Serum triglyceride concentration in dogs with epilepsy treated with phenobarbital or with phenobarbital and bromide

Elissa K. Kluger, BVSc; Richard Malik, DVSc, PhD; William J. Ilkin, BVSc; David Snow, DVSc, PhD; David R. Sullivan, MMed; Merran Govendir, BVSc, PhD

**Objective**—To compare serum triglyceride concentrations obtained after food had been withheld (ie, fasting concentrations) in dogs with epilepsy that had been treated long term (≥3 months) with phenobarbital or with phenobarbital and potassium bromide with concentrations in healthy control dogs.

**Design**—Cross-sectional study.

**Animals**—57 epileptic dogs that had been treated with phenobarbital (n = 28) or with phenobarbital and bromide (29) and 57 healthy, untreated control dogs matched on the basis of age, breed, sex, neuter status, and body condition score.

**Procedures**—Blood samples were collected after food had been withheld for at least 12 hours, and serum biochemical and lipid concentrations were determined. Oral fat tolerance tests were performed in 15 control dogs and 9 dogs with epilepsy treated with phenobarbital alone.

**Results**—19 of the 57 (33%) epileptic dogs had fasting serum triglyceride concentrations greater than the upper reference limit. Nine (16%) dogs had a history of pancreatitis, and 5 of the 9 had high fasting serum triglyceride concentrations at the time of the study. A significant relationship was found between body condition score and fasting serum triglyceride concentration in all dogs, but serum triglyceride concentration was not significantly associated with phenobarbital dosage or serum phenobarbital concentration.

**Conclusions and Clinical Relevance**—Results suggested that dogs treated long term with phenobarbital or with phenobarbital and bromide may develop hypertriglyceridemia. Fasting serum triglyceride concentration should be periodically monitored in dogs treated with phenobarbital because hypertriglyceridemia is a risk factor for pancreatitis. (J Am Vet Med Assoc 2008;233:1270–1277)

**Abbreviations**

| BCS | Body condition score |
| cPLI | Canine-specific pancreatic lipase immunoreactivity |
| HDL-c | High-density lipoprotein cholesterol |
| IQR | Interquartile range |
| LDL | Low-density lipoprotein |
| LPL | Lipoprotein lipase |
| VLDL | Very-low-density lipoprotein |

Phenobarbital is the most commonly administered agent to control seizure disorders in dogs. Grossly lipemic serum most commonly associated with hypertriglyceridemia has been observed from some dogs that have received phenobarbital long term. Reference has been made to hypertriglyceridemia causing pancreatitis or neurologic disturbances, particularly seizures; however, the validity and clinical importance of these observations remains unknown, as only a few studies involving dogs or people have reported this association.

In dogs from which food has been withheld, hypertriglyceridemia has most commonly been associated with obesity, diabetes mellitus, pancreatitis, and hyperadrenocorticism, although it may also be seen in dogs that have received glucocorticoids long term and in dogs that have been fed a diet high in fat or carbohydrate. Possible physiologic mechanisms accounting for hypertriglyceridemia in dogs from which food has been withheld include increased production of chylomicrons or VLDL or delayed clearance of chylomicrons or VLDL from the circulation by LPL. Lipoprotein lipase hydro-
lyzes triglycerides in chylomicrons and VLDL to long-chain fatty acids and glycerol, which are subsequently stored in adipose tissue or used for energy by skeletal and cardiac muscle. Because insulin and triiodothyronine modulate LPL expression, disorders reducing their concentrations may lead to hypertriglyceridemia. Additionally, phenobarbital is reported to increase hepatic VLDL production and decrease LPL activity in other species, with both mechanisms leading to an increase in serum triglyceride concentration; however, whether phenobarbital has this effect in dogs is unknown.

The purposes of the study reported here were to compare serum triglyceride concentrations obtained after food had been withheld (ie, fasting serum triglyceride concentrations) in dogs with epilepsy that had been treated long term with phenobarbital or with phenobarbital and potassium bromide with serum triglyceride concentrations in healthy control dogs and to determine whether fasting hypertriglyceridemia in dogs with epilepsy was associated with poor seizure control or pancreatitis.

