Received: 11 July 2022 | Accepted: 16 November 2022

DOI: 10.1111/jvim.16595

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Relationship between serum magnesium, calcium, and parathyroid concentrations in dogs with abnormally low serum 25-hydroxyvitamin D concentration and chronic or protein-losing enteropathy

Charles Jones [1] | Sara A. Jablonski [1] | Brian K. Petroff [2] | Daniel K. Langlois [1]

[1]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, USA

[2]Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, USA

Correspondence
Sara A. Jablonski, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA.
Email: sjw@msu.edu

Abstract

Background: The relationship between the development of SHPT and ionized magnesium (iMg) concentrations in blood of dogs with chronic gastrointestinal (GI) disease and abnormally low 25(OH)D is undefined.

Objectives: Evaluate relationships between ionized magnesium (iMg), PTH, ionized calcium (iCa), and 25(OH)D in dogs with chronic enteropathy (CE) with or without protein-losing enteropathy (PLE) and abnormal 25(OH)D. Determine whether dogs with CE or PLE, decreased 25(OH)D and SHPT have differences in iMg, iCa, or 25(OH)D when compared to dogs that do not have SHPT.

Animals: Fifty dogs with CE +/− PLE and abnormally low serum 25(OH)D.

Methods: Retrospective search of submissions database at a veterinary diagnostic laboratory for vitamin D profiles submitted in years 2017 to 2020. Cases were excluded if supplemented with Ca, Mg, or vitamin D. Spearman correlation was performed to evaluate relationships between iMg, PTH, 25(OH)D, and iCa. Ionized Mg, iCa, and 25(OH)D concentrations were compared between dogs with SHPT and those with normal PTH concentrations.

Results: Concentrations of iMg were weakly negatively correlated with PTH (rho, −.31; $P = .03$), and weakly positively correlated with serum 25(OH)D (rho, .34, $P = .02$) and iCa (rho, .42, $P = .003$). Ionized magnesium concentrations were lower in dogs with abnormally low 25(OH)D and SHPT compared to dogs with abnormally low 25(OH)D and normal parathyroid hormone concentrations ($P = .01$).

Conclusions and Clinical Importance: Hypomagnesemia might contribute to alterations in iCa and parathyroid hormone in dogs with CE +/− PLE and abnormally low 25(OH)D.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CE, chronic enteropathy; GI, gastrointestinal; iCa, ionized calcium; iMg, ionized magnesium; PHPT, primary hyperparathyroidism; PLE, protein-losing enteropathy; PTH, parathyroid hormone; SHPT, secondary hyperparathyroidism.

Preliminary data from this project was presented as a poster at the 2022 ACVIM Forum in Austin, TX.

KEYWORDS
25(oh)D, calcium, enteropathy, magnesium, parathyroid, protein-losing enteropathy

1 | INTRODUCTION

Abnormally low concentrations of serum 25-hydroxyvitamin D (25[OH]D) occur in some dogs with chronic enteropathy (CE), especially those with protein losing enteropathy (PLE).[1-5] The mechanism of low serum 25(OH)D in these dogs is not completely understood, but is generally hypothesized to be secondary to malabsorption, with particular attention given to malabsorption of fat.[2,5] Ionized hypocalcemia and elevated serum parathyroid hormone (PTH) concentrations are associated with abnormally low serum 25(OH)D concentrations in dogs with CE and PLE and are suggested to be the result of reduced vitamin D or calcium absorption, and a normal physiologic response to a vitamin D deficient state, respectively.[2,4] However, not all dogs with CE or PLE and low serum 25(OH)D concentrations develop ionized hypocalcemia, secondary hyperparathyroidism (SHPT), or a combination of these abnormalities.[2,3,4]

Secondary hyperparathyroidism is defined as an appropriate increase in PTH secretion, driven by either reduced serum calcium concentrations, increased serum phosphate concentrations, or decreased serum concentrations of active vitamin D.[6,7] The serum concentration of magnesium also affects secretion of PTH, with modest reductions in magnesium resulting in increased PTH secretion, while severely abnormally low serum magnesium concentrations induce a paradoxical block of PTH release.[8] Because magnesium also plays an important role in calcium homeostasis, hypomagnesemia might also worsen SHPT by inducing hypocalcemia and lowering serum concentrations of vitamin D metabolites.[7]

Total and ionized hypomagnesemia is documented in several dogs with PLE and ionized hypocalcemia.[9-11] The concurrent hypomagnesemia and hypocalcemia is likely the results of intestinal loss, malabsorption, and abnormal vitamin D metabolism.[10,11] In 1 of these dogs, a state of secondary hypoparathyroidism rather than SHPT was described, which was thought to be a consequence of severe magnesium depletion.[11] Gastrointestinal disease is the most common cause of concurrent ionized hypocalcemia and hypomagnesemia in dogs.[12]

Beyond these reports, there is minimal data on the interactions of magnesium with calcium and vitamin D, especially compared with the extensive data that are available concerning the interactions of vitamin D and calcium. More specifically, little is known about the influence of magnesium concentrations on the development of decreased 25(OH)D and SHPT in dogs with gastrointestinal disease. The relationship between serum ionized magnesium concentrations and PTH in the context of decreased 25(OH)D also has not been evaluated in a large number of dogs with CE +/− PLE.

The primary objective of this study was to evaluate the relationships between ionized magnesium and PTH, ionized calcium, serum albumin, and 25(OH)D concentrations in dogs with CE +/− PLE and decreased 25(OH)D concentrations.

