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# Evaluation of gallbladder motility assessed by ultrasonography in dogs with hyperlipidemia

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## Abstract

**Background:** The pathogenesis of gallbladder (GB) mucoceles in dogs is unknown. It has been proposed that hyperlipidemia could impair GB motility and contribute to GB mucocele formation.

**Hypothesis/Objectives:** The objective of this study was to compare GB motility in dogs with hyperlipidemia to control dogs using ultrasonography. We hypothesized that hyperlipidemic dogs will have decreased GB motility compared with controls.

**Animals:** Twenty-six hyperlipidemic and 28 healthy, age-matched control dogs were prospectively enrolled.

**Methods:** Cholesterol and triglyceride concentrations were measured in all dogs. Hyperlipidemia was defined as hypercholesterolemia (>332 mg/dL) and/or hypertriglyceridemia (>143 mg/dL) using a biochemical analyzer. Ultrasound was performed before feeding, and 60 and 120 minutes after ingestion of a high fat diet. Gallbladder volumes (GBV) and ejection fractions (EF) were calculated.

**Results:** Hyperlipidemic dogs had significantly larger GBVs (ml/kg) before feeding and 60 minutes after feeding of 1.2 (0.4-7.5; $P = .008$) and 0.6 (0.1-7.2; $P = .04$) compared with controls 0.6 (0.2-2.6) and 0.4 (0.1-1.9), respectively. Severely hyperlipidemic dogs had significantly larger GBV at baseline, 60 minutes, and 120 minutes of 1.7 (0.6-7.5; $P = .03$), 1.3 (0.4-7.2; $P = .02$), and 1.3 (0.2-8.2; $P = .04$), respectively compared with mildly hyperlipidemic dogs. EFs at 60 and 120 minutes between controls, hyperlipidemic, and severely hyperlipidemic were all 0.3 at 60 minutes and 0.5, 0.3, and 0.3 at 120 minutes, respectively which were not statistically different.

**Conclusions and Clinical Importance:** Hyperlipidemia leads to GB distention in dogs which could lead to retention of bile and gallbladder disease.

**KEYWORDS**
diabetes mellitus, gallbladder mucocele, hyperadrenocorticism, hypercholesterolemia, hypertriglyceridemia, hypothyroidism

**Abbreviations:** ALP, alkaline phosphatase; ALT, alanine aminotransferase; BD, bile duct; CCK, cholecystokinin; EF, ejection fraction; GB, gallbladder; GBM, gallbladder mucocele; GBV, gallbladder volume; GBV/kg, gallbladder volume per kilogram body weight; GGT, gamma-glutamyl transferase.

## 1 | INTRODUCTION

Gallbladder (GB) disease in dogs, in particular gallbladder mucocele (GBM), is common. Several breeds are at increased risk of developing GBMs, including Cocker Spaniels, Pomeranians, Miniature Schnauzers, Chihuahuas, and Shetland Sheepdogs.[1–3] Shetland Sheepdogs and Miniature Schnauzers are up to 14 and 6 times more likely, respectively, than other breeds to develop GBM.[1–3] Shetland Sheepdogs and Miniature Schnauzers are also predisposed to familial primary hypercholesterolemia and hypertriglyceridemia, respectively.[4,5] Hyperlipidemia and GB dysmotility are found in a small number of Shetland Sheepdogs before the formation of GBM.[1] The most common causes of secondary hyperlipidemia in dogs are endocrine disorders, such as hypothyroidism, diabetes mellitus, and hyperadrenocorticism.[5–14] These diseases are associated with an increased risk of GBM formation.[1,15–18]

Hyperlipidemia might play a role in the dysregulation of GB motor function and mucosal function, as well as bile composition, resulting in abnormal GB motility and, in some cases, GBM formation.[19,20] Gallbladder motor function can be assessed noninvasively using ultrasonography by measuring the GB volume before feeding and 60 and 120 minutes after a standardized meal.[19–21] If hyperlipidemic dogs have impaired GB motility, further evaluation of effective management of the lipid disorder in conjunction with vigilant monitoring for the development GB disease could be investigated to reduce morbidity and mortality, particularly in predisposed breeds.