Materials and Methods

Dogs—A convenience sample of 57 dogs with epilepsy that were being treated as outpatients at veterinary hospitals in New South Wales, Australia, participated in the study. In all dogs, the diagnosis of epilepsy had been made by the referring veterinarian on the basis of seizures commencing between 1 and 5 years of age, an initial unremarkable neurologic examination, and exclusion of a metabolic or toxic basis for seizure activity. Twenty-eight of the dogs were being treated with phenobarbital, with all dogs having received phenobarbital for at least 3 months (range, 3 to 120 months). The phenobarbital dosage ranged from 4 to 13 mg/kg/d (1.8 to 5.9 mg/lb/d; mean, 7.3 mg/kg/d [3.3 mg/lb/d]); all dogs received phenobarbital twice daily. The remaining 29 dogs had been treated with phenobarbital and bromide, with all dogs having received phenobarbital for at least 3 months (range, 3 to 144 months). The phenobarbital dosage ranged from 3 to 21 mg/kg/d (1.4 to 9.5 mg/lb/d; mean, 8.6 mg/kg/d [3.9 mg/lb/d]); the bromide dosage ranged from 5 to 90 mg/kg/d (2.3 to 40.9 mg/lb/d; mean, 28.8 mg/kg/d [13.1 mg/lb/d]). All dogs received phenobarbital twice daily and bromide either once or twice daily.

Information regarding dietary history, seizure activity, time of last anticonvulsant dose, and activity level was obtained from owners of the 57 dogs with epilepsy. None of the dogs had any history of seizures during the 24 hours prior to study enrollment. Seizure activity was scored on a scale from 1 to 4, where 1 = no seizures in the past year; 2 = 1 to 4 seizures/yr; 3 = 1 seizure/mo; and 4 = 1 seizure/wk. Activity level was classified as active (daily walks), moderately active (walks 3 to 5 times/wk), or minimally active (no walks or walks 1 to 2 times/wk). Diet consumed was classified as adult, light (< 10% fat content), or natural ingredient (ie, meat, rice, and vegetables). The diet category was chosen on the basis of what the dog was fed predominately. Breed was grouped according to the American Kennel Club breed groups (sporting, hound, working, terrier, toy, non-sporting, and herding). Age categories were defined as 1 = 1 to 3 years; 2 = 4 to 6 years; 3 = 7 to 9 years; and 4 = > 9 years of age. Referring veterinarians were contacted to determine whether any of the dogs had a history of pancreatitis; a diagnosis of pancreatitis was made on the basis of clinical signs (eg, vomiting and signs of abdominal pain) in conjunction with a 3-fold or higher increase in serum lipase or amylase activity and, in some instances, typical ultrasonographic abnormalities. At the time of enrollment in the study, the referring veterinarian was sent a standardized BCS chart and asked to assign a BCS ranging from 1 to 9 to each dog enrolled in the study (1 to 3 = too thin; 4 to 5 = ideal; and 6 to 9 = excessively fat).

Dogs with epilepsy were followed up for 18 months following study enrollment. Follow-up information concerning seizure activity was obtained through telephone interviews with owners or referring veterinarians.

For comparison purposes, a control group of 57 healthy dogs being treated as outpatients at veterinary hospitals in New South Wales that had no history of having been treated with phenobarbital was also included in the study. Control dogs were matched to epileptic dogs on the basis of age (47 dogs were within 1 year and the remainder were within 2 years of the age of the matched dog), sex, neuter status (with the exception of 1 dog), breed, and BCS. All were deemed healthy on the basis of history and results of a physical examination and serum biochemical testing.

All study procedures were approved by The University of Sydney Animal Ethics Committee.

Study protocol—Blood samples were collected from all dogs enrolled in the study by means of jugular venipuncture after food had been withheld for at least 12 hours (mean, 16 hours). A portion of each sample was placed in a plain plastic tube for subsequent determination of serum phenobarbital, bromide, and total thyroxine concentrations and serum biochemical testing. A second portion of each sample was placed in a tube containing EDTA for lipoprotein analysis, and the remainder of each sample was placed in a tube containing fluoride oxalate for determination of blood glucose concentration. Samples in plain plastic tubes and tubes containing EDTA were centrifuged at 2,500 × g for 10 minutes, and serum and plasma were collected. The standing plasma test was performed to detect the presence of chylomicrons.

Serum triglyceride, cholesterol, HDL-c, and phenobarbital concentrations were determined by use of commercially available assays. Serum total thyroxine concentration was determined with a commercial chemiluminescent immunoassay. Serum bromide concentration was determined with a spectrometer. Serum cPLI was determined with a commercial ELISA.

