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Proteinuria and lipoprotein lipase activity in Miniature schnauzer dogs with and without hypertriglyceridemia

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Abstract

Spontaneous hyperlipidemia in rats causes glomerular disease. Idiopathic hypertriglyceridemia (HTG) is prevalent in Miniature schnauzers, but its relationship with proteinuria is unknown. Decreased activity of major lipid metabolism enzymes, lipoprotein lipase (LPL) and hepatic lipase (HL), may play a role in the cyclic relationship between hyperlipidemia and proteinuria. These enzymes have also not been previously investigated in Miniature schnauzers. The aims of this study were to determine the relationship between HTG and proteinuria in Miniature schnauzers and to measure LPL and HL activities in a subset of dogs. Fifty-seven Miniature schnauzers were recruited (34 with and 23 without HTG). Fasting serum triglyceride concentrations and urine protein-to-creatinine ratios (UPC) were measured in all dogs, and LPL and HL activities were determined in 17 dogs (8 with and 9 without HTG). There was a strong positive correlation between triglyceride concentration and UPC ($r = 0.77-0.83$, $P < 0.001$). Proteinuria (UPC > 0.5) was present in 60% of dogs with HTG and absent from all dogs without HTG ($P < 0.001$). Proteinuric dogs were not azotemic or hypoalbuminemic. Dogs with HTG had a 65% reduction in LPL activity relative to dogs without HTG ($P < 0.001$); HL activity did not differ. Proteinuria occurs with HTG in Miniature schnauzers and could be due to lipid-induced glomerular injury. Reduced LPL activity may contribute to the severity of HTG, but further assay validation is required.

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Conflict of interest statement

None of the authors have any financial or personal relationship with people or organizations that could inappropriately influence or bias the content of the paper.

Keywords

Canine; Miniature schnauzer; Glomerular disease; Hyperlipidemia; Triglycerides

Introduction

Idiopathic hypertriglyceridemia (HTG) is common in Miniature schnauzers, affecting more than 75% of the breed by 10 years of age (Xenoulis et al., 2007). The disorder has been associated with pancreatitis (Xenoulis et al., 2010, 2011a), gall bladder mucoceles (Kutsunai et al., 2014), ocular lipid deposits (Crispin, 1993; Zarfoss and Dubielzig, 2007) and neurological abnormalities (Rogers et al., 1975; Bodkin, 1992). Miniature schnauzers with HTG have higher serum liver enzyme activities and fasting serum insulin concentrations than those without HTG (Xenoulis et al., 2008, 2011b).

Hyperlipidemia, including both HTG and hypercholesterolemia, can induce kidney injury in humans and rats, a concept termed 'lipid nephrotoxicity' (Gyebi et al., 2012). Spontaneously hyperlipidemic Imai rats and obese Zucker rats develop progressive focal segmental glomerulosclerosis (Kasiske et al., 1985; Kondo et al., 1995). In humans, hyperlipidemia has been associated with increased risk for, or more rapid progression of, renal disease (Gyebi et al., 2012). The relationship between hyperlipidemia and glomerular disease is complex and cyclic. Hyperlipidemia is a well-documented sequela of protein-losing nephropathies.

Urinary protein loss causes a decrease in lipoprotein lipase (LPL) activity and impairment of very low density lipoprotein binding to LPL (Shearer et al., 2001; Sato et al., 2002; Clement et al., 2014). Hepatic lipase (HL) activity may also be reduced (Liang and Vaziri, 1997) and hepatic genes coding for proteins involved in the biosynthesis of lipids are upregulated in nephrotic syndrome (Zhou et al., 2008). These changes result in decreased clearance and catabolism of triglyceride rich lipoproteins, and increased production of cholesterol, fatty acids and triglycerides.