The objective of this study was to compare GB motility in dogs with hyperlipidemia to healthy, control dogs via ultrasonography. We hypothesized that hyperlipidemic dogs would have decreased GB motility, intended as increased fasting gallbladder volume (GBV) and decreased GB ejection fractions at 60 and 120 minutes after a meal ($EF_{60}$ and $EF_{120}$) when compared with control dogs.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals

Fifty-four dogs, 26 hyperlipidemic and 28 healthy, age-matched control dogs >1 year of age were prospectively enrolled at the Virginia-Maryland College of Veterinary Medicine (VMCVM). Institutional Animal Care and Use Committee approval and informed owner consent were obtained. Hyperlipidemia was defined as serum hypercholesterolemia (>332 mg/dL) and/or hypertriglyceridemia (>143 mg/dL) using a biochemical analyzer (Beckman Coulter AU480, Brea, California). Dogs were arbitrarily assigned to a subgroup of severe hyperlipidemia if either triglyceride, cholesterol, or both concentrations were >500 mg/dL. Dogs with secondary causes (eg, hypothyroidism, hyperadrenocorticism, and diabetes mellitus) of hyperlipidemia were included provided they were receiving treatment that controlled the clinical signs associated with the primary disease. Control dogs were healthy based on history, physical examination, and serum biochemistry. Dogs receiving drugs that could alter GBM, including anticholinergics, erythromycin, loperamide, ondansetron, cisapride, cholestyramine, ursodiol, or SAM-e within 7 days before evaluation, were excluded. Dogs with co-morbidities that could affect GB motility such as GBM, acute pancreatitis, extrahepatic biliary obstruction, portosystemic shunt, cholangiohepatitis, or diffuse hepatobiliary neoplasia were also excluded. These dogs were excluded based on clinical evaluation and a complete abdominal ultrasound performed before feeding. Food was withheld from all dogs for at least 12 hours before collection of blood for biochemistry and pre-prandial ultrasound.

### 2.2 | Ultrasonography

Abdominal ultrasounds were performed by a board-certified radiologist who was blinded to the lipidemia status of the dogs using an ultrasound machine (Philips iU22, Philips Medical Systems, Bothell, Washington) equipped with a preset broad bandwidth operating frequency transducer (8-5 mHz microconvex) Abdominal ultrasound was performed on dogs in the fasted state as well as 60 and 120 minutes after ingesting 10 g/kg of Hill's a/d (Hills Prescription Diet a/d Topeka, Kansas). Transverse and longitudinal images of the GB were obtained with dogs in dorsal or lateral recumbency via subcostal, right-sided intercostal or both approaches. For all time sequences, 6 measurements of each dimension (length, width, and height) were made and averaged to get the final measurements for each GBV calculation. Three measurements were taken from still images, frozen at the subjective maximum dimension to most accurately average for final measurements. Three additional measurements were taken from videos frozen at the subjectively assessed maximum dimension. Length was assessed on sagittal images of the GB, measuring from apex to neck. Width was assessed on transverse images, frozen when the GB had an ovoid shape. Width was assessed at the widest aspect of the GB. Height was assessed on the same image, perpendicular to the width measurement. Measurements were made from the inner aspect of the GB wall in each case. If adherent sludge was present, the wall was measured through the sludge (Figure 1). Gallbladder volumes were calculated using the ellipsoid method (Volume $= 0.52 \times L \times W \times D$), as described by Atalan et al.[22] The $EF_{60}$ and $EF_{120}$ were calculated using the volume of the GB in mL ($V$) at each time point with the equation $EF = ([V_0 - V_{60,120}]/V_0) \times 100$.[20] Relative GBV was calculated by dividing the volume of the GB by kilograms of body weight, which was referred as the GBV (mL/kg) in this study.[20]

### 2.3 | Statistical analysis

A power analysis using the advanced repeated measures procedure of PASS (Hintze, J. [2011]. PASS 11. NCSS, LLC. Kaysville, Utah; www.ncss.com) showed that 26 dogs per group (healthy and hyperlipidemic) would be needed to detect an effect size of 25 for ejection fraction between the groups with a power of 100% and to detect an effect size of 0.4 for the within group effect (60 vs 120 minutes) with a

