Use of a meal challenge test to estimate peak postprandial triglyceride concentrations in dogs

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Objective—To develop a standardized meal challenge test by assessing associations between food-withheld preprandial (ie, fasting) and postprandial triglyceride concentrations, determining the most appropriate sampling time to detect the peak concentration (highest postprandial concentration), and estimating reference intervals for fasting and postprandial concentrations in healthy dogs.

Animals—12 lean healthy mixed-breed dogs.

Procedures—Dogs were fed a dry commercially available diet (fat, 31% metabolizable energy) for 3 weeks. After food was withheld for 23 to 24 hours, plasma triglyceride concentrations were measured 1 and 0.083 hours before and 1, 2, 3, 4, 5, 6, 9, and 12 hours after feeding of a standardized challenge meal (median amount eaten, 63 kcal/kg [127 kcal/kg0.75]). Correlation and agreement between concentrations at peak and other time points were assessed by use of correlation coefficients and Bland-Altman limits of agreement. Reference intervals were calculated by use of a robust method.

Results—Fasting and peak triglyceride concentrations were not closely associated. The highest concentration among samples obtained 2, 5, and 6 hours after meal consumption had closest agreement with peak concentration. In 5 of 12 dogs, concentrations 12 hours after eating were still significantly above baseline concentration (mean of each dog’s fasting concentrations).

Conclusions and Clinical Relevance—Fasting triglyceride concentration could not be used to accurately predict peak concentration. When estimating peak concentration, multiple samples should be collected 2, 5, and 6 hours after consumption of a standardized meal. Food may need to be withheld for > 12 hours when assessing fasting concentrations in healthy dogs. (Am J Vet Res 2011;72:161–168)

Hyperlipidemia in dogs is associated with pancreatic disease,1,2,3 and potential complications include hepatobiliary disease,2 seizures,3,4 ocular disease,4,5 and atherosclerosis.6 Hyperlipidemia in dogs can be primary or secondary to a number of other disorders.4,7,8 Primary hyperlipidemia is assumed when secondary causes are ruled out7; primary hyperlipidemia may be idiopathic7 or potentially hereditary.9,10 Secondary causes may also coexist with primary hyperlipidemia.

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ABBREVIATIONS
CI  Confidence interval
ME  Metabolizable energy
VLDL  Very–low-density lipoproteins

and exacerbate the hyperlipidemia in humans11,12 and possibly in dogs.8,10 When assessing dogs for hyperlipidemia, a diagnosis is usually based on detection of triglyceride concentrations, cholesterol concentrations, or both that exceed the respective reference intervals for healthy dogs after a minimum period of 12 hours of food withholding.13 Additional tests include qualitative visual assessment of the color of the plasma sample obtained initially after food withholding and later after the sample has been refrigerated for 8 to 14 hours7,14,15 and quantitative lipoprotein profile analysis. A hazy appearance of a plasma sample suggests triglyceride concentrations > 300 mg/dL (3.3 mmol/L), whereas the sample is opaque at concentrations > 600 mg/dL (6.6 mmol/L).9 Formation of a cream layer after refrigeration indicates the presence.
of excessive amounts of chylomicrons (exogenous triglycerides absorbed from the gastrointestinal tract), and cloudiness of the underlying plasma indicates the presence of excessive amounts of VLDLs (endogenous triglycerides). Lipoprotein profile analysis is infrequently performed for hyperlipidemic dogs. The ultracentrifugation and electrophoresis methods required for accurate analysis are not routinely offered by veterinary diagnostic laboratories. In contrast, assays for triglyceride and cholesterol concentrations are readily available.

Therefore, greater importance is placed on the measurement of the food-withheld preprandial (ie, fasting) triglyceride concentration for the diagnosis and clinical management of hyperlipidemia in dogs. However, fasting triglyceride concentrations in dogs may have low diagnostic sensitivity, which is similar to the situation reported in humans. A study that involved healthy, hyperlipidemic, and diabetic Miniature Schnauzers, some of the apparently healthy Miniature Schnauzers with fasting triglyceride concentrations within the reference range had previous histories of visible lipemia. This intradog variability in visible lipemia might have been attributable to variations in the amount of dietary fat fed before and during the study, variations in the duration of food withholding, or changes in the rate of VLDL clearance. In addition, when food withholding was continued for 12 hours, diagnostic sensitivity for detection of hypertriglyceridemia may have been reduced in some of the apparently healthy Miniature Schnauzers. These results suggest that a proportion of dogs with lipid disorders may not be detected when only fasting triglyceride concentrations are measured, and there is a need to determine a standardized period for food withholding.

