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Association of Postprandial Serum Triglyceride Concentration and Serum Canine Pancreatic Lipase Immunoreactivity in Overweight and Obese Dogs

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Background: Hypertriglyceridemia has been proposed to contribute to the risk of developing pancreatitis in dogs.

Objectives: To determine associations between postprandial serum triglyceride concentrations and canine pancreatic lipase immunoreactivity (cPLI) concentrations or pancreatic disease.

Animals: Thirty-five client-owned overweight (n = 25) or obese (n = 10) dogs weighing >10 kg.

Methods: Healthy dogs were prospectively recruited for a cross-sectional study. Serum triglyceride concentrations were measured before and hourly for 12 hours after a meal. Fasting cPLI and canine trypsin-like immunoreactivity (cTLI) concentrations were assayed. Cut-off values for hypertriglyceridemia were set *a priori* for fasting ($\geq$88, $\geq$177, $\geq$354, $\geq$885 mg/dL) and peak postprandial ($\geq$133, $\geq$442, $\geq$885 mg/dL) triglyceride concentrations. The association between hypertriglyceridemia and high cPLI concentrations was assessed by exact logistic regression. Follow-up was performed 4 years later to determine the incidence of pancreatic disease.

Results: Eight dogs had peak postprandial triglycerides >442 mg/dL and 3 dogs had fasting serum cPLI concentrations $\geq$400 μg/L. Odds of high cPLI concentrations were 16.7 times higher in dogs with peak postprandial triglyceride concentrations $\geq$442 mg/dL relative to other dogs ($P < .001$). Fasting triglyceride concentration was not significantly associated with cPLI concentrations. None of the dogs with high triglyceride concentrations and one of the dogs with low fasting and peak postprandial triglyceride concentrations developed clinically important pancreatic disease.

Conclusions and Clinical Importance: Overweight and obese dogs with peak serum postprandial triglyceride concentrations $\geq$442 mg/dL after a standard meal are more likely to have serum cPLI concentrations $\geq$400 μg/L, but did not develop clinically important pancreatic disease.

Key words: Hypertriglyceridemia; Lipid; Pancreatitis.

There is some evidence that hypertriglyceridemia might contribute to the pathogenesis of pancreatitis in dogs[1–4] and low-fat diets are recommended in many veterinary textbooks to prevent recurrence of pancreatitis[5,6] although there are no prospective studies to support this recommendation and hypertriglyceridemia might not be a contributing factor in all dogs with pancreatitis. Overweight and obese dogs are thought to be at increased risk of hypertriglyceridemia[7] and pancreatitis,[8] but a definitive association between hypertriglyceridemia and pancreatitis in dogs has not been established, and the relative contributions of fasting and postprandial triglyceride concentrations to pancreatitis risk have not been distinguished in existing studies.

Abbreviations:

cPLI	canine pancreatic lipase immunoreactivity
cTLI	canine trypsin-like immunoreactivity
95% CI	95% confidence interval

It is recommended that food be withheld for 18 hours before blood sampling for the diagnosis of hypertriglyceridemia, to avoid the effect of postprandial chylomicronemia on triglyceride concentrations.[9] However, in some people with delayed triglyceride clearance, an overnight fast can normalize serum triglyceride concentrations and abnormalities are only apparent after a meal[10] or a lipid tolerance test.[11] In dogs with significantly impaired chylomicron clearance, hypertriglyceridemia might not always be apparent after withholding food, and measurement of postprandial serum triglyceride concentrations might be more sensitive for detecting hypertriglyceridemia in dogs.[12,13]

The aims of this study were to establish whether fasting or postprandial triglyceride concentrations are associated with increased serum cPLI, increased or decreased serum cTLI concentrations, or both in overweight and obese dogs and to assess the associations between fasting serum triglyceride concentrations and selected peak postprandial triglyceride concentrations. A secondary aim was to assess whether dogs with high

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fasting and/or postprandial triglyceride concentrations are subsequently at increased risk of having clinically important episodes of pancreatic disease.

Material and Methods

Study Overview and Animals

A cross-sectional study was conducted with overweight and obese dogs. Dogs were recruited from veterinary general practices in Brisbane and in Townsville, Queensland, Australia between February 2006 and June 2007. Inclusion criteria were that the dogs were overweight or obese (body condition score[14] $\geq 6/9$), otherwise healthy based on historical and physical examination findings, and weighed more than 10 kg to facilitate blood sampling for this and a concurrent study.[15] Dogs were healthy on history and physical examination, but no laboratory testing to confirm health was performed. Dogs were not included if they were aggressive, frightened of veterinary clinics, currently on medications other than routine parasite prophylaxis, eating food prescribed by a veterinarian for a clinical condition, refused to eat the food provided during the acclimation period, or if their owners had noticed recent inappetence, lethargy, vomiting, diarrhea, or other signs of illness. Enrolled dogs that ate less than 90% of the test meal were subsequently excluded. The University of Queensland Animal Ethics Committee approved all animal use (SVS/591/06/UQ and SVS/230/05/UQ), and informed consent was obtained from all owners of the dogs at the time of recruitment.

