Hyperglycaemia but not hyperlipidaemia decreases serum amylase and increases neutrophils in the exocrine pancreas of cats

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Abstract

The goal of the study was to determine whether hyperglycaemia or hyperlipidaemia causes pancreatitis in cats and to assess the effect of excess serum glucose and lipids on amylase and lipase activity. Ten-day hyperglycaemic and hyperlipidaemic clamps were carried out in five and six healthy cats, respectively. Ten healthy cats received saline and served as controls. The activity of amylase was below the normal range in 4 of 5 hyperglycaemic cats by day 10. The activity of lipase did not vary in any of the cats. Samples of exocrine pancreas were normal on histological examination, but the number of tissue neutrophils was increased in hyperglycaemic cats (P<0.05). In a retrospective study 14 of 40 (35%) cats with naturally occurring diabetes mellitus had amylase activities below the reference range at the time of admission. Amylase activities normalised within 1 week of insulin therapy and subsequent glycaemic control. Lipase activity was increased in 26 of 40 (65%) diabetic cats and remained elevated despite glycaemic control. In conclusion, hyperglycaemia, but not hyperlipidaemia, increases pancreatic neutrophils in cats. However, because the histological morphology of the exocrine pancreas was normal, hyperglycaemia may play only a minor role in the pathogenesis of pancreatitis. Low amylase activities in diabetic cats may reflect an imbalance in glucose metabolism rather than pancreatitis.
HYPERGLYCEMIA BUT NOT HYPERLIPIDEMIA DECREASES SERUM AMYLASE AND INCREASES NEUTROPHILS IN THE EXOCRINE PANCREAS OF CATS

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Short title: Hyperglycemia and hyperlipidemia in cats
Abstract

Hypothesis: To evaluate if hyperglycemia or hyperlipidemia promotes pancreatitis in cats.

Because it is unclear whether pancreatitis is accompanied by increased or decreased serum amylase concentrations, we also evaluated the effects of excess glucose and lipids on amylase concentration.

Animals: 21 healthy cats and 40 cats with naturally occurring diabetes mellitus.

Procedures: Healthy cats were infused with glucose (n=5) or lipids (n=6) for 10 days to clamp them at the approximate concentration of untreated feline diabetes (glucose: 450-540 mg/dL; triglycerides: 265-620 mg/dL). Controls received saline (n=10). Amylase was measured in serum, pancreatic sections were examined by light microscopy and myeloperoxidase-stained neutrophils were quantified in the exocrine pancreas. Amylase concentration was measured in diabetic cats on admission and follow-up.

Results: Serum amylase decreased during glucose infusion and was below reference in 4 of 5 cats by day 10. Control and hyperlipidemic cats had normal amylase. Exocrine pancreas was morphologically normal in all cats. Compared to controls, hyperglycemic cats had 3-times more neutrophils in the exocrine pancreas (P<0.05). Hyperlipidemic cats had similar neutrophil counts as controls. In cats with naturally occurring diabetes amylase was below reference in 35% of cases at admission. Concentrations normalized within one week of glycemic control.

Conclusions and Clinical Relevance: Hyperglycemia but not hyperlipidemia increases pancreatic neutrophils in cats, but due to the normal morphology of the exocrine pancreas their role in the pathogenesis of pancreatitis may be negligible. Low amylase in diabetic cats may reflect imbalance of glucose metabolism rather than being a marker of pancreatitis.

Keywords: feline, diabetes, exocrine pancreas, amylase, neutrophils.
Diabetes mellitus is one of the most common endocrine diseases of cats and its prevalence is increasing due to increased frequency of predisposing factors, such as obesity and physical inactivity.\textsuperscript{1-3} In humans approximately 50% of type 1 and 2 diabetic patients show lesions characteristic for chronic pancreatitis, and a 3-fold increased risk of acute pancreatitis has been documented in type 2 diabetes.\textsuperscript{4,5} Pancreatitis has been hypothesized to play a permissive role for the development of diabetes in humans, possibly through spread of inflammation from the exocrine to the endocrine pancreas.\textsuperscript{5,6} Whether the same holds true for cats is not clear.

