Calcium and Phosphorus Homeostasis in Dogs with Spontaneous Chronic Kidney Disease at Different Stages of Severity

O. Cortadellas, M.J. Fernández del Palacio, J. Talavera, and A. Bayón

Background: Studies in dogs with experimental chronic kidney disease (CKD) have demonstrated that abnormalities of calcium-phosphorus (Ca-P) homeostasis occur frequently and have a negative effect on kidney function and survival. However, the prevalence of these alterations in dogs with naturally occurring CKD at different stages of severity has not yet been investigated.

Hypothesis: Abnormalities of Ca-P metabolism occur early in the course of CKD with an increased prevalence in more severe stages.

Animals: Fifty-four dogs with CKD and 22 healthy dogs.

Methods: Blood and urine samples were obtained for a CBC, biochemistry, determination of parathyroid hormone (PTH), calcitriol, and ionized calcium concentrations and urinalysis. Based on urine protein/creatinine ratio and serum creatinine concentration, dogs were grouped according to the IRIS classification for CKD.

Results: Hyperparathyroidism (HPTH) (PTH ≥ 48 pg/mL) was diagnosed in 41 (75.9%) dogs with CKD. Its prevalence increased from 36.4% (stage 1) to 100% (stage 4). Hyperphosphatemia (P > 5.5 mg/dL) was present in 37 (68.5%) dogs; increasing in prevalence from 18% (stage 1) to 100% (stage 4). Receiver-operating characteristic curve analysis showed that serum phosphorus concentration in the 4.5–5.5 mg/dL range correctly identified the presence of HPTH in most dogs. Calcitriol concentration progressively decreased in dogs with CKD and differences became statistically significant by stage 3.

Conclusion and Clinical Relevance: HPTH and hyperphosphatemia occur frequently in dogs with naturally occurring CKD, even at early stages of CKD in some dogs. These findings highlight the importance of monitoring these parameters early in the course of CKD.

Key words: Calcitriol; Canine; Hyperparathyroidism; Hyperphosphatemia.

Available evidence in human beings1–3 and cats with spontaneous chronic kidney disease (CKD)4 and in dogs5,6 with experimental CKD indicates that assessment of calcium and phosphorus metabolism abnormalities is an important part of the laboratory evaluation in these patients, because of their negative effects on kidney function and survival. In human patients treated with hemodialysis, serum phosphorus concentrations > 6.5 mg/dL1 or 7 mg/dL2 significantly increased the relative risk of mortality. In cats, for each 1 mg/dL increase in serum phosphorus concentration at time of diagnosis, there is an 11.8% increase in the risk of mortality.4 Similarly, in dogs with experimental CKD, hyperphosphatemia has been associated with a more rapid progression of CKD and decreased survival.5,6 In addition, secondary renal hyperparathyroidism (HPTH) has deleterious effects on many organ systems such as bones, kidneys, brain, heart, smooth muscle, lungs, erythrocytes, lymphocytes, pancreas, adrenal glands, and testes.7

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Chronologically, the sequence of events begins when glomerular filtration rate (GFR) decreases, leading to a reduction in renal phosphorus excretion. Hyperphosphatemia has a direct effect on promoting HPTH because phosphorus retention enhances parathyroid hormone (PTH) secretion. Moreover, excess serum phosphorus, by inhibiting the activity of renal 1α-hydroxylase, leads to decreases in 1,25-dihydroxycholecalciferol (calcitriol) and ionized calcium (iCa) concentrations that stimulate PTH secretion. This increase in PTH activity diminishes tubular phosphorus reabsorption, increasing the rate of phosphaturia per remaining nephron. Initially, this situation restores calcitriol and iCa concentrations but at expenses of PTH activity remaining increased. If PTH returns to its initial concentration, further phosphorus retention will occur. Each time GFR decreases, this cycle recurs, preserving phosphorus balance through an important part of course of CKD. Finally, maximal inhibition of tubular phosphorus reabsorption is exceeded and GFR continues to decrease. Ultimately, the magnitude of hyperphosphatemia and the progressive loss of renal tubular cells limit renal calcitriol synthesis and, consequently, calcitriol concentration remains low, despite the persistently increased PTH concentration and severe hyperphosphatemia.8,9