Meal Feeding Tests

Owners of enrolled dogs were given a bag of test food[a] providing 3791 kcal/kg with 23% of metabolizable energy as protein, 40% as fat, and 37% as carbohydrate. Fats were derived principally from poultry meat, unspecified animal fats, and poultry liver. Owners were instructed to feed the dog once daily in the morning, and to gradually reduce the dog's current food and introduce the test food in increments of 25% per day. The daily amount of the test diet to be offered was calculated to be isocaloric with each dog's current food intake. At least 4 days after the dog was eating 100% of its daily intake as the test food (7 days after the test food was introduced), the dog was admitted to its usual veterinary clinic after food had been withheld for 24 hours. A physical examination was performed by one of the authors (KV), including body condition score assessment and examination for abdominal pain. A cephalic vein catheter was placed, and blood samples were collected 10 and 5 minutes before the test meal was offered and hourly for 12 hours after the dog started eating the meal. The catheter and injection site were flushed with physiologic saline solution and then locked with saline with 1 i.u. heparin/mL after each blood collection.[16]

Dogs were offered a meal containing 50% of their daily maintenance energy requirement[17] calculated as kcal = 99 × (body weight in kg)$^{0.75}$, with body weight corrected for excess body fat content[14] by the formula:

$$BW_{corrected} = BW_{actual} \times \{1 - [(obese\,\%fat - lean\,\%fat)/100]\}$$

where BW is the body weight, obese%fat is the average percent body fat of dogs with the body condition score and sex of the obese dog,[14] and lean%fat is the average percent body fat of lean dogs (BCS midway between scores 4 and 5/9) of the relevant sex based on previously published body compositions.[14] Dogs that initially refused to eat were hand-fed. Dogs that still refused to eat were transported to their owner's home and fed by their owner, then returned to the veterinary clinic. Food that was uneaten 10 minutes after the dog started eating was removed and weighed.

Owners were instructed to gradually reintroduce the dog's previous food over 7 days after the test day.

Sample Handling and Assays

Blood samples (fasting samples 5 mL, postprandial samples 3 mL) were collected into plain tubes with serum separator gel,[b] allowed to clot for 10 minutes, and centrifuged (centrifugation details not available). Serum was aliquoted, immediately frozen, stored at −80°C until after all dogs were sampled, and transported on dry ice. Sample handling and transport conditions were within the guidelines of the assay manufacturer, or previously validated.[18,19] Serum triglyceride concentrations[c] were measured in all samples, but results from the fasting samples were averaged for the purposes of statistical analysis. Canine pancreatic lipase immunoreactivity (cPLI) and canine trypsin-like immunoreactivity (cTLI) concentrations were measured in a single fasting sample (collected 10 minutes before feeding) per dog at the Gastrointestinal Laboratory at Texas A&M University. Serum cPLI concentrations were assayed by the Spec cPL ELISA,[d] with inter- and intra-assay coefficients of variation (CVs) of 3.8% and 7.8% at concentrations in the lowest quintile of the reference interval and 11.2% and 5.6% at concentrations in the highest quintile of the reference range, respectively. Serum cTLI concentrations were measured by radioimmunoassay[e] with inter- and intra-assay CVs of 6.7% and 4.2% at 1.2 μg/L and 4.7% and 4.2% at 30 μg/L. All triglyceride concentrations were assayed in unicate in a single batch and cPLI and cTLI concentrations were assayed in duplicate in a single batch, 10–20 months after sample collection. Assays were performed by trained laboratory technicians who were blinded to the identity and body condition score of the dogs, as well as the concentrations of other analytes.

Hypertriglyceridemia was defined as a fasting or peak postprandial triglyceride concentration that equaled or exceeded the low, medium, high, and extreme cut-offs set *a priori* (Table 1). Triglyceride cut-offs were chosen arbitrarily based on concentrations previously proposed as being associated with pancreatitis[20] (extreme fasting cut-off and moderate and high peak postprandial cut-offs) and multiples of the upper limit of the fasting reference interval (other cut-offs). Increased cPLI was defined as a serum cPLI concentration ≥ 400 μg/L. Increased cTLI was defined as a serum cTLI concentration ≥ 50 μg/L. Exocrine pancreatic insufficiency was defined as a serum cTLI concentration ≤ 2.5 μg/L.

