

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

D.H.N. van den Broek , R.F. Geddes, T.L. Williams, Y.-M. Chang , J. Elliott, and R.E. Jepson

**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 ( $\tau_b = 0.14$ ;  $P = 0.31$ ;  $n = 33$ ), but significant positive correlation was evident within individual responders over time (within-subject correlation coefficient [ $r_{\text{within}}$ ], 0.83; 95% confidence interval [CI], 0.63–0.92). Calcitonin correlated negatively over time with plasma ALP ( $r_{\text{within}}$ ,  $-0.55$ ; 95% CI,  $-0.79$  to  $-0.16$ ).

**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,<sup>1</sup> with a disease prevalence of 30–80% in cats over 10 years old,<sup>2,3</sup> and is commonly associated with hypercalcemia.<sup>4–6</sup> The kidneys play an important role in calcium regulation,<sup>7,8</sup> but the etiology of hypercalcemia in cats with CKD is not completely understood.<sup>9</sup> 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.<sup>10–12</sup>

### Abbreviations:

$\tau_b$	Kendall's rank correlation coefficient tau-b
CKD	chronic kidney disease
FE	fractional excretion
$\text{HCO}_3^-$	venous bicarbonate concentration
IRIS	International Renal Interest Society
PTH	parathyroid hormone
$r_{\text{within}}$	within-subject correlation coefficient
SBP	systolic blood pressure
USG	urine specific gravity

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

Calcitonin is released by C-cells of the thyroid in response to an increased serum ionized calcium concentration.<sup>16,17</sup> It protects against hypercalcemia by inhibition of bone turnover<sup>17–19</sup> and renal reabsorption of calcium,<sup>20</sup> but does not appear to have an important physiologic role in all species.<sup>21</sup> Only 5 of 18 cats with ionized hypercalcemia had measurable plasma calcitonin concentrations,<sup>a</sup> 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.<sup>22</sup> 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.<sup>23</sup> 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.<sup>24,25</sup> Healthy and azotemic normocalcemic cats generally appear to have undetectable plasma calcitonin concentrations.<sup>b,22</sup>

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

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  $\geq 2$  mg/dL in conjunction with a urine specific gravity (USG)  $< 1.035$ , or a plasma creatinine concentration  $\geq 2$  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.<sup>26</sup> 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 amlodipine besylate were eligible to be included in the study. For all cats with azotemic CKD it was advised they be fed a clinical 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,<sup>27</sup> 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 in non-heparinized whole blood using a point-of-care analyzer,<sup>c</sup> blood was collected in heparinized and EDTA plasma tubes.

All samples were stored on ice for a maximum of 6 h until centrifugation and separation. Heparinized plasma was sent to an external laboratory for biochemical analysis.<sup>d</sup> Urine samples underwent in-house urinalysis including measurement of USG by refractometry, dipstick analysis, and urine sediment microscopic examination. Residual blood and urine samples were stored at  $-80^{\circ}\text{C}$  for future measurement of plasma PTH, calcidiol, calcitriol, and calcitonin concentrations, and urinary creatinine, calcium, and phosphate concentrations.

Parathyroid hormone was measured in EDTA plasma using a previously validated intact PTH immunoradiometric assay<sup>e</sup> with a lower limit of detection of 5.2 pg/mL.<sup>28</sup> Samples with a concentration below this value were assigned an arbitrary PTH concentration of 2.6 pg/mL. Results of calcidiol and calcitriol concentrations were available for some cats, and had been measured at an external laboratory.<sup>f</sup> Urine biochemistry was performed by a commercial laboratory<sup>d</sup> in order to calculate the fractional excretion (FE) values of calcium and phosphate using the spot sample method.<sup>g</sup>

