Association of hypertriglyceridemia with insulin resistance in healthy Miniature Schnauzers

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Objective—To determine whether hypertriglyceridemia in Miniature Schnauzers is associated with insulin resistance.

Design—Case-control study.

Animals—28 Miniature Schnauzers with hypertriglyceridemia and 31 Miniature Schnauzers for which serum triglyceride concentrations were within the reference range (control dogs).

Procedures—All dogs had no history of chronic disease, were free of clinical signs for at least 3 months prior to blood collection, and were not receiving any medications known to affect lipid metabolism or serum insulin concentration. Food was withheld from each dog for ≥12 hours; a 5- to 10-mL blood sample was collected and allowed to clot to obtain serum. Serum insulin and glucose concentrations were measured, and the homeostasis model assessment (HOMA) score was calculated (ie, [basal serum insulin concentration (mU/L) x basal serum glucose concentration (mmol/L)]/22.5).

Results—Median serum insulin concentration was significantly higher in hypertriglyceridemic Miniature Schnauzers (21.3 mU/L) than it was in control dogs (12.5 mU/L). The percentage of dogs with high serum insulin concentrations was significantly greater in the hypertriglyceridemic group (6.5%) than it was in the control group (2.8; 95% confidence interval, 1.1 to 30.2). Median HOMA score for hypertriglyceridemic Miniature Schnauzers (4.9) was significantly higher than that for control dogs (2.8).

Conclusions and Clinical Relevance—Results indicated that hypertriglyceridemia in Miniature Schnauzers is often associated with insulin resistance. Further studies are needed to determine the prevalence and clinical importance of insulin resistance in hypertriglyceridemic Miniature Schnauzers. (J Am Vet Med Assoc 2011;238:1011–1016)

Primary (idiopathic) hypertriglyceridemia is common in healthy Miniature Schnauzers in the United States. A recent study revealed hypertriglyceridemia in 32.8% of 192 healthy Miniature Schnauzers from which food had been withheld for at least 12 hours. Interestingly, the prevalence of this condition appears to be age related and, in the same study, hypertriglyceridemia was detected in >75% of Miniature Schnauzers ≥9 years old. These findings suggest that hypertriglyceridemia in Miniature Schnauzers is the most common breed-related primary lipid disorder in dogs. Hypertriglyceridemia in Miniature Schnauzers is characterized by abnormal accumulation of VLDLs or a combination of VLDLs and chylomicrons, with or without hypercholesterolemia. The genetic and metabolic bases of this condition have not yet been identified. Possible mechanisms involved include increased production and decreased clearance of VLDLs and chylomicrons.

Until recently, primary hypertriglyceridemia was considered to be a relatively benign condition in Miniature Schnauzers. An exception to this might be the development of pancreatitis, which has long been considered a complication of severe hypertriglyceridemia in dogs. However, recent evidence suggests that primary hypertriglyceridemia might not be as benign as initially thought. For example, results of recent studies indicate that hypertriglyceridemia might be associated with hepatobiliary disease in Miniature Schnauzers and other dog breeds. In one of those studies, otherwise healthy hypertriglyceridemic Miniature Schnauzers were 192 times as likely to have high serum alkaline phosphatase activity and 8 times as likely to have high serum ALT activity as were Miniature Schnauzers for which serum triglyceride concentrations were within the reference range. Gallbladder mucocoele and lipid-containing vacuole formation in hepatocytes have also been associated with hypertriglyceridemia in dogs. Primary hypertriglyceridemia in humans has been associated with a much wider range of pathological conditions, including metabolic syndrome, type 2 diabetes mellitus, NAFLD, hypertension, and cardiovascular disease.

Abbreviations

HOMA
NAFLD
T4
VLDL
Homeostasis model assessment
Nonalcoholic fatty liver disease
Thyroxine
Very-low-density lipoprotein

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mia in dogs may be associated with some of the complications that have occurred in humans.