2 | MATERIALS AND METHODS

2.1 | Case selection

The submissions database at the Michigan State University Veterinary Diagnostic Laboratory (MSU-VDL) was searched from February 20, 2017 to September 21, 2020 to identify cases in which serum samples from dogs had been submitted for Vitamin D profiles. These profiles consist of measurements of 25-hydroxyvitamin-D (25(OH)D), ionized calcium (iCa), and parathyroid hormone (PTH) concentrations. Although not externally reported, ionized magnesium (iMg) concentrations also are determined as part of this comprehensive profile. Panels were submitted by individual veterinarians according to the laboratory guidelines for clinical evaluation and not for the purposes of this study. The search was further refined by selecting cases in which the terms "malabsorption" or "impaired" or "inadequate" were contained in the endocrinology interpretation section. This refinement was used as it was considered likely to capture cases in which the Vitamin D profiles were performed because of gastrointestinal disease. Cases were excluded if concentrations of 25(OH)D were within the normal reference interval, or the results of the panel indicated a diagnosis of primary hyperparathyroidism. Next, submitting veterinarians and practices were contacted electronically to obtain permission for using patient data and to ask if they would be willing to share additional case information. Any cases in which the submitting veterinarian denied permission or did not respond to the electronic consent were excluded.

2.2 | Data collection

Submitting veterinary practices and veterinarians that consented to participation were contacted by email or phone or both, and then asked to complete a standardized electronic survey (Supporting Information File S1). Participants also were given the option to send complete medical records to the investigators for survey completion. The first 2 survey questions asked if the vitamin D profile was submitted for a disease process other than gastrointestinal disease or if the dog was receiving calcium, magnesium, or vitamin D supplementation at the time the profile was submitted. These questions were required. The survey was ended (and the cases excluded) if the panel was submitted for any process other than gastrointestinal disease or if the dog was receiving supplementation of calcium, magnesium, or any type of vitamin D at the time the profile was submitted. A history of clinical signs compatible with chronic enteropathy (eg, diarrhea, weight loss, vomiting, decrease or loss of appetite) for a minimum of 3 weeks duration or recurrent over a period of >3 weeks was required for inclusion. If requested information was unavailable or unknown to

the survey respondent, the incomplete sections of the survey were omitted from description or analysis, but all available information was retained.

Signalment, body condition score (1-9),[13] and duration of clinical signs were recorded. Duration of clinical signs was recorded as: less than 3 weeks without recurrent signs, less than 3 weeks though recurrent clinical signs, 3 weeks-3 months, 3 months-6 months, and greater than 6 months. Additional historical information gathered included clinical signs and dog diet, if known.

The survey was additionally designed to gather information concerning demographics, clinical features, diagnostic test results, treatments, and outcome. All information recorded was at the discretion of the survey respondent, based on the dogs medical records and diagnostic work-ups.

CBC and chemistry variables and associated reference intervals were recorded if performed, the most relevant of which were reported. Dogs with hypoalbuminemia (defined as serum albumin concentration ≤2.5 g/dL) were further assessed for performance of urinalysis and results of dipstick proteinuria, urine protein: creatinine ratio, and urine specific gravity. Results of bile acid stimulation tests were recorded if performed. If testing for hypoadrenocorticism was performed, those results were recorded. Performance of fecal analysis, prescription of directed or empirical anti-helminth therapy, and imaging modalities performed were also noted. Cobalamin, folate, canine pancreatic lipase immunoreactivity (cPLI), and trypsin-like immunoreactivity (TLI) values and associated reference intervals were noted if performed. If the dog underwent endoscopic or surgical gastrointestinal biopsies, the type of procedure performed and histologic diagnosis(es) obtained were recorded. For dogs that did not receive a histologic diagnosis, the clinicians working diagnosis and rationale was described.

Because of the difficulty of clearly outlining the therapy(ie, s) provided before submission of the vitamin D profile, interventions trialed were recorded in a nominal "yes/no" manner, the most relevant of which were reported herein. Vitamin D supplementation instituted at any time after results of the vitamin D profile was described. Disposition of the dog at the time of survey was categorized as: alive with resolution of gastrointestinal disease, alive with ongoing gastrointestinal disease, deceased either confirmed or suspected from gastrointestinal disease, deceased either confirmed or suspected not from gastrointestinal disease, or unknown.

2.3 | Laboratory assays

Measurements of serum concentrations of ionized calcium and magnesium, 25-hydoxyvitamin D (25[OH]D), and PTH were performed at the Michigan State University Veterinary Diagnostic Laboratory using validated assays that are used for both research and diagnostic purposes. All assays were performed on the same serum sample. The ionized calcium and magnesium concentrations were determined using ion-specific electrodes. Vitamin D was measured in canine sera with a commercially available radioimmunoassay (RIA) kit that provides reagents necessary for extraction and quantitation of the analyte (Supplemental Information File S2), and serum PTH concentrations were measured using a chemiluminescent immunometric assay (Supplemental Information File S3).

2.4 | Data and statistical analysis

Data distribution was assessed by Shapiro-Wilk testing and QQ plot inspection. Data that were not normally distributed was reported as median and range. Normally distributed data were reported as mean ± SD. Potential associations of iMg with 25(OH)D, iCa, and PTH were explored by calculating Spearman's rank correlation coefficients (rho). For Spearman testing, a statistically significant correlation score of (+/−) 0.3-0.5 was considered a weak correlation, (+/−) 0.5-0.7 a moderate correlation, and (+/−) 0.7-1.0 a strong correlation.[14] Biochemical variables (iMg, iCa, 25(OH)D and albumin) were also compared between dogs with normal and elevated serum PTH concentrations using a Mann-Whitney *U* test. A Mann-Whitney *U* was also performed to compare variables between dogs that were currently or previously receiving steroid therapy and those that were not. Statistical analyses were performed with commercially available statistical software (Graph Pad Prism version 9.2.0, GraphPad Software, Inc, San Diego, California), and $P < .05$ was considered significant for all comparisons.