A conventional enzymatic method was used to measure serum bile acids concentrations before and after a meal in 3 dogs with high serum triglyceride concentrations. Thyroid stimulating hormone concentration was measured in 5 control dogs and 8 dogs with epilepsy in which total thyroxine concentration was less than the lower reference limit.

Lipoprotein separation by agarose gel electrophoresis was performed on all plasma samples. Plasma was stored at 4°C (39.2°F) and analyzed between 3 and 5 days.
days after blood sample collection. Briefly, 5 µL of plasma was applied to a 0.6% agarose gel. Electrophoresis was performed for 45 minutes in Tris-barbital buffer (pH, 8.6) at 100 V.\(^m\) Gels were stained\(^m\) and analyzed with a scanning densitometer.

Because canine VLDL is not well differentiated from LDL by means of electrophoresis, ultracentrifugation to separate VLDL from LDL prior to electrophoresis was performed on samples from 7 dogs with epilepsy (4 treated with phenobarbital and 3 treated with phenobarbital and bromide) and 5 control dogs. In brief, saline (0.9% NaCl) solution was layered over the plasma sample, and the sample was ultracentrifuged at 500,000 \(X\) g at 15°C (59°F) for 2 hours. Electrophoresis was then performed on the top and bottom fractions. With this method, triglyceride-rich lipoproteins with a density < 1,006 g/L (chylomicrons and VLDL) were concentrated in the top fraction.\(^m\)

**Oral fat tolerance test**—Oral fat tolerance tests were performed in 9 dogs with epilepsy that had been treated with phenobarbital (4 with a history of high serum triglyceride concentrations) and 15 control dogs. Blood was collected after food had been withheld for 16 hours, and dogs were fed 1.6 g of fat/kg (0.7 g of fat/lb) in the form of thickened cream and 1 tablespoon each of chocolate syrup and skim milk powder. This provided a source of carbohydrate, protein, and fat to simulate the effects of a mixed meal.\(^m\) Overweight dogs were dosed according to estimated lean body mass. Additional blood samples were collected every 2 hours for the next 8 hours, and serum triglyceride concentration was determined. Results were interpreted by grouping dogs into 4 categories: control dogs with BCS ≤ 5, control dogs with BCS ≥ 6, dogs with epilepsy with BCS ≤ 5, and dogs with epilepsy with BCS ≥ 6.

**Statistical analysis**—Data were summarized as mean and SD or as median and IQR (25th to 75th percentile). Normality plots were used to determine whether data were normally distributed, and skewed data were logarithmically transformed prior to statistical analysis. The reference interval for serum triglyceride concentration was defined as the central 95% interval (ie, the 2.5th to 97.5th percentile) for the control dogs. Serum triglyceride concentration was also classified on the basis of a previously described system as normal (≤ 1.6 mmol/L), mildly elevated (1.7 to 4.4 mmol/L), moderately elevated (4.5 to 11.0 mmol/L), or markedly elevated (> 11.0 mmol/L).

A general linear model was used to determine whether serum triglyceride concentration differed significantly across all 114 dogs with respect to BCS, diet, or breed categories as well as among groups when dogs were grouped on the basis of treatment (ie, dogs treated with phenobarbital vs dogs treated with phenobarbital and bromide vs control dogs); age was tested in the model as a covariate. For dogs in which an oral fat tolerance test was performed, serum triglyceride concentration was plotted against time and a residual maximum likelihood procedure was performed. Area under the curve (corrected for a zero baseline), maximum serum triglyceride concentration, and time to reach maximum serum triglyceride concentration were also determined.

Correlations between serum phenobarbital concentration and each biochemical analyte were determined by calculating the Pearson correlation coefficient. Ordinal logistic regression was used to determine whether fasting serum triglyceride concentration was significantly associated with seizure activity score. Binary logistic regression was used to determine whether a prior episode of pancreatitis was associated with current serum triglyceride concentration, current cPLI, BCS, or group. The \(\chi^2\) test was used to determine whether activity level was significantly associated with group. All analyses were performed with standard software.\(^m\) Values of \(P < 0.05\) were considered significant.

