Effects of hyperlipidemia on gallbladder motility in dogs

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ABSTRACT

Background: The pathogenesis of gallbladder mucocele is unknown in the dog. It has been proposed that hyperlipidemia could impair gallbladder motility and contribute to gallbladder mucocele formation.

Objectives: The objective of this study was to compare gallbladder motility in dogs with hyperlipidemia to healthy, control dogs using ultrasonography. We hypothesized that hyperlipidemic dogs have decreased gallbladder motility, defined by increased fasting gallbladder volume (GBV) and decreased gallbladder ejection fractions at 60 (EF_{60}) and 120 minutes (EF_{120}) compared to controls.

Animals: 26 hyperlipidemic dogs, 28 healthy control dogs

Methods: Twenty-six hyperlipidemic and 28 healthy, age-matched control dogs were prospectively enrolled. Hyperlipidemia was defined as hypercholesterolemia (>332 mg/dL) and/or hypertriglyceridemia (>143 mg/dL). Dogs with both primary and secondary causes of hyperlipidemia were included. All dogs were fasted for at least 12 hours prior to collection of plasma biochemistry and pre-prandial ultrasound. Ultrasound was performed on dogs in the fasted state as well as at 60 and 120 minutes after being fed 10g/kg of a high fat diet (Hill's a/d diet; Hill's Pet Nutrition, Topeka, Kansas, USA). GBVs and EFs were calculated using the following formulas: GBV = (0.52 x L x W x H)/kg and EF = ((GBV_0- $GBV_{60,120}$)/GBV_0) x 100, respectively. GBV_0, GBV_{60}, GBV_{120}, EF_{60} and EF_{120} were compared between dogs with hyperlipidemia and controls using the Wilcoxon rank sum test. Statistical significance was set to $p<0.05$.

Results: Hypercholesterolemia and hypertriglyceridemia were present in 15/26 (58%) and 21/26 (81%) hyperlipidemic dogs, respectively and 10/26 (38%) had elevations in both parameters. The median age in both groups was 10 years. Median (range) cholesterol concentration was 346 mg/dL (181-1372 mg/dL)

and 238 mg/dL (153-324) in hyperlipidemic and control dogs, respectively. Median triglyceride concentration was 330 mg/dL (52-2213) and 65.5 mg/dL (32-142) in hyperlipidemic and control dogs, respectively. Eleven (42%) hyperlipidemic dogs were considered severely hyperlipidemic based on the triglyceride and/or cholesterol concentrations above 500 mg/dL.

There were significant differences in GBV_0 and GBV_{60} between hyperlipidemic and control dogs. Dogs with severe hyperlipidemia had significantly larger GBVs at all time points. Dogs with hypercholesterolemia also had significantly greater GBVs at all times compared to dogs without hypercholesterolemia. Median EF_{60} and EF_{120} were not significantly different between hyperlipidemic and control dogs nor severely hyperlipidemic and mildly hyperlipidemic dogs.

Conclusions: Hyperlipidemic dogs have significantly greater fasting and postprandial GBVs but similar ejection fractions when compared to control dogs. Gallbladder emptying is unaltered in hyperlipidemic dogs, but gallbladder volume is higher in hyperlipidemic dogs after feeding. This distention could contribute to bile retention of bile and potentially gallbladder disease.

LAY ABSTRACT

Gallbladder (GB) diseases are more frequently recognized in dogs as a significant cause of illness and potentially death. In particular, gallbladder mucoceles (GBM), the distention of the GB with mucus, can rupture and cause a critical condition that can quickly lead to death if not addressed immediately. Currently, the cause of GBM is unknown, making treatment and preventative strategies difficult. Dogs with GBM have poor GB motility and increased lipid levels, such as cholesterol and triglycerides, leading to the theory that increased lipid levels may lead to abnormal GB motility and eventually GBM formation. It has yet to be determined if increased lipid levels are associated with impaired GB motility in dogs. If increased lipid levels cause decreased gallbladder motility then diets and medications aimed to reduce lipid levels in conjunction with vigilant monitoring for the development GB disease may prove beneficial to prevent or reduce severity of illness and risk of death, particularly in predisposed breeds.

The purpose of this study was to evaluate gallbladder (GB) emptying in dogs with elevated lipid levels compared to healthy dogs. We are using ultrasonography to measure GB volumes (GBVs) in dogs at various time points before and after a meal. If dogs with elevated lipid levels have decreased gallbladder emptying, preventative and management strategies can be developed in dogs at risk for GB disease.

This study revealed that gallbladder emptying is unaltered in hyperlipidemic dogs, but gallbladder volume is higher in hyperlipidemic dogs after feeding. This distention could contribute to bile retention of bile and potentially gallbladder disease.

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ABBREVIATIONS

ALP - alkaline phosphatase

ALT - alanine aminotransferase

Apo - apolipoprotein

BD - bile duct

cAMP - cyclic adenosine monophosphate

CCK - cholecystokinin

DM - diabetes mellitus

EF - ejection fraction

EHBO - extrahepatic biliary obstruction

FFA - free fatty acids

GB - gallbladder

GBM - gallbladder mucocele

GBV - gallbladder volume

GBV/kg - gallbladder volume per kilogram body weight

GGT - gamma-glutamyl transferase

GN – glomerulonephritis

HDL - high density lipoprotein

LCAT - lecithin-cholesterol acyl transferase

LDL - low density lipoprotein

LPL - lipoprotein lipase

SOD - Sphincter of Oddi

UDCA - ursodeoxycholic acid

VLDL - very low-density lipoprotein

CHAPTER I: Literature Review

A. Canine hyperlipidemia

a. Introduction

Lipids are an essential compound for normal function of living organisms and play a major role in the storage of energy, intracellular messaging, and are an important component of the cellular membrane. Although, there are several groups of lipids, cholesterol and triglycerides are the most clinically important and easily measurable in animals. Major sources of cholesterol and triglycerides are the diet and endogenous production by the liver.[1]

b. Lipid physiology

The three major groups of clinically important lipids are free fatty acids (FFAs), sterols, and acylglycerols. Fatty acids are simple lipids composed of a straight chain of carbon and hydrogen atoms with a carboxyl group at one end. This simple structure makes FFAs an important component of many other lipids. Sterols are a subgroup of steroids with a more complicated multi-ring structure. Cholesterol, the main sterol, is present in the diet, and is endogenously produced by the liver.[2] Lastly, acylglycerols such as triglycerides are the most common form of stored energy in mammals.[2] Triglycerides are composed of 3 FFAs esterified to glycerol. Other less clinically important lipids found in the diet are phospholipids and fat-soluble vitamins. Phospholipids are important constituents of mixed micelles present in the bile. Fat-soluble vitamins A, D, E, and K are only present in trace amounts, but their absorption is critical for numerous body functions.[2,3]

Lipids are hydrophobic, organic substances that are soluble in organic solvents, such as cell membranes rather than aqueous solutions. Fatty acids are easily transported in plasma bound to proteins, particularly albumin. The more complex lipids are transported in aqueous plasma as macromolecular complexes called lipoproteins. Lipoproteins are spherical structures with a

hydrophobic, hydrophilic outer layer consisting of phospholipids, free cholesterol and proteins that encase the lipid core including triglycerides and cholesterol esters.[3-6]

There are four major classes of lipoproteins in cats and dogs based on the hydrated density after ultracentrifugation. These include chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).[2,7] Plasma lipoproteins are important for transportation of lipids, primarily triglycerides and cholesterol. Chylomicrons are responsible for the transfer of dietary triglycerides to adipose tissue, muscle, or liver. The other lipoproteins are endogenous and present during fasting. VLDLs transport triglycerides and cholesterol to the liver and triglycerides to muscle. VLDLs are precursors to LDLs which transfer cholesterol to peripheral tissues and back to the liver. HDLs are important for reverse transport of excessive cholesterol from peripheral tissues to the liver for storage, excretion, or redistribution in the liver.[8] HDLs are the major cholesterol-carrying lipoproteins.[6]

The protein components of the lipoproteins are known as apolipoproteins or apoproteins (apo) which play a significant role in lipid transportation, structure, and enzyme activation for lipid metabolism. There are several apoproteins present in lipoproteins are clinically important for lipid metabolism. Apoprotein B, specifically apoprotein-100 (apo B100) is the main protein constituent of VLDL and LDL, and apoprotein B-48 (apo B48) is a unique lipoprotein specific to chylomicron formation in the intestine.[9] The latter carries exogenous lipids in the diet to the liver and peripheral tissues. Apoprotein E (apo E) is a key regulator of plasma lipid levels and participates in the homeostasis of plasma and tissue lipid content.[10] It has been widely studied in relation to neurobiology in humans.[10] Apoprotein C-II (apo CII) is an important co-factor for lipid metabolism and works intimately with lipoprotein lipase.[11] Lipoprotein lipase (LPL) is an important enzyme located in capillaries which hydrolyzes triglycerides within the lipoproteins into FFAs, glycerides, and glycerol.[6,12] Another important enzyme involved in lipid metabolism is hepatic triglyceride lipase, also known as hepatic lipase which is located mostly in endothelial cells of the liver and is involved in the uptake of triglycerides and phospholipids from the

chylomicrons and VLDL.[2] Lastly, lecithin-cholesterol acyl transferase (LCAT) circulates bound to HDL and works with HDL to convert cholesterol into cholesterol esters for storage, excretion or redistribution of excess cholesterol.[2]

c. Lipid metabolism

Lipid metabolism can be divided into two pathways: exogenous and endogenous pathways.

The exogenous pathway is defined as the metabolism of dietary lipids. Dietary lipids are ingested, subsequently digested, and once in the duodenum undergo emulsification through hydrolysis by intestinal and pancreatic lipases.[2,3,13] The products of hydrolysis, FFAs and monoglycerides, are transferred to the intestinal epithelial cells in the form of micelles where they diffuse across the cell membrane into the intestinal mucosal cells. Once in the mucosal cells, the FFAs and monoglycerides reassemble to form triglycerides. These can combine with phospholipids, cholesterol and apoB48 to form chylomicrons. The formed chylomicrons contain primarily triglycerides that are secreted into the lymphatics and ultimately the circulation to acquire apo CII and E from HDL molecules, which in turn activates LPL.[14] At that tissue level, lipoprotein lipase hydrolyzes triglycerides into FFA and glycerol which can then be used for energy production in muscle or storage in adipose tissue.[2] The chylomicron remnants being the cholesterol-rich particles, return the apo CII to HDLs. These HDLs are recognized by specific hepatic apo E receptors that remove them from circulation where they are used for VLDL formation, bile acid formation, or storage.[13]

The endogenous pathway includes the metabolism of endogenously synthesized triglycerides and cholesterol. Endogenous triglycerides and cholesterol will combine with phospholipids, apoB100, and apo B48 to form VLDL.[6] VLDL combines with apo C-II and E from HDL and actives LPL leading to the hydrolysis of triglycerides and the production of FFAs and glycerol.[1,6,12] The VLDL remnants are either removed from the circulation or will form LDL

via enzymatic reaction with hepatic lipase or LPL.[6] LDL delivers cholesterol throughout the body to be used for steroid-derived hormone and cell membrane synthesis.[2,11]

HDL are primarily synthesized in the liver and act as donors and acceptors of apo C and E in the circulation.[6,11] HDL cholesterol is esterified by LCAT which allows more free cholesterol to be absorbed onto the surface of the lipoprotein. This action of LCAT is unique to dogs and the continued absorption of free cholesterol on HDL2 molecules leads to the formation of HDL1 molecules. The HDL1 molecules are transferred from the tissues to the liver for excretion or storage and not for continued circulation as LDL or VLDL (as occurs in humans).[2,6] This function of the HDL1 may account for the lower incidence of atherosclerosis in dogs compared to humans.[2,11,12,15]

Lipoprotein profiling is rarely done clinically, but the lipoprotein profile for several disease processes have been described in animals. Table 1 summarizes the most common diseases in dogs with altered lipid metabolism and the associated changes seen with the specific lipids and lipoproteins. The major physiologic changes that must occur in these diseases to result in hyperlipidemia or abnormal lipoprotein profiles include increased production of lipoproteins by hepatocytes, defective intravascular transport process of lipoproteins, and/or defective cellular uptake of lipoproteins.[2] The breakdown of lipids is also important and involves lipases such as gastric lipase, pancreatic lipase, lipoprotein lipase, hepatic lipase, hormone-sensitive lipase, and lysosomal acid lipase.[2]

