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Associations among serum insulin, calprotectin, and C-reactive protein concentrations in Miniature Schnauzers with idiopathic hyperlipidemia before and after feeding an ultra-low-fat diet

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Abstract

Background: Miniature Schnauzers (MS) commonly have idiopathic hypertriglyceridemia (HTGL), which is associated with insulin resistance (IR) and a subclinical inflammatory phenotype.

Objectives: Determine the association between indicators of IR and inflammatory biomarkers in MS with and without HTGL and identify how indicators of IR are affected by dietary intervention in MS with HTGL.

Animals: Seventy MS with HTGL and 79 MS without HTGL. In addition, 15 MS with HTGL were placed on a low-fat diet.

Methods: Serum concentrations of triglycerides, cholesterol, calprotectin, insulin, and glucose were compared between groups.

Results: Serum glucose and calprotectin concentrations (shown to be higher in MS with HTGL than in MS without HTGL) were inversely correlated ($\rho = -.28$; $P < .001$). After dietary intervention, median serum insulin concentrations were 8.1 mU/L compared to 20.8 mU/L before dietary intervention ($P = .06$). Dogs with complete resolution of HTGL after dietary intervention (5 dogs) had significantly lower serum insulin concentrations compared to baseline ($P = .03$).

Conclusion and Clinical Importance: The subclinical inflammatory phenotype in MS with HTGL appears to be associated with IR. Resolution of HTGL by dietary intervention is associated with a decrease in serum insulin concentrations. The implication of the increase in serum calprotectin concentrations after resolution of HTGL warrants further study.

KEYWORDS

dog, dyslipidemia, glucose homeostasis, hypertriglyceridemia, S100A8/A9

Abbreviations: BCS, body condition score; CETP, cholesterylester transfer protein; CRP, C-reactive protein; HCHOL, hypercholesterolemia; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of IR; HTGL, hypertriglyceridemia; IH, idiopathic hyperlipidemia; IR, insulin resistance; LDL, low-density-lipoprotein; MS, Miniature Schnauzers; RI, reference interval; VLDL, very-low-density lipoproteins.

1 | INTRODUCTION

Miniature Schnauzers (MS) are predisposed to the development of idiopathic or primary hyperlipidemia (IH), characterized by fasting hypertriglyceridemia (HTGL) with or without hypercholesterolemia (HCHOL).[1] Increased production of very-low-density lipoproteins (VLDL) and chylomicrons, decreased clearance of VLDL and chylomicrons, or both are suggested to be involved in the development of HTGL. Diagnosis of idiopathic HTGL is made after excluding other conditions that cause secondary hyperlipidemia, such as endocrinopathies and possibly pancreatitis.[2,3] The high prevalence of idiopathic HTGL in MS suggests a hereditary mechanism, but a definitive metabolic or genetic basis for idiopathic HTGL in MS has not yet been identified.[1]

The older hypothesis that HTGL in MS is a relatively benign condition has been challenged in the past 2 decades by reports about potential complications of HTGL, including pancreatitis,[3,4] proteinuria,[5,6] gallbladder dysmotility or mucocele development,[7] and insulin resistance (IR) characterized by increased serum insulin concentrations and high homeostatic model assessment of IR (HOMA-IR) scores.[1,4] Humans with familial forms of hyperlipidemia commonly have IR and are at a higher risk for diabetes mellitus compared to healthy controls with a 6.3 hazard ratio,[8] as well as cardiovascular disease[9] and other pathologic conditions. A partial genetic background has been proposed for explaining the decreased insulin sensitivity in some humans with familial hyperlipidemia.[10] The presence of IR further potentiates the metabolic consequences of HTGL. Insulin resistance leads to increased activity of hormone-sensitive lipase in the adipose tissue, resulting in increased lipolysis and increased fatty acid delivery to the liver.[11] Other consequences of IR include impaired activity of lipoprotein lipase, resulting in a decreased clearance of triglyceride-rich lipoproteins,[12] and an increase in cholesterol synthesis with a decrease in its absorption.[13] Ultra-low-fat diets defined by a fat content of ≤20 g/1000 kcal are typically the first line of treatment for HTGL in dogs, and in a recent study, feeding a commercial ultra-low-fat diet was effective in significantly decreasing serum triglyceride and cholesterol concentrations as well as improving lipoprotein profiles in MS with HTGL.[14] The effect of feeding an ultra-low-fat diet on IR indices in MS with HTGL has not been investigated previously.