Measuring postprandial and fasting triglyceride concentrations may increase diagnostic sensitivity and aid in the clinical management of dyslipidemia in dogs, which is analogous to the use of results of oral glucose tolerance tests and fasting blood glucose measurements for the diagnosis and clinical management of type 2 diabetes mellitus in humans. A standardized protocol for diagnosing postprandial hyperlipidemia in population subsets of dogs is needed because triglyceride concentrations may be affected by characteristics of a meal and the timing of sample collection, body condition score, and age.

The objectives of the study reported here were to provide assistance for the diagnosis and management of hyperlipidemia in dogs by assessing associations between fasting and postprandial triglyceride concentrations, determining the most appropriate time to obtain a sample to detect the highest postprandial triglyceride concentration in healthy dogs after they consumed a standard meal, and estimating reference intervals for fasting and postprandial triglyceride concentrations during a meal challenge test in dogs.

Materials and Methods

Animals—Twelve mixed-breed dogs (6 females and 6 males) that each weighed 12 to 23 kg and had a lean body condition (4 to 5 on a 9-point scale) were used in the study. All dogs were neutered or spayed; any dogs that were sexually intact at the time of enrollment were neutered or spayed 2 weeks before commencement of the study. All dogs were considered healthy on the basis of results of physical examination, routine urinalysis, hematologic analysis, and serum biochemical analysis and a negative result on a commercial heartworm test. Dogs were vaccinated with standard canine viral vaccines and were receiving medications monthly for heartworm and intestinal parasite prophylaxis. Accurate ages of the dogs were not known; however, it was estimated that the dogs were between 10 months and 3 years of age. Dogs were acquired from council animal shelters and adopted as pets at the conclusion of the study. The protocol was approved by The University of Queensland Animal Ethics Committee (425/04 and 570/05) and by the WALTHAM Ethical Review Committee.

Experimental protocol—A prospective descriptive study was conducted. A commercially available extruded dry adult maintenance diet was used as the standard meal to determine reference intervals. Total ME of the diet was 378 kcal/100 g of diet (dry-matter basis). Energy distribution was as follows: carbohydrate, 45% ME; fat, 31% ME; and protein, 24% ME. Total dietary fiber was 2 g/100 kcal. The major protein source was poultry, and the major soluble carbohydrate source was corn; the diet also contained beet pulp, yeast, vegetable oil, fish oil, mineral salts, egg powder, vitamins, and minerals (in order by decreasing percentage on an as-fed basis).

The daily initial food requirement was calculated and the amount of food fed was then adjusted each week to ensure that each dog maintained a lean body condition. All dogs were fed the diet for 3 weeks prior to a meal challenge test. Dogs were weighed prior to the meal challenge test, and the median quantity of food eaten on test days (on the basis of total ME) was 63 kcal/kg of body weight. The test meal had to be consumed by each dog within 15 minutes after it was fed (time test meal was fed was designated as time 0).

Blood samples (4 mL) were collected from each dog 1 and 0.083 hours (ie, 5 minutes) before and 1, 2, 3, 4, 5, 6, 9, and 12 hours after the standard test meal was fed. Samples were collected via an indwelling 20-gauge, 1.16-inch (1.1 X 30-mm) catheter placed percutaneously in a cephalic vein approximately 2 hours before the standard test meal was fed. Catheter patency was maintained by flushing each catheter with 5 mL of saline (0.9% NaCl) solution and 0.5 mL of heparinized saline solution (1 U of heparin/mL) after each sample collection. For each collection, 0.3 mL of heparin-diluted blood was removed and discarded, and the sample volume was then aspirated. To minimize activation of lipoprotein lipase by heparin and any subsequent effects on triglyceride concentrations, low doses of heparin were used and blood samples (except for those collected 0.083 hours before the standard test meal was fed) were collected 1 hour after catheters were flushed with heparinized saline solution.