### Measurement of Plasma Calcitonin Concentration

Calcitonin was measured in heparinized plasma by an immunoradiometric assay developed for human serum<sup>h</sup> for every visit where stored plasma and data on ionized calcium concentrations were available. This assay had previously been validated for use on feline heparinized plasma samples.<sup>22</sup> A standard of 3 pg/mL, derived by dilution of the 30 pg/mL standard with deionized water, was added to the standard curve. The lower limit of detection of the immunoradiometric assay, determined as the mean + 10 standard deviations (SD) of 8 consecutive measurements of the zero standard of the assay, was 1.2 pg/mL. Cats with plasma calcitonin concentrations below this value were assigned an arbitrary concentration of 0.6 pg/mL for statistical analysis. Heparinized plasma samples for measurement of calcitonin had been stored at  $-80^{\circ}\text{C}$  for a median of 1,055 [766, 2,239] days. No significant correlation was found between storage time and plasma calcitonin concentration for the cats included in this study ( $\tau_b = -0.10$ ;  $P = 0.45$ ;  $n = 33$ ).

### Statistical Analyses

Statistical analyses were performed using statistical software packages.<sup>i</sup> For all reported analyses 2-sided tests of significance were carried out with an alpha level of 0.05 defining statistical significance. Continuous clinical data are presented as mean (SD) or as median [25th, 75th percentiles] as appropriate. The distribution of numerical variables was assessed for normality by the Shapiro–Wilk test and by visual inspection of quantile–quantile plots. The assumption of equal variances was tested with Levene's test.

The study population was divided into 2 groups: cats that had a measurable increase in plasma calcitonin concentration in response to ionized hypercalcemia (responders) and cats that did not have an increase in plasma calcitonin concentration in response to ionized hypercalcemia (nonresponders).

### 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,<sup>29</sup> a marker of osteoblastic bone formation.<sup>30</sup> Bone-specific ALP is more reliable, but total ALP can be used to assess bone turnover,<sup>31–33</sup> 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 ( $\tau_b$ ),<sup>34</sup> which is a preferred nonparametric measure of correlation for analysis of data with ties or small sample size.<sup>35,36</sup>

### 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<sup>37</sup> of plasma calcitonin with ionized calcium using a bivariate linear mixed effects model.<sup>38</sup> 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 [ $\text{HCO}_3^-$ ], 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 hypercalcaemia 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  $\text{HCO}_3^-$  (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 ( $\tau_b = 0.14$ ;  $P = 0.31$ ;  $n = 33$ , Fig 3), nor in the group of responders when analyzed separately ( $\tau_b = -0.22$ ;  $P = 0.35$ ;  $n = 11$ ). Plasma calcitonin did not correlate either with plasma total ALP ( $\tau_b = -0.10$ ;  $P = 0.53$ ;  $n = 26$ ) or FE of calcium ( $\tau_b = 0.42$ ;  $P = 0.10$ ;  $n = 11$ ), 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

**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).

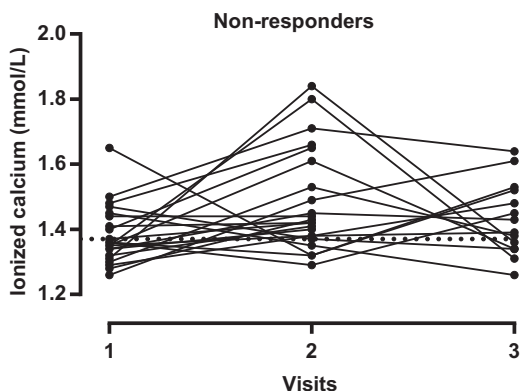
Variable (reference interval)	Nonresponders (n = 22)		Responders (n = 11)		P
	Median [25th, 75th Percentile]	n	Median [25th, 75th Percentile]	n	
Calcitonin (pg/mL)	0.6 [0.6, 0.6]	22	4.7 [2.1, 28.4]	11	
Ionized calcium (1.19–1.37 mmol/L)	1.48 [1.43, 1.65]	22	1.59 [1.46, 1.66]	11	0.22
Total ALP ( $\leq 60$ 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 ( $\geq 1.035$ )	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
Calcidiol (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 <sub>3</sub> <sup>-</sup> (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<sub>3</sub><sup>-</sup>, bicarbonate; PCV, packed cell volume; SBP, systolic blood pressure.

