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Untersuchungen zur idiopathischen Hypertriglyceridämie des Zwergschnauzers in Nordamerika

Investigations into idiopathic hypertriglyceridemia in the Miniature Schnauzer in North America

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This thesis is dedicated to

My parents Giorgos and Andromachi, my sister Maria, and Patricia

List of abbreviations

°C	degrees Celsius
95% CI	95% confidence interval
µg	microgram
ACTH	adenocorticotropic hormone
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CETP	cholesteryl ester transfer protein
dL	deciliter
EDTA	ethylenediaminetetraacetic acid
GGT	gamma-glutamyl transferase
HDL	high density lipoprotein
IDL	intermediate density lipoprotein
L	liter
LCAT	lecithin-cholesterol acyl transferase
LDL	low density lipoprotein
LPL	lipoprotein lipase
mg	milligram
min	minutes
mL	milliliter
NAFLD	non-alcoholic fatty liver disease
NEFA	nonesterified fatty acid
p	p value
PLN	protein-loosing nephropathy
rpm	revolutions per minute
VLDL	very low density lipoprotein
x g	centrifugal force, expressed as x gravity

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I Introduction

Hypertriglyceridemia, which refers to abnormally increased serum triglyceride concentrations, is a relatively common clinicopathologic finding in dogs. Idiopathic hypertriglyceridemia appears to be associated with specific breeds, most commonly the Miniature Schnauzer (ROGERS et al., 1975a; WHITNEY, 1992; FORD, 1993; WHITNEY et al., 1993; BAUER, 2004). Idiopathic hypertriglyceridemia has been reported in Miniature Schnauzers in the United States, but has not been documented in this breed elsewhere (ROGERS et al., 1975a; WHITNEY, 1992; FORD, 1993; WHITNEY et al., 1993). The cause of hypertriglyceridemia in Miniature Schnauzers remains unclear, but possible mechanisms include increased production of VLDL and chylomicrons, decreased clearance of VLDL and chylomicrons, or both. The fact that hypertriglyceridemia is more prevalent in a specific breed suggests a possible hereditary mechanism (ROGERS et al., 1975a; FORD, 1993; WHITNEY et al., 1993). Although it is generally accepted that idiopathic hypertriglyceridemia is a common disorder in Miniature Schnauzers in the United States, and despite the fact that it can potentially be associated with serious diseases (e.g., pancreatitis), it has received limited attention. The assumption that Miniature Schnauzers have a high incidence of primary hypertriglyceridemia compared to other breeds is largely anecdotal or based on small case series (ROGERS et al., 1975a; WHITNEY et al., 1993). Studies investigating the prevalence and clinical implications of this condition in large populations of Miniature Schnauzers are lacking. Also, the effects of age, sex, and reproductive status on serum triglyceride concentrations have not been sufficiently studied in Miniature Schnauzers.

In human beings, hypertriglyceridemia has been associated with the development of fatty liver and a condition known as non-alcoholic fatty liver disease (NAFLD) (ASSY et al., 2000; ANGULO, 2002; NEUSCHWANDER-TETRI & CALDWELL, 2003; DE BRUIN et al., 2004; BROUWERS et al., 2007). The prevalence of fatty liver in patients with hyperlipidemia, including both hypertriglyceridemia and hypercholesterolemia, has been reported to be about 50% (ASSY et al., 2000; BROUWERS et al., 2007). Most human patients with NAFLD remain asymptomatic

for long periods and many of these patients have only abnormally high serum hepatic enzyme activities as the initial manifestation of NAFLD (ASSY et al., 2000; ANGULO, 2002; NEUSCHWANDER-TETRI & CALDWELL, 2003). Liver enzyme activities are usually only mildly increased (i.e., < 2 times the upper reference limit), with high concentrations typically being identified during routine screening (ANGULO, 2002; NEUSCHWANDER-TETRI & CALDWELL, 2003). Studies investigating a possible association between hypertriglyceridemia, high serum liver enzyme activities, and liver disease in dogs have not been described.

The present study consisted of two parts. The hypothesis of the first part of this study was that hypertriglyceridemia is prevalent among Miniature Schnauzers that appear to be healthy. To prove or disprove this hypothesis, the aim of this first part of the study was to determine the prevalence of hypertriglyceridemia in a large population of healthy Miniature Schnauzers and to further characterize this condition in this breed.

The hypothesis of the second part of the present study was that hypertriglyceridemia in overtly healthy Miniature Schnauzers is associated with increased serum liver enzyme activities. To prove or disprove this hypothesis the goal of the second part of this study was to measure serum hepatic enzyme activities in healthy hypertriglyceridemic Miniature Schnauzers.

II Literature review

1. Lipids

Lipids are water-insoluble organic compounds, which are present in almost every living organism (RIFAI et al., 1999). They are highly polymorphic and chemically diverse biomolecules, which makes it difficult to define them structurally (RIFAI et al., 1999). Lipids are essential for many normal functions of living organisms: they are important components of cell membranes, they are used to store energy, and they play significant roles as enzyme co-factors, hormones, and intracellular messengers (RIFAI et al., 1999).

Of the many groups of lipids, three are most important from a clinical perspective: fatty acids, sterols (mainly cholesterol), and acylglyceroles (mainly triglycerides) (GINSBERG, 1998; RIFAI et al., 1999). Fatty acids are relatively simple lipids and are also important components of many other lipids. They are usually classified based on the length of the fatty acid chain (i.e., short chain, medium chain, and long chain fatty acids), and also based on their degree of saturation (i.e., saturated, monounsaturated, and polyunsaturated fatty acids) (GINSBERG, 1998; RIFAI et al., 1999). Cholesterol is the main sterol in animal tissues and is almost exclusively found in animals. Dietary intake is the major source of cholesterol, but it can also be synthesized endogenously by the liver and other tissues. It plays a fundamental role in central metabolic pathways, such as bile acid metabolism and steroid hormone and vitamin D synthesis (GINSBERG, 1998; RIFAI et al., 1999). Triglycerides are the most common and efficient form of stored energy in mammals. They can be derived from both dietary sources and endogenous (hepatic) production. Each triglyceride consists of glycerol and three fatty acids. Triglycerides are mostly stored in adipocytes and, when energy is needed, fatty acids are released from triglycerides through the catalytic action of lipases. Oxidation of these fatty acids provides large amounts of energy (GINSBERG, 1998; RIFAI et al., 1999).

2. Lipoproteins

Because lipids are water-insoluble molecules, they cannot be transported in aqueous solutions, such as plasma. For that reason, lipids are transported in plasma as macromolecular complexes known as lipoproteins (MAHLEY & WEISGRABER, 1974; WHITNEY, 1992; WATSON & BARRIE, 1993; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004; JOHNSON, 2005). Lipoproteins are spherical structures that consist of a hydrophobic core containing lipids (i.e., triglycerides and/or cholesteryl esters), and an amphophilic (i.e., both hydrophobic and hydrophilic) outer layer of phospholipids, free cholesterol, and proteins that forms a protective envelope surrounding the lipid core. The amphophilic nature of the outer layer allows it to bind to the lipid core on one side, while the hydrophilic surface allows the lipoprotein to be transported in plasma (MAHLEY & WEISGRABER, 1974; BAUER, 1996; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004; JOHNSON, 2005). The proteins that are part of the lipoproteins are known as apolipoproteins (or apoproteins) and play a significant role in lipid transport and metabolism (see apolipoproteins).

Plasma lipoproteins differ in their physical and chemical characteristics such as size, density, and composition. In addition, although lipoproteins of different species share common characteristics, important species differences exist (MAHLEY & WEISGRABER, 1974; DEMACKER et al., 1987; BAUER, 1992; BAUER, 1996; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004; JOHNSON, 2005). Canine lipoproteins can be divided based on their hydrated density (after ultracentrifugation) into four major classes (Table 1): chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) (BAUER, 1992; WATSON & BARRIE, 1993; MALDONADO et al., 2001). HDL can be further subdivided into HDL_1 (present only in dogs and possibly also in cats), HDL_2 (present in dogs, cats, and humans), and HDL_3 (present in dogs, cats, and humans) (MAHLEY & WEISGRABER, 1974; DEMACKER et al., 1987; BAUER, 1992; WATSON & BARRIE, 1993; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004; JOHNSON, 2005). In human beings, a class of intermediate density lipoproteins (IDL) has also been identified, but this class of lipoproteins has not been described in dogs (GINSBERG, 1998; RIFAI et al., 1999).

Table 1: Characteristics of major canine and feline plasma lipoproteins.

Lipoprotein	Species	Major lipids	Major apoliporpoteins	Size (nm)	Density (g/mL)
chylomicron	dog, cat	dietary triglycerides	B, C	75-1200	<0.960
VLDL	dog, cat	endogenous triglycerides	B, C, E	30-80	0.093-1.006
LDL	dog, cat	phospholipids cholesteryl esters	B	18-25	1.019-1.087
HDL1	dog, possibly cat	phospholipids cholesteryl esters	A, C, E	10-35	1.025-1.100
HDL2	dog, cat	phospholipids	A, C, E	9-12	1.063-1.100
HDL3	dog, cat	phospholipids	A, C	5-9	1.100-1.210

VLDL: very low density lipoproteins, LDL: low density lipoproteins, HDL: high density lipoproteins

3. Apolipoproteins

As mentioned earlier, apolipoproteins are the protein components of lipoproteins. The most important classes of apolipoproteins are apolipoprotein A (apo A), apolipoprotein B (apo B), apolipoprotein C (apo C), and apolipoprotein E (apo E) (GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004; JOHNSON, 2005). Each one of these classes can be further divided into specific apolipoproteins (Table 2). Lipoproteins can contain one or a variety of different apolipoproteins, which regulate their metabolic functions (BAUER, 2004).

Apolipoproteins are involved in several physiological functions of lipoproteins such as facilitation of lipid transport, maintenance of structural integrity, and activation of certain enzymes that play key roles in lipid metabolism (Table 2) (GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004; JOHNSON, 2005). For example, apo C-II is a necessary activator of lipoprotein lipase (the major enzyme that hydrolyzes the triglycerides in lipoproteins), while apo C-III inhibits lipoprotein lipase. Deficiencies of certain apolipoproteins have been documented mostly in humans, and usually result in a dysfunctional lipid metabolism.

Table 2: Classification and properties of major human plasma apolipoproteins.

Apolipoprotein	Molecular weight	Lipoproteins	Major metabolic function
Apo A-I	28 016	HDL, chylomicrons	structural, LCAT activator
Apo A-II	17 414	HDL, chylomicrons	unkown
Apo A-IV	46 465	HDL, chylomicrons	unkown
Apo B-48	264 000	Chylomicrons	secretion of chylomicrons from intestine
Apo B-100	540 000	VLDL, IDL, LDL	secretion of VLDL from the liver, structural
Apo C-I	6630	chylomicrons, VLDL, IDL, HDL	uncertain
Apo C-II	8900	chylomicrons, VLDL, IDL, HDL	activator of lipoprotein lipase
Apo C-III	8800	chylomicrons, VLDL, IDL, HDL	inhibitor of lipoprotein lipase
Apo E	34 145	chylomicrons, VLDL, IDL, HDL	facilitates uptake of chylomicron remnants
Apo (a)	250 000-800 000	Lp (a)	uncertain

Apo: apolipoprotein, HDL: high density lipoproteins, VLDL: very low density lipoproteins, IDL: intermediate density lipoproteins, LDL: low density lipoproteins, Lp (a): lipoprotein a

3.1. Apolipoprotein C-II

The presence of a factor in plasma that activates lipoprotein lipase was first suspected in 1955 by Korn (KORN, 1955). About 15 years later, apo C-II was identified as an activator of lipoprotein lipase in plasma (HAVEL et al., 1970; LAROSA et al., 1970). Human apo C-II is made up of a single polypeptide chain that consists of 79 amino acids. However, the mature apolipoprotein is 73 amino acids long and is a product of proteolytic cleavage of the original polypeptide chain (FOJO et al., 1986). The human apo C-II gene is located on chromosome 19, has 4 exons, and encodes a polypeptide chain of 101 amino acids that includes a 22 amino acid signal peptide (FOJO et al., 1984; FOJO et al., 1986; FOJO et al., 1987). Apolipoprotein C-II deficiency was first described in 1978 by Breckenridge et al. in a human patient with severe hypertriglyceridemia (BRECKENRIDGE et al., 1978). Since then several reports of apo C-II deficient patients have been published, and the abnormal apo C-II protein has been sequenced (YAMAMURA et al., 1979; MILLER et al., 1981; STALENHOEF et al., 1981; CATAPANO et al., 1983; SAKU et al., 1984; BAGGIO et al., 1986; CONNELLY et al., 1987a; CONNELLY et al., 1987b). However, it wasn't until 1988 when the DNA sequence of the apo C-II gene was described in human patients with hypertriglyceridemia (COX et al., 1988; FOJO et al., 1988b). To date, at least 16 mutations of the apo C-II gene have been reported in humans (COX et al., 1988; FOJO et al., 1988a; FOJO et al., 1988b; FOJO et al., 1989a; FOJO et al., 1989b; CRECCHIO et al., 1990; HEGELE et al., 1991; XIONG et al., 1991; PARROTT et al., 1992; INADERA et al., 1993; PULLINGER et al., 1993; STREICHER et al., 1996; DE GRAAF et al., 2000; WILSON et al., 2003; LAM et al., 2006). Mutations of the apo C-II gene have not been reported in dogs.

4. Plasma lipid enzymes

4.1. Lipoprotein lipase

Lipoprotein lipase is an enzyme that hydrolyzes triglycerides within lipoproteins into free fatty acids, mono- and diglycerides, and glycerol. Lipoprotein lipase is synthesized primarily in adipose tissue and skeletal muscle and is transported to the luminal surface of the capillary endothelial cells (NILSSON-EHLE et al., 1980;

CRYER, 1981; SEMENKOVICH et al., 1989; WANG & HARTSUCK, 1992). The adhesion of this enzyme to the endothelium is facilitated by heparan sulfated proteoglycans, which can be competed by heparin (CRYER, 1981; WANG & HARTSUCK, 1992). Apolipoprotein C-II (found in chylomicrons and VLDL) is an important co-factor of lipoprotein lipase (CRYER, 1981; AMEIS et al., 1992; WANG & HARTSUCK, 1992; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004; JOHNSON, 2005). Lipoprotein lipase activity is hormonally controlled and is regulated dependant on tissue energy requirements (BEN ZEEV et al., 1983). The crucial role of lipoprotein lipase in lipoprotein metabolism has been demonstrated in experimental animals (knockout mice), cats, dogs, and humans with lipoprotein lipase deficiency, which leads to severe hypertriglyceridemia (BAUM, 1969; LEES et al., 1973; BRUNZELL et al., 1986; JONES et al., 1986a; JONES et al., 1986b; HUBERT et al., 1987; PERITZ et al., 1990; HAYDEN et al., 1994; COLEMAN & SEIP, 1995; WEINSTOCK, 1995; BIJVOET & GAGNE, 1996; MURTHY et al., 1996; GINZINGER & CLEE, 1999).

4.2. Hepatic triglyceride lipase

Hepatic triglyceride lipase, also known as hepatic lipase, is a glycoprotein synthesized by hepatocytes and is located on endothelial cells of hepatic sinusoids, but also on endothelial cells of several extrahepatic tissues (FROST et al., 1982; LAPOSATA et al., 1987; CONNELLY, 1999). In contrast to lipoprotein lipase, hepatic lipase does not require the presence of a co-factor for enzymatic activity (LAROSA et al., 1972). It is involved in hepatic uptake of triglycerides and phospholipids from chylomicrons and VLDL remnants, and also in the conversion of VLDL to LDL (NILSSON-EHLE et al., 1980; JENSEN et al., 1982; CONNELLY, 1999). In addition, hepatic lipase may be the major enzyme responsible for the conversion of HDL_2 to HDL_3 (NIKKILA, 1981). Thus hepatic lipase plays a crucial role in triglyceride removal from the circulation and deficiency of this enzyme has been associated with the development of premature atherosclerosis (BRECKENRIDGE et al., 1982; CONNELLY & HEGELE, 1998).

4.3. Lecithin-cholesterol acyl transferase (LCAT)

LCAT is a glycoprotein synthesized primarily by the liver, which circulates in the blood mainly bound to HDL (TALL, 1990; FIELDING & FIELDING, 1995; JONAS, 2000). LCAT acts on HDL molecules to convert cholesterol into cholesteryl esters. The newly formed cholesteryl esters move to the core of the HDL molecule, thus allowing more cholesterol to be transferred from tissues into HDL molecules (TALL, 1990; FIELDING & FIELDING, 1995; JONAS, 2000; BAUER, 2004; JOHNSON, 2005). Through this function, LCAT plays a crucial role in a pathway known as reverse cholesterol transport. Reverse cholesterol transport is very important because, through this pathway, cholesterol is transferred from peripheral tissues to the liver for reuse or excretion (FIELDING & FIELDING, 1995). At least 40 mutations of the LCAT gene have been reported in humans, and are associated with greatly decreased plasma HDL concentrations and susceptibility to coronary heart disease (KUIVENHOVEN et al., 1997; CALABRESI et al., 2005).

4.4. Cholesteryl ester transfer protein (CETP)

In humans, CETP is synthesized by the liver and its major function is to transport triglycerides from VLDL and chylomicrons to HDL_2 and cholesteryl esters (produced by LCAT) from HDL_2 to VLDL and LDL (DECKELBAUM et al., 1982; TALL, 1990; FIELDING & FIELDING, 1995; GINSBERG, 1998; JOHNSON, 2005). This results in the formation of cholesteryl ester-rich LDL and VLDL molecules and triglyceride-rich HDL_2 (TALL, 1990; FIELDING & FIELDING, 1995; BAUER, 2004; JOHNSON et al., 2005). However, the presence of CETP has not been documented in dogs and is questionable in cats (DEMACKER et al., 1987; WATSON et al., 1995; TSUTSUMI et al., 2001; BAUER, 2004; JOHNSON, 2005). As a result, cholesteryl esters produced by LCAT accumulate in canine HDL_2 molecules leading to the formation of HDL_1, which is unique to dogs (BAUER, 2004; JOHNSON, 2005).

5. Lipid metabolism

Lipid metabolism can be divided into two basic pathways: the exogenous pathway, which is associated with the metabolism of exogenous (dietary) lipids, and the

endogenous pathway, which is associated with metabolism of endogenously produced lipids (GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004).

5.1. Exogenous pathway

The first step of dietary lipid metabolism (mainly metabolism of triglycerides, but also cholesterol and phospholipids) is digestion (BAUER, 1996; GUYTON & HALL, 2000). Dietary lipids that reach the duodenum undergo emulsification through the action of bile salts and lecithin. Emulsification is crucial for subsequent digestion of fat because, through this procedure, the interfacial tension of the fat decreases and large fat globules eventually break down into smaller aggregates. This greatly increases the total surface area of the fat and makes it accessible for hydrolysis by digestive enzymes, which are water-soluble (GUYTON & HALL, 2000).

Dietary triglycerides, cholesterol, and phospholipids are hydrolyzed by gastric and pancreatic lipases, cholesteryl ester hydrolase, and phospholipase A_2, respectively (BAUER, 1996; GUYTON & HALL, 2000; STEINER, 2000). Triglycerides are mainly split into free fatty acids and 2-monoglycerides. Hydrolysis products are then transferred to the microvilli of the intestinal epithelial cell brush border in the form of micelles. At this point the fat hydrolysis products diffuse through the epithelial cell membranes into the enteric mucosal cells (BAUER, 1996; GUYTON & HALL, 2000). In the intestinal mucosal cell, free fatty acids and monoglycerides reassemble to form new triglycerides, which then combine with phospholipids, free and esterified cholesterol, and apo B_{48} to form chylomicrons (BAUER, 1995; BAUER, 1996; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004).