Hyperlipidemia refers to disorders of lipid metabolism leading to increased concentrations of triglycerides, cholesterol, or both in the blood. Hyperlipoproteinemia is commonly clinically used interchangeably with hyperlipidemia. Lastly, the term lipemia is used to describe the opaque gross appearance of serum or plasma resulting from severe hypertriglyceridemia.[2,7]

d. Causes in dogs

i. Primary hyperlipidemia

Primary hyperlipidemias are uncommon lipid abnormalities occurring predominantly in a limited number of breeds. The pathogenesis and cause of primary hyperlipidemia in these dogs is largely unknown but is speculated to have a hereditary cause.[2]

Primary hypertriglyceridemia has been described in Miniature Schnauzers and Brittany Spaniels. Hypertriglyceridemia in Miniature Schnauzers is well-described in the United States and is further characterized by an accumulation of VLDL and chylomicrons.[5,7,13,16-19] In one study, more than 75% of Miniature Schnauzers greater than 9 years of age had increased serum triglyceride concentrations, and about 80% of Miniature Schnauzers with moderate to severe hypertriglyceridemia were 6 years of age or older.[19] The cause of hypertriglyceridemia in Miniature Schnauzers is unknown, but reduced LPL activity has recently been documented in this breed.[20] Primary hypertriglyceridemia has been reported affecting two related Brittany Spaniels, but the cause remains unknown in those dogs as well. [21,22]

Primary hypercholesterolemia without hypertriglyceridemia has been described in Shetland Sheepdogs, Briards, Rough Collies, Doberman Pinschers and Rottweilers.[23] Shetland Sheepdogs, specifically in Japan, have an increased incidence of hypercholesterolemia when compared to other breeds and the cause is thought to be from a primary disorder in lipoprotein metabolism but this is ultimately unknown.[24,25] Hypercholesterolemia in Briards and Rough Collies has been investigated due to the increased incidence of retinal pigment epithelial dystrophy and corneal lipidosis.[26,27] Electrophoresis can be used to further classify lipoproteins based on density of the lipoproteins. In both breeds, a marked increase in the intensity of the alpha-2 band on lipoprotein electrophoresis suggests that there may be an abnormal accumulation of HDL playing a role in the development of hypercholesterolemia.[24,26,27] Additionally, primary hypercholesterolemia has been reported in Doberman Pinschers and Rottweilers, but the mechanism is unknown.[15,28,29]

Primary hyperlipidemia characterized by both hypertriglyceridemia and hypercholesterolemia has been reported in a case series of two related Beagles. The mechanism is unknown in these two cases.[30]

ii. Secondary hyperlipidemia

Secondary hyperlipidemia can be physiologic or pathologic and is more common than primary hyperlipidemia in dogs.[2] Postprandial hyperlipidemia is a normal physiologic phenomenon and is the most common reason an otherwise healthy dog would have hyperlipidemia on plasma biochemical analysis. Postprandial hyperlipidemia typically resolves within 12 hours after a meal. A 12 hour fast should be done prior to determining lipid concentrations to ensure clinically relevant results.[5,7,31,32] Severe fasting hyperlipidemia likely reflects the presence of a primary or secondary hyperlipidemia and warrants further diagnostic investigation to find the underlying cause. Age has been shown to have varying effects on lipid parameters and may play a role in changes to lipid metabolism. A difference was noted in one study which demonstrated that aged dogs have a different lipid profile suggesting characteristics of a lipid metabolism disorder when compared to younger dogs, but it is unknown if this is of any clinical consequence.[33] Sex has not been thoroughly evaluated in dogs, but cholesterol and lipoprotein concentrations have been noted to be higher in intact females compared to intact males.[34]

Persistent fasting hyperlipidemia needs further investigation and may occur secondary to feeding a high fat diet, obesity, endocrine diseases, diestrus, nephrotic syndrome, pancreatitis, bile duct obstruction and certain drugs.[7] High fat diets have been experimentally shown to reduce insulin sensitivity and increase plasma triglyceride concentration through an increase in VLDL and HDL. In other studies, obesity resulted in a significant increase in plasma leptin, cholesterol, and triglyceride concentrations in dogs.[35-39]

The most common reasons for secondary pathologic hyperlipidemia is an endocrine disorder, such as hypothyroidism, diabetes mellitus, or hyperadrenocorticism.[16,28,34,40-45] Hypothyroid dogs frequently exhibit hypercholesterolemia and/or hypertriglyceridemia and are further characterized by increases in VLDL, LDL, and HDL.[16,28,42] Thyroid hormone deficiency leads to reduced hepatic LDL receptor and hepatic lipase activity causing hypercholesterolemia and reduced LPL activity causing hypertriglyceridemia.[16,34] Hyperadrenocorticism typically results in hypercholesterolemia through an increase in LDL.[34,40,43] Cortisol induces lipolysis and promotes the development of hyperglycemia by antagonizing the effects of insulin leading to increased hepatic gluconeogenesis and decreased peripheral glucose utilization.[46] Lipolysis causes hyperlipidemia by stimulating the synthesis of VLDL by hepatocytes and promoting the activity of hormone-sensitive lipases which results in the release of FFAs from adipose tissue and increased VLDL by the hepatocytes.[2] Hyperglycemia leads to defective lipoprotein metabolism by inducing overproduction of VLDL and a defect in VLDL clearance.[2,40,44,47] Cholesterol and triglycerides concentrations are typically elevated in dogs with uncontrolled diabetes mellitus.[46] Insulin is an inhibitor of lipolysis and FFA oxidation. Insulin deficiency results in decreased LPL activity and defective lipid clearance. Insulin deficiency also activates hormone-sensitive lipase, resulting in increased hepatic production of triglycerides and VLDL.[45,47] Activation of hormone-sensitive lipase also results in the release of large quantities of FFAs from adipose tissue into the blood.[46] These FFAs are converted to triglycerides and packaged as VLDL. The increased cholesterol concentrations also down-regulates the LDL receptor, and effectively decreases the clearance of circulating LDL leading to hypercholesterolemia.[47] Peripheral tissue utilization of glucose also signals mobilization of lipids and releases FFAs in the plasma resulting in increased VLDL and further hyperlipidemia.[34,41,46] Insulin therapy causes the lipid concentrations to decrease and the electrophoretic pattern to revert to near normal.[16] Diestrus can cause relative insulin resistance leading to reversible secondary diabetes mellitus and a lipoprotein profile similar to DM.[34]

Nephrotic syndrome is a rare complication of glomerulonephritis (GN) characterized by hypercholesteremia, hypoalbuminemia, edema, and proteinuria.[2] These abnormalities together are pathognomonic for glomerular disease, but all these abnormalities were only present in 15% of dogs with GN in one study with only 49% having hypercholesterolemia.[48-52] Dyslipidemia has been described in dogs with chronic kidney disease and those with nephrotic syndrome, characterized by a decrease in HDL and an increase in LDL, VLDL or both.[48-51] Hypercholesterolemia is thought to reflect a potential increase in lipoprotein synthesis as a compensatory means to increase oncotic pressure as proteinuria in these patients leads to low oncotic pressure secondary to hypoalbuminemia.[2] Hypercholesterolemia is also present as a result of defective lipolysis of lipoproteins in plasma.

In dogs with naturally occurring pancreatitis, mild to moderate hypercholesterolemia and hypertriglyceridemia is seen in only a small percentage of dogs (30%) and the majority (>70%) had serum triglyceride and cholesterol concentrations within the reference intervals.[53] Many dogs with naturally occurring pancreatitis also have concurrent diseases, including diabetes mellitus, hyperadrenocorticism, chronic renal failure, neoplasia, and autoimmune disorders which may also predispose to hyperlipidemia.[54,55] Dogs with experimentally-induced pancreatitis had higher LDL fractions and lower triglyceride-rich lipoprotein and HDL fractions than healthy dogs, but this did not result in significant hyperlipidemia.[56,57] Possible mechanisms for abnormal lipid metabolism with pancreatitis include decreased insulin and LPL activity, altered metabolism from inflammatory cytokines and defective lipid metabolism associated with cholestasis.[54,58,59]

Cholestasis results in moderate hypercholesterolemia, mild hypertriglyceridemia, and specifically an increase in HDL1 lipoproteins in dogs.[5,60,61] In dogs with experimentally-induced cholestasis, cholesteryl esters have been shown to be increased with an increase in the activity of lecithin:cholesterol acyltransferase (LCAT).[60] This enzyme converts free cholesterol to cholesterol esters for lipoprotein transport and metabolism. Cholestasis results in marked lipoprotein changes, and a deficient LCAT mechanism is suspected in dogs with cholestasis even

with normal to high plasma LCAT activity.[60,62,63] In comparison to humans, dogs with cholestasis have similar lipoprotein profiles but are different cholesterol and apoprotein composition which may suggest that the metabolism of cholesterol is affected differently in the dog than in the human.[60,61,64] Hypercholesterolemia in humans may also result from cholestatic disease due to the production of a lipoprotein similar to HDL1 called lipoprotein-X, but the evidence is conflicting whether this also occurs in dogs.[60,63] Cholestatic diseases that have been shown to cause hyperlipidemia include gallbladder mucoceles (GBM), extrahepatic neoplasia, cholecystitis, and cholelithiasis. [65-68] Dogs with endocrinopathies such as diabetes mellitus, hyperadrenocorticism, and hypothyroidism have been shown to be at an increased risk for GBM formation.[69,70] It is speculated that hyperlipidemia may play a role in dysregulation of GB motor function, mucosal function, and bile composition resulting in abnormal GB motility and subsequently, GBM formation.[71-75]

Other secondary causes of hyperlipidemia include drugs, infectious diseases such as leishmaniosis, and lymphoma. Drugs including phenobarbital, potassium bromide, and glucocorticoids have been associated with hyperlipidemia. The association between hyperlipidemia and anticonvulsants is unclear and may be related to altered lipid metabolism or polyphagia-induced obesity as a direct clinical side effect predisposing them to become overweight and subsequently hyperlipidemic.[16,76,77] Glucocorticoids are also a cause of mild to marked hypertriglyceridemia and hypercholesterolemia. The proposed mechanisms are thought to be similar to endogenous corticosteroids or hyperadrenocorticism.[2] Leishmaniosis is an infectious cause of mild to moderate hyperlipidemia.[78] Full lipoprotein profiling revealed defects in cholesterol transport which may be attributed to the consumptive evolution of the disease, immunocomplex deposits in cells, hepatic disorders and interactions between the parasite and the normal cholesterol metabolism of the host.[78] Lymphoma has also been demonstrated to cause an alteration in lipoprotein profiles resulting in hypertriglyceridemia with or without

hypercholesterolemia which persistent despite treatment.[79] The mechanism of hyperlipidemia in these dogs is unknown and likely not clinically relevant.

d. Diagnostics

Hyperlipidemia is diagnosed by measurement of fasting serum triglyceride and cholesterol concentrations. Moderate to severe hypertriglyceridemia can be suspected based on a turbid appearance of the serum or plasma, and usually occurs when triglyceride concentrations are equal or greater to 200 mg/dL.[17,26] A full diagnostic work-up is required in order to determine if the hyperlipidemia is primary or secondary to an underlying disease process. A complete blood count, chemistry panel, and urinalysis should be performed after a 12 hour fast if hyperlipidemia is persistent. Additional tests to rule out secondary causes of hyperlipidemia are undertaken if any abnormalities suggestive of an underlying disease are present on the blood work and urinalysis. Lipemia can falsely increase or decrease bilirubin, liver enzymes, amylase, lipase, electrolytes, proteins and glucose making it important to remember these changes when evaluating a serum chemistry for causes of hyperlipidemia.[80]

If primary hypertriglyceridemia is suspected, a chylomicron test can be performed. This crude test entails leaving lipemic serum undisturbed at 4 C for 12 hours. Chylomicrons are lower density and will move to the top of the sample and a cream layer will form indicating an excess of chylomicrons.[23] If no cream layer forms then the hypertriglyceridemia is due to an excess of other lipoproteins (usually VLDL).[2,5] Chylomicrons carry triglycerides from the intestine to the tissues, so are typically increased due to diet.[13,23] If a cream layer forms at the top, this indicates an increase in chylomicrons likely due to postprandial hyperlipemia. When hyperlipemia is due to increased VLDL, a fat layer does not form and the sample remains turbid, indicating a primary hypertriglyceridemia. The sample may also form a fat layer and be turbid, indicating an increase in both VLDL and chylomicrons. VLDL are produced in the liver from FFAs and are the main carrier of triglycerides in the fasting state.[2] This test is neither specific nor sensitive for specific

causes of hyperlipidemia. Miniature Schnauzers with primary hypertriglyceridemia have been shown to have increased VLDL with or without chylomicronemia suggesting that if the sample is turbid with or without a fat layer then it could be due to familial hypertriglyceridemia.[18]