3 | RESULTS

The original search query yielded 420 cases, 27 of which were consistent with primary hyperparathyroidism and were excluded. An additional 61 cases had serum concentrations of 25(OH)D within the reference interval and were excluded. Three hundred thirty-two cases remained from 160 submitting practices. Practices were contacted by VDL personnel. Forty-two submitting practices comprising 123 cases responded and granted permission for contact. All practices were then contacted by study investigators. Twenty-two practices responded and provided information on the cases (either through provision of medical records for study investigator examination, or direct filling out of the survey), leaving 74 cases. Nineteen cases were excluded because the vitamin D panel was submitted for evaluation of a disease process other than gastrointestinal disease (renal disease, n = 15; fracture n = 2; hypocalcemia following removal of apocrine gland anal sac adenocarcinoma n = 1; calcium deficient home cooked diet n = 1). Five cases were excluded because the dog was receiving calcium, magnesium, or any form of vitamin D supplementation at the time the panel was submitted (Figure 1).

Fifty dogs with CE +/-PLE and decreased serum 25(OH)D concentrations met all criteria and were included. Twenty-six were castrated males, 5 dogs were intact males, and the remainder were spayed females. Median age of the dogs was 7.5 (range, 1-16) years. Breeds included mixed breed (10), Yorkshire terrier (6), German shepherd dog (4), Labrador retriever (4), American bulldog (3), English