In one investigation in diabetic cats, chronic pancreatitis was a common concurrent diagnosis based on histopathology.\textsuperscript{7} In that study, pancreatitis was identified in 19 of 27 cats on necropsy, with 17 showing features of chronic and two of acute inflammation. Recently, it was reported that diabetic cats had higher serum concentrations of feline pancreatic lipase immunoreactivity (fPLI), a circulating marker of pancreatitis, and that the concentration positively correlated with fructosamine concentrations.\textsuperscript{8} Altogether, these results suggest a link between hyperglycemia and pancreatic inflammation in cats.

Because exposure of the exocrine pancreas to high concentrations of lipid solutions was shown to be toxic to feline acinar cells,\textsuperscript{9} and because sustained hyperlipidemia is a cause of pancreatitis in dogs and humans,\textsuperscript{10,11} excess lipids may directly contribute to pancreatic inflammation in cats. Therefore, and because diabetic cats are often hyperlipidemic,\textsuperscript{12} we hypothesized that high serum concentrations of triglycerides, in addition to hyperglycemia, may be involved in the pathogenesis of pancreatitis in feline diabetes.

Non-invasive diagnosis of pancreatitis is difficult in cats because available circulating markers of pancreatitis show limited sensitivity, especially for less severe cases.\textsuperscript{13-16} Among them, feline trypsin-like immunoreactivity (fTLI), amylase and lipase have been studied in addition to fPLI in cats.\textsuperscript{13-16} Diagnostic value of amylase and lipase is low because first, cats with pancreatitis often present with normal concentrations and second, common disorders,
such as renal failure, can cause false positive results.\textsuperscript{14,15,17} However, regarding amylase in cats with naturally occurring pancreatitis, only concentrations above the normal range have been considered.\textsuperscript{18} On the other hand, in an experimental model of pancreatitis, amylase was decreased soon after induction of pancreatic injury.\textsuperscript{9} Concentration of amylase was also reduced following surgical excision of pancreatic biopsies in healthy cats.\textsuperscript{19} In diabetic humans amylase concentrations are often low and this has been suggested to reflect exocrine pancreatic dysfunction due to chronic pancreatitis or to lack of insulin’s trophic effects on acinar cells.\textsuperscript{4,6,20} Thus, we hypothesized that decreased rather than increased amylase may be a marker of pancreatic disease in diabetic cats.

The present study was conducted to test whether sustained glucose or lipid infusion causes pancreatitis in healthy cats, based on histopathology. Circulating markers of pancreatic disease were measured to identify variations expected to occur during pancreatitis, with particular attention to low amylase concentration. In addition, amylase and lipase concentrations were retrieved from a group of diabetic cats that underwent therapy to assess whether improving glycemia leads to normalization of their amylase values.
Materials and Methods

Animals

Twenty-one neutered male, 15-18 months old, healthy domestic-shorthair cats were used following principles of laboratory animal care (Veterinary Office of Zürich, Switzerland, permission nr. 51/2007, 116/2007). The cats were divided into 3 groups and infused over 10 days through a jugular catheter. Five cats received 50% glucose added to saline. Blood glucose was evaluated 6-12 times per day and the infusion rate was adjusted to target concentrations at 450-540 mg/dL. Six cats received a lipid solution. Blood triglycerides were measured 2-3 times per day to target concentrations at 265-620 mg/dL. Concentrations of glucose and lipids were those found in untreated diabetic cats. The remaining 10 cats served as controls and were infused with saline. The present experiment was carried-out with samples obtained from 16 cats (i.e., 5 glucose-, 6 lipid-, and 5 saline-infused cats) used in a previous investigation, and 5 saline-infused cats recently employed in an unpublished study. The number of control cats was augmented (i.e., 10 saline-infused cats) to increase consistency of results. All cats were of same age, gender and breed. Body weight and baseline blood work results were similar between groups (data not shown). To reduce bias, all laboratory analyses described below have been performed at the same time.

From the database of the Clinic for Small Animal Internal Medicine, University of Zürich, Switzerland, records of cats that had been diagnosed with diabetes mellitus between the years 2000 and 2008 were reviewed. Forty treatment naïve cats having amylase and lipase concentrations measured on first admission were included in the study. When available, follow-up of the two enzymes and results of creatinine and fructosamine concentrations were also retrieved. Median age of the 40 cats was 11 years (range: 4-16 years). Among them, 29 (72.5%) were neutered males, 9 (22.5%) were spayed females and in two cats sex was not recorded. Twenty-four cats (60.0%) were domestic short- or longhair, three (7.5%) were Main Coon, and one each of Birman, Norwegian forest and Persian breed. Breed was not available
in 8 cats. Median body weight was 5.3 kg (range: 3.2-8.2 kg). Concurrent disorders were diagnosed on admission in 13 cats (32.5%). Of these, two cats each had acromegaly, bacterial cystitis, cholangiohepatitis and hypertrophic cardiomyopathy, and one cat each had corneal ulcer, idiopathic epilepsy, lung carcinoma, skin abscess and severe stomatitis. Five other cats had diabetic ketoacidosis.