Hypertriglyceridemia in humans also has been associated with subclinical systemic inflammation. Patients with HTGL were shown to have increased concentrations of serum markers of systemic inflammation, such as calprotectin and C-reactive protein (CRP).[15,16] In addition, improvement of HOMA-IR scores to a level associated with remission of diabetes mellitus in humans is accompanied by a decrease in serum calprotectin and CRP concentrations.[17] Recently, an association between HTGL and subclinical inflammation in MS also has been reported.[18] In that study, MS with HTGL had increased serum calprotectin concentrations compared to normolipidemic MS.[18] However, serum calprotectin concentrations did not decrease after dietary intervention despite a significant improvement in triglyceride and cholesterol concentrations, and the relationship between markers of inflammation and IR in MS has not yet been reported.

We hypothesized (a) that indices of IR and markers of systemic inflammation would be correlated in MS with and without HTGL; and (b) that IR indices would be improved after feeding an ultra-low-fat diet to MS with HTGL. We aimed to evaluate: (a) indices of IR and markers of systemic inflammation, as well as their association, in MS with and without HTGL; and (b) potential changes in indicators of IR in relation to serum markers of systemic inflammation after feeding a commercially available ultra-low-fat diet to MS with HTGL.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The protocol for the collection of serum samples from healthy MS for evaluation and further study of IH was reviewed and approved by the Texas A&M University Clinical Research Review Committee (CRRC# 2008-37, approved 11-05-2008).

2.2 | Healthy MS with and without HTGL

Data and serum samples from 149 clinically healthy MS with or without idiopathic HTGL also were included in the study. These serum samples had been collected as part of a different study.[18] The inclusion criteria for dogs in this group were: (a) HTGL for dogs that were diagnosed with idiopathic HTGL or normal serum triglyceride concentrations for dogs without idiopathic HTGL; (b) consuming diets that were not labeled as "low-fat"; (c) absence of any clinical signs at the time of initial blood collection; (d) no history of diseases or current use of drugs known to affect lipid metabolism; (e) body condition score (BCS) between 4 and 6 on a scale of 1 to 9 (BCS was assessed by the referring veterinarian using a printed guide with pictures and instructions that was provided to each referring veterinarian); and (f) willingness of the dog's owner to enroll the animal into the study.

Diagnostic testing included a CBC, serum biochemistry profile, baseline cortisol concentration, urinalysis, thyroid profile (ie, serum concentrations total thyroxine [T4], canine thyroid stimulation hormone [cTSH], free T4 by equilibrium dialysis) and measurement of canine pancreatic lipase immunoreactivity (cPLI, as measured by Spec cPL, Idexx Laboratories, Westbrook, ME). Testing was performed to evaluate dogs for the possibility of having HTGL secondary to other conditions including hypothyroidism, diabetes mellitus, hyperadrenocorticism, and pancreatitis. No function testing for hyperadrenocorticism was performed, but consistent clinical signs together with clinicopathological abnormalities for this condition were not identified in any of the dogs included in the study. Based on the historical information for each dog and the results of the tests performed, secondary causes of hyperlipidemia were excluded with reasonable certainty, and therefore all hypertriglyceridemic dogs enrolled in the study were classified as having primary HTGL.

Dogs in this group were enrolled on a 1-time basis as part of a previous study,[18] and no follow-up samples were collected. Blood

samples were used for measurement of serum triglyceride, cholesterol, glucose, and calprotectin concentrations.

2.3 | MS with HTGL included in the dietary trial

2.3.1 | Animals

Fifteen MS with HTGL of different severity were included in this group. A subset of these dogs had been enrolled in a previous study.[14] The inclusion criteria for dogs in this group were: (a) HTGL; (b) consuming diets that were not labeled as "low-fat"; (c) absence of any clinical signs at the time of initial blood collection; (d) no history of diseases or current use of drugs known to affect lipid metabolism; (e) body condition score (BCS) between 4 and 6 on a scale of 1 to 9; and (f) willingness of the dog's owner to enroll the animal into the study. The same diagnostic tests described above were performed in the dogs of this group, and based on the historical information for each dog and the results of the tests performed, secondary causes of hyperlipidemia were excluded with reasonable certainty, and therefore all hypertriglyceridemic dogs enrolled in the study were presumed to have primary HTGL.

Each of the dogs in this group had 4 blood samples collected. The first sample (sample 1) was used to investigate the presence of HTGL and select which dogs would be candidates for enrollment into the study. A second blood sample (sample 2) was collected 1 to 2 mo after collection of the initial blood sample to confirm the presence of HTGL. If HTGL was confirmed in the second sample, the dogs were placed on the study diet (see below). Approximately 7-9 wk after the dogs had been exclusively fed the study diet, a third blood sample (sample 3) was collected. Finally, a fourth sample (sample 4) was collected approximately 2-4 wk after the third sample, and while the dogs were still exclusively being fed the study diet. All 4 samples collected from these dogs were used to measure serum concentrations of triglycerides, cholesterol, calprotectin, CRP, and insulin, as well as to calculate HOMA-IR scores; the first and last samples from each dog were used for data and statistical analyses.