In human beings, it is accepted that phosphorus retention starts at the earliest stages of the disease.10 In these patients, PTH concentrations begin to increase when serum phosphorus concentration is still within the reference range.10 There are few studies about when phosphorus retention and increased PTH concentration occur in dogs. Available data indicate that hyperphosphatemia is a common finding in dogs with CKD, with prevalence ranging from 44.2 to 94.9%.11–14 However, only 1 of these studies14 assessed hyper-
phosphatemia in relation to the severity of the disease. In that study, hyperphosphatemia \((P > 5.5 \text{ mg/dL})\) was present in 12, 50, 76.9, and 100% of dogs at stages 1, 2, 3, and 4 (IRIS classification), respectively. On the other hand, Gerber et al\(^\text{12}\) reported that HPTH was present in 100% of dogs with moderate or severe CKD, while calcitriol was significantly lower compared with a control group, but still in the reference range in most dogs. Nevertheless, to the author’s knowledge, the relationship between these clinicopathologic parameters at the different stages of CKD has not yet been investigated. Considering these facts, the aim of the current study was to prospectively evaluate abnormalities of calcium-phosphorus (Ca-P) metabolism in dogs with naturally occurring CKD at different stages, according to the IRIS classification.

**Materials and Methods**

**Dogs**

The study was carried out at the Clínica Veterinaria Germanías (Gandía-Valencia, Spain) and the Veterinary Teaching Hospital of the University of Murcia (Murcia, Spain) between April 2007 and September 2008. Fifty-four dogs with newly diagnosed and untreated CKD at different stages according to the IRIS classification\(^\text {13}\) were included in the study. The diagnosis of CKD was based on (a) presence of persistent proteinuria (urine protein/creatinine ratio (UPC) \(\geq 0.5\)) of renal origin assessed at least twice during a 3-week period, or (b) presence of clinical signs of CKD with associated azotemia (serum creatinine concentration [SCr] \(\geq 1.4 \text{ mg/dL}\)) and urine specific gravity (USG) < 1.025.

During the same period, 22 healthy adult dogs presented for elective surgeries or health screening were used as a control group. These dogs were determined to be normal on the basis of history and complete physical examination and had results within the reference ranges for CBC, biochemistry, and complete urinalysis (including microalbuminuria).

**Initial Clinical and Laboratory Evaluation**

At admission, all dogs underwent complete history and clinical examination. A blood sample was obtained from all dogs for CBC and serum biochemistry (alanine aminotransferase, albumin, alkaline phosphatase, total calcium (tCa), cholesterol, SCr, globulins, glucose, phosphorus, total proteins, and urea) after at least a 12-hour fasting period. A urine sample obtained by cystocentesis at the same time was used for urinalysis, which consisted of urine dipstick analysis, USG, sediment microscopic examination, UPC, and microalbuminuria testing\(^\text{a}\) only control dogs. If the attending clinician noticed that the animal was clinically dehydrated, the dog was rehydrated and laboratory tests were performed with samples obtained after dehydration had been corrected. After the initial evaluation, healthy dogs were assigned to the control group and dogs with CKD were staged (IRIS classification) according to their SCr and UPC as follows: stage 1: SCr: < 1.4 mg/dL; UPC: > 0.5; stage 2: SCr: 1.4–2 mg/dL; stage 3: SCr: 2.1–5 mg/dL; stage 4: SCr > 5 mg/dL.

