Journal of Veterinary Internal Medicine
Open Access

Standard Article

J Vet Intern Med 2017;31:1740–1748

Clinical Consequences of Hypertriglyceridemia-Associated Proteinuria in Miniature Schnauzers

R.E. Smith, J.L. Granick, C.D. Stauthammer, D.J. Polzin, D.A. Heinrich, and E. Furrow

Background: Primary hypertriglyceridemia is a common condition in older Miniature Schnauzers that recently has been associated with proteinuria and underlying glomerular pathology, particularly glomerular lipid thromboemboli. Consequences of glomerular disease can include hypertension, thromboembolic disease, and cardiac disease. The incidence of these sequelae in Miniature Schnauzers with hypertriglyceridemia-associated proteinuria (HTGP) is unknown.

Objective: To investigate prevalence of hypertension, decreased antithrombin III activity, and cardiac disease in Miniature Schnauzers with and without HTGP.

Animals: Thirty-two Miniature Schnauzers ≥7 years old.

Methods: Prospective case-control study. Data collected from dogs included a CBC, biochemistry panel, urinalysis, urine protein-to-creatinine ratio, urine cortisol-to-creatinine ratio, serum total thyroxine concentration, fasting serum triglyceride concentration, indirect blood pressure, antithrombin III activity, and serum cardiac troponin I concentration. Results from dogs with HTGP (serum triglyceride concentration ≥ 100 mg/dL and urine protein-to-creatinine ratio >0.5) were statistically compared to normotriglyceridemic, nonproteinuric dogs.

Results: Eighteen of the 32 dogs (56%) had primary hypertriglyceridemia. Of those dogs, 8 of 18 had proteinuria. None of the HTGP dogs were azotemic or hypoalbuminemic. Serum albumin concentration, alkaline phosphatase activity, and cholesterol concentration were significantly increased in dogs with HGTP compared to those without HGTP. No increased risk of hypertension, decreased antithrombin III activity, or cardiac disease was noted. Limited data from 8 dogs with HTGP showed no development of hypoalbuminemia or azotemia over a median follow-up period of 18 months.

Conclusions and Clinical Importance: Geriatric Miniature Schnauzers with HGTP may have a good prognosis overall, and are not typically azotemic or hypoalbuminemic.

Key words: Glomerular disease; Hyperlipidemia; Renal; Urine protein-to-creatinine ratio.

Primary hypertriglyceridemia (HTG) is a common disorder in Miniature Schnauzers.[1] The prevalence increases with age with >75% of the breed affected by 10 years of age. Many potential sequelae of HTG have been identified in the breed, including pancreatitis,[2] increased liver enzyme activity,[3] and gall bladder mucoceles.[4] Recently, proteinuria was shown to have a strong positive association with HTG in Miniature Schnauzers,[5] and evaluation of renal biopsy samples from proteinuric Miniature Schnauzers with HTG disclosed glomerular lipid thromboemboli, often in conjunction with focal segmental glomerular sclerosis.[6]

Abbreviations:

ACE	angiotensin-converting enzyme
ALP	alkaline phosphatase
ATIII	antithrombin III
BP	blood pressure
cTnI	cardiac troponin I
HTG	hypertriglyceridemia
HTGP	HTG-associated proteinuria
IRIS	International Renal Interest Society
MEA	mean electrical axis
NTGNP	normotriglyceridemic, nonproteinuric
T4	total thyroxine
UCCR	urine cortisol-to-creatinine ratio
UMN VMC	University of Minnesota Veterinary Medical Center
UPC	urine protein-to-creatinine ratio
USG	urine specific gravity

Most Miniature Schnauzers reported to have HTG-associated proteinuria (HTGP) have not been azotemic or hypoalbuminemic.[5,6] Therefore, it is unknown whether they suffer from other important clinical consequences of proteinuria, or whether this condition is similar to other endocrine-induced proteinuric syndromes, such as those associated with hyperadrenocorticism or diabetes mellitus,[7,8] in which no overt progression of renal disease occurs.[9] Knowledge of whether or not clinical consequences are present in Miniature Schnauzers with HTGP is important for developing monitoring and treatment recommendations.

Three important consequences of glomerular disease are thromboembolic tendencies, hypertension, and cardiac disease. In 1 study, the majority (71%) of dogs with non-nephrotic glomerular disease had decreased

From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN (Smith, Granick, Stauthammer, Polzin, Heinrich, Furrow).

This work was performed at the University of Minnesota, Veterinary Medical Center.