Lipoproteins can be further classified by electrophoresis, ultracentrifugation, and anion-exchange high performance chromatography based on the mobility or density of the lipoprotein relative to water which is done in research and has limited clinical value.[5,80-82] Electrophoresis was used for lipid profiling in dogs treated for diabetes mellitus. Treatment of the diabetes mellitus resolved the hyperlipidemia and the abnormal electrophoretic pattern.[16] Lipoprotein electrophoresis does not appear to be more sensitive than serum lipid evaluation, and thus does not provide additional clinically relevant information. It is infrequently utilized in dogs with hyperlipidemia. Ultracentrifugation has been evaluated in Miniature Schnauzers with and without hyperlipidemia, revealing that these groups of dogs had significantly different lipoprotein profiles.[83] The clinical significance of this finding is unknown however. Ultracentrifugation may be a more sensitive test for predicting the development of hyperlipidemia, but this needs further evaluation for clinical application.

e. Treatment

Treatment for hyperlipidemia depends on whether it is a primary condition or secondary to an underlying disease process. Treatment of the primary disease will typically resolve the hyperlipidemia within 4-6 weeks.[2,5,17,80]

Treatment for primary hyperlipidemia is typically a stepwise clinical approach and is recommended if the serum triglycerides and/or cholesterol concentration is greater than 500 mg/dL due to the possible complications.[5,7,84] This recommendation comes from clinical, anecdotal experience from experts in the field, but is not supported by evidence in dogs. Treatment options include low fat diets, omega-3 fatty acids, fibric acid derivatives, niacin, and statins.[2,84] Medical management is typically reserved for patients that do not respond solely to a

low-fat diet.[2,7,17,26] Omega-3 fatty acid supplementation lowers serum lipoprotein concentrations in humans and other animal models.[85,86] A study in healthy dogs revealed that omega-3 fatty acid supplementation led to a significant reduction in serum triglyceride concentrations with no major side effects.[87] Fibric acid derivatives such as gemfibrozil, bezafibrate, and fenofibrate have been reported to reduce serum triglyceride concentrations. These drugs are weak agonists of peroxisome proliferator-activated receptor-alpha, a transcription factor that regulates lipid and lipoprotein synthesis and catabolism.[2] Gemfibrozil was one of the first fibrates to be used in dogs with hypertriglyceridemia, but there are no studies evaluating the efficacy and safety of gemfibrozil in dogs.[13] In a recent study, bezafibrate was successful in reducing triglyceride and cholesterol concentrations in dogs with primary or secondary causes of hyperlipidemia with no adverse effects.[88] Fenofibrate has also been shown to improve lipid parameters in obese dogs with no major side effects anecdotally.[89] Niacin, a form of vitamin B_3, is thought to inhibit hormone-sensitive lipase activity and diacylglycerol acyltransferase, an enzyme that decreases triglyceride biosynthesis.[13] Statins are reversible inhibitors of the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), the rate limiting step for cholesterol biosynthesis.[90] Due to the lack of evidence on efficacy for hyperlipidemia in dogs, niacin and statins are typically reserved for the cases that do not respond to traditional therapies.

f. Consequences

Hyperlipidemia should be further evaluated and treatment initiated if the serum triglyceride and/or cholesterol concentration is greater than 500 mg/dL. Although a direct correlation has not been established. Severe hyperlipidemia may lead to or predispose to the development of other clinically relevant diseases.

Hyperlipidemia, specifically hypertriglyceridemia has been associated with the development of pancreatitis. The proposed mechanism involves an increase in chylomicrons (typically associated with hypertriglyceridemia) and hydrolysis of the triglycerides by pancreatic

lipase leading to excess FFAs which activate trypsinogen and triggers inflammation, increasing plasma viscosity and ischemia in pancreatic tissues.[54,59,91] An increase in serum FFAs may also aggregate into micellar structures and further damage the pancreas and vascular epithelium.[53,55,56,91] Hypertriglyceridemia appears to be a risk factor for the development of pancreatitis in Miniature Schnauzers. Miniature Schnauzers that were hypertriglyceridemic prior to the clinical diagnosis of pancreatitis were 5 times more likely to develop pancreatitis than Miniature Schnauzers without hypertriglyceridemia.[19,50,92]

Insulin resistance also occurs with hyperlipidemia and may worsen glycemic control in dogs with diabetes mellitus. In one study, about 30% of Miniature Schnauzers with hyperlipidemia had insulin resistance determined by increased serum insulin levels.[93]

Atherosclerosis is arterial wall thickening as a result of lipid deposition and is rarely seen in dogs. It is primarily associated with hypercholesterolemia secondary to hypothyroidism or diabetes mellitus.[15,44,94-96] In humans, atherosclerosis is a well-described complication of hyperlipidemia. Dogs appear to be more resistant to atherosclerosis due to their lipoprotein composition.[2,4,8,15,44,97] Dogs have a unique lipoprotein, HDL1, that transfers cholesterol esters from tissues to the liver for disposal or reuse, and not to peripheral tissues to accumulate within blood vessels. It has been suggested that this accounts for the lower incidence of atherosclerosis.[4,15,44,95,97] Dogs with atherosclerosis were over 53 times more likely to have concurrent diabetes mellitus and 51 times more likely to have hypothyroidism than dogs without, but no correlation was seen between hyperadrenocorticism and atherosclerosis.[44] One possible consequence of hyperlipidemia is the development of neurologic signs due to atherosclerosis and thromboembolic events.[98,99]

Proteinuria is associated with hyperlipidemia, but dogs with hypertriglyceridemia and proteinuria did not appear predisposed to azotemia, hypoalbuminemia, hypertension, decreased antithrombin, or cardiac disease.[100] In one study, all proteinuric dogs that were diagnosed with

glomerular lipid thromboemboli were Miniature Schnauzers with hyperlipidemia, but the clinical significance is unknown.[100]

Lipid accumulation in the eyes is a secondary manifestation of hyperlipidemia. Recently, solid intraocular xanthogranuloma formation was reported in Miniature Schnauzers.[101,102]

Hyperlipidemia in dogs is associated with primary hepatobiliary disease, specifically vacuolar hepatopathy and gallbladder mucocele formation. In one study, about 50% of Miniature Schnauzers with primary hyperlipidemia had increased liver enzymes, primarily an increased alkaline phosphatase (ALP).[103] Vacuolar hepatopathy may occur secondary to hyperlipidemia and is characterized by hepatocellular accumulation of triglycerides and/or glycogen within the hepatocytes and appears to be of little clinical significance.[92,103] Gallbladder mucoceles (GBM) appear to develop more commonly in dogs with primary or secondary hyperlipidemia.[72] Kutsunai et al. demonstrated that dogs with hypercholesterolemia and hypertriglyceridemia were 3 and 3.5 times more likely respectively to develop GBM.[104] Shetland Sheepdogs and Miniature Schnauzers are predisposed to primary hypercholesterolemia and hypertriglyceridemia, respectively, and these breeds are also more likely to develop GBM.[25,72,105] GBM can lead to a surgical emergency if not monitored and fatal complications of GB disease include rupture and subsequent bile peritonitis. The causes of GB dysmotility and GBM development are unknown. It is speculated that hyperlipidemia may play a role in dysregulation of GB motility, bile composition, and subsequently, GBM formation.

B. Biliary system physiology

a. Anatomy of the biliary system

The biliary system consists of the gallbladder (GB), cystic duct, bile duct, hepatic ducts, interlobular ducts, intralobular ducts, bile ductules, and bile canaliculi. Bile is actively formed by the liver and flow is directed from the liver to the duodenum. Bile is actively secreted by the hepatocytes continuously which subsequently drains into the bile canaliculi, and then flows

through the intralobular ducts and the interlobar ducts. Bile canaliculi are specialized structures in the hepatocyte cell membrane that form tight junctions to separate bile from the sinusoidal blood and ultrafiltrate of the liver. The lobar ducts merge into the right and left hepatic ducts which eventually merge to form the common hepatic duct. The cystic duct joins the hepatic duct and after this connection becomes the bile duct. The cystic duct transports the bile into the GB. Animals have a variable number of hepatic ducts and anastomoses with the common hepatic and cystic ducts.[106] The anastomosis of the common hepatic duct and cystic duct forms the bile duct, which transfers bile to the duodenum as shown in Figure 1. The sphincter of Oddi (SOD) is a muscular thickening around the entrance into the duodenum which helps mediate flow of bile in the GB in humans, but there is question whether this structure exists in animals. In dogs, the bile duct enters the duodenum at the major duodenal papilla near the pancreatic duct. The accessory pancreatic duct enters the duodenum at the minor duodenal papilla, located 1-2 cm aborad to the major duodenal papilla.[107] Bile from the GB is expelled into the duodenum via the cystic and bile ducts. A small percentage of hepatic bile bypasses the GB and enters directly into the duodenum.

The GB is a teardrop-shaped distensible, muscular organ located in the cranioventral abdomen and lies within a fossa between the right medial and quadrate liver lobes.[107] The structure of the GB starting from the apical aspect includes the fundus, body, and lastly the neck which tapers to the cystic duct. The GB wall is composed of four layers: mucosa (innermost layer), lamina propria, muscularis, connective tissue and serosa (outermost layer).[107] Mucous glands within the mucosal layer of the GB secrete mucin which serves as a protective mechanism against cytotoxic substances.[107] Blood supply to the GB is entirely from the cystic artery. The innervation of the GB is through the vagus and splanchnic nerves which make up the parasympathetic and sympathetic innervation respectively.[107]

b. Function of the gallbladder

The GB stores hepatic bile that is modified and eventually expelled into the duodenum. The mucosal layer of the GB is lined with microvilli resulting in a surface area with high absorptive capacity. The hepatic ducts transfer bile without modifying the composition significantly, other than the addition of mucus, which helps protect the epithelium from cytokines and cytotoxic, hydrophobic bile acids. Once the bile reaches the GB, it is stored and concentrated until the next GB contraction.

The ability to concentrate bile is an important function. The concentration of bile salts and bile pigments can increase substantially because of water and electrolyte absorption.[108-110] This is an active process that does not generate an electrical potential difference within the GB. It is highly dependent on the presence of either Cl- or HCO3-, and it appears that Na+ transport is coupled with the transport of either anion making the electrolyte transfer electrically neutral.[108] In contrast, water absorption is entirely passive and relies on the active absorption of NaCl and NaHCO3.[110] The absorption of water and electrolytes cause an increased concentration in other ions such as K+, Ca++, and bile salts, leading to micellar formation.[3] Micellar formation in the small intestine function to emulsify dietary fats and facilitate their intestinal absorption. The formation of micelles also acts to lower the bile salt concentrations, reducing the toxic detergent effects of bile salts on the biliary epithelium.[108]

GB contraction occurs about 30 minutes after a meal, expelling bile into the duodenum. These contractions are mediated by several factors including vagal stimulation, cholecystokinin (CCK) release, and motilin release from the duodenum.[108] The most potent stimulator of GB contraction is CCK which acts by binding to CCK1 receptors distributed in the GB smooth muscle.[108] Acetylcholine release via vagal stimulation is a weak stimulator of GB contraction. The GB musculature contracts in response to CCK released from enterocytes.[108,110] CCK release from enterocytes occurs in response to fatty acids and amino acids entering the duodenum. CCK also causes relaxation of the SOD and the release of pancreatic digestive enzymes.[107] Secretin also enhances SOD relaxation. Conversely to contraction, GB relaxation is not completely

understood. Adrenergic and cholinergic nerves mediate the active relaxation induced by incoming bile.[111] Somatostatin is secreted by the delta cells of the pylorus, duodenum, pancreas, and nervous system, and is thought to play a role in GB relaxation.[110] Somatostatin release is stimulated by gastric acid secretion after a meal. Somatostatin's actions are primarily inhibitory including suppression of GI and pancreatic hormones releases, decreased gastric emptying, and smooth muscle contraction.[112] Somatostatin also inhibits GB contraction and enhances GB relaxation through direct effects on the neural activity, inhibition of acetylcholine, and inhibition of CCK and other GI peptides that would stimulate GB contraction.[113] The inhibition of CCK appears to play the largest role in GB relaxation allowing the GB to fill with bile.[114]

c. Bile formation and secretion

Bile acids are synthesized as end products of cholesterol metabolism which occurs in the liver. Bile acid formation occurs by two different pathways. The initial and rate-limiting step in bile acid formation is the hydroxylation of cholesterol at the 7 position by the enzyme cholesterol 7α hydrolase.[112,115] The newly formed 7α hydroxycholesterol can be acted on by C27 hydroxylase to yield the bile acid chenodeoxycholic acid or hydroxylation by 12α hydroxylase prior to C27 hydroxylase active, yielding cholic acid.[115] These two are the most common primary bile acids.[112] These primary bile acids are released into the small intestine or colon and are altered by bacterial enzymes to yield secondary bile acids such as lithocholic acid, ursodeoxycholic acid (UDCA), and deoxycholic acid.[112] Bile acids are secreted continuously by the liver, but the rate of secretion can vary.[109] Bile conjugation takes place by the liver after secretion. It requires the addition with an amino group such as glycine and taurine which renders the bile acids more water-soluble.[109] Once the bile acids are conjugated, they are referred to as bile salts. Conjugation also decreases the pKa, making them "charged" or anions. Due to this charge, conjugated bile acids can cross cell membranes by active transport for secretion or uptake.[109] This permits the body to use bile acids for digestion when needed, such as after a

meal.[112] Once secreted by hepatocytes, they are stored in the GB between meals, with small amounts being delivered to the small intestine due to small GB interdigestive contraction.[110] After a meal, the GB will empty the bile into the duodenum in a more continuous pattern.[112] The rate of secretion varies and depends widely on the amount of bile acids delivered to the liver. The bile salts that enter the small intestine are integral to the absorption and digestion of lipids.[112] Most bile acids are absorbed from the intestine (ileum), travel through the portal blood to the liver, and return to the liver where they can again be secreted by the liver. This pathway from GB to intestine and back to the liver via the portal vein is called enterohepatic circulation.[107,112]