**Additional Laboratory Evaluation**

Three milliliters of serum samples used to classify the severity of CKD were frozen at \(-70^\circ\text{C}\) within 30 minutes after blood was drawn. Samples, preserved in dry ice, were shipped to a reference laboratory\(^b\) for determination of PTH, calcitriol, and iCa concentrations. Serum PTH was determined by a solid-phase, 2-site chemiluminescent enzyme-labeled immunometric assay.\(^\text{2}\) The inter-assay variability of this assay in our laboratory was <7.9%; the intraassay variability was <6.3%. A quantitative radioimmunoassay\(^\text{3}\) was performed to determine calcitriol concentration. The interassay variability of this assay in our laboratory was <12.7%; the intraassay variability was <9.3%. An ion-selective electrode system\(^\text{4}\) was used to determine iCa. All samples were assayed in duplicate.

**Statistical Analysis**

All statistical analyses were performed with a statistical software package\(^\text{5}\). Nonparametric statistical tests were used after variables were evaluated for normality with the Shapiro-Wilk and the Kolmogorov-Smirnov tests. The Kruskal-Wallis one-way analysis of variance and the Mann-Whitney tests were used to investigate differences in serum concentrations of calcium, iCa, phosphorus, Ca-P product, calcitriol, and PTH at different stages of CKD. For the purpose of the study, reference intervals for iCa, iCa, Ca-P product, calcitriol, and PTH were defined as the central 95% interval bounded by the 2.5th and 97.5th percentiles of the results obtained in the control group. The Spearman rank correlation test \((p)\) was applied to identify correlations among serum phosphorus, iCa, iCa, Ca-P product, calcitriol, and PTH. A regression analysis was performed to evaluate the strength of the relationship between serum phosphorus and serum PTH concentrations. Receiver-operating characteristic curves (ROC) were generated to assess the specificity and sensitivity of different concentrations of serum phosphorus for detecting increased concentrations of PTH. Positive and negative likelihood ratios for the different serum phosphorus cut-off concentrations were also calculated. Statistical significance was set at \(P < .05\).

**Results**

The group of dogs with CKD comprised 54 dogs (23 males and 31 females) with a median age of 9 years (range, 1–16 years). Their median weight was 17 kg (range, 2–38 kg). Fourteen breeds were represented in this group, including mixed breed dogs \((n = 27)\), Boxer \((n = 5)\), Toy Poodle \((n = 4)\), and West Highland White Terrier \((n = 3)\) as the most commonly represented breeds. The control group consisted of 22 dogs (13 males and 9 females) of 11 different breeds. Mixed breed dogs \((n = 5)\) and Giant Schnauzer \((n = 3)\) were most frequently represented. The median weight of control group dogs was 28 kg (range, 7–45 kg) and their ages ranged from 1 to 13 years, with a median of 5.5 years. Compared with the control group, dogs with CKD were significantly older \((P = .003)\) and weighed significantly less \((P = .02)\). The distribution of dogs according to the severity of CKD and the main results of the laboratory evaluation are presented in Table 1.

Serum PTH and serum phosphorus concentrations increased with severity of CKD, but differences with control dogs only reached a statistical significance \((P < .001)\) at stage 3 (Table 1, Figs 1 and 2). HPTH (PTH \(\geq 48 \text{ pg/mL}\)) was diagnosed in 41 (75.9%) of dogs and its prevalence increased from 36.4% (stage 1) to 100% (stage 4) (Table 2). Hyperphosphatemia \((P > 5.5 \text{ mg/dL})\) was present in 37 (68.5%) dogs with CKD, with an increasing prevalence (from 18% at stage 1 till 100% at stage 4) according to CKD stage (Table 2). There were 6 dogs (2 at stage 1, 2 at stage 2, and 2 at stage 4).
with increased PTH concentrations and normal phosphorus concentrations. In contrast, 2 dogs with serum P > 5.5 mg/dL had a PTH within the reference range. The statistical analysis showed a significant correlation between serum phosphorus and PTH concentrations ($r = 0.781; P < .001$) and the regression analysis demonstrated a strong relationship ($r^2 = 0.79$; $\text{PTH} = -113.96 + 31.7 \times \text{P}$) between these 2 variables (Fig 3). Results of the ROC analysis (AUC = 0.933) for the ability of different serum phosphorus cut-off concentrations to distinguish between dogs with normal versus increased concentrations of PTH showed that optimal results were reached for serum phosphorus concentrations ranging between 4.5 and 5.5 mg/dL (Table 3).