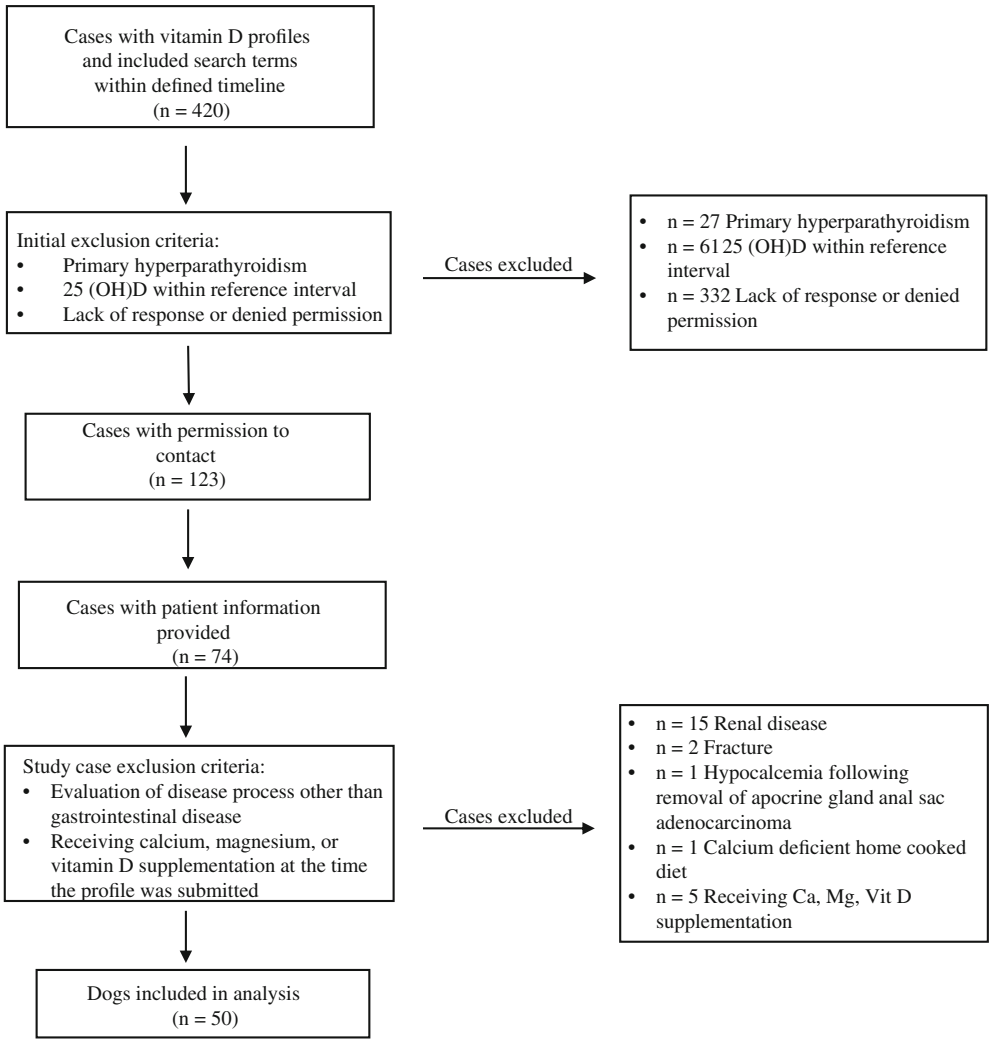

FIGURE 1 Flow diagram detailing case enrollment, removal, and completion

bulldog (3), Australian shepherd (2), Bernese mountain dog (2), Jack Russell terrier (2), and 1 each of American Eskimo dog, American pit bull terrier, Airedale terrier, Boston terrier, Brussels griffon, cavalier King Charles spaniel, Chihuahua, miniature dachshund, Rottweiler, toy poodle, treeing Walker coonhound, vizsla, Welsh terrier, and West Highland terrier. Body condition score (1–9) was available for 48 dogs. Median BCS for these 48 dogs was 3 (range, 2-8). The duration of clinical signs was recorded as less than 3 weeks (but recurrent) for 3/50 (6%) dogs, 3 weeks-3 months for 20/50 (40%) dogs, 3 months-6 months for 9/50 (18%) dogs, and greater than 6 months for 18/50 (36%) dogs. Forty-eight dogs (96%) were reported to have diarrhea (small bowel, large bowel, or mixed bowel), 26/50 (52%) were vomiting, and 23/50 (46%) were exhibiting hyporexia. One dog (2%) was reported to be anorexic. Weight loss was noted as a feature of disease in 33/50 (66%) of dogs. Current diet being fed was available for 47 dogs. At the time of profile submission, diets being consumed included GI-prescription based for 32/47 dogs (68%), commercial over-the-counter diets for 11/50 (22%) dogs, and a home-cooked diet for 3/50 (6%) dogs. CBC and chemistry variables are reported in Table 1. Median serum albumin concentration for all dogs was 1.6 g/dL (range, 0.8 g/dL-3.9 g/dL). Forty-six (92%) dogs had a serum albumin concentration ≤ 2.5 g/dL. Serum globulin concentrations were available for 41/46 dogs that had serum albumin concentration <2.5 g/dL. According to the provided reference intervals 28/41 (68%) of those dogs had concurrent hypoglobulinemia. Thirty-six (78%) dogs with serum albumin concentration <2.5 g/dL had relevant proteinuria excluded with a negative urine dipstick test or urine protein: creatinine ratio < 0.5. Of note, 7/10 dogs that did not have relevant proteinuria excluded had concurrent hypoglobulinemia; the other 3 dogs did not have serum globulin concentrations recorded. Ten (22%) dogs with serum albumin ≤2.5 g/dL had normal nonfed bile acid concentrations. According to the provided reference intervals, serum cholesterol concentrations were abnormally low in 43/48 (90%) dogs. Thirty-nine (78%) dogs had a fecal analysis performed with no parasites detected, and 8/11 (72%) dogs that did not have fecal analysis performed were prescribed empiric anthelminthic medications. Hypoadrenocorticism was excluded as a cause of clinical signs and biochemical abnormalities in 29/50 (58%) dogs. Forty-eight (96%) dogs had abdominal ultrasonography performed. Of the 42 dogs that had serum cobalamin concentrations measured, 31 (74%) were below the reference interval, and the remainder were within the reference interval. The same 42 dogs had serum folate concentrations

TABLE 1 Selected hemostatic and biochemical data from dogs with CE +/− PLE and abnormally low 25(OH)D concentrations

Variable	Mean ± SD or median (range)	% below RI	% above RI	% within RI
HCT (%)[a]	45.4 ± 8.8	20%	6%	74%
Absolute reticulocyte count ($\times 10^3/\mu L$)[b]	61 (4.2-279)	0%	26%	74%
Absolute lymphocyte count ($\times 10^3/\mu L$)[c]	1.2 (0.0-3.5)	46%	0%	54%
Absolute neutrophil count ($\times 10^3/\mu L$)[d]	13.4 (4-30.4)	0%	63%	37%
Platelet count ($\times 10^3/\mu L$)[d]	405 (132-1044)	7%	40%	53%
BUN (mg/dL)[e]	14 (3-73)	10%	6%	84%
Creatinine (mg/dL)[f]	0.6 (0.2-1.5)	34%	0%	64%
Total calcium (mg/dL)[e]	7.3 (3.1-9.7)	90%	0%	10%
Albumin (g/dL)	1.6 (0.8-3.9)	96%	0%	4%
Globulin (g/dL)[e]	1.7 (1.1-3.8)	48%	0%	52%
Cholesterol (mg/dL)[e]	90 (35-354)	85%	0%	15%

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BUN, blood urea nitrogen; CE, chronic enteropathy; HCT, hematocrit; PLE, protein-losing enteropathy; RI, reference interval.
[a]Data available for n = 45.
[b]Date available for n = 27.
[c]Data available for n = 41.
[d]Date available for n = 30.
[e]Data available for n = 48.
[f]Data available for n = 47.

measured, and 20 (48%) were below the reference interval, 2 (5%) were above the reference interval, and the remainder measured within the reference interval. Thirty-six (72%) dogs had exocrine pancreatic insufficiency excluded as a cause of their clinical signs with a concentration of serum trypsin-like immunoreactivity concentration >5.7 ng/L. Small intestinal biopsies were obtained in 38/50 (76%) dogs; endoscopic biopsies were performed in all cases except 1. Twenty-nine of the 38 dogs (76%) had biopsies obtained of both duodenum and ileum, the remaining dogs had duodenum sampled only. Lymphoplasmacytic inflammation of the small intestine (duodenum, ileum, or both) was noted in all dogs with an eosinophilic and/or neutrophilic component noted in 12/38 (32%) and 11/38 (29%) of cases, respectively. Intestinal lymphangiectasia (IL) was noted in 24/38 (63%) of cases. Degree of IL was noted as mild, moderate, or severe in 12/24 (50%), 10/24 (42%), and 2/24 (8%) cases, respectively. The cause of chronic enteropathy in the remaining 12 dogs was unknown.

Before submission of the vitamin D profile, 34/50 (68%) dogs were treated with a diet trial of at least 2 weeks duration, 43/50 (86%) dogs were treated with at least 1 antibiotic, and 21/50 (42%) dogs had been treated with a corticosteroid. Of the 21 who had been treated with a corticosteroid, 14 (66%) had corticosteroid therapy discontinued before submission of vitamin D panel, and the remaining 7/21 (33%) dogs were receiving corticosteroids at the time of sample collection. Steroid therapy was discontinued a median of 9.5 (range, 7-18) days before sample collection in the 8 dogs this data was available for. For 4/7 dogs the steroid type and dose was known and all dogs were being treated with prednisone or prednisolone at 0.4-1.2 mg/kg, PO, q24h or divided q12h. Additionally, 8/50 (16%) of dogs had been treated with a nonsteroidal immunosuppressive medication. All but 1 of these 8 dogs had been treated with cyclosporine; the remaining dog had been treated with azathioprine.