The primary aim of this study was to evaluate the relationship between fasting serum triglyceride concentration and urine protein-to-creatinine ratio (UPC) in Miniature schnauzers. We hypothesized that there would be a positive correlation between HTG and proteinuria. Two independent groups were used to test the primary hypothesis. A secondary aim was to compare LPL and HL activities in a subgroup of Miniature schnauzers with and without HTG. Due to the critical role of LPL and HL in lipoprotein conversion via hydrolysis of core triglycerides, as well surface phospholipids, we hypothesized that a deficiency in lipase activity may be present.

Materials and methods

Study population

The first group comprised Miniature schnauzers recruited to the Ohio State University (OSU) Veterinary Medical Center from 2001 to 2003. No restrictions were placed on age or reproductive status. The second group comprised Miniature schnauzers recruited to the University of Minnesota (UMN) Veterinary Medical Center from 2012 to 2015 for both this

study and concurrent genetic studies in the breed. Only neutered dogs 7 years of age were recruited to the UMN group in order to determine whether the findings in the initial OSU group could be replicated in a population where potentially confounding factors were minimized. Dogs were excluded if they had a diagnosis of hyperadrenocorticism, hypothyroidism or diabetes mellitus, or were receiving corticosteroids. Owner written informed consent was obtained for each study participant. The study protocol was approved by the OSU Veterinary Teaching Hospital Executive Committee (2000-46) and the UMN Institutional Animal Care and Use Committee (1509-33019A).

Triglyceride and biochemical measurements

Dogs were fasted for 12–18 h prior to sample collection. Blood was collected in tubes without additives. Serum was separated and analyzed immediately (48 samples) for triglyceride concentration (OSU group: Roche Hitachi 911 Chemistry analyzer, Roche Diagnostics; UMN group: AU480 Chemistry analyzer, Beckman Coulter) or stored at -80°C (nine samples) for up to 18 months prior to analysis (Matthan et al., 2010). HTG was defined as a triglyceride concentration >75 mg/dL (OSU group) or >85 mg/dL (UMN group) based on the respective laboratory reference ranges and was characterized as mild (76–400 mg/dL OSU group; 86–400 mg/dL UMN group), moderate (401–800 mg/dL) or severe (>800 mg/dL) (Xenoulis et al., 2007, 2010). The OSU group and a subset of the UMN group had chemistry panels performed on fresh serum (OSU group: COBAS c501 Chemistry analyzer, Roche Diagnostics; UMN group: AU480 Chemistry analyzer, Beckman Coulter). The UMN group had blood collected into a syringe with dry lithium heparin for determination of creatinine, glucose and electrolytes using a blood gas analyzer (i-STAT 1, Abbott Point of Care).

Endocrine testing

The OSU dogs had plasma cortisol concentrations (Immulin analyzer, Immulin Diagnostic Products Corporation) measured before and 1 h after administration of synthetic adrenocorticotrophic hormone (5 $\mu\text{g/kg}$ IV Cortrosyn, Organon). Serum total thyroxine, total triiodothyronine, free thyroxine, thyroid stimulating hormone, and triiodothyronine and thyroxine autoantibody concentrations, were also measured in the OSU dogs (Michigan State University Animal Health Diagnostic Laboratory). The owners of the UMN dogs with HTG were asked to return for a second study visit for determination of serum total thyroxine concentration (AU480 Chemistry analyzer, Beckman Coulter) and a urine cortisol-to-creatinine ratio (Marshfield Labs Veterinary Services), but these tests were not required for study participation. For two UMN dogs, urine cortisol-to-creatinine ratios were determined on samples stored at -80°C for up to 18 months (Miki and Sudo, 1998).

Urinary protein determination

Urine was collected by cystocentesis or mid-stream free-catch into a sterile container. Samples were stored at room temperature and a urinalysis was performed within 4 h. Hematuria was defined as >30 erythrocytes/high power field (HPF; $40\times$ objective), pyuria as >5 leukocytes/HPF and bacteriuria as the presence of any microscopically detected bacteria; dogs with any of these findings were excluded from the proteinuria analyses. A UPC was performed the same day (48 samples) or on an aliquot that had been stored at -80°C (nine

samples) for up to 18 months (Parekh et al., 2007). Urine protein was determined using a colorimetric method and urine creatinine was determined by a modified Jaffe procedure on an automated chemistry analyzer (OSU group: Roche Hitachi 911 Chemistry analyzer, Roche Diagnostics; UMN group: AU480 Chemistry analyzer, Beckman Coulter). Proteinuria was defined as a UPC ≥ 0.5 and characterized as mild (0.5–0.9), moderate (1.0–1.9) or severe (≥ 2.0) (Lees et al., 2005).