Calcitriol concentration was measured in 47 dogs with CKD and its concentration progressively decreased with the severity of CKD. Differences achieved statistical significance by stage 3 ($P < .001$) (Table 1, Fig 4). Ten dogs (5 at stage 3 and 5 at stage 4) had calcitriol concentrations below the lower reference value (22.5 pg/mL).

### Table 1. Median (range) of urine protein to creatinine ratio (UPC), serum creatinine (SCr), serum urea, and mineral metabolism parameters measured in 22 control dogs and 54 dogs with chronic kidney disease staged according to the IRIS staging scheme.

<table>
<thead>
<tr>
<th>Laboratory Parameter</th>
<th>Control ($n = 22$)</th>
<th>Stage 1 ($n = 11$)</th>
<th>Stage 2 ($n = 10$)</th>
<th>Stage 3 ($n = 25$)</th>
<th>Stage 4 ($n = 8$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dL)</td>
<td>3.3 (2.9–3.9)</td>
<td>2.4 (1.4–3.1)</td>
<td>2.7 (1.3–3.1)</td>
<td>2.1 (1.2–3.1)</td>
<td>1.9 (1.3–2.4)</td>
</tr>
<tr>
<td>UPC (mg/dL)</td>
<td>0.1 (0.01–0.18)</td>
<td>0.1 (0.7–8.5)</td>
<td>0.1 (0.1–10.5)</td>
<td>0.1 (0.7–10.8)</td>
<td>5.7 (2.9–13.8)</td>
</tr>
<tr>
<td>SCr (mg/dL)</td>
<td>0.8 (0.5–1.3)</td>
<td>1 (0.6–1.3)</td>
<td>1.7 (1.5–2)</td>
<td>3.2 (2.2–4.8)</td>
<td>7.6 (5.1–15.5)</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>27.8 (19–41)</td>
<td>21 (18–39)</td>
<td>57 (14–124)</td>
<td>155 (86–520)</td>
<td>395.5 (255–540)</td>
</tr>
<tr>
<td>iCa (mg/dL)</td>
<td>4.3 (4–6.1)</td>
<td>4.5 (3.8–5.4)</td>
<td>4.3 (4.1–4.9)</td>
<td>4.2 (2.1–5.8)</td>
<td>3.3 (2.2–4)</td>
</tr>
<tr>
<td>Ca (mg/dL)</td>
<td>10.7 (9–12)</td>
<td>10.1 (8.7–11.9)</td>
<td>10.5 (8.9–11.8)</td>
<td>11.2 (7.5–13.9)</td>
<td>9.8 (8.2–14.2)</td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>4.5 (3.3–5.5)</td>
<td>4.9 (2.8–6.3)</td>
<td>5.3 (3–8.1)</td>
<td>7 (3–15.2)</td>
<td>17.8 (7.8–23.8)</td>
</tr>
<tr>
<td>Ca × P (mg²/dL²)</td>
<td>46 (36.9–62)</td>
<td>50.5 (33–66.3)</td>
<td>54 (31.2–95.6)</td>
<td>81.6 (31.9–154.8)</td>
<td>166.5 (84.2–255.6)</td>
</tr>
<tr>
<td>Calcitriol (pg/mL)</td>
<td>60.1 (22.5–99.2)</td>
<td>49.7 (37.4–77.2)</td>
<td>48.8 (34.9–84.6)</td>
<td>34.2 (2.4–89.6)</td>
<td>18.9 (5–38.2)</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>15.8 (6.7–47.9)</td>
<td>33.7 (8.1–96.6)</td>
<td>48.2 (9.4–161)</td>
<td>99 (22.6–416)</td>
<td>303 (169–851)</td>
</tr>
</tbody>
</table>

* Differences are statistically significant compared with healthy dogs and dogs in stages 1, 2, and 3.