In humans, severe hypertriglyceridemia is thought to be related to insulin resistance and the development of diabetes mellitus.6–11 Insulin resistance appears to be an important component of familial forms of hyperlipidemia in humans and is implicated in the metabolic alterations observed in those patients.8,12 Families with hereditary hypertriglyceridemia have a high incidence of type 2 diabetes mellitus (21% in one 10-year study).5 In those families, hyperlipidemic family members were more likely to develop type 2 diabetes mellitus, and high serum triglyceride concentration was shown to be a risk factor for glucose intolerance and type 2 diabetes mellitus.9 In another study,11 severely high serum triglyceride concentration was shown to induce insulin-resistant diabetes mellitus in humans. In addition, correction of hypertriglyceridemia in obese humans with diabetes mellitus improves glucose utilization and enhances insulin sensitivity.10 Collectively, the aforementioned data suggest that hypertriglyceridemia can lead to insulin resistance and type 2 diabetes mellitus in humans. Insulin resistance has been recognized as an important factor predisposing patients to pathological conditions such as metabolic syndrome, NAFLD, gallbladder disease, hyperlipidemia, hypertension, hyperglycemia, and cardiovascular disease.12,13 To the authors’ knowledge, the role of hypertriglyceridemia as a risk factor for the development of insulin resistance and diabetes mellitus in dogs has not yet been investigated.

The purpose of the study reported here was to determine whether hypertriglyceridemia in Miniature Schnauzers is associated with insulin resistance. To this end, serum insulin concentrations—a marker for insulin sensitivity—in healthy Miniature Schnauzers with and without hypertriglyceridemia were compared. The hypothesis was that primary hypertriglyceridemia in healthy Miniature Schnauzers is associated with insulin resistance.

Materials and Methods

Dogs—Healthy Miniature Schnauzers that belonged to Miniature Schnauzer breeders or owners from different parts of the United States were considered for enrollment in the study. Serum samples had been collected from these Miniature Schnauzers as part of a separate study.1 The breeders and owners were affiliated with the American Miniature Schnauzer Club and had been randomly selected and notified about this study. All participating breeders and owners signed and returned an informed owner consent form. The study1 was reviewed and approved by the Clinical Research Review Committee at Texas A&M University.

Blood sample collection—All Miniature Schnauzer breeders and owners who decided to participate in the study were sent a package containing ice packs and materials necessary for blood sample collection and were asked to schedule an appointment with their veterinarian for collection of a blood sample. Breeders and owners were instructed not to feed their dogs for at least 12 hours prior to the scheduled blood collection appointment. Veterinarians were instructed to collect 5 to 10 mL of blood into a blood collection tube containing no additive, centrifuge the sample immediately after clot formation, separate the serum from the clot, transfer the serum into another additive-free blood collection tube, and send the samples on ice to the Gastrointestinal Laboratory via overnight courier.

Questionnaire and inclusion criteria—Owners were asked to complete a questionnaire for each dog. Information gathered for each dog included date of birth, sex, reproductive status, body weight, current diet, current medications, and current and past health status. Completed questionnaires from all dog owners were reviewed. A dog was included in the study if certain inclusion criteria were met: absence of clinical signs of any kind for at least 3 months prior to blood sample collection, no history of chronic diseases that might affect lipid metabolism and circulating insulin or glucose concentrations (eg, endocrine disorders), and no current treatment with medications that may affect lipid metabolism or circulating insulin and glucose concentrations.

Serum sample analysis and group allocation—Upon receipt at the Gastrointestinal Laboratory, serum samples were immediately aliquoted and stored frozen at –80°C until further use. Samples were analyzed for serum triglyceride, glucose, and insulin concentrations by use of enzymatic assays (triglyceride and glucose concentrations) and a commercially available radioimmunoassay (insulin concentration). Most of the commercially available immunoassays for measurement of insulin concentration are intended for use in humans. However, because the amino acid sequence of human and canine insulins are almost identical, these kits are also suitable for the determination of serum insulin concentrations in dogs as long as they have been properly validated.13 The immunoassay used in the present study has been validated for use in dogs, and reference ranges have been established.14