Lipoprotein and hepatic lipase activities

LPL and HL activities were determined in a subset of the OSU group after a 12 h fast and after IV administration of sodium heparin (100 IU/kg, UPS Elkins-Sinn). Blood was obtained via jugular venipuncture prior to and 10 min after heparin administration and placed into tubes containing ethylene diamine tetra-acetic acid (EDTA) on ice. Plasma samples were frozen immediately (-70°C) and processed within 3 months. Total plasma LPL and HL activities were measured using a radio-isotope labeled emulsion protocol (Ostlund-Lindqvist and Iverius, 1975; Iverius and Brunzell, 1985; Babirak et al., 1989) that has been used to determine lipase activity in human beings and multiple animal species (Ginzinger et al., 1996; Matsusue et al., 2003; Lupia et al., 2012). A monoclonal antibody (5D2) was added that inhibits active LPL but not HL. The decrease in total lipase activity after addition of 5D2 indicates LPL activity, while the remaining activity reflects HL. Results are expressed as fatty acids (nmol) released/min/mL plasma. A bovine milk lipase and a human post-heparin standard were included with each assay.

Statistical analysis

Data distribution was inspected with Q-Q plots and assessed for normality with the Shapiro–Wilk test. Normally distributed data are reported as mean $\pm$ standard deviation. The triglyceride and UPC data required logarithmic transformations (logTG and logUPC, respectively) for analysis; the data are reported as median (range). Body condition score (BCS, 1–5 scale) is also reported as median (range). For each study group, differences between dogs with and without HTG were determined with the Student's *t* test or the Wilcoxon rank-sum test. Pearson correlation coefficients (*r*) were calculated to assess relationships between variables of interest. Associations of age (years), sex (male versus female) and BCS were analyzed in a multivariable regression with logTG as the outcome. LogUPC was analyzed as a parallel outcome. A simple regression was used to test the relationship between serum cholesterol and UPC; cholesterol was not included in the multivariable regressions due to missing values. Significance was assessed using Type II tests and the coefficient of determination (R^2) was calculated to assess model fit. Fisher's exact test was used to compare the prevalence of proteinuria, and a χ^2 test was performed to compare reproductive status between dogs with and without HTG. Analyses were performed using R software for statistical computing.¹ $P < 0.05$ was considered to be significant.

¹See: <http://www.R-project.org/>.

Results

Fifty-seven dogs were enrolled, comprising 30 OSU and 27 UMN dogs. Signalment and biochemical data are presented in Table 1. In the OSU group, dogs with HTG were older and more likely to be neutered than those without ($P < 0.001$ for both). The UMN group was composed entirely of neutered dogs ≥ 7 years of age (as per the inclusion criteria for this group), and age was not different between dogs with and without HTG ($P = 0.092$).

Fasting HTG was present in 16/30 (53%) OSU dogs and 18/27 (67%) UMN dogs (Table 1). Cholesterol was measured in 46 dogs, and hypercholesterolemia was detected in 11/29 (38%) dogs with HTG and 1/17 (6%) dogs without HTG. Two OSU dogs with HTG were receiving phenobarbital. Due to effects of this medication on serum triglyceride concentrations (Kluger et al., 2008), these dogs were excluded from the regression analyses for logTG.