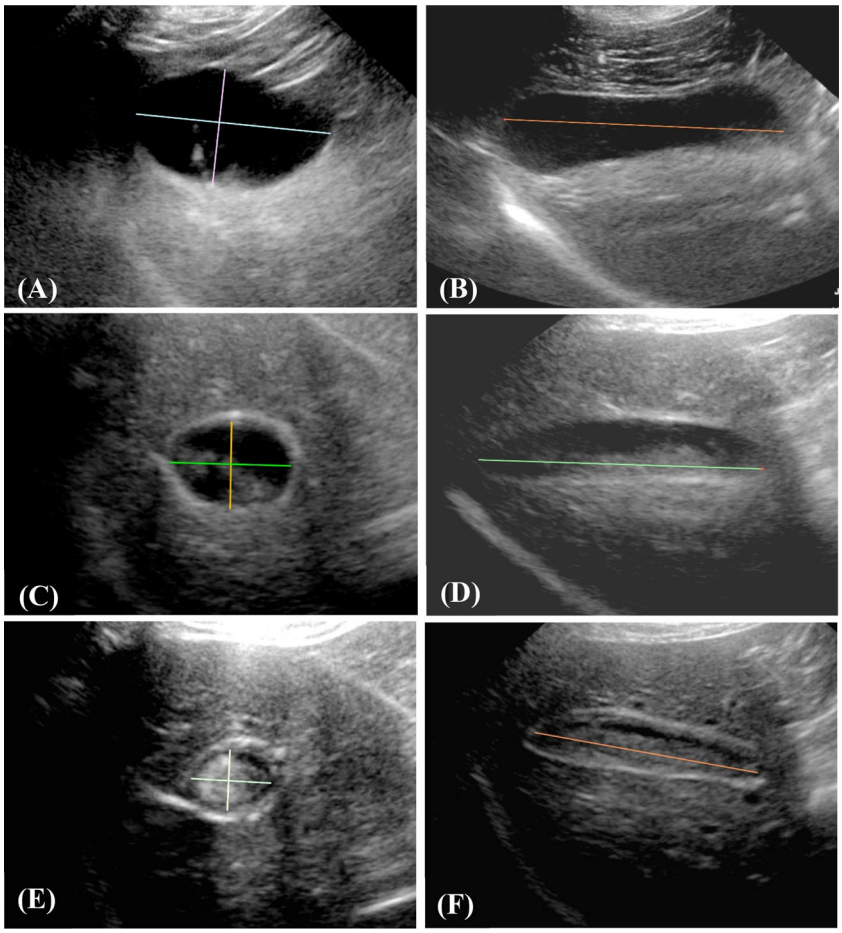


**FIGURE 1** Gallbladder measurements before feeding, 60 and 120 minutes after meal. (A) Time 0: Width and height (transverse), (B) Time 0: Length (sagittal), (C) Time 60: Width and height (transverse), (D) Time 60: Length (sagittal), (E) Time 120: Width and height (transverse).

power of 80.7%. Mean ejection fractions used for this analysis were: 51.7% for healthy dogs at 60 minutes, 20.2% for hyperlipidemic dogs at 60 minutes, 64.4% for healthy dogs at 120 minutes, and 8.3% for hyperlipidemic dogs at 120 minutes.[19] Smaller values for the hyperlipidemic group contributed to the large between group effect while the within group effect was the limiting factor.

Outcomes were $GBV_0$, $GBV_{60}$, $GBV_{120}$, $EF_{60}$, and $EF_{120}$. Potential risk factors that were evaluated for changes to GBV and EF were age, sex, breed, hyperlipidemia (hypercholesterolemia and hypertriglyceridemia), and clinicopathologic abnormalities such as bilirubin, alanine transaminase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) above the reference range. Hyperlipidemia was divided into mild and severe (>500 mg/kg) and Kruskal-Wallis test followed by Dunn procedure was used for multiple comparisons. Normal probability plots showed that all continuous variables age, sex, triglycerides, cholesterol, total bilirubin, ALT, ALP, GGT, $GBV_0$, $GBV_{60}$, $GBV_{120}$, $EF_{60}$, and $EF_{120}$ were skewed. Accordingly, data were summarized as median (range) for continuous variables and counts and percentages for the categorical variables. Associations between outcomes (1 outcome at a time) and risk factors were assessed using the Wilcoxon rank sum test (for the continuous variables) and Fisher's exact test (for the categorical variables). Statistical significance was determined at $P < .05$. All analyses were performed using SAS version 9.4 (Cary, North Carolina).

## 3 | RESULTS

### 3.1 | Study cohort

Twenty-seven dogs with hyperlipidemic dogs were evaluated for the study. Two dogs were excluded, with 1 dog being excluded because had a GBM and the other was no longer hyperlipidemic at evaluation. Thirty-two control dogs were evaluated, with 3 excluded because of the presence of mild hyperlipidemia and 1 because of temperament affecting evaluation. This resulted in 26 dogs in the hyperlipidemic and 28 in the control groups, respectively.