### Results

**Signalment and activity**—Many breeds were represented in both the epilepsy and control groups. For the dogs with epilepsy, the most common breeds were Labrador Retriever (n = 5), Golden Retriever (4), Maltese (4), and Poodle (4). Mean ± SD body weight was 18.3 ± 12.0 kg (40.2 ± 26.4 lb) for the 28 dogs treated with phenobarbital, 21.2 ± 12.0 kg (46.6 ± 26.4 lb) for the 29 dogs treated with phenobarbital and bromide, and 18.7 ± 12.0 kg (41.1 ± 26.4 lb) for the 57 control dogs. Body weight did not differ significantly (\(P = 0.582\)) among groups. Mean ± SD age was 7.3 ± 2.9 years for the dogs treated with phenobarbital, 7.9 ± 3.1 years for the dogs treated with phenobarbital and bromide, and 7.7 ± 3.1 years for the control dogs. Age did not differ significantly (\(P = 0.712\)) among groups. The epilepsy group consisted of 26 neutered males, 6 sexually intact males, 24 spayed females, and 1 sexually intact female. The control group consisted of 27 neutered males, 5 sexually intact males, 24 spayed females, and 1 sexually intact female. Control dogs were significantly (\(P = 0.030\)) more active than epileptic dogs, but activity level was not significantly (\(P = 0.200\)) different between the 2 groups of dogs with epilepsy. Fasting serum triglyceride concentration was not significantly associated with age (\(P = 0.255\)), sex (\(P = 0.794\)), breed (\(P = 0.747\)), or diet (\(P = 0.679\)).

**Serum lipid and lipoprotein concentrations**—Fasting serum triglyceride concentration was significantly (\(P = 0.010\)) higher in dogs treated with phenobarbital (median, 0.9 mmol/L; IQR, 0.6 to 1.6 mmol/L) than in the control dogs (median, 0.6 mmol/L; IQR, 0.4 to 0.9 mmol/L) and was significantly (\(P < 0.001\)) higher in dogs treated with phenobarbital and bromide (median, 1.2 mmol/L; IQR, 0.8 to 3.6 mmol/L) than in the control dogs but did not differ significantly between the 2 groups of dogs with epilepsy (Figure 1). The reference range for fasting serum triglyceride concentration, calculated on the basis of values for the 57 control dogs, was 0.4 to 1.6 mmol/L, and 19 (33%) of the 57 dogs with epilepsy had fasting serum triglyceride concentrations higher than the upper reference limit (as did 7/28 [25%] dogs treated with phenobarbital and 12/29 [41%] dogs treated with phenobarbital and bromide). Only 1 control dog had a high serum triglyceride concentration (1.7 mmol/L); this was a Pug with a BCS of 7.

Of the 19 dogs with epilepsy with high fasting serum triglyceride concentrations, 11 had mildly elevated...
concentrations (ie, 1.7 to 4.4 mmol/L), 5 had moderately elevated concentrations (ie, 4.5 to 11.0 mmol/L), and 3 had markedly elevated concentrations (ie, > 11.0 mmol/L). Serum cholesterol concentration did not differ significantly among the 3 groups (Table 1), but serum HDL-c concentrations were significantly higher in the 2 groups of dogs with epilepsy than in the control group.

According to the referring veterinarians, 3 of the dogs with epilepsy had had extremely high fasting serum triglyceride concentrations within 1 month after treatment with phenobarbital had been instituted (44.0, 68.5, and 88.8 mmol/L). Two of these dogs had fasting serum triglyceride concentrations within reference limits before treatment with phenobarbital had begun; fasting serum triglyceride concentration had not been measured in the other dog before treatment with phenobarbital had begun. One of these dogs subsequently developed pancreatitis and was eventually euthanized. The second had consistently high fasting serum triglyceride concentrations over a 12-month period. The third dog was euthanized because of uncontrolled seizure activity, although fasting serum triglyceride concentration was within reference limits at the time of euthanasia.

Six of the 7 dogs treated with phenobarbital and all 12 dogs treated with phenobarbital and bromide that had high fasting serum triglyceride concentrations had intensely staining chylomicron bands on electrophoretic gels. Four of the 21 dogs treated with phenobarbital and 4 of the 17 dogs treated with phenobarbital and bromide that had fasting serum triglyceride concentrations within reference limits had lightly staining chylomicron bands. Two control dogs (serum triglyceride concentrations of 1.2 and 1.7 mmol/L) also had faintly staining chylomicron bands. The 5 dogs with epilepsy and hypertriglyceridemia in which ultracentrifugation was performed prior to electrophoresis had moderate chylomicron and VLDL bands and faint LDL bands on electrophoretic gels. The 2 dogs with epilepsy and the 5 control dogs with normal serum triglyceride concentrations in which ultracentrifugation was performed prior to electrophoresis had faint or no VLDL bands and no chylomicron bands.