All 16 OSU dogs and 10/18 UMN dogs with HTG underwent thyroid testing and screening for hyperadrenocorticism. The results were within laboratory reference intervals. Seven out of the 8 UMN dogs with HTG that did not have these tests performed had only mild HTG (87–267 mg/dL); one dog had severe HTG (1024 mg/dL). None of these dogs had clinical signs consistent with either hypothyroidism or hyperadrenocorticism, and their owners declined returning for a second visit to test for these conditions. All dogs had serum glucose measured. Two dogs with HTG and one without HTG had mild hyperglycemia (119–143 mg/dL).

Regression analyses for logTG revealed a strong positive relationship between age and logTG in the OSU group ($r = 0.75$, $P < 0.001$; Fig. 1, Table 2). The relationship between age and logTG in the UMN group was not significant (Fig. 1, Table 2). Neither sex nor BCS had a significant correlation with logTG in either group. Reproductive status was not included in the regression analyses because it had a strong relationship with age (all intact dogs except one were ≥ 3 years of age, whereas all neutered dogs were ≥ 4 years of age), which was expected to confound the results.

Three dogs with active urine sediment (two bacteriuria and one hematuria) were excluded from the UPC analyses. One additional dog was excluded due to treatment with enalapril; this dog had moderate HTG and proteinuria (TG = 697 mg/dL, UPC = 1.5). Overall, 60% of the dogs with HTG had proteinuria compared to none of the dogs with normal triglyceride concentrations ($P < 0.001$; Table 1). Eleven of 13 (85%) dogs with moderate to severe HTG were proteinuric, and 7/17 (41%) dogs with mild HTG were proteinuric (Fig. 2). There was a strong correlation between logTG and logUPC in the OSU and UMN groups ($r = 0.83$ and 0.77 , respectively; $P < 0.001$ for both groups; Fig. 3). Cholesterol also correlated with UPC in both groups ($r = 0.57$ – 0.66 ; $P = 0.0026$ – 0.0053); however, whereas all proteinuric dogs had HTG, only 7/18 had hypercholesterolemia. Age was correlated with logUPC in the simple regression analysis (OSU group: $r = 0.66$, $P < 0.001$; UMN group: $r = 0.39$, $P = 0.042$); however, in the multivariable regression analysis, logTG was the only significant predictor of logUPC ($P < 0.001$ for both groups; Table 3). Neither sex nor BCS were associated with UPC. All dogs in both study groups had BUN and creatinine concentrations

within the respective laboratory reference interval. Serum albumin concentrations were available for all proteinuric dogs and were within reference intervals.

Plasma LPL and HL activities were measured in eight OSU dogs with HTG (median 1504 mg/dL, range 373–5510 mg/dL) and nine OSU dogs with normal triglyceride concentrations (median 36 mg/dL, range 24–66 mg/dL). In the regression analyses for LPL activity (Fig. 4, Table 4), logTG and age had strong negative correlations with LPL activity. Mean LPL activity was substantially lower in dogs with HTG (129 ± 39 η mol/mL/min) compared to those without (370 ± 81 η mol/mL/min; $P < 0.001$). There was also a strong negative correlation between LPL activity and logUPC ($r = -0.78$, $P < 0.001$). The HL activity did not significantly correlate with logUPC ($r = 0.44$, $P = 0.076$), and was not different between dogs with and without HTG (136 ± 80 and 105 ± 34 η mol/mL/min, respectively; $P = 0.35$). No predictor had a significant relationship with HL activity in the regression analyses (Fig. 4, Table 4).

Discussion

The present study demonstrates a strong relationship between HTG and proteinuria in Miniature schnauzers. Eighty-five percent of the Miniature schnauzers with moderate to severe HTG and 41% with mild HTG had elevated UPC values, and most were of a severe magnitude (> 2.0). Despite the prevalence and severity of proteinuria, no dog had hypoalbuminemia or azotemia. This study did not test for causality, but the findings suggest that a subclinical lipid nephrotoxicity might occur in Miniature schnauzers with HTG.