Blood samples were placed into EDTA tubes that contained aprotinin (0.01 mg of aprotinin/mL of blood), chilled on ice for 8 minutes, and then centri-
fuged at 1,500 × g for 10 minutes. Plasma was harvest-
ed and placed in aliquots in micro test tubes; plasma samples were frozen at −20°C for 12 to 24 hours and then stored at −70°C until assayed to measure the tri-
glyceride concentration. Samples were tested by use of an automated analyzer via a commercial test kit in a commercial laboratory with maximum intra-assay and interassay coefficients of variation of 12% and 9%, re-
respectively. The estimated upper limit of the commer-
cial laboratory’s reference interval was 136 mg/dL (1.5 mmol/L).

Statistical analysis—Peak postprandial triglycer-
ide concentration was defined as the highest triglyceride concentration measured for each dog. The correlation between fasting triglyceride concentration measured 0.083 hours before the standard test meal was fed and the peak postprandial triglyceride was assessed by use of the Spearman rank correlation coefficient with asso-
ciated 95% CIs calculated via a Fisher transformation.
To establish the most appropriate time at which to col-
clect samples to detect the peak postprandial triglyceride concentration, agreement between peak postprandial triglyceride concentration and the triglyceride concentra-
tion at each time point after eating and between peak postprandial triglyceride concentration and the highest triglyceride concentration at each time point after eating and between peak postprandial triglyceride concentration returned to the baseline concentration for each dog was estimated by use of the 90% range of differences. For each dog, the plasma triglyceride concentration was considered to differ significantly (P < 0.05) from the baseline triglyceride concentration at each time point at which the observed concentration was higher than the baseline concentration for that dog multiplied by the 90% range of differences. The 90% range of differences was calculated as described elsewhere except that the within-
dog variance was calculated as the residual mean square from results of an ANOVA after the fasting triglyceride data were logarithmically transformed before analysis. An ANOVA was performed with a commercial software package. Because triglyceride data were logarithmically trans-
formed prior to calculating the 90% range of dif-
ferences on the logarithmic scale, the back-transformed 90% range of differences was interpreted as a multipli-
cative factor. The baseline concentration for each dog was multiplied by the back-transformed 90% range of differences, and the plasma postprandial concentration was considered to differ from the baseline concentra-
tion at each time point at which it was higher than this product. The back-transformed 90% range of differ-
ences for plasma triglyceride concentration was 1.374.

Amount of time for which the postprandial triglyc-
erde concentration exceeded the baseline concentra-
tion was defined for each dog as the interval from feed-
ing until the first time point at which the triglyceride concentration was significantly (P < 0.05) higher than the baseline concentration. Amount of time to return to the baseline concentration was similarly defined as the interval from feeding to the first time point after the peak triglyceride concentration at which the triglycer-
ide concentration was not significantly (P ≥ 0.05) dif-
ferent from the baseline concentration. Linear interpo-
lation between time points was used to determine the time to exceed and the time to return to the baseline concentration for each dog.
Results

Fasting triglyceride concentration measured 0.083 hours prior to feeding of the standard test meal was not closely associated with peak postprandial triglyceride concentration (Spearman r, –0.05; 95% CI, –0.61 to 0.54; P = 0.879) nor with the triglyceride concentration at 3, 4, 5, 6, or 12 hours after eating (Table 1).

The highest of the triglyceride concentrations at 2, 5, and 6 hours after eating had the closest agreement with peak postprandial concentration (Lin concordance correlation coefficient, 0.99; 95% CI, 0.96 to 1.00; P < 0.001; Table 2). The highest of the triglyceride concentrations at 5 and 6 hours after eating had acceptable agreement (Lin concordance correlation coefficient, 0.89; 95% CI, 0.68 to 0.97; P < 0.001). For concentrations at single time points, the closest agreement with peak postprandial concentration was at 6 hours after eating, but the agreement was too low to be acceptable (Lin concordance correlation coefficient, 0.74; 95% CI, 0.44 to 0.90; P < 0.001).