Chylomicrons are the lipoprotein class responsible for transfer of dietary (exogenous) lipids. After formation in enterocytes, chylomicrons, which mainly contain triglycerides, are secreted into the lacteals and enter the lymphatic and later the blood circulation where they acquire apolipoproteins C and apo E from circulating HDL molecules (BAUER, 1995; BAUER, 1996; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004). Apolipoprotein C-II, which is exposed on the chylomicron surface, activates the lipoprotein lipase attached to the capillary beds in adipose and muscle tissues, which then hydrolyzes triglycerides into free fatty acids and glycerol

(BAUER, 1995; BAUER, 1996; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004). Free fatty acids enter the muscle cells (where they are used for energy production) and/or adipocytes (for storage). The remaining particles, which are rich in cholesterol (chylomicron remnants), return their apo C-II molecule to HDL and are recognized by specific hepatic apo E receptors that rapidly remove them from the circulation by way of endocytosis (BAUER, 1995; BAUER, 1996; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004). The cholesterol found in chylomicron remnants can be used for lipoprotein (VLDL) and/or bile acid formation, or stored as cholesteryl ester (BAUER, 1995; BAUER, 1996).

5.2. Endogenous pathway

While chylomicrons are responsible for transport of dietary lipids, VLDL, LDL, and HDL are mainly involved in the metabolism of endogenously produced lipids (BAUER, 1996). As mentioned earlier, both triglycerides and cholesterol can be synthesized by the liver. Endogenously synthesized triglycerides and cholesterol (and cholesteryl esters) combine with phospholipids and apo B_{100} to form VLDL (BAUER, 1996; GINSBERG, 1998; RIFAI et al., 1999). In dogs, apo B_{48} is also present in VLDL molecules (BAUER, 2004). After VLDL molecules reach the vasculature they acquire apolipoproteins C and apo E from HDL (BAUER, 1995; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004). As is the case for chylomicrons, VLDL apo C-II activates lipoprotein lipase located in capillary beds, which in turn leads to hydrolysis of triglycerides and the production of free fatty acids and glycerol. The VLDL molecules remaining after hydrolysis of VLDL triglycerides (VLDL remnants) are either removed from the circulation by the liver or undergo further transformation by lipoprotein lipase and/or hepatic lipase to form LDL (BAUER, 1995; BAUER, 1996; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004; JOHNSON, 2005).

LDL, which contains mainly cholesteryl esters, circulates in the blood and binds to specific receptors that are widely distributed throughout tissues. This way, cholesterol is delivered to peripheral tissues in order to be used for the synthesis of steroid hormones and cell membranes, and for hepatic metabolism (BAUER, 1996; GINSBERG, 1998; RIFAI et al., 1999).

HDLs, which are synthesized primarily in the liver, play an important role as donors and acceptors of apolipoproteins C, apo E, and various lipids from other lipoproteins in the circulation (TALL, 1990; WATSON & BARRIE, 1993; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004). They play a fundamental role in the metabolic pathway known as the reverse cholesterol transport pathway (WATSON & BARRIE, 1993; FIELDING & FIELDING, 1995; GINSBERG, 1998; BAUER, 2004). Through this metabolic pathway, cholesterol is transferred from peripheral tissues to the small circulating discoid HDL molecules, thus converting them to nascent HDL_3 molecules. Cholesterol is then esterified by the action of LCAT and cholesteryl esters move to the core of the HDL_3 molecule thus allowing more free cholesterol to get absorbed into their surface. Continued absorption of free cholesterol and subsequent esterification by LCAT leads to the formation of the larger, cholesteryl ester-rich HDL_2. Cholesteryl esters of HDL_2 molecules are eventually delivered to the liver for reuse or excretion (FIELDING & FIELDING, 1995; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004). With this pathway, cellular and lipoprotein cholesterol is transferred back to the liver for reuse or disposal. In humans, an additional enzyme (cholesteryl ester transfer protein) is involved in this process but, as mentioned earlier, the presence of this enzyme has not been documented in dogs and its existence in questionable in cats (DEMACKER et al., 1987; WATSON & BARRIE, 1993; WATSON et al., 1995; GINSBERG, 1998; TSUTSUMI et al., 2001; BAUER, 2004; JOHNSON, 2005). The role of this enzyme is to transfer triglycerides from LDL, VLDL, and chylomicrons to HDL_2 in exchange for cholesteryl esters. This results in the production of cholesteryl ester-rich LDL and triglyceride-rich HDL_2 molecules (WATSON & BARRIE, 1993; GINSBERG, 1998; RIFAI et al., 1999; BAUER, 2004; JOHNSON, 2005). The absence of this enzyme in dogs might be responsible for certain differences in lipoprotein metabolism between this species and human beings. Due to absence of this enzyme, HDL_2 molecules continuously acquire cholesteryl esters, resulting in the formation of HDL_1 molecules, which are unique to dogs. On HDL_1, cholesteryl esters are transferred from tissues to the liver for disposal or reuse rather than in LDL or VLDL molecules, which transfer cholesterol to peripheral tissues (WATSON & BARRIE, 1993; BAUER, 2004; JOHNSON, 2005). Thus, it is this function of HDL_1 that accounts for the lower incidence of atherosclerotic disorders in dogs compared to humans (JOHNSON, 2005).

6. Disorders of lipid metabolism

6.1. Definitions

The term hyperlipidemia refers to increased concentrations of lipids (usually triglycerides, cholesterol, or both) in the blood (WATSON & BARRIE, 1993; FORD, 1996; JOHNSON, 2005). More specifically, an increased blood concentration of triglycerides is referred to as hypertriglyceridemia, while an increased blood concentration of cholesterol is referred to as hypercholesterolemia. The term hyperlipoproteinemia refers to increased blood concentrations of lipoproteins, but it is often used interchangeably with the term hyperlipidemia (BAUER, 1995). However, the term hyperlipoproteinemia should ideally be limited to cases where measurements of lipoprotein concentrations have been conducted (BAUER, 1995; JOHNSON, 2005).

The term lipemia is used to describe turbid or lactescent appearance of serum or plasma (WATSON & BARRIE, 1993; FORD, 1996; JOHNSON, 2005). Lipemia is a result of hypertriglyceridemia, but not hypercholesterolemia (WATSON et al., 1993; BAUER, 1995; FORD, 1996; JOHNSON, 2005). Mild hypertriglyceridemia does not cause lipemia. Usually, lipemia is apparent when serum triglyceride concentrations exceed 200 - 300 mg/dL (BAUER, 1995). As serum triglyceride concentrations increase, serum becomes turbid (cloudy) and then lactescent (milky).

Hypertriglyceridemia is a relatively common clinicopathologic finding in dogs. In a study of 1,022 blood samples from both healthy and diseased dogs of various breeds, 5.4% had increased serum triglyceride concentrations, but the study did not include grossly lipemic samples (COMAZZI et al., 2004). Postprandial hypertriglyceridemia is normal and transient, and typically resolves within 7-12 hours after a meal, depending on the fat content of the meal (DOWNS et al., 1997; BAUER, 2004). Persistent fasting hypertriglyceridemia is always considered abnormal and can either be primary (most commonly idiopathic) or be secondary to other diseases or drug administration (BAUER, 2004).

6.2. Laboratory evaluation of lipid disorders

6.2.1. Serum turbidity

Visual evaluation of plasma or serum usually offers the first estimate of a sample's triglyceride concentration. Samples that have normal or near normal serum triglyceride concentrations are clear, while samples with increased serum triglyceride concentrations are lipemic (turbid or lactescent; Figure 1) (WATSON & BARRIE, 1993; FORD, 1996; JOHNSON, 2005). In general, turbidity appears when serum triglyceride concentrations are above to 200 - 300 mg/dl and lactescent serum is seen when serum triglyceride concentrations are above 1,000 mg/dL (BAUER, 1995; JOHNSON, 2005). However, these guidelines provide only a rough estimate of the actual serum triglyceride concentration, and measurement of the actual serum triglyceride concentration is required. In addition, the presence of hypercholesterolemia cannot be excluded on the basis of a clear serum sample because hypercholesterolemia does not cause increased serum turbidity (WHITNEY, 1992; JOHNSON, 2005).

Figure 1: Lipemia

Serum samples with normal serum triglyceride concentrations are clear (left tube). As serum triglyceride concentration increases, the serum becomes turbid (middle tube) and ultimately lactescent (right tube).

6.2.2. Refrigeration test

The refrigeration test (or chylomicron test) is performed on lipemic samples, and is a simple test to determine the specific form of lipoprtotein that is responsible for hypertriglyceridemia (ROGERS, 1977; WHITNEY, 1992; JOHNSON, 2005). Serum samples are refrigerated and left undisturbed for 10 to 12 hours. Due to their lower density, chylomicrons tend to move to the top of the sample and form a “cream layer” (Figure 2). When a cream layer is formed, chylomicrons account (partially or solely) for the hypertriglyceridemia (ROGERS, 1977; WHITNEY, 1992; JOHNSON, 2005). If there is no cream layer formation, the hypertriglyceridemia and lipemia are due to an excess of other lipoproteins (usually VLDL, because these contain high concentrations of triglycerides) (WHITNEY, 1992; JOHNSON, 2005). When a cream layer forms, the serum below the cream layer can either be clear or turbid. In the first case, hyperchylomicronemia is most likely solely responsible for the hypertriglyceridemia, while in the second case other lipoproteins are also present in excess (Figure 2) (WHITNEY, 1992; JOHNSON, 2005).

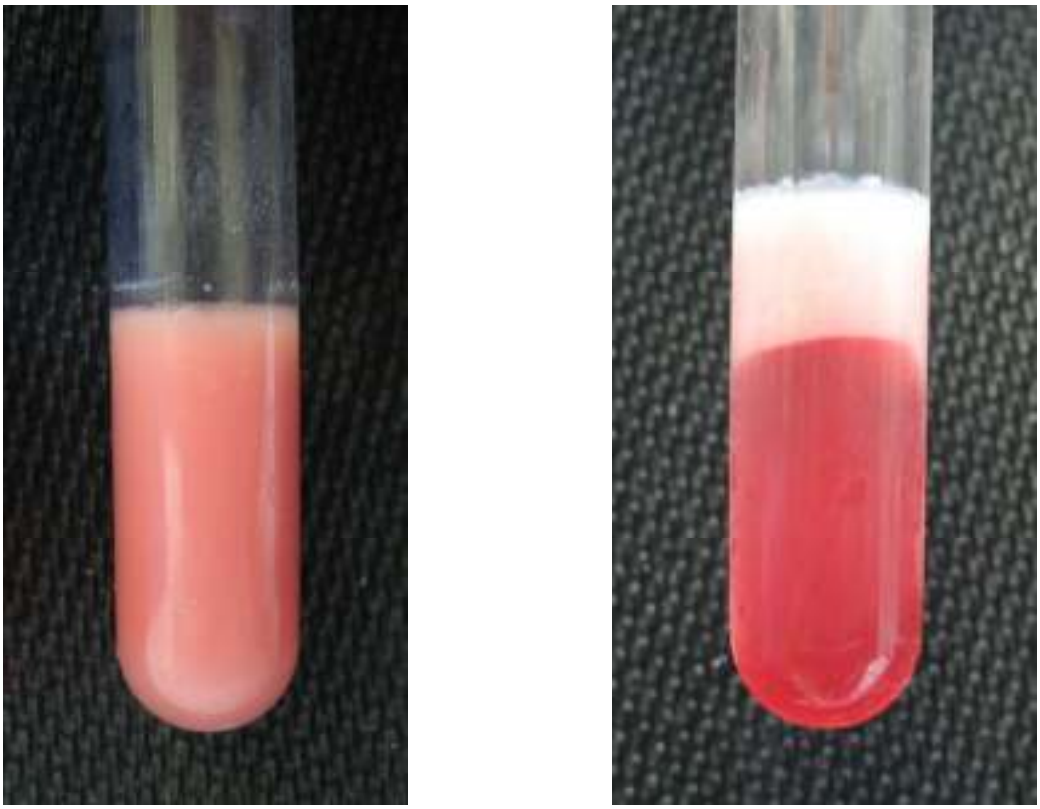

Figure 2: Chylomicron test.

The picture on the left shows a lipemic serum sample from a dog with hypertriglyceridemia right after separation of the serum from the clot. The same serum sample is shown on the right after overnight refrigeration (chylomicron test). The formation of a cream layer is obvious. The remaining serum below the cream layer is clear (although hemolytic), which suggests that other classes of lipoproteins are not increased in this patient.

6.2.3. Methods for quantification and characterization of lipids in blood

Routine quantitative assessment of total cholesterol and triglyceride concentrations in serum or plasma is usually achieved by use of spectrophotometric or enzymatic methods (NELSON et al., 2004). Lipoprotein electrophoresis has been commonly used to further characterize lipid abnormalities in dogs and other animals, but has limited use in the routine clinical evaluation of hyperlipidemic dogs (WHITNEY, 1992; NELSON et al., 2004). Some differences of serum lipoprotein and fatty acid concentrations among dog breeds have been reported (DOWNS et al., 1993; SMITH et al., 2008). Finally, ultracentrifugation has been used to separate serum or plasma lipoproteins based on their density. However, due to its high cost, this method is not routinely used in veterinary medicine.

6.3. Causes of hyperlipidemia

Postprandial hypertriglyceridemia is physiologic and transient, and typically resolves within 7-12 hours after a meal, depending on the fat content of the meal (WHITNEY, 1992; DOWNS et al., 1997; BAUER, 2004; JOHNSON, 2005). For that reason, determination of serum lipid concentrations should always follow a fast of at least 12 hours. Persistent fasting hypertriglyceridemia is always considered abnormal and can either be primary (idiopathic) or secondary due to other diseases or drug administration.

6.3.1. Secondary causes of hyperlipidemia

Secondary hyperlipidemia is the most common pathologic form of hyperlipidemia in dogs (NELSON et al., 2004). Several diseases have been reported to cause hyperlipidemia.

6.3.1.1. Endocrine Diseases

Most commonly, canine hyperlipidemia is the result of an endocrine disorder, such as hypothyroidism, diabetes mellitus, or hyperadrenocorticism (ROGERS et al., 1975b; ROGERS, 1977; WHITNEY, 1992; BAUER, 2004; FELDMAN & NELSON, 2004; JOHNSON, 2005). Increases of both serum triglyceride and cholesterol

concentrations have been reported in dogs with hypothyroidism (ROGERS et al., 1975b; BARRIE et al., 1993; PANCIERA, 1994; DIXON et al., 1999; SCHENCK et al., 2004). In one study, hypertriglyceridemia and hypercholesterolemia were found in 88% and 78% of dogs with hypothyroidism, respectively (DIXON et al., 1999). Usually, lipid abnormalities resolve after treatment of hypothyroidism (ROGERS et al., 1975b). Dogs with diabetes mellitus commonly have hyperlipidemia, which is most commonly associated with hypertriglyceridemia, although hypercholesterolemia might also be present (ROGERS et al., 1975b; WILSON et al., 1986; WHITNEY, 1992; BARRIE et al., 1993; FELDMAN & NELSON, 2004; JOHNSON, 2005). Hypertriglyceridemia usually resolves after successful treatment of diabetes, but hypercholesterolemia might persist despite therapy (GLEESON et al., 1990; WHITNEY, 1992). Finally, both naturally occurring and iatrogenic hyperadrenocorticism have been associated with hyperlipidemia (hypertriglyceridemia and hypercholesterolemia) in dogs (LING et al., 1979; WHITNEY, 1992; BARRIE et al., 1993; HUANG et al., 1999; BAUER, 2004; FELDMAN & NELSON 2004; JOHNSON, 2005). Hypertriglyceridemia appears to be present more frequently than hypercholesterolemia in dogs with hyperadrenocorticism, and increases of both types of lipids are usually mild or moderate (BAUER, 2004; FELDMAN & NELSON 2004; JOHNSON, 2005).

6.3.1.2. Pancreatitis

The presence of hyperlipidemia (hypertriglyceridemia and, to a lesser degree, hypercholesterolemia) has long been associated with naturally occurring pancreatitis in dogs (ANDERSON & LOW, 1965; ANDERSON & STRAFUSS, 1971; ROGERS et al., 1975b; ROGERS, 1977; WHITNEY, 1992; COOK et al., 1993; HESS et al., 1998; HESS et al., 1999; BAUER, 2004; JOHNSON, 2005; WILLIAMS & STEINER, 2005). However, it remains uncertain whether hyperlipidemia develops as a result of pancreatitis or can be the cause of pancreatitis in some cases (WHITNEY, 1992; WILLIAMS & STEINER, 2005). In one study where acute pancreatitis was experimentally induced in dogs by intraductal infusion of bile and lyophilized trypsin, neither hypertriglyceridemia nor hypercholesterolemia developed up to 96 hours after induction of pancreatitis (WHITNEY et al., 1987). Similar results were reported in an

older study where pancreatitis was induced in dogs by ligation of the major and minor pancreatic ducts (BASS et al., 1976). In this study, pancreatitis did not result in hypertriglyceridemia or hypercholesterolemia up to 14 days after induction (BASS et al., 1976). In a more recent study, mild increases in serum triglyceride concentrations were noted in dogs after induction of pancreatitis with oleic acid (CHIKAMUNE et al., 1998). Although serum triglyceride concentrations significantly increased, they seemed to remain within the reference range even after induction of pancreatitis (CHIKAMUNE et al., 1998). However, this is not clearly discussed in this report (CHIKAMUNE et al., 1998). Based on these studies, it can be concluded that hypertriglyceridemia is not a feature of experimentally induced pancreatitis in dogs. No conclusive statement regarding the role of naturally occurring pancreatitis in the development of secondary hyperlipidemia can be made. Given the documented association between naturally occurring pancreatitis and hyperlipidemia, it is possible that either hyperlipidemia is a preexisting abnormality in some dogs with naturally occurring pancreatitis, which may or may not contribute to the development of the disease, or that naturally occurring pancreatitis differs from the experimental models of pancreatitis used in the above studies in its ability to produce hyperlipidemia.

6.3.1.3. Obesity

Increased serum triglyceride and/or cholesterol concentrations have been observed in obese dogs (CHIKAMUNE et al., 1995; BAILHACHE et al., 2003; JEUSETTE et al., 2005). The most profound lipid abnormalities were associated with severe chronic obesity (JEUSETTE et al., 2005). Weight loss in obese dogs leads to significant decreases of both serum triglyceride and cholesterol concentrations (DIEZ et al., 2004; JEUSETTE et al., 2005).

