = 1), insufficient follow-up (n = 20), or not having a stored residual plasma sample available for measurement of calcitonin (n = 7), allowing 33 cats for analysis. These cats were of the following breeds: domestic shorthair (n = 23), domestic longhair (n = 3), Persian (n = 2), Burmese (n = 2), and 1 each of Abyssinian, British blue, and Cornish rex. Seventeen cats were of male sex, of which 1 was entire, and 16 cats were female neutered. According to International Renal Interest Society guidelines (http://iris-kidney.com/guidelines/staging.html), 26 cats had Stage 2 CKD, 6 had Stage 3 CKD, and 1 had Stage 4 CKD. Three cats were treated PO with aluminum hydroxide, 1 cat with chitosan, and 5 cats with amlodipine besylate.

Based on each cat’s visit with the highest ionized calcium concentration, median whole blood ionized calcium concentration of the 33 hypercalcemic cats was 1.50 [1.45, 1.65] mmol/L (range, 1.39–1.93). Followed over time, no response of calcitonin to ionized hypercalcemia could be detected in 22 cats (nonresponders; Fig 1), as plasma calcitonin concentrations were persistently below the lower limit of detection on all available visits (58 clinic visits: 8 cats with 2 visits and 14 cats with 3 visits available). Measurable plasma concentrations of calcitonin were observed in 11 of 33 hypercalcemic cats, and the highest plasma calcitonin concentrations (median, 4.7 [2.1, 28.4] pg/mL; range, 1.7–87.2) coincided with the highest ionized calcium concentrations in these responders (31 available visits: 4 cats with 2, 5 cats with 3, and 2 cats with 4 visits available). In 3 responders, measurable plasma calcitonin concentrations were observed not only while being hypercalcemic but also while their ionized calcium concentration was within reference interval.

**Comparison of Responders versus Nonresponders**

No evidence was found for a relationship between the frequency of showing a calcitonin response and IRIS stage ($P = 0.76$), with 10 of 26 cats with IRIS Stage 2 CKD and 1 of 6 cats with IRIS Stage 3 CKD showing a response of calcitonin to ionized hypercalcemia. Six of 11 responders were male, and no relationship was found between sex and the frequency of showing a calcitonin response by Fisher’s exact test ($P = 1$). The Mann–Whitney $U$-test indicated that the median maximum ionized calcium concentration of responders (median, 1.59 [1.46, 1.66] mmol/L; range, 1.42–1.93) was not significantly different from that of the 22 nonresponders (median, 1.48 [1.43, 1.65] mmol/L; range, 1.39–1.84; $P = 0.22$; Table 1). Mean plasma total ALP activity was not significantly different between nonresponders (mean, 26 IU/L; SD, 9.6; n = 18) and responders (mean, 22 IU/L; SD, 2.8; n = 8; $P = 0.13$). Median FE of calcium values did not differ significantly between groups ($P = 0.11$; Fig 2), although could be calculated only for a small number of cats because availability of urine samples was limited to 7 nonresponders and 4 responders. Responders did not differ significantly from nonresponders in any clinicopathological variable other than a higher HCO\textsubscript{3}\textsuperscript{−} (responders: mean, 22 mEq/L; SD, 2.1; n = 11, and nonresponders: mean, 19 mEq/L; SD, 3.5; n = 20; $P = 0.034$). Storage time of plasma samples was not significantly different between responders (median, 925 [724, 1,561] days) and nonresponders (median, 1,080 [775, 2,353] days; $P = 0.35$).

No correlation between plasma calcitonin and blood ionized calcium concentration was evident in hypercalcemic cats ($r_{\text{bivariate}} = -0.14; P = 0.31; n = 33$, Fig 3), nor in the group of responders when analyzed separately ($r_{\text{bivariate}} = -0.22; P = 0.35; n = 11$). Plasma calcitonin did not correlate either with plasma total ALP ($r_{\text{bivariate}} = -0.10; P = 0.53; n = 26$) or FE of calcium ($r_{\text{bivariate}} = 0.42; P = 0.10; n = 11$), nor with any other clinicopathological variable (results not shown).

