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Serum trypsin-like immunoreactivity in dogs with diabetes mellitus

Kristen Hamilton[1] | Allison L. O'Kell[2] | Chen Gilor[2]

[1]Small Animal Hospital, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA

[2]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA

Correspondence
Allison L. O'Kell, Box 100126, 2015 SW 16th Avenue, Gainesville, FL 32608, USA.
Email: aokell@ufl.edu

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Abstract

Background: Concurrent exocrine pancreatic dysfunction and decreased pancreatic organ size are common findings in various stages of human type 1 diabetes mellitus (DM). Exocrine pancreatic insufficiency (EPI) is incompletely described in diabetic dogs.

Objective: To compare canine trypsin-like immunoreactivity (cTLI) of diabetic dogs with that of healthy controls. A secondary aim was to evaluate the correlation between duration of DM and cTLI.

Animals: Thirty client-owned diabetic dogs and thirty client-owned control dogs.

Methods: Cross-sectional study. Diabetic and healthy control dogs were included if they had no clinical evidence of pancreatitis and if serum samples obtained after food was withheld were available. Serum cTLI was measured at a reference laboratory and compared between groups. Canine pancreatic lipase immunoreactivity (cPLI) was analyzed concurrently as an indicator of pancreatitis.

Results: The median cTLI concentration in all diabetic dogs (36.4 μg/L [range, 7.0-288 μg/L]) did not differ from control dogs (28.7 μg/L [range, 12.8-58.6 μg/L]) ($P = .07$; difference −7.8 μg/L [95% Confidence Interval (CI), −23.5 to 0.6 μg/L]). There was still no difference in cTLI between groups after exclusion of dogs with cPLI consistent with pancreatitis (n = 8 diabetic dogs). There was no correlation between cTLI and DM duration in all diabetic dogs ($r = -0.07$, [95% CI, −0.43 to 0.3], $P = .7$).

Conclusions and Clinical Importance: There was no evidence of EPI as evaluated using cTLI in this cohort of diabetic dogs, but concurrent increases in cPLI suggest cTLI might not be the optimal indicator of exocrine pancreatic dysfunction in dogs with DM.

KEYWORDS
dog, pancreatic lipase immunoreactivity, pancreatitis, trypsin-like immunoreactivity

Abbreviations: cPLI, canine pancreatic lipase immunoreactivity; cTLI, canine trypsin-like immunoreactivity; DKA, diabetic ketoacidosis; DM, diabetes mellitus; EPI, exocrine pancreatic insufficiency; T1DM, type 1 diabetes mellitus.

1 | INTRODUCTION

Diabetes mellitus (DM) is an endocrine disease common in dogs with a prevalence of 0.26% to 0.6%.[1,2] Diabetes mellitus in dogs is often compared to human type 1 DM (T1DM),[3] a condition of absolute insulin deficiency characterized by islet-directed autoimmunity.[4] However, evidence for autoimmunity in DM in dogs is inconsistent and it is now recognized to be a heterogeneous syndrome.[5-8] Other factors that might contribute to the development of DM in dogs include other endocrinopathies, exogenous steroid or progesterone administration, and exocrine pancreatic disorders such as pancreatitis.[6,9,10]

Diabetes mellitus in humans is a disease of the entire pancreas, with exocrine pancreatic involvement occurring as either a cause or effect of T1DM.[11-14] The lack of insulin trophic effects might have adverse effects on exocrine pancreatic size and function.[15] Pancreatic organ size is lower in T1DM in diabetic patients, prediabetic patients, and first degree relatives compared to nondiabetic controls,[13,16-18] and there are a lower number of acinar cells in the pancreata of T1DM patients.[19] Additionally, fecal elastase 1 concentrations, a marker of exocrine pancreatic function used commonly in humans, are abnormally low in 13% to 57% of diabetic patients, with a higher prevalence in T1DM compared to type 2 DM patients although clinical signs of exocrine pancreatic insufficiency (EPI) are not always present.[13,20-23] Serum trypsinogen, another biomarker of exocrine pancreatic function in humans, is also lower in patients with T1DM as well as pre-type 1 DM (multiple autoantibodies) compared with nondiabetic control patients.[24] Diabetes caused by exocrine pancreatic disease such as pancreatitis is also associated in people with concurrent EPI.[25] This form of diabetes could be a relevant parallel to some cases of DM in dogs as the development of EPI secondary to chronic pancreatitis occurs, with some dogs also having concurrent DM.[26,27]