6.3.1.4. Protein losing nephropathy (PLN)

Proteinuria associated with PLN, regardless of the cause, is often associated with hyperlipidemia in dogs (CHEW et al., 1983; CENTER et al., 1987; DIBARTOLA et al., 1989; DIBARTOLA et al., 1990; REUSCH et al., 1994; COOK & COWGILL, 1996; DE MORAIS et al., 1996; LITTMAN et al., 2000). The most commonly reported lipid abnormality in dogs with PLN is hypercholesterolemia, which is usually

mild or moderate (CHEW et al., 1983; CENTER et al., 1987; DIBARTOLA et al., 1989; DIBARTOLA et al., 1990; REUSCH et al., 1994; COOK & COWGILL, 1996; DE MORAIS et al., 1996; LITTMAN et al., 2000). Hypercholesterolemia in dogs with PLN is usually part of a more complex syndrome, the nephrotic syndrome, which in addition to hypercholesterolemia, is characterized by hypoalbuminemia, proteinuria, and ascites (BAUER, 2004; JOHNSON, 2005). Hypercholesterolemia has been reported with varying frequencies in dogs with acquired glomerular disease (amyloidosis or glomerulonephritis) and proteinuria, as well as several hereditary forms of PLN (Chinese Shar-peis, Golden Retrievers, Soft-coated Wheaten Terriers, Doberman Pinschers, and Bernese Mountain dogs) (CHEW et al., 1983; CENTER et al., 1987; DIBARTOLA et al., 1989; DIBARTOLA et al., 1990; REUSCH et al., 1994; COOK & COWGILL, 1996; DE MORAIS et al., 1996; LITTMAN et al., 2000).

6.3.1.5. Cholestasis

Cholestasis has been reported to lead to mild or moderate hypercholesterolemia and mild hypertriglyceridemia in dogs (DANIELSSON et al., 1977; WHITNEY, 1992; CHUANG et al., 1995). Changes in lipoproteins, mainly excessive esterification of lipoprotein cholesterol, have also been reported in dogs with experimentally induced cholestasis (BLOMHOFF et al., 1978; WALLI & SEIDEL, 1984).

6.3.1.6. Other causes

Several other causes of hyperlipidemia have been reported or suspected to exist in dogs. These include high fat diets, lymphoma, infection with *Leishmania infantum*, congestive heart failure due to dilated cardiomyopathy, and administration of certain drugs (e.g., glucocorticoids) (NIETO et al., 1992; OGILVIE et al., 1994; BURKHARD & MEYER, 1995; DOWNS et al., 1997; TIDHOLM & JONSSON, 1997). Finally, in a recent study, significantly increased serum triglyceride concentrations were reported in association with other lipid abnormalities in dogs with parvoviral enteritis (YILMAZ & SENTURK, 2007).

6.3.2. Primary causes of hyperlipidemia in dogs

In general, primary lipid abnormalities have not been sufficiently studied in dogs. They appear to be rare and are usually, but not always, associated with specific breeds. Primary hyperlipidemia in Miniature Schnauzers was the first breed-related primary lipid disorder described in dogs, and is believed to be the most common (ROGERS et al., 1975a; WHITNEY, 1992; FORD, 1993; WHITNEY et al., 1993; BAUER, 1995; BAUER, 2004). This condition was first reported more than 30 years ago in Miniature Schnauzers in the United States, but has not been documented elsewhere (ROGERS et al., 1975a; WHITNEY, 1992; FORD, 1993; WHITNEY et al., 1993; BAUER, 1995; BAUER, 2004). It is characterized by abnormal accumulation of VLDL or a combination of VLDL and chylomicrons (FORD, 1993; WHITNEY et al., 1993). Although hypercholesterolemia may also be present, this finding is not consistent (WHITNEY, 1992; FORD, 1993; WHITNEY et al., 1993). The cause of hypertriglyceridemia in Miniature Schnauzers remains unclear, but possible mechanisms include increased production and/or decreased clearance of VLDL and chylomicrons (FORD, 1993; BAUER, 1995; FORD, 1996). The fact that hypertriglyceridemia is prevalent within a single breed suggests a possible hereditary mechanism (ROGERS et al., 1975a; FORD, 1993; WHITNEY et al., 1993). Because lipoprotein lipase is the major enzyme involved in triglyceride clearance, deficiency of this enzyme has been considered as a possible cause of hypertriglyceridemia in this breed. Two studies that included both Miniature Schnauzers and dogs of other breeds with primary hyperlipidemia, found that lipoprotein lipase activity was significantly lower in plasma from hyperlipidemic dogs than in healthy control dogs (SCHENCK, 2002; JAEGER et al., 2003). However, a recent study in Miniature Schnauzers with hypertriglyceridemia and pancreatitis failed to identify any mutations of the lipoprotein lipase gene, suggesting that inherited lipoprotein lipase dysfunction is not the cause of hypertriglyceridemia in this breed (SCHICKEL, 2005a). Further studies are warranted to identify the genetic basis of idiopathic hypertriglyceridemia in Miniature Schnauzers.

Primary hypercholesterolemia without hypertriglyceridemia has been well documented in 15 Briards from the UK (WATSON et al., 1993). A similar condition

has recently been described in a family of rough Collies also from the UK (JEUSETTE et al., 2004) A slightly different condition of primary hypercholesterolemia with or without concurrent hypertriglyceridemia has been reported in Shetland Sheepdogs in Japan (SATO et al., 2000). The cause of these lipid abnormalities that mainly involve cholesterol metabolism cannot be determined based on these studies, but hereditary factors have been suspected (SATO et al., 2000; WATSON et al., 1993). In addition, primary hypercholesterolemia has been anecdotally reported in Doberman Pinschers and Rottweilers (ARMSTRONG & FORD, 1989).

Primary hyperlipidemia with hypercholesterolemia and hypertriglyceridemia has been reported in two related Beagles (WADA et al., 1977). Also, primary hypertriglyceridemia has been reported in two related adult Brittany Spaniels and one mixed-breed 28-day old puppy. In all 3 cases, lipoprotein lipase deficiency was suspected based on a deficient or absent lipoprotein lipase activity after heparin administration (BAUM, 1969; HUBERT et al., 1987).

6.4. Clinical signs and complications of hyperlipidemia

In general, dogs with secondary hyperlipidemia display clinical signs associated with the primary disorder. Dogs with primary lipid disorders are often asymptomatic for long periods or even throughout their lives. However, in some cases, dogs with primary hyperlipidemia develop secondary diseases as a result of hyperlipidemia that may account for the development of specific clinical signs. Whether or not dogs with primary hyperlipidemia will develop clinical signs depends on many factors, including the type and severity of hyperlipidemia, and the presence or absence of other diseases.

6.4.1. Pancreatitis

An association between hyperlipidemia and pancreatitis was first noted by Speck in 1865 (SPECK, 1865). Today, severe hypertriglyceridemia is a known risk factor for the development of pancreatitis in humans (CAMERON et al., 1974; TOSKES, 1990;

FORTSON et al., 1995; YADAV & PITCHUMONI, 2003). Although hyperlipidemia has been reported in up to 38% of human patients with pancreatitis, in the vast majority of these patients, hyperlipidemia is mild and is likely the result rather than the cause of pancreatitis (TOSKES, 1990; FORTSON et al., 1995; YADAV & PITCHUMONI, 2003). An increased risk for pancreatitis from hyperlipidemia has been shown to exist when serum triglyceride concentrations exceed 1,000 mg/dL, which is most commonly associated with primary defects in lipid metabolism (TOSKES, 1990; FORTSON et al., 1995; YADAV & PITCHUMONI, 2003). Lipid disorders most commonly associated with pancreatitis in humans are lipoprotein lipase or apo C-II deficiency, familial combined hyperlipidemia, and familial hypertriglyceridemia (TOSKES, 1990; YADAV & PITCHUMONI, 2003). The latter 2 conditions occur much more frequently than lipoprotein lipase or apo C-II deficiency, but the underlying genetic defects have not yet been determined (TOSKES, 1990; YADAV & PITCHUMONI, 2003). In cases where secondary hyperlipidemia is severe (i.e., >1,000 mg/dL) or if it is combined with a primary lipid disorder, it can potentially also lead to pancreatitis. For example, hyperlipidemic pancreatitis has been found to occur more commonly in patients with poorly controlled or untreated diabetes or diabetic ketoacidosis (FORTSON et al., 1995; NAIR et al., 2000). Hypercholesterolemia does not constitute a risk factor for pancreatitis in humans (TOSKES, 1990). The mechanism by which hypertriglyceridemia (primary or secondary) induces pancreatitis is not clear. A possible mechanism is that serum triglycerides are hydrolyzed by the action of pancreatic lipase, leading to excessive production of free fatty acids, which are toxic to the pancreas (HAVEL, 1969; SAHARIA et al., 1977). To date, there are no means to predict which hyperlipidemic patients will develop pancreatitis, and the clinical course of hyperlipidemic pancreatitis is no different from other forms of pancreatitis in humans (TOSKES, 1990; YADAV & PITCHUMONI, 2003).

A similar relationship between hypertriglyceridemia and pancreatitis has been suggested in dogs (ROGERS et al., 1975a; ROGERS, 1977; WHITNEY, 1992; FORD, 1993; BAUER, 1995; FORD, 1996; WILLIAMS, 1996; BAUER, 2004; WILLIAMS & STEINER, 2005). In addition, the clinical impression of a high prevalence of pancreatitis in Miniature Schnauzers has been attributed to the fact that

dogs of this breed commonly develop hyperlipidemia (WHITNEY, 1992; FORD, 1996; WILLIAMS, 1996; WILLIAMS & STEINER, 2005). Available clinical and experimental data to support this hypothesis are limited, however. Pancreatitis has been shown to develop in dogs after feeding high fat, low protein diets, and is more severe when induced in dogs being fed a high fat diet (LINDSAY et al., 1948; GOODHEAD, 1971). Also, experimental data in isolated canine pancreas showed that high triglyceride concentrations can induce pancreatitis, possibly through the release of free fatty acids (SAHARIA et al., 1977). Clinical studies have shown an association between hyperlipidemia and pancreatitis in dogs, although it was not determined whether hyperlipidemia was the cause or the result of pancreatitis, or just an incidental finding in some cases (ROGERS et al., 1975a; ROGERS et al., 1975b; WHITNEY et al., 1987; COOK et al., 1993; HESS et al., 1998; HESS et al., 1999; WILLIAMS & STEINER, 2005). Secondary hyperlipidemia seen in patients with some endocrinopathies (e.g., hypothyroidism, hyperadrenocorticism, or diabetes mellitus), may be responsible for the increased risk for pancreatitis associated with these diseases (HESS et al., 1999; HESS et al., 2000). Also, hyperlipidemia has been observed in obese dogs, suggesting a possible explanation for why obese dogs are more prone to pancreatitis (CHIKAMUNE et al., 1995; HESS et al., 1999). Based on these studies, an association between hypertriglyceridemia and pancreatitis in dogs is obvious, but a cause-and-effect relationship cannot be established. Further studies are needed in order to evaluate hypertriglyceridemia as a risk factor for pancreatitis in dogs.

6.4.2. Atherosclerosis

Hyperlipidemia, and especially hypercholesterolemia, is recognized as a major risk factor for the development of atherosclerosis in humans (SCHOEN & COTRAN, 1999). Although dogs are resistant to atherosclerosis due to their lipoprotein composition and metabolism, they have been reported to develop atherosclerosis in both experimental and clinical studies (MAHLEY et al., 1974; MAHLEY et al., 1977; LIU et al., 1986; KAGAWA et al., 1998; HESS et al., 2003). Spontaneous atherosclerosis has been reported in dogs mainly in association with secondary hyperlipidemia (LIU et al., 1986; HESS et al., 2003). In these cases, atherosclerosis

has been thought to develop as a result of hypercholesterolemia (KAYSEN et al., 1986; HESS et al., 2003). The most commonly reported disorders associated with spontaneous atherosclerosis in dogs are endocrinopathies that are associated with hypercholesterolemia (HESS et al., 2003; VITALE & OLBY, 2007). In one study, 60% of 30 dogs with atherosclerosis had hypothyroidism, and 20% had diabetes mellitus (HESS et al., 2003). However, in the same study, 23% of the dogs with atherosclerosis did not have any endocrine disorder associated with hypercholesterolemia, which suggests that atherosclerosis can also develop secondary to other diseases (HESS et al., 2003). It is currently not known whether primary hyperlipidemia can also be associated with atherosclerosis in dogs (HESS et al., 2003).

6.4.3. Insulin resistance and diabetes mellitus

In human beings, severe hypertriglyceridemia has been reported to be related to insulin resistance and the development of diabetes mellitus (SANE & TASKINEN, 1993; MINGRONE et al., 1997; MINGRONE et al., 1999). Families with hereditary hypertriglyceridemia have a high incidence of type 2 diabetes (21% in one 10-year study) (SANE & TASKINEN, 1993). In these families, hyperlipidemic family members were more likely to develop type 2 diabetes, and increased serum triglyceride concentrations were a risk factor for glucose intolerance and type 2 diabetes (SANE & TASKINEN, 1993). In another study, severely increased serum triglyceride concentrations were shown to induce insulin-resistant diabetes mellitus in humans (MINGRONE et al., 1999). In addition, correction of hypertriglyceridemia in obese patients with diabetes has been shown to improve glucose utilization and enhance insulin sensitivity (MINGRONE et al., 1997). In vitro studies show that increased nonesterified fatty acid (NEFA) concentrations result in insulin hypersecretion by cultured islet cells at low glucose concentrations (MILBURN et al., 1995). Collectively, above data suggest that hypertriglyceridemia and/or increased NEFA concentrations can lead to insulin resistance and type 2 diabetes mellitus in humans. The role of hypertriglyceridemia as a risk factor for the development of diabetes mellitus has not been studied in dogs.

6.4.4. Liver disease

In human beings, hypertriglyceridemia has been associated with the development of fatty liver and a condition known as NAFLD (ASSY et al., 2000; ANGULO, 2002; NEUSCHWANDER-TETRI & CALDWELL, 2003; DE BRUIN et al., 2004; BROUWERS et al., 2007). This condition is characterized by excessive lipid deposition in hepatocytes with or without concurrent inflammation, fibrosis, and cirrhosis in the absence of alcohol abuse (ANGULO, 2002). The pathogenesis of fatty liver and NAFLD is not completely understood, but is believed to involve several factors, including hypertriglyceridemia, that can potentially lead to substantial lipid deposition in hepatocytes (ANGULO, 2002; NEUSCHWANDER-TETRI & CALDWELL, 2003). The prevalence of fatty liver in patients with hyperlipidemia, including both hypertriglyceridemia and hypercholesterolemia, has been reported to be about 50% (ASSY et al., 2000; BROUWERS et al., 2007). However, hypertriglyceridemia has been found to be a more useful predictor of fatty liver and, in 1 study, about 70% of patients with hypertriglyceridemia were found to have ultrasonographic evidence of fatty infiltration of the liver or NAFLD (ASSY et al., 2000). Most human patients with NAFLD remain asymptomatic for long periods, and many of these patients have only abnormally high liver enzyme activities as the initial manifestation of NAFLD (ASSY et al., 2000; ANGULO, 2002; NEUSCHWANDER-TETRI & CALDWELL, 2003). Liver enzyme activities are usually only mildly increased (i.e., < 2 times the upper reference limit) and are typically identified during routine screening (ANGULO, 2002; NEUSCHWANDER-TETRI & CALDWELL, 2003). Studies investigating a possible association between hypertriglyceridemia, high serum liver enzyme activities, and liver disease in dogs have not been described.

Clinical studies and anecdotal observations suggest that two conditions of the liver might be associated with hypertriglyceridemia in dogs: vacuolar hepatopathy and gallbladder mucocele (CENTER, 1996b; SCHERK & CENTER 2005). Vacuolar hepatopathy shares some common characteristics with certain types of NAFLD, such as the fact that both can be asymptomatic for long periods and that histopathologically they are characterized by vacuole formation of hepatocytes (CENTER, 1996b; NEUSCHWANDER-TETRI & CALDWELL, 2003; HUBSCHER, 2006).

Gallbladder mucocele has been commonly reported in dog breeds that are predisposed to idiopathic hyperlipidemia (e.g., Miniature Schnauzers and Shetland Sheepdogs) and hyperlipidemia has been implicated in gallbladder disease in humans (BOLAND et al., 2002; PIKE et al., 2004; AGUIRRE et al., 2007). A clear association between the presence of hyperlipidemia and gallbladder mucocele formation had not been described in dogs, however. In a recent study, an association between gallbladder mucocele formation and dyslipidemias (hypertriglyceridemia and hypercholesterolemia) was described in Shetland Sheepdogs (AGUIRRE et al., 2007). In this study, many of the dogs with a gallbladder mucocele were found to have no clinical signs or biochemical abnormalities, except for an increased serum ALP activity in some cases (AGUIRRE et al., 2007). The relationship between different forms of hyperlipidemia and liver and gallbladder diseases in dogs needs further investigation.

6.4.5. Ocular disease

Lipemia retinalis has been reported to occur as a result of severe (typically >1,000 mg/dL) primary or secondary hypertriglyceridemia in humans (SHAH et al., 2001; LU et al., 2005). This condition is characterized by lipid accumulation in the retinal vessels, which turn white and can be differentiated only by their size (SHAH et al., 2001; LU et al., 2005). Vision is usually not affected in this condition (SHAH et al., 2001; LU et al., 2005). Cases of lipemia retinalis have been reported mainly in cats with hypertriglyceridemia due to lipoprotein lipase deficiency, and in one dog with pancreatitis (BRIGHTMAN et al., 1980; JONES et al., 1986a; JONES et al., 1986b). Other ocular manifestations of hyperlipidemia, such as lipemic aqueous and lipid keratopathy have also been reported in dogs (CRISPIN, 1993). Recently, solid intraocular xanthogranuloma formation was reported as a unique disorder of hyperlipidemic Miniature Schnauzers (ZAFROSS & DUBIELZIG, 2007). Diabetic Miniature Schnauzers with hyperlipidemia were also considered to be at increased risk for the development of uveitis and glaucoma (ZAFROSS & DUBIELZIG, 2007).

6.4.6. Other possible complications of hyperlipidemia

Seizures have been reported to occur as a result of hypertriglyceridemia in dogs (ROGERS et al., 1975a; BODKIN, 1992; BAUER, 1995). In a recent study, severe hyperlipidemia (both hypertriglyceridemia and hypercholesterolemia) was reported as a possible cause of neurologic signs in 4 Labrador Retrievers with hypothyroidism (VITALE & OLBY, 2007). In this study, diagnostic imaging studies related neurologic signs to vascular lesions in 2 dogs (VITALE & OLBY, 2007). However, the relationship between hyperlipidemia and neurologic disorders remains obscure in dogs.

Some authors report that hyperlipidemia can cause clinical signs of abdominal pain, lethargy, vomiting, and/or diarrhea, without evidence of pancreatitis or other diseases (FORD, 1993; FORD, 1996). This is highly speculative, however, because published reports are lacking and, given the difficulty in diagnosing pancreatitis especially in past decades, pancreatitis could easily have been missed. Finally, cutaneous xanthomas and peripheral neuropathies have also been reported, mainly in cats with lipoprotein lipase deficiency (JONES et al., 1986b).

6.5. Treatment of hyperlipidemia

The first step in the treatment of canine hyperlipidemia is the determination of whether the patient has a primary or a secondary lipid disorder (ROGERS, 1977; FORD, 1996; JOHNSON, 2005). Thus, specific diagnostic investigation should be performed in order to diagnose or rule out diseases that can cause secondary hyperlipidemia. Treatment of secondary hyperlipidemia relies on successful treatment of the primary disorder, after which hyperlipidemia usually resolves (ROGERS, 1977; WHITNEY, 1992; FORD, 1996; JOHNSON, 2005).