Serum triglyceride concentration and seizure activity—Seventeen of the 28 (61%) dogs treated with phenobarbital had not had any seizures in the preceding 12 months (seizure activity score of 1) or had only 1 to 4 seizures/y (seizure activity score of 2). In contrast, 21 of the 29 (72%) dogs treated with phenobarbital and bromide had at least 1 seizure/mo (seizure activity score of 3 or 4). Fasting serum triglyceride concentration was not significantly (P = 0.511) associated with seizure activity score (Figure 2). Although serum phenobarbital concentration was significantly (P = 0.010) higher in dogs treated with phenobarbital and bromide (mean ± SD, 109.3 ± 46.8 µmol/L) than in dogs treated with phenobarbital alone (83.4 ± 21.8 µmol/L), we did not detect significant correlations between fasting serum triglyceride concentration and serum phenobarbital concentration (P = 0.676), between fasting serum triglyceride concentration and serum bromide concentration in dogs treated with phenobarbital and bromide (P = 0.807), between fasting serum triglyceride concentration and phenobarbital dosage (P = 0.069), or between fasting serum triglyceride concentration and bromide dosage in dogs treated with phenobarbital and bromide (P = 0.230). Mean ± SD serum bromide concentration in dogs treated with phenobarbital and bromide was 12.7 ± 3.8 mmol/L.

Figure 1—Scatterplots of serum triglyceride concentration measured after food had been withheld for at least 12 hours (ie, fasting concentration) in 57 healthy control dogs and 57 dogs with epilepsy that had been treated long term with phenobarbital (PB; n = 28) or with phenobarbital and bromide (PB/Br; 29). Horizontal lines represent median concentration for each group.

Table 1—Results of serum biochemical analyses in dogs with epilepsy treated long term with phenobarbital (n = 28) or with phenobarbital and bromide (29) and in healthy, untreated control dogs matched on the basis of age, sex, neuter status, breed, and body condition score (57).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Phenobarbital</th>
<th>Phenobarbital and bromide</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>6.5 ± 1.9</td>
<td>6.6 ± 2.4</td>
<td>6.1 ± 1.1</td>
<td>0.372</td>
</tr>
<tr>
<td>HDL-c (mmol/L)</td>
<td>5.5 ± 1.5*</td>
<td>5.4 ± 1.3*</td>
<td>4.8 ± 0.8</td>
<td>0.021</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>34 ± 5*</td>
<td>32 ± 5*</td>
<td>38 ± 3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>60 (38–104)*</td>
<td>52 (44–69)*</td>
<td>29 (22–60)</td>
<td>0.007</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>133 (72–598)*</td>
<td>273 (118–1,586)*</td>
<td>43 (28–68)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>47 ± 21*</td>
<td>46 ± 15*</td>
<td>25 ± 7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Amylase (U/L)</td>
<td>716 (541–866)</td>
<td>751 (600–1,166)*</td>
<td>573 (472–732)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lipase (U/L)</td>
<td>73 (50–148)</td>
<td>94 (64–314)*</td>
<td>75 (54–125)</td>
<td>0.017</td>
</tr>
<tr>
<td>Total Thyroxine (nmol/L)</td>
<td>15.2 ± 10.0†</td>
<td>10.7 ± 6.3†</td>
<td>20.3 ± 6.8</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Significantly different from value for control dogs. †Significantly different from value for dogs treated with phenobarbital and bromide.

ALT = Alanine aminotransferase. ALP = Alkaline phosphatase. AST = Aspartate aminotransferase.
Serum triglyceride concentration, serum cPLI, and pancreatitis—None of the dogs had clinical signs of pancreatitis at the time blood samples were collected for the present study. Of the 57 dogs with epilepsy, 9 (16%; 4 treated with phenobarbital and 5 treated with phenobarbital and bromide) had previously had clinical signs consistent with pancreatitis. Four of the 9 had undergone abdominal ultrasonography, and all 4 had ultrasonographic findings consistent with pancreatitis. Of the 9 dogs with a history of pancreatitis, 5 (3 treated with phenobarbital and 2 treated with phenobarbital and bromide) had high fasting serum triglyceride concentrations at the time of this study. Dogs treated with phenobarbital and bromide were not significantly (P = 0.638) more likely to have a history of pancreatitis than were dogs treated with phenobarbital alone. However, epileptic dogs with a history of pancreatitis were 2.2 times (95% confidence interval, 1.04 to 4.12) as likely to have hypertriglyceridemia as were epileptic dogs without any history of pancreatitis.