The HOMA score, which provides a measure of assessment of insulin resistance,15,16 was calculated by use of a formula as follows:

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\text{HOMA score} = (\frac{\text{Basal serum insulin concentration} \text{[mU/L]}}{22.5}) \times (\frac{\text{Basal serum glucose concentration} \text{[mmol/L]}}{18})
\]

The HOMA score has been extensively used for the assessment of insulin resistance in humans and experimental animals17,18 and has recently also been applied for studies in dogs19,20 and cats.21 The HOMA score has proven to be very useful for the early detection of insulin resistance in population-based studies and clinical practice because it is simple to determine, accurate, and only requires analysis of a single blood sample.18

For purposes of the study, the Miniature Schnauzers were allocated to 1 of 2 groups on the basis of their serum triglyceride concentration. Miniature Schnauzers with hypertriglyceridemia (reference range, 26 to 108 mg/dL) were allocated to group 1, and Miniature Schnauzers that had serum triglyceride concentrations within the reference range were allocated to group 2 (control dogs). To determine whether hypothyroidism was a cause of hypertriglyceridemia in the dogs of group 1, serum total T4 concentrations were measured in 8 dogs in this group for which a sufficient volume of serum was available.
Statistical analysis—All data were tested for normal distribution by use of the Kolmogorov-Smirnov test. Median serum triglyceride concentrations were calculated for each group. The mean ± SD age and body weight of each group were calculated, and values were compared between groups by use of Student t tests. The median basal serum insulin concentration and the median HOMA score of each group were calculated, and values were compared between groups by use of Mann-Whitney U tests. To investigate whether a systematic change in serum insulin concentrations and HOMA scores occurred with increasing serum triglyceride concentrations, data were analyzed for correlation between these variables by use of the Spearman rank correlation. The percentage of Miniature Schnauzers with basal serum insulin concentrations that exceeded the upper limit of the reference range were compared between groups by use of a Fisher exact test. All statistical analyses were performed by use of a statistical software package4; significance was set at a value of P < 0.05.

Results

Dogs—Seventy-three Miniature Schnauzers were initially considered for enrollment in the study. Of those 73 dogs, 14 were nonpregnant sexually intact females in unknown stages of the estrous cycle. Because one of the most common causes of secondary diabetes mellitus in dogs is diestrus6 and it was likely that at least some of the sexually intact female Miniature Schnauzers were in diestrus, all nonpregnant sexually intact female dogs were excluded from analysis. Thus, a total of 59 Miniature Schnauzers were included in the study.

On the basis of their serum triglyceride concentration (determined after food withholding), 28 Miniature Schnauzers with hypertriglyceridemia were allocated to group 1 and 31 Miniature Schnauzers with serum triglyceride concentrations within reference range were allocated to group 2 (control dogs). Group 1 dogs included 15 spayed females, 3 sexually intact males, and 9 castrated males. Group 2 dogs included 14 spayed females, 8 sexually intact males, and 8 castrated males. One dog in each group was of unknown sex.

The ages of 57 of the 59 dogs were obtained from the questionnaires (ages of 1 dog from each group were not provided); the mean ± SD age of dogs in group 1 was 8.2 ± 3.3 years, whereas the mean age of dogs in group 2 was 6.7 ± 2.6 years. Mean ages did not differ significantly (P = 0.083) between the 2 groups. Body weights were available for 56 of the 59 dogs (weights of 1 dog in group 1 and 2 dogs in group 2 were not provided); there was no significant (P = 0.074) difference in mean body weights between dogs in group 1 (8.6 ± 1.9 kg [18.9 ± 4.2 lb]) and dogs in group 2 (7.7 ± 1.4 kg [16.9 ± 3.1 lb]).