There was no difference in age ($P = .63$) or sex between the control (median 10, range 4-14 years) and hyperlipidemic dogs (median 10, range 5-14 years) between the groups. All dogs were either spayed or neutered in both groups. Of the 17 breeds included in the study, mixed breed (n = 9), Shetland Sheepdog (n = 6), and Miniature Schnauzer (n = 4) were most common in the hyperlipidemic group. The most common breeds in the control group included mixed breed (n = 17) and Shetland Sheepdog (n = 3). There was 1 breed of each including Cavalier King Charles Spaniel, Corgi, English Springer Spaniel, Jack Russell, Labrador Retriever, Pomeranian, and Shih Tzu in the hyperlipidemic group. The control group included 1 of each including Boxer, Chihuahua, Husky, Jack Russell, Pug, Schipperke, Staffordshire terrier and a mixed breed dog.

**TABLE 1** Association of biochemical indices, gallbladder volumes, and ejection fractions in dogs with and without hyperlipidemia.

| Variable | Control group | Hyperlipidemia group | HTG group | HC group | Severe hyperlipidemic group |
|---|---|---|---|---|---|
| ALT | 43.5 (25.0 to 130.0) | 58.0 (11.0 to 566.0) | 59.0 (11.0 to 384.0) | 44.0 (11.0 to 384.0) | 65.0 (19.0 to 384.0) |
| ALP | 28.0 (6.0 to 415.0) | 97.5 (17.0 to 2482.0)* | 219.0 (17.0 to 2482.0)* | 88.0 (17.0 to 2482.0) | 219.0 (24.0 to 2482.0)* |
| GGT | 3.0 (0.0 to 7.0) | 4.5 (0.0 to 17.0)* | 6.0 (0.0 to 17.0)* | 5.0 (0.0 to 17.0) | 6.0 (1.0 to 17.0)* |
| Tbili | 0.2 (0.1 to 0.5) | 0.2 (0.1 to 0.5) | 0.2 (0.1 to 0.5) | 0.2 (0.1 to 0.5) | 0.2 (0.1 to 0.5) |
| Chol | 238.0 (153.0 to 324.0) | 346.0 (181.0 to 1372.0)* | 322.0 (181.0 to 1372.0)* | 437.0 (340.0 to 1372.0)* | 437.0 (222.0 to 1372.0)* |
| Trig | 65.5 (34.0 to 142.0) | 330.0 (52.0 to 2213.0)* | 466.0 (193.0 to 2213.0)* | 275.0 (52.0 to 2213.0)* | 910.0 (275.0 to 2213.0)* |
| $GBV_0$/kg | 0.6 (0.2 to 2.6) | 1.0 (0.4 to 7.5)* | 1.1 (0.4 to 7.5)* | 1.2 (0.4 to 7.5)* | 1.7 (0.6 to 7.5)* |
| $GBV_{60}$/kg | 0.4 (0.1 to 1.9) | 0.6 (0.1 to 7.2)* | 0.7 (0.1 to 7.2)* | 1.0 (0.1 to 7.2) | 1.3 (0.4 to 7.2)* |
| $GBV_{120}$/kg | 0.3 (0.1 to 1.9) | 0.5 (0.1 to 8.2) | 0.6 (0.1 to 8.2) | 1.0 (0.1 to 8.2)* | 1.3 (0.2 to 8.2)* |
| $EF_{60}$ | 0.3 (−0.5 to 0.7) | 0.3 (−0.4 to 0.8) | 0.3 (−0.5 to 0.7) | 0.2 (−0.4 to 0.7) | 0.3 (−0.1 to 0.6) |
| $EF_{120}$ | 0.5 (−1.6 to 0.9) | 0.3 (−0.3 to 0.9) | 0.5 (−0.3 to 0.9) | 0.2 (−0.3 to 0.9) | 0.3 (−0.3 to 0.7) |

Abbreviations: ALP, alkaline phosphatase (U/L); ALT, alanine transaminase (U/L); Chol, cholesterol (mg/dL); $EF_{0,60,120}$, ejection fraction before feeding, 60, and 120 minutes after test meal; $GBV_{0,60,120}$, gallbladder volumes per kg body weight prior, 60, and 120 minutes after test meal; GGT, gamma-glutamyl transferase (U/L); HC, hypercholesterolemic group; HTG, hypertriglyceridemic group; Tbili, total bilirubin (mg/dL); Trig, triglyceride (mg/dL).
*Significance set to $P < .05$, all variables compared with controls or non-hyperlipidemic dogs.