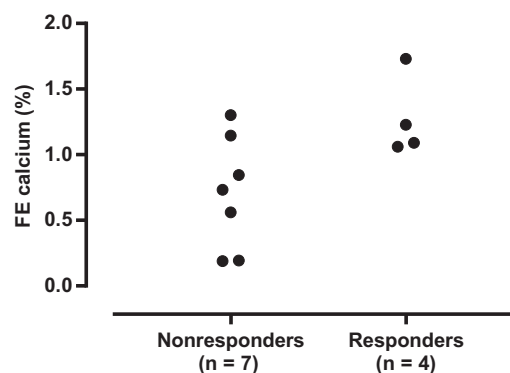
hypercalcemia occurred in a third of cats with azotemic CKD. In this group of responders, a positive relationship between plasma calcitonin and blood ionized calcium concentration and an inverse relationship between plasma calcitonin concentration and total ALP activity appeared to exist within individual cats over time. No difference in severity of ionized hypercalcemia was observed between cats that

showed an increase in plasma calcitonin and their nonresponding counterparts.

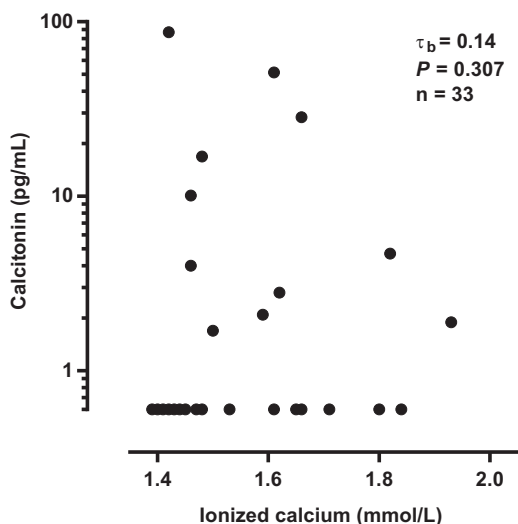
Plasma calcitonin concentrations were generally low in cats with azotemic CKD that developed ionized hypercalcemia. Previously we reported low plasma calcitonin concentrations in normocalcemic cats with renal azotemia<sup>b</sup> ( $< 1.2$  pg/mL in all 15 cats) and in hyperthyroid cats ( $\geq 1.2$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



**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 ( $P = 0.11$ ).



**Fig 3.** Scatter plot illustrating the relationship between plasma calcitonin and blood ionized calcium concentrations in hypercalcemic cats with azotemic CKD. Kendall's rank correlation indicated that no relationship was apparent between the 2 variables in the group of hypercalcemic cats as a whole ( $n = 33$ ), nor when the subgroup of responders was analyzed separately ( $\tau_b = -0.22$ ;  $P = 0.349$ ;  $n = 11$ ).