2.3.2 | Study diet

The diet selected was a commercially available therapeutic diet labeled as "low-fat" in dry form (Royal Canin Gastrointestinal Low-fat, Royal Canin USA Inc., St. Charles, MO). The fat content of the study diet was 18.6 g of fat per 1000 kcal.

2.4 | Sample analysis

2.4.1 | Sample collection and handling

All blood samples were collected after withholding food for at least 12 hours. Blood samples were placed into additive-free tubes, allowed to clot for 20 min, centrifuged, and serum was stored at −80°C until analysis.

2.4.2 | Assays

Serum triglyceride (reference interval [RI], 26-108 mg/dL) and cholesterol (RI, 124-335 mg/dL) concentrations were measured, and serum biochemistry profiles were performed using analytically validated automated enzymatic assays (Roche/Hitachi MODULAR ANALYTICS D 2400 module, Roche Diagnostics, Indianapolis, IN). Serum cPLI was measured by Spec cPL (RI, ≤200 μg/L) using an analytically validated immunoassay as described elsewhere.[19] Serum total T4 concentration was measured using a solid-phase chemiluminescent competitive assay (Immulite 2000 Canine Total T4, Siemens Healthcare Diagnostics, Deerfield, IL), serum free T4 concentration using a commercial equilibrium dialysis radioimmunoassay (Free T4 [by ED], Antech Diagnostics, Irvine, CA), and serum cTSH concentration using a solid-phase, 2-site chemiluminescent immunometric assay (Immulite 2000 Canine TSH, Diagnostic Products Corporation, Los Angeles, CA, USA). Baseline serum cortisol concentration was measured using a competitive chemiluminescent immunoassay (Immulite 2000 Cortisol, Siemens Healthcare Diagnostics, Deerfield, IL). Serum calprotectin concentration (RI, 72-234 μg/L) was determined using a validated species-specific in-house radioimmunoassay.[20] Serum insulin concentration was measured using a radioimmunoassay designed for humans and validated for use in dogs (Insulin RIA IDSL 1600, Diagnostics Systems Laboratories, Webster, TX). Serum glucose concentrations were only available from pre-treatment (baseline) samples and, along with insulin, were used to calculate HOMA-IR scores based on the formula: HOMA-IR score = (serum insulin concentration [mU/L] × serum glucose concentration [mmol/L]/22.5).[4] Serum CRP concentrations (RI, 0-7.6 mg/L) were measured using a solid-phase sandwich immunoassay (Tri-Delta Phase CRP, Tri-Delta Diagnostics, Boonton Township, NJ).

2.5 | Statistical analysis

A commercial statistical software package was used for all statistical analyses (JMP v13.0, SAS Institute Inc., Cary, NC). Data were evaluated for normality by use of a Shapiro-Wilk test. Statistical analyses were performed using tests for non-parametric 2-group comparisons of unpaired (Wilcoxon rank-sum test) or paired data (Wilcoxon signed-rank test). Possible associations were assessed using a Fisher's exact or likelihood ratio test with 95% confidence intervals (95% CI) reported, and the non-parametric Spearman correlation coefficient ρ was used for correlation testing. Summary statistics are presented as medians and ranges. Multiple comparisons were Bonferroni adjusted and statistical significance was set at $P < .05$.

3 | RESULTS

3.1 | MS with and without HTGL ($n = 149$)

Serum was available from 149/150 MS from our previous study.[18] Seventy of the 149 (47%) healthy MS in this study were diagnosed

with HTGL. The 149 dogs had a median age of 7.2 y (range, 0.7-12.9 y), a median body weight (available for 142 dogs) of 7.7 kg (range, 5.0-12.9 kg), and a median BCS of 5/9 (range, 3/9-7/9). Sex status was available for 145 dogs; 89 (61%) were females (43 intact) and 56 (39%) were males (28 intact).

Fifty (71%) of the dogs diagnosed with HTGL had isolated HTGL with a median serum triglyceride concentration of 264 mg/dL (range, 114-1224 mg/dL). Seven (10%) had isolated HCHOL with median serum cholesterol concentration of 361 mg/dL (range, 340-544 mg/dL). Thirteen dogs (19%) had combined HTGL and HCHOL with median serum triglyceride concentration of 515 mg/dL (range, 146-5077 mg/dL) and cholesterol concentration of 492 mg/dL (range, 359-783 mg/dL). Median serum glucose concentration in MS with IH was 5.6 mmol/L (range, 2.9-7.3 mmolL) and in healthy MS was 5.8 mmol/L (range, 4.0-11.8 mmol/L).