Follow-up

Medical records were reviewed and owners of participating dogs were contacted 4–5.5 years after the meal feeding tests to determine if the dogs had developed clinical signs of severe acute pancreatitis or end-stage exocrine pancreatic disease. Severe acute pancreatitis was defined as suspected if the dog was hospitalized with signs of vomiting and abdominal pain and as confirmed if in addition to these clinical signs any of the following diagnostic test results were present: amylase or lipase activity greater than 3 times the upper limit of the reference range, cPLI concentrations in the diagnostic range for pancreatitis, or ultrasonographic imaging consistent with pancreatic disease. Pancreatitis was not regarded as suspected if vomiting and abdominal pain were attributable to another confirmed disease process. End-stage exocrine pancreatic disease was defined as diabetes mellitus (assumed to be the result of chronic pancreatitis) or exocrine pancreatic

Table 1. The *a priori*-specified cut-offs for fasting and peak postprandial serum triglyceride concentrations. Cut-offs were chosen arbitrarily based on multiples of the upper limit of the fasting reference range and concentrations previously proposed as being associated with pancreatitis.

	Fasting Serum Triglyceride Concentration	Peak Postprandial Serum Triglyceride Concentration
Low cut-off	≥88 mg/dL (≥1 mmol/L)	≥133 mg/dL (≥1.5 mmol/L)
Moderate cut-off	≥177 mg/dL (≥2 mmol/L)	≥442 mg/dL (≥5 mmol/L)
High cut-off	≥354 mg/dL (≥4 mmol/L)	≥885 mg/dL (≥10 mmol/L)
Extreme cut-off	≥885 mg/dL (≥10 mmol/L)	Not evaluated

insufficiency. Diabetes mellitus was defined as the presence of unintended weight loss and substantial hyperglycemia or glucosuria requiring treatment with exogenous insulin. Exocrine pancreatic insufficiency was defined as a cTLI concentration ≤2.5 μg/mL. Follow-up data were gathered by one of the authors (SG), who was blinded to the results of the initial meal response tests, by a standardized interview and review of medical records. Disease definitions were designed to be sensitive rather than specific. A veterinarian (KV) reviewed the suspected cases and excluded cases attributable to diseases other than exocrine pancreatic disease.

Statistical Analyses

Assuming a prevalence of hypertriglyceridemia of between 0.25 and 0.5, and a prevalence of high cPLI concentrations of 0.1 in nonhypertriglyceridemic dogs, we estimated the power of this study to be between 0.20 and 0.75 to detect a significant difference at the 0.05 level if the true odds ratio is between 2 and 5, with a sample size of 35 dogs. Peak postprandial and fasting serum triglyceride concentrations were calculated for each dog, and categorical variables created based on the *a priori* cut-offs. The odds ratio for having increased serum cPLI concentrations in dogs with triglyceride concentrations at or above the *a priori* cut-offs relative to those with triglyceride concentrations below the *a priori* cut-offs was calculated.[21]

Associations between triglyceride category and the presence of each of increased serum cTLI or increased serum cPLI were intended to be evaluated, but the analysis of increased cTLI was not presented because of insufficient numbers. Conditional maximum likelihood estimates were used except where these were infinite, in which case median unbiased estimates were used. *P*-values were calculated as twice the probability of the sufficient statistic. The *P*-values and confidence intervals were calculated by the mid-*P* rule.[22] Statistically significant associations on these univariable analyses were also analyzed with body condition score (treated as a continuous variable) included in the regression model as a covariate to account for any confounding on the association between hypertriglyceridemia and cPLI, cTLI, or both concentrations caused by effects of obesity.

The association between fasting serum triglyceride concentrations and peak postprandial triglyceride concentrations was assessed by Pearson's correlation coefficient with the –pwcorr– command (with 95% confidence intervals by the -corrci- command) in Stata.[f] Normality of fasting and peak postprandial triglyceride concentration data was assessed by quantile-normal and normal probability plots, and tests for skewness and kurtosis by Stata's -sktest- command.[23] Linearity and the presence of outliers were assessed by inspection of a scatter plot. Because the data were not normally distributed, Spearman's signed rank correlation coefficient was also calculated by the -spearman- command with confidence interval calculated based on Fisher's transformation, by the -ci2- command. The discriminatory ability of fasting triglyceride concentrations for detecting peak postprandial triglyceride concentrations ≥442 mg/dL (≥5 mmol/L) was assessed by the receiver operating characteristics (ROC) curve. The maximum-likelihood model assuming a binormal distribution of the latent variable by Stata's -rocfit- command failed to converge, and so nonparametric analysis was performed by Stata's -roctab- command. The precision of the estimated area under the curve was described by an exact binomial 95% confidence interval. Sensitivity and specificity were reported at the cut-off with the highest percentage of dogs that were correctly classified; associated exact 95% confidence intervals were calculated by Stata's -cii- command. Positive and negative predictive values for these sensitivity and specificity estimates were reported for prior probabilities of having peak postprandial triglyceride concentrations ≥442 mg/dL (≥5 mmol/L) of 10%, 30%, and 50%.