Table 1—Spearman rank correlation coefficients (associated 95% CI) and P values for the analysis of associations between the triglyceride concentration after food was withheld for 23 to 24 hours (fasting triglyceride concentration) and the peak postprandial triglyceride concentration and the triglyceride concentration at each time point after feeding of a standard test meal to 12 healthy dogs.

<table>
<thead>
<tr>
<th>Triglyceride variable</th>
<th>r (95% CI)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak postprandial</td>
<td>–0.05 (–0.61 to 0.58)</td>
<td>0.879</td>
</tr>
<tr>
<td>1 h after eating</td>
<td>0.44 (–0.18 to 0.81)</td>
<td>0.151</td>
</tr>
<tr>
<td>2 h after eating</td>
<td>0.51 (–0.09 to 0.84)</td>
<td>0.092</td>
</tr>
<tr>
<td>3 h after eating</td>
<td>0.13 (–0.46 to 0.65)</td>
<td>0.694</td>
</tr>
<tr>
<td>4 h after eating</td>
<td>–0.04 (–0.60 to 0.53)</td>
<td>0.936</td>
</tr>
<tr>
<td>5 h after eating</td>
<td>–0.12 (–0.65 to 0.49)</td>
<td>0.709</td>
</tr>
<tr>
<td>6 h after eating</td>
<td>–0.17 (–0.68 to 0.45)</td>
<td>0.596</td>
</tr>
<tr>
<td>9 h after eating</td>
<td>–0.45 (–0.81 to 0.17)</td>
<td>0.144</td>
</tr>
<tr>
<td>12 h after eating</td>
<td>0.01 (–0.56 to 0.58)</td>
<td>0.965</td>
</tr>
</tbody>
</table>

*The P value for assessing the hypothesis that the actual correlation coefficient is 0; values were considered significant at P < 0.05.

Table 2—Lin concordance correlation coefficients (associated 95% CI) and P values for analysis of the agreement between logarithmically transformed peak postprandial triglyceride concentration and the peak postprandial triglyceride concentration and the highest triglyceride concentration for multiple time points.

<table>
<thead>
<tr>
<th>Triglyceride variable</th>
<th>Lin coefficient (95% CI)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h after eating</td>
<td>0.04 (–0.11 to 0.18)</td>
<td>0.616</td>
</tr>
<tr>
<td>2 h after eating</td>
<td>0.12 (–0.20 to 0.42)</td>
<td>0.475</td>
</tr>
<tr>
<td>3 h after eating</td>
<td>0.37 (0.03 to 0.64)</td>
<td>0.036</td>
</tr>
<tr>
<td>4 h after eating</td>
<td>0.43 (0.06 to 0.70)</td>
<td>0.025</td>
</tr>
<tr>
<td>5 h after eating</td>
<td>0.67 (0.26 to 0.87)</td>
<td>0.004</td>
</tr>
<tr>
<td>6 h after eating</td>
<td>0.74 (0.44 to 0.90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>9 h after eating</td>
<td>0.46 (0.10 to 0.71)</td>
<td>0.013</td>
</tr>
<tr>
<td>12 h after eating</td>
<td>0.09 (–0.09 to 0.26)</td>
<td>0.527</td>
</tr>
<tr>
<td>Highest of concentrations 5 and 6 h after eating</td>
<td>0.89 (0.58 to 0.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Highest of concentrations 2 and 6 h after eating</td>
<td>0.87 (0.56 to 0.96)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Highest of concentrations 2, 5, and 6 h after eating</td>
<td>0.99 (0.96 to 1.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Highest of concentrations 2, 4, and 6 h after eating</td>
<td>0.89 (0.70 to 0.97)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*The P value for assessing the hypothesis that the actual correlation coefficient is 0; values were considered significant at P < 0.05.

