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Serum Lipase Activity and Canine Pancreatic Lipase Immunoreactivity (cPLI) Concentration in Dogs with Experimentally Induced Chronic Renal Failure

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Abstract: Assays for the measurement of pancreatic lipase concentration in dog serum (cPLI) have been suggested to be useful for the diagnosis of canine pancreatitis. Clinical signs of pancreatitis and renal failure can overlap. Previously, serum lipase activity has been reported to be increased in dogs with renal failure but the influence of renal failure on serum cPLI concentration has not been evaluated. The goal of this project was to examine the influence of experimentally induced Chronic Renal Failure (CRF) on serum lipase activity and cPLI concentration. Serum samples were collected from 17 dogs with experimentally-induced CRF and were analyzed for creatinine concentration, lipase activity and cPLI concentration. One of the dogs showed extreme results for both serum lipase activity and cPLI concentration but was shown to have histological evidence of pancreatitis and was removed from further analysis. Serum lipase activities and cPLI concentrations of the 16 remaining dogs were compared to the reference intervals for these parameters. Serum lipase activity was within the reference interval in all 16 dogs with experimentally induced chronic renal failure. Serum cPLI concentration was outside the reference interval for serum cPLI (2.2-02.1 μg L^{-1}) concentration for two dogs but below the suggested diagnostic cut-off value for pancreatitis (200 μg L^{-1}) in all 16 dogs. Dogs with experimentally induced chronic renal failure studied here did not have clinically relevant increases in serum lipase activity or serum cPLI concentration. Further studies in dogs with spontaneous renal failure are necessary and ongoing.

Key words: cPLI, chronic renal failure, pancreatitis, lipase activity, nephrectomy, diagnostic test

INTRODUCTION

Estimation of serum lipase has been used for the diagnosis of pancreatitis in humans and dogs for the last three decades (Brobst *et al*., 1970; Mia *et al*., 1978b; Strombeck *et al*., 1981; Kolars *et al*., 1984; Ventrucci *et al*., 1989). Most studies use the measurement of serum lipase activity rather than that of the mass concentration of pancreatic lipase. However, enzymatic assays may not be suitable to distinguish between lipases of different cellular origins.

The suspicion that lipase activity measured in serum does not solely originate from the exocrine pancreas is supported by studies that show that total pancreatectomy in dogs does not lead to total depletion of serum lipase activity and that the median serum lipase activity in dogs with EPI is not decreased when compared to healthy dogs (Eto *et al*., 1969; Simpson *et al*., 1991; Steiner *et al*., 2006).

Several studies have shown that dogs with experimental or spontaneous pancreatitis can have an increased serum lipase activity (Brobst and Brester, 1967; Brobst *et al*., 1970; Mia *et al*., 1978a, b; Strombeck *et al*., 1981; Whitney *et al*., 1986). However, other dogs with spontaneous pancreatitis may display no or only mild increases of serum lipase activity (Strombeck *et al*., 1981). Also, several non-pancreatic conditions have been identified in dogs that are associated with an increase in serum lipase activity (Strombeck *et al*., 1981). Dogs with experimental as well as spontaneous chronic renal failure have been reported to have increased serum lipase activities (Wagner and Macy, 1982; Polzin *et al*., 1983).

It is interesting to note that no correlation of this increase with inulin clearance, serum creatinine concentration or serum urea nitrogen concentration could be demonstrated and reduced renal excretion of serum lipase activity may therefore not be the sole explanation

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for this increase (Polzin *et al*., 1983). Heat stress and muscular exercise has also been described to lead to an increase in serum lipase activity (Bedrak, 1965). In addition, administration of dexamethasone has been reported to cause an increase in serum lipase activity in normal dogs (Parent, 1982). Finally, several dogs without any identifiable lesion of the exocrine pancreas by light microscopy had significantly increased serum lipase activities (Strombeck *et al*., 1981).