** Differences are statistically significant compared with healthy dogs and dogs in stages 1 and 2.

iCa, ionized calcium; PTH, parathyroid hormone.
No significant differences in tCa concentration were detected between control and diseased dogs, whereas dogs in stage 4 had iCa concentrations lower than the other groups (P < 0.001) (Fig 5). Hypercalcemia (Ca > 12 mg/dL) was detected in 5 dogs (9.2%) whereas 4 dogs (7.4%) were hypocalcemic (Ca < 9 mg/dL). In contrast, ionized hypercalcemia (iCa > 6.1 mg/dL) was not present in any of the dogs with CKD and ionized hypocalcemia (iCa < 4 mg/dL) was observed in 18 (33.3%) dogs (Table 2). Three of the 5 hypercalcemic and 2 of the 4 hypocalcemic dogs had iCa within the reference interval, whereas iCa was below the lower reference value in the other dogs with high and low Ca. Only 2/18 dogs with low iCa concentration had tCa > 9 mg/dL.

The Ca-P product also increased with the severity of CKD, and differences from control dogs reached statistical significance (P < .001) at stage 3 (Table 1, Fig 6).

Twenty-four (44.4%) dogs had a Ca-P product > 72 mg^2/dL^2 (Table 2).

The Spearman rank correlation test showed that iCa, Ca-P product and calcitriol significantly correlated with PTH. However, the regression analysis demonstrated that the relationship between PTH and these variables (except Ca-P product and urea) was quite weak (Table 4). Total serum Ca was the only studied variable that did not have any statistical relationship with the other parameters of Ca-P homeostasis.

**Discussion**

This study has reported for the first time the prevalence of abnormalities in Ca-P homeostasis in a population of dogs with naturally occurring CKD staged according to the IRIS classification. An important concern when evaluating Ca-P metabolism in dogs is the lack of standardized reference values for some of the evaluated parameters, which results in great variability in the reported normal values. For example, Gerber et al. reported a reference range for PTH of 8–45 pg/mL; Ramsey et al. and Gear et al. used a normal range of 10–60 pg/mL and Aguilera-Tejero et al. reported a reference range of 15–65 pg/mL. Recently, a range of 9.7–73 pg/mL has been reported by a rapid assay.

<table>
<thead>
<tr>
<th>Cut-off Value (mg/dL)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>+LR</th>
<th>−LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>100</td>
<td>15.4</td>
<td>1.18</td>
<td>0</td>
</tr>
<tr>
<td>3.5</td>
<td>97.6</td>
<td>30.8</td>
<td>1.41</td>
<td>0.08</td>
</tr>
<tr>
<td>4</td>
<td>97.6</td>
<td>38.5</td>
<td>1.59</td>
<td>0.06</td>
</tr>
<tr>
<td>4.5</td>
<td>95.1</td>
<td>61.5</td>
<td>2.47</td>
<td>0.08</td>
</tr>
<tr>
<td>5</td>
<td>90.2</td>
<td>69.2</td>
<td>2.93</td>
<td>0.14</td>
</tr>
<tr>
<td>5.5</td>
<td>85.4</td>
<td>84.6</td>
<td>5.45</td>
<td>0.17</td>
</tr>
<tr>
<td>6</td>
<td>70.7</td>
<td>100</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>6.5</td>
<td>61</td>
<td>100</td>
<td>0.39</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Number and percentage (%) of dogs with abnormal results in the mineral metabolism parameters.