Incidence rates for clinical pancreatitis during the follow-up period were calculated for all dogs pooled, and separately for dogs with peak postprandial triglyceride concentrations less than and ≥442 mg/dL (≥5 mmol/L) by the –cii– command in Stata assuming the number of occurrences of clinical pancreatitis has a Poisson distribution, with dog-years at risk (the sum of times for each dog from enrollment in study to follow-up date) fitted as the exposure variable. Exact 95% confidence intervals (95% CI) for incidence rates were calculated by the –cii– command in Stata.

Results

Study Population

Of the 107 owners contacted, 9 owners were not contactable, 22 owners declined to participate, 17 dogs were no longer overweight, 5 were injured or ill, 3 were aggressive or anxious in a veterinary clinic, and 10 refused to eat the food during the acclimation period. Forty-one dogs were enrolled but of these 6 were subsequently excluded because they did not consume >90% of the food on the test day. The final study population consisted of 25 overweight and 10 obese dogs comprising 11 castrated males, 1 intact male, and 23 spayed females with a median age of 7 years (range: 2–12 years). Twenty breeds and their crosses were represented, including 5 Labrador Retrievers, 4 Golden Retrievers, 4 Beagles (including 1 Beagle cross), and 4 Border Collies (including 2 crosses). One dog was a Miniature Schnauzer.

Fasting and Postprandial Triglycerides

Peak postprandial serum triglyceride concentrations occurred at any time between 1 and 11 hours after feeding. In 2 dogs, serum triglyceride concentrations before the meal were higher than any of the postprandial triglyceride concentrations. Eight dogs (4 overweight and 4 obese) had peak postprandial triglyceride

concentrations $\geq$442 mg/dL ($\geq$5 mmol/L). At none of the sampling times were more than 25% of all dogs at peak triglyceride concentration, but of the dogs whose peak postprandial triglyceride concentration was $\geq$442 mg/dL ($\geq$5 mmol/L), 75% (6/8), 88% (7/8), and 88% (7/8) had a serum triglyceride concentration $\geq$442 mg/dL ($\geq$5 mmol/L) at 2, 3, or 4 hours postprandial, respectively (Fig 1). All were identified when samples were evaluated at 2 and 4 hours, or at 3 and 4 hours.

Fasting serum triglyceride concentration was significantly positively associated with peak postprandial serum triglyceride concentration (Pearson correlation coefficient 0.95; 95% CI 0.90 to 0.97, $P < .001$). This association was marginally less close when 1 dog with very high triglyceride concentrations was excluded (Pearson correlation coefficient 0.87; 95% CI 0.75 to 0.93, $P < .001$). The relationship between fasting and peak postprandial triglyceride concentration appeared to be linear (Fig 2) but the distribution of both variables deviated substantially from normality (results not shown). There was significant rank correlation between fasting and peak postprandial triglyceride concentrations (Spearman's signed rank correlation coefficient 0.85, 95% CI 0.72 to 0.92; $P < .001$). Fasting triglyceride concentration was highly discriminatory for detecting peak postprandial triglyceride concentrations $\geq$442 mg/dL ($\geq$5 mmol/L). The ROC curve is shown in Figure 3. The area under the ROC curve was 0.97 (95% CI 0.85 to 1.00). The highest percentage of dogs that were correctly classified was 33/35 or 94.3%; this occurred at a fasting triglyceride concentration cut-off of $\geq$310 mg/dL ($\geq$3.5 mmol/L). Sensitivity and specificity at this cut-off were 7/8 or 87.5% (95% CI: 47.3 to 99.7%) and 26/27 or 96.3% (95% CI 81.0 to 99.9%), respectively. At prior probabilities of having peak postprandial triglyceride concentrations $\geq$442 mg/dL ($\geq$5 mmol/L) of 10%, 30%, and 50%, positive predictive values at these sensitivity and specificity estimates were 72.4%, 91.0%, and 95.9%, respectively. Corresponding negative predictive values were 98.6%, 94.7%, and 88.5%, respectively.

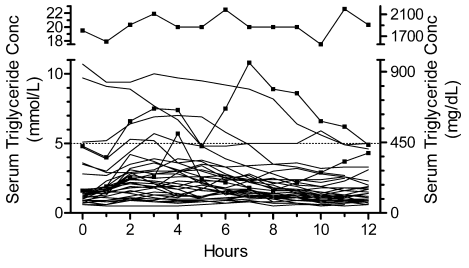


Fig 1. Serum triglyceride concentrations in 25 overweight and 10 obese dogs before and for 12 hours after a standard meal that provided 50% of the daily metabolizable energy requirement, with 40% of energy as fat. Eight dogs had postprandial triglyceride concentrations $\geq$442 mg/dL ($\geq$5 mmol/L; dotted line) at one or more time points. Three dogs (closed squares) had canine pancreatic lipase immunoreactivity concentrations >400 μg/L.