## 3.2 | Serum biochemical variables

Hypercholesterolemia and hypertriglyceridemia were present in 15/26 and 21/26 hyperlipidemic dogs, respectively, and 10/26 had elevations of both analytes. Dogs with well-controlled, previously diagnosed, secondary causes of hyperlipidemia included 2 with hyperadrenocorticism (7%) and 4 with diabetes mellitus (15%). Secondary causes of hyperlipidemia were previously diagnosed by the referring veterinarian and were being treated for their underlying diseases by the referring veterinarian. Further evaluation of diagnosis and treatment were deemed appropriate but further investigation was not under the scope of this study. Median and range of biochemical abnormalities for each group are listed in Table 1. The median cholesterol concentration was 346 and 238 mg/dL, in dogs in the hyperlipidemia and control groups, respectively. The median triglyceride concentration was 330 and 65.5 mg/dL in the hyperlipidemia and control groups, respectively. Eleven (42%) hyperlipidemic dogs were severely hyperlipidemic with a median cholesterol concentration of 437 mg/dL and a median triglyceride concentration of 910 mg/dL compared with mildly hyperlipidemic dogs with median concentrations of 272 and 73 mg/dL of cholesterol and triglycerides, respectively. Of the 15 dogs with hypercholesterolemia, median cholesterol concentration was 437 mg/dL and median triglyceride concentration was 275 mg/dL. Of the 21 dogs with hypertriglyceridemia, median cholesterol concentrations was 322 mg/dL and triglyceride concentration was 466 mg/dL. In the 6 dogs with secondary hyperlipidemia, 3 dogs with diabetes mellitus had severe hyperlipidemia. The breeds of dogs with severe hyperlipidemia included 4 mixed breed, 3 Miniature Schnauzers, and 1 Shih Tzu, Jack Russell, Pomeranian, and Shetland Sheepdog.

Dogs with hyperlipidemia had significantly higher serum ALP ($P = .0001$) and GGT ($P = .02$) compared with controls. There were no significant differences in ALT ($P = .63$), and total bilirubin ($P = .39$) between the 2 groups. Dogs with severe hyperlipidemia had significantly higher ALP ($P = .009$) and GGT ($P = .007$) activities, but no differences in ALT activity ($P = .06$) and total bilirubin concentration ($P = .27$) compared with dogs with mild hyperlipidemia (Table 1).

Dogs with hypertriglyceridemia had significant differences in ALP ($P = .0003$), GGT ($P = .02$), and cholesterol ($P = .002$) compared with controls. Dogs with hypercholesterolemia did not have any significant differences in serum biochemical variables from control dogs.

## 3.3 | Gallbladder volume

Median and range GBVs at all-time points are listed in Table 1. Hyperlipidemic dogs had a significantly larger $GBV_0$ ($P = .008$) and $GBV_{60}$ ($P = .04$), but not $GBV_{120}$ ($P = .12$) compared with controls (Table 1). Severely hyperlipidemic dogs had significantly larger GBV at baseline, 60 18 minutes, and 120 minutes of 1.7 (0.6-7.5; $P = .03$), 1.3 (0.4-7.2; $P = .02$), and 1.3 (0.2-8.2; $P = .04$), respectively, compared with mildly hyperlipidemic dogs. There were no statistically significant differences in the GBVs between mildly hyperlipidemic and control dogs. Boxplot representation of the GBVs in controls, mildly hyperlipidemic and severely hyperlipidemic dogs are seen in Figures 2 to 4. GBVs were not statistically significantly different between dogs with or without hypertriglyceridemia. Dogs with hypercholesterolemia had significantly larger $GBV_0$ ($P = .02$), $GBV_{60}$ ($P = .01$) and $GBV_{120}$ ($P = .02$) when compared with dogs without hypercholesterolemia.[1]