Results of the vitamin D profile including serum 25(OH)D, iCa, PTH, and iMg concentrations and associated reference intervals are reported in Table 2. Concentrations of iMg were weakly positively correlated with serum iCa (rho, .42, $P = .003$) and 25(OH)D (rho, .34, $P = .02$) concentrations, and weakly negatively correlated with PTH concentrations (rho, −.31; $P = .03$; Figure 2). Twenty-four (48%) dogs had iMg concentrations below the reference interval, 3/50 (6%) dogs had iMg concentrations above the reference interval, and the remainder (23/50; 46%) had iMg concentrations within the reference interval. Twenty-six (52%) dogs had elevated serum PTH concentrations, consistent with SHPT, the remaining 24 (48%) dogs had serum PTH concentrations within the reference interval. Ionized magnesium concentrations were lower in dogs with abnormally low 25(OH)D and SHPT when compared to dogs with abnormally low 25(OH)D and normal serum PTH concentrations ($P = .01$; Figure 3). Serum ionized calcium ($P = .02$; Figure 4) and 25(OH)D ($P = .006$; Figure 5) concentrations were also different in dogs with decreased 25(OH)D and SHPT when compared to dogs with abnormally low 25(OH)D and normal serum PTH concentrations. Concentrations of serum 25(OH)D and PTH were not different between dogs treated with a corticosteroid (previously or currently; n = 21; $P = .69$ and $P = .16$, respectively). Serum 25(OH)D and PTH concentrations were also compared between dogs who were receiving a corticosteroid (prednisone or prednisolone; n = 7) at the time of sample collection and were similarly not different between groups ($P = .64$ and $P = .49$, respectively).

Eleven dogs had abnormally low serum iCa concentrations without the expected physiologic response of increased PTH concentrations. Of these 11 dogs, 7 (64%) had decreased serum iMg concentrations. Finally, the 2 dogs with the lowest serum iMg concentrations (0.19 and 0.2 mmol/L) both had normal serum PTH concentrations.

TABLE 2 25(OH)D, ionized calcium, ionized magnesium, and parathyroid hormone concentrations in dogs with CE +/− PLE and abnormally low 25(OH)D concentrations

Variable	Median (range)	Reference interval (RI)	% below RI	% above RI	% within RI
25(OH)D (nmol/L)	19 (6-95)	109-423	NA	NA	NA
Ionized calcium (mmol/L)	1.2 (0.5-1.42)	1.25-1.45	64%	0%	36%
Ionized magnesium (mmol/L)	0.43 (0.19-1.17)	0.43-0.6	48%	6%	46%
Parathyroid hormone (pmol/L)	6.15 (0.6-139.7)	0.5-5.8	0%	52%	48%

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CE, chronic enteropathy; PLE, protein-losing enteropathy; RI, reference interval.

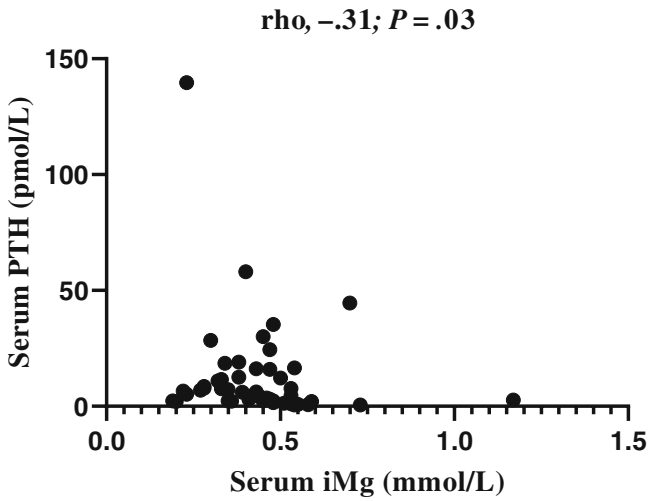

FIGURE 2 Scatter plot showing relationship between PTH and iMg in dogs with CE +/− PLE and abnormally low 25(OH)D. iMg, ionized magnesium; PTH, parathyroid hormone

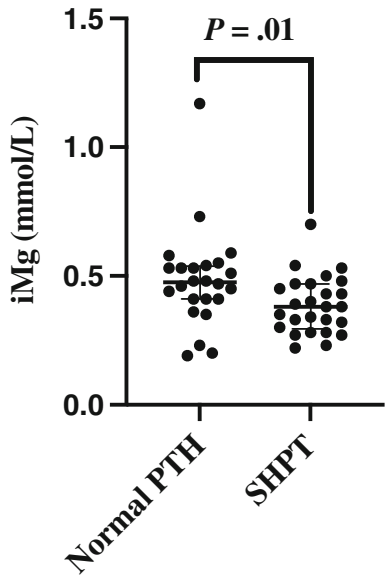

FIGURE 3 Dot plot of iMg in CE +/− PLE dogs with abnormally low 25(OH)D and normal PTH versus SHPT. Horizontal bar represents median. Interquartile range also shown. CE, chronic enteropathy; iMg, ionized magnesium; PLE, protein-losing enteropathy; PTH, parathyroid hormone; SHPT, secondary hyperparathyroidism