**Longitudinal Analysis of Responders**

Within individual responders, changes in plasma calcitonin concentration were generally paralleled by changes in blood ionized calcium concentration over time (Fig 4). The within-subject correlation coefficient of plasma calcitonin and ionized calcium was 0.83 (95% CI, 0.63–0.92; n = 11), which is considered statistically significant as the 95% CI did not include zero. Plasma calcitonin moreover showed a statistically significant positive within-subject correlation with plasma total calcium (0.81; 95% CI, 0.59–0.92; n = 11), and a significant inverse within-subject correlation with plasma total ALP (−0.55; 95% CI, −0.79 to −0.16; n = 8). Limited information was available for calculation of the within-subject correlation coefficient of calcitonin with FE of calcium, which was apparent by the wide CI that included zero (0.46; 95% CI, −0.08 to 0.79; n = 4). Results from the bivariate linear mixed effects models can be found in Table 2.

**Discussion**

In our retrospective study a measurable increase in plasma calcitonin concentration in response to ionized...
hypercalcemia occurred in a third of cats with azotemic CKD. In this group of responders, a positive relationship between plasma calcitonin and blood ionized calcium concentration and an inverse relationship between plasma calcitonin concentration and total ALP activity appeared to exist within individual cats over time. No difference in severity of ionized hypercalcemia was observed between cats that showed an increase in plasma calcitonin and their non-responding counterparts.

Plasma calcitonin concentrations were generally low in cats with azotemic CKD that developed ionized hypercalcemia. Previously we reported low plasma calcitonin concentrations in normocalcemic cats with renal azotemia (< 1.2 pg/mL in all 15 cats) and in hyperthyroid cats (≥1.2 pg/mL in 4 of 37 cats with a maximum concentration of 2.4 pg/mL). Others

Table 1. Selected clinicopathological variables of hypercalcemic cats grouped according to whether a calcitonin response to ionized hypercalcemia was observed (responders, n = 11) or not (nonresponders, n = 22).

| Variable (reference interval) | Nonresponders (n = 22) | Responders (n = 11) |  
|------------------------------|------------------------|---------------------|-----------------|
|                              | Median [25th, 75th Percentile] | n   | Median [25th, 75th Percentile] | n   | P     |
| Calcitonin (pg/mL)           | 0.6 [0.6, 0.6]         | 22  | 4.7 [2.1, 28.4]               | 11  |       |
| Ionized calcium (1.19–1.37 mmol/L) | 1.48 [1.43, 1.65]     | 22  | 1.59 [1.46, 1.66]             | 11  | 0.22  |
| Total ALP (≤60 IU/L)         | 26 [16, 32]            | 18  | 22 [19, 23]                   | 8   | 0.13  |
| FE calcium (%)               | 0.73 [0.19, 1.15]      | 7   | 1.16 [1.07, 1.60]             | 4   | 0.11  |
| Age (years)                  | 13.4 [11.7, 16.3]      | 22  | 12.5 [10.1, 16.6]             | 11  | 0.85  |
| Weight (kg)                  | 4.08 [3.29, 4.71]      | 22  | 4.51 [3.14, 4.85]             | 11  | 0.64  |
| Albumin (2.5–4.5 g/dL)       | 3.23 [2.97, 3.36]      | 21  | 3.10 [2.94, 3.41]             | 11  | 0.57  |
| Creatinine (0.23–2.00 mg/dL) | 2.2 [2.0, 2.9]         | 21  | 2.7 [2.3, 2.7]                | 11  | 0.39  |
| USG (≥1.055)                 | 1.021 [1.018, 1.026]   | 10  | 1.019 [1.017, 1.022]          | 6   | 0.29  |
| Phosphate (2.79–6.81 mg/dL)  | 4.27 [3.72, 4.57]      | 21  | 3.93 [3.47, 4.03]             | 11  | 0.061 |
| FE phosphate (%)             | 29 [19, 52]            | 7   | 29 [23, 38]                   | 4   | 0.79  |
| tCa (8.2–11.8 mg/dL)         | 11.7 [11.1, 12.6]      | 21  | 12.5 [11.4, 13.0]             | 11  | 0.40  |
| Total protein (6.0–8.0 g/dL) | 7.85 [7.53, 8.37]      | 21  | 7.85 [7.48, 8.32]             | 11  | 0.78  |
| PTH (2.6–17.6 pg/mL)         | 2.6 [2.6, 2.6]         | 7   | 2.6 [2.6, 2.6]                | 4   | 1     |
| Calciol (65–170 nmol/L)      | 85 [78, 149]           | 5   | 139 [79, 159]                 | 4   | 0.56  |
| Calcitriol (90–342 pmol/L)   | 108 [65, 161]          | 5   | 132 [59, 170]                 | 4   | 0.84  |
| HCO$_3^-$ (17–24 mEq/L)      | 19 [17, 22]            | 20  | 22 [21, 23]                   | 11  | 0.034 |
| Venous pH (7.21–7.44)        | 7.34 [7.27, 7.38]      | 20  | 7.33 [7.32, 7.38]             | 11  | 0.46  |
| PCV (30–45%)                 | 35 [30, 38]            | 22  | 33 [31, 36]                   | 11  | 0.51  |
| Sodium (145–157 mEq/L)       | 152 [151, 155]         | 19  | 155 [153, 157]                | 10  | 0.33  |
| Potassium (3.5–5.5 mEq/L)    | 4.1 [3.7, 4.4]         | 19  | 4.3 [4.0, 4.4]                | 10  | 0.59  |
| Chloride (100–124 mEq/L)     | 120 [118, 123]         | 19  | 119 [118, 121]                | 10  | 0.58  |
| SBP (<160 mmHg)              | 138 [118, 145]         | 22  | 142 [120, 146]                | 11  | 0.88  |