The upper limit of the 95% reference interval for baseline triglyceride concentration (mean of the 2 concentrations measured 1 and 0.083 hours before feeding of the standard test meal) after food was withheld for 23 to 24 hours was 85 mg/dL (0.94 mmol/L; Table 3). For the highest of the triglyceride concentrations at 2, 5, and 6 hours after eating, the upper limit of the 95% reference interval was 233 mg/dL (2.56 mmol/L), and for the highest of the triglyceride concentrations at 5 and 6 hours after eating, the upper limit was 219 mg/dL (2.41 mmol/L). In general, the 90% CIs for the upper limits of the reference intervals were wide, which indicated that these were imprecise estimates.

The upper limits of the 95% reference interval for triglyceride concentrations at 4 and 6 hours after eating were 108 and 148 mg/dL (1.18 and 1.63 mmol/L), respectively. These intervals were calculated with values for only 11 dogs because one of the dogs was categorized as an outlier at 4 and 6 hours after eating by use of predefined criteria for outlier detection via a macro in the spreadsheet program prior to calculation of reference intervals for triglyceride concentrations; thus, data for this dog were excluded when estimating reference intervals for these time points. The peak postprandial triglyceride concentration and the highest of concentrations at 2, 5, and 6 hours after eating for this dog were substantially higher at 207 and 205 mg/dL (2.28 and 2.25 mmol/L), respectively, than those for the dog with the next highest concentrations of 144 mg/dL (1.58 mmol/L). This dog also had consistently higher concentrations 4 to 9 hours after eating (range, 178 to 207 mg/dL [1.96 to 2.28 mmol/L]) than those for the dogs with the next highest concentrations (range, 92 to 136 mg/dL [1.01 to 1.50 mmol/L]). However, the baseline triglyceride concentration of 29 mg/dL (0.32 mmol/L) for this dog was similar to those of the other dogs (median, 31 mg/dL [0.34 mmol/L]; range, 27 to 76 mg/dL [0.30 to 0.84 mmol/L]). The values for this dog were not categorized as outliers for any other reference interval calculations, including the highest of the concentrations at 2, 5, and 6 hours after eating. Results for the correlation between the fasting triglyceride concentra-
tration measured 0.083 hours before feeding the standard test meal and the peak postprandial triglyceride concentration remained low (ie, they were not closely correlated) after excluding this dog (Spearman \( r = -0.20; 95\% \text{ CI}, -0.45 \text{ to } 0.72; P = 0.552\)).

Triglyceride concentration increased rapidly after eating and was first significantly higher than the baseline concentration at a median of 1.1 hours after eating (range, 0.5 to 2.0 hours). Median time to peak postprandial concentration was 5 hours after eating (range, 2 to 9 hours). The median time to return to the baseline concentration was 11.2 hours (range, 2.4 to 11.2 hours; Figure 1). After the peak postprandial concentration was reached, triglyceride concentrations in 7 of 12 dogs returned to the baseline concentration within 12 hours after eating. In the remaining 5 dogs, triglyceride concentrations were still significantly higher than their baseline concentration at 12 hours after eating (ranges, 42 to 90 mg/dL [0.46 to 0.99 mmol/L] and 27 to 31 mg/dL [0.30 to 0.35 mmol/L], respectively), although none had triglyceride concentrations above the estimated upper limit of the commercial laboratory’s fasting triglyceride reference interval.

