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

E. Furrow [a,*], J.Q. Jaeger [b,1], V.J. Parker [b], K.W. Hinchcliff [b,2], S.E. Johnson [b], S.J. Murdoch [c], I.H. de Boer [d], R.G. Sherding [b], J.D. Brunzell [c]

[a] Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55113, USA
[b] Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210, USA
[c] Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, University of Washington, Seattle, WA 98195, USA
[d] Division of Nephrology, Department of Medicine, University of Washington, Seattle, WA 98104, USA

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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.

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

* Corresponding author. Tel.: +1 612 6257493.
E-mail address: furro004@umn.edu (E. Furrow).
[1] Present address: Carolina Veterinary Specialists, Charlotte, NC 28104, USA.
[2] Present address: Faculty of Veterinary Science, University of Melbourne, VIC 3010, Australia.

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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 (Immulite analyzer, Immulite Diagnostic Products Corporation) measured before and 1 h after administration of synthetic adrenocorticotropic hormone (5 µg/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× 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.

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.

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 ± 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.

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] See: http://www.R-project.org/.