These dogs were diagnosed with renal diseases such as glomerulosclerosis and glomerulonephritis, hepatic diseases such as hepatic necrosis, hepatic fatty degeneration, hepatocellular carcinoma, bile duct carcinoma and lymphosarcoma or other diseases such as hemangiosarcoma of the heart, adenocarcinoma of the small intestine, lymphosarcoma of the gastrointestinal tract or amyloidosis of multiple organs (Strombeck *et al*., 1981). Thus, serum lipase activity lacks specificity for exocrine pancreatic origin. An assays for the measurement of classical pancreatic lipase concentration in serum, canine Pancreatic Lipase Immunoreactivity (cPLI) has recently been developed and analytically validated (Steiner *et al*., 2003).

This assay is an ELISA for which a reference interval of 2.2-102.1 μg L^{-1} has been established in 74 healthy control dogs (Steiner *et al*., 2003). In contrast to serum lipase activity, serum cPLI concentration was significantly decreased in 25 dogs with exocrine pancreatic insufficiency, suggesting that this marker is specific for exocrine pancreatic origin (Steiner *et al*., 2006). However, serum concentration of a marker molecule is not only dependant on addition of that molecule by secretion but also on removal through metabolism and excretion. Thus, renal failure may cause an increase in serum cPLI concentration especially since a previous study reported an elevation of serum lipase activity in dogs with chronic renal failure (Polzin *et al*., 1983). Therefore, the aim of this study was to determine whether experimentally induced renal failure would increase serum lipase activity and/or cPLI concentrations.

MATERIALS AND METHODS

Chronic renal failure was induced by 15/16 nephrectomy in 18 healthy dogs for an unrelated study in the early 80 ties (Finco *et al*., 1985). Serum samples from these dogs were collected at different stages during their disease and were stored frozen at -80°C. No animal research was conducted for the purpose of the study reported here. Serum samples from 17 of the 18 dogs originally studied were still available and were analyzed for creatinine concentration, lipase activity and cPLI concentration. All measurements were made as a batch analysis for all 17 samples within the same assay run. Serum creatinine concentrations were analyzed using an automated serum chemistry analyzer[1]. Serum lipase activity was measured using an automated serum chemistry analyzer and a commercially available lipase assay kit[2].

This assay uses a 1, 2-diglyceride as a substrate and colipase and deoxycholate as activators and is based on the colorimetric detection of a quinone dye that is formed in a cascade of reactions following the hydrolysis of the substrate to 2-monoglyceride and fatty acids by lipase at pH 6.8. Serum canine pancreatic lipase immunoreactivity was measured by an in-house ELISA (Steiner *et al*., 2003).

A commercially available software package[3] was used for data analysis and a $p<0.05$ was considered evidence for statistical significance. Initially, the data were analyzed for normality by the D'Agostino and Pearson omnibus normality test. For parametric data sets the means and standard deviations were calculated while the median was calculated for non-parametric datasets.

One of the dogs showed extreme results for both serum lipase activity and cPLI concentration. The Grubb's test for outliers was suggestive of outlier data points for both serum lipase activity and serum cPLI concentration but not for serum creatinine concentration, suggesting that other factors may be responsible for this data point. This notion was further supported by an extreme serum trypsin-like immunoreactivity concentration of 9,702 μg L^{-1} (reference range: 5-35 μg L^{-1}) measured in the same serum sample. Therefore this dog was excluded from further analysis.

Serum lipase activities and cPLI concentrations of the 16 remaining dogs were compared to the reference interval for each parameter. Also, serum lipase activities and cPLI concentrations were statistically compared to those from a group of 74 healthy dogs that had previously been used to calculate the reference interval for both serum lipase activity and serum cPLI concentration using Mann-Whitney tests (data sets failed normality testing).