Biochemical measurements

Daily concentrations of glucose, triglycerides and insulin were measured in the 21 cats as described. In the same cats, serum concentration of amylase and lipase, total protein and calcium were measured on day 10 with colorimetric assays according to the manufacturers’ instructions. In the group of glucose-infused cats concentration of amylase was also measured during the 10-day infusion from 3 samples, each formed by pooling equal aliquots collected from each cat on 3 consecutive days, as follows: day 1-3, day 4-6 and day 7-9. The amylase assay quantifies all isoenzymes. In all 21 cats, serum concentrations of fTLI were determined on day 10 with an enzyme-linked immuno sorbent assay (ELISA) using the method previously described; serum fPLI was measured with a validated cat-specific radioimmunoassay.

Histopathology

From the 21 experimental cats one aliquot of the left lobe of the pancreas was surgically removed under sterile conditions after euthanasia with barbiturates, formalin-fixed for 24 h and paraffin-embedded. An additional aliquot collected from the same lobe was snap-frozen in liquid nitrogen and stored at −80°C. Paraffin sections were stained with hematoxylin and eosin using standard protocols, and examined at light microscopy by two expert pathologists (FG, AP) to identify morphologic changes compatible with pancreatitis, such as acinar cell necrosis, and parenchymal hemorrhage, edema or fibrosis. Additional slides were
immunohistochemically stained for myeloperoxidase, a neutrophil marker, using a polyclonal rabbit antibody anti-human myeloperoxidase following antigen retrieval with cell conditioning solution, as described for cats. Neutrophils were counted in the exocrine pancreas in 100 randomly selected microscopic fields at 40×-magnification, excluding those cells in ducts, interlobular connective tissue, and large or small vessels (i.e., neutrophils surrounded by endothelial cells). The number of neutrophils was similarly counted in samples collected from other abdominal organs, including the liver and visceral fat. This served to verify whether a possible difference observed in the exocrine pancreas was accompanied by similar variations in the other tissues. Further pancreatic sections were immunostained for the lymphocyte antigen CD3 as previously reported. Lymphocytes were counted as described for neutrophils. Histological slides were evaluated in a blinded manner.

**Real-time PCR**

Total RNA from pancreas was extracted and reverse-transcribed according to standard protocols. Pancreatic cDNA was subjected to quantitative real-time PCR with feline-specific oligonucleotides we designed for pancreatic amylase and the macrophage protein F4/80, and using accessible sequences for the control gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Detection of pancreatic amylase was achieved with forward primer 5′-

TACAAACTGGTTCCCCGAAG-3′, reverse primer 5′-TCACACAGCATTCCAAG-GA-3′

and probe 5′-TGGGTGGAGGCAAATCCAAGCA-3′. For detection of F4/80, forward primer 5′-CTGAACACAGAGAGGGTTCGT-3′, reverse primer 5′-

AGTCAGGAGGATGGAATTGATCA-3′ and probe 5′-

AGCTTCTGGGACCAGTCTCGGCAGG-3′ were used. Primers and probes were developed using known partial mRNA sequences of pancreatic amylase (FJ715294) and F4/80 (EF685707). Probes were labelled at the 5′-end with FAM (6-carboxyfluorescein) and at the 3′-end with TAMRA (5,6-tetramethylrhodamine). PCR reactions consisted of 12.5 µL qPCR
Mastermix, primers and probe to final concentrations of 900 nM and 250 nM, respectively, and 5 µL of cDNA diluted 1:50 in a 25 µL total reaction volume. Cycling parameters were an initial denaturation of 10 min at 95 °C, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. cDNA samples were run in triplicate and transcripts were quantified using the relative standard curve method. A template-free control and a sample without reverse transcriptase were included in each amplification run. Gene expression was normalized to the respective quantities of GAPDH. PCR product identity was confirmed by DNA sequencing.