Both HTG and hypercholesterolemia have been demonstrated to cause podocyte injury and focal segmental sclerosis in rats (Kamanna and Kirschenbaum, 1993; Joles et al., 2000). Low density lipoprotein can be oxidized by mesangial cells and stimulates pro-inflammatory and pro-fibrotic cytokines, vasoactive substances, and apoptosis (Dalrymple and Kaysen, 2008). In vitro studies demonstrate that disruption of fatty acid oxidation increases apoptosis, intracellular lipid deposition and dedifferentiation in renal tubular epithelial cells (Kang et al., 2015).

Glomerular protein loss can also cause hyperlipidemia. In the nephrotic syndrome, plasma cholesterol has a direct correlation with urinary protein and an inverse correlation with serum albumin (Appel et al., 1985). Abnormal triglyceride metabolism can also occur. Glomerular protein loss causes an increase in the ratio of free fatty acids to albumin that triggers secretion of angiopoietin-like 4 (Angptl4). Circulating Angptl4 serves as an inhibitor of LPL and results in HTG (Clement et al., 2014). This cascade does not occur until proteinuria is moderate to severe; mild proteinuria does not cause increased triglyceride concentrations. There are minimal data available on HTG in dogs with glomerular disease, but disease appears to be mild, with median triglyceride concentrations of 79 mg/dL in nephrotic syndrome and 85 mg/dL in non-nephrotic glomerular disease (Klosterman et al., 2011). Alterations in relative proportions of lipoproteins are reported in dogs with chronic kidney disease (Behling-Kelly, 2014), but it is unknown whether they result in HTG.

The chronological relationship between HTG and proteinuria was not determined in this study. However, Miniature schnauzers have an atypical lipoprotein density profile, independent of whether or not HTG is present (Xenoulis et al., 2013). This suggests that dyslipidemia in the breed is a primary disturbance rather than secondary to renal disease. Hypercholesterolemia commonly accompanied HTG in this study and also correlated with UPC, but most proteinuric dogs had normal serum cholesterol concentrations. The cause-and-effect relationship between HTG, with and without concurrent hypercholesterolemia, and proteinuria warrants investigation.

Lower LPL activity was observed in Miniature schnauzers with HTG. Human patients with loss of function mutations in the LPL gene have close to zero LPL activity and have severe HTG, but those with partial deficiencies (25–75% normal activity) have normal to mildly elevated serum triglycerides (Babirak et al., 1989). The HTG dogs in the present study had decreased LPL activities, but no dog had a complete deficiency. Therefore, the extent of the contribution of reduced LPL activity to the genesis of the HTG is not clear. A negative correlation between LPL activity and proteinuria was noted, and the low LPL activity could be secondary to the effects of urinary protein loss on increasing Angptl4 rather than a primary LPL defect.

Idiopathic HTG in Miniature schnauzers is presumed to be genetic, but animal and environmental factors can influence the disease. In this study, age had a positive relationship with HTG. This is consistent with previous studies. The presence and degree of HTG in Miniature schnauzers is strongly associated with age (Xenoulis et al., 2007) and a positive relationship has also been observed in other breeds (Usui et al., 2014).

There was no association between BCS and triglyceride concentration in this study. Obesity has been inconsistently documented to have an association with HTG in dogs (Jeusette et al., 2005; Kluger et al., 2008; Jericó et al., 2009). There was also no relationship between BCS and proteinuria. An obesity-related glomerulopathy is well described in human beings (Praga and Morales, 2006) and experimentally-induced obesity in dogs results in glomerular lesions (Henegar et al., 2001). However, a recent study did not find any relationship between obesity and proteinuria in client-owned dogs; 20 dogs were overweight or obese, and all had UPC values <0.2 (Tefft et al., 2014). Most of the dogs in this study had an ideal BCS, which could have hindered the ability to identify a relationship between obesity and HTG or proteinuria.