Miniature Schnauzers with HTGL were significantly older (median, 8.8 y; range, 2-14.2 y) and had significantly higher body weight (8.14 kg; range, 4.26-16.3 kg) compared to normolipidemic MS (for age: median 5.4 y, range 1-12.9; for body weight: median 7.54 kg, range, 5.2-11.6 kg; both $P < .001$). However, no significant difference was found in BCS between the 2 groups. In addition, MS with HTGL had significantly higher serum calprotectin concentrations (median, 200 μg/L; range, 82-1336 μg/L) compared to normolipidemic MS (median, 165 μg/L; range, 28-371 μg/L; $P < .001$). A weak positive correlation was observed between serum triglyceride and calprotectin concentrations ($\rho = .34$, $P < .001$) and between serum cholesterol and calprotectin concentrations ($\rho = .17$, $P = .04$). A weak inverse correlation was detected between serum glucose and calprotectin concentrations ($\rho = -.28$, $P < .001$), but serum glucose concentrations did not differ between hypercalprotectinemic dogs and dogs with normal serum calprotectin concentrations in both the hyperlipidemic ($P = .1$) and normolipidemic groups ($P = .50$). No other significant differences were found between MS with HTGL and normolipidemic MS (sex, $P = .50$; BCS, $P = .50$; glucose, $P = .08$).

3.2 | MS with HTGL included in the dietary trial (n = 15)

Fifteen dogs were placed on the ultra-low-fat diet for a median of 18 wk (range, 14-26 wk). The median body weight was 8.4 kg (range, 5-10.9 kg), median BCS was 5/9 (range, 4/9-6.5/9), and median age was 8.3 y (range, 6.7-11.8 y). Of these 15 animals, 9 were female,

TABLE 1 Serum triglyceride, cholesterol, calprotectin, insulin, and C-reactive protein (CRP) concentrations in Miniature Schnauzers (MS) with HTGL before and after dietary intervention with an ultra-low fat diet

	Before dietary intervention		After dietary change		
Parameter	Median [range]	% of values above the RI	Median [range]	% of values above the reference interval	*P* value
Triglycerides (in mg/dL)	602 [181-1320]	100	179 [77-745]	67	<.001
Cholesterol (in mg/dL)	366 [158-944]	60	259 [122-917]	20	.001
Calprotectin (in μg/L)	199 [94-570]	27	228 [64-387]	40	.72
C-reactive protein (in mg/L)	0.1 [0.1-8.5]	7	0.1 [0.1-7.2]	0	.94
Insulin (in mU/L)	20.8 [3.3-39.8]	-[a]	8.1 [3.8-48.3]	-[a]	.06

[a]No reference interval established.