Serum canine trypsin-like immunoreactivity (cTLI) is pancreatic specific and is the most sensitive and specific test available for exocrine pancreatic function and detection of EPI in dogs, including subclinical disease.[28,29] The relationship between decreased exocrine pancreatic function and DM in dogs has not been extensively studied. The objective of the current study was to investigate exocrine pancreatic function in diabetic dogs without evidence of concurrent pancreatitis by comparing cTLI in diabetic dogs and nondiabetic healthy control dogs. We hypothesize that dogs with DM will have lower serum cTLI concentrations as compared to nondiabetic control dogs. In addition, given that in human T1DM decreased pancreatic volume and low fecal elastase are associated with longer duration of diabetes in some studies[20,30-32] and given the potential for progressive chronic pancreatitis in dogs leading to EPI,[26,27] we also expect for cTLI concentration to be inversely correlated with time since diagnosis of DM.

2 | MATERIALS AND METHODS

2.1 | Study design and dog selection

Blood samples were obtained between the years of 2016 and 2020 from client owned dogs that presented to the University of Florida Small Animal Hospital as part of a study evaluating DM biomarkers, pathogenesis, and metabolism. The study was approved by the Institutional Animal Care and Use Committee and the Veterinary Hospital Research Review Committee. Owners provided informed consent before study participation. Diabetic dogs were eligible for study enrollment if they had a confirmed diagnosis of DM (hyperglycemia and glucosuria concurrent with polyuria/polydipsia), were older than 1 year of age, had a body weight > 3 kg, and collection of an additional blood sample was considered safe by the attending clinician. Healthy control dogs were eligible for enrollment if they were aged >1 year, had a body weight > 3 kg, had no major medical conditions and no history of pancreatic or immune mediated disease, did not receive medications other than routine parasite preventatives, and were confirmed to be normoglycemic. Minor health conditions, such as incidental heart murmurs, periodontal disease, and seasonal allergies, were not exclusionary. Samples were retrospectively selected for analysis from the DM group if obtained after food was withheld for a minimum of 8 hours and if there was no clinical suspicion of active pancreatitis. An increased pancreatic lipase immunoreactivity or ultrasonographic evidence of pancreatitis (based on retrospective review of ultrasound reports) combined with acute gastrointestinal signs at the time of sample collection was considered consistent with active pancreatitis and an exclusion criterion. These tests were performed at the discretion of the attending clinician during the dog's visit and therefore not performed in all dogs. Data recorded included age at sample collection, sex, body weight, duration since diagnosis of DM, and medical history. Nondiabetic control samples were retrospectively selected if a sample obtained after food was withheld for a minimum of 8 hours was obtained. Serum samples were stored at −80°C between time of collection and submission to Texas A&M University Gastrointestinal Laboratory at which all laboratory analysis described below were performed.

2.2 | Laboratory methods

Assessment of serum cTLI (Immulite 2000 Canine TLI Kit; Siemens Healthcare Diagnostics Inc, Tarrytown, New York) and canine pancreatic lipase immunoreactivity (cPLI) via specific canine pancreatic lipase (IDEXX Laboratories, Westbrook, Maine) were performed using kits and reagents provided by the manufacturers. The laboratory reference interval for cTLI is 5.7 to 45.2 μg/L; results for values >50 μg/L were obtained by dilution.[33] Serum cTLI values above 50.0 μg/L might be associated with acute or chronic pancreatitis in dogs. The cPLI was also performed to evaluate for evidence of active pancreatic injury that may cause temporary concurrent increases in cTLI even in dogs with EPI.[34] The laboratory reference interval is 0 to 200 μg/L; between 201 and 399 μg/L is a questionable range and >400 μg/L is consistent with pancreatitis.[35]

2.3 | Data and statistical analysis

A sample size calculation based on pilot study cTLI data (unpublished) of 6 diabetic and 10 healthy control dogs with normal cPLIs using the

parameters $\alpha = 0.05$, power of 90%, effect size of 11 μg/L, and SD of 11 μg/L indicated that 22 dogs per group would be required to detect a significant difference between groups. We estimated that up to 25% of cases might eventually be excluded because of increased cPLI, and therefore aimed to recruit at least 30 cases per group. Quantitative data were assessed for normality using visual inspection of scatter and QQ plots along with the D'Agostino and Pearson test, and parametric or nonparametric tests were then performed accordingly. Age and body weight were compared between DM and control groups with an unpaired *t* test and Mann-Whitney *U* test, respectively, and sex distribution was compared using the Fisher's exact test. For cTLI concentrations, Mann-Whitney *U* tests were used to compare DM and control dogs in the following categories (a) all dogs, (b) only dogs with cPLI <400 μg/L, and (c) only dogs with cPLI <200 μg/L. The relationship between cTLI and DM duration was evaluated using Spearman correlation for each of the categories described in (a), (b), and (c) above.