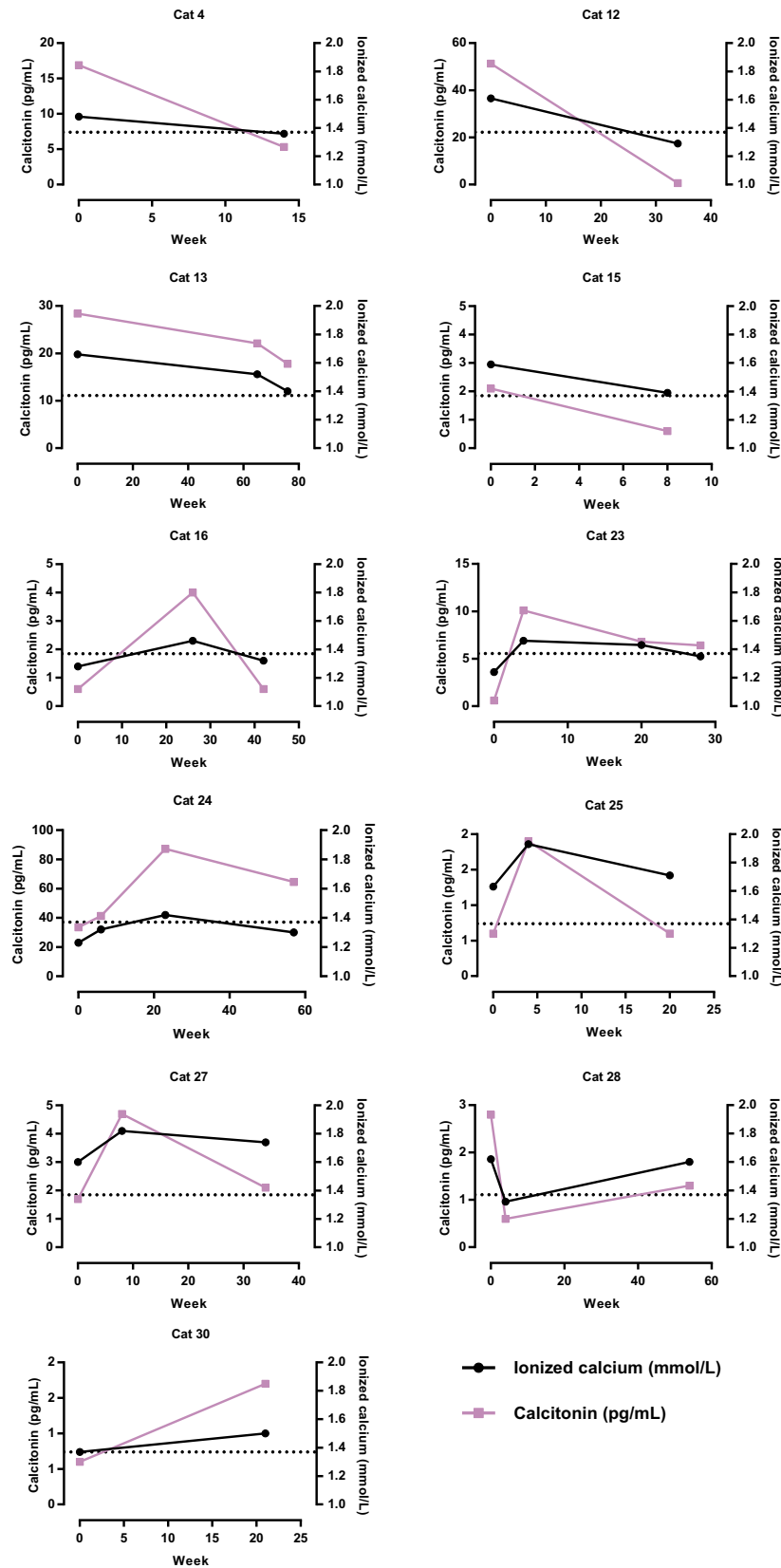
have observed low plasma calcitonin values in normocalcemic healthy cats (maximum, 3.2 pg/mL).<sup>22</sup> Low circulating calcitonin concentrations (<10 pg/mL) are considered normal in healthy humans, but up to 30% of human CKD patients have increased plasma calcitonin concentrations in the absence of hypercalcemia.<sup>21,24,39-42</sup> This secondary hypercalcitoninemia could either be explained by decreased degradation,<sup>21</sup> as calcitonin is predominantly degraded by the kidneys,<sup>23</sup> or more likely, by increased secretion, as C-cell hyperplasia is commonly associated with hypercalcitoninemia in humans with CKD.<sup>25,41,43</sup> 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.<sup>43,44</sup>

The foremost function of calcitonin is thought to be lowering of blood calcium concentration, and so to protect against hypercalcemia.<sup>21</sup> 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)<sup>22</sup> 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).<sup>a</sup> Moreover, loss of calcitonin secretory ability following bilateral thyroidectomy does not result in hypercalcemia in cats.<sup>45</sup> Absence of a calcitonin response has also been observed in humans with experimentally induced hypercalcemia, predominantly in women.<sup>46,47</sup> 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.<sup>22</sup> 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,<sup>22</sup> 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.<sup>48</sup>