### Table 3

<table>
<thead>
<tr>
<th>Triglyceride variable</th>
<th>Concentration</th>
<th>Lower limit</th>
<th>Upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline*</td>
<td>mg/dL</td>
<td>0 (0–0)</td>
<td>81 (54–137)</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>0 (0–0)</td>
<td>0.94 (0.60–1.51)</td>
</tr>
<tr>
<td>0.083 h before eating</td>
<td>mg/dL</td>
<td>0 (0–0)</td>
<td>79 (56–92)</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>0 (0–0)</td>
<td>0.87 (0.52–1.01)</td>
</tr>
<tr>
<td>2 h after eating</td>
<td>mg/dL</td>
<td>0 (0–31)</td>
<td>155 (100–189)</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>0 (0–0.34)</td>
<td>1.71 (1.10–2.08)</td>
</tr>
<tr>
<td>3 h after eating</td>
<td>mg/dL</td>
<td>28 (0–31)</td>
<td>136 (116–147)</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>0.31 (0–0.34)</td>
<td>1.50 (1.27–1.62)</td>
</tr>
<tr>
<td>4 h after eating†</td>
<td>mg/dL</td>
<td>0 (0–43)</td>
<td>108 (97–116)</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>0 (0–0.47)</td>
<td>1.18 (1.06–1.30)</td>
</tr>
<tr>
<td>5 h after eating</td>
<td>mg/dL</td>
<td>52 (0–57)</td>
<td>210 (123–267)</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>0.58 (0–0.62)</td>
<td>2.31 (1.35–2.93)</td>
</tr>
<tr>
<td>6 h after eating†</td>
<td>mg/dL</td>
<td>56 (40–65)</td>
<td>148 (98–178)</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>0.62 (0.43–0.72)</td>
<td>1.63 (1.08–1.96)</td>
</tr>
<tr>
<td>9 h after eating</td>
<td>mg/dL</td>
<td>0 (0–0)</td>
<td>215 (109–281)</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>0 (0–0)</td>
<td>2.36 (1.20–3.09)</td>
</tr>
<tr>
<td>12 h after eating</td>
<td>mg/dL</td>
<td>0 (0–81)</td>
<td>103 (78–117)</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>0 (0–0.09)</td>
<td>1.13 (0.86–1.29)</td>
</tr>
<tr>
<td>Highest of concentrations 2, 5, and 6 h after eating</td>
<td>mg/dL</td>
<td>64 (57–70)</td>
<td>223 (156–271)</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>0.70 (0.63–0.77)</td>
<td>2.56 (1.75–2.98)</td>
</tr>
<tr>
<td>Highest of concentrations 5 and 6 h after eating</td>
<td>mg/dL</td>
<td>62 (55–69)</td>
<td>219 (138–267)</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td>0.68 (0.61–0.76)</td>
<td>2.41 (1.52–2.94)</td>
</tr>
</tbody>
</table>

*Baseline value was the mean of the 2 triglyceride concentrations at 1 and 0.083 hours before dogs were fed a standard test meal. 1 dog was categorized as an outlier, and the data for that dog were removed from the calculation of the reference intervals at this time point.

### Discussion

An important finding for the study reported here was that fasting triglyceride concentration was not closely associated with peak postprandial triglyceride concentration nor with triglyceride concentrations measured 3 to 6 hours after eating. This is important for clinicians because it indicates that fasting concentrations are not predictive of the highest postprandial triglyceride concentrations. This was highlighted in our study by 1 dog that had both higher peak postprandial concentration and concentrations 4 to 9 hours after eating than those for the dogs with the next highest corresponding concentrations; however, the fasting triglyceride concentration was comparable with those of the other dogs. Thus, the measurement of postprandial triglyceride concentration after consumption of a standard test meal may be important for detecting postprandial hypertriglyceridemia and perhaps for evaluating response to dietary fat restriction in dogs with fasting normotriglyceridemia; however, this requires further investigation in dogs with lipid disorders.

The situation is similar in humans. In a study of 20 patients with hyperlipidemic pancreatitis, one-third had fasting triglyceride concentrations within or nearly
within the reference interval but postprandial triglyceride concentrations were > 300 mg/dL (5.5 mmol/L).

In a study of 17 patients with markedly high peak postprandial triglyceride concentrations, the diagnostic sensitivity for the fasting triglyceride concentration was only 47% for the detection of these high responders. Studies are needed to determine the prevalence of markedly high postprandial triglyceride concentrations in dogs. In such a study in humans, 17 of 133 (13%) had markedly high peak postprandial triglyceride concentrations after administration of a standardized lipid load.

A second important finding in the present study was that of the methods assessed, the most appropriate method for the detection of peak postprandial triglyceride concentration in healthy dogs was to determine the single highest concentration in the samples obtained 2, 3, and 6 hours after feeding of the standardized test meal. Indeed, there was almost perfect agreement between peak triglyceride concentration and the highest of the triglyceride concentrations at 2, 5, and 6 hours after eating. This was because for most of these dogs, the peak concentration (defined as the highest observed concentration) was detected at one of these time points. The single highest triglyceride concentration at 5 and 6 hours after eating had lower agreement with peak postprandial concentration. For concentrations at single time points, the highest agreement was at 6 hours after eating, but the agreement with peak postprandial concentration was considered too low to be acceptable.