Post hoc analysis to further investigate cPLI results was performed. cPLI was compared using the Mann-Whitney *U* test between all dogs in the diabetic group and the control group as well as after excluding dogs with diabetic ketoacidosis (DKA) (n = 3) from the diabetic group due to the high percentage of increased cPLI results reported in dogs with DKA.[36] The relationship between cPLI and DM duration in all diabetic dogs and after excluding dogs with DKA was evaluated using Spearman correlation. Additionally, the relationship between cPLI and cTLI in all dogs was evaluated using Spearman correlation. A *P* value of <.05 was considered statistically significant. All calculations were performed using GraphPad Prism version 8.0 (La Jolla, California).

3 | RESULTS

Sixty dogs (30 diabetic, 30 control) were included in the study. Among diabetic dogs, 22/30 (73%) were neutered males and 8/30 (27%) were spayed females, which did not differ from the control group (15/30 neutered male, 15/30 spayed female) ($P = .11$). Dogs in the DM group were older than controls (mean ± SD = 9.1 ± 2.2 years vs 5.9 ± 2.7 years, respectively) ($P \leq .001$; difference −3.1 years [95% CI, −4.5 to −1.9 years]). There was no difference in body weight between diabetic and control groups (median 11.7 kg [range, 5.4-41.6 kg] vs 9.8 kg [range, 4.4-45.9 kg], respectively) ($P = .53$; difference −1.9 kg [95% CI, −4.8 to 3.0 kg]). The DM group included 8 mixed breed dogs, 3 Labrador retrievers, 2 Chihuahuas, 2 Yorkshire terriers, and 1 each of the following breeds: miniature Shetland sheepdog, Lhasa Apso, toy poodle, silky terrier, Maltese, shih tzu, Pembroke Welsh corgi, pug, miniature poodle, beagle, Siberian husky, miniature schnauzer, Pomeranian, rottweiler, and Australian shepherd. The control group included 11 mixed breed dogs, 2 Labrador retrievers, and 1 of each of the following breeds: coonhound, West Highland white terrier, Shetland sheepdog, dachshund, greyhound, Cairn terrier, shih tzu, Pembroke Welsh corgi, pug, miniature poodle, beagle, Siberian husky, miniature schnauzer, Pomeranian, rottweiler, Chihuahua, and Australian shepherd.

The median duration of DM at the time of sample collection was 3 months (range, 2 days to 18 months). Three diabetic dogs were clinically ill with a diagnosis of DKA and newly diagnosed DM at the time of the study, and 3 other diabetic dogs were hospitalized due to clinical illness without ketoacidosis due to uncharacterized hepatopathy and newly diagnosed DM (n = 1), symptomatic hypoglycemia (n = 1), and unregulated DM, urinary tract infection, and inappetence (n = 1). All other diabetic dogs visited the hospital as outpatients. Descriptive statistics for all group comparisons are presented in Table S1. The median cTLI concentration in all diabetic dogs (36.4 μg/L [range, 7.0-288 μg/L]) did not differ from the control group (28.7 μg/L [range, 12.8-58.6 μg/L]) ($P = .07$; difference −7.8 μg/L [95% CI, −23.5 to 0.6 μg/L]). Two control dogs (7%) and 9 diabetic dogs (30%) had serum cTLI measurements greater than 50.0 μg/L. When only dogs with cPLI <400 μg/L were included (n = 22 diabetic dogs, n = 30 control dogs), the median cTLI in the DM group (32.7 μg/L [range, 7.0-83.3 μg/L]) was not different from the control group (28.7 μg/L [range, 12.8-58.6 μg/L]) ($P = .3$; difference −4.1 μg/L [95% CI, −15.6 to 4.5 μg/L]). Similarly, median cTLI concentration was not different between DM and control dogs when only dogs with cPLI <200 μg/L were included (DM [n = 17] = 30.4 μg/L [range, 7.01-64.5 μg/dL], control [n = 29] = 28.3 μg/L [range, 12.8-58.6 μg/L]) ($P = .64$; difference −2.1 μg/L [95% CI, −11.9 to 6.8 μg/L]) (Figure 1). There was no correlation between cTLI concentrations and DM duration for all DM dogs ($r = -0.07$, [95% CI, −0.43 to 0.3], $P = .7$), diabetic dogs with cPLI <400 μg/L ($r = -0.26$, [95% CI, −0.61 to 0.19], $P = .25$) (Figure 2), or diabetic dogs with cPLI <200 μg/L ($r = -0.23$, [95% CI, −0.65 to 0.3], $P = .36$).