Serum sample analysis data—The median serum triglyceride concentration was 356.0 mg/dL (range, 252 to 3,125 mg/dL) for group 1 and 63.0 mg/dL (range, 49 to 95 mg/dL) for group 2 (upper reference limit for this variable, 108 mg/dL). Serum glucose concentration was measured in 58 of 59 dogs (assessment was not made for 1 dog in group 2); the median serum glucose concentration in groups 1 (90.0 mg/dL) and 2 (88.1 mg/dL) did not differ significantly (P = 0.663). Only 1 of these 58 dogs (from group 1) had slightly high serum glucose concentration (140 mg/dL; reference range, 60 to 120 mg/dL).

In dogs of group 1, the median serum insulin concentration (21.3 mU/L) was significantly (P < 0.001) higher than that in the control dogs of group 2 (12.5 mU/L; reference range, 8.4 to 33.0 mU/L; Figure 1). Also, the median HOMA score for dogs of group 1 was significantly (P = 0.002) higher than that for dogs of group 2 (4.9 and 2.8, respectively). Eight of 28 (28.6%) dogs in group 1 had serum insulin concentrations that exceeded the upper limit of the reference range (control dogs [group 2]). Basal insulin and glucose concentrations were determined in serum samples obtained after food had been withheld from each dog for at least 12 hours. The HOMA score was calculated as ([basal serum insulin concentration (mU/L) X basal serum glucose concentration (mmol/L)]/22.5). For each box, the horizontal line represents the median value and the upper and lower boundaries represent the 25th and 75th percentiles, respectively. Whiskers represent the minimum and maximum values. Median basal serum insulin concentration and median HOMA score differed significantly (P < 0.001 and P = 0.002, respectively) between the 2 groups.

Figure 1—Box-and-whisker plots of basal serum insulin concentrations (A) and HOMA scores (B) in 28 Miniature Schnauzers with hypertriglyceridemia (group 1) and 31 Miniature Schnauzers for which basal serum triglyceride concentrations were within the reference range (control dogs [group 2]). Basal insulin and glucose concentrations were determined in serum samples obtained after food had been withheld from each dog for at least 12 hours. The HOMA score was calculated as ([basal serum insulin concentration (mU/L) X basal serum glucose concentration (mmol/L)]/22.5). For each box, the horizontal line represents the median value and the upper and lower boundaries represent the 25th and 75th percentiles, respectively. Whiskers represent the minimum and maximum values. Median basal serum insulin concentration and median HOMA score differed significantly (P < 0.001 and P = 0.002, respectively) between the 2 groups.
per reference limit for this variable, whereas only 2 of 31 (6.5%) control dogs had serum insulin concentrations that exceeded the upper reference limit for this variable ($P = 0.036$; odds ratio, 5.8; 95% confidence interval, 1.1 to 30.2). There was a significant but weak positive correlation between serum triglyceride and insulin concentrations for all dogs ($r = 0.403; P = 0.002$; Figure 2). There was no significant correlation between serum triglyceride concentration and HOMA score for all dogs ($P = 0.150$).

Serum total $T_4$ concentrations were within the reference range in 7 of 8 dogs in group 1 for which that variable was assessed (mean ± SD, 2.01 ± 0.52 µg/dL). One dog had borderline low serum total $T_4$ (1.52 µg/dL); in that dog, serum free $T_4$ concentration was measured by use of equilibrium dialysis and was within the reference range.

**Discussion**

To the authors’ knowledge, this is the first study to investigate a possible association between hypertriglyceridemia and insulin resistance in dogs. The results of the present study indicated that Miniature Schnauzers with hypertriglyceridemia often have evidence of insulin resistance, indicated by higher basal (fasting) serum insulin concentrations and higher HOMA scores, compared with nonhypertriglyceridemic control dogs.

In insulin-resistant states, the normal effect of insulin on tissues is impaired. In humans and other animals that have a healthy pancreas, compensation for insulin resistance occurs via overproduction of insulin by pancreatic islet cells and subsequent development of hyperinsulinemia. This, in turn, leads to a normal or near normal glucose tolerance. Type 2 diabetes mellitus develops when the level of compensatory hyperinsulinemia is inadequate for maintenance of normal glucose homeostasis. In the present study, with the exception of 1 dog in which serum glucose concentration was mildly high but serum insulin concentration was within reference range, all dogs had serum glucose concentrations that were considered normal, indicating that type 2 diabetes mellitus had not developed in those dogs. This is in agreement with findings of a study in humans with insulin resistance, which indicated that most affected humans maintain normal glucose metabolism.