Values are presented as median [25th, 75th percentile], and were derived at each cat’s available visit with the highest ionized calcium concentration. Group comparisons were made by independent sample t-test or Mann–Whitney U-test. All cats had azotemic CKD. ALP, alkaline phosphatase; FE, fractional excretion; USG, urine specific gravity; PTH, parathyroid hormone; HCO$_3^-$, bicarbonate; PCV, packed cell volume; SBP, systolic blood pressure.

Fig 1. Changes in blood ionized calcium concentrations in the subgroup of nonresponders over time. Even though blood ionized calcium concentration exceeded 1.37 mmol/L on at least one visit in all 22 cats, plasma calcitonin concentration remained below the lower limit of detection of the immunoradiometric assay (1.2 pg/mL) throughout follow-up.

Fig 2. Scatter dot plot illustrating the fractional excretion (FE) values of calcium in the subgroups of responders (n = 4) and nonresponders (n = 7). Fractional excretion values were obtained by the spot sample approach. The Mann–Whitney U-test indicated that the median fractional excretion values of calcium did not differ significantly between the 2 groups (P = 0.11).
have observed low plasma calcitonin values in normocalcemic healthy cats (maximum, 3.2 pg/mL). Low circulating calcitonin concentrations (<10 pg/mL) are considered normal in healthy humans, but up to 30% of human CKD patients have increased plasma calcitonin concentrations in the absence of hypercalcemia. This secondary hypercalcitoninemia could either be explained by decreased degradation, as calcitonin is predominantly degraded by the kidneys, or more likely, by increased secretion, as C-cell hyperplasia is commonly associated with hypercalcitoninemia in humans with CKD. If secondary hypercalcitoninemia was to occur in feline CKD, this may not have been identified in our study because the majority of cats had IRIS Stage 2 CKD, whereas secondary hyperplasia might arise only in more advanced disease.