This work was supported by a Small Companion Animal Research Grant from the University of Minnesota and by a Gray Lady Foundation Grant. Partial support for EF was provided by an NIH ORIP K01 Mentored Research Scientist Development Award (1K01OD019912-02).

Corresponding author: R.E. Smith, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, 1352 Boyd Ave, St. Paul, MN 55108;
e-mail: resmith128@gmail.com.

Submitted March 10, 2017; Revised July 27, 2017; Accepted August 21, 2017.

DOI: 10.1111/jvim.14833

antithrombin III (ATIII) activity,[10] but other studies have reported decreased ATIII activity to be less common than other indicators of hypercoagulability.[11,12] Thromboembolic complications occur in 7–22% of dog with glomerular disease,[11–13] and the current clinical consensus is that dogs with persistent proteinuria should be treated with antithrombotic drugs such as acetylsalicyclic acid or clopidogrel.[14] Hypertension is another common complication of glomerular disease in dogs caused by complex mechanisms including activation of the renin-angiotensin system, sodium retention, and stimulation of the sympathetic nervous system[15]; antihypertensive treatment is instituted for patients with moderate to severe hypertension.[14] Data on cardiovascular complications of glomerular disease in dogs are limited, but microalbuminuria is associated with left ventricular hypertrophy, electrocardiographic (ECG) changes indicative of myocardial ischemia, and cardiovascular morbidity and mortality in people.[16] Proposed mechanisms for the concurrence of proteinuria and cardiovascular disease include generalized endothelial dysfunction, abnormalities of coagulation or fibrinolysis, systemic inflammation, or renal hypertension. Atherosclerosis is also an important factor in the development of cardiovascular disease. Although atherosclerosis is rare in dogs, it has been reported in dogs with glomerulopathies.[17]

The primary objective of our study was to compare populations of Miniature Schnauzers with and without HTGP with regard to sequelae of proteinuria as assessed by measurements of serum ATIII activity, indirect blood pressure (BP), and serum cardiac troponin I concentrations (cTnI). We also evaluated whether other biochemical abnormalities (eg, azotemia, hypoalbuminemia) were associated with HTGP in the breed. A secondary aim was to evaluate long-term outcomes in a subset of dogs with HTGP. These data should help determine the need for monitoring or treatment of complications attributed to HTGP.

Materials and Methods

Animals

Client-owned or fostered Miniature Schnauzers were prospectively screened for enrollment at the University of Minnesota Veterinary Medical Center (UMN VMC) between March 2015 and August 2016. Dogs were selected for the study as outlined by the inclusion and exclusion criteria described below. The study was approved by the University of Minnesota Institutional Animal Care and Use Committee (protocol #150933019A), and owner written informed consent was obtained for each study participant.

Selection Criteria

Dogs were included if they were >7 years of age; this cutoff was selected because the manifestations of HTG and proteinuria are age-dependent.[1,5] Dogs were not considered for enrollment if they had a history of previously diagnosed diabetes mellitus, hyperadrenocorticism, or hypothyroidism; dogs also were screened for these conditions during the study (see Procedures below). To be enrolled, dogs could not have any history of medication with drugs known to alter urinary protein concentration, including corticosteroids, angiotensin-converting enzyme (ACE) inhibitors, or antihypertensive agents. Dogs also were not recruited if they had clinical signs consistent with lower urinary tract disease (eg, pollakiuria, gross hematuria, stranguria) within the prior 2 weeks. No dog was reported to have a history of *Borrelia burgdorferi* or other infectious diseases affecting the kidneys, but screening was not a requirement for enrollment. In dogs discovered to be proteinuric during study enrollment, *B. burgdorferi* antibody testing[a] was recommended to the primary veterinarian and results were recorded when made available.

Procedures

Owners were requested to withhold food from dogs for 12–18 hours before scheduled study appointment time. Indirect BP was evaluated at the start of the study appointment with the owner present, and American College of Veterinary Internal Medicine (ACVIM) consensus statement guidelines were followed.[18] In brief, dogs were positioned in lateral or sternal recumbency, and measurements were obtained by a Doppler blood pressure device, a sphygmomanometer, and an appropriately sized blood pressure cuff (based on comparison with the diameter of the right forelimb). Three BP readings were obtained, and the average was used as the final data point. Hypertension was defined as a systolic indirect BP ≥150 mmHg, and was further characterized as mild at 150–159 mmHg, moderate at 160–179 mmHg, or severe at ≥180 mmHg.[18]