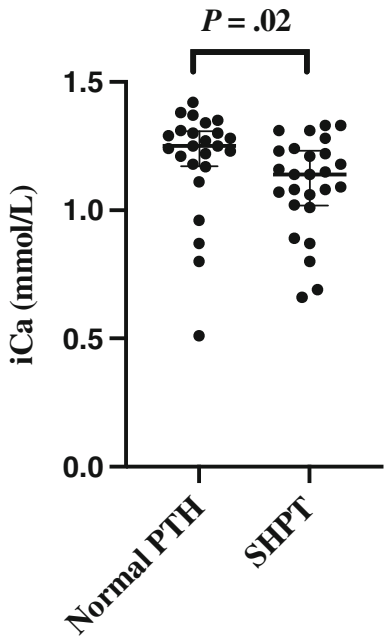

FIGURE 4 Dot plot of iCa in CE +/− PLE dogs with abnormally low 25(OH)D and normal PTH versus SHPT. Horizontal bar represents median. Interquartile range also shown. CE, chronic enteropathy; iCa, ionized calcium; PLE, protein-losing enteropathy; PTH, parathyroid hormone; SHPT, secondary hyperparathyroidism

Following the results of the vitamin D profile, 25/50 (50%) dogs received some type of vitamin D supplementation. Calcitriol was administered in 23/25 (92%) cases, oral cholecalciferol was administered in the remaining 3/25 (12%) cases. Dose of calcitriol was available for 19 dogs. Median dose of calcitriol administered per day was .03 mcg/kg/day (range, 0.01-0.07 mcg/kg/day). Doses of oral cholecalciferol or formulations utilized were not reported.

Dog outcomes were reported as alive with resolution of GI disease (2/50; 4%), alive with ongoing management of GI disease (11/50; 22%), deceased, suspected or confirmed from GI disease (20/50; 40%), deceased not related to GI disease (1/50; 2%), or unknown (16/50; 32%).

4 | DISCUSSION

Ionized hypomagnesemia was common in a group of dogs with CE +/− PLE and abnormally low 25(OH)D concentrations. In addition,

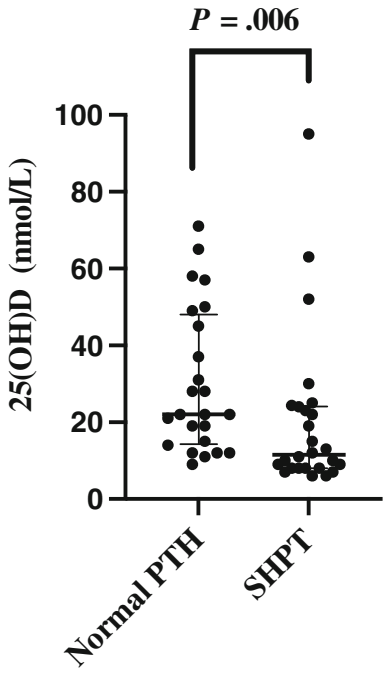

FIGURE 5 Dot plot of 25(OH)D in CE +/− PLE dogs with abnormally low 25(OH)D and normal PTH versus SHPT. Horizontal bar represents median. Interquartile range also shown. 25(OH)D, 25-hydroxyvitamin D; CE, chronic enteropathy; PLE, protein-losing enteropathy; PTH, parathyroid hormone; SHPT, secondary hyperparathyroidism

serum ionized Mg concentrations were correlated to iCa, PTH, and 25(OH)D concentrations in this study group, which suggests that iMg could contribute to alterations in iCa and PTH in dogs with CE +/− PLE and decreased 25(OH)D.

Magnesium is a crucial electrolyte for a wide array of cellular processes, as the second most abundant cation in vertebrates.[8,15] Alterations in magnesium concentrations have been increasingly identified in veterinary medicine, especially in animals with critical illness.[16] Ionized hypomagnesemia occurs in dogs with PLE,[9-11] however, the relationships between magnesium, calcium, 25(OH)D, and PTH are not described in a large cohort of dogs.

Serum ionized magnesium and ionized calcium were correlated in our group of dogs with CE +/− PLE. This is expected, as magnesium and calcium are similarly decreased as a consequence of malabsorption and intestinal loss, and GI disease is considered the most common cause of concurrent hypocalcemia and hypomagnesemia in dogs.[12] Additionally, magnesium affects calcium homeostasis through its role in the production and modulation of PTH.[8] Mild hypomagnesemia appropriately inhibits G-protein coupled receptors leading to increased PTH secretion. However, severe hypomagnesemia could lead to production of defective cyclic adenosine monophosphate in the parathyroid glands, leading to paradoxical block of PTH secretion, resulting in or contributing to hypocalcemia.[8] This relationship between magnesium and PTH is made even more complex by the influence of calcium concentrations. In vitro work using intact rat parathyroid glands found that magnesium was able to reduce PTH when exposed to moderately low calcium concentrations, but not at normal to high calcium concentrations.[17]

In our group of dogs with CE +/− PLE, serum ionized magnesium concentrations were weakly negatively correlated with serum PTH concentrations. Additionally, dogs with ionized hypomagnesemia and low 25(OH)D concentrations were more likely to have SHPT when compared to dogs with normal or high magnesium concentrations and low 25(OH)D concentrations. Because a negative correlation was observed, it is likely that in the majority of our cases hypomagnesemia appropriately triggered increased PTH secretion, perhaps along with calcium in cases where calcium was concurrently decreased. Of the 32 dogs in our study with ionized hypocalcemia, 11 (34%) did not have the expected physiologic response of increased PTH. Hypomagnesemia was present in 7/11 (64%) of those dogs. Additionally, the dogs in our study with the lowest concentrations of ionized magnesium (<0.2 mmol/L) had PTH concentrations within the reference interval. It is possible that if we had more dogs in our study with severe ionized hypomagnesemia, we would have observed no correlation, or even a positive correlation between iMg and PTH. It is also possible that the negative correlation between iMg and PTH would have been stronger if our cohort had not included dogs with severe hypomagnesemia and normal PTH concentrations. In other words, it is likely that a complex relationship exists between magnesium concentration and the development of SHPT, rather than a simple positive to negative correlation.