The foremost function of calcitonin is thought to be lowering of blood calcium concentration, and so to protect against hypercalcemia. However, a calcitonin response appears to be absent in the majority of cats with azotemic CKD as only a 3rd showed an increase in plasma calcitonin in response to naturally occurring ionized hypercalcemia. Previously, 6 of 13 cats responded to acute hypercalcemia in an experimental study (maximum plasma calcitonin concentration, 43.5 pg/mL) and measurable calcitonin values were observed in 5 of 18 cats included in a cross-sectional study of cats with naturally occurring ionized hypercalcemia (maximum, 22.9 pg/mL). Moreover, loss of calcitonin secretory ability following bilateral thyroidectomy does not result in hypercalcemia in cats. Absence of a calcitonin response has also been observed in humans with experimentally induced hypercalcemia, predominantly in women. Except for higher numbers of calcitonin-positive C-cells in thyroid tissue of cats that showed a calcitonin response, the above-mentioned experimental study found no difference in sex or any other characteristic between responders and nonresponders. In agreement with this, no clear differences between the 2 groups were identified in our population of hypercalcemic cats with azotemic CKD. It could be speculated that individual variation in the number of calcitonin-expressing C-cells explains why some cats appear able to increase plasma calcitonin in response to hypercalcemia, while others appear unable to, and it is interesting in this respect that nonresponders in our study did not have measurable plasma calcitonin concentrations at any visit. However, no thyroid tissue was available for histopathologic examination to explore calcitonin expression in cats included in our study. Increases in plasma calcitonin in response to hypercalcemia could have been missed in nonresponders. The sensitivity of the assay may have been too low to detect an actual change in calcitonin in many cats. Perhaps increases in plasma calcitonin were detected only in those cats with the most marked increase in calcitonin which exceeded the lower limit of detection, and there might be a subset of cats with smaller increases in calcitonin that could not be identified. In addition, chronic hypercalcemia might temporarily use up the calcitonin content of the thyroid gland and thereby prevent further secretion.

No correlation between plasma calcitonin and ionized calcium was observed in our cross-section of hypercalcemic cats with CKD, nor in the group of responders when analyzed separately. This is in agreement with the results of the previous cross-sectional study among cats with ionized hypercalcemia. It has been questioned if plasma calcitonin is actually related to calcium metabolism. Nonetheless, the mentioned experimental study showed that some cats respond directly to calcium infusion with an increase in plasma calcitonin. Their observation that this response is heterogeneous between cats may explain the lack of correlation found between calcitonin and ionized calcium or any other clinicopathological variable. This study found sufficient evidence to support a positive within-subject correlation between plasma calcitonin and ionized calcium. Thus, within an individual cat these 2 variables were likely to change in parallel over time. Calcitonin secretion therefore appeared to be stimulated by ionized calcium, albeit only in a minority of cats. Severity of hypercalcemia did not differ significantly between responders and nonresponders in this study. Even though the query whether increases in blood ionized calcium concentration were limited by calcitonin release in responders could not be assessed because of the observational design of our study, this result is in agreement with the previous finding that absence of calcitonin secretion did not result in more severe hypercalcemia in cats infused with calcium chloride. Therefore, the role of calcitonin in the prevention and restriction of ionized hypercalcemia in cats remains to be elucidated.

Calcitonin is thought to exert its hypocalcemic effect mainly through its inhibitory action on bone turnover. It presumably inhibits osteoclast-mediated stimulation of osteoblast activity, and thereby indirectly reduces bone formation. In addition, high or potent doses of calcitonin were shown to directly inhibit osteoclastic bone resorption. Data on bone turnover markers were not available for cats included in this study, except for plasma total ALP activity. A significant inverse relationship between plasma...
Fig 4. Changes in plasma calcitonin and blood ionized calcium concentrations in the 11 individual responders over time. Plasma calcitonin concentration (pink squares, left y-axis) and blood ionized calcium concentration (black dots, right y-axis) tended to change in parallel over time within each individual cat, resulting in a significant within-subject correlation (0.83; 95% CI, 0.63–0.92; n = 11) as calculated with a bivariate linear mixed effects model.
calcitonin and total ALP was apparent within individual cats over time, which might suggest a possible inhibitory effect of calcitonin on bone formation in hypercalcemic cats. It must be pointed out, however, that the observed changes in plasma total ALP activity over time were small, and so the effect of calcitonin on bone, if any exists, appears minor. Moreover, the observed association of calcitonin with plasma total ALP over time may have been caused by a variable other than calcitonin. Calcium itself negatively regulates the activity and release of ALP from osteoblasts.52–54 Thus, it might be that instead of plasma calcitonin, changes in ionized calcium, or another variable such as PTH, instigated the observed change in plasma total ALP, but it should be remembered that the observational design of this study does not allow for any conclusions on causation.