The enterohepatic circulation involves both passive and active transport processes that are dictated by bile composition. Hydrophobic and hydrophilic bile acids are absorbed by passive and active transport within the intestine, respectively. The uptake of hydrophilic bile acids occurs in the ileum and includes sodium-dependent transporter proteins, binding proteins, and sodium-independent anion exchange. This system delivers >90% of the bile acids to the portal blood. The uptake of bile acids into hepatocytes is mediated by a sodium-dependent transporter protein and several binding proteins for the bile acids to reach the canalicular membrane. All bile acids within the portal blood are removed from circulation after one passage through the liver.[3,7,107]

Bilirubin is derived from the reticuloendothelial system breakdown of hemoglobin found in aged red blood cells.[116] Hemoglobin is first split into heme and globin. The heme ring is opened and oxidized, and iron is removed ultimately to form bilirubin which is transported from the reticuloendothelial tissues to the hepatocytes.[116] In transit, bilirubin is highly bound to plasma albumin. Hepatocytes extract the bilirubin, conjugate it with glucuronic acid and secrete the conjugated product into the bile.[116] Bilirubin is not absorbed from the intestine in any noticeable amount, but bacteria can reduce bilirubin to urobilinogen, which is converted to stercobilin and excreted in the feces.[116] Bilirubin can also be absorbed into the portal blood and returned to the liver in the unconjugated form. Some of the urobilinogen continues through the circulation and is excreted by the kidneys. It is oxidized in the urine to form urobilin.[116]

Bile acid secretion also promotes water secretion into bile. Amphipathic anions synthesized by the liver are conjugated to taurine or glycine in the dog, forming micelles.[109] Active uptake of bile acids in the ileum and enterohepatic circulation makes this a highly efficient system.[109] Bile salt-dependent bile flow has a powerful choleretic influence at the bile canaliculus. Transcellular transport of bile salts with micelle formation maintains a concentration gradient between bile and blood which promotes choleresis. Water secretion occurs due to the osmotic gradient produced from passive accompaniment of cations.[109,112] Electrolytes are also secreted into the bile by bile acid-independent mechanisms involving active transport of glutathione.[117] Glutathione is an endogenous anion that promotes canalicular bile formation under physiologic conditions. Glutathione is hydrophilic and has a strong osmotic influence which is amplified by hydrolysis to three amino acids (cysteine, glutamate, glycine), tripling its osmolar impact.[110] This draws water and electrolytes into bile through paracellular pathways.[110] In dogs, s-adenosylmethionine (SAMe), a glutathione donor, is a medication used for its' choleretic properties. The secretory activity of these epithelial cells are under hormonal control, specifically by secretin.[3,118]

The flow of bile from the canaliculi to the small intestine is primarily dictated by the secretory pressure generated by the hepatocytes and cholangiocytes. Bile is secreted from the liver at a relatively high pressure of 25-30 mmHg.[112] This pressure is produced by bile flow itself but also the contractile forces of the liver including the stellate cells and myofibroblasts that constrict portions of the biliary tree.[112] The gallbladder pressure is low at rest due to GB relaxation but also active stimulation of gallbladder filling which occurs in response to bile acids in the gallbladder lumen.[112] Bile secretion not only requires a structurally and functionally intact biliary tree, but also requires active transport systems within the hepatocytes and cholangiocytes (bile duct cells). This active transport produces osmotic and electrochemical gradients which allows passive diffusion of inorganic ions.[110] Hepatocytes and cholangiocytes active secretion functions are highly regulated by meal-induced hormone release.[108] During fasting, bile is

secreted periodically in synchrony with the migrating motor complex, a physiologic pattern of electrical activity observed in the gastrointestinal tract in a regular cycle during fasting. [119] The GB contracts, expelling bile, shortly before and during the period of duodenal contractions. The pathways responsible for GB contraction shortly after eating involve the cholinergic nervous system. CCK, is released from the mucosa of the duodenum during food digestion and is carried in the blood to the GB. CCK binds to receptors directly on the GB smooth muscle cells, which in turn stimulates the musculature to contract. CCK's action are mediated through extrinsic vagal and intrinsic cholinergic nerves. Gastrin also stimulates GB contraction, but only at levels that are above the physiologic range. The gastrointestinal hormone, secretin, antagonizes the effects of CCK and inhibits GB contractions. Pancreatic polypeptide and somatostatin both have also been shown to inhibit GB contractions and may be an important component in certain disease states.[3]

d. Bile composition

Bile is a mixture of organic and inorganic components and is normally a homogenous and stable solution. However, this stability depends on the various components within the bile. It is primarily composed of cholesterol, lecithin, other phospholipids, and bile salts.[106] The appropriate bile composition is important for fat emulsification and hydrochloric acid neutralization in digested food.[106] Bile acids are the major organic component, accounting for approximately 50% of the solid component of bile. They are composed of a hydroxyl and carboxyl group located on one side of the molecule with the remainder containing a nucleus and several methyl groups. This allows bile acids to be amphipathic with the hydroxyl and carboxyl group being hydrophilic and the methyl grouping being hydrophobic. The behavior of bile acids in a solution also depends on their concentration. At high concentrations, aggregates are formed between the bile acids creating micelles. Micelles aids in bile acid transport to the enterocyte for lipid digestion. Cholic acid is the major primary bile acid in the dog, constituting 62% of all bile

acids, whereas chenodeoxycholic acid is the major acid in humans, constituting 46% of the bile acid pool.[120] Bile acids are different in their solubility based on the number of hydroxyl groups and terminal carboxyl group present. Prior to bile acid secretion, hepatocytes conjugate bile acids with amino acids taurine or glycine. This creates the eight different conjugated bile salts that make up bile composition.[109] Bile acid conjugation make the bile acids more water-soluble and therefore can emulsify fats. Secondary bile acids are formed from bacterial deconjugation or dihydroxylation, which removes the amino acid from the bile salts which creates four more types of bile acids. These bile acids are absorbed into the bloodstream and brought back to the liver via the enterohepatic circulation to be resecreted by the hepatocytes leading to bile acid recycling.[109] Taurine conjugates are predominant in the dog, whereas glycine conjugates are predominant in the human.[120]

The second group of organic compounds in bile are the phospholipids, including lecithins. Phospholipids are also amphipathic but are not soluble in water and form liquid crystals. In the presence of bile salts, the liquid crystals are broken up and become a component of the micelles.[3] Phospholipid composition of bile is generally similar between species with 90–95% being lecithins.[121]

Cholesterol is the third organic component of bile, which is about 4% of the total solids. Cholesterol appears in the non-esterified form and on its' own is insoluble in water. In the presence of bile salts and phospholipids, cholesterol becomes the interior hydrophobic part of the micelles.[3] Cholesterol is the precursor of all steroid hormones, cholesterol esters and bile acids. Cholesterol is derived from the diet and is synthesized in the liver. Primary bile acids are synthesized from cholesterol in the liver. Figure 2 shows bile acid synthesis from cholesterol in humans. Human bile is rich in cholesterol as compared with dog bile.[115] In fact, human gallstones can be dissolved in canine bile due to the low cholesterol saturation index of canine bile compared to human bile.[122] The 2 primary bile acids of dogs, cholic acid and chenodeoxycholic acid are conjugated with taurine or glycine in the hepatocyte. After a meal, conjugated, primary

bile salts are released from the GB into the intestines, where they are deconjugated and converted to secondary bile acids (deoxycholic acid, ursodeoxycholic acid, and lithocholic acid) by the intestinal microbiota. There are species differences in cholesterol derived bile composition as well. Cholic acid is the major primary bile acid in the dog whereas chenodeoxycholic acid is the predominant acid in the human.[120] This likely accounts for the different diseases that affect the GB in each species. Bile is the major route for cholesterol elimination.[114] Cholesterol homeostasis is largely mediated by the transporters, ABCG5 and ABCG8 that move cholesterol from the hepatocytes into bile canaliculi in humans.[112,123] The liver also actively secretes cholesterol into the bile via VLDL.[124] The secretion of cholesterol is also largely dependent on the rate of secretion of bile acids.[3,108]

Lastly, bile pigments are the fourth organic component of bile. These constitute only 2% of the total solids, with bilirubin being the most important. In the free form, it is insoluble in water, but usually are conjugated with glucuronic acid. This makes them soluble in a solution. They do not take part in the micelle formation, but provide pigment properties.[3]

Several inorganic ions are found in bile, including cations (Na+, K+ and Ca++) and anions (Cl- and HCO3-). Normally the number of inorganic cations exceeds the number of anions. Bile acids possess a net negative charge at the pH values found in bile, which accounts for the differences in charge. Bile is isosmotic with plasma even though the number of cations far exceeds the anions.[3]

C. Gallbladder dysmotility

a. Pathophysiology

GB contraction and secretion is facilitated by neurohormonal stimulation after a meal. Gastric distension, FFAs, and amino acids lead to vagal stimulation and CCK release from duodenal enteroendocrine cells.[112] Cholecystokinin is the most potent stimulus for GB contraction and acts by binding to CCK1 receptors distributed in the GB smooth muscle.[71,125-127]

During the interdigestive phase, the GB contracts in response to the migrating motor complex of the intestine via motilin.[107,128] Disruption of the neurohormonal regulation of the GB can result in dysmotility.

GB dysmotility, also referred to as biliary dyskinesia, has been recently recognized in dogs both experimentally and clinically, but the pathophysiology is unknown.[72,73,107] There are several disease processes associated with GB dysmotility. Conditions such as gallbladder mucoceles (GBM), cholelithiasis, and biliary sludge have been associated with GB dysmotility, and the hypothesized mechanisms include impaired smooth muscle contractility, increased endogenous steroid hormones, increased bile cholesterol concentrations, CCK receptor dysfunction, abnormally semi-solid GB contents, or biliary obstruction.[73,107,126,129] An increase in unconjugated, hydrophobic bile acids has been documented in inflammatory, neoplastic, and cholestatic conditions in humans and dogs.[130,131] Unconjugated, hydrophobic bile acids impair GB smooth muscle contractility leading to increased GB volumes.[132] Excessive cholesterol in bile has also been shown to impair smooth muscle contraction. [75,133] Bile composition is significantly different in dogs when compared to humans. In healthy dogs, taurocholic acid, taurodeoxycholic acid, and taurochenodeoxycholic acid account for approximately 72.8%, 20.3%, and 6.2% of the total bile acids, respectively.[134] In dogs, increases in bile acids derived from cholesterol has not been demonstrated like in humans, but increased hydrophobic bile acids have been shown in dogs being fed high-fat-high-cholesterol diets with gallbladder mucoceles.[135] This bile acid composition has been proposed to decrease GB motility.[75,135] Normal GB contraction involves CCK binding to its receptors on the plasma membrane, thereby activating G-proteins for generation of an action potential. There are several mechanisms for GB hypomotility in humans with cholesterol predominant choleliths. GB hypomotility in humans with cholesterol rich choleliths persists after stimulation by CCK, indicating affected individuals may have decreased CCK receptor sensitivity or decreased number of CCK receptors.[136-138] Cholesterol supersaturation in bile may facilitate cholesterol diffusion across the GB epithelium where it is

incorporated into the GB smooth muscle. This in turn may decrease the action potentials and voltage-activated Ca2+ currents in the GB smooth muscle cells.[139] Cholesterol may also impair binding of other hormones important for GB motility such as prostaglandin E2.[140,141] Lastly, alteration in the bile salts may generate free radicals that damage CCK receptors and inhibit contraction.[142] In humans, endocrinopathies such as hypothyroidism and hyperadrenocorticism have been associated with GB dysmotility by decreasing bile flow due to changes in bile composition, decreased bile secretion, and alterations in cholesterol homeostasis.[143-145] An increase in endogenous steroid hormones, such as with hyperadrenocorticism, may cause GB dysmotility by decreasing GB smooth muscle contractility through inhibition of several signaling pathways. Other hormones such as sex hormones, including progesterone, testosterone, and dihydrotestosterone have also been shown to inhibit GB motility. [72,146-148] In people, hypothyroidism decreases bile flow and predisposes to cholelith formation.[143-145] In rodent models, hydrophobic bile acids attenuate GB contractility via the activation of GPBAR1, a G-coupled protein bile acid receptor, which ultimately results in hyperpolarization and decreased smooth muscle contractility.[132] In mice, serum cholesterol and triglyceride concentrations were negatively correlated with GB contractility.[149] People with elevated triglyceride concentrations were also found to have an increased risk for GB disease.[150] People with hypertriglyceridemia (HTG) may have impaired CCK response or CCK receptor dysfunction thus resulting in GB dysmotility, which has been hypothesized to occur in dogs as well.[151,152]