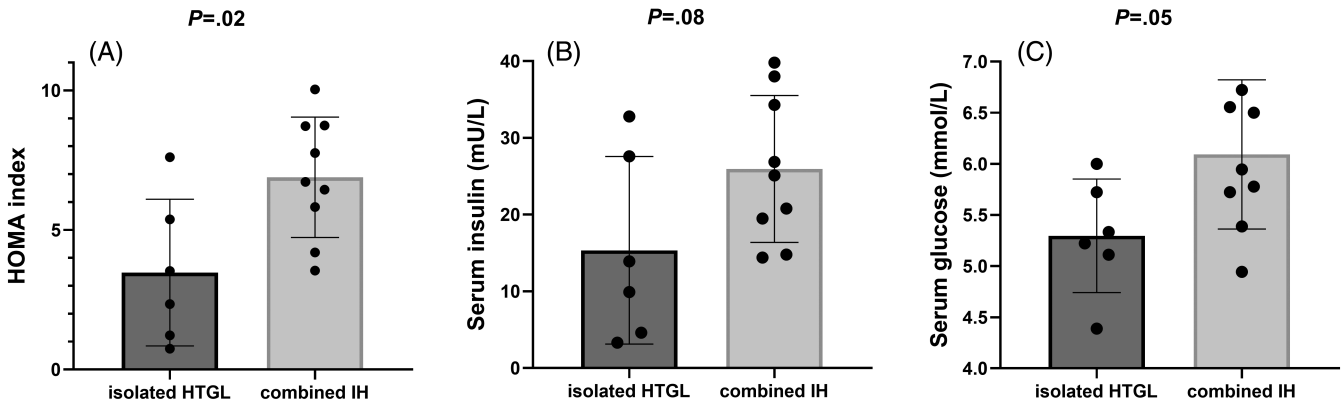

FIGURE 1 A) Miniature Schnauzers with combined IH ($n = 9$) had significantly higher pre-treatment HOMA-IR scores (median: 6.7, range: 3.5-10.0) and a higher proportion of dogs with increased serum calprotectin concentrations (44%) compared to dogs with isolated hypertriglyceridemia ($n = 6$; median HOMA-IR score: 2.9, range: 0.8-7.6; proportion of hypercalprotectinemic dogs: 0%). B) and C) whereas the difference in serum insulin (median: 25.1 mU/L, range: 14.4-39.8 mU/L vs median: 11.9 mU/L, range: 3.3-32.8 mU/L) and glucose concentrations (median: 5.9 mmol/L, range: 4.9-7.3 mmol/L vs median: 5.3 mmol/L, range: 4.4-6.0 mmol/L) did not reach statistical significance

5 were male, and for 1 sex status was unknown. In 5/15 dogs, HTGL completely resolved, whereas in 2/15 dogs serum triglyceride concentrations decreased but these dogs remained hyper-triglyceridemic, with 7/15 (47%) dogs experiencing a decrease in serum triglyceride concentration with dietary intervention. Serum triglyceride ($P < .001$) and cholesterol ($P = .001$) concentrations significantly decreased after dietary intervention (Table 1).[18]

Six of 15 dogs had HTGL only whereas 9 dogs also had HCHOL. Dogs with combined HTGL and HCHOL had significantly higher pre-treatment HOMA-IR scores (median, 6.7; range, 3.5-10.0) than dogs

TABLE 2 Comparisons of baseline variables between Miniature Schnauzers (MS) with HTGL resolution and MS without HTGL resolution after intervention with an ultra-low fat diet

	MS with HTGL resolution	MS without HTGL resolution	
Baseline parameters	**Median [range]**	**Median [range]**	***P* value**
Body weight (in kg)	9.1 [5.0-9.5]	8.3 [6.8-10.9]	.96
BCS	4 [4-7]	5 [4-6]	.21
Age (in years)	7.7 [7.2-10.3]	8.3 [6.7-11.8]	.59
Sex	3 F/2 M	6 F/3 M	.74
Triglycerides (in mg/dL)	403 [218-998]	709 [181-1320]	.21
Cholesterol (in mg/dL)	454 [158-530]	328 [241-944]	.77
Glucose (in mmol/L)	6.0 [4.9-6.7]	5.6 [4.4-7.3]	.31
Insulin (in mU/L)	19.5 [4.6-39.8]	23.8 [4.4-7.3]	.86
HOMA-IR	5.8 [1.2-8.7]	6.1 [0.7-10.0]	.95
Calprotectin (in μg/L)	206.2 [163.9-266.5]	189.5 [93.9-570.1]	.59
C reactive protein (in mg/L)	0.1 [0.1-3.3]	0.1 [0.1-7.2]	.86