The presented results were derived from a low number of observations, and data for variables of interest were not available for all cats at every visit because of the retrospective design of our study. This should be carefully considered when extrapolating the results of this study to the general feline CKD population. Limited availability of information resulted in within-subject correlation coefficients with wide CIs often including zero and may explain a lack of statistically significant findings. The underlying causes of hypercalcemia for cats included in our study remain unknown, because often no further investigation had been performed. No signs of neoplasia were recorded although cats included in this study did not undergo diagnostic imaging.

The physiological value of calcitonin in mammals has been debated and appears distinct among different species.21,49,50,55,56 It appears to play an important role in calcium homeostasis in horses, for example, but less so in humans in which neither hypercalcitoninemia nor low circulating calcitonin concentrations have been associated with calcium derangements.21 Our study in azotemic cats with ionized hypercalcemia found calcitonin to change in parallel with calcium in a subset of cats. Although its restrictive effect on incidence and severity of hypercalcemia could not be properly assessed, calcitonin release did not appear to prevent hypercalcemia in cats and no significant difference was observed in maximum blood ionized calcium concentration between responders and nonresponders. Therefore, the relative importance of calcitonin in calcium homeostasis in cats with azotemic CKD appears minor.

Footnotes

a Maniaki E, Pineda C, Dunbar Z, Finch N. Calcitonin response to naturally occurring hypercalcemia in cats. J Small Anim Pract 2016;57:32–33 (abstract)
b Van den Broek DHN, Geddes RF, Williams TL, et al. Calcitonin response to naturally occurring ionized hypercalcemia in cats with chronic kidney disease. J Small Anim Pract 2016;57:32 (abstract)
c iSTAT 1 point-of-care analyzer, Abbott Point of Care Inc, Princeton, NJ
   d Idexx Laboratories, Wetherby, United Kingdom
   e Total intact PTH immunoradiometric assay—coated bead version, 3KG600, Scantibodies, Santee, CA
   f Michigan State University Diagnostic Center for Population and Animal Health, Lansing, MI
   g FE of analyte (%) = 100 × ([urine analyte][serum creatinine]/ [serum analyte][urine creatinine])
   h Calcitonin immunoradiometric assay—coated tube technology, 3KG556, Scantibodies, Santee, CA
   i IBM SPSS Statistics for Windows, Version 22, IBM Corp., Armonk, NY and GraphPad Prism 7, GraphPad Software, La Jolla, CA
   j Williams TL, Van den Broek DHN, Elliott J, Syme HM. Does hypercalcitoninism contribute to hypocalcemia in hyperthyroid cats? J Vet Intern Med 2017;31:221–222 (abstract)

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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.
References