<table>
<thead>
<tr>
<th>Parameter (units) (reference range)</th>
<th>Ca (mg/dL) (9–12)</th>
<th>iCa (mg/dL) (4–6.1)</th>
<th>P (mg/dL) (3.3–5.5)</th>
<th>Ca × P (mg^2/dL^2) (&lt;72)</th>
<th>Calcitriol (pg/mL) (22.5–99.2)</th>
<th>PTH (pg/mL) (6.7–47.9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage of CKD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1 (n = 11)</td>
<td>Increased 0 (0)</td>
<td>0 (0)</td>
<td>2 (18)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (36.4)</td>
</tr>
<tr>
<td></td>
<td>Decreased 1 (9.1)</td>
<td>1 (9.1)</td>
<td>1 (9.1)</td>
<td></td>
<td></td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stage 2 (n = 10)</td>
<td>Increased 0 (0)</td>
<td>0 (0)</td>
<td>4 (40)</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>5 (50)</td>
</tr>
<tr>
<td></td>
<td>Decreased 1 (10)</td>
<td>0 (0)</td>
<td>1 (10)</td>
<td></td>
<td></td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stage 3 (n = 25)</td>
<td>Increased 4 (16)</td>
<td>0 (0)</td>
<td>23 (92)</td>
<td>15 (60)</td>
<td>0 (0)</td>
<td>24 (96)</td>
</tr>
<tr>
<td></td>
<td>Decreased 1 (4)</td>
<td>10 (40)</td>
<td>1 (4)</td>
<td></td>
<td>5 (22.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stage 4 (n = 8)</td>
<td>Increased 1 (12.5)</td>
<td>0 (0)</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>0 (0)</td>
<td>8 (100)</td>
</tr>
<tr>
<td></td>
<td>Decreased 1 (12.5)</td>
<td>7 (87.5)</td>
<td>0 (0)</td>
<td></td>
<td>5 (62.5)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*Two dogs at stages 1 and 2, and 3 dogs at stage 3 had no calcitriol determined because of an insufficient blood sample.

CKD, chronic kidney disease; iCa, ionized calcium; PTH, parathyroid hormone.

![Fig 3](image-url) Scatterplot, linear regression line, and 95% confidence interval of serum phosphorus against parathyroid hormone (PTH).
However, other authors consider that the upper normal limit for PTH can be as high as 130 pg/mL.\textsuperscript{20,21}

Regarding calcitriol concentrations, although Gerber et al\textsuperscript{12} reported a reference range of 23–112 pg/mL, Berry et al\textsuperscript{22} reported a range of 16–40 pg/mL. Considering these facts, we defined our own reference range for the current study. Our reference values for these parameters were quite similar to those reported previously by Gerber et al.\textsuperscript{12}

The results of the current study indicate that secondary renal HPTH is the most common abnormality of Ca-P metabolism, affecting to 75.9\% of dogs. A previous study in dogs with moderate and severe CKD reported a prevalence of rHPTH of 100\%.\textsuperscript{12} However, that study did not include dogs with mild kidney dysfunction making comparison difficult. In the current study, however, the prevalence of HPTH in dogs with moderate and severe disease (stages 3 and 4) was $\geq 96\%$. We also found that the prevalence of renal HPTH increases with the severity of the disease. This finding is in agreement with a previous study performed in cats with CKD that reported an increasing prevalence of renal HPTH from 47\% in asymptomatic persistently azotemic cats to 100\% in cats with end-stage disease. The overall prevalence of HPTH in that study was 84\%.\textsuperscript{23}

