Serum calcium concentrations are inversely correlated with pancreatic lipase concentrations in dogs

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OBJECTIVE
(1) Determine if a relationship exists between ionized calcium (iCa) and pancreatic lipase (cPLI) concentration in dogs, and (2) assess for correlation between resolving hypercalcemia and cPLI concentrations in dogs after treatment for primary hyperparathyroidism (PHPT).

SAMPLES
Phase I, 44 residual serum samples (collected April 2023) from client-owned dogs with a clinical indication for cPLI quantification. Phase II, 24 residual serum samples (collected August 2022 through February 2023) from client-owned dogs with PHPT pre- and postcorrection of hypercalcemia.

METHODS
Serum cPLI and iCa concentrations were measured via the Spec cPL assay and a spectrophotometric method respectively. Spearman’s rank correlation coefficients were used to investigate if there was a correlation between serum calcium and cPLI concentrations. A paired t-test was used to investigate the effect of the resolution of hypercalcemia on serum cPLI concentrations.

RESULTS
Phase I, serum cPLI concentrations were negatively correlated with serum iCa concentrations ($r = -.429$, 95% CI $[-.64, -.14]$, $P = .005$) in dogs with a clinical indication for cPLI quantification. Phase II, median serum cPLI concentrations were higher before (median: 228.5 μg/L, IQR: 351.3 μg/L) than after (median: 141.0 μg/L, interquartile ranges (IQR): 279.5 μg/L) management of hypercalcemia (PHPT model). However, the decrease in cPLI concentration was not statistically significant ($P = .70$).

CLINICAL RELEVANCE
Calcium depletion may result in an inverse relationship between serum cPLI and iCa concentrations in dogs with a clinical indication for cPLI quantification. Hypercalcemia may be associated with an above reference interval cPLI concentration in some dogs.

Keywords: hypercalcemia, pancreatic acinar cell, cPLI, lipase, saponification

Acute pancreatitis (AP) is often deemed idiopathic; however, limited research into the etiology of pancreatitis has been performed in dogs and this may limit the detection of underlying causes. One proposed underlying etiology is hypercalcemia, but limited information exists on the role of calcium in the etiology of AP in dogs. Animal models show that hypercalcemia induces pancreatic injury via the development of an apical block (rat), premature activation of digestive zymogens (rat), and subsequent acinar cell damage (cat). Alternatively, hypercalcemia may induce pancreatitis via the development of calculi and obstruction of the pancreatic duct. The association between hypercalcemia and pancreatitis in people is evidenced by up to 8% of hyperparathyroid cases having concurrent pancreatitis. While some studies have questioned the significance of this association, it is clear that...
surgical correction of primary hyperparathyroidism (PHPT) can reduce the recurrence of pancreatitis in people, suggesting a causal relationship between hypercalcemia and the development of AP at least in people.7,8 The effect of the resolution of hypercalcemia on pancreatic lipase (cPLI) concentrations in dogs is unknown. Humans with recurrent pancreatitis are also often evaluated for PHPT and hypocalcemia.9 Also, calcium infusion is used to experimentally induce acute pancreatitis in cats and other species.10,11

Calcium derangements are not only restricted to the inciting phases of pancreatitis but are also noted during the clinical phases of pancreatitis. Hypocalcemia is commonly reported during pancreatitis in dogs and may be a negative prognostic indicator.12 However, the correlation between cPLI concentrations and hypocalcemia in dogs is unknown.

Thus, the objectives of our study were to determine whether serum cPLI and iCa concentrations are correlated in dogs with clinically suspected pancreatitis and to determine whether treatment of hypercalcemia in dogs with primary hyperparathyroidism lowers cPLI concentration in dogs, via a PHPT model of hypercalcemia.