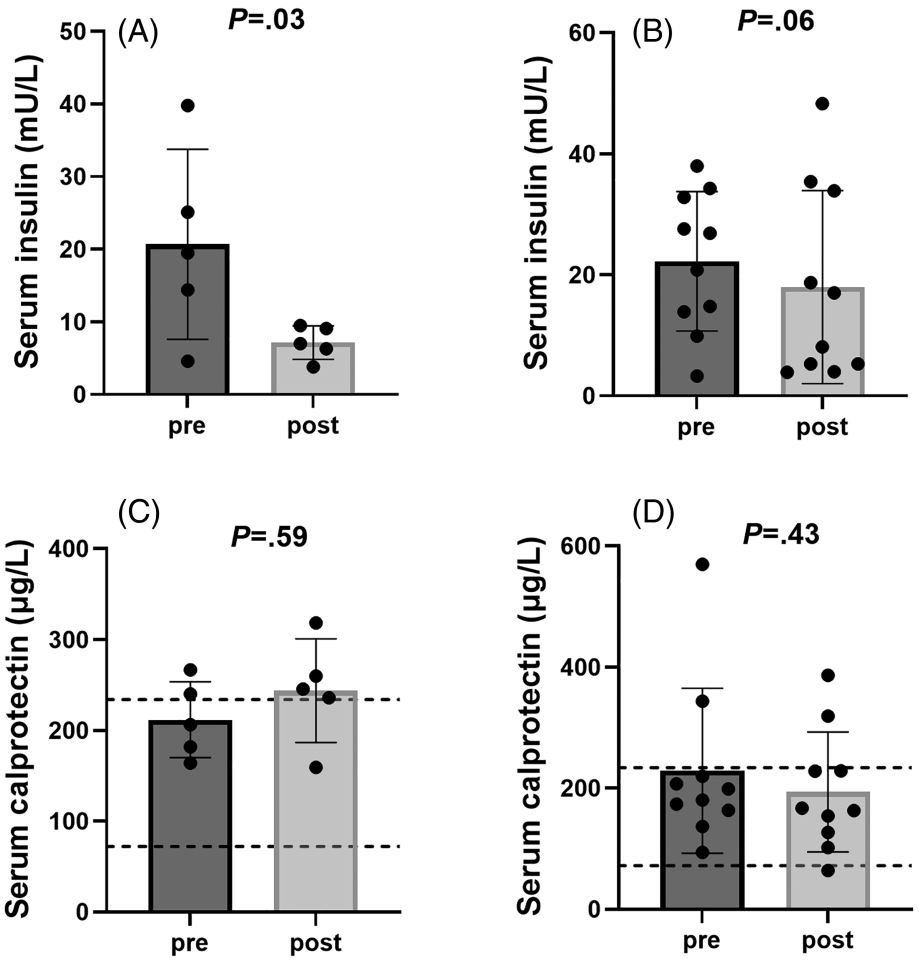

FIGURE 2 Changes in serum insulin (A and B) and calprotectin (C and D) concentrations in Miniature Schnauzers (MS) with hypertriglyceridemia (HTGL) included in the ultra-low fat dietary trial. (A) Serum insulin concentrations in MS with HTGL resolution (5/15) following dietary intervention. (B) Serum insulin concentrations in MS without HTGL resolution (10/15) following dietary intervention. (C) Serum calprotectin concentrations in MS with HTGL resolution (5/15) following dietary intervention. (D) Serum calprotectin concentrations in MS with HTGL resolution (10/15) following dietary intervention

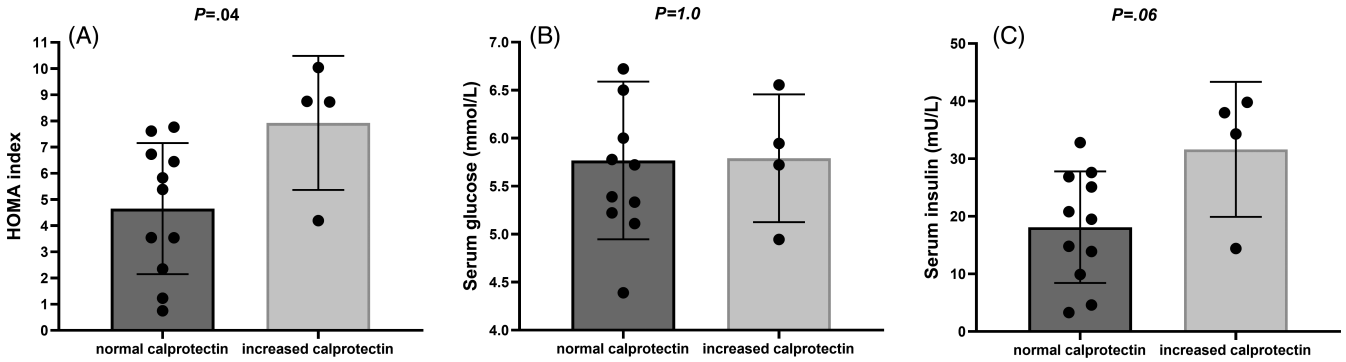

FIGURE 3 A) Miniature Schnauzers (MS) with hypertriglyceridemia (HTGL) and hypercalprotectinemia ($n = 4$) had significantly higher HOMA-IR scores (median: 8.7, range: 4.2-10.0) compared to MS with HTGL and normal serum calprotectin concentrations ($n = 11$; median: 5.4, range: 0.8-7.8). B) and C) The difference in serum insulin concentrations (median: 36.2 mU/L, range: 14.4-39.8 mU/L vs median: 19.5 mU/L, range: 3.3-32.8 mU/L) did not reach statistical significance, and there was no difference in serum glucose concentrations (median: 5.8 mmol/L, range: 4.9-6.6 mmol/L vs median: 5.7 mmol/L, range: 4.4-7.3 mmol/L)

with isolated HTGL (median, 2.9; range, 0.8-7.6; $P = .02$; Figure 1). The difference in serum insulin ($P = .08$) and serum glucose concentrations ($P = .05$) between these groups did not reach statistical significance. No significant association was found between HOMA-IR scores and serum triglyceride concentrations ($\rho = .421$, $P = .12$).