In the present study, median serum insulin concentrations were significantly higher in hypertriglyceridemic Miniature Schnauzers, compared with values in control dogs, indicating an insulin-resistant state in at least some of the hypertriglyceridemic dogs. In addition, the percentage of Miniature Schnauzers that had hyperinsulinemia among group 1 dogs (ie, dogs with hypertriglyceridemia; 28.6%) was considerably greater than the percentage among group 2 dogs (ie, dogs with serum triglyceride concentrations that were within reference range; 6.5%), further suggesting insulin resistance in some hypertriglyceridemic dogs. Basal serum insulin concentrations are considered to be useful for the assessment of insulin resistance in both dogs and cats. High basal serum insulin concentrations suggest early stages of secondary diabetes mellitus in dogs because, with the exception of a few conditions (eg, functional beta-cell tumor or postprandial hyperinsulinemia), there are no other causes of high basal serum insulin concentration. Basal circulating insulin concentrations are also considered to be useful for the assessment of insulin resistance in nondiabetic humans. Basal serum insulin concentration has been reported to be at least as accurate as the HOMA score, insulin-to-glucose concentration ratio, or other indicators for the assessment of insulin resistance in both normoglycemic humans and cats. In fact, results of 1 study have suggested that when testing normoglycemic human populations, any method to predict insulin sensitivity should be compared with measurement of fasting insulin concentration. It should be noted that aside from hypertriglyceridemia in some dogs, the dogs in the present study were healthy and had serum glucose concentrations that were within reference range. This is important because the suppressive effect of hyperglycemia on beta-cell function often interferes with accurate interpretation of serum insulin concentration data.

The hypertriglyceridemic Miniature Schnauzers in the present study also had significantly higher HOMA scores, compared with scores for the nonhypertriglyceridemic control Miniature Schnauzers; this finding further supports the presence of an insulin-resistant state in the hypertriglyceridemic dogs. Simplified methods used for the assessment of insulin sensitivity in humans, such as the HOMA index, have been recently introduced for the assessment of insulin sensitivity in dogs. In 1 study, the HOMA index was used to estimate insulin sensitivity in comparison with the euglycemic hyperinsulinemic clamp technique in obese dogs, and findings suggested that HOMA score determination might be useful for the assessment of insulin sensitivity in dogs. In addition, the HOMA index has been extensively used for evaluation of insulin sensitivity in several other species and has been found to provide valuable information.
er methods exist for the evaluation of insulin sensitivity in humans, however, in dogs, only few of these methods have been evaluated. Those methods, such as the euglycemic hyperinsulinemic clamp technique, are very accurate but rather laborious and impractical for use in client-owned veterinary patients, as they require multiple blood sample collections and injections of insulin and glucose.

The clinical consequences of insulin resistance in hypertriglyceridemic Miniature Schnauzers are unknown. It is important to note that all dogs enrolled in the present study were healthy (aside from hypertriglyceridemia in some); thus, the prevalence of insulin resistance as a result of hypertriglyceridemia in Miniature Schnauzers was perhaps underestimated because dogs with diseases that might be potentially associated with insulin resistance were excluded from the study. For the same reason, the study was not designed to determine the relationships of hypertriglyceridemia with diseases that might be associated with insulin resistance.