Methods

Study overview

In phase I of the study, residual serum samples were collected from the Texas A&M Gastrointestinal Laboratory in April 2023. These samples were submitted to the laboratory due to a clinical indication for cPLI quantification as assessed by their primary veterinarian. Residual serum samples were selected on a convenience basis from dogs with cPLI (Spec cPL, Texas A&M Gastrointestinal Laboratory) concentrations across the dynamic range of the assay. These samples were then shipped overnight on ice to the Michigan State University Veterinary Diagnostic Laboratory. Serum iCa (NOVA electrolyte analyzer, Nova Biomedical) concentrations were then quantified. An IACUC exemption was approved by the Michigan State University IACUC committee due to the use of residual serum samples.

In phase II of the study, residual serum samples from 24 client-owned dogs with primary hyperparathyroidism (PHPT) were utilized. Residual serum samples were available from baseline (diagnosis of PHPT) and 4 weeks after surgical parathyroidectomy or ultrasound-guided ethanol ablation of parathyroid lesions. In the prior study, blood was collected via jugular venipuncture into plain glass tubes before being allowed to clot for 30 minutes. Blood was then centrifuged at 2500 RPM for 10 minutes before the supernatant (serum) was collected. Samples were collected between August 2022 and February 2023. Medical records from the 24 dogs were reviewed for clinical signs and for any treatments that were administered during the prior study period. Diagnosis of PHPT was based on a serum iCa > 1.55 mmol/L (NOVA electrolyte analyzer, Nova Biomedical), with a mid to high normal or high parathyroid hormone concentration (Intact parathyroid hormone kit, Immunodiagnostic Systems), and a serum phosphorous (multiple analyzers) concentration within or below the reference interval. Dogs with PHPT were used in this study as a naturally occurring model of the effects of hypercalcemia on cPLI concentrations. Serum cPLI (Spec cPL, Texas A&M Gastrointestinal Laboratory) concentrations were measured at baseline (hypercalcemia) and 4 weeks after successful treatment of hypercalcemia. IACUC exemption was approved by the Michigan State University IACUC committee.

Diagnostic assays

Serum iCa (NOVA electrolyte analyzer, Nova Biomedical) concentrations were measured using an externally validated assay with results corrected to a standard pH of 7.40.13 Serum cPLI concentrations were measured using an analytically validated assay (Spec cPL, Texas A&M Gastrointestinal Laboratory) that has been shown to be highly sensitive and specific for a diagnosis of pancreatitis.14–17 This assay has been shown to be unaffected by extra-pancreatic lipases.18,19

Statistical analysis

Sample size calculations revealed that 36–47 samples were needed for phase I of the study given an assumed correlation of 0.40 to 0.45, \( \alpha = 0.05 \), and power of 0.80. Data sets were assessed for normality by Shapiro-Wilk testing. Normally distributed data were reported as means ± SD, whereas non-normally distributed data were reported as medians and interquartile ranges (IQR). In phase I, Spearman’s rank correlation coefficients were calculated to investigate potential relationships between cPLI and iCa concentrations. For Spearman testing a significant correlation score of ± 0.3 to 0.5 was considered a weak correlation, ± 0.5 to 0.7 was considered a moderate correlation, and ± 0.7 to 1.0 was considered a strong correlation.20 The Kruskal-Wallis rank sum test was used to investigate iCa differences among cPLI groups (< 200 µg/L, 201–399 µg/L, and ≥ 400 µg/L). Where a significant difference was detected by Kruskal-Wallis testing, post hoc pairwise comparisons were performed using the Wilcoxon rank sum test. In phase II, a paired t-test was used to evaluate the effect of the resolution of hypercalcemia on serum cPLI concentrations. All statistical analyses were performed using commercially available software (R Statistical Software v4.2.1, R Foundation for Statistical Computing), and \( P < .05 \) was considered significant for all comparisons.