Serum cPLI was measured in 17 dogs treated with phenobarbital, 16 dogs treated with phenobarbital and bromide, and 17 control dogs and was high in 3 of the 17 (18%) dogs treated with phenobarbital, 6 of the 16 (38%) dogs treated with phenobarbital and bromide, and 2 of the 17 (12%) control dogs. These 2 control dogs also had concurrently high serum lipase activities. We did not identify an association between cPLI and fasting serum triglyceride concentration, although there was a strong positive correlation (r = 0.97; P < 0.001) between cPLI and serum lipase activity. Ten of the 33 dogs with epilepsy in which cPLI was measured had high fasting serum triglyceride concentrations, and 4 of these 10 dogs had high cPLI.

Sequential changes in serum triglyceride concentration during oral fat tolerance tests in 15 control dogs, 4 dogs treated with phenobarbital with a BCS ≥ 6, and 5 dogs treated with phenobarbital with a BCS ≥ 6 were plotted (Figure 4). Examination of results revealed that there was no significant effect of group (control dogs vs epileptic dogs treated with phenobarbital) on serum triglyceride concentration at each time point; however, both control and epileptic dogs with a BCS ≥ 6 had significantly higher (P < 0.050) serum triglyceride concentrations at each time point, compared with control and epileptic dogs with a BCS ≤ 5. Epileptic dogs with a BCS ≤ 5, epileptic dogs with a BCS ≥ 6, and control dogs with a BCS ≥ 6 had higher serum triglyceride concentrations at 8 hours than did control dogs with a BCS ≤ 5. Area under the serum triglyceride concentration versus time curve was significantly (P ≤ 0.001) greater in control and epileptic dogs with a BCS ≥ 6, compared with control and epileptic dogs with a BCS ≤ 5; however, no significant (P = 0.610) difference was found between epileptic dogs with a BCS ≥ 6 and control dogs with a BCS ≥ 6.

Figure 2—Scatterplots of fasting serum triglyceride concentration as a function of seizure activity score (1 = no seizures in the past year; 2 = 1 to 4 seizures/y; 3 = 1 seizure/mo; and 4 = 1 seizure/wk) in 57 dogs with epilepsy treated long term with phenobarbital (n = 28) or with phenobarbital and bromide (29). Horizontal dotted lines represent cutoffs for determining whether serum triglyceride concentration was mildly elevated (1.7 to 4.4 mmol/L), moderately elevated (4.5 to 11 mmol/L), or markedly elevated (> 11 mmol/L).

Figure 3—Scatterplots of the natural logarithm of fasting serum triglyceride concentration as a function of BCS in 57 healthy control dogs and 57 dogs with epilepsy that had been treated long term with phenobarbital (n = 28) or with phenobarbital and bromide (29). Lines represent fitted regression lines for dogs treated with phenobarbital and bromide (dashed line), dogs treated with phenobarbital alone (dotted line), and control dogs (solid line). See Figure 1 for remainder of key.

Figure 4—Mean serum triglyceride concentration before and after a meal in 15 healthy control dogs (8 with BCS ≤ 5 and 7 with BCS ≥ 6) and 9 dogs with epilepsy treated with phenobarbital (4 with BCS ≤ 5 and 5 with BCS ≥ 6). Error bars represent SEM. See Figure 1 for remainder of key.
≥ 6. The time to reach maximum serum triglyceride concentration was higher in dogs treated with phenobarbital (6 hours), compared with control dogs (4 hours); however, this difference was not significant (P = 0.073). The maximum serum triglyceride concentration was significantly (P = 0.001) higher in dogs with a BCS ≥ 6, but was not significantly (P = 0.610) higher in dogs treated with phenobarbital than in control dogs with a BCS ≥ 6.

Serum biochemical findings—There was a significant negative correlation (r = -0.53; P < 0.001) between serum phenobarbital concentration and serum albumin concentration and a significant positive correlation (r = 0.55; P < 0.001) between fasting serum triglyceride concentration and serum alkaline phosphatase activity. Total thyroxine concentration was significantly different between control dogs and dogs treated with phenobarbital and between control dogs and dogs treated with phenobarbital and bromide (Table 1). Furthermore, dogs treated with phenobarbital and bromide had significantly (P = 0.040) lower serum total thyroxine concentration than did dogs treated with phenobarbital, and there was a significant negative correlation (r = -0.40; P = 0.002) between serum total thyroxine concentration and serum phenobarbital concentration. Serum total thyroxine concentration was not significantly correlated with serum bromide concentration, serum triglyceride concentration, or seizure activity score.