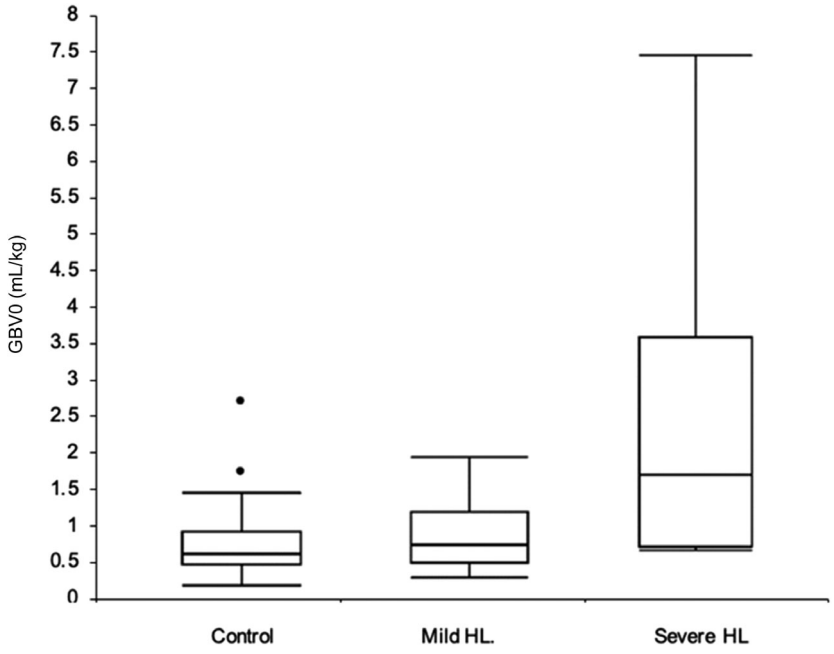


**FIGURE 2** Box-and-whisker plot of $GBV_0$ of healthy, mildly hyperlipidemic, and severely hyperlipidemic dogs. Lines in box plot represent the median value, the box represents the 25th to 75th percentiles and the whiskers represent the 5th to 95th percentiles. Individual outliers are represented by a dot. $GBV_0$ gallbladder volume per kg body weight.

### 3.4 | Ejection fraction

EFs at 60 and 120 minutes between controls, hyperlipidemic, and severely hyperlipidemic were all 0.3 at 60 minutes and 0.5, 0.3, and 0.3 at 120 21 minutes, respectively which were not statistically significantly different among groups. Median EFs at all-time points are listed in Table 1. The median EFs for 60 and 120 minutes were not statistically significantly different between any comparison at any time point. Ejection fractions at 120 min were <25% in 9 (35%) and 7 (25%) hyperlipidemic and control dogs, respectively. Of the control dogs with an EF at 120 min <25%, 2 had EF >25% at 60 minutes.

A full abdominal ultrasound was performed before the test meal in each dog. In the hyperlipidemic group, 2 solitary liver masses and 1 focal gastric mass were incidentally found. In the control group, ultrasound revealed an incidental apical bladder mass and 2 dogs with hepatic nodules. One of the dogs with a liver mass had an abnormal $EF_{120}$, but the remaining dogs with these findings had a normal $EF_{120}$.

## 4 | DISCUSSION

Gallbladder motility as assessed via EF is unaltered in hyperlipidemic dogs but GBV remains high in hyperlipiemic dogs before and after feeding indicating GB dysmotility. Furthermore, dogs with severe hyperlipidemia had persistent GB distention not observed in dogs with mild hyperlipidemia. It has been theorized that gallbladder distention and bile retention could represent an early phase in a continuum leading to changes in bile composition and ultimately GBV formation.[1,2,20] As such, dogs with severe hyperlipidemia might be predisposed to develop GB disease such as GBM as previously suggested.[1,2,20] As such, the dogs with severe hyperlipidemia might be more likely to develop GB disease.[1,2,20] A decrease in GB motility after feeding a high-fat, high-cholesterol diet was evident when using a pharmacologic method to assess GB motility, involving a cholecystokinin (CCK) infusion in sedated dogs.[23] However, it is unclear if this methodology could be compared with the GB contraction induced by feeding in the present study. The effects of motilin, acetylcholine, secretin, gastrin, and other factors involved in GB motility are likely to be preserved in the methods we used.

The pathogenesis of GBM formation is unknown, but decreased GB motility is present in dogs with GBM.[19] Dogs with GBM have increased GBVs as well as reductions in EFs at all-time points compared with control dogs when using the same method of GB motility used in the present study.[19] However, this study did not look at dogs with hyperlipidemia before GBM formation. In humans, GB distension occurs with hyperlipidemia and predisposes to bile stasis and development of GB disease.[22,24] Bile stasis is hypothesized to lead to retained mucus secretions and predisposes to compromised local blood circulation to the GB mucosa and wall, leading to impaired absorption of water and electrolytes, which further aggravates the dilatation.[25] Prolonged exposure to concentrated, hydrophobic bile acids are cytotoxic on GB epithelial cells and promote mucous hypersecretion which ultimately leads to GB distention and bile stasis.[26,27] GB distention and bile stasis will increase the contact with the hydrophobic bile acids which can have cytotoxic effects on GB epithelial cells and might contribute to GBM formation.[26,27] Hypercholesterolemia in humans leads to elevated bile cholesterol concentration and subsequently an increased cholesterol in GB smooth muscle cells which can affect smooth muscle contraction, but has not been investigated in dogs.[27] The latter could be another mechanism on how hypercholesteremia leads to GB dysmotility in dogs. Dogs with GBMs have decreased GB motility and abnormal lipid and energy metabolism.[28] This might be related to a predominance of the disorder in specific breeds that could have other