Healthy dogs fed a high fat, high cholesterol diet have been shown to have impaired GB contractility along with increased plasma cholesterol and increased unconjugated bile acids in bile.[135] Thus, in dogs with hyperlipidemia, it is speculated that similar mechanisms affecting the GB smooth muscle would result in GB hypomotility and pose a risk for GBM formation. Gallbladder motility in dogs has not been directly evaluated with these specific conditions, but GB dysmotility is frequently implicated in GBM formation in dogs with hypothyroidism and hyperadrenocorticism.

b. Diagnostics for gallbladder motility

Clinical evaluation of the GB is difficult and often lacks sensitivity and specificity. The typical diagnostic approach to biliary disease includes biochemical analysis and imaging such as abdominal ultrasonography or rarely nuclear scintigraphy. Gallbladder motility is not evaluated clinically due to the limited diagnostic and treatment value. Experimentally, assessment of GB motility is useful to further define a link between the afore mentioned conditions in the development of GBMs.

Functional ultrasonography or cholescintigraphy can be used to assess GB volumes and/or ejection fractions to aid in the diagnosis of GB dysmotility.[73,153-158] Scintigraphy is rarely used in the evaluation of canine biliary disease as it is more invasive and time consuming compared to abdominal ultrasonography. Currently, ultrasound is the screening method of choice for canine GB diseases. Ultrasonography is readily available and a non-invasive modality that can be used to assess GB function as well as structural changes. Functional ultrasonography entails performing ultrasound after a 12 hour fast followed by periodic ultrasound exams performed within 2 hours of either a test meal or administration of a cholagogue. The GB volume is determined at multiple intervals and an ejection fraction is calculated which represents the degree to which the GB empties. After ingestion of a meal, the canine GB secretes up to 60% of its contents within 60 minutes and 100% within 120 minutes. [153,159,160] Normal GB motor function is defined as a fasted GBV less than 1 mL/kg or > 25% reduction in GB volume.[153] Dogs with fasted GB volumes of < 1 mL/kg are presumed to have normal GB emptying. Those with GB volumes > 1 mL/kg are fed a test meal with serial GB volumes recorded for up to 2 hours. Ejection fractions < 25% represent decreased GB emptying.[153]

c. Consequences

In dogs, GB dysmotility has been implicated in GBM formation. Although the current etiology is unknown, other risk factors of GBM formation include breed (Shetland Sheepdogs,

Miniature Schnauzers, and Cocker Spaniels), endocrinopathies (hyperadrenocorticism, hypothyroidism), hyperlipidemias (hypercholesterolemia and hypertriglyceridemia), and GB dysmotility.[69,70,161] Although the pathophysiology of GBM formation is unknown, it has been speculated that GB dysmotility and mucus hypersecretion promote GBM formation. [69,70,161] Similar perturbations of the GB lead to the development of cholesterol gallstones in humans.[71] Impaired GB motility in dogs with GBMs and the finding of GB dysmotility in hyperlipidemic dogs preceding the development of GBM in a few cases supports the hypothesis that dysmotility is a factor in GBM formation.[72,73] Complications of GBM formation includes rupture and subsequent peritonitis, both requiring urgent surgical intervention. Necrosis and rupture of the GB wall has been reported in up to 61% of dogs at the time of surgery.[162] Perioperative mortality rates are as high as 40% in dogs with GBM requiring emergency surgery.[66,69,70,105,162,163]

CHAPTER II: EVALUATION OF GALLBLADDER MOTILITY ASSESSED VIA ULTRASONOGRAPHY IN DOGS WITH HYPERLIPIDEMIA

A. Introduction

GB disease in dogs, in particular GBM, is common in veterinary medicine. Several breeds are at increased risk of developing GBMs, including Cocker Spaniels, Pomeranians, Miniature Schnauzers, Chihuahuas, and Shetland Sheepdogs. Shetland Sheepdogs and Miniature Schnauzers are up to 14 and 6 times more likely, respectively, than other breeds to develop GBM.[72,104,164] Interestingly, Shetland Sheepdogs and Miniature Schnauzers are also predisposed to familial primary hypercholesterolemia and hypertriglyceridemia, respectively.[2] Hyperlipidemia and GB dysmotility have been found in a small number of Shetland Sheepdogs preceding the formation of GBM.[72] The most common causes of secondary pathologic hyperlipidemia in dogs are endocrine disorders, such as hypothyroidism, diabetes mellitus, and hyperadrenocorticism.[16,28,34,40-45] These diseases have also been associated with an increased risk

of GBM formation. [70,72,162,165,166] It is speculated that hyperlipidemia might play a role in the dysregulation of GB motor function, mucosal function, and bile composition, resulting in abnormal GB motility and, subsequently, GBM formation. The effect of hyperlipidemia on GB motility in dogs is unknown.

Gallbladder motor function can be assessed noninvasively using ultrasonography by measuring the GB volume prior to, and 60 and 120 minutes after a standardized meal.[73,153] If hyperlipidemic dogs have impaired GB motility, effective management of the lipid disorder in conjunction with vigilant monitoring for the development GB disease could 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, specifically 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 to control dogs.

B. Materials and Methods

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 hypercholesterolemia (>332 mg/dL) and/or hypertriglyceridemia (>143 mg/dL) using a biochemical analyzer (Beckman Coulter AU480, Brea, CA). 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 (e.g. 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

plasma biochemistry. Dogs receiving drugs that could alter GBM, including anticholinergics, erythromycin, loperamide, ondansetron, cisapride, cholestyramine, ursodiol, or SAM-e within 7 days prior to 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 prior to feeding. All dogs were fasted for at least 12 hours prior to collection of plasma biochemistry and pre-prandial ultrasound.

All ultrasounds were performed by a board-certified radiologist (ML) who was unaware of the hyperlipidemia status of the dogs using an ultrasound machine (Philips iU22, Philips Medical Systems, Bothell, WA) equipped with a preset broad bandwidth operating frequency transducer (8-5 mHz microconvex). 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, USA). Transverse and longitudinal images of the GB were obtained with dogs in dorsal or lateral recumbency via subcostal and/or right-sided intercostal approaches. For all time sequences, 6 measurements of each dimension (length, width, height) were made. Three measurements were taken from still images, frozen at the subjective maximum dimension. 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 3). Gallbladder volumes were calculated using the ellipsoid method (Volume = 0.52 x L x W x D).[167] Relative GBV was calculated by dividing the GBV by kilograms of body weight, which was used as the GBV in this study.[153] The EF_{60} and EF_{120} were calculated using the GBV at each time

point with the equation EF = ((GBV_0- $GBV_{60,120}$)/GBV_0) x 100, which represented GB emptying.[153]

C. Statistical analysis

Statistical analysis was performed using commercially available software. A power analysis using the advanced repeated measures procedure of PASS (Hintze, J. (2011). PASS 11. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com) showed that 26 dogs per group (healthy and hyperlipidemic) would be needed to detect an effect size of 25.29 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 minutes vs 120 minutes) with a 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.[73] 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 hyperbilirubinemia, increased ALT, ALP, and GGT. Hyperlipidemia was divided into mild and severe (>500 mg/kg) and Kruskal-Wallis test followed by Dunn's 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 (one 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 $P < 0.05$. All analyses were performed using SAS version 9.4 (Cary, NC, USA).

D. Results

Study population

Twenty-eight dogs with hyperlipidemic dogs were evaluated for the study, with 2 dogs being excluded, because one had a GBM and the other was no longer hyperlipidemic at evaluation. Thirty-two control dogs were evaluated, with three excluded due to the presence of mild hyperlipidemia and one due to 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=0.63) between the control (median 10, range 4-14 years) and hyperlipidemic (median 10, range 5-14 years) or sex (P=1) between the groups (Table 2). Of the 17 breeds included in the study, mixed breed (n=9), Shetland Sheepdog (n=9), and Miniature Schnauzer (n=4) were most common in the hyperlipidemic group (Table 2).

Hyperlipidemia

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 (15.4%). Median and range of biochemical abnormalities in each group are listed in Table 3. The median cholesterol concentration was 346 mg/dL and 238 mg/dL, in dogs in the hyperlipidemia and control groups, respectively. The median triglyceride concentration was 330 mg/dL 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 to mildly hyperlipidemic dogs with median concentrations of 272 mg/dL 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.

Biochemical abnormalities

Dogs with hyperlipidemia had significantly higher serum ALP (P = 0.0001) and GGT (P = 0.018) compared to controls. There were no significant differences in ALT (P = 0.627), and total bilirubin (P = 0.391) between the two groups. Dogs with severe hyperlipidemia (cholesterol and/or triglyceride >500 mg/dL) had significantly higher ALP (P = 0.009) and GGT (P = 0.007), but no differences in ALT (P = 0.064) and total bilirubin (P = 0.267) compared to dogs with mild hyperlipidemia. (Table 3).

Dogs with hypertriglyceridemia had significant differences in ALP (P = 0.0003), GGT (P = 0.021), and cholesterol (P = 0.002) compared to controls. Dogs with hypercholesterolemia did not have any significant differences in serum biochemical variables from control dogs.

Gallbladder Volumes

Median and range GBVs at all time points are listed in Table 4. Hyperlipidemic dogs had a significantly larger GBV_0 ($P = 0.008$) and GBV_{60} ($P = 0.043$), but not GBV_{120} (P = 0.123) compared to controls (Table 4). Severely hyperlipidemic dogs had a significantly larger GBV_0 ($P = 0.026$), GBV_{60} ($P = 0.021$), and GBV_{120} ($P = 0.004$) compared to mildly hyperlipidemic dogs. There were no significant differences between mildly hyperlipidemic and control dogs. Boxplot representation of the GBVs in controls, mildly hyperlipidemic and severely hyperlipidemic dogs are seen in Figure 4, 5, and 6. GBVs were not significantly different between dogs with or without hypertriglyceridemia. Dogs with hypercholesterolemia had significantly larger GBV_0 (P

= 0.02), GBV_{60} ($P = 0.01$) and GBV_{120} ($P = 0.02$) when compared to dogs without hypercholesterolemia.

Ejection Fractions

Median EFs at all time points are listed in Table 4. The median EFs for 60 and 120 minutes were not significantly different between any comparison at any time point.

Abdominal ultrasound

A full abdominal ultrasound was performed prior to the test meal in each dog. In the hyperlipidemic group, two solitary liver masses and one focal gastric mass were incidentally found. In the control group, ultrasound revealed an incidental apical bladder mass and two 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}.

D. Discussion

Gallbladder emptying is unaltered in hyperlipidemic dogs, but GBV remains high in hyperlipidemic dogs prior and after feeding. This distention indicating bile retention could represent an early phase in a continuum leading to changes in bile composition and ultimately GBM formation. A recent study by Kakimoto et al. revealed a decrease in GB motility after feeding a high-fat, high-cholesterol diet using a pharmacologic method to assess GB motility, involving a CCK infusion in sedated dogs.[135] However, it is unclear if this methodology is valid compared with the GB contraction induced by feeding in the present study. The effects of motilin, acetylcholine, seretin, gastrin, and other factors involved in GB emptying are likely to be preserved in the methods we used. The present study found that dogs with hyperlipidemia do not have reduced GB emptying but had significantly larger fasted and post prandial GBVs. Furthermore, dogs with severe hyperlipidemia had significantly greater GB distention at all time points compared to dogs with mild hyperlipidemia which could suggest that these dogs are more likely to develop GB disease.

