Serum lipase activities and pancreatic lipase immunoreactivity concentrations in dogs with exocrine pancreatic insufficiency

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Objective—To determine serum lipase activities and pancreatic lipase immunoreactivity (PLI) concentrations in dogs with exocrine pancreatic insufficiency (EPI).

Animals—74 healthy dogs and 25 dogs with EPI.

Procedures—A diagnosis of EPI was made on the basis of clinical signs, low serum trypsin like immunoreactivity (TLI) concentration, and response to treatment with enzyme replacement. Median values for fasting serum lipase activity and serum PLI concentrations were compared between the 2 groups with a Mann-Whitney U test.

Results—Median fasting serum lipase activity was not significantly different between dogs with EPI (366.0 U/L) and healthy dogs (294.5 U/L), and only 1 dog with EPI had a serum lipase activity less than the lower limit of the reference range. Median serum PLI concentration was significantly lower in dogs with EPI (0.1 µg/L) than in healthy dogs (16.3 µg/L). All dogs with EPI had serum PLI concentrations less than the lower limit of the reference range.

Conclusion and Clinical Relevance—Serum lipase activity is not limited to the exocrine pancreas in origin, whereas serum PLI is derived only from the exocrine pancreas. Unlike in serum TLI concentrations, there was a small degree of overlap in serum PLI concentrations between healthy dogs and dogs with EPI. Serum TLI concentration remains the test of choice for diagnosis of EPI. (Am J Vet Res 2006;67:84–87)

Exocrine pancreatic disease is common in dogs. In a study of abnormal findings in dogs necropsied during an 11-year period at the University of Munich, 1.7% of 9,342 dogs had clinically important lesions of the exocrine pancreas. The most common exocrine pancreatic disorder reported in that and other studies was pancreatitis, followed by exocrine pancreatic neoplasia and lesions suggestive of EPI. Despite the frequency of its occurrence, pancreatitis can be difficult to diagnose, especially in animals in which the disease is mild. This is chiefly because the clinical features can be variable and nonspecific in nature, the disease is highly localized, and there is an absence of sensitive and specific diagnostic markers for pancreatitis.

Determinant of serum lipase activity has been used for diagnosis of pancreatitis in humans and dogs for several decades. However, in both species, it is well recognized that serum lipase activity is neither sensitive nor specific for diagnosis of pancreatitis. Serum lipase activity decreases in dogs after pancreatectomy, indicating that some lipase activity in serum originates from the exocrine pancreas. However, considerable serum lipase activity remains in dogs after pancreatectomy, indicating that the lipase activity measured in serum also has nonpancreatic sources. There are many cell types that synthesize and secrete lipases. Lipases of different cellular origins share a common function and thus cannot be differentiated by use of catalytic assays, such as those used to measure lipase activity in serum.

Recently, a new assay for measurement of PLI was developed and validated. An immunolocalization study revealed that only pancreatic acinar cells stain for pancreatic lipase, suggesting that the analyte measured with that assay is limited to exocrine pancreatic origin.

Exocrine pancreatic insufficiency is caused by a lack of synthesis of pancreatic digestive enzymes. The most common cause of the condition in dogs is pancreatic acinar atrophy, a disorder most often diagnosed in German Shepherd Dogs and Collies. In contrast, EPI in humans and cats is most commonly caused by chronic pancreatitis, which can also cause EPI in dogs. Whatever the cause, it has been suggested that in humans, > 90% of the secretory reserve of the pancreas must be lost before clinical signs of EPI become apparent. Common clinical signs in dogs with EPI are weight loss, diarrhea, discolored feces that may be malodorous, and polyphagia.

Diagnosis of EPI in dogs was simplified by the development of a radioimmunoassay for the measurement of TLI in serum. In 1 study, this assay was 100% sensitive and 100% specific for diagnosis of EPI in dogs suspected of having EPI. The test remains the gold standard for diagnosing EPI in dogs.

Although serum TLI concentration is considered the gold standard for diagnosing EPI, there are few noninvasive options for diagnosis of pancreatitis that are both sensitive and specific. To evaluate whether the origin of analytes measured by use of a commonly used serum lipase activity assay and a newly available diagnostic test (serum PLI concentration) was limited to...