Of the diabetic dogs, 13/30 (43%) had cPLI concentrations >200 μg/L, of which 8/30 (27%) had cPLI concentrations >400 μg/L. In the control group, 1/30 (3%) had cPLI concentrations >200 μg/L and none had cPLI concentrations >400 μg/L. Of the diabetic dogs with a cPLI >200 μg/L, 3/13 (23%) were hospitalized with DKA at the time of sample collection, of which 2 of the dogs with DKA had a cPLI >400 μg/L. Twenty-four diabetic dogs had samples collected during outpatient hospital visits, and of those, 11/24 (46%) had cPLI concentrations >200 μg/L, including 6 with cPLI >400 μg/L (6/24, 25%). Serum cPLI concentrations were higher in the dogs with DM (median 124 μg/L; range, 29-2001 μg/L) than that of the control group (median 29 μg/L; range, 29-334 μg/L) ($P < .001$; difference −95 μg/L [95% CI, −196 to −10 μg/L]). When dogs with DKA were removed from the diabetic group (n = 3), the cPLI concentration in the diabetic group (median 68 μg/L; range, 29-2001) remained higher from that of the control group (median 29 μg/L; range, 29-334 μg/L) ($P < .001$; difference −39 μg/L [95% CI, −157 to −6 μg/L]). There was a moderate positive correlation ($r = 0.5$, [95% CI, 0.28-0.68], $P < .001$) between cTLI and cPLI concentrations in all dogs). There was also a moderate negative correlation between cPLI concentrations and DM duration in all diabetic dogs ($r = -0.42$, [95% CI, −0.69 to −0.06], $P = .02$). When dogs with DKA (n = 3) were removed from this analysis, this correlation was no longer significant ($r = -0.27$, [95% CI, −0.60 to 0.14], $P = .18$).

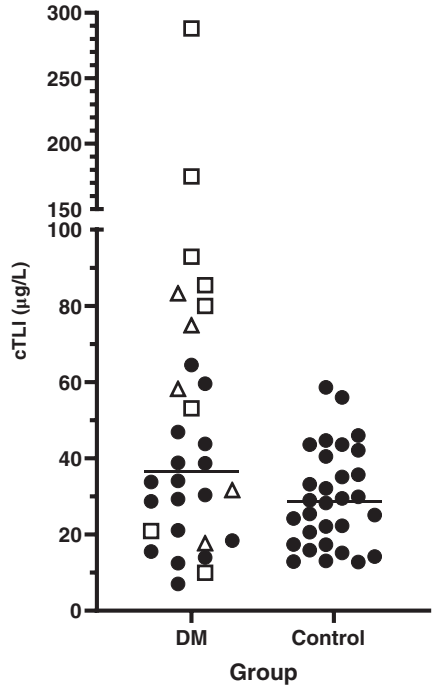

FIGURE 1 Canine trypsin-like immunoreactivity (cTLI) concentration in diabetic (DM) (n = 30) and control (n = 30) dogs. In the DM group, black circles are dogs with concurrent cPLI concentrations <200 µg/L, open triangles are dogs with concurrent cPLI 201 to 400 µg/L, and open squares are dogs with concurrent cPLI >400 µg/L. The horizontal line represents the median. cPLI, canine pancreatic lipase immunoreactivity