Association between Triglycerides and cPLI

Three (2 overweight and 1 obese) of the 35 dogs had serum cPLI concentrations $\geq$400 μg/L (so, the odds of having high cPLI in the study population were 0.09); one of these also had serum cTLI concentration $\geq$50 μg/L. Two dogs had cTLI $\leq$5.7 μg/L (5.4 and 5.7 μg/L) but none were in the diagnostic range for exocrine pancreatic insufficiency ($\leq$2.5 μg/L).

Eight dogs had peak postprandial triglyceride concentrations at or above the moderate cut-off [$\geq$442 mg/dL ($\geq$5 mmol/L)] with 3 of these dogs having high cPLI concentrations, whereas none of the 27 dogs with peak postprandial triglycerides below this cut-off had high cPLI concentrations. Peak postprandial triglyceride concentrations $\geq$442 mg/dL ($\geq$5 mmol/L) significantly increased the odds of hav-

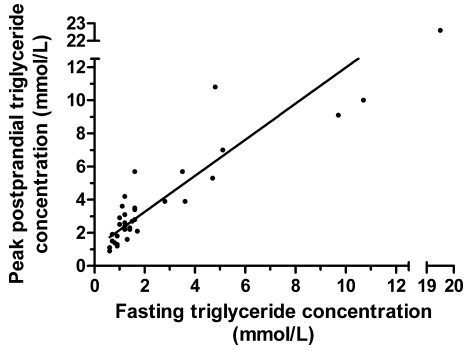


Fig 2. Fasting and peak postprandial triglyceride concentrations in 25 overweight and 10 obese dogs. There was a significant positive association between fasting and peak postprandial triglyceride concentrations (Pearson correlation coefficient 0.95, 95% CI 0.90 to 0.97, $P < .001$). The line shows the reduced major axis of best fit.

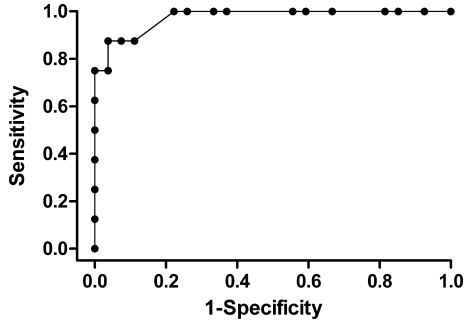


Fig 3. Nonparametric receiver operating characteristics curve showing the relationship between sensitivity and specificity of fasting triglyceride concentrations for the detection of peak postprandial triglyceride concentrations $\geq$442 mg/dL ($\geq$5 mmol/L) at various fasting triglyceride concentration cut-offs. The highest percentage of dogs that were correctly classified was 33/35 or 94.3%; this occurred at a fasting triglyceride concentration cut-off of $\geq$310 mg/dL ($\geq$3.5 mmol/L). Sensitivity and specificity at this cut-off were 7/8 or 87.5% (95% CI 47.3–99.7%) and 26/27 or 96.3% (95% CI 81.0–99.9%), respectively.

ing serum cPLI concentrations $\geq$400 μg/L relative to dogs with peak triglyceride concentrations below this cut-off (odds ratio 16.7, 95% CI 2.3 to infinity; $P < .01$) (Table 2, Fig 4). Two dogs had peak postprandial triglyceride concentrations at or above the high cut-off [$\geq$885 mg/dL ($\geq$10 mmol/L)] and both had serum cPLI concentrations $\geq$400 μg/L whereas only 1 of the 33 dogs below this triglyceride cut-off had high cPLI concentrations (odds ratio 41.6, 95% CI 2.1 to 1855; $P = .02$) (Table 2, Fig 4). The significance of the associations was not altered when body condition score was included in the regression models, but the odds ratio for the high postprandial triglyceride cut-off was substantially lower (odds ratio 16.2, 95% CI 1.8 to infinity, $P = .02$). There was no significant association between increased serum triglyceride concentrations and the risk of increased serum cPLI and cTLI concentrations when the low or moderate fasting triglyceride cut-offs or low peak postprandial triglyceride concentrations were assessed ($P = .13$–$.83$).