The pathogenesis of GBM formation is unknown, but decreased GB motility has been previously demonstrated in dogs with GBM.[73] Tsukagoshi et al. demonstrated that dogs with GBM had increased GBVs as well as reductions in EFs at all time points compared to control dogs when using the same method of GB function used in the present study.[73] However, this study did not look at dogs with hyperlipidemia prior to GBM formation. In humans, GB distension has been documented with hyperlipidemia and predisposes to bile stasis and furthers GB disease.[152,168] Bile stasis leads 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. Prolonged exposure to concentrated, hydrophobic bile acids has been shown to have cytotoxic effects on GB epithelial cells and promotes mucous hypersecretion which ultimately leads to GB distention and bile stasis. 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 be a mechanism of GBM formation.[169,170] Elevated bile cholesterol concentration leads to increased cholesterol in GB smooth muscle cells which can affect smooth muscle contraction in people, but has not been investigated in dogs.[171] Dogs with GBMs have not only been shown to have decreased GB motility but also have abnormal lipid and energy metabolism.[75] This may be related to a predominance of the disorder in specific breeds that could have other abnormalities of the GB such as altered mucosal secretory and absorptive function.[75]

Primary hyperlipidemia or breed-related hyperlipidemia was prevalent in our study population (50 %) including Miniature Schnauzers or Shetland Sheepdogs with hypertriglyceridemia and hypercholesterolemia, respectively. Secondary hyperlipidemias made up a smaller proportion of the study population. Dogs in the present study were not evaluated extensively for underlying causes of hyperlipidemia, including hypothyroidism and hyperadrenocorticism, but were for diabetes. Dogs in neither group had clinical findings suggestive of these diseases, but there was a difference in GGT and ALP. ALP and GGT was

significantly higher in hyperlipidemic dogs compared to 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 due to primary hypertriglyceridemia as seen in previous studies.[19,74,100,103] Screening tests for hyperadrenocorticism or hypothyroidism were not done to rule out these diseases in these cases. ALP and GGT are inducible enzymes bound to cell membranes of biliary epithelial cells and are indicators of corticosteroid excess or intrahepatic cholestasis. Hyperlipidemia has been associated with hepatocellular accumulation of triglycerides and glycogen leading to vacuolar hepatopathy and is likely of little clinical consequence.[2,103] 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 physiologically 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. Based on these findings an ejection fraction of >25% has been referred to as normal gallbladder emptying in dogs. If we applied this standard to our population of dogs, 5/28 (18%) of the control group would have been considered to have decreased GB emptying. The use of a control group provided a reference for affected dogs under physiologic conditions making the use of CCK analogues unnecessary.

There were several limitations to our study. Based on our power analysis, we met the criteria of 26 hyperlipidemic dogs to detect a significant difference in variables between hyperlipidemic dogs and controls. Eleven dogs within the hyperlipidemic group had severe hyperlipidemic 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 incidentally found intra-abdominal

masses, and only one of the dogs with a liver mass had abnormal GB motility. These masses were unlikely to result in changes to GB motility directly. Finally, ultrasonography has been shown to be a reliable, noninvasive method to assess GB function in dogs, but there are limitations to two-dimensional ultrasonography to assess functionality. Interobserver variability was limited by averaging six measurements from different images and having one radiologist perform all the ultrasounds and measurements. A more physiologic method to assessing GB function was used in our study and erythromycin or CCK analogues were not used to stimulate a stronger GB contraction.

Gallbladder emptying is unaltered in hyperlipidemic dogs, but GBV remains high in hyperlipidemic dogs prior to and after feeding. While it is not clear that this retention of bile leads to GB dysmotility and diseases such as GBM, our findings warrant further investigation of the association between severe hyperlipidemia and GB function. Evaluation of GB function and bile composition in a larger group of dogs with severe hyperlipidemia over time would be useful. Further studies are warranted assessing GB motility in a larger population of dogs with severe hyperlipidemia or persistent hyperlipidemia. An increase in cholesterol and hydrophobic, conjugated bile acids in hyperlipidemic dogs could alter the GB's secretory and motor functions in a continuum and further studies assessing bile composition in naturally occurring pathologic hyperlipidemia is warranted.

CHAPTER III: CONCLUSION

Dogs with hyperlipidemia did not have delayed GB emptying when compared to controls dogs, but the gallbladder volume was significantly higher. This represents abnormal GB distension and bile stasis. The dogs with severe hyperlipidemia in this study did not reveal a significant difference in GB emptying, but 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. Bile composition in hyperlipidemic dogs requires further investigation.

In conclusion, hyperlipidemic dogs do not exhibit decreased GB emptying but do appear to have GB distension. This was the first study to evaluate GB motility using non-invasive ultrasonography in dogs with pathologic hyperlipidemia. Additional studies are warranted to provide more information on the pathogenesis of GBM and the role of hyperlipidemia.

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FIGURES

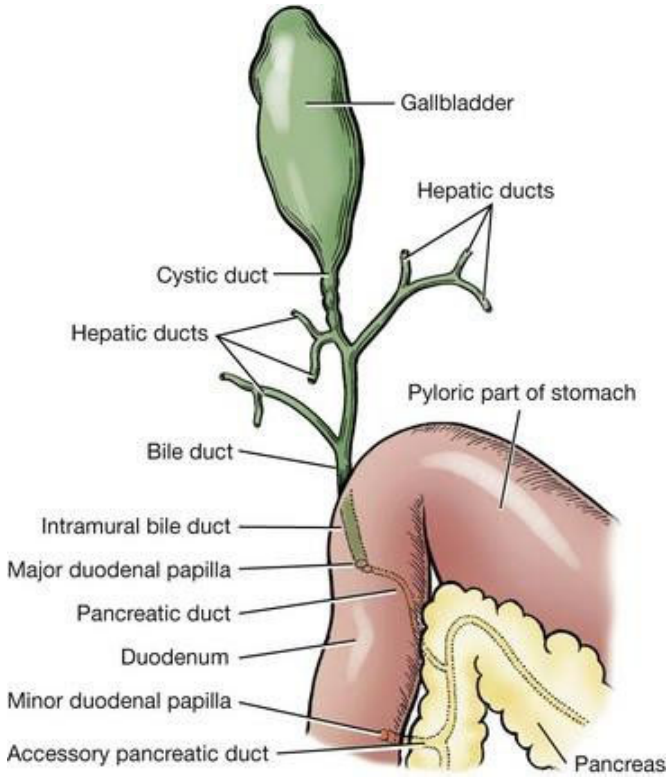

Figure 1. **Anatomic arrangement of the hepatic ducts, common bile duct, and pancreatic ducts.**

(From Evans HE, de Lahunta A: Miller's anatomy of the dog, ed 4, St Louis, in press, Saunders/Elsevier.)

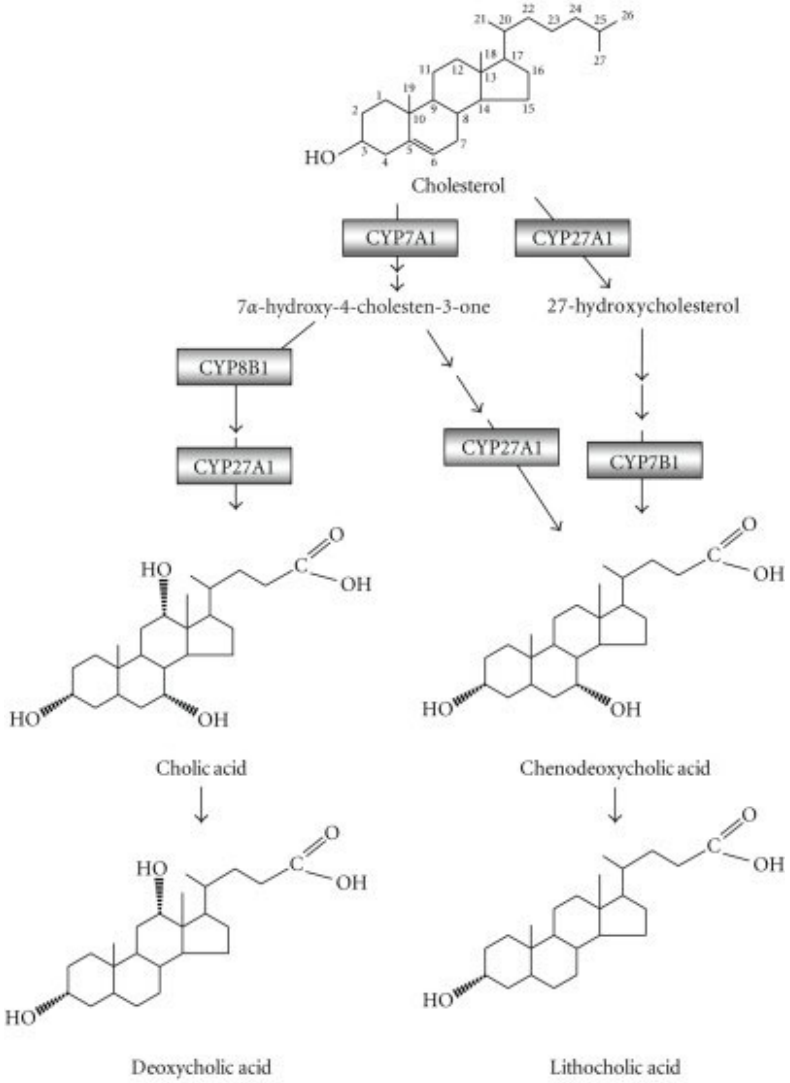

Figure 2. Human bile acid synthesis from cholesterol from two pathways: the classic pathway and the alternative pathway[123]

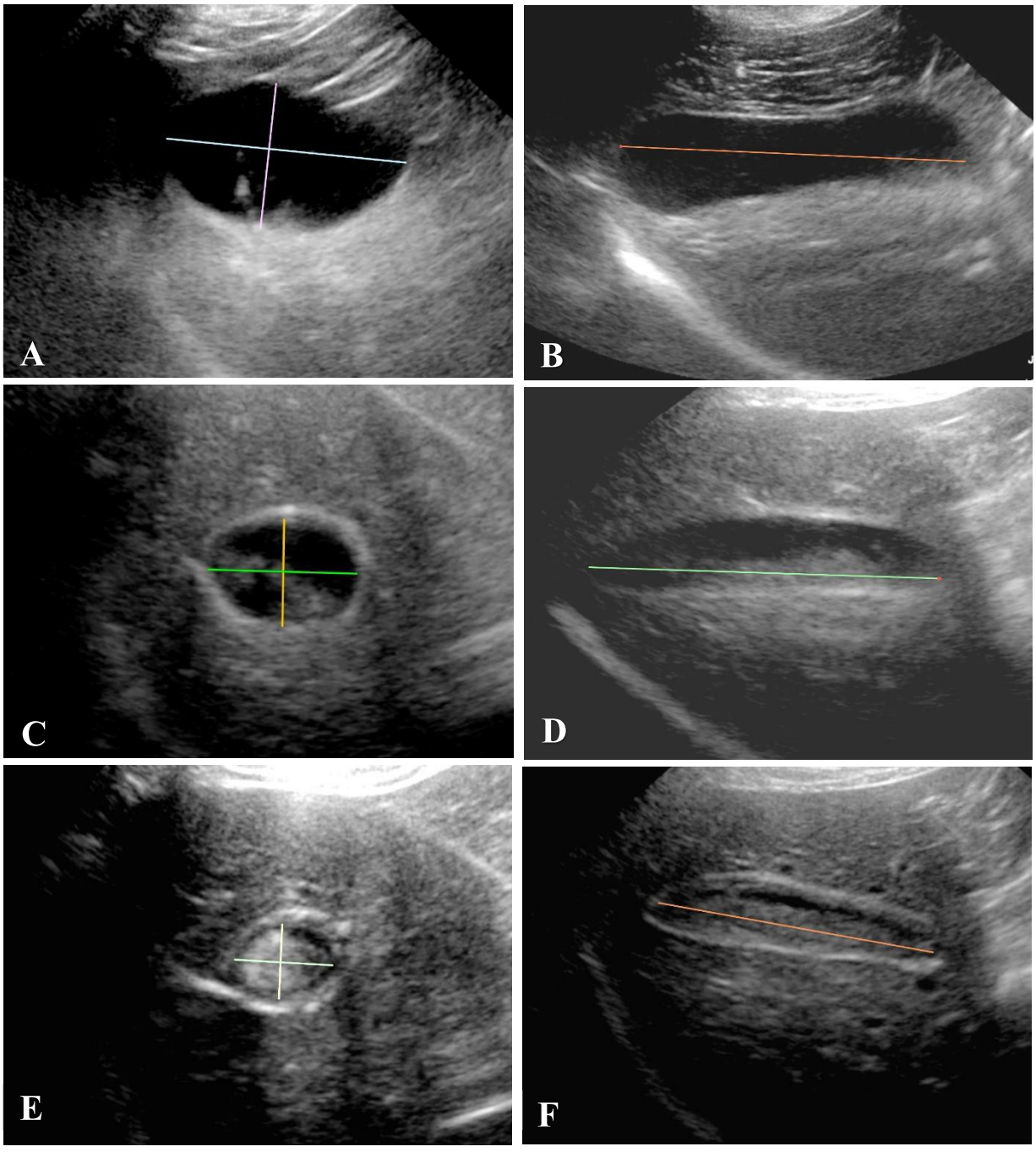

Figure 3. Gallbladder measurements before, 60 and 120 minutes after meal. A. Time 0: Width and height (transverse), B. Time 0: Length (sagittal), C: Time 60: Width and height (transverse), D: Time 60: Length (sagittal), E: Time 120: Width and height (transverse)