Discussion

Results of the present study suggested that hypertriglyceridemia was common in dogs with epilepsy being treated with phenobarbital or with phenobarbital and bromide, which was consistent with previous observations. Age, sex, breed, and diet did not appear to account for the fasting hypertriglyceridemia in the present study.

For all 3 groups in the present study (dogs with epilepsy treated with phenobarbital, dogs with epilepsy treated with phenobarbital and bromide, and healthy control dogs), fasting serum triglyceride concentration increased as BCS increased. This was consistent with findings of a previous study in which fasting serum triglyceride concentration was significantly different between normal weight (BCS ≤ 5) and overweight (BCS 7 or 8) dogs. It is not surprising that some dogs treated with phenobarbital become overweight, in that commonly reported adverse effects of phenobarbital include polyphagia and lethargy, which together may result in weight gain. Anecdotally, it appears that bromide may also cause polyphagia, which is consistent with the finding in the present study that dogs treated with phenobarbital and bromide had higher BCSs than did dogs treated with phenobarbital alone. Because control dogs were matched with dogs with epilepsy on the basis of age, sex, neuter status, breed, and BCSs, differences in fasting serum triglyceride concentration among groups were most likely directly attributable to the effects of phenobarbital.

Most of the dogs with epilepsy that had high fasting serum triglyceride concentrations had intensely staining chylomicron bands on electrophoretic gels, suggesting that clearance of chylomicrons from the circulation was delayed, given that blood samples were collected after food had been withheld for a prolonged period and most dogs were fed a low-fat diet. Dogs with epilepsy and hypertriglyceridemia in which samples were tested after ultracentrifugation all had moderate VLDL bands on electrophoretic gels. In people, it has been shown that obesity alone can result in hepatic overproduction of VLDL secondary to insulin resistance and subsequent delayed clearance of chylomicrons and VLDL. Oral fat tolerance studies performed before and after feeding in dogs before, during, and after initiation of phenobarbital treatment while maintaining baseline body weight are required to determine whether phenobarbital itself, rather than obesity, has a direct effect on delaying triglyceride clearance.

Alterations in serum triglyceride concentration attributable to phenobarbital administration have been studied in other species. In rabbits and guinea pigs, phenobarbital increases hepatic VLDL production secondary to microsomal enzyme induction, which can result in high serum triglyceride concentrations. Excess VLDL may overload LPL receptors, causing an accumulation of triglyceride-rich chylomicrons in the blood. Phenobarbital is also known to decrease LPL activity in other species, so it is possible that VLDL overproduction or decreased LPL activity caused by phenobarbital could have contributed to the hypertriglyceridemia in the present study. Microsomal enzyme induction can also increase serum HDL-c concentration in people, providing a possible mechanism to also explain the high serum HDL-c concentrations in dogs with epilepsy in the present study.

Pancreatitis is reported to occur in dogs that have been treated long term with phenobarbital and bromide or with bromide alone. The present study, 9 of the 57 (16%) dogs with epilepsy had a history of pancreatitis and 9 of 33 (27%) dogs with epilepsy in which cPLI was measured had high values, which may have been consistent with ongoing pancreatic inflammation. Although the sample size was small, a significant relationship was found between a history of pancreatitis and current fasting serum triglyceride concentration, in that dogs with a history of pancreatitis were 2.2 times as likely to have hypertriglyceridemia as were dogs without any history of pancreatitis. Importantly, fasting hypertriglyceridemia has been associated with high postprandial serum triglyceride concentrations, a known risk factor for pancreatitis.

There was a strong positive correlation between cPLI and serum lipase activity in the present study. Canine-specific pancreatic lipase immunoreactivity has been reported to have 95% sensitivity and specificity for the diagnosis of acute pancreatitis in dogs, whereas serum lipase activity has 55% sensitivity and 73% specificity. In most species, the pancreas contains phenobarbital-inducible P450 enzymes, although this has yet to be demonstrated in dogs. This might explain the strong correlation in serum lipase activity and cPLI in the present study, although we can only speculate as to whether a link exists between induction of P450 enzymes and development of pancreatitis.