1. O’Neill DG, Church DB, McGreevy PD, et al. Prevalence of disorders recorded in cats attending primary-care veterinary practices in England. Vet J 2014;202:286–291.
2. Lulich JP, Osborne CA, Obrien TD, et al. Feline renal-failure - Questions, answers, questions. Compend Contin Educ Pract Vet 1992;14:127.
3. 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.
4. 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.
5. Schenck PA, Chew DJ. Prediction of serum ionized calcium concentration by serum total calcium measurement in cats. Can Vet J 2010;51:448–451.
6. Van den Broek DHN, Chang YM, Elliott J, et al. Chronic kidney disease in cats and the risk of total hypercalcemia. J Vet Intern Med 2017;31:465–475.
7. Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. J Am Soc Nephrol 2015;10:1257–1272.
8. Schenck PA, Chew DJ, Nagode LA, et al. Disorders of calcium: Hypercalcemia and hypocalcemia. In: DiBartola SP, ed. Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice, 4th ed. Saint Louis, MO: Saunders; 2012:120–194.
9. Finch NC. Hypercalcaemia in cats: The complexities of calcium regulation and associated clinical challenges. J Feline Med Surg 2016;18:387–399.
10. 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.
11. 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.
12. Finch NC, Syme HM, Elliott J. Parathyroid hormone concentration in geriatric cats with various degrees of renal function. J Am Vet Med Assoc 2012;241:1326–1335.
13. Polzin DJ. Chronic kidney disease. In: Ettinger SJ, Feldman E.C, eds. Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat. St. Louis, MO: Saunders Elsevier; 2010:1990.
14. Bolliger AP, Graham PA, Richard V, et al. Detection of parathyroid hormone-related protein in cats with humoral hypercalcaemia of malignancy. Vet Clin Pathol 2002;31:3–8.
15. Barber PJ, Rawlings JM, Markweu PJ, et al. Effect of dietary phosphate restriction on renal secondary hyperparathyroidism in the cat. J Small Anim Pract 1999;40:62–70.
16. Hirsch PF, Voelkel EF, Munson PL. Thyrocalcitonin: Hypocalcemic hypophosphatemic principle of the thyroid gland. Science 1964;146:412–413.
17. Peare AG. The cytochemistry of the thyroid C cells and their relationship to calcitonin. Proc R Soc Lond B Biol Sci 1966;164:478–487.
18. Chambers TJ, Moore A. The sensitivity of isolated osteoclasts to morphological transformation by calcitonin. J Clin Endocrinol Metab 1983;57:819–824.
19. Keller J, Catala-Lehnen P, Huebner AK, et al. Calcitonin controls bone formation by inhibiting the release of spongiosine 1-phosphate from osteoclasts. Nat Commun 2014;5:5215.
20. Cochran M, Peacock M, Sachs G, et al. Renal effects of calcitonin. Br Med J 1970;1:135–137.
21. Felsenfeld AJ, Levine BS. Calcitonin, the forgotten hormone: Does it deserve to be forgotten? Clin Kidney J 2015;8:180–187.
22. Pineda C, Aguilera-Tejero E, Rayà AI, et al. Assessment of calcitonin response to experimentally induced hypercalcemia in cats. Am J Vet Res 2013;74:1514–1521.
23. Simmons RE, Hjelte JT, Mahoney C, et al. Renal metabolism of calcitonin. Am J Physiol 1988;254:F593–F600.
24. Messa P, Mioni G, Turin D, et al. The calcitonin-calcium relation curve and calcitonin secretory parameters in renal patients with variable degrees of renal function. Nephrol Dial Transplant 1995;10:2259–2265.
25. Borchhardt KA, Horl HW, Sunder-Plassmann G. Reversibility of 'secondary hypercalcitoninemia' after kidney transplantation. Am J Transplant 2005;5:1757–1763.
26. Geddes RF. Calcium-Phosphate Homeostasis in Feline Chronic Kidney Disease. London: Royal Veterinary College, University of London; 2014. PhD Thesis.
27. Bijsmans ES, Jepson RE, Chang YM, et al. Changes in systolic blood pressure over time in healthy cats and cats with chronic kidney disease. J Vet Intern Med 2015;29:855–861.
28. 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.
29. Horney BS, Farmer AJ, MacKenzie A, et al. Alkaline phosphatase isoenzymes in feline serum using an agarose gel alkaline phosphatase kit method. Can Vet J 1992;56:373–375.
30. Brown JP, Albert C, Nassar BA, et al. Bone turnover markers in the management of postmenopausal osteoporosis. Clin Biochem 2009;42:929–942.
31. Ureña P, Hruby M, Ferreira A, et al. Plasma total versus bone alkaline phosphatase as markers of bone turnover in hemodialysis patients. J Am Soc Nephrol 1996;7:506–512.