PLI Pancreatic lipase immunoreactivity
EPI Exocrine pancreatic insufficiency
TLI Trypsin like immunoreactivity

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the exocrine pancreas, we evaluated serum lipase activities and serum PLI concentrations in dogs with EPI.

Materials and Methods

Animals—Sera from 2 groups of dogs were used for the study. The first group comprised 74 healthy dogs obtained from different sources that had previously been used to establish the reference range for serum PLI concentration. None of the study dogs had a history of clinical signs suggestive of gastrointestinal tract or other disease, but no further diagnostic tests other than physical examination were performed to exclude subclinical disease in any of the dogs.

The second group included 25 dogs with EPI that had been enrolled in an unrelated study. Those dogs had clinical signs of EPI, including weight loss, diarrhea, discolored feces with or without malodorous smell, and polyphagia. Dogs also had serum TLI concentrations $\leq 2.0$ µg/L and had responded favorably to treatment with enzyme replacement.

Assays—Blood for serum samples was collected from all dogs. Veterinarians enrolling dogs were instructed to collect a blood sample after withholding food for at least 8 hours, but compliance was not verified. Serum TLI concentration, serum lipase activity, and serum PLI concentration were evaluated. Serum TLI concentration was measured by means of a commercially available radioimmunoassay. Serum lipase activity was measured by use of an automated serum chemistry analyzer and a lipase assay kit. The assay kit included 1,2-diglyceride as substrate and colipase and deoxycholate as activators, and results were interpreted on the basis of colorimetric detection of a quinone dye formed in a cascade of reactions associated with hydrolysis of the substrate to 2-monoglyceride and fatty acids by lipase at a pH of 6.8. Serum PLI was measured with an in-house ELISA.

Statistical analysis—Analyses were performed by use of commercial statistical software packages. Values of $P < 0.05$ were considered significant. Initially, all data sets were analyzed by testing the data set for normality with the D'Agostino and Pearson omnibus normality test. For variables that failed the test for normality in either healthy dogs or dogs with EPI, median values were compared between the 2 data sets by use of a 2-tailed Mann-Whitney U test. The number of dogs with EPI that had serum lipase activity or serum PLI concentration outside a previously determined reference range was also determined.

Results

Exocrine pancreatic insufficiency was conclusively diagnosed in 25 dogs by means of a clinical history compatible with EPI, serum TLI concentrations $\leq 2.0$ µg/L, and a favorable response to enzyme replacement treatment. Serum lipase activities and PLI concentrations were determined in the 25 dogs with EPI.

In dogs with EPI, data sets for serum TLI concentration and serum lipase activity passed the test for normality. In contrast, data sets for serum TLI in healthy dogs, serum lipase activity in healthy dogs, serum PLI in healthy dogs, and serum PLI in dogs with EPI failed the normality test.

Median serum TLI concentration was significantly lower in dogs with EPI (0.7 µg/L; range, 0.5 to 1.5 µg/L) than in healthy dogs (10.7 µg/L; range, 5.0 to 46.0 µg/L; $P = 0.001$; Figure 1). By definition, all 25 dogs with EPI had serum TLI concentrations less than 2.5 µg/L; 8 of those dogs had concentrations less than the detection limit (0.5 µg/L).

Median serum lipase activity was not significantly different between healthy dogs (294.5 U/L; range, 32 to 557 U/L) and dogs with EPI (366.0 U/L; range, 87 to 788 U/L; $P = 0.273$; Figure 2). Only 1 of 25 (4%) dogs with EPI had serum lipase activity less than the lower limit of the reference range of 93.5 to 625.4 U/L.
The median serum PLI concentration was significantly lower in dogs with EPI (0.1 μg/L; range, 0.1 to 1.4 μg/L) than in healthy dogs (16.3 μg/L; range, 1.4 to 270.6 μg/L; P < 0.001; Figure 3). Dogs with EPI had serum PLI concentrations less than the lower limit of the reference range (2.2 to 102.1 μg/L), and 20 had serum PLI concentrations less than the detection limit of 0.1 μg/L. The 5 lowest values for TLI and PLI concentration in the 74 healthy dogs and the 5 highest values for serum TLI and PLI concentrations in the dogs with EPI were summarized (Figure 4), and there was no overlap of serum TLI concentrations between healthy dogs and dogs with EPI.