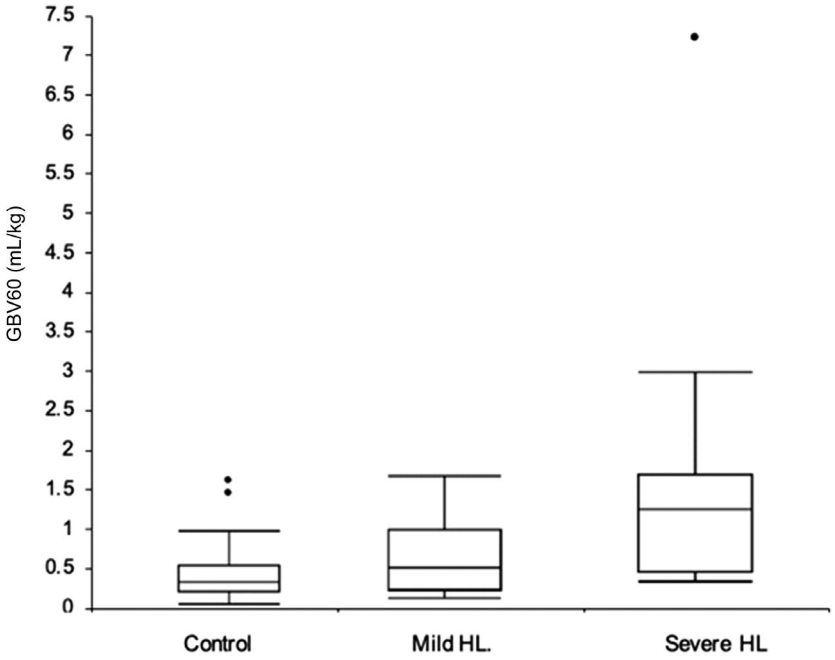


**FIGURE 3** Box-and-whisker plot of $GBV_{60}$ of healthy, mildly hyperlipidemic, and severely hyperlipidemic dogs. Lines in box plot represent the median value, the box represents the 25th to 75th percentiles and the whiskers represent the 5th to 95th percentiles. Individual outliers are represented by a dot. $GBV_{60}$ gallbladder volume per kg body weight.

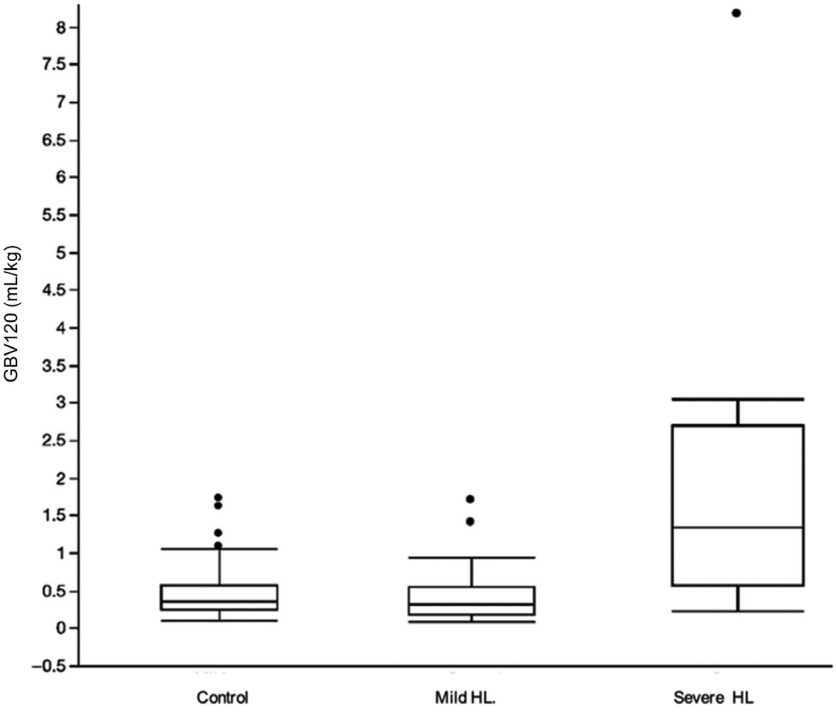


**FIGURE 4** Box-and-whisker plot of $GBV_{120}$ of healthy, mildly hyperlipidemic, and severely hyperlipidemic dogs. Lines in box plot represent the median value, the box represents the 25th to 75th percentiles and the whiskers represent the 5th to 95th percentiles. Individual outliers are represented by a dot. $GBV_{120}$ gallbladder volume per kg body weight.