In order to exert its biologic functions in the body, vitamin D needs to be activated from its storage form. The various conversions required are performed by enzymes that are magnesium-dependent.[18] Furthermore, magnesium deficiency can also decrease the number of available vitamin D receptors in target tissues.[19] Consequently, magnesium status is well understood to affect vitamin D concentrations. In our group of dogs with CE +/− PLE, ionized magnesium concentrations were weakly positively correlated to serum 25(OH)D concentrations, suggesting that decreased concentrations of ionized magnesium may affect serum 25(OH)D concentrations. It is also possible that the decreased concentrations of serum 25(OH)D could occur first, and subsequently influence serum ionized magnesium concentrations. Furthermore, critically ill animals could also simply be affected by both lower ionized magnesium concentrations and 25(OH)D without a relationship between the 2.[19,20] Whether supplementation with magnesium in hypomagnesemic dogs with decreased 25(OH)D is necessary to improve 25(OH)D concentrations is unknown. In 1 human study, serum calcitriol concentrations were reported to remain low in human patients with magnesium deficiency despite intake of cholecalciferol.[21]

Fifty percent of dogs in this study received vitamin D supplementation for treatment of abnormally low 25(OH)D concentrations. Calcitriol was the most common form of vitamin D supplemented (23/25; 92%). Currently, there is little to no information regarding whether vitamin D supplementation is beneficial or effective in dogs with CE +/− PLE.

Hypomagnesemia has been linked to increased morbidity and mortality in both human and veterinary patients.[12,19,22] Given the

concerns for morbidity and mortality and the known influence of magnesium on calcium, PTH and 25(OH)D concentrations, increased monitoring for hypomagnesemia should be considered in dogs with GI disease. It is unknown whether directed therapy for hypomagnesemia would be beneficial, though it is known that refractory hypocalcemia might improve with magnesium supplementation.[15]

Dogs with PLE and low serum 25(OH)D concentrations have poorer outcomes when compared to dogs with PLE and normal serum 25(OH)D concentrations.[4] In our study, outcome data were available for 34/50 (68%) dogs. Of these dogs for which outcomes were known, 20/34 (59%) died or were euthanized as a result of their GI disease. This is similar to reports in the veterinary literature of 54.2% mortality rate in PLE.[23] Given the retrospective nature of this study and incomplete data set, it is difficult to comment on whether hypomagnesemia was related to outcome in the group of dogs in our study.

Our study had limitations, many of which are related to the retrospective design. Survey responses were based on interpretation of the medical record, which was not always completed by the primary clinician. Submission of the vitamin D panel due to concern for gastrointestinal disease was a requirement for inclusion. It is unclear however which biochemical abnormality, in conjunction with clinical signs prompted submission by the clinician. Hypovitaminosis D can be secondary to a variety of disease processes, including infectious, inflammatory, and neoplastic disease.[24-27] Since concurrent disorders could not be excluded in all cases, it is possible that low serum 25(OH)D was not entirely due to GI disease in all cases. Ileal histopathology was included in 29 of 38 dogs, and since lesions can differ among sections[28,29] this could have altered the diagnosis. Similarly, IL can be segmented and confined to deeper layers of the intestines and endoscopic biopsies may have underestimated the numbers of dogs with this lesion.[30] Some dogs (7/50) were receiving glucocorticoid therapy at the time of sample collection which could have affected 25(OH)D or PTH concentrations.[31] Additionally the time since the last dose of corticosteroid was only available in 8/14 dogs that had a history of previous steroid administration. Previous doses administered were also unknown.

In conclusion, dogs with CE +/− PLE and decreased 25(OH)D commonly have ionized hypomagnesemia. The pathophysiology of CE +/− PLE remains complex, and results of our study suggest magnesium is associated with alterations to iCa, PTH, and 25(OH)D.

ACKNOWLEDGMENT

No funding was received for this study.

CONFLICT OF INTEREST DECLARATION

The authors are employees of Michigan State University where the diagnostic assays are performed, however, none of the authors benefit financially from submission of samples for the described assays.

OFF-LABEL ANTIMICROBIAL DECLARATION

Author's declare no off-label use of antimicrobials.

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

Authors declare IACUC approval was not needed for this study.

HUMAN ETHICS APPROVAL DECLARATION

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

ORCID

Sara A. Jablonski https://orcid.org/0000-0002-6486-3644
Daniel K. Langlois https://orcid.org/0000-0001-7726-6307