A third important finding was that at 12 hours after eating, 5 of the 12 dogs still had triglyceride concentrations significantly higher than their baseline concentrations; the concentrations at 12 hours were at least 1.374 times as high as the baseline concentrations. The clinical importance of this finding needs to be investigated in a larger population of dogs. In only 1 dog did the triglyceride concentration at 12 hours after eating exceed the fasting triglyceride reference interval established in our study (Table 3), and none of the dogs had concentrations above the commercial laboratory's reference interval. However, the commercial laboratory could not provide information on the duration of food withholding nor on the composition and energy content of the meal fed to the dogs used to establish their reference interval. Therefore, on the basis of the results of the study reported here, reference intervals for triglyceride concentration may be influenced by the previous meal if food withholding is not longer than 12 hours. Analysis of our results suggested that there may be a need to withhold food for > 12 hours to reduce the effect of the previous meal before assessing fasting triglyceride concentration in dogs, and effects of duration of food withholding require further investigation.

Our postprandial results are consistent with those in other studies. In a study,15 meal size influenced the duration of the dietary effect on postprandial free fatty acid concentrations in healthy dogs fed once daily. For dogs fed approximately 88 kcal/kg (fat content, 30%), the effects lasted up to 19 hours. In another study,16 postprandial leptin concentrations did not return to basal values until at least 19 hours after eating. Importantly, in Miniature Schnauzers with a history of lipemia after food withholding, the lowest fasting triglyceride concentrations were not observed until as long as 17 to 24 hours after feeding,17,18 whereas in humans with type 2 diabetes mellitus, postprandial triglyceride concentrations were still significantly higher than the baseline values 11 hours after eating,19 which reinforces the need to determine the appropriate period of food withholding in dogs with lipid disorders. Additional studies are needed to determine the appropriate withholding period before feeding meals with a standardized fat content to obtain accurate fasting concentrations in healthy dogs and dogs with lipid disorders.

For the protocol used in the present study, the highest of the postprandial triglyceride concentrations at 2, 5, and 6 hours after eating can be considered outside of the reference interval and consistent with postprandial hyperlipidemia if > 233 mg/dL (2.56 mmol/L).

However, as indicated by the wide 90% CI, this estimate of the upper limit of the reference interval is imprecise and should be interpreted only as indicative of hyperlipemia until more precise estimates are available. In addition, the estimated upper limits of all reference intervals should be used only with the protocol that was used in the present study. For triglyceride concentrations 4 and 6 hours after eating, the estimated upper limits for the reference intervals were 108 and 148 mg/dL (1.18 and 1.63 mmol/L), respectively; these were considerably lower than the upper limits of all other reference intervals. This is probably because the dog with the markedly higher postprandial concentration at 4 and 6 hours after eating was excluded from those calculations on the basis of outlier-detection criteria but was not excluded from calculation of the other reference intervals, including the interval for the single highest concentration in the samples obtained 2, 5, and 6 hours after eating. The cause of the markedly higher postprandial triglyceride concentrations in this dog is unknown; therefore, it was decided that it could not be removed from the other reference interval calculations on a post hoc basis. The use of outlier identification is controversial. The purpose of outlier detection is to avoid inclusion of values from subjects that are not part of the target population (eg, subjects that are not healthy) and would result in incorrectly wide reference intervals without excluding values from healthy subjects.

The use of a standard test meal as described here is likely to be advantageous in detecting dogs with abnormal lipid metabolism when fasting triglyceride concentrations are not markedly increased, comparable to the oral glucose tolerance test used in the identification of prediabetic41 and type 2 diabetic humans with fasting blood glucose concentrations within the reference interval.19–21,42 For a meal challenge test, it is also important to standardize the composition and formulation of the meal fed. A moderate to high dietary fat content is likely to be most appropriate,22–24 such as that in the commercially available maintenance diet used in the study reported here. Investigators in an earlier study43 determined the importance of the dietary fat content for increasing detection of dyslipidemia. In that study,14 of 56 related racing Huskies had significantly in-
creased fasting mean cholesterol concentrations when fed a high-fat (39% ME), high-protein (61% ME), low-carbohydrate (0% ME) diet, yet no hypercholesterolemia was detected when the same dogs were fed a moderate-fat (22% ME), high-protein (23% ME), high-carbohydrate (53% ME) diet. Those authors proposed that the 14 huskies had a diet-induced hyperresponsive trait that resulted in abnormal cholesterol metabolism and dietary fat intolerance.