RESULTS AND DISCUSSION

Serum creatinine concentration, lipase activity and cPLI concentration for 17 dogs with experimentally induced chronic renal failure are shown in Table 1. As mentioned above the data points for one of the dogs were removed from further analysis. Mean±SD serum

Table 1: Serum creatinine concentrations, lipase activities and cPLI concentrations in 17 dogs with experimentally induced chronic renal failure

ID number	Creatinine (mg dL^{-1})	Lipase activity (U L^{-1})	cPLI (µg L^{-1})
1	2.1	198.0	26.2
2	2.3	337.0	78.3
3	2.9	396.0	123.3
4	3.5	240.0	32.6
5	3.7	93.0	12.5
6	3.7	376.0	34.0
7	3.8	270.0	35.2
8	3.9	394.0	27.8
9	4.1	181.0	53.9
10	4.1	340.0	67.1
11	4.3	86.0	14.3
12	4.6	528.0	57.0
13	5.3	263.0	33.1
14	5.5	260.0	61.6
15	6.2	453.0	132.0
16	8.0	128.0	51.6
17	9.3	12710.0	5398.4
Mean	4.3	283.9	52.5
SD	1.5	128.7	34.7
Median	4.0	266.5	43.4

The data points from dog #17 were deleted from analysis and are not included in the values calculated for mean, median and SD

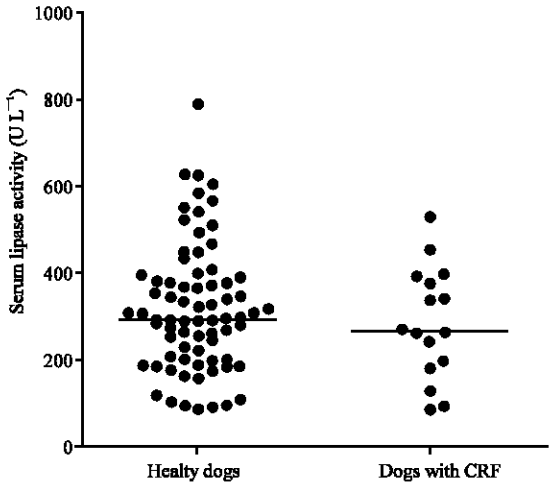

Fig. 1: Serum lipase activities in dogs with CRF. This figure shows the serum lipase activities in a group of 74 healthy dogs and 16 dogs with experimentally induced chronic renal failure. The lines show the medians for each dataset

creatinine concentration in the remaining 16 dogs was 4.3±1.5 mg dL^{-1} with a range of 2.1-8.0 mg dL^{-1}. Serum lipase activity was within the reference range (94-625 U L^{-1}) for all dogs with experimentally induced chronic renal failure. Serum lipase activity was not significantly different between dogs with experimentally induced chronic renal failure (median: 266.5 U L^{-1}) and a group of 74 healthy dogs (median: 294.5 U L^{-1}; p = 0.463; Fig. 1). Serum cPLI concentration was

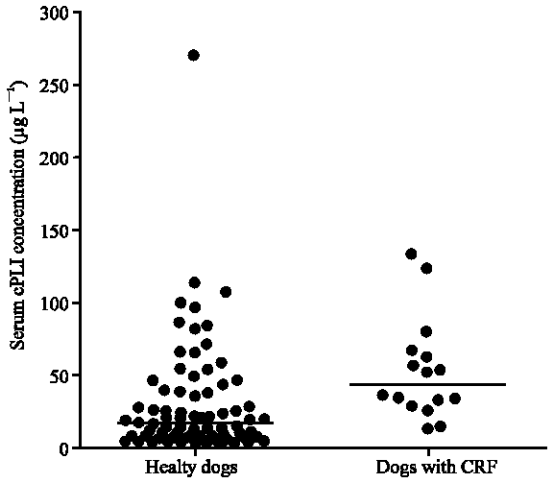

Fig. 2: Serum cPLI concentrations in dogs with CRF. This figure shows serum cPLI concentrations in a group of 74 healthy dogs and 16 dogs with experimentally-induced chronic renal failure. The lines show the medians for each dataset

above the upper limit of the reference interval (102.1 µg L^{-1}) in 2 of the 16 dogs with experimentally-induced renal failure (data points: 123.3 and 132.0 µg L^{-1}) but was below the suggested cut-off value for a diagnosis of pancreatitis (200 µg L^{-1}) in all 16 dogs (Steiner, 2003).