After secondary causes of hyperlipidemia have been ruled out, a presumptive diagnosis of a primary lipid disorder can be made (WHITNEY, 1992). It has been recommended that hypertriglyceridemia that exceeds 500 mg/dL should be treated in order to avoid possible complications (WHITNEY, 1992; FORD, 1996). It also has

been recommended that the treatment goal should be to keep serum triglyceride concentrations below 500 mg/dL (FORD, 1996). Primary hypercholesterolemia is usually associated with less severe complications compared to hypertriglyceridemia. Therefore, correction of hypercholesterolemia is only recommended when serum cholesterol concentrations are severely increased (FORD, 1996).

6.5.1. Dietary management

Typically, the first step in the management of primary hyperlipidemia is dietary modification (ROGERS, 1977; WHITNEY, 1992; FORD, 1996; JOHNSON, 2005). Dogs with primary hyperlipidemia should be offered a low fat diet throughout their lives (FORD, 1996). Especially dogs with hyperchylomicronemia (i.e., most Miniature Schnauzers with primary hyperlipidemia) will typically benefit from low fat diets because chylomicrons are a result of dietary fat absorption (ROGERS, 1977; BAUER, 1995; FORD, 1996). Diets that contain less than 20% fat on a metabolic energy basis are recommended (FORD, 1996; ELLIOTT, 2005; JOHNSON, 2005). Many commercially available diets are suitable for dogs with primary hyperlipidemia. Treats and table scraps should be avoided unless they are low in fat (ELLIOTT, 2005). Serum lipid concentrations should be re-evaluated after feeding a low fat diet for about 4 weeks (FORD, 1996). If serum triglyceride concentrations have decreased to < 500 mg/dL, dietary therapy should be continued and the new diet should be offered for the rest of the animal's life, and serum triglyceride concentrations should be re-evaluated every 6 to 12 months (FORD, 1996). However, some animals will not sufficiently respond to low fat diets. In these cases, an ultra low fat home-made diet (e.g., 10% of fat on a metabolic energy basis) can be offered, or medical treatment can be initiated (ELLIOTT, 2005).

6.5.2. Medical management

Some dogs with primary hyperlipidemia will not sufficiently respond to feeding a low or extra low fat diet alone, especially when hypertriglyceridemia is due to endogenously formed triglycerides (FORD, 1993; WATSON & BARRIE, 1993; BAUER, 1995; FORD, 1996). In these cases, medical treatment is required in

addition to the low fat diet in an effort to effectively reduce serum lipid concentrations (FORD, 1993; WATSON & BARRIE, 1993).

Polyunsaturated fatty acids of the n-3 series (omega-3 fatty acids) are abundant in marine fish (LOGAS et al., 1991). Omega-3 fatty acid supplementation, usually in the form of fish-oil, has been shown to lower serum lipoprotein concentrations in humans with primary hypertriglyceridemia, normal humans, and experimental animals (ILLINGWORTH et al., 1989; SANDERS et al., 1989; FROYLAND et al., 1995; FROYLAND et al., 1996; ADAN et al., 1999; STALENHOEF et al., 2000; OKUMURA et al., 2002). Proposed mechanisms of action include decreased production of VLDL, which contain high concentrations of triglycerides, and inhibition of triglyceride synthesis (LEBLANC et al., 2005). In a recent study of healthy dogs, fish-oil supplementation led to a significant reduction of serum triglyceride concentrations, suggesting that fish oil supplementation could play a role in the treatment of primary canine hypertriglyceridemia (LEBLANC et al., 2005). No major side effects were observed (LEBLANC et al., 2005). However, studies evaluating the efficacy of fish-oil supplementation in dogs with primary hyperlipidemia are lacking and clinical experience is limited. Because side effects are rarely reported and efficacy of omega-3 fatty acids is likely, it is recommended by some authors that fish-oil should be administered to dogs with primary hypertriglyceridemia that do not respond to a low fat diet alone (LOGAS et al., 1991; BAUER, 1995; JOHNSON, 2005; LEBLANC et al., 2005). Menhaden fish-oil capsules have been successfully used at doses ranging from 220 to 330 mg/kg of body weight once a day (BAUER, 1995; LEBLANC et al., 2005). Periodic retesting of serum triglyceride concentrations is recommended during the treatment period.

Gemfibrozil belongs to the group of fibric acid derivatives, and has been reported to reduce serum triglyceride concentrations in both healthy humans and patients with hypertriglyceridemia (BHATNAGAR et al., 1992; SPENCER & BARRADELL, 1996; STALENHOEF et al., 2000). In dogs its use is anecdotal and it is usually administered at a fixed dose of 200 mg/day (BAUER, 1995). Because side effects are believed to be minimal and occur rarely, gemfibrozil is commonly recommended in

combination with dietary therapy when the latter fails to lower serum triglyceride concentrations below 500 mg/dL (BAUER, 1995; WHITNEY, 1992).

Niacin is a vitamin that has been used successfully for the treatment of hyperlipidemia in humans for many years (KASHYAP et al., 2002). In dogs, niacin treatment has been reported in very few patients with primary hypertriglyceridemia. Niacin reduced serum triglyceride concentrations in dogs for several months without causing any side effects (WHITNEY, 1992; BAUER, 1995; JOHNSON, 2005). However, large clinical trials regarding the efficacy and safety of niacin use in dogs with primary hypertriglyceridemia are lacking. As in humans, niacin administration in dogs is potentially associated with side effects such as erythema and pruritus (BAUER, 1995; KASHYAP et al., 2002). Niacin is usually administered at the dose of 25 to 100 mg/day (BAUER, 1995).

III Publications

1. Investigation of hypertriglyceridemia in healthy Miniature Schnauzers

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Running title: Hypertriglyceridemia in Miniature Schnauzers

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1.1. Abstract

Background: Idiopathic hypertriglyceridemia has been reported in Miniature Schnauzers (MS). However, studies investigating the prevalence of this disorder in a large population of MS are lacking.

Hypothesis: Our hypothesis was that hypertriglyceridemia is prevalent in healthy MS.

Animals: 192 healthy MS and 38 healthy dogs of other breeds (control dogs).

Methods: Serum triglyceride and cholesterol concentrations were measured and statistically compared in both the MS and control group. Dogs were categorized based on their age, and median serum triglyceride concentrations were compared among different age groups.

Results: A total of 63 (32.8%) of the 192 MS had serum triglyceride concentrations above the reference range. In contrast, of the 38 control dogs, only 2 (5.3%) had serum triglyceride concentrations above the reference range. The median serum triglyceride concentration in MS was 73.5 mg/dL, which was significantly higher compared to that of the control group (median, 55 mg/dL; $p=0.0005$). Serum cholesterol concentration was above the reference range in 9 (9.0%) of 100 MS and in 2 (5.3%) of the control dogs. Mean serum cholesterol concentrations were not significantly different between the 2 groups ($p=0.1374$). Median serum triglyceride concentrations in MS increased significantly with age ($p<0.0001$), and there was a significant positive correlation between serum triglyceride concentrations and age (Spearman $r=0.47$; $p<0.0001$). There was no difference in serum triglyceride concentrations between male and female MS ($p=0.48$).

Conclusion: Healthy Miniature Schnauzers have a high prevalence of hypertriglyceridemia compared to healthy dogs of other breeds. Both the prevalence and severity of hypertriglyceridemia increase with age.

1.2. Key words

Idiopathic, hypercholesterolemia, hyperlipidemia, dog, prevalence, age.

1.3. Introduction

Hypertriglyceridemia, which refers to abnormally increased serum triglyceride concentrations, is a relatively common clinicopathologic finding in dogs.[1] In a study of 1,022 blood samples from both healthy and diseased dogs of various breeds, 5.4% had increased serum triglyceride concentrations, but the study did not include grossly lipemic samples.[1] Postprandial hypertriglyceridemia is normal and transient, and typically resolves within 7-12 hours after a meal, depending on the fat content of the meal.[2,3] Persistent fasting hypertriglyceridemia is always considered abnormal and can either be primary (idiopathic) or secondary to other diseases or drug administration.[2] Secondary hypertriglyceridemia, which is the most common form in dogs, usually is the result of an endocrine disorder such as hypothyroidism, diabetes mellitus, or hyperadrenocorticism.[4,5] Other possible causes of secondary hypertriglyceridemia include pancreatitis,[6] obesity,[7,8] lymphoma,[9] or administration of certain drugs (e.g., glucocorticoids).[10] A presumptive diagnosis of idiopathic hypertriglyceridemia can only be made when other causes of secondary hypertriglyceridemia have been ruled out. Idiopathic hypertriglyceridemia appears to be associated with specific breeds, most commonly Miniature Schnauzers.[2,11-14] Other breeds, however, as well as mixed breed dogs, also can be affected.[2,15,16] Dogs with severe hypertriglyceridemia might be at increased risk for the development of pancreatitis,[2,11,17] seizures,[11,18] or both, although the relationship between these disorders and hypertriglyceridemia has not been proven.

Idiopathic hypertriglyceridemia has been reported in Miniature Schnauzers in the United States, but has not been documented elsewhere.[11-14] It is characterized by abnormal accumulation of very low density lipoproteins (VLDL) or a combination of VLDL and chylomicrons.[12,13] Although hypercholesterolemia may also be present, this finding is not consistent.[12-14] The cause of hypertriglyceridemia in Miniature Schnauzers remains unclear, but possible mechanisms include increased production of VLDL and chylomicrons, decreased clearance of VLDL and chylomicrons or both. The fact that hypertriglyceridemia is prevalent within a single breed suggests a possible hereditary mechanism.[11-13]

Although it is generally accepted that idiopathic hypertriglyceridemia is a common disorder in Miniature Schnauzers in the United States, and despite the fact that it can potentially be associated with serious diseases (e.g., pancreatitis), it has received limited attention. The assumption that Miniature Schnauzers have a high incidence of primary hypertriglyceridemia compared to other breeds is largely anecdotal or based on small case series.[11,13] Studies investigating the prevalence and clinical implications of the disease in large populations of Miniature Schnauzers are lacking. Also, the effects of age, sex, and reproductive status on serum triglyceride concentrations have not been sufficiently studied in Miniature Schnauzers.

The aim of this study was to determine the prevalence of hypertriglyceridemia in a large population of healthy Miniature Schnauzers, and to further characterize this condition in this breed.

1.4. Materials and Methods

Serum samples were collected from healthy Miniature Schnauzers (Miniature Schnauzer group). A total of 118 Miniature Schnauzer breeders affiliated with the American Miniature Schnauzer Club located in the United States were randomly selected, contacted by e-mail, and notified about the study. Several other Miniature Schnauzer breeders and owners were informed through the breeders initially contacted. Dog owners and breeders interested in this study were asked to contact the Gastrointestinal Laboratory to have their dogs enrolled. Breeders and owners who decided to participate in the study were sent a package containing an ice pack and materials necessary for blood collection and were asked to schedule an appointment with their veterinarian for blood collection. Veterinarians were instructed to collect 5-10 ml of blood into a tube containing no additive, centrifuge the sample after clot formation, separate the serum from the blood clot, transfer the serum into another tube, and send the samples to the Gastrointestinal Laboratory packaged on ice by overnight courier. Breeders and owners were instructed not to feed their dogs for at least 12 hours before the scheduled blood collection. In addition, they were asked to complete a questionnaire for each dog. Information gathered included date of birth, sex, reproductive status (i.e., intact or neutered), body weight, current diet(s), current medication(s), and current and past health status of the dog. Finally, all breeders and

owners signed and returned an informed owner consent form to the Gastrointestinal Laboratory.

Upon receipt, serum samples were immediately aliquoted and stored at -80°C until analysis. Samples were analyzed for serum triglyceride concentration using an enzymatic assay.[a] Samples from some of the Miniature Schnauzers also were analyzed for serum cholesterol concentration using an enzymatic assay.[a] Questionnaires from all dogs were reviewed. Inclusion criteria consisted of absence of clinical signs of any disease for at least 3 months before blood collection, absence of a history of chronic diseases that might affect lipid metabolism (e.g., endocrine disorders), and absence of current medications that may affect lipid metabolism (e.g., glucocorticoids).

A total of 38 healthy dogs served as controls. Of the 38 dogs, 20 were pure bred dogs consisting of 12 breeds, and 18 dogs were mixed breed dogs. The breeds in the control group included Labrador Retriever (3), Boxer (2), Border Collie (2), Boston Terrier (2), Golden Retriever (2), German Shepherd (1), English Mastiff (1), Alaskan Malamute (1), Basset Hound (1), Bloodhound (1), Redbone Coonhound (1), Weimaraner (1), Blue Lacy (1), Whippet (1) and 18 mixed breed dogs. Due to the fact that Beagles have been reported to have a familial form of hyperlipoproteinemia, dogs of this breed were not included in this study.[2,16] Control dogs were owned by students and staff of the College of Veterinary Medicine and Biomedical Sciences at Texas A&M University. Owners had been asked not to feed their dogs for at least 12 hours before blood collection. In addition, owners were asked to fill out a short questionnaire similar to the one used for the Miniature Schnauzers and were asked to sign an informed owner consent form. Blood samples were collected and, after clot formation and centrifugation at 3,000 rpm for 15 min, serum was transferred to a separate tube and stored at - 80°C until further use. Samples were analyzed for the same constituents as were the samples from the Miniature Schnauzers.

For statistical analyses, the total number of Miniature Schnauzers and dogs of the control group with serum triglyceride concentrations above the upper limit of the reference range were recorded. Also, the total number of Miniature Schnauzers and

dogs of the control group with serum cholesterol concentrations above the upper limit of the reference range were recorded. For the purposes of this study, Miniature Schnauzers were divided into 3 subgroups based on their serum triglyceride concentration: Miniature Schnauzers with normal (reference range: 26-108 mg/dL), mildly increased (109-400 mg/dL), and moderately to severely increased (>400 mg/dL) serum triglyceride concentrations. Although generally accepted criteria for categorization of the severity of hypertriglyceridemia are not available, a previously suggested categorization was used here.[19] All data were tested for normal distribution using the Kolmogorov-Smirnov test. Median serum triglyceride and mean serum cholesterol concentrations were compared between the Miniature Schnauzers and the control group using a Mann - Whitney test. The proportion of Miniature Schnauzers with serum triglyceride concentrations above the upper limit of the reference range was compared with the proportion of control dogs with serum triglyceride concentrations above the reference range by use of a Fisher's exact test. Odds ratios with their 95% confidence intervals for having an increased serum triglyceride concentration also were calculated for the above data.

Miniature Schnauzers and control dogs were categorized by age into 5 subgroups (group 1: < 1 year, group 2: 1 to 3 years, group 3: 3 to 6 years, group 4: 6 to 9 years, and group 5: > 9 years). The number of Miniature Schnauzers in each age group was: 26 for group 1, 51 for group 2, 52 for group 3, 23 for group 4, and 30 for group 5; the number of control dogs in each age group was: 4 for group 1, 12 for group 2, 11 for group 3, 3 for group 4 and 3 for group 5. Comparison of serum triglyceride concentrations among the age classes was based on a Kruskal-Wallis test followed by a Dunn's multiple comparison test. The percentages of Miniature Schnauzers and control dogs with serum triglyceride concentrations above the upper limit of the reference range, as well as above 400 mg/dL, in each age group were recorded. Also, to investigate whether a systematic change in serum triglyceride concentrations occurred with age, data were analyzed for correlation between serum triglyceride concentrations and age in both the Miniature Schnauzers and the control group. A Fisher's exact test was used to determine whether serum triglyceride concentration was associated with sex or reproductive status.

For analysis of data sets that were normally distributed, unpaired t-tests and one way ANOVAs were used. All statistical analyses were performed using a statistical software package[b] and a p value of < 0.05 was considered significant.

1.5. Results

Serum samples were collected from a total of 213 Miniature Schnauzers. Of those, 192 were suitable (based on their medical history) for enrollment into the study. The 21 dogs that were not enrolled in the study had clinical signs at the time of blood collection or within <3 months before blood collection. Data on sex and reproductive status were available for 187 of the 192 dogs; 114 (61%) of these dogs were female and 73 (39%) were male. Forty one (36%) females were spayed and 34 (46.6%) males were castrated. Age was available for 182 Miniature Schnauzers and the median age was 45 months (range, 4-161 months). Of the 38 dogs in the control group, 17 were female (1 intact and 16 spayed) and 21 were male (2 intact and 19 castrated). Age was available for 33 dogs (5 dogs were rescue dogs of unknown age), and the median age was 42.5 months (range, 2-138 months). There was no significant difference between the median ages of the Miniature Schnauzer and control groups (p=0.7106).

A total of 63 (32.8%) of the 192 Miniature Schnauzers had serum triglyceride concentrations above the upper limit of the reference range. Of the 38 control dogs, only 2 (5.3%) had serum triglyceride concentrations above the upper limit of the reference range. Forty one (21.3%) of the 192 Miniature Schnauzers had mild increases in serum triglyceride concentration (109-400 mg/dL), and 22 (11.5%) had moderate to severe increases in serum triglyceride concentrations (>400 mg/dL). None of the control dogs had moderately to severely increased serum triglyceride concentrations (>400 mg/dL). The odds ratio for Miniature Schnauzers to have serum triglyceride concentrations above the upper limit of the reference range when compared to the control group was 8.8 (p=0.0003, 95% CI 2.1 – 37.7).

Serum cholesterol concentrations were measured in a total of 100 Miniature Schnauzers. Sixty of them had normal serum triglyceride concentrations, 20 had mildly increased serum triglyceride concentrations, and 20 had moderately to severely increased serum triglyceride concentrations. A total of 9 (9%) Miniature Schnauzers

had serum cholesterol concentrations above the upper limit of the reference range (reference range, 124-335 mg/dL), but none (0%) of the 60 Miniature Schnauzers with normal serum triglyceride concentrations had a serum cholesterol concentration above the upper limit of the reference range. Of the 20 Miniature Schnauzers with mildly increased serum triglyceride concentrations only 1 (5%) had a serum cholesterol concentration above the upper limit of the reference range, whereas of the 20 Miniature Schnauzers with moderately to severely increased serum triglyceride concentrations 8 (40%) had serum cholesterol concentrations above the upper limit of the reference range. In the control group, 2 (5.3%) dogs had serum cholesterol concentrations above the upper limit of the reference range; one of these dogs also had a serum triglyceride concentration above the reference range.

All data sets, except for serum cholesterol concentrations, failed normality testing and non-parametric methods were used for further analysis of the data sets. The median serum triglyceride concentration in the Miniature Schnauzer group was 73.5 mg/dL (range, 24 – 3,125 mg/dL), which was significantly higher than the median serum triglyceride concentration of the control group (median, 55 mg/dL; range, 24 – 205 mg/dL; $p=0.0005$; Figure 1). Mean serum cholesterol concentration in Miniature Schnauzers was 214 mg/dL, which was not significantly different from the mean serum triglyceride concentration in the control group (mean, 233 mg/dL; $p=0.1374$; Figure 2). However, one-way ANOVA analysis showed that the mean serum cholesterol concentration in the 20 Miniature Schnauzers with moderately to severely increased serum triglyceride concentrations was significantly higher than the mean serum cholesterol concentration in the 60 Miniature Schnauzers with normal serum triglyceride concentrations ($p<0.001$) and that of the control group ($p<0.001$); differences between the 20 Miniature Schnauzers with moderately to severely increased serum triglyceride concentrations and the 20 Miniature Schnauzers with mildly increased serum triglyceride concentrations were not significant (Figure 3).