Hypertriglyceridemia in dogs is of clinical concern. Various authors have suggested that dogs4,44 and humans1,11,45,46 are at increased risk of developing acute pancreatitis if the fasting triglyceride concentration is > 1,000 mg/dL (11.0 mmol/L) and have recommended dietary intervention to lower the triglyceride concentration to < 500 mg/dL (5.5 mmol/L). Results of recent studies support this hypothesis. Fasting hypertriglyceridemia > 300 mg/dL (5.5 mmol/L) in diabetic dogs b and > 500 mg/dL (9.9 mmol/L) in Miniature Schnauzers were associated with laboratory evidence of exocrine pancreatic disease. 1 Among overweight and obese dogs provided a standard meal that contained 40% ME dietary fat that was fed at 50% of resting energy requirement, peak postprandial hypertriglyceridemia ≥ 445 mg/dL (5.5 mmol/L) was associated with a 6-fold increase in the risk of having laboratory evidence of exocrine pancreatic disease. b Importantly, there was no significant association between fasting triglyceride concentrations and pancreatic disease in the latter study. b Interestingly, in a study 46 of human patients with acute pancreatitis and hyperlipidemia, 91% had postprandial triglyceride concentrations > 500 mg/dL (5.5 mmol/L) and 45% had postprandial triglyceride concentrations > 1,000 mg/dL (11.0 mmol/L) more than 2 months after diagnosis. It was suggested that lipid abnormalities may have been involved in the pathogenesis of previously diagnosed acute pancreatitis.

Considerable research has been conducted in human patients to investigate the hypothesis that there is atherogenesis during the postprandial period as a result of chylomicron or VLDL (or both) remnants binding to arterial endothelium. 57,48 It was reported that increased postprandial triglyceride concentrations provide a more accurate assessment of the risk of cardiovascular disease in humans than do fasting triglyceride concentrations. 49,50 Importantly, postprandial hypertriglyceridemia was associated with increased endothelial dysfunction and oxidative stress. 51,52 Various authors have suggested the use of an oral triglyceride tolerance test with a standardized test meal to detect postprandial hypertriglyceridemia.22–24 Although dogs are considered resistant to atherosclerosis, 53 the clinical relevance of postprandial hyperlipidemia in the pathogenesis of endothelial dysfunction may be relevant to some diseases in dogs, including lipid and macrophage–rich uveitis in hyperlipidemic diabetic Miniature Schnauzers 4 and exocrine pancreatic disease. b On the basis of the adverse health effects of postprandial hyperlipidemia in humans and the prolonged increase in triglyceride concentrations after eating in dogs, it might be important in dogs to ensure that both the fasting and postprandial triglyceride concen-

trations remain < 500 mg/dL (5.5 mmol/L). Dietary intervention is important for management of primary hyperlipidemia, but it may be only partially successful in dogs. 9 The determination of both fasting and peak postprandial triglyceride concentrations in response to consumption of a meal of a dog’s current diet may also assist in predicting the efficacy of dietary intervention in hyperlipidemic dogs. 9

We concluded that fasting triglyceride concentrations do not predict the highest postprandial triglyceride concentration in healthy dogs. When determining peak postprandial triglyceride concentration, the highest of the concentrations measured 2, 5, and 6 hours after feeding of the standard test meal is superior to values at any single time point for healthy dogs, provided the same protocol and test meal (fat content, 31% ME) are used. Food may need to be withheld for > 12 hours when assessing fasting triglyceride concentrations in healthy dogs, and effects of the duration of food withholding require further investigation. Studies that involve larger number of subjects are required to obtain precise estimates of reference interval limits and to obtain reference intervals for other populations.

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