Bacteremia
To exclude that sustained infusion with glucose or lipid solutions led to bacteremia, possibly contributing to tissue inflammation, detection of bacteria was assessed in blood samples collected at day 10 in the 21 cats with PCR amplification of the 16S rDNA using universal primers.

Statistical analysis
Biochemical measurements, neutrophil and lymphocyte counts and real-time PCR results were compared between cats infused with glucose, lipids and saline using the Kruskal-Wallis test followed by Dunn’s correction. Concentrations of amylase measured during infusion were compared between all time points with Friedman test followed by Dunn’s correction. In diabetic cats the frequency of decreased amylase and increased lipase concentrations was calculated on admission. A possible association between these two markers of pancreatitis and the degree of glycemic control based on fructosamine concentration was assessed with Spearman correlation. Because renal failure may increase the concentration of amylase and lipase, their association with fructosamine was also assessed accounting for the effect of creatinine with partial correlation. The same test was used to account for the confounding effect of body weight, assuming that obese cats may have tissue inflammation possibly
involving the pancreas; obese cats have been shown to have increased cytokine expression in the subcutaneous fat, but this has not been tested in the pancreas. In addition, because diabetic cats with ketoacidosis or concurrent disorders may have activation of the inflammatory response, to avoid potential bias the correlation analysis was repeated by excluding those cases. Significance was set at $P<0.05$. Data were analyzed with a software.
Results

Hyperglycemic and hyperlipidemic clamps in cats

Plasma glucose and lipid concentrations were successfully targeted throughout infusion; cats rendered hyperglycemic had insulin concentrations that decreased close to the detection limit by the end of the study.\textsuperscript{21} On day 10, serum amylase was normal in most control and lipid-infused cats but clearly below the reference range in 4 of 5 glucose-infused cats (fig. 1). Of note, in the group of cats infused with glucose, amylase increased from day 1-3 to 4-6 in 4 of the 5 cats, from day 4-6 to 7-9 decreased in all 5 cats and from day 7-9 to 10 decreased further in 4 cats (fig. 2). However, amylase was found significantly different only between day 4-6 and 10 [P<0.01; amylase on day 4-6 (median and range): 1245 U/L (942-1389 U/L); amylase on day 10 (median and range): 654 U/L (576-948 U/L)]. At the end of the infusion period, concentrations of fPLI, fTLI and lipase as well as total calcium and proteins were all within normal limits in all groups.

On day 10, based on histopathological assessment, the exocrine pancreas of all cats was morphologically normal, without any evidence of acinar cell necrosis or vacuolization, parenchymal hemorrhage and inter-and intralobular edema or fibrosis. The number of neutrophils counted in the exocrine pancreas was approximately 3-fold higher in glucose-compared to saline- [P<0.05; neutrophils per high-power field in glucose-cats (median and range): 1.37 (0.94-1.41); neutrophils per high-power field in saline-cats (median and range): 0.44 (0.22-1.10)] or lipid-infused cats [P<0.001; neutrophils per high-power field in lipid-cats (median and range): 0.37 (0.10-0.76)] (fig. 3A). The two latter groups had similar counts. In cats receiving glucose, neutrophils were not clustered within pancreatic lobuli, did not surround acinar cells with apparent abnormal morphology and were not preferentially found nearby pancreatic ducts or pancreatic islets (fig. 3B). The number of neutrophils in the liver and visceral fat did not differ between groups. Because the size of adipocytes may change due to lipid accumulation and that of hepatocytes due to lipid or glycogen storage, the number of
neutrophils was also normalized for the number of cells counted per microscopic field; similar
to the above, no differences were observed (data not shown). The number of lymphocytes in
the exocrine pancreas was similar in all groups.

Expression of pancreatic amylase mRNA and F4/80 mRNA transcripts did not differ between
groups; transcripts quantities of the housekeeping gene GAPDH were also equal irrespective
of the solution used. Based on amplification of the 16S rDNA, none of the cats had bacteria
identified in blood.