When considering all 15 dogs, no significant differences in baseline variables were found between MS that experienced complete resolution of HTGL compared to MS in which triglycerides remained above the reference interval (Table 2). Post-treatment serum insulin concentrations were lower than pre-treatment serum insulin concentrations (Table 1), but this difference was not significant ($P = .06$). When considering only those dogs that had complete resolution of HTGL after dietary intervention (5 dogs), a significant decrease in serum insulin concentrations occurred after dietary intervention ($P = .03$; Figure 2). Pre-treatment and post-treatment median serum insulin concentration in these dogs were 19.5 mU/L (range, 4.6-39.8 mU/L) and 7.0 mU/L (range, 3.8-9.5 mU/L), respectively.

A higher proportion of dogs with combined HTGL and HCHOL had increased serum calprotectin concentrations (4/9) compared to dogs with isolated HTGL at baseline (0/6; $P = .03$). The HOMA-IR scores were significantly higher in dogs with increased serum calprotectin concentrations compared to dogs with normal serum calprotectin concentrations ($P = .04$; Figure 3), but no significant differences were detected in serum insulin ($P = .06$) or glucose ($P = 1.0$) concentrations (Figure 3). Changes in serum calprotectin and CRP in dogs with HTGL resolution did not change significantly ($P = .59$ for calprotectin, $P = .50$ for CRP; Table 1).

4 | DISCUSSION

Evidence of IR has been reported previously in MS with HTGL.[4] Our study provides evidence that complete resolution of HTGL in MS (ie, return of serum triglyceride and cholesterol concentrations to within their respective RI) while feeding an ultra-low-fat diet leads to a significant decrease in serum insulin concentrations. In addition, dogs with combined HTGL and HCHOL had significantly higher pre-treatment HOMA-IR scores compared to dogs with isolated HTGL. Finally, calprotectin was weakly negatively correlated with serum glucose concentrations.

Baseline serum insulin concentration is considered comparable to HOMA-IR scores for evaluation of IR in non-diabetic humans and possibly dogs.[4] Increased serum insulin concentrations reflect a compensatory mechanism for resolution of peripheral IR.[21] Although trends in HOMA-IR scores could not be evaluated in our study, the findings suggest that resolution of HTGL by feeding an ultra-low-fat diet results in an improvement in insulin sensitivity in MS. This finding is in agreement with studies in humans, where improvement of lipid profiles is associated with a decrease in serum insulin concentrations.[22,23]

The reason for the decrease in serum insulin concentrations and potentially improved insulin sensitivity after dietary intervention in MS in our study cannot be determined. Insulin concentrations were significantly decreased only in MS with complete resolution of HTGL and not in MS with partial resolution of hyperlipidemia. This finding suggests that IR, as indicated by serum insulin concentrations, is more likely to result from improved serum lipid concentrations rather than simply the consumption of a low-fat diet itself. However, this hypothesis needs to be tested in experimental studies. Hypertriglyceridemia is associated with IR in both humans and dogs, and it would be expected that resolution of HTGL would lead to improvement of IR.[22] Studies in humans also have shown that improvement of hyperlipidemia improves IR.[22]

Several mechanisms have been proposed to explain the associations between hyperlipidemia and IR, mainly based on studies in humans or gnotobiotic animals. One such mechanism is the effect of abnormal concentrations of hepatic diacylglycerol, an intermediate of triglyceride synthesis,[24,25] and ceramides, precursors of sphingolipid synthesis,[26] on modifying the action of insulin receptors, which results in a decrease in adipose tissue insulin responsiveness.[25] Other mechanisms include specific haplotypes of the lipoprotein lipase gene that increase the risk of hepatic IR manifestation,[27] and experimental

infusions of triglycerides induce muscle IR through the glucose-fatty acid cycle.[11] Finally, HTGL in humans has been associated with subclinical inflammation and increased production of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α).[28,29] In obese humans, TNF-α production by abnormal adipose tissue infiltrated by macrophages and other immunocytes induces IR, but this link has not been proven in humans with HTGL.[30,31]

The clinical importance of persistence of higher insulin concentrations in MS that remained hypertriglyceridemic compared to MS in which triglyceride concentrations returned to normal after dietary management remains to be determined. Insulin resistance has been associated with increased risk for several diseases in humans (mostly diabetes mellitus, metabolic syndrome, atherogenesis, and heart disease)[9,11,12,32] whereas in dogs, limited data is available.[33] Therefore, correction of IR by use of low-fat diets, lipid-lowering medications or both could be recommended. Whether MS with increased serum insulin concentrations truly benefit from these interventions remains to be determined.