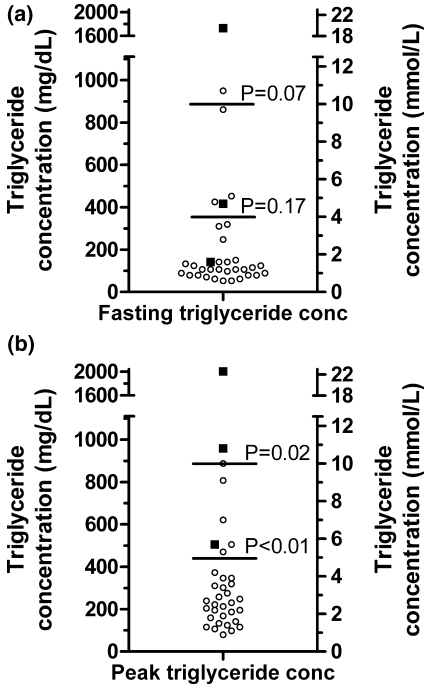


Fig 4. The association between fasting canine pancreatic lipase immunoreactivity (cPLI) concentration category and each of (**a**) fasting and (**b**) peak postprandial triglyceride concentrations on the same day. Open circles show dogs with fasting cPLI concentrations that were <400 μg/L, closed squares show dogs with fasting cPLI concentrations $\geq$400 μg/L. The horizontal lines show (a) the high and extreme fasting and (b) moderate and high peak postprandial triglyceride cut-offs specified *a priori*. *P*-values indicate the significance of the association between having triglyceride concentrations above the respective cut-offs and having high cPLI concentrations. The dog with the highest fasting and postprandial triglyceride concentrations was a Miniature Schnauzer that also had canine trypsin-like immunoreactivity (cTLI) concentrations $\geq$50 μg/L.

Follow-up

Seven owners were not contactable. Follow-up information was available for 28 dogs [including all dogs with peak postprandial triglyceride concentrations $\geq$442 mg/dL ($\geq$5 mmol/L)] for a median of 4 years 3 months (range 9 months to 5 years 4 months). Nine dogs had died (3 of neoplasia, 1 with central neurologic disease, 5 of unrecorded causes) and 29 were still alive at the time of follow-up.

One dog was hospitalized 5 years 4 months after the meal response test with signs of vomiting and abdominal pain. Serum lipase activity was 15 times higher than the upper limit of the reference range, so this dog was regarded as having confirmed pancreatitis. This dog had fasting and peak postprandial triglyceride concentrations of 62 and 133 mg/dL (0.7 and 1.5 mmol/L), respectively, at the time of the meal response test. The overall incidence rate of pancreatitis for all dogs was 0.85 cases per 100 dog-years at risk (95% CI 0.02 to 4.7 cases per 100 dog-years). The incidence rate in dogs with peak postprandial triglyceride concentrations <442 mg/dL (<5 mmol/L) was 1.1 cases per 100 dog-years (95%CI 0.03 to 6.4 cases per 100 dog-years) and in dogs with peak triglycerides $\geq$442 mg/dL ($\geq$5 mmol/L), the incidence rate was 0 cases per 100 dog-years (95% CI 0 to 12 cases per 100 dog-years). None of the dogs for which follow-up was available were reported to have developed exocrine pancreatic insufficiency or diabetes mellitus.

Discussion

This study demonstrates that those dogs with high peak postprandial serum triglyceride concentrations are more likely to have serum cPLI concentrations $\geq$400 μg/L, but dogs with hypertriglyceridemia did not subsequently develop clinically important pancreatic disease. Overweight and obese dogs in this study with peak postprandial serum triglyceride concentrations $\geq$442 mg/dL ($\geq$5 mmol/L) or higher had more than 16-fold higher odds (with the 95% confidence interval including at least a doubling of odds) of having serum cPLI concentrations $\geq$400 μg/L compared with dogs with lower serum triglyceride concentrations. This extends the findings of a previous study in Miniature Schnauzers[24] to overweight and obese dogs of other breeds. High serum triglyceride concentrations at the time of diagnosis of acute clinical pancreatitis have been anecdotally reported in dogs[20,25,26] and in case series in people.[27–29] The results of the present study indicate that an association between hypertriglyceridemia and biochemical markers of pancreatitis also exists in spontaneously overweight and obese dogs. However, long-term follow-up results did not support an association between hypertriglyceridemia and the subsequent development of clinically important episodes of acute pancreatitis.

The basis of this study was the hypothesis that postprandial serum triglyceride concentrations might be useful to determine which dogs would benefit from

Table 2. Percentages of dogs above or below selected *a priori*-determined triglyceride concentration cut-offs, and odds ratios for having fasting serum cPLI $\geq$400 μg/L in overweight and obese dogs with fasting or peak postprandial serum triglyceride concentrations above relative to below cut-offs. One dog with serum cPLI concentration $\geq$400 μg/L also had a serum cTLI concentration $\geq$50 μg/L. None of the fasting triglyceride concentrations categorized by the low or moderate cut-offs, or peak postprandial triglyceride concentrations categorized by the low cut-offs in Table 1 were significantly associated with either increased serum cPLI or cTLI concentrations.