Diabetic cats

Amylase and lipase concentrations were available in 40 diabetic cats on the day of admission.
Fourteen of these (35.0%) had amylase below the reference range and 26 (65.0%) had
increased lipase concentrations. Seven of the 26 cats with elevated lipase had decreased
amylase concentration. A negative correlation was observed between serum amylase and
fructosamine in the 40 cats (r=-0.38; P<0.01; CI95%: -0.62 – -0.10) (fig. 4); the same was
found when excluding cats with ketoacidosis (n=5) and those affected by concurrent disorders
(n=13) (r=-0.38; P<0.05; CI95%: -0.67 – -0.06), or when controlling for creatinine (r=-0.45;
P<0.01) and body weight (r=-0.48; P<0.05). No correlation was found between lipase and
fructosamine concentrations, excluding cats with ketoacidosis and those with concurrent
disorders, and after correction for creatinine or body weight.

In 5 cats with low amylase and 5 with normal amylase at first diagnosis, a follow-up was
available. In cats with low concentrations, amylase increased back into the normal range
already after one week of insulin therapy in all cases and then remained stable for up to 12-16
weeks. Increased amylase was accompanied by decreased serum fructosamine (fig. 5A). In
cats with normal concentrations at first diagnosis, amylase remained within normal limits and
fructosamine tended to decrease (fig. 5B). In addition, 5 cats with increased lipase
concentrations on admission were followed-up; lipase remained elevated over the successive
12-16 weeks despite declining fructosamine (fig. 5C). In 2 cats lipase decreased to normal concentrations after 12-16 weeks of treatment. At this time, creatinine was increased in one of the cats that had lipase returned to normal; creatinine was also increased in another cat that maintained elevated lipase concentrations.
Discussion

Pancreatitis is a common concurrent diagnosis in cats with diabetes.\textsuperscript{3,8} However, whether the former triggers the latter or vice versa has not been explored. Here, we show that experimental hyperglycemia maintained for 10 days at the typical concentration of untreated diabetes led to a 3-fold increase in the number of neutrophils within the exocrine pancreas but not the liver or visceral fat in healthy cats. In addition, neutrophils were evenly spread between pancreatic acinar cells and not preferentially located in proximity to ducts or islets. Even though these features may suggest a pancreatic-specific inflammatory condition, possibly involving acinar cells as the primary target, the number of lymphocytes did not differ and morphology of the exocrine pancreas appeared normal at light microscopy. During the acute phase of experimentally-induced pancreatitis in rats,\textsuperscript{30,31,32} the amount of pancreatic neutrophils was 3- to 5-fold increased, which is similar to findings in our hyperglycemic cats. However, beside increased neutrophils, lesions of acinar cells, represented by vacuolization or necrosis, and interstitial hemorrhage or edema were consistently documented in cats with pancreatitis.\textsuperscript{31,32} Because these important findings were not identified in any hyperglycemic cat, the importance of an increased number of pancreatic neutrophils may be questionable. Unfortunately, current histopathological criteria to diagnose acute pancreatitis in cats do not set limits for the number of pancreatic neutrophils in affected versus healthy cats.\textsuperscript{17,33}

The role of hyperglycemia in the pathogenesis of inflammation of the exocrine pancreas has recently been addressed in a rodent model of type 2 diabetes;\textsuperscript{34} it has been shown that the pancreas of affected mice had no more exocrine-associated granulocytes than that of controls. However, diabetic mice had higher mRNA concentrations of the chemokine KC (the rodent functional homolog of IL-8) which is expected to promote chemotaxis and increase the number of neutrophils. In that same study, the number of infiltrating macrophages was similar in diabetic and healthy rats.\textsuperscript{34} In keeping with this observation, we also did not find an increased number of macrophages in the pancreas of hyperglycemic cats, as assessed by
quantification of F4/80 transcripts. However, our results may be biased, in particular by the
fact that the analysis was carried out on whole pancreas, thereby including acinar cells, ducts,
interlobular connective tissue and islets that may contain macrophages. Furthermore,
specificity of the assay was not evaluated, thus whether the F4/80 antigen is also expressed by
other cell types in cats is unknown. The reason why hyperglycemia triggered selective
neutrophil infiltration of the exocrine pancreas in cats is uncertain. It is possible that excess
glucose induced exocrine pancreas expression of chemokines as in diabetic mice. However,
because the accumulation of neutrophils was mild and not accompanied by other features
typical of pancreatic inflammation, it seems likely that these cells did not induce or contribute
to overt pancreatitis in hyperglycemic cats, at least not within the time frame of this
experiment. Unfortunately, pancreatic biopsies were not collected prior to infusion; this
would have excluded baseline differences between groups. Baseline pancreatic biopsies were
not performed because they may contribute to pancreatic inflammation in cats.¹⁹
Different from hyperglycemia, 10-day lipid infusion did not result in an increase in the
number of neutrophils in exocrine pancreas. Hyperlipidemia is a known cause of acute
pancreatitis in humans and dogs.¹⁰,¹¹ Based on experimental animal models, hyperlipidemia
leads to accumulation of free fatty acids in the pancreas due to pancreatic lipase hydrolysis.
Free fatty acids in turn produce acinar cell injury by causing an acidic environment, ischemia,
and activation of the inflammatory cascade.¹¹ In humans, pancreatitis is more frequent if
plasma triglycerides are above 1000 mg/dL.¹¹ We therefore cannot exclude that exocrine
pancreatic inflammation would have occurred in cats if lipids had been clamped at higher
concentrations or for a longer period.
Of note, to perform the present study only samples collected from the left lobe of the pancreas
were assessed. However, because pancreatitis may have a patchy distribution in cats,²³ it
cannot be excluded that differing findings in other parts of the pancreas, in terms of neutrophil
and lymphocyte counts and quantification of F4/80 transcripts in pancreas, remained unrecognized. Samples from other parts of the organ were not available for analysis.