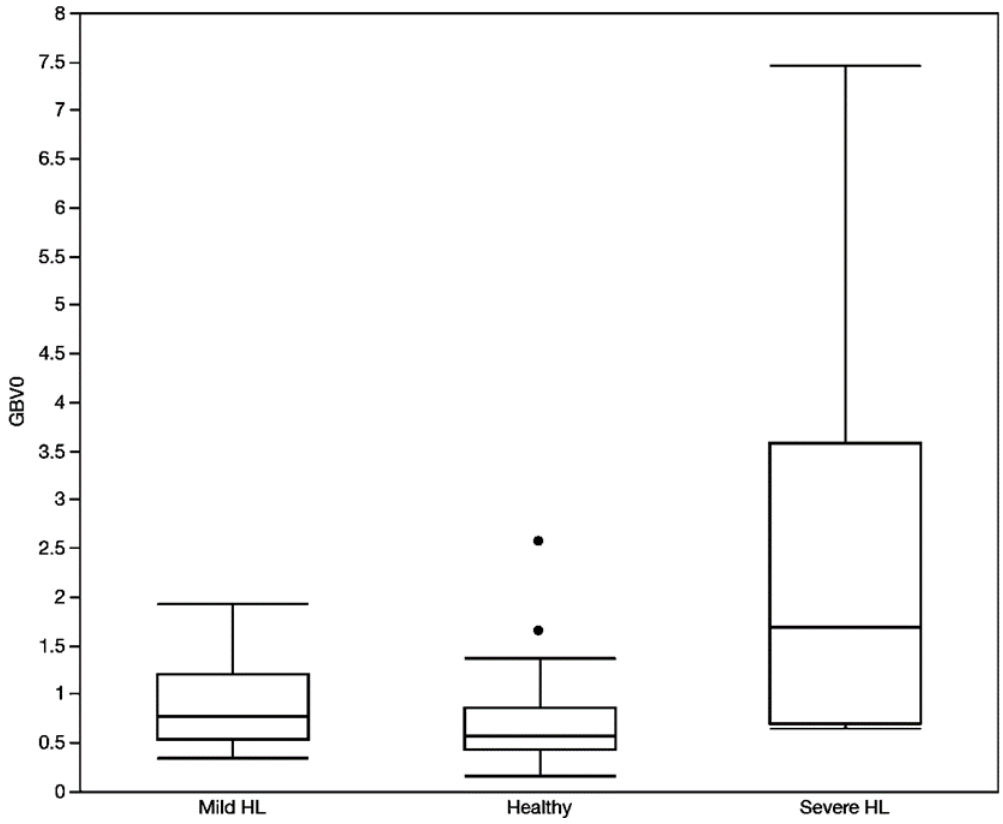

Figure 4. Box-and-whisker plot of GBV_0 of healthy, mildly hyperlipidemic and severely hyperlipidemic dogs. Lines in box plot represents the median value, the box represents the 25th to 75th percentiles and the whiskers represent the 5th to 95th percentiles. Individual outliers are represented by a dot.

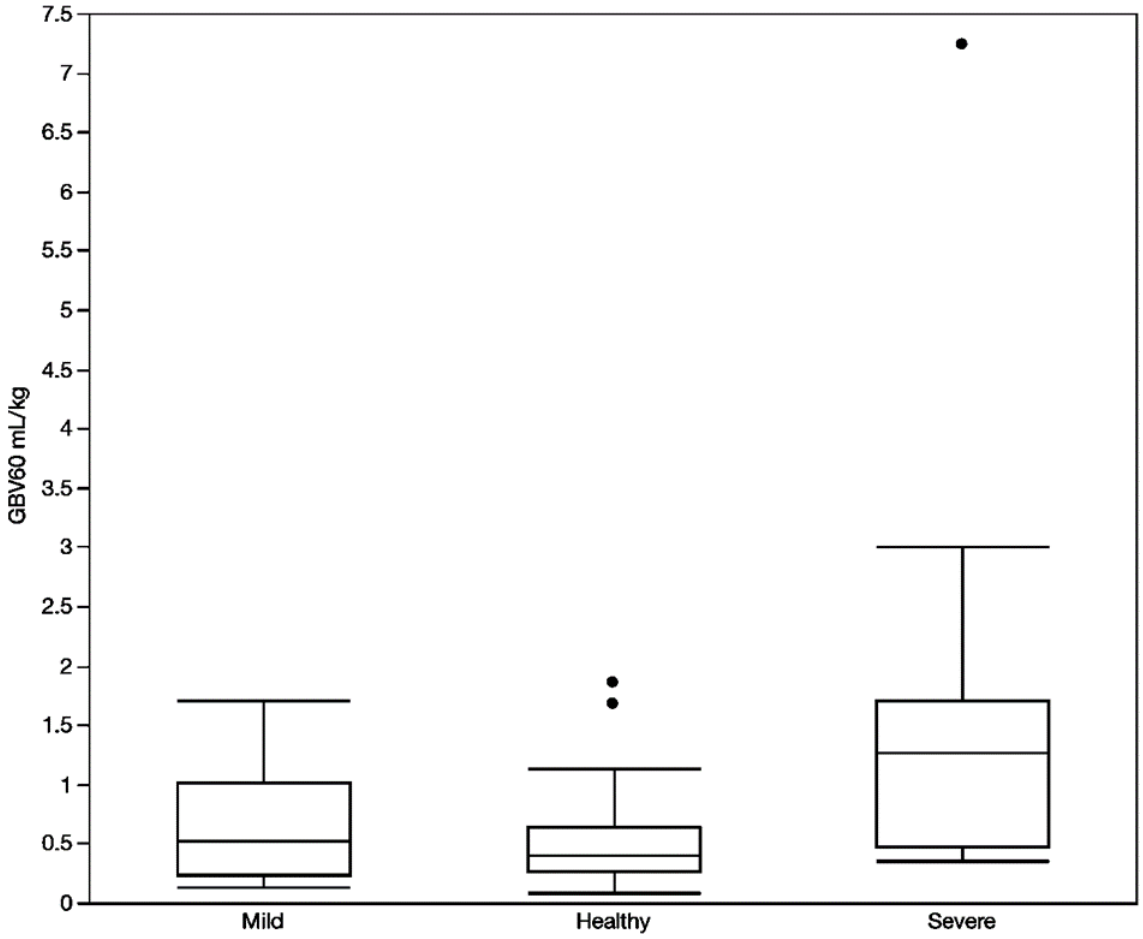

Figure 5. Box-and-whisker plot of GBV_{60} of healthy, mildly hyperlipidemic and severely hyperlipidemic dogs. Lines in box plot represents the median value, the box represents the 25th to 75th percentiles and the whiskers represent the 5th to 95th percentiles. Individual outliers are represented by a dot.

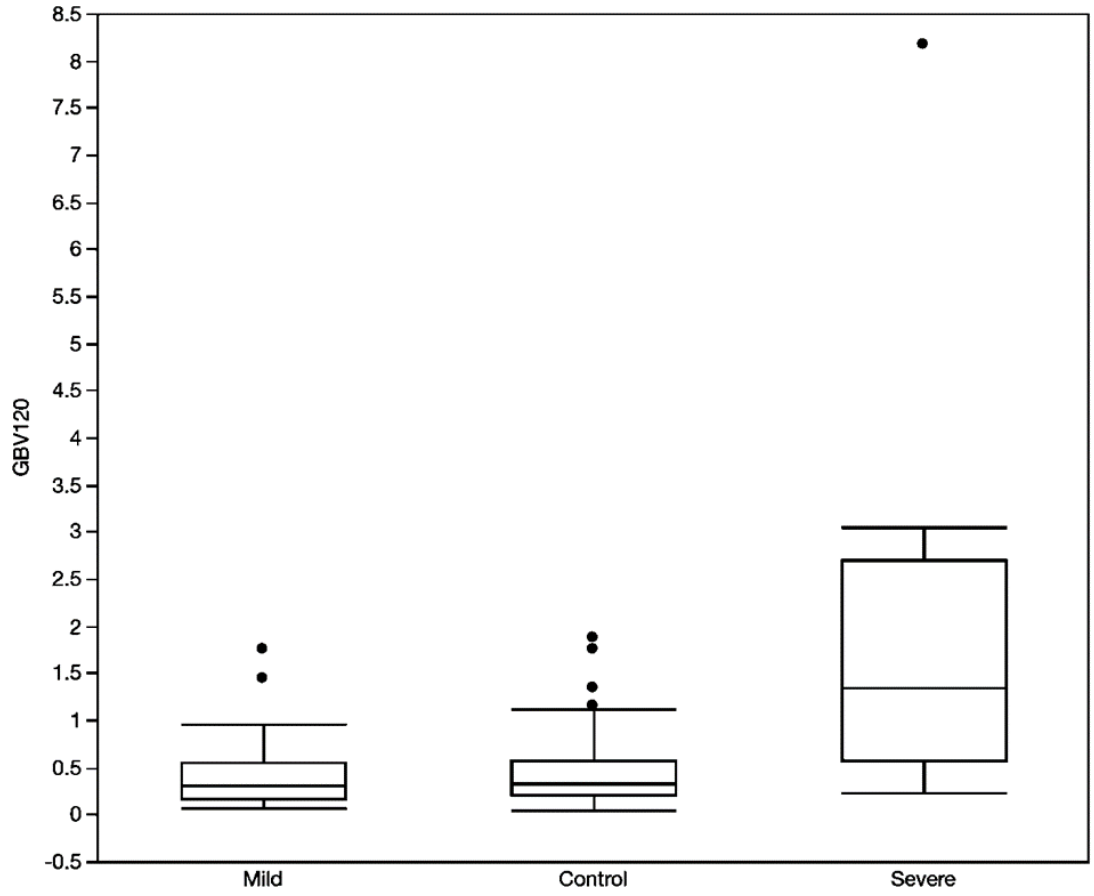

Figure 6. Box-and-whisker plot of GBV_{60} of healthy, mildly hyperlipidemic and severely hyperlipidemic dogs. Lines in box plot represents the median value, the box represents the 25th to 75th percentiles and the whiskers represent the 5th to 95th percentiles. Individual outliers are represented by a dot.