Serum cPLI concentrations were significantly higher in dogs with experimentally induced chronic renal failure (median: 43.4 µg L^{-1}) than in a group of healthy dogs (median: 16.3 µg L^{-1}; p = 0.0007; Fig. 2). However, serum cPLI concentrations did not correlate with serum creatinine concentrations (Pearson r = 0.2377; p = 0.3753; Fig. 3).

Clinical signs of renal failure and pancreatitis can overlap in some cases. Also, renal failure has been described as a complication of severe pancreatitis (Steiner, 2008). Thus, it is important for any clinically useful test for pancreatitis to not be affected by renal failure. At the same time, determination of a lack of pancreatitis in dogs with spontaneous renal failure is tentative at best. Pancreatitis has been reported to be highly localized and definitive exclusion of pancreatitis in a given patient would require removal, systematic sectioning and histopathological evaluation of the entire pancreas (Newman *et al*., 2004). Naturally this is not possible for dogs with spontaneous renal failure unless death ensues from the renal failure and necropsy is consented for by the owner. Thus, the study of the effect of compromised renal function on serum lipase activity and cPLI concentration is extremely difficult in dogs with spontaneous disease. Therefore, studies in

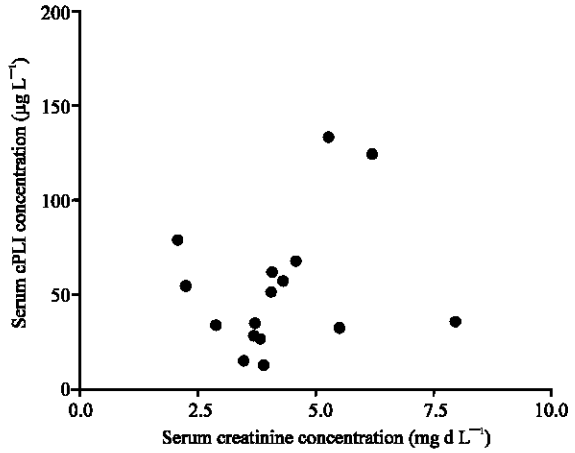

Fig. 3: Correlation of serum cPLI and creatinine concentrations. This figure shows the correlation of serum cPLI and creatinine concentrations in 16 dogs with experimentally induced chronic renal failure. There was no statistically significant correlation between these two parameters (Pearson r = 0.2377; p-value = 0.3753)

dogs with experimentally induced renal failure by subtotal nephrectomy would be expected to have some benefits for the study of the effect of decreased renal function on serum these parameters. To the researchers knowledge, no studies of experimentally-induced renal failure by subtotal nephrectomy are currently being performed in dogs. Thus, samples from a previous study that had been conducted almost 20 years previously had to be utilized for this study. The researchers fully acknowledge that because of this limitation, the study design was predetermined and could not be optimized for the purpose of this study. Ideally, a base-line serum sample would have been collected and analyzed before the experimental induction of renal failure but such samples were not available.

Ideally, the stability of serum lipase activity and period of >20 years but methodology for serum lipase activity has changed multiple times over the last 20 years and the assay for the measurement of serum cPLI concentration had only been introduced in 2000. However, it should be pointed out that even at room temperature, serum cPLI concentration has been reported to be stable for at least 21 days (Steiner *et al*., 2009). This shows an unusual resilience of this parameter as many proteins deteriorate quite quickly at room temperature (Berghoff *et al*., 2006). Also, serum cPLI concentration was extremely high in one dog of this study (5,398.4 µg L^{-1}) which would further suggest that this parameter is extremely stable. As indicated, one of 17 dogs was excluded from analysis. This dog had a serum creatinine concentration of 9.3 mg dL^{-1} and was euthanized a few hours after the serum sample had been collected. Serum lipase activity and serum concentrations for cTLI and cPLI were all extremely high (Table 1). A Grubb's test for outlier values was performed on the serum lipase activity (dataset passed normality testing with the D'Agostino and Pearson omnibus normality test) and serum creatinine (dataset passed normality testing with the D'Agostino and Pearson omnibus normality test) and cPLI concentrations (dataset did not pass normality testing with the D'Agostino and Pearson omnibus normality test; however, a case for utilization of outlier statistics on non parametric datasets has been made in the literature (Zimmerman, 1995) and was suggestive of an outlier value for this dog for both serum lipase activity and serum cPLI concentration but not for serum creatinine concentration.