	Serum Triglyceride Cut-off		Percentage of Dogs above or below Each Triglyceride Cut-off with cPLI $\geq$ 400 μg/L	*P*-Value, Odds Ratio (95% CI)
Fasting triglyceride concentration	$\geq$354 mg/dL ($\geq$4 mmol/L)	Dogs below triglyceride cut-off	4% (1/29 dogs)	*P* = .07,
		Dogs above triglyceride cut-off	33% (2/6 dogs)	12.3 (0.8 to 424)
	$\geq$885 mg/dL ($\geq$10 mmol/L)	Dogs below triglyceride cut-off	6% (2/33 dogs)	*P* = .17,
		Dogs above triglyceride cut-off	50% (1/2 dogs)	12.9 (0.3 to 618)
Peak postprandial triglyceride concentration	$\geq$442 mg/dL ($\geq$5 mmol/L)	Dogs below triglyceride cut-off	0% (0/27 dogs)	*P* < .01,
		Dogs above triglyceride cut-off	38% (3/8 dogs)	16.7 (2.3 to infinity)
	$\geq$885 mg/dL ($\geq$10 mmol/L)	Dogs below triglyceride cut-off	3% (1/33 dogs)	*P* = .02,
		Dogs above triglyceride cut-off	100% (2/2 dogs)	41.6 (1.8 to 1855)

cPLI, canine pancreatic lipase immunoreactivity; cTLI, canine trypsin-like immunoreactivity.

dietary fat restriction. Although postprandial serum triglyceride concentrations gave a better indication than fasting triglyceride concentrations of which dogs had serum cPLI concentrations $\geq$400 μg/L on the same day, none of the hypertriglyceridemic dogs subsequently developed clinically significant pancreatitis or clinical evidence of end-stage subclinical chronic pancreatitis. A longitudinal study with a larger number of hypertriglyceridemic dogs is required to verify whether hypertriglyceridemia increases the risk of clinically important pancreatitis. Although in one recent study, dietary fat content did not alter cPLI concentrations in healthy dogs,[30] a controlled trial is still required to assess whether dietary fat restriction reduces the risk of clinically important pancreatic disease.

All dogs with fasting triglyceride concentrations $\geq$354 mg/dL ($\geq$4 mmol/L) had peak postprandial triglyceride concentrations $\geq$452 mg/dL ($\geq$5 mmol/L), but some dogs with high peak postprandial triglyceride concentrations did not have high fasting triglyceride concentrations. The practical implication of these findings is that a meal challenge test is not warranted to detect hypertriglyceridemia in dogs that have fasting serum triglyceride concentrations $\geq$354 mg/dL ($\geq$4 mmol/L), but meal challenge tests will identify additional dogs that experience hypertriglyceridemia for parts of each day. Postprandial samples can be collected hourly for 4 or more hours to determine if serum triglyceride concentrations exceed $\geq$442 mg/dL ($\geq$5 mmol/L). The significant association between fasting and peak postprandial triglyceride concentrations in this study contrasts with those of another recent study in healthy lean dogs[13] with fasting triglyceride concentrations in the range 0–81 mg/dL (0 to 0.94 mmol/L) and peak postprandial triglyceride concentrations in the range 159–271 mg/dL (1.75–2.98 mmol/L). One likely explanation for the closer correlation in the present study is that a wider range of fasting and peak triglyceride concentrations was present in the overweight and obese dogs than in the lean dogs. Correlation is weaker in data with a narrower data range even if the underlying relationship between the variables is the same.[31]

One of the limitations of this study is that the sensitivity of the cPLI assay for pancreatic disease in asymptomatic dogs such as those in this study is not known. Previous studies have estimated the sensitivity at 64%[32] and 93%.[33] Specificity of the cPLI assay in a healthy population, such as that in this study, has been estimated to be 97%,[34] but was lower (78%) in a hospitalized population of dogs with clinical signs compatible with pancreatitis but where this disease was considered not to be present by an expert panel reviewing clinical records and ultrasonographic images.[33] Canine TLI concentrations can be increased during clinical pancreatitis[35,36] but the sensitivity of cTLI concentrations for the diagnosis of pancreatitis is low.[35] However, the relevance of high serum cPLI or cTLI concentrations in asymptomatic dogs is currently unknown. Although high serum cPLI and cTLI concentrations might be the result of increased leakage of enzymes from the pancreatic acini and indicate pancreatic inflammation, other possibilities exist. Increased cPLI and cTLI concentrations might also indicate increased production of pancreatic enzymes or reduced clearance of pancreatic enzymes from circulation, as has been demonstrated for liver enzyme activities.[37] Some asymptomatic people with increased serum pancreatic enzyme activities have chronic pancreatitis, but increased amylase and lipase activities have also been reported in people with no identifiable pancreatic pathology found with endoscopic ultrasound or magnetic resonance cholangiopancreatography,[38] tests regarded as sensitive for pancreatic pathology. Such patients represent around half of asymptomatic people with increases of pancreatic enzyme activities. It is currently unclear whether these observations in people are applicable to dogs, because different test methodologies are applied to diagnose pancreatitis. Although there is no evidence in dogs that high serum cPLI or cTLI concentrations in