**Discussion**

Measurement of serum TLI concentration has been established as a gold-standard diagnostic test for diagnosis of EPI in dogs. All 25 dogs with EPI in our study had low serum TLI concentrations. Similarly, serum PLI concentration was low in dogs with EPI, and serum PLI concentration was below the lower limit of the reference range in all 25 dogs. This suggests that, as is true for serum TLI concentration, serum PLI concentration is derived only from an analyte of exocrine pancreatic origin. Initially, evaluation of these data may suggest that measurement of serum PLI concentration is a viable alternative to determination of serum TLI concentration for diagnosis of EPI in dogs. However, a closer inspection of the data indicated that, in this group of dogs with EPI, determination of serum TLI concentration was superior to determination of serum PLI concentration for diagnosis of EPI. The 5 lowest values for TLI and PLI concentration in the 74 healthy dogs and the 5 highest values for serum TLI and PLI concentrations in the dogs with EPI were summarized, and there was no overlap of serum TLI concentrations between healthy dogs and dogs with EPI. The lowest serum TLI concentration among the healthy dogs was 5.0 μg/L, whereas all dogs with EPI (by definition) had serum TLI concentrations ≤ 2.0 μg/L. In contrast, there was a small degree of overlap in measurements of serum PLI concentrations in that the lowest serum PLI concentration in a healthy dog (1.4 μg/L) also represented the highest serum PLI concentration in the group of dogs with EPI. This degree of overlap is small but suggests that serum TLI concentration was a superior marker for EPI, compared with serum PLI concentration, in dogs in our study. It could be argued that this finding was inevitable, given that serum TLI concentrations were used to verify a diagnosis of EPI. However, healthy control dogs were not selected on the basis of a serum TLI concentration within the reference range, suggesting that fewer healthy dogs have serum TLI concentrations below the lower limit of the reference range than dogs that have serum PLI concentrations below the lower limit of the reference range. This finding supports the possibility that serum TLI concentration is a better test than serum PLI concentration for diagnosis of EPI. The small overlap in serum TLI concentrations may be a result of the shorter serum half-life of trypsinogen, compared with that of pancreatic lipase. In healthy dogs or dogs with EPI, almost all of the analyte detected via the assay for serum TLI is trypsinogen, as opposed to trypsin or trypsin that is bound to scavenging proteins (such as α1-proteinase inhibitor); the latter may theoretically also be detected by the TLI assay but is virtually absent in healthy individuals. Canine cationic trypsinogen, the trypsinogen detected by the commercially available TLI assay, is relatively small in molecular size (approx 24 kd) and is positively charged. In contrast, the pancreatic lipase molecule in dogs is much larger (approx 50.7 kd) and negatively charged. Thus, whereas pancreatic lipase would be repulsed from the glomerular filter, a larger portion of trypsinogen would be filtered and excreted by the kidney, resulting in a shorter serum half-life.

Humans with EPI rarely have selective pancreatic enzyme deficiencies, particularly pancreatic lipase deficiencies. Affected patients have clinical signs of fat malabsorption, but serum TLI concentrations remain within reference range. Specific evaluation of serum PLI concentration may be useful for diagnosis of congenital lipase deficiency in dogs. However, because dogs with deficiencies of specific pancreatic enzymes have not been reported, routine evaluation of serum PLI concentration for diagnosis of isolated pancreatic lipase deficiency does not appear to be warranted.

Our data indicate that, unlike serum lipase activity, the analyte measured in serum as PLI is limited in origin to the exocrine pancreas because there is near-total absence of detectable serum PLI concentrations in dogs with EPI. The poor specificity of serum lipase activity has been reported, and our findings were in agreement with those. This finding is germane to the potential clinical usefulness of the measurement of serum PLI concentration for diagnosing pancreatitis in dogs. Further studies are warranted to determine the sensitivity and specificity of this new diagnostic marker for pancreatitis in dogs.

**Figure 4—Serum TLI and PLI concentrations in healthy dogs and dogs with EPI.** Data points represent the 5 lowest serum TLI and PLI concentrations in 74 healthy dogs and the 5 highest serum TLI and PLI concentrations in 25 dogs with EPI.
References