This would suggest that other factors, unrelated to the renal failure such as acute pancreatitis or yet other factors may have affected these parameters. Fortunately, the pancreas from this dog had been saved and was available for histopathological analysis which showed necrotic acinar cells and fibrin exudation which does explain the results for serum lipase activity and serum cTLI and cPLI concentrations.

Unexpectedly, any increases of serum lipase activity in dogs with experimentally induced chronic renal failure was not found as had been described previously for dogs with spontaneous renal failure (Polzin *et al*., 1983). In fact, the data set not only failed to show a statistically significant difference between dogs with experimentally induced CRF and a group of healthy dogs but also did not show a trend towards higher serum lipase activities in the dogs with CRF. One possible explanation would be the relatively mild degree of azotemia in several of the dogs in this study. However, serum lipase activity was not higher in dogs with more severe azotemia refuting such hypothesis.

For example, the dog with the highest serum creatinine concentration of 8.0 mg dL^{-1} had one of the lowest serum lipase activities of 128 U L^{-1}. Another, more likely explanation for the disagreement between these two studies would be the use of different lipase activity assays. There are many different lipases of different cellular origins (Petersen and Drablos, 1994; Svendsen, 1994). While all of these lipases have the same general function, the hydrolysis of apolar lipids mainly triglycerides into more polar lipolysis products many lipases show slightly different optimal hydrolysis conditions and slightly different affinities towards different substrates (Jensen *et al*., 1982). Thus different

lipase assays which often differ in the substrate used and hydrolysis conditions may show different results in the same patient. It is possible that whatever lipase led to an increase in serum lipase activity in the previous study was not detected by the lipase activity assay used in this study. Finally, researchers possible explanation for the finding would be that catalytic activity of serum lipase activity was not stable during the storage period. However, the researchers do not believe that this explanation is very likely. If indeed the catalytic activity of serum lipase activity would have deteriorated it would be likely that after such a long storage period all assays would have resulted in unmeasurable serum lipase activities which was not the case.

Serum cPLI concentration was above the upper limit of the reference interval in two dogs and serum cPLI concentrations were significantly higher in dogs with experimentally induced chronic renal failure than in a group of healthy dogs. However this increase was considered to be not clinically relevant. The highest cPLI concentration of any dog with CRF was 132.0 $\mu g\ L^{-1}$ while a second highest dog had a serum cPLI concentration of 123.3 $\mu g\ L^{-1}$. Both of these values are well below the cut-off value of 200 $\mu g\ L^{-1}$ that has been recommended for this assay for a diagnosis of pancreatitis (Steiner, 2003). It is also interesting to note that serum cPLI concentrations did not correlate with serum creatinine concentrations. This would suggest that decreased renal function is not responsible for this mild increase in serum cPLI concentration in the dogs with CRF studied here. This is not surprising as canine pancreatic lipase has a molecular weight of approximately 50 kDa and is slightly anionic and would therefore not be freely filtered through the glomerular filter in the kidney (Steiner and Williams, 2002). As a result, removal of pancreatic lipase from the vascular space should not deteriorate due to decreased renal function. It is possible that other factors, such as a mild degree of dehydration that could be present in dogs with chronic renal failure could be responsible for the slight increase in serum cPLI concentration observed in these dogs.

CONCLUSION

In this study, the dogs with experimentally-induced chronic renal failure studied here had no increases in serum lipase activity and had significant, yet clinically unimportant increases in serum cPLI concentrations. Further studies in dogs with spontaneous acute and chronic renal failure are necessary and are currently in progress.

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