REFERENCES

1. Titmarsh H, Gow AG, Kilpatrick S, et al. Association of vitamin D status and clinical outcome in dogs with a chronic enteropathy. *J Vet Intern Med*. 2015;29:1473-1478.
2. Wennogle SA, Priestnall SL, Suárez-Bonnet A, et al. Comparison of clinical, clinicopathologic, and histologic variables in dogs with chronic inflammatory enteropathy and low or normal serum 25-hydroxycholecalciferol concentrations. *J Vet Intern Med*. 2019;33:1995-2004.
3. Gow AG, Else R, Evans H, Berry JL, Herrtage ME, Mellanby RJ. Hypovitaminosis D in dogs with inflammatory bowel disease and hypoalbuminaemia. *J Small Anim Pract*. 2011;52:411-418.
4. Allenspach K, Rizzo J, Jergens AE, Chang YM. Hypovitaminosis D is associated with negative outcome in dogs with protein losing enteropathy: a retrospective study of 43 cases. *BMC Vet Res*. 2017;13:96.
5. Mellanby RJ, Mellor PJ, Roulois A, et al. Hypocalcaemia associated with low serum vitamin D metabolite concentrations in two dogs with protein-losing enteropathies. *J Small Anim Pract*. 2005;46:345-351.
6. Muppidi M, Meegada SR, Rehman A. Secondary hyperparathyroidism. *StatPearls*. Treasure Island, FL: StatPearls Publishing; 2022. https://www.ncbi.nlm.nih.gov/books/NBK557822/. Accessed July 6, 2022.
7. Bargagli M, Arena M, Naticchia A, et al. The role of diet in bone and mineral metabolism and secondary hyperparathyroidism. *Nutrients*. 2021;13:2328-2345.
8. Vetter T, Lohse MJ. Magnesium and the parathyroid. *Curr Opin Nephrol Hypertens*. 2002;11:403-410.
9. Whitehead J, Quimby J, Bayliss D. Seizures associated with hypocalcemia in a Yorkshire terrier with protein-losing enteropathy. *J Am Anim Hosp Assoc*. 2015;51:380-384.
10. Kimmel SE, Waddell LS, Michel KE. Hypomagnesemia and hypocalcemia associated with protein-losing enteropathy in Yorkshire terriers: five cases (1992-1998). *J Am Vet Med Assoc*. 2000;217:703-706.
11. Bush WW, Kimmel SE, Wosar MA, Jackson MW. Secondary hypoparathyroidism attributed to hypomagnesemia in a dog with protein-losing enteropathy. *J Am Vet Med Assoc*. 2001;219:1732-1708.
12. Woods GA, Oikonomidis IL, Gow AG, et al. Investigation of hypomagnesaemia prevalence and underlying aetiology in a hospitalised cohort of dogs with ionised hypocalcaemia. *Vet Rec*. 2021;189(9):e301.
13. Laflamme DRPC. Development and validation of a body condition score system for dogs. *Canine Pract*. 1997;22:10-15.
14. Mukaka MM. A guide to appropriate use of correlation coefficient in medical research. *Mal Med J*. 2012;24:69-71.
15. Na D, Tao G, Shu-Ying L, et al. Association between hypomagnesemia and severity of primary hyperparathyroidism: a retrospective study. *BMC Endocr Disord*. 2021;21:170-178.
16. Schulz N, Güssow A, Bauer N, et al. Magnesium in dogs and cats—physiology, analysis, and magnesium disorders. *Tierarztl Prax Ausgabe K Kleintiere—Heimtiere*. 2018;46:21-32.
17. Rodríguez-Ortiz ME, Canalejo A, Herencia C, et al. Magnesium modulates parathyroid hormone secretion and upregulates parathyroid

receptor expression at moderately low calcium concentration. *Nephrol Dial Transplant*. 2014;29:282-289.
18. Uwitonze AM, Razzaque MS. Role of magnesium in vitamin D activation and function. *J Am Osteopath Assoc*. 2018;118:181-189.
19. Martin LG, Matteson VL, Wingfield WE, Pelt DR, Hackett TB. Abnormalities of serum magnesium in critically ill dogs: incidence and implications. *J Vet Emerg Crit Care*. 1994;4:15-20.
20. Jaffey JA, Backus RC, McDaniel KM, et al. Serum vitamin D concentrations in hospitalized critically ill dogs. *PLoS One*. 2018;13:e0194062.
21. Reddy P, Edwards LR. Magnesium supplementation in vitamin D deficiency. *Am J Ther*. 2019;26:e124-e132.
22. Zafar MS, Wani JI, Karim R, Mir MM, Koul PA. Significance of serum magnesium levels in critically ill-patients. *Int J Appl Basic Med Res*. 2014;4:34-37.
23. Craven MD, Washabau RJ. Comparative pathophysiology and management of protein-losing enteropathy. *J Vet Intern Med*. 2019;33:383-402.
24. Kraus MS, Rassnick KM, Wakshlag JJ, et al. Relation of vitamin D status to congestive heart failure and cardiovascular events in dogs. *J Vet Intern Med*. 2014;28:109-115.
25. Gerber B, Hässig M, Reusch CE. Serum concentrations of 1,25-dihydroxycholecalciferol and 25-hydroxycholecalciferol in clinically normal dogs and dogs with acute and chronic renal failure. *Am J Vet Res*. 2003;64:1161-1166.
26. Selting KA, Sharp CR, Ringold R, Thamm DH, Backus R. Serum 25-hydroxyvitamin D concentrations in dogs—correlation with health and cancer risk. *Vet Comp Oncol*. 2016;14:295-305.
27. Rosa CT, Schoeman JP, Berry JL, Mellanby RJ, Dvir E. Hypovitaminosis D in dogs with spirocercosis. *J Vet Intern Med*. 2013;27:1159-1164.
28. Procoli F, Motskula PF, Keyte SV, et al. Comparison of histopathologic findings in duodenal and ileal endoscopic biopsies in dogs with chronic small intestinal enteropathies. *J Vet Intern Med*. 2013;27:268-274.
29. Casamian-Sorrosal D, Willard MD, Murray JK, Hall EJ, Taylor SS, Day MJ. Comparison of histopathologic findings in biopsies from the duodenum and ileum of dogs with enteropathy. *J Vet Intern Med*. 2010;24:80-83.
30. Larson RN, Ginn JA, Bell CM, Davis MJ, Foy DS. Duodenal endoscopic findings and histopathologic confirmation of intestinal lymphangiectasia in dogs. *J Vet Intern Med*. 2012;26:1087-1092.
31. Ramsey IK, Tebb A, Harris E, et al. Hyperparathyroidism in dogs with hyperadrenocorticism. *J Small Anim Pract*. 2005;46:531-536.

SUPPORTING INFORMATION

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

How to cite this article: Jones C, Jablonski SA, Petroff BK, Langlois DK. Relationship between serum magnesium, calcium, and parathyroid concentrations in dogs with abnormally low serum 25-hydroxyvitamin D concentration and chronic or protein-losing enteropathy. *J Vet Intern Med*. 2023;37(1):101-109. doi:10.1111/jvim.16595