Results

Phase I

Serum samples from 60 dogs were initially evaluated; however, there was insufficient residual volume for quantification of iCa in 16 dogs, leaving 44 dogs for inclusion in phase I of the study. Seventeen dogs had cPLI concentrations within the reference interval (< 200 µg/L). Thirteen dogs had cPLI concentrations in the diagnostic gray zone (200–399 µg/L),
and 14 dogs had a cPLI concentration ≥ 400 μg/L that is consistent with a diagnosis of pancreatitis. The median iCa concentration was 1.36 mmol/L (IQR: 0.1 mmol/L) (Reference interval [RI]: 1.25-1.45 mmol/L). Thirty-seven (37) dogs had a within RI iCa concentration. Three (3) dogs had a below RI iCa concentration, and 4 dogs had an above RI iCa concentration. There was a weak negative correlation between serum cPLI and iCa concentration (r = −.429, 95% CI [−.644, −.136], P = .005; Figure 1) in this population of dogs with a clinical indication for cPLI quantification. There was a significant difference in median iCa concentration between dogs with different cPLI concentration groupings (P = .02). Dogs with cPLI concentrations ≥ 400 μg/L had a lower median iCa concentration (1.30 mmol/L, IQR: 0.08 mmol/L) than those with a cPLI concentration < 200 μg/L (1.37 mmol/L, IQR: 0.08 mmol/L; P = .005). No other pairwise comparisons were significant (Figure 2).

Phase II

In phase II, the median iCa concentration at diagnosis was 1.68 mmol/L (IQR: 0.13 mmol/L), which significantly reduced to 1.38 mmol/L (IQR: 0.08 mmol/L; P < .001) 4 weeks after surgical parathyroidectomy or ultrasound-guided ethanol ablation. All dogs had resolution of their hypercalcemia with treatment.

In phase II, the median cPLI concentration at the time of PHPT diagnosis was 228.5 μg/L (IQR: 351.3 μg/L), which significantly reduced to 141.0 μg/L (IQR: 0.08 mmol/L; P < .001) 4 weeks after surgical parathyroidectomy or ultrasound-guided ethanol ablation. All dogs had resolution of their hypercalcemia with treatment.

Figure 1—The correlation between pancreatic lipase (cPLI) and ionized calcium (iCa) concentrations in 44 residual serum samples from client-owned dogs with a clinical indication for cPLI testing (original sample collection: April 2023). The blue line represents the fitted correlation between cPLI and iCa (based on a linear regression model) while the gray shading indicates the 95% CI for predictions made by the model. There is a weak negative correlation between serum cPLI and iCa concentration (r = −.429, 95% CI [−.644, −.136], P = .005).

Figure 2—The median and IQR of ionized calcium (iCa) concentrations between client-owned dogs with baseline pancreatic lipase (cPLI) concentrations < 200 μg/L (17 dogs), cPLI concentrations of 201-399 μg/L (13 dogs), and cPLI concentrations ≥ 400 μg/L (14 dogs). Dogs with cPLI concentrations ≥ 400 μg/L had a lower median iCa concentration (1.30 mmol/L, IQR: 0.08 mmol/L) than those with a cPLI concentration < 200 μg/L (1.37 mmol/L, IQR: 0.08 mmol/L; P = .005).

Figure 3—Pancreatic lipase (cPLI) concentrations in 24 client-owned primary hyperparathyroidism (PHPT) dogs at baseline and 4 weeks after surgical parathyroidectomy or ultrasound-guided ethanol ablation of parathyroid lesions. Figure 3B includes only those dogs that had an elevated baseline cPLI concentration (> 200 μg/L) (13 dogs). Dogs with truncated cPLI concentrations at baseline (ie, > 2,000 μg/L) were excluded from this figure.
Discussion

In this study, we used 2 populations of dogs to investigate the various dynamics of calcium in pancreatitis. In phase I we utilized serum samples from dogs that had had cPLI quantification performed by the attending veterinarian. Thus, the results of phase I were designed to reflect calcium dynamics in the clinical phases of AP, although clinical disease cannot be definitively determined based on the study design. In phase II, we utilized a population of dogs with PHPT as a naturally occurring model of the effects of hypercalcemia on cPLI concentrations. We propose that this population is best suited for evaluating whether hypercalcemia could be an inciting cause of pancreatitis in dogs and the effect of treatment of hypercalcemia on the resolution of pancreatitis.