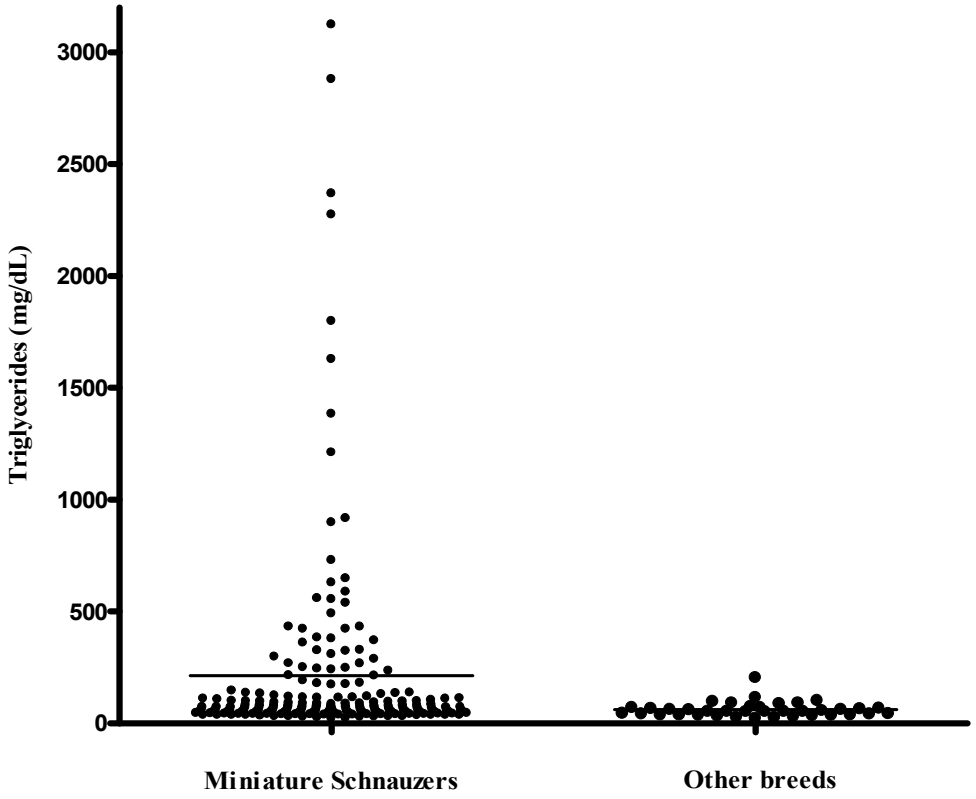

Figure 1. Distribution of serum triglyceride concentrations.

Scatter plot showing the distribution of serum triglyceride concentrations in Miniature Schnauzers and in a group of dogs of other breeds. The line represents the median of each group (p=0.0005).

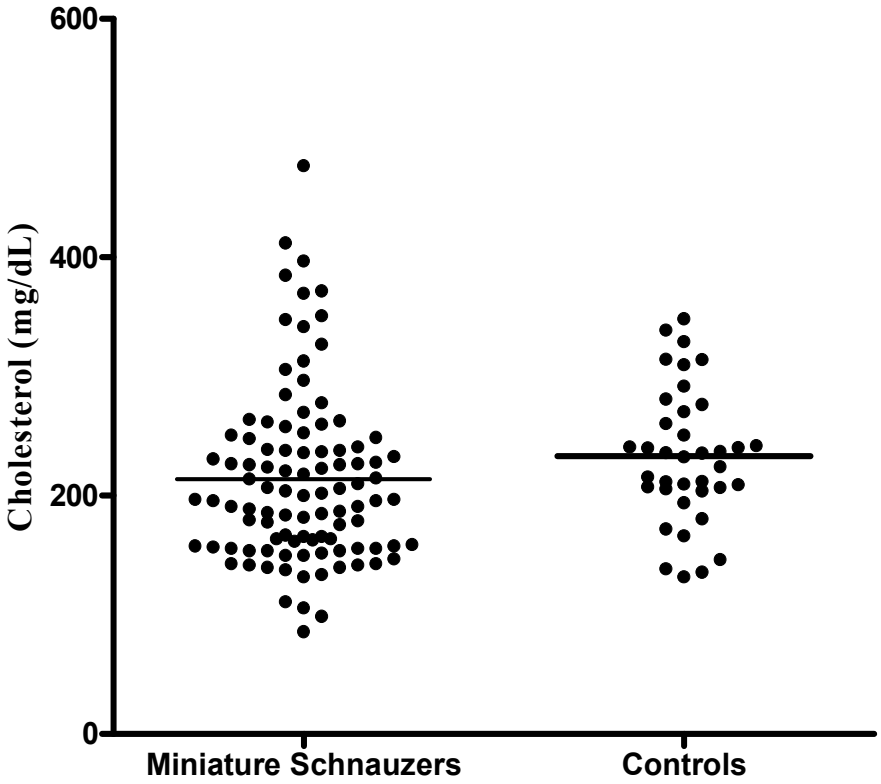

Figure 2. Distribution of serum cholesterol concentrations.

Scatter plot showing the distribution of serum cholesterol concentrations in Miniature Schnauzers and in a group of dogs of other breeds. The line represents the mean of each group (p=0.1374).

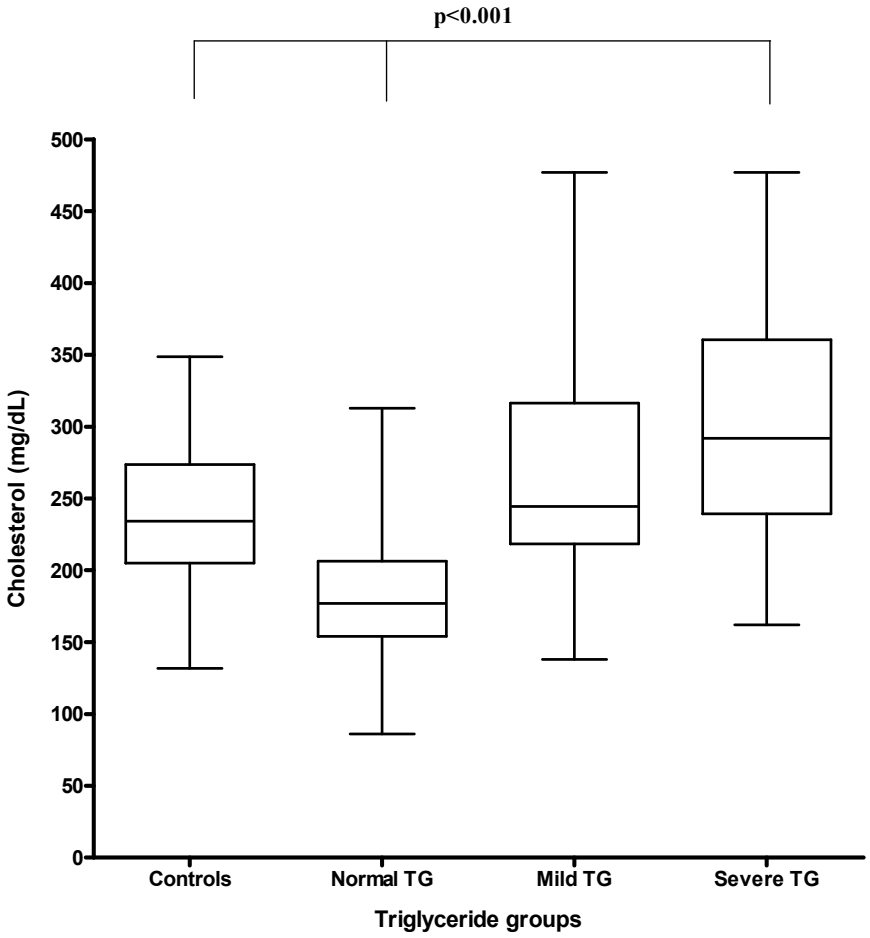

Figure 3. Differences in serum cholesterol concentrations among groups.

Boxplots of serum cholesterol concentrations (mg/dL) for different triglyceride (TG) groups in Miniature Schnauzers. The line in the rectangle corresponds to the mean, the upper and lower limits of the rectangle correspond to the 25th and 75th percentile, respectively, and the whiskers correspond to the lowest and highest values. The p-values show the significance of the differences in serum cholesterol concentrations between groups compared to the group with severely increased serum triglyceride concentrations (Severe TG).

The Kruskal-Wallis test indicated that median serum triglyceride concentrations in Miniature Schnauzers differed significantly among the age classes ($p<0.0001$; Figure 4). In particular, median serum triglyceride concentrations of Miniature Schnauzers in the age class > 9 years differed significantly from the age classes of < 1 year ($p<0.001$), 1 to 3 years ($p<0.001$), and 3 to 6 years ($p<0.001$). In addition, median serum triglyceride concentrations of Miniature Schnauzers in the age class < 1 year differed significantly from the age class 6 – 9 years (Figure 4). Also, the percentages of Miniature Schnauzers with serum triglyceride concentrations above the reference range appeared to increase with age (Figure 5). Only 15.4% of dogs < 1 year of age and 15.7% of dogs 1 to 3 years of age had serum triglyceride concentrations above the reference range, whereas 43.5% of dogs 6 to 9 years of age and 76.7% of dogs > 9 years of age did. In addition, there appeared to be an association between the degree of hypertriglyceridemia and age. Only 3.9% and 3.8% of dogs 1 to 3 and 3 to 6 years of age, respectively, had serum triglyceride concentrations > 400 mg/dL, whereas 17.4% of dogs 6 to 9 years of age and 46.7% of dogs > 9 years of age did (Figure 6). Also, 18 of 22 (81.8%) Miniature Schnauzers with moderate to severe hypertriglyceridemia were ≥ 6 years of age, and 14 (63.6%) were ≥ 9 years of age. There was a significant positive correlation between serum triglyceride concentration and age in Miniature Schnauzers ($p<0.0001$, Spearman $r = 0.47$). The Kruskal-Wallis test did not identify significant differences of median serum triglyceride concentrations among the age classes in the control group ($p=0.2235$). There was no significant correlation between serum triglyceride concentration and age in the control dogs ($p=0.6697$; Spearman $r= -0.077$).

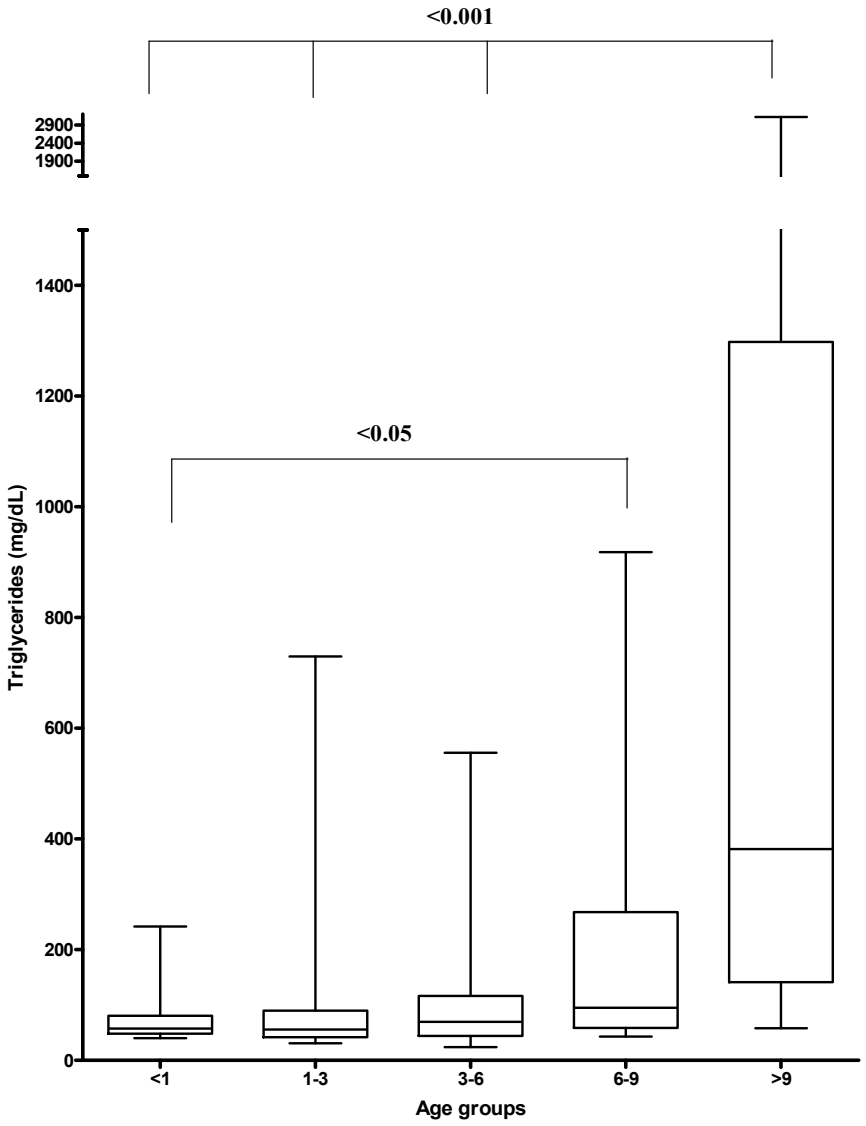

Figure 4. Serum triglyceride concentrations in different age groups in Miniature Schnauzers.

Boxplots of serum triglyceride concentrations (mg/dL) for different age groups in Miniature Schnauzers. The line in the rectangle corresponds to the median, the upper and lower limits of the rectangle correspond to the 25th and 75th percentile, respectively, and the whiskers correspond to the lowest and highest values. The p-values show the significance of the differences in serum triglyceride concentrations among age groups (Note: the Y-axis is split).

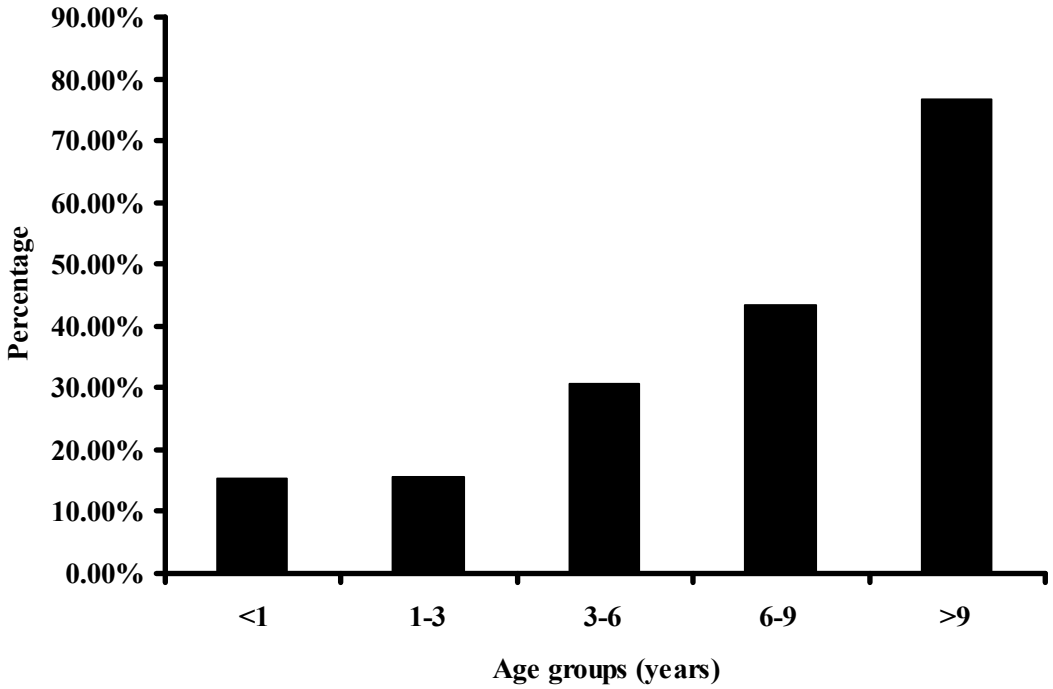

Figure 5. Percentages of healthy Miniature Schnauzers with hypertriglyceridemia in different age groups.

Percentages of healthy Miniature Schnauzers with hypertriglyceridemia (serum triglyceride concentrations > 108 mg/dL) in different age groups.

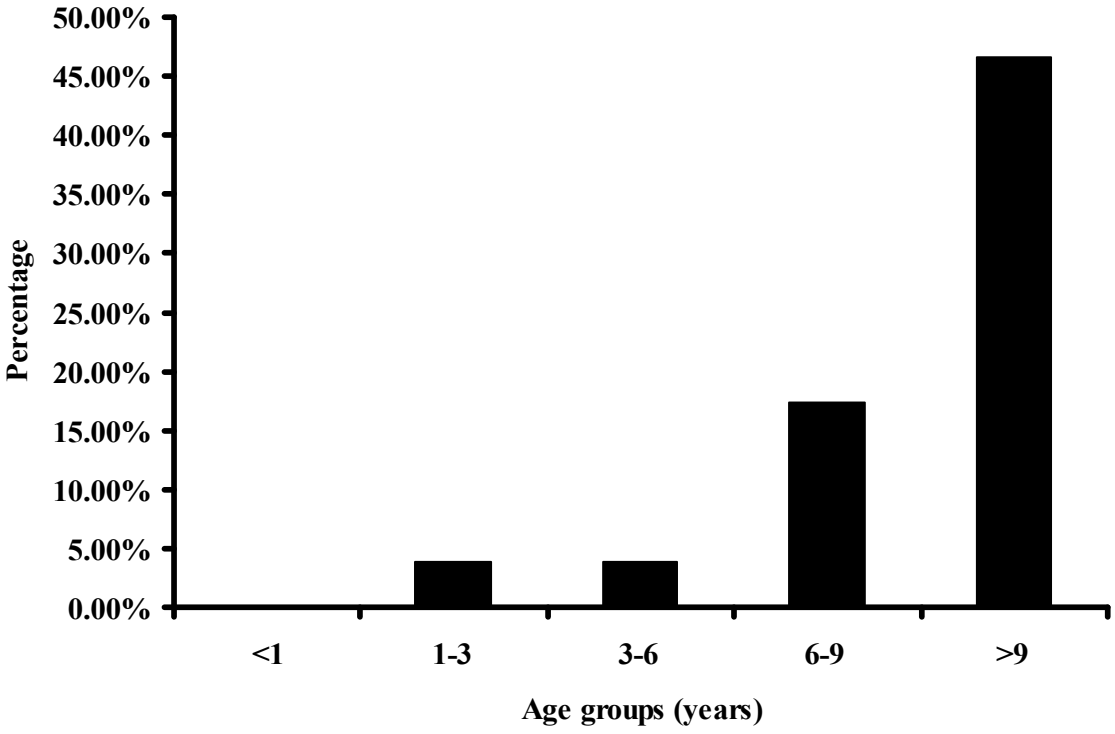

Figure 6. Percentages of healthy Miniature Schnauzers with moderate to severe hypertriglyceridemia in different age groups.

Percentages of Miniature Schnauzers with moderate to severe hypertriglyceridemia (triglycerides > 400 mg/dL) in different age groups.

There was no difference in proportions of Miniature Schnauzers with serum triglyceride concentrations above the upper limit of the reference range ($p = 0.48$) or in median serum triglyceride concentrations ($p = 0.46$) between male and female Miniature Schnauzers. There were significant differences in the proportions of Miniature Schnauzers with serum triglyceride concentrations above the upper limit of the reference range between spayed and intact females ($p<0.0001$) and between castrated and intact males ($p = 0.0166$). However, there were significant differences in median ages between these groups (spayed and intact females: $p<0.0001$; castrated and intact males: $p = 0.0248$), with neutered animals being older than the intact ones.

