

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

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

Design—Cross-sectional study.

Animals—65 Miniature Schnauzers with serum triglyceride concentrations within the reference range (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. (*J Am Vet Med Assoc* 2008;232:63–67)

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 inflamma-

ABBREVIATIONS

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

tion, 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

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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 serum triglyceride concentrations within the reference range.

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 study⁴ 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°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 allotted 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 slight-

ly 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); 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 $20,000 \times g$ 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 exact test. Similarly, the Fisher 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 were calcu-

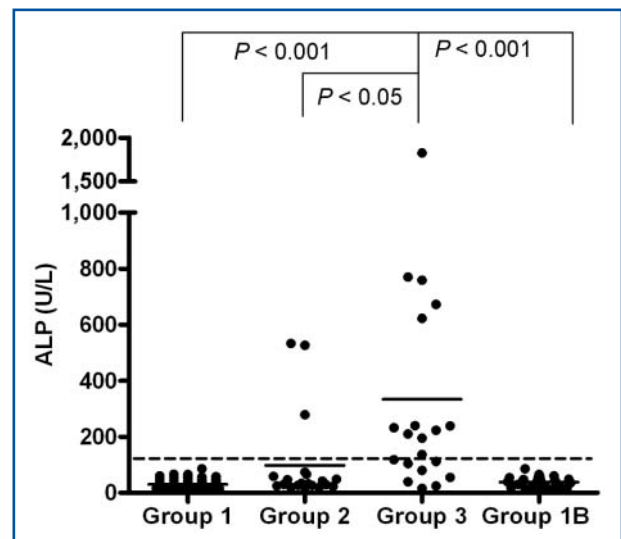


Figure 1—Scatterplots of serum ALP activities in 65 healthy Miniature Schnauzers with serum triglyceride concentrations within the reference range (group 1), 20 healthy Miniature Schnauzers with slightly high serum triglyceride concentrations (group 2), and 20 healthy Miniature Schnauzers with moderately 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).

lated for proportions of dogs with serum liver enzyme activities greater than the upper reference limit. To determine whether there was a systematic change in serum liver enzyme activities 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 method. 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.

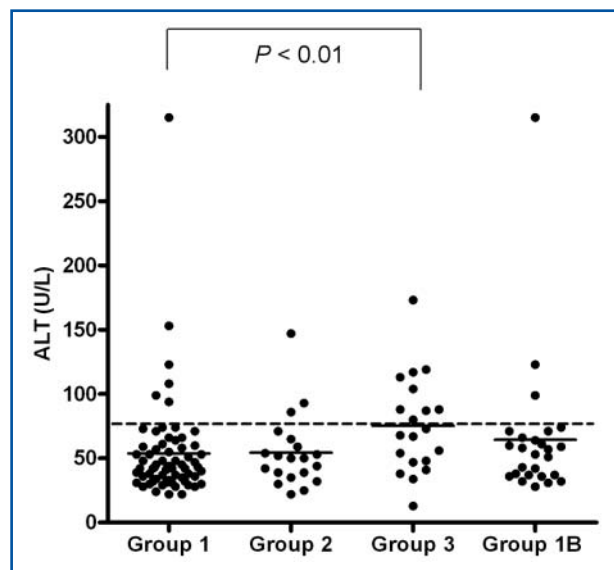


Figure 2—Scatterplots of serum ALT activities in healthy Miniature Schnauzers. The horizontal dashed line represents the upper reference limit (79 U/L). See Figure 1 for key.

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 serum triglyceride concentrations within the reference range (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.

Variable	Group 2 vs group 1		Group 3 vs group 1		Group 3 vs group 1B	
	OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> 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

CI = Confidence interval. See Table 1 for group designations.

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

The 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).

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.

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 as likely to have a high serum ALP activity as were Miniature Schnauzers with serum triglyceride concentrations within the reference range. In addition, Miniature Schnauzers with moderate to severe hypertriglyceridemia were 31 times as likely to have ≥ 2 high serum liver enzyme activities as were Miniature Schnauzers with serum triglyceride concentrations within the reference range.

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 develop 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 common, and a recent study¹⁷ in humans revealed 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 hyperlipidemia.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 an animal must have hypertriglyceridemia 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 also have 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.

- Roche/Hitachi Modular Analytics D2400 module, Roche Diagnostics, Indianapolis, Ind.
- Roche/Hitachi Modular Analytics P800 module, Roche Diagnostics, Indianapolis, Ind.
- Prism5, GraphPad, San Diego, Calif.

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