Interestingly, dogs with combined HTGL and HCHOL had significantly higher pre-treatment HOMA-IR scores compared to dogs with isolated HTGL. This observation has not been made previously in dogs. In humans, some studies have found no differences between HTGL types and the severity of IR,[34] whereas others have found that the severity of IR is associated with the lipid phenotype.[32]

The finding of opposite changes in serum insulin and calprotectin concentrations after successful dietary intervention, as already shown in our previous study,[18] differs from studies in humans with obesity and weight loss,[35,36] and indicates that subclinical inflammation is not strictly associated with IR in MS with HTGL.

Serum CRP concentration was normal in all but 1 dog both before and after dietary intervention. Notably, this dog had combined IH and the highest serum cholesterol concentrations compared to all of the other MS. Increases in serum CRP concentrations in humans have been associated with development of atherosclerosis, and CRP is deposited in the arterial wall during atheromatic plaque formation.[37,38] In addition, in humans, increased serum low-density lipoprotein (LDL) concentrations, which mainly transfer cholesterol to the tissues and are the primary cause of atherogenesis, are associated with increases in serum CRP concentrations.[39] However, unlike humans, atherogenesis is not a common clinical problem in dogs because of their unique lipoprotein metabolism and lack of cholesteryl ester transfer protein (CETP) enzyme.[3] Also, compared to humans, LDL molecules are not as highly abundant in canine serum because most of their cholesterol is found in high-density lipoprotein (HDL) molecules. Therefore, differences in lipoprotein metabolism between humans and dogs might explain the lack of significant differences in serum CRP concentrations in dogs with HTGL. Alternatively, it is possible that the CRP assay used in our study was not sensitive enough to detect small differences in CRP concentrations. In addition, the high inter-individual variability in baseline serum CRP concentrations[40] must be considered when comparing serum CRP concentrations among dogs or to a population-based RI.

Lack of significant serum glucose variation in clinically healthy MS with or without hypercalprotectinemia agrees with studies in non-obese people where parameters of glucose homeostasis were not correlated with serum calprotectin concentrations,[41] but it contrasts with results in diabetic or obese patients.[15] However, the association of hypercalprotectinemia with higher HOMA-IR scores in MS with HTGL agrees with studies in people with metabolic syndrome.[15,42] Altogether, these findings suggest that subclinical systemic inflammation in MS with HTGL is associated with IR, but that normoglycemia is maintained in affected MS despite IR and increased hypercalprotectinemia, indicating neutrophil and macrophage activation.

Our study had some limitations. First, although not very likely, the possibility of some conditions causing secondary hyperlipidemia cannot be definitively ruled out. Second, it is unknown how long it takes for markers of IR, such as HOMA-IR and serum insulin concentrations, and inflammatory biomarkers to improve after correction or improvement of HTGL. Therefore, changes in these markers may require a longer time period to occur, and may have been missed in our study. In addition, obtaining single serum samples for evaluating insulin concentrations is another limitation of the study, given the large variation of insulin concentrations throughout the day. The lack of availability of serum glucose concentrations in MS with IH completing the dietary trial did not allow the follow-up measurement of HOMA-IR scores. The HOMA-IR score is not considered the gold standard for evaluating insulin sensitivity in dogs, and no RI exists in this species. In addition, the lipid content of the initial diets might have affected baseline concentrations of triglycerides in dogs with IH, but in our study, the exact lipid content of these diets could not be determined because many of the dogs were consuming combinations of different diets and treats. Finally, the small sample size for the dietary trial part of the study could have led to type II error.

5 | CONCLUSION AND CLINICAL IMPORTANCE

Resolution of HTGL with dietary intervention is associated with a decrease in insulin concentrations and potentially improvement in insulin sensitivity. The subclinical inflammatory phenotype in MS with HTGL appears to be associated with IR and might contribute to the development of pancreatitis or other conditions. Calprotectin might be a useful biomarker for IR in MS with HTGL and likely has ambivalent proinflammatory and anti-inflammatory properties in dogs. Additional controlled studies investigating a possible link between innate immunity and metabolic pathways in MS with HTGL are warranted.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

The authors declare no off-label use of antimicrobials.

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

The protocol for the collection of serum samples from healthy Miniature Schnauzers for evaluation and further study of idiopathic hyperlipidemia was reviewed and approved by the Texas A&M University Clinical Research Review Committee (CRRC# 2008-37, approved 11-05-2008).

HUMAN ETHICS APPROVAL DECLARATION

The authors declare human ethics approval was not needed for this study.

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