1.6. Discussion

Based on the results of the present study, it can be concluded that hypertriglyceridemia is a common finding in healthy Miniature Schnauzers in the United States, because about one-third of the enrolled Miniature Schnauzers had serum triglyceride concentrations above the upper limit of the reference range. Also, 11.5% of the Miniature Schnauzers in this study had moderate to severe hypertriglyceridemia which, based on some authors, is more likely to be associated with complications such as pancreatitis or seizures.[12] Hypertriglyceridemia does not appear to be a common finding in healthy dogs of other breeds. In addition, healthy control dogs had only mild increases in serum triglyceride concentrations if they had a serum triglyceride concentration above the upper limit of the reference range.

Miniature Schnauzers enrolled in this study came from many different locations within the United States, thus minimizing the possibility of selection of populations that were more inbred than the general Miniature Schnauzer population. Thus, findings of this study are believed to reflect the general population of Miniature Schnauzers in the United States.

Although the possibility that hypertriglyceridemia in some of the Miniature Schnauzers in the present study might have been the result of secondary causes (e.g., hyperadrenocorticism) cannot be excluded, we feel that this explanation is unlikely because all dogs were free of clinical signs for at least 3 months before blood collection, had no known chronic diseases, and were not receiving any medications

that are known to or suspected to affect serum triglyceride concentrations. In addition, dogs of the control group, which were enrolled based on the same inclusion criteria, had a significantly lower prevalence of hypertriglyceridemia.

Information regarding each dog's current diet (as well as the length of time each dog had received that diet) was available for the majority of the dogs in this study. However, many dogs were on home made or multiple diets, and it was not possible to determine the lipid content of these diets. It is possible that some Miniature Schnauzers with normal serum triglyceride concentrations were on low fat diets, and that the true percentage of Miniature Schnauzers with hypertriglyceridemia is even higher than reported here. Information regarding the body condition score of each dog would have been interesting because obesity might affect serum triglyceride concentrations,[7] but this information was not available in the present study.

Most of the affected Miniature Schnauzers had mildly increased serum triglyceride concentrations, but a considerable percentage (11.5%) had moderate to severely increased serum triglyceride concentrations. As mentioned earlier, dogs with severe hypertriglyceridemia might be at increased risk for the development of pancreatitis,[2,11,17] seizures,[11,18] or both, although the relationship between these disorders has not been proven. In addition, because hypertriglyceridemia is a common feature of other (mostly endocrine) diseases, the findings of this study are important to veterinarians who may be evaluating hypertriglyceridemia in Miniature Schnauzers. A large proportion of healthy Miniature Schnauzers have mild, moderate, or even severe increases in serum triglyceride concentrations, and further investigation of hypertriglyceridemia (e.g., ACTH stimulation test, low dose dexamethasone suppression test, determination of serum thyroid hormone concentration) may not be indicated in the absence of compatible clinical and clinicopathological findings in Miniature Schnauzer dogs.

There currently is no evidence to support the hypothesis that the source (rather than the severity) of hypertriglyceridemia can affect the risk for developing secondary disorders. It also should be noted that if the severity of hypertriglyceridemia correlates with the potential for developing complications secondary to hypertriglyceridemia,

Miniature Schnauzers that have idiopathic hypertriglyceridemia might be at even higher risk for developing complications of hypertriglyceridemia if they develop diseases that lead to secondary hypertriglyceridemia (e.g., diabetes mellitus) as this occurrence would potentially lead to additional increases in serum triglyceride concentrations.

In Miniature Schnauzers, serum cholesterol concentrations above the upper limit of the reference range were noted only in association with moderate to severe hypertriglyceridemia and, in 1 dog, with mild hypertriglyceridemia. None of the Miniature Schnauzers with normal serum triglyceride concentrations had serum cholesterol concentrations above the upper limit of the reference range. Also, many Miniature Schnauzers with hypertriglyceridemia had normal serum cholesterol concentrations. These findings indicate that hypercholesterolemia is variably present in Miniature Schnauzers with hypertriglyceridemia. Hypercholesterolemia may be a secondary feature that accompanies moderate to severe hypertriglyceridemia in Miniature Schnauzers resulting from decreased clearance, increased production, or both, of chylomicrons and VLDL because these 2 molecules also contain small amounts of free and esterified cholesterol.[2]

Both the percentages of Miniature Schnauzers with hypertriglyceridemia and the degree of hypertriglyceridemia increased with age. More than 75% of Miniature Schnauzers ≥ 9 years of age had increased serum triglyceride concentrations, and the vast majority (>80%) of Miniature Schnauzers with moderate to severe hypertriglyceridemia were 6 years or older. This observation suggests that later development of hypertriglyceridemia cannot be excluded in young Miniature Schnauzer dogs found to have normal serum triglyceride concentrations. A genetic marker may help identify affected dogs before development of hypertriglyceridemia. However, although not very common, some young dogs may present with hypertriglyceridemia.

Due to the fact that only 1 serum sample was obtained from each dog in the present study, it was not possible to determine whether idiopathic hypertriglyceridemia is consistently present in affected Miniature Schnauzers. However, in the authors'

experience and based on a few published cases,[13] most affected Miniature Schnauzers remain hypertriglyceridemic throughout their lives unless low fat diets are fed consistently. In order to better understand the evolution of serum triglyceride concentrations in aging Miniature Schnauzers, further studies in selected groups of Miniature Schnauzers are required.

The almost even distribution of hypertriglyceridemia between male and female Miniature Schnauzers in the present study suggests that idiopathic hypertriglyceridemia is not a sex-linked disorder. X-linked inheritance would lead to many more affected males than females, whereas Y-linked inheritance would produce only affected males. However, such is not the case for idiopathic hypertriglyceridemia in this study. Although significant differences in serum triglyceride concentrations were detected between neutered and intact animals, this finding seems to be the result of age differences between the 2 groups.

In human medicine, hereditary causes of hypertriglyceridemia are well documented.[20] Familial hypertriglyceridemia (transmitted as an autosomal dominant disorder of unknown etiology), lipoprotein lipase deficiency (transmitted as an autosomal recessive disorder), and familial apolipoprotein C-II deficiency (transmitted as an autosomal recessive disorder) are disorders with a genetic basis that all lead to increases in serum triglyceride concentrations due to increased concentrations of VLDL, chylomicrons, or both.[20] Hypertriglyceridemia in human beings often is asymptomatic but also has been associated with coronary heart disease, pancreatitis, non-alcoholic hepatic lipidosis, and metabolic syndrome.[20]

The cause of idiopathic hypertriglyceridemia in Miniature Schnauzers has not yet been identified. Regardless of the mechanism, an underlying genetic defect is suspected.[11-13] Because lipoprotein lipase is the major enzyme involved in triglyceride clearance, deficiency of this enzyme has been considered a possible cause of hypertriglyceridemia. Two studies that included both Miniature Schnauzers and dogs of other breeds with idiopathic hyperlipidemia found that lipoprotein lipase activity was significantly reduced in hyperlipidemic dogs compared to healthy controls.[c,d] However, a recent study in Miniature Schnauzers with hypertriglyceridemia and

pancreatitis failed to identify any mutations of the lipoprotein lipase gene, suggesting that inherited lipoprotein lipase dysfunction may not be the cause of hypertriglyceridemia in this breed.[e] Further studies are warranted in order to identify the genetic basis of idiopathic hypertriglyceridemia in Miniature Schnauzers.

In conclusion, results of the present study suggest that idiopathic hypertriglyceridemia is a common finding in healthy Miniature Schnauzers in the United States. There is no difference between male and female Miniature Schnauzers with regard to the prevalence of hypertriglyceridemia. Both the prevalence and severity of hypertriglyceridemia in Miniature Schnauzers increase with age. Due to the high prevalence of this disorder in Miniature Schnauzers, extensive diagnostic work-up in order to identify the cause of hypertriglyceridemia probably is not necessary in otherwise healthy Miniature Schnauzer dogs. However, on the basis of the results of the present study, all Miniature Schnauzers in the United States should potentially be evaluated for hypertriglyceridemia while they are healthy, because this information may be useful for the avoidance of misinterpretation of increased serum triglyceride concentrations when the dogs are presented sick. In addition, by knowing the serum triglyceride status of the dog, veterinarians might consider offering low fat diets to affected dogs in order to avoid possible complications of hypertriglyceridemia. Due to the fact that hypercholesterolemia was found only in association with hypertriglyceridemia, the presence of hypercholesterolemia alone in Miniature Schnauzers might require additional diagnostic investigation. Genetic studies are warranted in order to obtain further information about the genetic basis of this disorder. In addition, the association between hypertriglyceridemia and diseases such as pancreatitis and seizures remains to be determined.

1.7. Footnotes

[a]Roche/Hitachi MODULAR *ANALYTICS* D 2400 module, Roche Diagnostics, Indianapolis, IN

[b]Prism4, GraphPad, San Diego, CA

[c]Schenck PA. Lipoprotein lipase and hepatic lipase activity in dogs with primary hyperlipoproteinemia. J Vet Int Med 2002, 17:386 (abstract)

[d]Jaeger JQ, Jonson S, Hinchcliff KW, Sherding R, Jensen W, Brunzell J, Murdock S. Characterization of biochemical abnormalities in idiopathic hyperlipidemia of Miniature Schnauzer dogs. J Vet Int Med 2003, 16:394 (abstract)

[e]Schickel R. Identification of the nucleotide sequence of the lipoprotein lipase gene as well as its role in the developmet of hyperlipidemia and pancreatitis in the Miniature Schnauzer. 2005. Ludwig-Maximilians University (Dr.med.vet. thesis)

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2. Serum liver enzyme activities in healthy Miniature Schnauzers with and without hypertriglyceridemia

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2.1. Abstract

Objective—To determine whether hypertriglyceridemia in healthy Miniature Schnauzers was associated with high serum liver enzyme activities.

Design—Cross-sectional study.

Animals—65 Miniature Schnauzers with normal serum triglyceride concentrations (group 1), 20 Miniature Schnauzers with slightly high serum triglyceride concentrations (group 2), and 20 Miniature Schnauzers with moderately to severely high serum triglyceride concentrations (group 3).

Procedures—Questionnaires regarding each dog's medical history were completed, and serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT) activities were measured.

Results—Median serum ALP activity was significantly higher in group 3 than in group 1 or 2 dogs, but was not significantly higher in group 2 than in group 1 dogs. Median serum ALT activity was significantly higher in group 3 than in group 1 dogs, but was not significantly different between any of the other groups. Compared with group 1 dogs, group 2 and 3 dogs were significantly more likely to have high serum ALP activity (odds ratio, 26.2 and 192.6, respectively). Group 3 dogs also were significantly more likely to have high serum ALT activity (odds ratio, 8.0), serum AST activity (odds ratio, 3.7), and serum GGT activity (odds ratio, 11.3), compared with group 1 dogs. Group 3 dogs were significantly more likely (odds ratio, 31.0) to have ≥ 2 high serum liver enzyme activities than were group 1 dogs.

Conclusions and Clinical Relevance—Results suggested that moderate to severe hypertriglyceridemia was associated with high serum liver enzyme activities in Miniature Schnauzers.

2.2. Abbreviations

VLDL	Very low density lipoproteins
NAFLD	Nonalcoholic fatty liver disease
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase

GGT γ-Glutamyltransferase

2.3. Introduction

Persistent hypertriglyceridemia is reportedly common in healthy Miniature Schnauzers in the United States,[1-4] with hypertriglyceridemia identified in 63 of 192 (32.8%) healthy Miniature Schnauzers in 1 study.[4] Hypertriglyceridemia in Miniature Schnauzers is characterized by an abnormal accumulation of VLDL or a combination of VLDL and chylomicrons, with or without hypercholesterolemia.[2,3] The cause of this condition remains unclear, but possible mechanisms include increased production or decreased clearance of VLDL and chylomicrons.[2] Dogs with severe hypertriglyceridemia have been suspected to be at increased risk for development of pancreatitis[1,5-8] and seizures,[1,9] although a relationship between hypertriglyceridemia and these disorders has not been proven.

In human beings, hypertriglyceridemia has been associated with development of fatty liver and a condition known as NAFLD.[10-14] This condition is characterized by excessive lipid deposition in the hepatocytes with or without concurrent inflammation, fibrosis, and cirrhosis in the absence of alcohol abuse.[10] The pathogenesis of fatty liver and NAFLD is not completely understood but is believed to involve several factors, including hypertriglyceridemia, that can potentially lead to substantial lipid deposition in hepatocytes.[10,11] The prevalence of fatty liver in patients with hyperlipidemia, including both hypertriglyceridemia and hypercholesterolemia, has been reported to be about 50%.[12,14] However, hypertriglyceridemia has been found to be a more useful predictor of fatty liver and, in 1 study,[12] about 70% of patients with hypertriglyceridemia were found to have ultrasonographic evidence of fatty infiltration of the liver or NAFLD. Most human patients with NAFLD remain asymptomatic for long periods, and many of these patients have only abnormally high liver enzyme activities as the initial manifestation of NAFLD.[10-12] Liver enzyme activities are usually only mildly high (ie, < 2 times the upper reference limit), with high values typically identified during routine screening.[10,11] To the authors' knowledge, studies investigating a possible association between hypertriglyceridemia, high serum liver enzyme activities, and liver disease in dogs have not been described.

Our hypothesis was that hypertriglyceridemia in overtly healthy Miniature Schnauzers might be associated with high serum liver enzyme activities. Thus, the purpose of the study reported here was to determine whether hypertriglyceridemia in healthy Miniature Schnauzers was associated with high serum liver enzyme activities. Specifically, we wanted to determine whether serum liver enzyme activities in Miniature Schnauzers with hypertriglyceridemia were significantly different from activities in Miniature Schnauzers with normal serum triglyceride concentrations.

2.4. Materials and Methods

Serum samples from 105 healthy Miniature Schnauzers were used in the study. All samples had been collected as part of a separate study[4] of healthy Miniature Schnauzers from various parts of the United States. Breeders and owners who had agreed to participate in the previous study were sent a package containing an ice pack and materials necessary for blood collection and were asked to schedule an appointment with their veterinarian for blood collection. Veterinarians were instructed to collect 5 to 10 mL of blood and to submit serum samples on ice to the Gastrointestinal Laboratory at Texas A&M University by overnight courier. Breeders and owners were instructed not to feed their dogs for at least 12 hours before blood samples were collected. In addition, they were asked to complete a questionnaire for each dog that requested information regarding date of birth, sex, neuter status, body weight, current diet, current medications, and current and past health status of the dog. Finally, all breeders and owners were requested to sign and return an informed consent form.

On receipt, serum samples were immediately divided into aliquots and stored at -80^{o}C until analyzed. Serum triglyceride concentration was measured with an enzymatic assay,[4,a] and serum ALT, AST, ALP, and GGT activities were measured by means of spectrophotometric methods with automated equipment.[b] Questionnaires were reviewed, and dogs were included in the study only if they had not had any clinical signs of disease for at least 3 months prior to blood collection, did not have any history of chronic diseases that might affect lipid metabolism (eg, endocrine

disorders), did not have any history of liver disease, and were not currently receiving any medications that may affect lipid metabolism or liver enzyme activities.

For purposes of the present study, Miniature Schnauzers were divided into 3 groups on the basis of serum triglyceride concentration. Group 1 consisted of 65 Miniature Schnauzers in which serum triglyceride concentration was within reference limits (26 to 108 mg/dL), group 2 consisted of 20 Miniature Schnauzers in which serum triglyceride concentration was slightly high (109 to 400 mg/dL), and group 3 consisted of 20 Miniature Schnauzers in which serum triglyceride concentration was moderately to severely high (> 400 mg/dL). Because liver enzyme activities might increase with age, a subgroup of group 1 (group 1B) was created that consisted of 26 dogs that were ≥ 5 years old so that median age of group 1B dogs was similar to median age for group 3 dogs.

Of the 65 dogs in group 1, 43 were female (16 spayed), and 21 were male (8 castrated); sex of 1 dog was not reported. Twelve group 2 dogs were female (7 spayed), and 8 were male (5 castrated), and 13 group 3 dogs were female (12 spayed), and 7 were male (5 castrated). Sixteen of the group 1B dogs were female (8 spayed), and 10 were male (4 castrated).

Because lipemia reportedly can interfere with certain serologic assays, resulting in falsely high or low results, 8 lipemic samples from group 3 dogs were tested for serum triglyceride concentrations and liver enzyme activities before and after centrifugation at 20,000 × *g* for 15 minutes.

Statistical analysis—Data were tested for normal distribution by use of the Kolmogorov-Smirnov test. Because data were not normally distributed, the Kruskal-Wallis test followed by the Dunn multiple comparison procedure was used to compare median age and median serum ALP, ALT, AST, and GGT activities among groups. Proportions of dogs in each group with serum ALP, ALT, AST, or GGT activities greater than the upper reference limit were compared among groups by use of the Fisher's exact test. Similarly, the Fisher's exact test was used to compare proportions

of dogs with ≥ 2 serum liver enzyme activities greater than the upper reference limit among groups. Odds ratios and their 95% confidence intervals (CI) were calculated for proportions of dogs with serum liver enzyme activities greater than the upper reference limit. To determine whether a systematic change in serum liver enzyme activities occurred with increasing serum triglyceride concentrations, data were analyzed for correlations between serum activity of each enzyme and serum triglyceride concentrations by means of the Spearman correlation. Finally, paired *t* tests were used to analyze serum liver enzyme activities obtained for the 8 lipemic samples before and after centrifugation. All statistical analyses were performed with standard statistical software.[c] Values of $P < 0.05$ were considered significant.

2.5. Results

For the 8 lipemic samples, no significant differences were found between mean ALP ($P = 0.444$), ALT ($P = 0.882$), AST ($P = 0.101$), and GGT ($P = 0.509$) activities obtained before and after centrifugation.

Median serum triglyceride concentration was 60 mg/dL (range, 24 to 105 mg/dL) for dogs in group 1, 247 mg/dL (range, 113 to 380 mg/dL) for dogs in group 2, 690 mg/dL (range, 423 to 3,125 mg/dL) for dogs in group 3, and 72 mg/dL (range, 30 to 100 mg/dL) for dogs in group 1B. Median age was 51 months (range, 7 to 151 months) for dogs in group 1, 70 months (range, 8 to 151 months) for dogs in group 2, 112 months (range, 18 to 161 months) for dogs in group 3, and 88 months (range, 61 to 151 months) for dogs in group 1B. There was no significant difference in median age between groups 1 and 2, but there was a significant ($P < 0.001$) difference in median age between groups 1 and 3. Median age for group 1B was not significantly ($P = 0.071$) different from median age for group 3.

Median serum ALP activity was significantly higher in group 3 dogs (202.5 U/L) than in group 1 dogs (27 U/L; $P < 0.001$), group 1B dogs (36 U/L; $P < 0.001$), or group 2 dogs (33 U/L; $P < 0.05$), but was not significantly higher in group 2 dogs than in group 1 dogs **(Figure 1)**. Median serum ALT activity was significantly higher in group 3 dogs (70.5 U/L) than in group 1 dogs (43 U/L; $P < 0.01$), but was not

significantly different between any of the other groups **(Figure 2)**. No significant differences were found in median serum AST and GGT activities between any of the groups.