In phase I, serum cPLI concentrations were weakly correlated with iCa concentrations, that is, dogs with higher cPLI concentrations having lower iCa concentrations. This is consistent with human medicine in which hypocalcemia is common in severe pancreatitis. A negative correlation between iCa and cPLI concentrations, the overall incidence of hypocalcemia was low, and the vast majority of dogs were normocalcemic, as seen in prior studies of dogs. A negative correlation between iCa and serum lipase activity has also been reported in the early stage of pancreatitis in people. Additionally dogs with high cPLI concentrations had lower median iCa concentrations than those with normal cPLI concentrations. Hypocalcemia in AP may be due to saponification of fat and calcium deposition, release of free fatty acids, or transient hypoparathyroidism and hypomagnesemia. Given these findings the presence of hypercalcemia in a dog with AP would be considered unusual and likely warrants further diagnostic investigation as it could be an underlying cause of pancreatitis.

In phase II, the majority of dogs had a reduction in cPLI concentration following the management of PHPT. The median cPLI concentration at PHPT diagnosis (ie, the time of hypercalcemia) was also numerically higher than the median cPLI concentration 4 weeks after correction of PHPT (ie, normocalcemia). While this was not statistically significant, the reduction in cPLI concentration noted after the correction of hypercalcemia could be of clinical significance, particularly in individual dogs with recurrent AP. Thus, the authors propose that iCa should be measured in dogs with recurrent AP to evaluate for a potential underlying cause of AP. Further studies are needed to determine whether the management of PHPT in dogs is protective against recurrent AP, as seen in humans. This study had limitations predominantly related to the limited patient data available for the dogs from which the residual serum samples originated. In phase I, clinical indication for cPLI quantification, and the cPLI concentration itself were used to indicate potential pancreatitis. Abdominal imaging is commonly included in reaching a clinical diagnosis of AP, but was not available for dogs enrolled in this study. While this is a limitation, this study design also offered the advantage of being able to assess the correlation between cPLI and iCa across a broad range of cPLI concentrations. The authors cannot exclude that an unmeasured confounding factor could be a cause for the negative correlation between cPLI and iCa, although this is considered less likely. Serum also underwent storage before measurement of iCa concentration. While all samples were stored frozen and handled in an expedited manner, we cannot rule out the potential that storage could have impacted the measured iCa concentrations. This could impact the results of our study. In phase II, we used PHPT as a naturally occurring model of hypercalcemia. It is also possible that a cause other than the resolution of hypercalcemia resulted in the decreased cPLI concentration in the PHPT dogs, although this is considered less likely. Some dogs had increases in cPLI concentrations between baseline and the 4-week sample, and it is possible that these dogs developed pancreatic injury secondary to hypercalcemia.

In conclusion, calcium depletion may result in an inverse relationship between serum cPLI and iCa concentration in dogs with a clinical indication for cPLI quantification. Thus, dogs with high serum cPLI concentrations should have iCa monitored to assist in the detection of hypocalcemia. Dogs with high serum cPLI concentrations and concurrently elevated iCa concentrations should be considered atypical and may warrant diagnostic investigation into the cause of hypercalcemia. Hypercalcemia may result in above reference interval cPLI concentrations, and cPLI concentrations may decrease after correction of hypercalcemia, but this was not significant in our population. This area requires additional exploration.

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None reported.

Disclosures

Dr. Steiner is associated with the Gastrointestinal Laboratory at Texas A&M University, which offers Spec cPL testing on a fee-for-service basis. Dr. Steiner acts as a paid consultant and Speaker for Idexx Laboratories, the manufacturer of the Spec cPL assay that also offers Spec cPL testing on a fee-for-service basis. No AI-assisted technologies were used in the generation of this manuscript.

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References