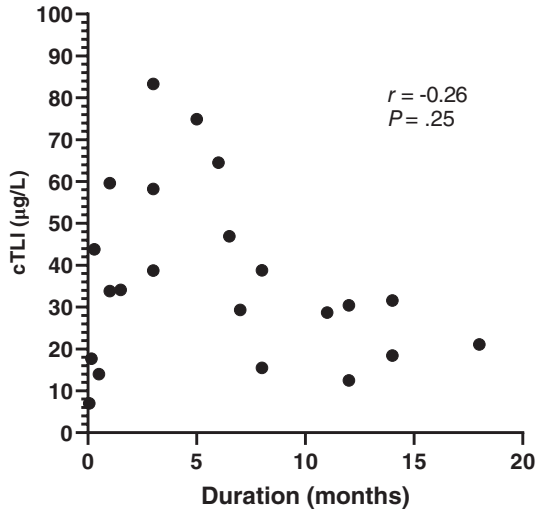

FIGURE 2 Relationship between canine trypsin-like immunoreactivity (cTLI) concentrations and diabetes duration in diabetic dogs with cPLI concentrations <400 µg/L. cPLI, canine pancreatic lipase immunoreactivity

4 | DISCUSSION

The present study did not find evidence of EPI as evidenced by cTLI concentration in diabetic dogs when compared to control dogs. Additionally, there was no correlation between cTLI concentration and duration since diagnosis in this population of diabetic dogs. An unexpected finding was that 25% of diabetic dogs without clinical signs of pancreatitis had cPLI concentrations consistent with pancreatitis.

Our findings contrast with recent human studies showing exocrine pancreatic dysfunction and decreased pancreas volume in T1DM.[13,16-18,24] It is possible that our study was underpowered to detect a difference between groups and that the findings are due to type 2 error.

Some cases of DM in dogs are suspected to be secondary to pancreatitis with concurrent EPI present,[27] but further studies to determine the similarity in the mechanisms of endocrine failure to humans are required. Given the apparent heterogeneity of the canine disease, it is possible that a narrow subset of dogs with DM has a component of exocrine pancreatic dysfunction or insufficiency and that our population represented a wide variety of disease etiologies. To the authors' knowledge, 1 other study investigating exocrine pancreatic function specifically in diabetic dogs with no clinical signs of pancreatitis is available only in abstract form; this study followed well-controlled diabetic dogs without a history or diagnostic imaging consistent with pancreatitis and found that in 2 out of 12 dogs the cTLI was consistent with EPI (<2.5 µg/L) without clinical signs and in 2 other dogs the cTLI was questionable for EPI on 1 occasion over 6 months.[37] Other studies focusing on dogs with chronic pancreatitis have identified concurrent DM and EPI in 2/4[26] and 2/14[27] dogs. In our study, no dogs had cTLI concentrations either consistent with EPI or in the questionable range, which could potentially be due differences in the study population and inclusion criteria leading to a more heterogenous group of dogs.

Because pancreatic injury might increase cTLI concentrations,[38] we measured cPLI in each dog as an additional indicator of pancreatic injury in an attempt to exclude dogs with active pancreatitis. Even when dogs with cPLI concentrations consistent with pancreatic injury were excluded, there was no evidence of a difference in cTLI concentration between diabetic and control groups. It remains difficult to definitively diagnose pancreatitis in dogs and a combination of clinical findings, laboratory results, and imaging are typically required.[39] In dogs, the cPLI is currently the most accurate test of pancreatitis when compared to the gold-standard of histopathological diagnosis, but its sensitivity is still low for mild pancreatitis (21% and 43% with a cutoff of 400 µg/L and 200 µg/L, respectively) compared with that for moderate to severe pancreatitis (71%).[40] In that study, the sensitivity of cTLI for diagnosis of mild pancreatitis was similar (30%).[40] It is therefore possible that mild or clinically undetected pancreatitis that increased cTLI above the baseline level for each dog was present in some diabetic dogs with a cPLI within the reference interval, and therefore masked differences in cTLI between the groups. Other noninvasive and accurate methods to measure exocrine pancreatic function in dogs that are not affected by pancreatitis are currently not available. In humans, fecal elastase-1 is a commonly used method to assess exocrine pancreatic function that is sensitive for detecting severe EPI, but false positives could result in some circumstances

where patients have a low probability of disease.[41] A canine-specific fecal elastase-1 assay is available but has a high rate of false-positive results that limit its clinical utility.[42]