32. Magnusson P, Sharp CA, Magnusson M, et al. Effect of chronic renal failure on bone turnover and bone alkaline phosphatase isoforms. Kidney Int 2001;60:257–265.
33. Archer FJ, Taylor SM. Alkaline phosphatase bone isoenzyme and osteocalcin in the serum of hyperthyroid cats. Can Vet J 1996;37:735–739.
34. Kendall MG. A new measure of rank correlation. Biometrika 1938;30:81–93.
35. Kendall MG. The treatment of ties in ranking problems. Biometrika 1945;33:239–251.
36. Arndt S, Turvey C, Andreasen NC. Correlating and predicting psychiatric symptom ratings: Spearman r versus Kendalls tau correlation. J Psychiatr Res 1999;33:97–104.
37. Bland JM, Altman DG. Calculating correlation coefficients with repeated observations: Part 1–Correlation within subjects. Br Med J 1995;310:446.
38. Hamlett A, Ryan L, Serrano-Trespalacios P, et al. Mixed models for assessing correlation in the presence of replication. J Air Waste Manag Assoc 2003;53:442–450.
39. Silva OL, Becker KL, Shallhoub RJ, et al. Calcitonin levels in chronic renal disease. Nephron 1977;19:12–18.
40. Kotzmann H, Schmidt A, Scheuba C, et al. Basal calcitonin levels and the response to pentagastrin stimulation in patients after kidney transplantation or on chronic hemodialysis as indicators of medullary carcinoma. Thyroid 1999;9:943–947.
41. Kratzsch J, Petzold A, Raue F, et al. Basal and stimulated calcitonin and procalcitonin by various assays in patients with and without medullary thyroid cancer. Clin Chem 2011;57:467–474.
42. d’Herbomez M, Caron P, Bauters C, et al. Reference range of d’Herbomez M, Caron P, Bauters C, et al. Reference range of calcitonin and procalcitonin by various assays in patients with and without medullary thyroid cancer. Clin Chem 2011;57:467–474.
43. Borchhardt KA, Heinzl H, Gessler A, et al. Calcitonin concentrations in patients with chronic kidney disease and medullary thyroid carcinoma or c-cell hyperplasia. Kidney Int 2006;70:2014–2020.
44. Mulder H, Silberbusch J, Hackeng WH, et al. Enhanced calci-tolin release in chronic renal failure depending on the absence of severe secondary hyperparathyroidism. Nephron 1982;31:123–128.
45. Naan EC, Kooistra HS, et al. Results of thyroidectomy in 101 cats with hyperthyroidism. Vet Surg 2006;35:287–293.
46. Silva OL, Snider RH, Becker KL. Radioimmunoassay of calci-tonin in human plasma. Clin Chem 1974;20:337–339.
47. Heath H 3rd, Sizemore GW. Plasma calcitonin in normal man. Differences between men and women. J Clin Invest 1977;60:1135–1140.
48. Raue F, Deutschle I, Kuntzel C, et al. Reversible diminished calcitonin secretion in the rat during chronic hypercalcemia. Endocrinology 1984;115:2362–2367.
49. Hirsch PF, Lester GE, Talmage RV. Calcitonin, an enigmatic hormone: Does it have a function? J Musculoskeletal Neuronal Interact 2001;1:299–305.
50. Davey RA, Findlay DM. Calcitonin: Physiology or fantasy? J Bone Miner Res 2013;28:973–979.
51. Monier-Faugere M-C, Geng Z, Qi Q, et al. Calcitonin prevents bone loss but decreases osteoblastic activity in ovariohysterectomized beagle dogs. J Bone Miner Res 1996;11:446–455.
52. Anh DJ, Dimai HP, Hall SL, et al. Skeletal alkaline phosphatase activity is primarily released from human osteoblasts in an insoluble form, and the net release is inhibited by calcium and skeletal growth factors. Calcif Tissue Int 1998;62:332–340.
53. Dvorak MM, Siddiqua A, Ward DT, et al. Physiological changes in extracellular calcium concentration directly control osteoblast function in the absence of calcitropic hormones. Proc Natl Acad Sci USA 2004;101:5140–5145.
54. Farley JR, Hall SL, Tanner MA, et al. Specific activity of skeletal alkaline phosphatase in human osteoblast-line cells regulated by phosphate, phosphate esters, and phosphate analogs and release of alkaline phosphatase activity inversely regulated by calcium. J Bone Miner Res 1994;9:497–508.
55. Rosol TJ, Chew DJ, Nagode LA, et al. Pathophysiology of Calcium Metabolism. Vet Clin Pathol 1995;24:49–63.
56. Hirsch PF, Baruch H. Is calcitonin an important physiological substance? Endocrine 2003;21:201–208.
57. Rourke KM, Kohn CW, Levine AL, et al. Rapid calcitonin response to experimental hypercalcemia in healthy horses. Domest Anim Endocrinol 2009;36:197–201.