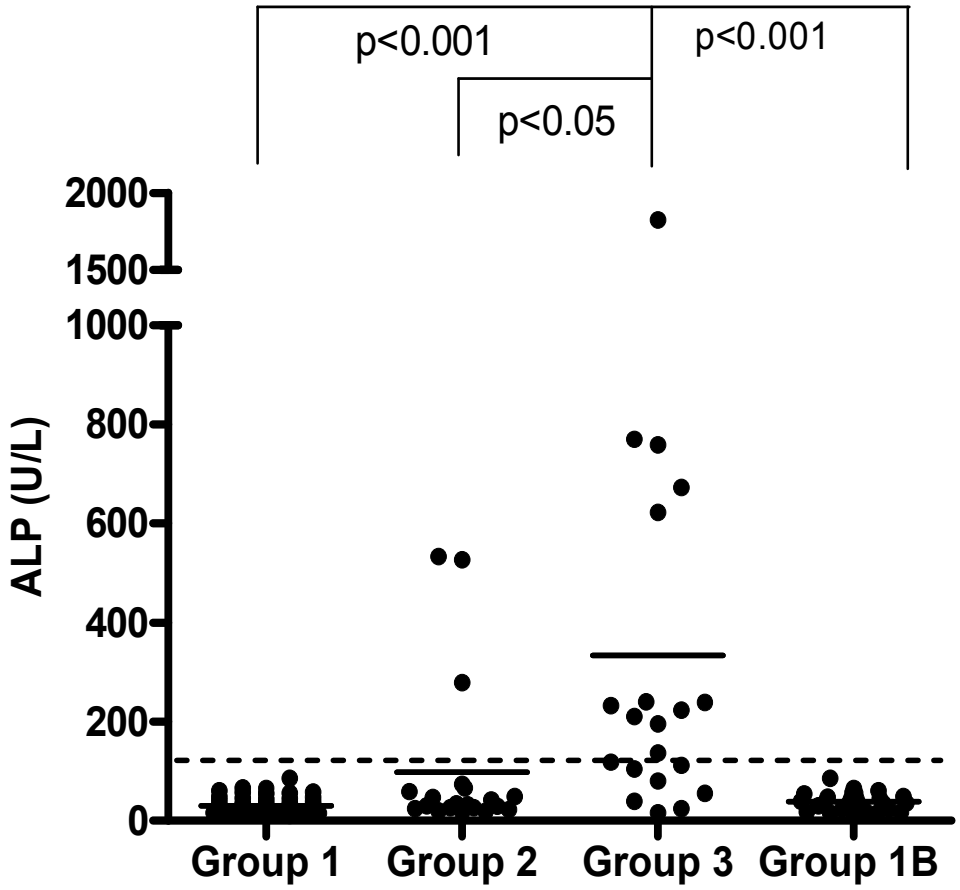

Figure 1—Serum ALP activities in healthy Miniature Schnauzers.

Scatterplots of serum ALP activities in 65 healthy Miniature Schnauzers with normal serum triglyceride concentrations (group 1), 20 healthy Miniature Schnauzers with slightly high serum triglyceride concentrations (group 2), and 20 healthy Miniature Schnauzers with moderately to severely high serum triglyceride concentrations (group 3). Values for a subset of group 1 dogs ≥ 5 years old (group 1B) are also shown. For each group, the solid horizontal line represents the median; the horizontal dashed line represents the upper reference limit (122 U/L).

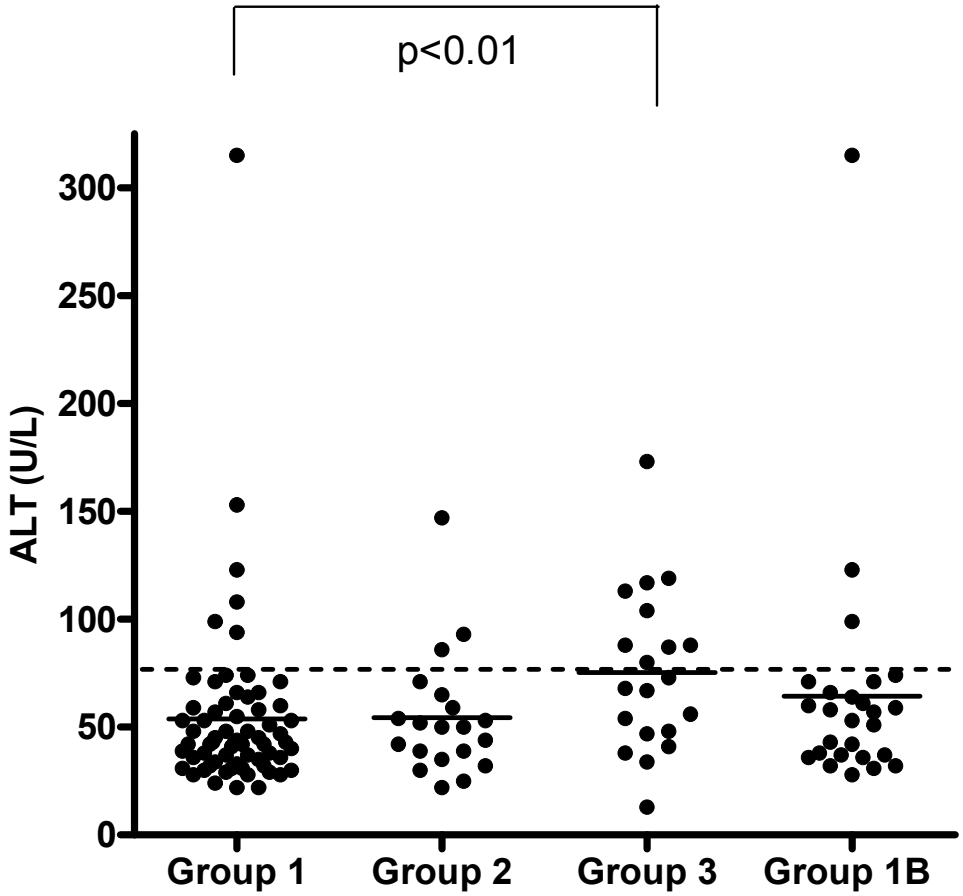

Figure 2— Serum ALT activities in healthy Miniature Schnauzers.

Scatterplots of serum ALT activities in healthy Miniature Schnauzers. *See* Figure 1 for key.

Proportions of dogs with serum liver enzyme activities greater than the upper reference limit were substantially higher for group 3 than for group 1 **(Table 1)**. Compared with group 1 dogs, group 2 and group 3 dogs were significantly more likely to have high serum ALP activity (ie, serum ALP activity greater than the upper reference limit; odds ratio, 26.2 and 192.6, respectively; **Table 2**). Group 3 dogs also were significantly more likely to have high serum ALT activity (odds ratio, 8.0), serum AST activity (odds ratio, 3.7), and serum GGT activity (odds ratio, 11.3), compared with group 1 dogs. Group 3 dogs were significantly more likely (odds ratio, 31.0) to have ≥ 2 high serum liver enzyme activities than were group 1 dogs. Compared with group 1B dogs, dogs in group 3 were significantly more likely to have high serum ALP activity (odds ratio, 77.9), serum ALT activity (odds ratio, 6.3), and serum AST activity (odds ratio, 5.1) and to have ≥ 2 high serum liver enzyme activities (odds ratio, 37.5).

Table 1—Proportions of healthy Miniature Schnauzers with and without hypertriglyceridemia that had high serum liver enzyme activities.

Group	ALP	ALT	AST	GGT	≥ 2 high enzyme activities
1 (n = 65)	0 (0)	6 (9)	10 (15)	1 (2)	3 (5)
1B (n = 26)	0 (0)	3 (12)	3 (12)	1 (4)	1 (4)
2 (n = 20)	3 (15)	3 (15)	2 (10)	1 (5)	3 (15)
3 (n = 20)	12 (60)	9 (45)	8 (40)	3 (15)	12 (60)

Data are given as number (%) of dogs with high activities (ie, activities greater than the upper reference limit) and represent values for 65 healthy Miniature Schnauzers with normal serum triglyceride concentrations (group 1), 20 healthy Miniature Schnauzers with slightly high serum triglyceride concentrations (group 2), 20 healthy Miniature Schnauzers with moderately to severely high serum triglyceride concentrations (group 3), and a subset of group 1 dogs that were ≥ 5 years old (group 1B).

Table 2—Likelihood of high serum liver enzyme activities in healthy Miniature Schnauzers with and without hypertriglyceridemia.

	Group 2 vs group 1		Group 3 vs group 1		Group 3 vs group 1B	
Variable	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
ALP	26.2 (1.3–531.8)	0.012	192.6 (10.4–3,559)	< 0.001	77.9 (4.2–1,461)	< 0.001
ALT	1.7 (0.4–7.7)	0.434	8.0 (2.4–27.2)	< 0.001	6.3 (1.4–27.9)	0.017
AST	0.6 (0.12–3.1)	0.723	3.7 (1.2–11.2)	0.028	5.1 (1.1–22.9)	0.038
GGT	3.4 (0.2–56.5)	0.417	11.3 (1.1–115.6)	0.039	4.4 (0.4–46.1)	0.303
≥ 2 high enzyme activities	3.6 (0.7–19.7)	0.139	31 (17.2–134)	< 0.001	37.5 (14.2–335.2)	< 0.001

See Table 1 for group designations.

There was a significant positive correlation between serum ALP activity and serum triglyceride concentration ($r = 0.53$; $P < 0.001$) and between serum ALT activity and serum triglyceride concentration ($r = 0.29$; $P = 0.003$). In contrast, serum AST ($r = 0.17$; $P = 0.236$) and GGT activities ($r = –0.17$; $P = 0.085$) were not significantly correlated with serum triglyceride concentration.

2.6. Discussion

Results of the present study indicated that moderate to severe hypertriglyceridemia (ie, serum triglyceride concentration > 400 mg/dL) was associated with high serum liver enzyme activities in Miniature Schnauzers. Although significant associations between moderate to severe hypertriglyceridemia and high serum liver enzyme activities were found for all enzymes studied, the most profound association involved serum ALP activity, in that Miniature Schnauzers with moderate to severe hypertriglyceridemia were 192.6 times more likely to have a high serum ALP activity than Miniature Schnauzers with normal serum triglyceride concentration. In addition, Miniature Schnauzers with moderate to severe hypertriglyceridemia were 31 times as likely to have ≥ 2 high serum enzyme activities as were Miniature Schnauzers with normal serum triglyceride concentration.

There was a significant difference in median ages of dogs in groups 1 and 3 in the present study. Because liver enzyme activities might increase with age as a result of benign conditions of the liver that tend to occur in older animals (eg, nodular hyperplasia),[15] this difference in age between groups could potentially have biased our results. However, when only those dogs in group 1 that were ≥ 5 years old (ie, group 1B dogs) were compared with group 3 dogs, similar associations between moderate to severe hypertriglyceridemia and serum liver enzyme activities were still found.

The etiology of high serum liver enzyme activities in Miniature Schnauzers with hypertriglyceridemia was not determined in the present study. In human beings, hypertriglyceridemia has been associated with fatty infiltration of the liver and asymptomatic increases in serum liver enzyme activities.[10,12,16] In these patients, increases in serum liver enzyme activities are usually mild and involve various

combinations of increases in ALT, AST, ALP, and GGT activity.[10,12] Although high serum ALT activities seem to be more commonly reported in human patients with fatty liver or NAFLD, high serum ALP activity is also very common, and a recent study[17] in humans showed that a subset of patients with histopathologically confirmed NAFLD, regardless of etiology, have only high serum ALP activity. It is not known whether high serum liver enzyme activities in Miniature Schnauzers in the present study with hypertriglyceridemia were associated with fatty infiltration of the liver, but this is quite likely. The fact that serum ALP activity, and not serum ALT activity, had the strongest association with hypertriglyceridemia in the present study may suggest that the underlying liver condition in Miniature Schnauzers with hypertriglyceridemia differs from NAFLD in humans.

Alkaline phosphatase is a membrane-bound enzyme, and its activity increases in serum as a result of increased enzyme production stimulated by impaired bile flow or various drugs.[18] Fatty infiltration of the liver can potentially lead to cholestasis and increases in serum ALP and, to a lesser degree, GGT activities.[18] In contrast, ALT and AST are cytosolic enzymes that leak from hepatocytes following injury and altered permeability of the hepatocellular membrane.[18] Hepatocellular injury following fatty infiltration and inflammation of the liver might explain increases of these enzyme activities in Miniature Schnauzers with hypertriglyceridemia.

The pathogenesis of fatty liver and NAFLD in hypertriglyceridemic human patients remains uncertain.[10] However, insulin resistance and hyperinsulinemia are believed to play a key role in the pathogenesis of NAFLD,[10] and it has been reported that in humans, hypertriglyceridemia is associated with insulin resistance and hyperinsulinemia.[19] In dogs, any association between hypertriglyceridemia and resistance to insulin remains to be determined.

Anecdotal observations suggest that 2 conditions of the liver might be associated with hypertriglyceridemia in Miniature Schnauzers: vacuolar hepatopathy and gallbladder mucocele.[20,21] The first shares some common characteristics with NAFLD, such as the fact that both can be subclinical for long periods and that histologically they are characterized by vacuole formation within hepatocytes.[11,21,22] Gallbladder mucocele has been commonly reported in dog breeds that are predisposed to idiopathic

hyperlipidemia, such as Miniature Schnauzers and Shetland Sheepdogs, and has also been described in humans with hypelipidemia.[23-25] However, a clear association between the presence of hyperlipidemia and gallbladder mucocele formation has not been identified in dogs. In a recent study,[24] an association between gallbladder mucocele formation and dyslipidemias (hypertriglyceridemia and hypercholesterolemia) in Shetland Sheepdogs was described. In that study,[24] many of the dogs with gallbladder mucocele were found to have no clinical signs or biochemical abnormalities, except for high serum ALP activity in some cases. Whether this could explain the high serum liver enzyme activities in Miniature Schnauzers with hypertriglyceridemia remains to be determined. Further studies involving histologic and ultrasonographic examination of the liver are underway in an attempt to identify concurrent liver diseases in Miniature Schnauzers with hypertriglyceridemia.

The possibility that high serum liver enzyme activities, especially high ALP and AST activities, in dogs with hypertriglyceridemia in the present study were a result of extrahepatic diseases (eg, hyperadrenocorticism) cannot be excluded, because diagnostic tests to exclude other diseases were not performed. However, this possibility seems unlikely, in that all dogs were free from clinical signs for at least 3 months prior to blood collection and did not have any history of chronic diseases that might affect serum liver enzyme activities. In addition, serum activity of ALT, which is considered specific to the liver, was high in many of the dogs with hypertriglyceridemia, which would suggest that these dogs had a primary hepatic process. It is of interest that when serum cholesterol concentrations, which have been reported to be high in 90% of all dogs with hyperadrenocorticism,[26] were measured in the 40 Miniature Schnauzers in the present study with hypertriglyceridemia, only 9 (23%) were found to have high serum cholesterol concentrations.[4] Thus, it seems unlikely that underlying hyperadrenocorticism played a role in the high serum ALP activities among Miniature Schnauzers with hypertriglyceridemia in the present study. In addition, dogs were not receiving any medications known to affect serum liver enzyme activities.

Similarly, although there have been concerns that lipemia may have affected serum liver enzyme values, the fact that we found no significant differences in mean ALP,

ALT, AST, or GGT activities before and after centrifugation for 8 samples with lipemia suggested that lipemia did not substantially interfere with assays used to measure serum liver enzyme activities in the present study.

It is not known how long hypertriglyceridemia must be present before serum liver enzyme activities will start to increase. Also, it is unknown whether increases in serum liver enzyme activities are persistent or whether correction of hypertriglyceridemia in Miniature Schnauzers would lead to normalization of serum liver enzyme activities. Clinicians should be aware of the potential that hypertriglyceridemia in Miniature Schnauzers, especially when serum triglyceride concentrations are > 400 mg/dL, can be associated with high serum liver enzyme activities. Whether these patients require additional diagnostic testing to identify liver disorders remains to be determined. In human beings, isolated increases in ALT activity are generally considered to be benign, but have also been associated with cirrhosis in 10% to 17% of cases and have been identified in an even higher proportion of patients with clinically important fibrosis.[16] Anecdotal observations suggest that some Miniature Schnauzers with hypertriglyceridemia might develop hepatic insufficiency secondary to severe vacuolar hepatopathy.[21] Also, gallbladder mucoceles, which might be associated with hypertriglyceridemia, can often lead to death or euthanasia.[24] Given the fact that most dogs in the present study had activities for > 1 liver enzyme that would be considered clinically important (ie, > 2 times the upper reference limit), additional diagnostic testing would seem appropriate..

2.7. Footnotes

a. Roche/Hitachi Modular Analytics D2400 module, Roche Diagnostics, Indianapolis, Ind.

b. Roche/Hitachi Modular Analytics P800 module, Roche Diagnostics, Indianapolis, Ind.

c. Prism5, GraphPad, San Diego, Calif.

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IV Discussion

Idiopathic hypertriglyceridemia is considered to be a relatively common disorder in Miniature Schnauzers in the United States (ROGERS et al., 1975a; FORD, 1993; WHITNEY et al., 1993; FORD, 1996). However, the true prevalence of this disorder in Miniature Schnauzers remains unknown, because the studies that have been conducted so far are limited to small case series (WHITNEY et al., 1993). In addition, studies investigating the clinical implications of idiopathic hypertriglyceridemia in large populations of Miniature Schnauzers, as well as the effect of age, sex, and reproductive status, on serum triglyceride concentrations are lacking.

In the first part of the present study, determination of the prevalence of hypertriglyceridemia in a relatively large population of healthy Miniature Schnauzers, as well as further characterization of this condition, was attempted. Based on the results of this part of the study, it can be concluded that hypertriglyceridemia is common in healthy Miniature Schnauzers in the United States. Overall, about one-third of the enrolled Miniature Schnauzers had serum triglyceride concentrations above the upper limit of the reference range. However, the percentage of Miniature Schnauzers with hypertriglyceridemia was much greater in older dogs, with 75% of Miniature Schnauzers ≥ 9 years old being affected.

Miniature Schnauzers enrolled in this study came from many different locations within the United States, thus minimizing the possibility of selection of populations that were more inbred than the general Miniature Schnauzer population. Thus, findings of this study are believed to reflect the general population of Miniature Schnauzers in the United States.

Most of the affected Miniature Schnauzers had mildly increased serum triglyceride concentrations, but a considerable percentage (11.5%) had moderately to severely increased serum triglyceride concentrations. As mentioned earlier, dogs with severe hypertriglyceridemia might be at an increased risk for the development of complications such as pancreatitis and/or seizures (BODKIN, 1992; BAUER, 1995; FORD, 1996; BAUER, 2004; WILLIAMS & STEINER, 2005). In addition, because hypertriglyceridemia is a common feature of other (mostly endocrine) diseases, the

findings of this study are important for veterinarians who may be evaluating Miniature Schnauzers with hyperlipidemia. A large proportion of healthy Miniature Schnauzers in the United States are expected to have mild, moderate, or even severe increases in serum triglyceride concentrations, and further investigation of the etiology of hypertriglyceridemia in Miniature Schnauzers may not be indicated in the absence of clinical or clinicopathological findings.