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.<sup>a</sup> It has been questioned if plasma calcitonin is actually related to calcium metabolism.<sup>a,49</sup> Nonetheless, the mentioned experimental study showed that some cats respond directly to calcium infusion with an increase in plasma calcitonin.<sup>22</sup> 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.<sup>22</sup> 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.<sup>50</sup> It presumably inhibits osteoclast-mediated stimulation of osteoblast activity, and thereby indirectly reduces bone formation.<sup>19,51</sup> In addition, high or potent doses of calcitonin were shown to directly inhibit osteoclastic bone resorption.<sup>18</sup> 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.

**Table 2.** Within-subject correlation of log-transformed plasma calcitonin concentration with other clinicopathologic variables in the group of responders calculated with a bivariate linear mixed effects model.

	$r_{\text{within}}$	95% CI	n
Ionized calcium	0.83	0.63 to 0.92	11
Total ALP	-0.55	-0.79 to -0.16	8
FE Calcium	0.46	-0.08 to 0.79	4
Total calcium	0.81	0.59 to 0.92	11
ln(Calcitriol)	-0.33	-0.84 to 0.50	4
HCO <sub>3</sub> <sup>-</sup>	0.26	-0.17 to 0.61	11
pH	-0.20	-0.57 to 0.24	11
ln(PTH)	-0.19	-0.69 to 0.44	4
Calcidiol	-0.15	-0.74 to 0.56	4
Phosphate	-0.12	-0.50 to 0.30	11
FE Phosphate	-0.01	-0.43 to 0.41	4

Statistically significant positive correlations of plasma calcitonin with both blood ionized and plasma total calcium concentrations were evident within individual responders over time. A statistically significant negative correlation was apparent between plasma calcitonin and plasma total ALP activity within individual responders over time.

$r_{\text{within}}$ , within-subject correlation coefficient; 95% CI, 95% confidence interval; ALP, alkaline phosphatase; ln, natural logarithm; FE, fractional excretion; HCO<sub>3</sub><sup>-</sup>, bicarbonate.

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.<sup>52-54</sup> 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.<sup>21,49,50,55,56</sup> It appears to play an important role in calcium homeostasis in horses, for example,<sup>57</sup> but less so in humans in which neither hypercalcitoninemia nor low circulating calcitonin concentrations have been associated with

calcium derangements.<sup>21</sup> 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

<sup>a</sup> Maniaki E, Pineda C, Dunbar Z, Finch N. Calcitonin response to naturally occurring hypercalcaemia in cats. *J Small Anim Pract* 2016; 57:32-33 (abstract)

<sup>b</sup> Van den Broek DHN, Geddes RF, Williams TL, et al. Calcitonin response to naturally occurring ionized hypercalcaemia in cats with chronic kidney disease. *J Small Anim Pract* 2016;57:32 (abstract)

<sup>c</sup> iSTAT 1 point-of-care analyzer, Abbott Point of Care Inc, Princeton, NJ

<sup>d</sup> Idexx Laboratories, Wetherby, United Kingdom

<sup>e</sup> Total intact PTH immunoradiometric assay—coated bead version, 3KG600, Scantibodies, Santee, CA

<sup>f</sup> Michigan State University Diagnostic Center for Population and Animal Health, Lansing, MI

<sup>g</sup> FE of analyte (%) = 100 × ([urine analyte]/[serum creatinine]/[serum analyte]/[urine creatinine])

<sup>h</sup> Calcitonin immunoradiometric assay—coated tube technology, 3KG556, Scantibodies, Santee, CA

<sup>i</sup> IBM SPSS Statistics for Windows, Version 22, IBM Corp., Armonk, NY and GraphPad Prism 7, GraphPad Software, La Jolla, CA

<sup>j</sup> 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.

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