Of the clinically stable diabetic dogs evaluated as outpatients in the present study, 6/24 (25%) had cPLI concentrations consistent with pancreatitis without concurrent supportive clinical signs. The study described above found an even higher percentage (67%) of increased cPLI in diabetic dogs despite a lack of current increases in c-reactive protein, clinical signs, or ultrasound findings that would suggest clinical pancreatitis.[37] Previous studies evaluating cPLI for the diagnosis of clinically suspected acute pancreatitis found specificities of 74% to 81%[43] and 77%,[44] indicating that false-positive results for a clinical diagnosis of pancreatitis are not uncommon in dogs with other diseases. Additionally, 35% of dogs with hyperadrenocorticism and no clinical signs of pancreatitis had cPLI >400 μg/dL in 1 study, although the absence of pancreatitis in these dogs was not confirmed histopathologically.[45-47] No dogs in the present study had confirmed hyperadrenocorticism, but the diagnostic workup for underlying diseases in diabetic dogs was not standardized, and the contribution of this disease to increases in cPLI in some cases cannot be completely ruled out. All 3 dogs diagnosed with DKA at the time of sample collection had cPLI concentrations consistent with pancreatitis, which supports previous evidence that pancreatic injury is common in dogs with DKA.[36] These results raise questions about the relationship of pancreatic injury to DM. It is difficult to ascertain whether DM is the precursor or sequela to pancreatitis, however. In humans, type 2 DM increases the risk for acute pancreatitis, although pathogenic mechanisms have not been clearly defined[48]; conversely, acute and chronic pancreatitis also increase the risk of developing DM in humans.[49,50] In diabetic dogs, pancreatitis has been found to be a common comorbid condition diagnosed clinically,[9,51] but the diagnosis of pancreatitis heavily relies on increased pancreatic lipase and there is wide variability among studies regarding histological confirmation of pancreatitis in diabetic dogs.[7,52-54] Additionally, some dogs with chronic pancreatitis have impaired beta cell function,[55] and pancreatitis might lead to secondary destruction of pancreatic islets.[10] To the authors' knowledge, although associations between DM and subsequent pancreatic injury have been demonstrated,[37] there are no studies in dogs evaluating DM as a direct cause of pancreatic injury.

Some evidence exists that suggests a correlation between the duration of DM and pancreatic atrophy in humans, while other studies found no such correlation.[17,30,56] The reduced pancreatic volume in newly diagnosed patients with T1DM and those with autoantibodies at risk for T1DM suggests that exocrine pancreatic involvement might begin before clinical manifestation of DM.[14,16,17] In the present study, no correlation was found between the duration of DM and cTLI concentration in dogs. However, the majority of dogs in the current study had a relatively short duration of disease. It is possible that if dogs with a longer history of DM were examined a greater effect on cTLI concentration might be observed.

Several limitations exist in the present study. Dogs with increased cPLI might have had pancreatitis and this could have affected cTLI as well. The medical workup was not standardized and only 4/30 diabetic dogs had an ultrasound near the time of sample collection as an additional method of evaluating for pancreatitis. No dogs had histopathology of pancreatic tissue. Given that the sensitivity of ultrasonography for a clinical diagnosis of pancreatitis varies between 42% and 89%[57] and that it has only fair correlation with cPLI,[58] it would be difficult to rule out concurrent pancreatitis as a cause for our cTLI results without histopathology even if ultrasound results were available in all cases. In addition, dogs in the diabetic group were significantly older than those in the control group, and, to the authors' knowledge, the effects of age on cTLI are unknown in dogs. It is also possible that cTLI concentration is an insufficiently sensitive indicator of mild exocrine pancreatic dysfunction or decreased exocrine pancreatic volume in dogs and that a more sensitive test might identify difference between groups similarly to those described in T1DM.

In conclusion, this study did not provide evidence to support the presence of decreased exocrine pancreatic function in dogs with DM. A larger sample size of dogs with more restrictive exclusion criteria against concurrent pancreatic injury might be necessary to rule this out as a cause of increases in cTLI concentrations above what might be considered a baseline in diabetic dogs. Assessment of pancreatic volume using advanced imaging (eg, magnetic resonance imaging) would provide further insight into the role of the exocrine pancreas in this disease. In addition, it is possible that only a small subset of dogs with an autoimmune component to their disease or those with end-stage chronic pancreatitis have concurrent exocrine pancreatic dysfunction.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

Approved by the University of Florida IACUC (#201609360 and #201810124) and the Veterinary Hospital Research Review Committee.

HUMAN ETHICS APPROVAL DECLARATION

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

ORCID

Allison L. O'Kell https://orcid.org/0000-0002-3412-0575
Chen Gilor https://orcid.org/0000-0003-0393-4135

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SUPPORTING INFORMATION

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

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