Hyperphosphatemia was the second most prevalent abnormality in the current study, affecting to 68.5\% of dogs. This percentage is higher than the 44.2\% we recently reported in a retrospective study on the prevalence of hyperphosphatemia in dogs with CKD secondary to leishmaniasis.\textsuperscript{14} However, that study also included a group of minimally (UPC: 0.2–0.5) proteinuric dogs among the dogs with CKD, which is not the case in the current study. If those dogs were excluded from the statistical analysis, the prevalence of hyperphosphatemia would increase to 51.9\%. The different nature of these 2 studies, retrospective versus prospective, also can affect the obtained results. Changes in serum phosphorus concentration mirrored those of PTH, with hyperphosphatemia also increasing in prevalence with the severity of CKD. However, the prevalence of hyperphosphatemia at stage 1 was 18\%, whereas 36.4\% of these dogs had renal HPTH. Moreover, 6 dogs included in the study had increased PTH concentrations in presence of normal serum phosphorus concentrations. Two conclusions can be made from these results. One is that in some dogs, phosphorus retention begins during stage 1 of CKD as has been reported in human beings.\textsuperscript{10} The other is that in dogs with naturally occurring CKD, renal HPTH may precede development of hyperphosphatemia. These results are in agreement with studies performed by Slatopolsky et al\textsuperscript{8} in experimental dogs. This sequence of events also has been reported in cats\textsuperscript{23} and occurs in human beings.\textsuperscript{10} However, veterinary practitioners do not routinely measure PTH in dogs with CKD because of its technical requirements and the high cost of the assay. This situation prompted us to investigate the use of serum phosphorus concentration as a surrogate marker of the presence of
renal HPTH. According to our results, serum P in the 4.5–5.5 mg/dL range predicts fairly accurately the existence of renal HPTH. These results support the recent recommendation of maintaining serum phosphorus concentrations below 4.5 mg/dL in dogs at stage 2, below 5 mg/dL in dogs at stage 3, and below 6 mg/dL in dogs at stage 4.24

A deficit in calcitriol synthesis has been shown to be a major factor in promoting development of secondary renal HPTH.25 However, there are few reports of calcitriol concentrations in dogs with CKD. In the current study, calcitriol concentrations progressively decreased with the severity of CKD and differences achieved statistical significance by stage 3. However, only 10 of the 47 dogs that had calcitriol measured had concentrations below the lower reference value. Another study performed in dogs with moderate and severe CKD, also found that a majority of dogs still had calcitriol concentrations within the reference range.12 On the other hand, in cats with CKD, low calcitriol concentrations were found mainly in animals with end-stage disease, whereas calcitriol concentration was normal in azotemic asymptomatic cats.23 In contrast with these findings, studies in humans have shown that calcitriol concentrations may decrease very early in the course of CKD, even before PTH increases substantially.26 These differences could be a consequence of earlier diagnosis of CKD in human beings, because human patient classification is based on an estimation of GFR using prediction equations, which is a more accurate index of GFR than Scr.27 As disease progresses, renal HPTH appears, stimulating calcitriol synthesis and allowing some patients with moderate or even severe disease to have seemingly normal calcitriol concentrations.26 This fact could explain why we found that a majority of dogs at stages 1, 2, and 3 had calcitriol concentrations within the reference range. Despite severe HPTH, at the most advanced stages of CKD calcitriol synthesis cannot be maintained in the normal range and serum concentrations remains low,9,26 as was the case for dogs in stage 4 in this study.

Regarding the prevalence of hypercalcemia and hypocalcemia, we found great discrepancies when the results obtained by measuring tCa were compared with those obtained by iCa determination. tCa overestimated the prevalence of hypercalcemia and underestimated the prevalence of hypocalcemia. These results agree with previous studies in dogs28 and cats23 with CKD. Another study in dogs with CKD12 showed that tCa underestimated both the prevalence of hyper- and hypocalcemia when compared with results obtained by determination of iCa.

Our study has some limitations. First, the control group was not age-matched with the diseased group. It has been shown that clinically healthy older dogs have PTH concentrations within the reference range (15–65 pg/mL) but significantly higher than concentrations obtained in a group of healthy dogs between 2 and 5 years of age.18 However, the statistical analysis performed in the current study demonstrated that no significant differences in PTH concentration existed when old control dogs (>5 years olds) and young control dogs were compared (P = .606).