With regard to circulating markers of pancreatitis in cats infused with glucose and lipids, we did not observe any increase in serum fPLI, fTLI, lipase or amylase on day 10. The sensitivity of fPLI to identify pancreatitis is considered higher than that of fTLI, especially for more severe cases, whereas it is accepted that the diagnostic value of lipase and amylase is low. However, with regard to the latter enzyme, little attention has been paid so far to a potential reduction rather than increase in amylase concentrations as a diagnostic marker.

Indeed, in 4 of 5 hyperglycemic cats in our study, the concentration of amylase was below the reference range; amylase concentrations progressively decreased during the 10 days of glucose infusion. This would be in line with two investigations in cats showing low amylase following pancreatic injury caused by free fatty acid injection or by surgical biopsies. Of note, in both cases amylase decreased 1-2 days after injury had been induced, and concentrations remained low for up to one week, suggesting that early phases of pancreatitis in cats may be accompanied by low rather than high amylase concentrations. However, because concentration of amylase changed but not that of fPLI, fTLI or lipase, it is unlikely that this laboratory finding was associated with pancreatitis in hyperglycemic cats.

The clinical data retrieved from diabetic cats may also argue against the usefulness of low amylase as a marker of pancreatitis in cats with high glucose concentrations. Diabetic cats with low amylase concentrations were frequent on first admission, representing 35% of cases. Most interestingly, a negative correlation was observed between amylase and fructosamine concentrations, that was not influenced by body weight, concurrent disorders and ketoacidosis, or serum creatinine. Furthermore, in cats with low amylase concentrations on admission, insulin therapy that improved glycemic control was accompanied by normalization of enzymatic activity already within one week of treatment. Serum amylase remained normal with appropriate insulin therapy. Therefore, independent of pancreatitis amylase
concentration may be directly associated to the degree of glucose homeostasis in cats, with poorer glycemic control leading to lower serum amylase.

In type 1 and 2 diabetic humans it has been demonstrated that the exocrine pancreas secretes less amylase which may result in exocrine pancreatic insufficiency, malnutrition and weight loss. On admission, approximately 10-15% of affected humans show low amylase concentrations and, based on more sensitive assays, the frequency of exocrine pancreatic insufficiency reaches approximately 45%. Dysfunction may arise from the lack of insulin’s trophic effect on pancreatic acinar cells. It has been shown in rodents that insulin promotes transcription of different pancreatic enzymes, particularly amylase. In diabetic rodents amylase concentrations and pancreatic transcripts were reduced, and insulin administration gradually restored the amount of enzyme to normal values. It is therefore possible that low amylase concentrations in cats with diabetes reflects imbalance of glucose metabolism, rather than pancreatitis. The biological significance of low amylase in diabetic cats may stem from the fact that the organism exposed to hyperglycemia tries to avoid further increases of circulating glucose by decreasing its absorption from the intestine. This may represent an adaptive mechanism to avoid uncontrolled rise of plasma glucose, which would quickly lead to cat’s death.