TABLES

Table 1. Lipid disorders in dogs with lipid/lipoprotein abnormalities[2]

Causes of hyperlipidemia	Lipid affected	Lipoprotein class mainly affected
Primary		
Primary triglyceride defect (Miniature Schnauzers, Brittany Spaniels)	Triglycerides	VLDL, chylomicrons
Primary cholesterol defect (Briards, Rough collies, Shetland Sheepdogs)	Cholesterol	HDL, VLDL, LDL
Secondary		
Pancreatitis	Triglycerides, maybe cholesterol	VLDL, chylomicrons, LDL, HDL
Hypothyroidism	Triglycerides, cholesterol	HDL, VLDL, LDL
Hyperadrenocorticism	Triglycerides, cholesterol	VLDL, LDL
Diabetes mellitus	Triglycerides, cholesterol	VLDL
Protein Losing Nephropathy	Cholesterol, less Triglycerides	Unknown
Cholestasis	Cholesterol, triglycerides	LDL
Obesity	Triglycerides, cholesterol	VLDL, LDL, HDL
High fat diets	Triglycerides, cholesterol	HDL

HDL high density lipoprotein, LDL low density lipoprotein, VLDL very low-density lipoprotein

Table 2. Signalment of dogs with hyperlipidemia, severe hyperlipidemia, hypercholesterolemia, hypertriglyceridemia

Outcome	Predictor	Category	Number	Yes (%)	No (%)	*P* Value*
Hyperlipidemia	Sex	FS	23	11 (42.3)	12 (42.9)	1.0000
		MN	31	15 (57.7)	16 (57.1)	
	Breed	Boxer	1	0 (0.0)	1 (3.6)	0.0425
		CKCS	1	1 (3.8)	0 (0.0)	
		Chihuahua	1	0 (0.0)	1 (3.6)	
		Corgi	1	1 (3.8)	0 (0.0)	
		English Springer Spaniel	1	1 (3.8)	0 (0.0)	
		Goldendoodle	1	0 (0.0)	1 (3.6)	
		Husky	1	0 (0.0)	1 (3.6)	
		Jack Russell	2	1 (3.8)	1 (3.6)	
		Labrador Retriever	1	1 (3.8)	0 (0.0)	
		Miniature Schnauzer	4	4 (15.4)	0 (0.0)	
		Mixed	26	9 (34.6)	17 (60.7)	
		Pomeranian	1	1 (3.8)	0 (0.0)	
		Pug	1	0 (0.0)	1 (3.6)	
		Schipperke	1	0 (0.0)	1 (3.6)	
		Sheltie	9	6 (23.1)	3 (10.7)	
		Shih Tzu	1	1 (3.8)	0 (0.0)	
		Staffordshire Terrier	1	0 (0.0)	1 (3.6)	
Severe hyperlipidemia	Sex	FS	23	5 (45.5)	18 (41.9)	1.0000
		MN	31	6 (54.5)	25 (58.1)	
	Breed	Boxer	1	0 (0.0)	1 (2.3)	0.1949
		CKCS	1	0 (0.0)	1 (2.3)	
		Chihuahua	1	0 (0.0)	1 (2.3)	
		Corgi	1	0 (0.0)	1 (2.3)	
		English Springer Spaniel	1	0 (0.0)	1 (2.3)	
		Goldendoodle	1	0 (0.0)	1 (2.3)	
		Husky	1	0 (0.0)	1 (2.3)	
		Jack Russell	2	1 (9.1)	1 (2.3)	
		Labrador Retriever	1	0 (0.0)	1 (2.3)	
		Miniature Schnauzer	4	3 (27.3)	1 (2.3)	

Outcome	Predictor	Category	Number	Yes (%)	No (%)	*P* Value*
		Mixed	26	4 (36.4)	22 (51.2)	
		Pomeranian	1	1 (9.1)	0 (0.0)	
		Pug	1	0 (0.0)	1 (2.3)	
		Schipperke	1	0 (0.0)	1 (2.3)	
		Sheltie	9	1 (9.1)	8 (18.6)	
		Shih Tzu	1	1 (9.1)	0 (0.0)	
		Staffordshire Terrier	1	0 (0.0)	1 (2.3)	
Hypercholesterolemia	Sex	FS	23	6 (40.0)	17 (43.6)	1.0000
		MN	31	9 (60.0)	22 (56.4)	
	Breed	Boxer	1	0 (0.0)	1 (2.6)	0.7691
		CKCS	1	0 (0.0)	1 (2.6)	
		Chihuahua	1	0 (0.0)	1 (2.6)	
		Corgi	1	0 (0.0)	1 (2.6)	
		English Springer Spaniel	1	0 (0.0)	1 (2.6)	
		Goldendoodle	1	0 (0.0)	1 (2.6)	
		Husky	1	0 (0.0)	1 (2.6)	
		Jack Russell	2	0 (0.0)	2 (5.1)	
		Labrador Retriever	1	0 (0.0)	1 (2.6)	
		Miniature Schnauzer	4	2 (13.3)	2 (5.1)	
		Mixed	26	7 (46.7)	19 (48.7)	
		Pomeranian	1	0 (0.0)	1 (2.6)	
		Pug	1	0 (0.0)	1 (2.6)	
		Schipperke	1	0 (0.0)	1 (2.6)	
		Sheltie	9	6 (40.0)	3 (7.7)	
		Shih Tzu	1	0 (0.0)	1 (2.6)	
		Staffordshire Terrier	1	0 (0.0)	1 (2.6)	
Hypertriglyceridemia	Sex	FS	23	9 (42.9)	14 (42.4)	1.0000
		MN	31	12 (57.1)	19 (57.6)	
	Breed	Boxer	1	0 (0.0)	1 (3.0)	0.0292
		CKCS	1	1 (4.8)	0 (0.0)	
		Chihuahua	1	0 (0.0)	1 (3.0)	
		Corgi	1	1 (4.8)	0 (0.0)	
		English Springer Spaniel	1	1 (4.8)	0 (0.0)	

Outcome	Predictor	Category	Number	Yes (%)	No (%)	*P* Value*
		Goldendoodle	1	0 (0.0)	1 (3.0)	
		Husky	1	0 (0.0)	1 (3.0)	
		Jack Russell	2	1 (4.8)	1 (3.0)	
		Labrador Retriever	1	1 (4.8)	0 (0.0)	
		Miniature Schnauzer	4	4 (19.0)	0 (0.0)	
		Mixed	26	8 (38.1)	18 (54.5)	
		Pomeranian	1	1 (4.8)	0 (0.0)	
		Pug	1	0 (0.0)	1 (3.0)	
		Schipperke	1	0 (0.0)	1 (3.0)	
		Sheltie	9	2 (9.5)	7 (21.2)	
		Shih Tzu	1	1 (4.8)	0 (0.0)	
		Staffordshire Terrier	1	0 (0.0)	1 (3.0)	

*Significance set to $P < 0.05$

Table 3. Association of biochemical indices in dogs with hyperlipidemia, severe hyperlipidemia. Hypercholesterolemia, and hypertriglyceridemia

Predictor	Outcome	Category of predictor	Age	Median (Range)	*P* Value*
Hyperlipidemia	ALT (U/L)	no	28	43.5 (25.0-130.0)	0.1468
		yes	26	58.0 (11.0-566.0)	
	ALP (U/L)	no	27	28.0 (6.0-415.0)	0.0001
		yes	26	97.5 (17.0-2482.0)	
	GGT (U/L)	no	28	3.0 (0.0-7.0)	0.0184
		yes	26	4.5 (0.0-17.0)	
	Tbili (mg/dL)	no	28	0.2 (0.1-0.5)	0.3913
		yes	26	0.2 (0.1-0.5)	
	Chol (mg/dL)	no	28	238.0 (153.0-324.0)	0
		yes	26	346.0 (181.0-1372.0)	
	Trig (mg/dL)	no	28	65.5 (34.0-142.0)	0
		yes	26	330.0 (52.0-2213.0)	
Severe hyperlipidemia	ALT (U/L)	no	43	44.0 (11.0-566.0)	0.0641
		yes	11	65.0 (19.0-384.0)	
	ALP (U/L)	no	42	38.5 (6.0-1353.0)	0.0091
		yes	11	219.0 (24.0-2482.0)	
	GGT (U/L)	no	43	3.0 (0.0-7.0)	0.0073
		yes	11	6.0 (1.0-17.0)	
	Tbili (mg/dL)	no	43	0.2 (0.1-0.5)	0.2666
		yes	11	0.2 (0.1-0.5)	
	Chol (mg/dL)	no	43	272.0 (153.0-446.0)	0.0089
		yes	11	437.0 (222.0-1372.0)	
	Trig (mg/dL)	no	43	73.0 (34.0-466.0)	0
		yes	11	910.0 (275.0-2213.0)	
Hypercholesterolemia	ALT (U/L)	no	39	44.0 (11.0-384.0)	0.1328
		yes	15	59.0 (19.0-566.0)	
	ALP (U/L)	no	38	38.5 (6.0-1353.0)	0.0676
		yes	15	88.0 (17.0-2482.0)	
	GGT (U/L)	no	39	3.0 (0.0-16.0)	0.0641
		yes	15	5.0 (0.0-17.0)	
	Tbili (mg/dL)	no	39	0.2 (0.1-0.5)	0.0523

Predictor	Outcome	Category of predictor	Age	Median (Range)	*P* Value*
		yes	15	0.2 (0.2-0.5)	
	Chol (mg/dL)	no	39	248.0 (153.0-324.0)	0
		yes	15	437.0 (340.0-1372.0)	
	Trig (mg/dL)	no	39	78.0 (34.0-2174.0)	0.0179
		yes	15	275.0 (52.0-2213.0)	
Hypertriglyceridemia	ALT (U/L)	no	33	43.0 (25.0-566.0)	0.183
		yes	21	59.0 (11.0-384.0)	
	ALP (U/L)	no	32	30.0 (6.0-415.0)	0.0003
		yes	21	219.0 (17.0-2482.0)	
	GGT (U/L)	no	33	3.0 (0.0-7.0)	0.0211
		yes	21	6.0 (0.0-17.0)	
	Tbili (mg/dL)	no	33	0.2 (0.1-0.5)	0.7479
		yes	21	0.2 (0.1-0.5)	
	Chol (mg/dL)	no	33	255.0 (153.0-446.0)	0.0024
		yes	21	322.0 (181.0-1372.0)	
	Trig (mg/dL)	no	33	63.0 (34.0-142.0)	0
		yes	21	466.0 (193.0-2213.0)	

ALT alanine transaminase, ALP alkaline phosphatase, GGT gamma-glutamyl transferase, Tbili total bilirubin, Chol cholesterol, Trig triglyceride

*Significance set to $P < 0.05$

Table 4. Associations between EF_{60}, EF_{120}, GBV_0, GBV_{60}, and GBV_{120} in dogs with hyperlipidemia, severe hyperlipidemia, hypertriglyceridemia, and hypercholesterolemia

Predictor	Outcome	Category of predictor	n	Median (Range)	*P* Value*
Hyperlipidemia	EF_{60}	no	28	0.3 (-0.5-0.7)	0.9451
		yes	26	0.3 (-0.4-0.8)	
	EF_{120}	no	28	0.5 (-1.6-0.9)	0.7435
		yes	26	0.3 (-0.3-0.9)	
	GBV_0/kg	no	28	0.6 (0.2-2.6)	0.0088
		yes	26	1.0 (0.4-7.5)	
	GBV_{60}/kg	no	28	0.4 (0.1-1.9)	0.0426
		yes	26	0.6 (0.1-7.2)	
	GBV_{120}/kg	no	28	0.3 (0.1-1.9)	0.123
		yes	26	0.5 (0.1-8.2)	
Severe hyperlipidemia	EF_{60}	mild	15	0.3 (-0.4-0.8)	0.9377
		control	28	0.3 (-0.5-0.7)	
		severe	11	0.3 (-0.1-0.6)	
	EF_{120}	mild	15	0.5 (0.1-0.9)	0.1615
		control	28	0.5 (-1.6-0.9)	
		severe	11	0.3 (-0.3-0.7)	
	GBV_0/kg	mild	15	0.8 (0.4-1.9)	0.0028
		control	28	0.6 (0.2-2.6)	
		severe	11	1.7 (0.6-7.5)	
	GBV_{60}/kg	mild	14	0.5 (0.1-1.7)	0.0104
		control	29	0.4 (0.1-1.9)	
		severe	11	1.3 (0.4-7.2)	
	GBV_{120}/kg	mild	14	0.4 (0.1-1.8)	0.0036
		control	29	0.3 (0.1-1.9)	
		severe	11	1.3 (0.2-8.2)	
Hypercholesterolemia	EF_{60}	no	39	0.3 (-0.5-0.8)	0.1915
		yes	15	0.2 (-0.4-0.7)	
	EF_{120}	no	39	0.5 (-1.6-0.9)	0.0564
		yes	15	0.2 (-0.3-0.9)	
	GBV_0/kg	no	39	0.7 (0.2-3.6)	0.0222
		yes	15	1.2 (0.4-7.5)	
	GBV_{60}/kg	no	39	0.4 (0.1-1.9)	0.0141

Predictor	Outcome	Category of predictor	n	Median (Range)	*P* Value*
		yes	15	1.0 (0.1-7.2)	
	GBV_{120}/kg	no	39	0.3 (0.1-1.9)	0.0192
		yes	15	1.0 (0.1-8.2)	
Hypertriglyceridemia	EF_{60}	no	33	0.3 (-0.5-0.7)	0.4333
		yes	21	0.3 (-0.1-0.8)	
	EF_{120}	no	33	0.4 (-1.6-0.9)	0.9507
		yes	21	0.5 (-0.3-0.9)	
	GBV_0 /kg	no	33	0.6 (0.2-2.6)	0.0062
		yes	21	1.1 (0.4-7.5)	
	GBV_{60} /kg	no	33	0.5 (0.1-1.9)	0.0832
		yes	21	0.7 (0.1-7.2)	
	GBV_{120} /kg	no	33	0.3 (0.1-1.9)	0.1029
		yes	21	0.6 (0.1-8.2)	

$EF_{0,60,120}$ ejection fraction prior, 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

*Significance set to $P < 0.05$