Another potential limitation of our study is the relatively low number of animals included. A power calculation test performed by the authors during the design of the study indicated that the sample size was sufficient to identify the existence of significant differences among the study groups (ie, stages of renal disease). However, confidence intervals for the different prevalences reported in the current study showed a wide range of values, specially at stages 1 and 2 (data not shown). Thus, these results must be interpreted with caution and additional studies including larger numbers of dogs in various IRIS stages of CKD are indicated.

Although not strictly a limitation, a comment about the nature of CKD in dogs included in stage 1 should be made. All of them had persistent proteinuria of renal origin. Four of these dogs had UPC between 0.5 and 2, which could be indicative of pathologic tubular or glomerular proteinuria.29 The other 7 dogs had UPC > 2, compatible with pathologic glomerular proteinuria.29 The evaluation of this group of dogs showed that 6 of them had been previously treated for leishmaniasis. Despite none of these dogs having clinical signs of this infectious disease when CKD was diagnosed and the fact that all were serologically negative for leishmaniasis, the authors still believe leishmaniasis was the most probable cause of CKD in these dogs. Leishmaniasis is a recognized cause of CKD of glomerular origin30,31 and, in our experience, it is not unusual to find that treated dogs remain clinically asymptomatic, but chronically proteinuric (Cortadellas et al, personal communication). In the other 5 dogs, neither the medical history nor the complete clinical examination and laboratory evaluation showed any evidence of an extrarenal disease that could cause persistent renal proteinuria. This is in agreement with a previous study in dogs with protein-losing glomerular disease, that only identified a concomitant medical problem in 52% of dogs.32 Renal histopathology would have allowed better characterization of the lesions present in these dogs, but biopsies were not performed in any of them. Thus, more studies would be necessary to investigate if the results of the current study can be extrapolated to other causes of stage 1 CKD, especially in nonproteinuric animals.

In summary, this study shows that HPTH is common in dogs with naturally occurring CKD. The fact that HPTH was detected in one-third of dogs at stage 1 supports the hypothesis that serum phosphorus retention starts at the earliest stages of CKD. Our results also have shown that a

### Table 4. Results of the Spearman rank correlation test (r) and the regression analysis (r²) between, ionized calcium (iCa), serum calcium-phosphorus product (Ca-P) and calcitriol with parathyroid hormone (PTH).

<table>
<thead>
<tr>
<th>Variables</th>
<th>P Value</th>
<th>r</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>iCa-PTH</td>
<td>.001</td>
<td>−0.372</td>
<td>0.22</td>
</tr>
<tr>
<td>P-PTH</td>
<td>&lt;.001</td>
<td>0.781</td>
<td>0.79</td>
</tr>
<tr>
<td>Ca-P-PTH</td>
<td>&lt;.001</td>
<td>0.772</td>
<td>0.78</td>
</tr>
<tr>
<td>Calcitriol-PTH</td>
<td>&lt;.001</td>
<td>−0.462</td>
<td>0.18</td>
</tr>
</tbody>
</table>
serum phosphorus concentration in the 4.5–5.5 mg/dL range predicts the existence of HPTH in dogs with CKD quite accurately. Together, these findings support the idea that early and frequent therapeutic monitoring of serum phosphorus in the course of CKD could allow an early intervention to maintain serum phosphorus concentration below the 4.5–5.5 mg/dL range.

### Footnotes

1. E.R.D-Healthscreen Canine Urine test; Heska, Loveland, CO
2. IDEXX-Vetlab, Barcelona, Spain
3. Immulate intact PTH, Siemens Medical Solutions Diagnostics, Los Angeles, CA
4. 1,25(OH)2-VIT.D-RIA-CT; Biosource, Europe, Belgium
5. Ilyte; Instrumentation Laboratory, Barcelona, Spain
6. SPSS 15.0 for Windows; Chicago, IL

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### References