The development of diseases that cause secondary hypertriglyceridemia (e.g., diabetes mellitus) in Miniature Schnauzers that also have primary hypertriglyceridemia, would be expected to potentially lead to additional increases in serum triglyceride concentrations. This means that, if the severity of hypertriglyceridemia correlates with the potential for developing complications secondary to hypertriglyceridemia, Miniature Schnauzers with both primary and secondary hypertriglyceridemia might be at higher risk for developing complications.

In the Miniature Schnauzers enrolled in the present study, serum hypercholesterolemia was noted almost exclusively in association with moderate to severe hypertriglyceridemia. None of the Miniature Schnauzers with normal serum triglyceride concentrations had hypercholesterolemia. Also, many Miniature Schnauzers with hypertriglyceridemia had normal serum cholesterol concentrations. These findings indicate that hypercholesterolemia is variably present in Miniature Schnauzers with hypertriglyceridemia. Hypercholesterolemia may be a secondary feature that accompanies moderate to severe hypertriglyceridemia in Miniature Schnauzers, resulting from decreased clearance, increased production, or both, of chylomicrons and VLDL, as these 2 lipoprotein molecules also contain small amounts of free and esterified cholesterol (RIFAI et al, 1999).

Both the percentages of Miniature Schnauzers with hypertriglyceridemia and the degree of hypertriglyceridemia increased with age. More than 75% of Miniature Schnauzers $\geq$ 9 years of age in this study had increased serum triglyceride concentrations, and the vast majority (>80%) of Miniature Schnauzers with moderate to severe hypertriglyceridemia were 6 years or older. This observation suggests that later development of hypertriglyceridemia cannot be excluded in young Miniature Schnauzer dogs found to have normal serum triglyceride concentrations. A genetic

marker may help identify affected dogs before the development of hypertriglyceridemia. However, some young Miniature Schnauzers may present with hypertriglyceridemia.

In human beings, the presence of hypertriglyceridemia has been associated with the development of fatty liver, increased serum liver enzyme activities, and a condition known as nonalcoholic fatty liver disease (NAFLD) (ASSY et al., 2000; ANGULO, 2002; DE BRUIN et al., 2004; BROUWERS et al., 2007). Studies investigating a possible association between hypertriglyceridemia, increased serum liver enzyme activities, and liver disease have not been described in dogs. Thus, the aim of the second part of the study presented here was to investigate serum liver enzyme activities in Miniature Schnauzers with hypertriglyceridemia and to compare them with those of Miniature Schnauzers with normal serum triglyceride concentrations.

Results of this second part of the study showed that moderate to severe hypertriglyceridemia (>400 mg/dL) was significantly associated with increased serum liver enzyme activities in Miniature Schnauzers. Although significant associations between moderate to severe hypertriglyceridemia and high serum liver enzyme activities were found for all enzymes tested, the most profound associations involved serum ALP activity (Miniature Schnauzers with moderate to severe hypertriglyceridemia were 192.6 times more likely to have an increased serum ALP activity). In addition, there was a strong association between hypertriglyceridemia and concurrent elevation of two or more serum liver enzyme activities (Miniature Schnauzers with moderate to severe hypertriglyceridemia were 31 times more likely to have elevations of two or more serum liver enzyme activities).

The etiology of elevated serum liver enzyme activities in hypertriglyceridemic Miniature Schnauzers cannot be determined based on the results of the present study. In human beings, hypertriglyceridemia has been associated with fatty infiltration of the liver leading to asymptomatic increases in serum liver enzyme activities (ASSY et al., 2000; ANGULO, 2002; CLARK et al., 2003; KICHIAN et al., 2003; DE BRUIN et al., 2004; IOANNOU et al., 2006; PANTSARI & HARRISON, 2006; BROUWERS et al., 2007). In these cases, increases in serum liver enzyme activities are usually mild and involve different combinations of increases in ALT, AST, ALP,

and GGT (CLARK et al., 2003; IOANNOU et al., 2006; PANTSARI & HARRISON, 2006). Although increases in serum ALT activity are more commonly reported in human patients with fatty liver or NAFLD, increases in ALP activity are also very common, and a recent study in humans showed that a subset of patients with histopathologically confirmed NAFLD present with isolated elevated serum ALP activities (CLARK et al., 2003). It is not known whether the increased serum liver enzyme activities identified in hypertriglyceridemic Miniature Schnauzers in the present study are associated with fatty infiltration of the liver, but this is quite likely. In the present study, high serum ALP, but not ALT (as is the case in humans) activity, showed the most common and strongest association with hypertriglyceridemia. This might indicate that the possible underlying hepatic change in hypertriglyceridemic Miniature Schnauzers differs from NAFLD in humans.

ALP is a membrane-bound enzyme and its activity increases in serum as a result of increased enzyme synthesis stimulated by impaired bile flow and/or drug induction (CENTER, 1996a). Fatty infiltration of the liver can potentially lead to cholestasis and increases in serum ALP, and, to a lesser degree, GGT activities (CENTER, 1996a). In contrast, ALT and AST are cytosolic enzymes that leak from hepatocytes following injury and altered permeability of the hepatocellular membrane (CENTER, 1996a). Hepatocellular injury following fatty infiltration and inflammation of the liver might explain increases of these enzymes in hypertriglyceridemic Miniature Schnauzers.

Anecdotal observations suggest that two conditions of the liver might be associated with hypertriglyceridemia in Miniature Schnauzers: vacuolar hepatopathy and gallbladder mucocele (CENTER, 1996b; SCHERK & CENTER, 2005). Vacuolar hepatopathy shares some common characteristics with some forms of NAFLD, such as the fact that both can be asymptomatic for long periods, and that histopathologically they are characterized by vacuole formation of hepatocytes (CENTER, 1996b; ANGULO, 2002; SCHERK & CENTER, 2005; HUBSCHER, 2006). Gallbladder mucocele has been commonly reported in dog breeds that are predisposed to idiopathic hyperlipidemia (e.g., Miniature Schnauzers and Shetland Sheepdogs) and has also been described in humans with hyperlipidemia (BOLAND et al., 2002; AGUIRRE et al., 2007). A clear association between the presence of hyperlipidemia and a gallbladder mucocele formation has not been described in dogs.

However, in a recent study, an association between gallbladder mucocele formation and dyslipidemias (i.e., hypertriglyceridemia and hypercholesterolemia) was described in Shetland Sheepdogs (AGUIRRE et al., 2007). In this study, many of the dogs with gallbladder mucocele were found to have no clinical signs or biochemical abnormalities, except for an increased serum ALP activity in some cases (AGUIRRE et al., 2007). Whether this is one or the only cause of high serum liver enzyme activities in Miniature Schnauzers with hypertriglyceridemia remains to be determined. Further studies involving histopathologic and ultrasonographic examination of the liver are underway in order to confirm and further characterize potential concurrent liver disease in Miniature Schnauzers with hypertriglyceridemia.

It is unknown how long hypertriglyceridemia must be present in order to lead to increases in serum liver enzyme activities. Also, it is unknown whether increases of serum liver enzyme activities are persistent or whether correction of hypertriglyceridemia in Miniature Schnauzers would lead to normalization of serum liver enzyme activities. Clinicians should be aware of the potential that hypertriglyceridemia in Miniature Schnauzers (especially when serum triglyceride concentrations are above 400 mg/dL) can be associated with increased serum liver enzyme activities. Whether or not these patients require any additional diagnostic work-up towards the diagnosis of hepatic disorders is unknown. In human beings, isolated aminotransferase elevations are generally considered to be benign, but have also been associated with cirrhosis in 10-17% of the cases and, in an even higher proportion of patients, with significant fibrosis (CLARK et al., 2003; ADAMS et al., 2005; EKSTEDT et al., 2006). Anecdotal observations suggest that some Miniature Schnauzers with hypertriglyceridemia might develop hepatic insufficiency due to severe vacuolar hepatopathy (CENTER, 1996b). Also, gallbladder mucoceles which might be associated with hypertriglyceridemia can often lead to death or euthanasia of the animal (AGUIRRE et al., 2007). Given the fact that in the present study most dogs had serum elevations of more than one liver enzyme activity that were considered significant (i.e., > 2 times the upper limit of the reference range), additional diagnostic work-up for patients with hyperlipidemia and increased serum hepatic enzyme activities would seem appropriate. Retesting serum liver enzyme activities could be an alternative approach.

Because idiopathic hypertriglyceridemia appears to be common in Miniature Schnauzers in the United States, and because Miniature Schnauzers with hypertriglyceridemia might be at increased risk for the development of secondary diseases (e.g., liver disease, pancreatitis, neurologic disease), determination of the etiology of hypertriglyceridemia is highly desirable in this breed as it could facilitate the prevention and management of this condition. In human beings, hereditary causes of hypertriglyceridemia are well documented (HEGELE, 2001; YUAN et al., 2007). Familial hypertriglyceridemia (transmitted as an autosomal dominant disorder of unknown etiology), lipoprotein lipase deficiency (transmitted as an autosomal recessive disorder), and familial apolipoprotein C-II deficiency (transmitted as an autosomal recessive disorder) are disorders with a genetic basis that all lead to increases in serum triglyceride concentrations due to increased concentrations of VLDL, chylomicrons, or both (HEGELE, 2001; YUAN et al., 2007). The cause of idiopathic hypertriglyceridemia in Miniature Schnauzers has not yet been identified, but an underlying genetic defect is suspected. A recent study in Miniature Schnauzers with hypertriglyceridemia and pancreatitis failed to identify any mutations of the lipoprotein lipase gene, suggesting that inherited lipoprotein lipase dysfunction is not the cause of hypertriglyceridemia in this breed (SCHICKEL, 2005a, SCHICKEL et al, 2005b). In another recent study, the gene encoding the apolipoprotein C-II was sequenced in Miniature Schnauzers with idiopathic hypertriglyceridemia, Miniature Schnauzers with normal serum triglyceride concentrations, and dogs of other breeds (XENOULIS, 2008). However, no mutations that co-segregated with hypertriglyceridemia were identified (XENOULIS, 2008). Further studies are needed and are underway in order to determine the underlying genetic defect responsible for hypertriglyceridemia in Miniature Schnauzers.

In conclusion, idiopathic hypertriglyceridemia is common in healthy Miniature Schnauzers in the United States. There is no difference between male and female Miniature Schnauzers with regards to the prevalence of hypertriglyceridemia. Both the prevalence and severity of hypertriglyceridemia increase with age in Miniature Schnauzers. Due to the high prevalence of idiopathic hypertriglyceridemia in Miniature Schnauzers, extensive diagnostic testing aiming in identifying the cause of hypertriglyceridemia may not be necessary in otherwise healthy Miniature Schnauzers. All Miniature Schnauzers in the United States should potentially be

evaluated for hypertriglyceridemia while they are healthy, because this information may be useful for avoidance of misinterpretation of increased serum triglyceride concentrations when the dogs become sick. In addition, veterinarians might consider offering low fat diets to hypertriglyceridemic dogs in order to avoid possible complications of hypertriglyceridemia. Due to the fact that hypercholesterolemia was found only in association with hypertriglyceridemia, the presence of hypercholesterolemia alone in Miniature Schnauzers might require additional diagnostic investigation. Clinicians should be aware of the potential that hypertriglyceridemia in Miniature Schnauzers, especially when serum triglyceride concentrations are > 400 mg/dL, can be associated with increased serum hepatic enzyme activities. Possible causes of increased serum liver enzyme activities in hypertriglyceridemic Miniature Schnauzers include fatty infiltration of the liver and/or gallbladder mucocele formation. Additional diagnostic work-up or retesting of serum liver enzyme activities should be recommended in hypertriglyceridemic Miniature Schnauzers with high serum liver enzyme activities.

V Summary

Idiopathic hypertriglyceridemia has been reported in Miniature Schnauzers. However, studies investigating the prevalence of this disorder in a large population of Miniature Schnauzers are lacking. 192 healthy Miniature Schnauzers and 38 healthy dogs of other breeds (control dogs) were enrolled in this study. Serum triglyceride and cholesterol concentrations were measured and statistically compared between the Miniature Schnauzers and the control group. Dogs were categorized based on their age, and median serum triglyceride concentrations were compared among different age groups. A total of 63 (32.8%) of the 192 Miniature Schnauzers had serum triglyceride concentrations above the upper limit of the reference range. In contrast, of the 38 control dogs, only 2 (5.3%) had serum triglyceride concentrations above the upper limit of the reference range. The median serum triglyceride concentration in Miniature Schnauzers was 73.5 mg/dL, which was significantly higher compared to that of the control group (median: 55 mg/dL; $p=0.0005$). Serum cholesterol concentration was above the upper limit of the reference range in 9 (9.0%) of 100 Miniature Schnauzers and in 2 (5.3%) of the control dogs. Mean serum cholesterol concentrations were not significantly different between the 2 groups ($p=0.1374$). Median serum triglyceride concentrations in Miniature Schnauzers increased significantly with age ($p<0.0001$), and there was a significant positive correlation between serum triglyceride concentration and age (Spearman $r=0.47$; $p<0.0001$). There was no difference in serum triglyceride concentrations between male and female Miniature Schnauzers ($p=0.48$). Healthy Miniature Schnauzers had a high prevalence of hypertriglyceridemia compared to healthy dogs of other breeds. Both the prevalence and severity of hypertriglyceridemia increased with age.

To determine whether hypertriglyceridemia in healthy Miniature Schnauzers was associated with increased serum liver enzyme activities, 65 Miniature Schnauzers with normal serum triglyceride concentrations (group 1), 20 Miniature Schnauzers with slightly increased serum triglyceride concentrations (group 2), and 20 Miniature Schnauzers with moderately to severely increased serum triglyceride concentrations (group 3) were evaluated. Questionnaires regarding each dog's medical history were collected, and serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT) activities were

measured. Median serum ALP activity was significantly higher in group 3 than in group 1 or 2, but was not significantly higher in group 2 than in group 1. Median serum ALT activity was significantly higher in group 3 than in group 1, but was not significantly different between any of the other groups. Compared with group 1, group 2 and 3 were significantly more likely to have an increased serum ALP activity (odds ratio, 26.2 and 192.6, respectively). Group 3 was also significantly more likely to have an increased serum ALT activity (odds ratio, 8.0), serum AST activity (odds ratio, 3.7), or serum GGT activity (odds ratio, 11.3), than group 1. Group 3 was significantly more likely (odds ratio, 31.0) to have ≥ 2 high serum liver enzyme activities than was group 1. Results suggested that moderate to severe hypertriglyceridemia was associated with high serum liver enzyme activities in Miniature Schnauzers.

VI Zusammenfassung

Die idiopathische Hypertriglyzeridämie des Zwergschnauzers wurde bereits zuvor in der Literatur beschrieben. Es fehlen jedoch Studien, welche die Prävalenz der Hypertriglyzeridämie in einer größeren Gruppe von Hunden dieser Rasse untersuchen. Die folgende Studie umfasste 192 klinisch gesunde Zwergschnauzer und 38 klinisch gesunde Hunde anderer Rassen (Kontrollgruppe). Die Triglyzerid- und Cholesterolkonzentrationen im Serum wurden in beiden Gruppen gemessen und statistisch ausgewertet. Hunde wurden in verschiedene Altersgruppen eingeteilt und die Triglyzeridkonzentration im Serum wurde zwischen den einzelnen Gruppen verglichen. Insgesamt wiesen 63 (32,8 %) der 192 Zwergschnauzer Triglyzeridkonzentrationen im Serum außerhalb des Referenzbereichs auf. Dagegen hatten von den insgesamt 38 Hunden der Kontrollgruppe nur 2 (5,3 %) Hunde Triglyzeridkonzentrationen im Serum außerhalb des Referenzbereichs. Der Medianwert der Triglyzeridkonzentration im Serum betrug bei Zwergschnauzern 73,5 mg/dL, und war damit signifikant höher als der Medianwert der Kontrollgruppe (Medianwert: 55 mg/dL; p=0,0005). Die Cholesterolkonzentration im Serum lag bei 9 (9,0 %) der 100 Zwergschnauzer und bei 2 Hunden (5,3 %) der Kontrollgruppe über dem Referenzbereiches. Der Mittelwert der Cholesterolkonzentration im Serum war nicht signifikant unterschiedlich zwischen beiden Gruppen (p=0,1374). Der Medianwert der Triglyzeridkonzentration im Serum der Zwergschnauzer war bei älteren Hunden signifikant höher (p<0,0001), und eine positive Korrelation wurde zwischen der Triglyzeridkonzentration im Serum und dem Alter der Tiere beobachtet (Spearman r=0,47; p<0,0001). Die Triglyzeridkonzentration im Serum war zwischen weiblichen und männlichen Tieren nicht signifikant verschieden (p=0,48). Die Ergebnisse dieser Studie ließen darauf schließen, dass klinisch gesunde Zwergschnauzer eine höhere Prävalenz von Hypertriglyzeridämie im Vergleich zu klinisch gesunden Hunden anderer Rassen aufweisen, und dass die Prävalenz sowie der Grad dieser Abnormalität mit steigendem Alter zunehmen.

Der zweite Teil dieser Arbeit untersuchte die Frage, ob Hypertriglyzeridämie möglicherweise mit erhöhten Leberenzymwerten assoziiert ist. Diese Studie umfasste 65 Zwergschnauzer mit physiologischer Triglyzeridkonzentration im Serum (Gruppe 1), 20 Zwergschnauzer mit leicht erhöhter Triglyzeridkonzentration im Serum

(Gruppe 2), and 20 Zwergschnauzer mit mittelgradig bis hochgradig erhöhter Triglyzeridkonzentration im Serum (Gruppe 3). Fragebögen bezüglich des Gesundheitszustandes der Hunde wurden ausgewertet, und die Serumaktivität der folgenden Leberenzyme wurde gemessen: Alkalische Phosphatase (ALP), Alaninaminotransferase (ALT), Aspartataminotransferase (AST), und γ-Glutamyltransferase (GGT). Die Hunde der Gruppe 3 hatten einen signifikant höheren Medianwert der ALP-Serumaktivität verglichen mit Hunden der Gruppen 1 und 2. Zwischen den Gruppen 1 und 2 gab es allerdings keinen signifikanten Unterschied bezüglich der Höhe der ALP-Serumaktivität. Die Hunde der Gruppe 3 hatten einen signifikant höheren Medianwert der ALT-Serumaktivität verglichen mit Gruppe 1. Im Vergleich zur Gruppe 1 hatten die Hunde der Gruppe 2 und 3 ein größeres Risiko für eine erhöhte ALP-Serumaktivität (odds ratio 26,2 bzw. 192,6). Zusätzlich hatten die Hunde der Gruppe 3 im Vergleich zu Gruppe 1 ein größeres Risiko für eine erhöhte Serumaktivität der ALT (odds ratio 8,0), AST (odds ratio 3,7) und GGT (odds ratio 11,3). Im Vergleich zu Gruppe 1 hatten Hunde der Gruppe 3 weiterhin ein größeres Risiko (odd ratio 31,0) für eine Erhöhung von mindestens zwei der untersuchten Leberenzymaktivitäten. Die Ergebnisse dieser Studie deuteten darauf hin, dass mittelgradig bis hochgradig erhöhte Triglyzeridkonzentrationen im Serum bei Zwergschnauzern oft mit einer erhöhten Serumaktivität der Leberenzyme assoziiert sind.

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VIII Legends for tables and figures

Legends for tables and figures in the publications

1. Investigation of hypertriglyceridemia in healthy Miniature Schnauzers

2. Serum liver enzyme activities in healthy Miniature Schnauzers with and without hypertriglyceridemia

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