In humans, insulin resistance has been implicated in the pathogenesis of several conditions, including type 2 diabetes mellitus, NAFLD, and gallbladder disease. These associations are attributed not only to the insulin resistance itself but also to the resulting compensatory hyperinsulinemia, which results in increased insulin action on unaffected pathways (differential insulin sensitivity). It has been reported that Miniature Schnauzers are at increased risk for the development of diabetes mellitus (although the dogs typically develop type 1 and not type 2 diabetes). Whether this predisposition of Miniature Schnauzers to diabetes mellitus is associated with the predisposition of Miniature Schnauzers to hypertriglyceridemia, which leads to insulin resistance, remains to be determined. It may also be possible that diabetes mellitus is more difficult to control in hypertriglyceridemic Miniature Schnauzers that are insulin resistant. Hypertriglyceridemia-induced insulin resistance might also play a role in the possible association between hypertriglyceridemia and hepatobiliary disease (eg, gallbladder disease or vacuolar hepatopathy) in dogs. Finally, in humans, it is established that insulin resistance can lead to hypertriglyceridemia through the impairment of lipoprotein lipase activity and overactivation of the hormone-sensitive lipase. This is very important because insulin resistance in hypertriglyceridemic Miniature Schnauzers might contribute to or worsen primary hypertriglyceridemia, resulting in an escalation in severity of both conditions in those animals. The possibility that insulin resistance might be the initial cause of hypertriglyceridemia in some Miniature Schnauzers may be also valid, although this is probably unlikely because many of the Miniature Schnauzers with hypertriglyceridemia in the present study did not have high serum insulin concentrations.

One limitation of the present study was that the body condition scores of the dogs enrolled were not available. This is important because insulin resistance might develop as a result of obesity in dogs. However, all dogs in the present study were of the same breed, which means that body weights alone can be somewhat useful in determining differences in the body condition of these animals. There was no significant difference in weight between the 2 groups and in the control group, only 2 of 31 (6.5%) dogs had basal serum insulin concentrations that exceeded the upper reference limit. Thus, although there is a possibility that obesity might have influenced the results of the present study, the fact that there was no difference in the mean body weights between the 2 groups would suggest that obesity did not play an important role in the study findings. In addition, circulating insulin concentration might not always be affected by obesity; in 2 studies in which insulin resistance was detected by use of the euglycemic hyperinsulinemic clamp technique in dogs with experimentally induced obesity, basal insulin concentration was not significantly different between obese and nonobese control dogs.

Hypertriglyceridemia and insulin resistance may be the result of hyperadrenocorticism in some dogs. Specific testing to exclude this condition in the dogs of the present study was not performed, but it is unlikely that the enrolled Miniature Schnauzers had hyperadrenocorticism because they were free of clinical signs for at least 3 months prior to blood sample collection and there was no obvious reason that would justify testing for this disease. Other secondary causes of hypertriglyceridemia, such as hypothyroidism and diabetes mellitus, were also considered unlikely to account for the hypertriglyceridemia in dogs of group 1 because all dogs in this group were apparently healthy (ie, there was no indication to test for those diseases) and almost all dogs (57/59) had serum glucose concentrations that were within the reference range. In addition, in the 8 dogs of group 1 in which serum total T4 concentration was measured, the results were within the reference range in all but 1 dog; however, the serum free T4 concentration in that dog was within the reference range. This, in conjunction with the fact that these dogs had no clinical signs suggestive of hypothyroidism, practically eliminates the possibility that hypothyroidism accounted for the hypertriglyceridemia.

The time that elapses between development of hypertriglyceridemia and development of insulin resistance in dogs is not known. Also, it is unknown whether insulin resistance is persistent or whether correction of hypertriglyceridemia in Miniature Schnauzers would lead to normalization of insulin sensitivity. Although only dogs with presumed primary hypertriglyceridemia were included in the present study, it is possible that secondary hypertriglyceridemia (eg, that attributable to hypothyroidism) would also affect insulin sensitivity. Studies involving use of the euglycemic hyperinsulinemic clamp technique are needed to further investigate the development of insulin resistance as a result of hypertriglyceridemia in dogs. In addition, investigation of the association of hypertriglyceridemia-induced insulin resistance with other pathological conditions in dogs is warranted.

a. Roche/Hitachi Modular Analytics D 2400 module, Roche Diagnostics, Indianapolis, Ind.
b. Insulin RIA IDS 1600, Diagnostic Systems Laboratories, Webster, Tex.
d. Prism3, GraphPad, San Diego, Calif.
References