the absence of clinical signs exclusively indicate the presence of inflammation of the pancreas, it is plausible that a portion have subclinical chronic pancreatitis. Future studies are needed to clarify interpretation of increased serum cPLI and cTLI concentrations in asymptomatic dogs, such as those in this study. Other tests such as pancreatic ultrasonography, fecal elastase-1 assays, and markers of systemic inflammation might help to distinguish between dogs with asymptomatic pancreatitis and benign increases in cPLI.

The cut-offs for serum triglyceride concentrations that were shown to be associated with serum cPLI concentrations ≥ 400 μg/L in this study might not be applicable to groups of dogs that are not overweight or obese, or dogs fed diets differing in fat content or the amount fed. Overweight and obese dogs were recruited to ensure that the prevalences of both hypertriglyceridemia and pancreatitis in the study population were higher than in lean populations,[8] but obesity might increase the risk of subclinical pancreatic pathology by mechanisms independent of hypertriglyceridemia, and this might alter the association between serum triglyceridemia and the prevalence of serum cPLI concentrations ≥ 400 μg/L. Similarly, this study included both male and female dogs pooled, and predominantly neutered dogs. We had insufficient power to assess male and female dogs separately, and it is possible that differences between males and females exist. The relationships studied might also differ between sexually intact and neutered dogs. Further work is required to determine what cut-offs are appropriate for use in other groups of dogs to identify those more likely to have serum cPLI concentrations ≥ 400 μg/L.

This study used a standardized meal to reduce bias and variation associated with dietary fat content. The adaptation period was chosen to minimize the risk of gastrointestinal adverse events associated with a dietary change, and was longer than the minimum requirement stated by the American Association of Feed Control Officials for digestibility studies.[39] However, we cannot be certain that residual effects from previous diets or inadequate adaptation to the test diet did not affect the results. This study used a standardized food from one batch of one brand of dog food with 40% of energy provided as fat. Further work is needed to determine whether another food with a similar fat content or a dog's usual food represents an appropriate test diet.

When interpreting associations between time-varying variables assessed by cross-sectional studies, the possibility of "reverse causation" should be considered. We do not know what degree of hypertriglyceridemia was present before the day of the study. However, it seems likely that dogs that had high postprandial serum triglyceride concentrations after the meal on the test day had a persistent, pre-existing defect in triglyceride metabolism and hence had experienced prior episodes of postprandial hypertriglyceridemia. A longitudinal study is required to determine if dogs with high peak postprandial serum triglyceride concentrations are at increased risk of subsequently developing serum cPLI concentrations ≥ 400 μg/L or clinical pancreatitis, and the degree of postprandial hypertriglyceridemia that increases the subsequent risk if any, and a controlled trial is required to assess whether dietary fat restriction reduces this risk.

In conclusion, after a standardized meal, overweight and obese dogs with peak postprandial serum triglyceride concentrations ≥ 442 mg/dL (≥ 5 mmol/L) are much more likely to have serum cPLI concentrations ≥ 400 μg/L than those with lower peak concentrations. However, none of the hypertriglyceridemic study dogs subsequently developed clinical signs of acute pancreatitis, diabetes mellitus, or chronic pancreatitis. Postprandial serum triglyceride concentrations ≥ 442 mg/dL (≥ 5 mmol/L) in overweight and obese dogs are typically observed 2–4 hours after a meal with 40% of metabolizable energy from fat fed at 50% of daily energy requirements but can occur earlier or later than this. Only 1 dog developed clinical acute pancreatitis over 4 years after the meal test, but this dog was not hypertriglyceridemic when tested. This information will inform future studies aiming to establish whether dogs with high triglyceride concentrations are at risk of developing clinically important pancreatitis, and whether dietary fat restriction is of benefit to these dogs.

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Footnotes

[a] Royal Canin Size Nutrition Medium Sensible, Aimargues, France
[b] Vacuette, 2 mL: Greiner bio-one, Tokyo, Japan
[c] Synchron CX Triglyceride GPO kit. Catalogue number 442850, Beckman Instruments, Inc, Brea, CA
[d] Spec cPL® Idexx Laboratories, Westbrook, ME
[e] Double Antibody Canine TLI, Siemens Medical Solutions Diagnostics, Los Angeles, CA
[f] STATA/IC v10.1, StataCorp, College Station, TX

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