At variance with findings in diabetic rodents, pancreatic amylase mRNA transcripts were not decreased in the pancreas of hyperglycemic cats. Infusion of glucose for 10 days may not affect transcription of pancreatic amylase. Of note, however, we used the entire pancreas rather than isolated acinar cells for analysis of amylase mRNA transcripts which may have biased the results. Alternatively, it is possible that low serum amylase resulted from decreased synthesis in organs other than the pancreas. Liver has been hypothesized to be an important source of amylase in cats, as described in rats.

At the time of admission, serum lipase was increased in 65% of diabetic cats, and insulin treatment was not accompanied by decreasing concentrations. Chronic pancreatitis or other
concurrent disorders may have contributed to increased lipase concentrations in some of these cats.

In conclusion, because light microscopy of the exocrine pancreas and the number of tissue lymphocytes and macrophages were normal in hyperglycemic cats, the mild increase of pancreatic neutrophils alone does not suggest subclinical or overt pancreatitis. Based on the fact that hyperglycemia decreased serum amylase in healthy cats and that correction of hyperglycemia in diabetic cats was associated with normalization of serum amylase, we assume that there is a link between circulating amylase and glycemic control. Thus, low amylase may not represent a marker of pancreatitis in diabetic cats.

Acknowledgment

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Footnotes

a. Charles River Laboratories, L'arbresle, France
b. Glukose 50%, Kantonspatthek, Zürich, Switzerland
c. Lipovenoes 10%, Fresenius-Kabi, Bad Homburg, Germany
d. α-Amylase EPS ver.2, e. Lipase colorimetric, f. Total Protein gen.2, g. Calcium, Roche Diagnostics, Vienna, Austria
h. Trypsin-Like Immunoreactivity, i. Feline Pancreatic Lipase Radioimmunoassay,
Gastrointestinal Laboratory, Texas A&M University, USA
j. Myeloperoxidase (MPO) Ab-1, Neomarkers-Lab Vision, Newmarket, UK
k. CC1, Ventana Medical Systems, Illkirch, France
l. qPCR MasterMix Plus Low ROX, Eurogentec, Seraing, Belgium
m. GraphPad Prism 4.0, GraphPad, San Diego, CA, USA
References


Figure legends

Fig. 1. Individual serum concentrations of amylase in saline-, glucose- and lipid-infused cats on day 10. Four of the 5 cats infused with glucose had decreased concentrations. The amylase reference range in cats is 700-1538 U/L (grey area).

Fig. 2. Individual serum concentrations of amylase in the 5 cats that received glucose infusion during 10 days. Aliquots collected from 3 consecutive days were pooled (day 1-3, day 4-6, day 7-9) in each cat. Amylase increased from day 1-3 to 4-6 in 4 cats, decreased from day 4-6 to 7-9 in all 5 cats and decreased further to day 10 in 4 cats. The amylase reference range in cats is 700-1538 U/L (grey area).

Fig. 3. (A) Neutrophil counts in the exocrine pancreas of saline-, glucose- and lipid-infused cats. Cats infused with glucose had significantly more neutrophils than the other groups. Median values are reported. (B) Myeloperoxidase (dark-brown) immunostaining detection of 3 neutrophils (arrows) in the exocrine pancreas of a glucose-infused cat (40×); the neutrophil closest to the bottom was excluded from the count because in a large vessel. Slides are counterstained with hemalum.

Fig. 4. Correlation analysis between serum amylase and fructosamine in diabetic cats on admission. Amylase concentration was inversely correlated with fructosamine.

Fig. 5. (A) Follow-up of 5 diabetic cats with serum amylase concentration below the reference range on admission. Amylase (white circles) returned to normal after one week of insulin therapy and fructosamine (black circles) progressively decreased, indicating improved glycemic control. Values are expressed as median and range. Dotted and shaded areas
illustrate the reference range of amylase (700-1538 U/L) and fructosamine (203-299 µmol/L), respectively. **(B)** Follow-up of 5 diabetic cats with serum amylase concentration within the reference range on admission; amylase (white circles) remained normal and fructosamine (white circles) tended to decrease. Values are expressed as median and range. Dotted and shaded areas illustrate the reference range of amylase and fructosamine, respectively. **(C)** Follow-up of 5 diabetic cats with serum lipase concentration above the reference range on admission. Lipase concentrations (white circles) remained increased and fructosamine (black circles) progressively decreased. Values are expressed as median and range. Dotted and shaded areas illustrate the reference range of amylase (8-26 U/L) and fructosamine, respectively.