A strength of this study was the use of two independent study groups. In the OSU group, there were differences in reproductive status and age between dogs with and without HTG, but these factors were controlled for in the UMN group. The findings in both groups provide strong evidence for an HTG associated proteinuria in the breed. Limitations of the study include differences in the diagnostics performed for the groups and a lack of dietary assessments. Additionally, though the dogs were clinically healthy, they were not screened for subclinical pancreatitis. HTG increases the risk for pancreatitis (Scherer et al., 2014) and proteinuria is common in pancreatitis (Zuidema et al., 2014). Thus, we cannot rule out that the link between proteinuria and HTG occurs via a mediator variable. The dogs in this study were not screened for other causes of proteinuria and no dog underwent a renal biopsy.

Another limitation of the study is that the assay used to measure LPL and HL activity has not been validated in dogs. In support of the accuracy of LPL activity measurement, the 5D2 antibody used in the assay was raised against bovine LPL and validated for human LPL (Ostlund-Lindqvist and Iverius, 1975; Iverius and Brunzell, 1985); canine LPL is highly homologous to LPL of both species (Holmes et al., 2011). Importantly, 25/26 amino acid residues in the epitope recognized by the 5D2 antibody are identical in dogs and humans, including all eight residues believed to be critical for epitope binding (Chang et al., 1998). This degree of homology for the 5D2 epitope is even greater than for other species (e.g. cow, pig, cat and chicken) with confirmed reactivity. Furthermore, the results were similar to previously reported ranges for dogs (Muller et al., 1985; Watson et al., 1995). Despite this evidence to support the application to canine samples, validation of the assay is needed to confirm the findings of this study.

An additional limitation in the interpretation of the LPL and HL results is that enzyme activities were only determined in a subset of dogs, and those with and without HTG differed in age and reproductive status. These signalment imbalances could have confounded the relationship between enzyme activity and HTG or age. However, data from other species suggest that the impact would be minor (Brodows and Campbell, 1972; Peinado-Onsurbe et al., 1993).

Conclusions

HTG-associated proteinuria occurs in Miniature schnauzers and appears to be associated with a decrease in LPL activity. The clinical significance is unknown, but the adverse consequences of hyperlipidemia on renal disease are well described in other species. Longitudinal studies are needed to further investigate HTG-associated proteinuria and determine the best clinical management for the condition.

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Fig. 1.

Relationship of age with fasting serum triglyceride concentration (TG) in Miniature schnauzers. Plots are shown for (A) untransformed and (B) log transformed TG (logTG). In the Ohio State University (OSU) group (solid line and dots), there was a positive relationship between age and logTG ($r = 0.75$, $P < 0.001$). The relationship in the University of Minnesota (UMN) group (dashed line and open dots) was not significant ($r = 0.31$, $P = 0.11$).

Fig. 2.

Plot of urinary protein-to-creatinine ratios (UPC) in Miniature schnauzers with normal serum triglyceride concentrations and mild, moderate or severe hypertriglyceridemia (HTG). Proteinuria was defined as a UPC $\geq$ 0.5 (dashed line).

Fig. 3. Relationship of fasting triglyceride concentration (TG) with urinary protein-to-creatinine ratio (UPC) in Miniature schnauzers. Plots are shown for (A) untransformed and (B) log-transformed data (logTG and logUPC). LogTG was a positive predictor of logUPC in both the Ohio State University (OSU) group (solid line and dots) and University of Minnesota (UMN) group (dashed line and open dots) ($r = 0.83$ and 0.77 , respectively; $P < 0.001$). r , Pearson correlation coefficient.

Fig. 4.

Relationship of fasting serum triglyceride concentration (TG) with lipoprotein lipase (LPL) and hepatic lipase (HL) activities in Miniature schnauzers. Plots are shown for LPL with (A) untransformed TG and (B) log-transformed TG (logTG) and HL with (C) untransformed TG and (D) logTG. There was a negative relationship between logTG and LPL activity ($r = -0.85$, $P < 0.001$). No significant relationship was detected between logTG and HL ($r = 0.22$, $P = 0.39$). r , Pearson correlation coefficient.

Table 1

Signalment and biochemical data for study groups.