abnormalities of the GB such as altered mucosal secretory and absorptive function.[28]

Primary hyperlipidemia or breed-related hyperlipidemia appeared prevalent in our study sample (50%) including Miniature Schnauzers or Shetland Sheepdogs with hypertriglyceridemia and hypercholesterolemia, respectively. Although a thorough investigation was not done in all dogs in evaluate for other causes of hyperlipidemia and an assumption was based on breed which is a limitation of the current study. Secondary hyperlipidemias (hypothyroidism, diabetes mellitus, and hyperadrenocorticism) made up a smaller proportion of the study sample. Dogs in neither group had clinical signs suggestive of these diseases. ALP and GGT were significantly higher in hyperlipidemic dogs compared with control dogs. Hyperlipidemic dogs, and more specifically in the hypertriglyceridemia group had a significantly increased ALP which could be related to an underlying endocrinopathy, or because of primary hypertriglyceridemia as seen in previous studies.[29–32] Hypertriglyceridemia has been associated with hepatocellular accumulation of triglycerides and glycogen leading to

intrahepatic cholestasis and is likely of little clinical consequence. Intrahepatic cholestasis may be the underlying reason for the significant difference seen in cholestatic enzymes between hyperlipidemic and control dogs.[4,31] It is also possible that vacuolar hepatopathy or hepatic inflammatory diseases such as chronic hepatitis or could result in intrahepatic and biliary cholestasis.

Our study relied on the GB contraction after ingestion of a high fat meal which represents normal physiology in dogs. One study reported >90% of healthy dogs had an EF of >25% within 120 min of a meal ingestion or stimulation via erythromycin.[21] Erythromycin was administered to dogs that did not have an EF >25% with a meal alone and this was necessary in 4 of the 6 dogs to achieve an EF >25%. Based on these findings an ejection fraction of >25% has been referred to as normal gallbladder emptying in dogs. This criterion of GB dysmotility has been used in 2 other studies assessing GB disease in dogs where GB dysmotility occurred in the absence of gallbladder sludge or mucoceles in dogs with an altered appetite.[33,34] In our study, both control dogs and hyperlipidemic dogs had a similar prevalence of EF <25% suggesting that hyperlipidemia also does not impact EF using this criterion. However, aforementioned studies that used this criterion did not give erythromycin to prove that dogs could achieve an EF of >25%. Therefore, using this criterion for GB dysmotility in our population and other studies that did not use erythromycin could lead to overdiagnosis of dysmotility. The use of a control group in the current study provided a reference for affected dogs and all dogs were in a home environment. All dogs were presumed healthy with no signs of apparent underlying GI disease, but further investigation was not within the scope of this study. Administration of CCK analogues, such as erythromycin, was not done to better compare all groups under physiologic conditions (meal induced GB contraction), thus providing direct clinical relevance.

There were several limitations to our study. Eleven dogs within the hyperlipidemic group had severe hyperlipidemia which could have decreased our ability to detect significant differences in EF between severely hyperlipidemic dogs and controls. The duration of hyperlipidemia was not assessed in these dogs, thus the effect of longstanding hyperlipidemia on GB motility requires further investigation. It is possible that dogs with more severe hyperlipidemia or with documented persistent hyperlipidemia could have greater effects on GB emptying than the dogs in this study, and further GBM formation. Six dogs had incidental intra-abdominal masses, but only 1 of the dogs with a liver mass had abnormal GB motility. Based on this, these masses were unlikely to result in changes to GB motility directly. Finally, ultrasonography is a reliable, noninvasive method to assess GB function in dogs, but there are limitations to 2-dimensional ultrasonography to assess functionality. Interobserver variability was limited by averaging 6 measurements from different images and having 1 radiologist perform all the ultrasounds and measurements.

Gallbladder volumes being greater in hyperlipidemic dogs represents abnormal GB distension and bile stasis. Although severely hyperlipidemia retained normal emptying in this study, future studies assessing GB motility in a larger population of dogs with severe hyperlipidemia is warranted. Other hypothesized theories for GBM formation include a change in bile composition, specifically increased hydrophobic bile acids and cholesterol, in hyperlipidemic dogs. These changes to bile could alter the GB's secretory and motor functions.

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## CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

## OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by Virginia-Maryland College of Veterinary Medicine IACUC 18-280.

## HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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