The finding that 3 dogs in the present study had a substantial increase in serum triglyceride concentra-
tions shortly after phenobarbital treatment was begun was interesting, although its importance could not be determined. However, 1 of these 3 dogs did develop clinical signs consistent with pancreatitis 5 days later. In dogs, postprandial lipemia usually resolves within 8 to 10 hours but can still be present up to 12 hours after consumption of a fatty meal.13 Owners of all dogs in the present study were instructed to withhold food for at least 12 hours before blood samples were collected for measurement of serum lipid and lipoprotein concentrations. However, because all dogs were privately owned, we could not determine how well owners complied with this request.

Potential secondary causes of hypertriglyceridemia in dogs in the present study were largely excluded on the basis of history, diet, physical examination findings, and results of serum biochemical analyses. Hypothyroidism, hyperadrenocorticism, and cholestatic liver disease can cause increases in fasting serum triglyceride concentrations, although these conditions are more likely to be associated with high serum cholesterol concentrations.24 Dogs treated with phenobarbital had low serum total thryoxine concentrations and high serum alkaline phosphatase activities, representing well-known effects of phenobarbital treatment,25,26 but did not have any clinical signs or repeatable biochemical abnormalities consistent with hypothyroidism or hyperadrenocorticism. Serum bile acids concentrations were measured before and after a meal in 3 dogs with fasting hypertriglyceridemia (4.1, 9.0, and 12.4 mmol/L) and were within reference limits.

In conclusion, results of the present study suggested that fasting hypertriglyceridemia was common in dogs with epilepsy treated with phenobarbital or with phenobarbital and bromide and that in most dogs, hypertriglyceridemia was attributable to delayed clearance of chylomicrons, most likely as a result of reduced LPL activity or hepatic VLDL overproduction causing LPL saturation. The lack of associations between serum triglyceride concentration and phenobarbital dosage, serum phenobarbital concentration, and seizure activity score suggested that this phenomenon may be idiosyncratic or multifaceted. In addition, 3 dogs in the present study were verified to have developed severe hypertriglyceridemia shortly after treatment with phenobarbital was begun, although we were unable to determine whether these dogs had persistent hypertriglyceridemia because only 1 of them was available for long-term follow-up. The delayed triglyceride clearance in these dogs might suggest that they have an increased risk of developing pancreatitis. We recommend therefore that dogs treated with phenobarbital be fed a low-fat diet and provided regular exercise to maintain a healthy BCS. Further, we recommend that fasting serum triglyceride concentration be periodically monitored. Finally, we recommend that care be taken when adjusting phenobarbital dosages, as serum phenobarbital concentration may be falsely elevated if serum triglyceride concentration is > 11.3 mmol/L.27

References


1276 Scientific Reports JAVMA, Vol 233, No. 8, October 15, 2008
Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Oral bioavailability of etoposide after administration of a single dose to tumor-bearing dogs
Andrea B. Flory et al

Objective—To characterize oral bioavailability and pharmacokinetic disposition of etoposide when the IV formulation was administered orally to dogs.

Animals—8 tumor-bearing dogs.

Procedures—An open-label, single-dose, 2-way crossover study was conducted. Dogs were randomly assigned to initially receive a single dose of etoposide (50 mg/m²) IV or PO. A second dose was administered via the alternate route 3 to 7 days later. Medications were administered before IV administration of etoposide to prevent hypersensitivity reactions. Oral administration of etoposide was prepared by reconstituting the parenteral formulation with 0.9% NaCl solution and further diluting the reconstituted mixture 1:1 with a sweetening agent. Plasma samples were obtained after both treatments. Etoposide concentrations were measured with a high-performance liquid chromatography assay, and plasma etoposide concentration–time profiles were analyzed by use of noncompartmental methods.

Results—4 dogs had hypersensitivity reactions during IV administration of etoposide. No adverse effects were detected after oral administration. Plasma etoposide concentrations were undetectable in 2 dogs after oral administration. Oral administration of etoposide resulted in significantly lower values for the maximum plasma concentration and the area under the plasma etoposide concentration–versus-time curve, compared with results for IV administration. Oral bioavailability of etoposide was low (median, 13.4%) and highly variable among dogs (range, 5.7% to 57.3%).

Conclusions and Clinical Relevance—Vehicle-related toxicosis can limit the IV administration of etoposide in dogs. The parenteral formulation of etoposide can be safely administered orally to dogs, but routine use was not supported because of low and variable oral bioavailability in this study. (Am J Vet Res 2008;69:1316–1322)