	Ohio State University (OSU) group		University of Minnesota group	
	Normal (14 dogs)	HTG (16 dogs)	Normal (9 dogs)	HTG (18 dogs)
Age	2.1 ± 1.3	8.5 ± 3.9 *	9.2 ± 1.4	10.3 ± 1.6
Sex	6 MI, 1 MU, 6 FI, 1 FU	7 MN, 7 FS, 1 FI, 1 FU *	5 MN, 4 FS	10 MN, 8 FS
BCS	3 (2–4)	3 (2–5)	3 (3–4)	3 (3–4)
TG	45 (4–69)	575 (108–5510) *	68 (14–81)	303 (87–2089) *
Cholesterol	209 ± 71	310 ± 98 *	228 ± 22	327 ± 109 *
UPC ^a	0.1 (0.1–0.2)	0.6 (0.1–5.7) *	0.1 (0.1–0.4)	0.6 (0.1–4.8) *
<0.5	14 (1.00)	4 (0.25)	9 (1.00)	8 (0.44)
0.5–0.9	0 (0.00)	2 (0.125)	0 (0.00)	2 (0.11)
1.0–1.9	0 (0.00)	2 (0.125)	0 (0.00)	1 (0.06)
2.0	0 (0.00)	4 (0.25)	0 (0.00)	7 (0.39)

HTG, hypertriglyceridemia; MN, male neutered; MI, male intact; MU, male unreported reproductive status; FS, female spayed; FI, female intact; FU, female unreported reproductive status; BCS, body condition score (1–5 scale); TG, serum triglyceride concentration; UPC, urine protein-to-creatinine ratio.

Values are mean ± standard deviation for age (years) and cholesterol (mg/dL), median (range) for BCS, TG (mg/dL) and UPC, and count (proportion) for UPC subcategories. Significant differences between dogs with and without HTG within study groups are denoted with

* $P < 0.05$.

^a UPC data is only reported for 12/16 dogs in the OSU group with HTG; see the text for explanation.

Table 2

Effects of animal factors on serum triglyceride concentrations.

	Estimate	Standard error	F value	P value	R ²
Ohio State University group					0.60
Age	0.27	0.06	22.6	<0.001	
Sex (male)	-0.38	0.49	0.6	0.44	
BCS	0.31	0.34	0.8	0.37	
University of Minnesota group					0.13
Age	0.24	0.16	2.3	0.15	
Sex (male)	-0.48	0.50	0.9	0.35	
BCS	0.22	0.52	0.2	0.67	

R², coefficient of determination; BCS, body condition score.

Table 3
Effects of serum triglyceride concentration and animal factors on urinary protein-to-creatinine ratios.

	Estimate	Standard error	F value	P value	R ²
Ohio State University group					0.72
logTG	0.52	0.12	17.4	<0.001	
Age	0.09	0.06	1.9	0.19	
Sex (male)	0.30	0.35	0.7	0.41	
BCS	-0.16	0.24	0.4	0.52	
University of Minnesota group					0.68
logTG	0.92	0.16	34.6	<0.001	
Age	0.12	0.12	0.8	0.34	
Sex (male)	0.61	0.38	2.5	0.13	
BCS	0.25	0.39	0.4	0.52	

R², coefficient of determination; logTG, log transformed triglyceride concentration; BCS, body condition score.

Table 4

Effects of serum triglyceride concentration and animal factors on lipoprotein lipase (LPL) and hepatic lipase (HL) activity.

	Estimate	Standard error	F value	P value	R ²
LPL					0.82
logTG	-35.1	11.8	8.7	0.012	
Age	-13.4	5.6	5.8	0.033	
Sex (male)	-28.0	35.2	0.6	0.44	
BCS	-11.1	24.6	0.2	0.66	
HL					0.24
logTG	-4.9	10.5	0.2	0.65	
Age	7.5	4.9	2.4	0.15	
Sex (male)	-6.8	31.1	0.1	0.83	
BCS	-8.9	21.7	0.2	0.69	

R², coefficient of determination; LPL, lipoprotein lipase; HL, hepatic lipase; logTG, log transformed triglyceride concentration; BCS, body condition score.