A history was obtained from the owner, and a physical examination was performed. Body weight was measured, and each dog was assigned a body condition score on a 9-point scale.[19] Blood was collected from the jugular vein for CBC and serum biochemistry, along with measurement of serum triglyceride, ATIII,[b] cTnI,[b] and total thyroxine (T4) concentrations. Hypertriglyceridemia was defined based on 12- to 18-hour fasting blood concentrations ≥100 mg/dL, with severity classified as mild (100–400 mg/dL), moderate (401–800 mg/dL), or severe (>800 mg/dL).[1,5] The upper end of the laboratory reference range was 85 mg/dL. However, in our study, normal fasting serum triglyceride concentrations were defined as <100 mg/dL; this decision was made because geriatric populations of healthy dogs have higher mean fasting serum triglyceride concentrations,[20] and the cutoff of 100 mg/dL more closely matches definitions of HTG used in other studies.[1–3] Decreased ATIII activity was defined as <74% and increased cTnI was defined as >70 ng/L, according to reference laboratory ranges (Table 1). Discrepancy was encountered when evaluating reference ranges for total T4. This test was performed at the UMN VMC clinical pathology laboratory for all dogs, but a subset of samples was tested by an outside reference laboratory.[b] The lower limit of the reference range for total T4 differed between the 2 laboratories (1.0 and 0.7 μg/dL,[b] respectively); the lower value (0.7 μg/dL) was selected for characterizing a dog as euthyroid because our population was geriatric, and total T4 is reported to decrease with age.[21] Postenrollment exclusions based on total T4 or other factors are described in the results. Urine was obtained in hospital by ultrasound-guided cystocentesis for urinalysis, urine protein-to-creatinine ratios (UPCs), and urine cortisol-to-creatinine ratio (UCCR).[b] A second urine sample obtained at home by the owner by midstream voiding was requested but was not a requirement for study participation. When an at-home sample was provided (n = 13 dogs), it was pooled with the in-hospital sample to minimize UPC variability, with equal aliquots used of each. Sterile containers were provided to the owner for this use, and owners were instructed on appropriate at-home urine sampling techniques. Proteinuria was defined as a UPC ≥ 0.5, with severity evaluated as mild (0.5–0.9), moderate (1–1.9),

Table 1. Blood pressure and clinicopathologic characteristics of the normotriglyceridemic, nonproteinuric (NTGNP) group and the group with hypertriglyceridemia-associated proteinuria (HTGP).

						P Value	
Variable	Reference Range	NTGNP Dogs	HTGP Dogs	Estimate	95% CI	Raw	Adjusted
BP	≤150 mmHg	150 (110–193) 5/11	152 (132–200) 4/8	7	−20 to 34	0.77	0.85
BUN	9–31 mg/dL	16 (10–26) 0/12	15 (11–28) 0/8	1	−4 to 6	0.59	0.81
Creatinine	0.6–1.6 mg/dL	0.9 (0.6–1.2) 0/12	0.7 (0.5–1.2) 0/8	−0.2	−0.3 to 0	0.059	0.16
Albumin	2.7–3.7 g/dL	3.0 (2.7–3.2) 0/12	3.6 (2.9–3.8) 0/8	0.5	0.2–0.7	0.0080	0.035
Cholesterol	143–373 mg/dL	159 (132–226) 0/12	247 (148–457) 1/8	86	28–154	0.0050	0.035
Glucose	75–117 mg/dL	98 (87–132) 1/12	100 (78–133) 1/8	2	−9 to 11	0.70	0.85
ALP	8–139 U/L	28 (12–159) 1/12	291 (14–1,646) 5/8	206	30–780	0.010	0.035
Platelet count	129–395 × 10^3/μL	287 (196–585) 3/12	399 (267–532) 4/8	104	1–219	0.082	0.18
cTn1	0–70 ng/L	55 (12–125) 3/11	64 (20–168) 4/8	16	−32 to 45	0.48	0.76
ATIII	74–142%	143 (111–155) 0/11	141 (97–187) 0/8	−1	−30 to 32	0.93	0.93
USG	NA	1.030 (1.006–1.045)	1.021 (1.008–1.034)	−0.009	−0.019 to 0.002	0.15	0.28

BP, blood pressure; cTn1, cardiac troponin 1; ALP, alkaline phosphatase; ATIII, antithrombin; USG, urine specific gravity; estimate, estimate for difference in means between groups.

Median (range) is reported as well as the proportion of dogs (number/total) above (BP, BUN, creatinine, cholesterol, glucose, ALP, platelet count, and cTn1) or below (albumin, ATIII) the reference range.

and severe (≥2).[22] Dogs were excluded postenrollment if they had bacteria, WBC (>5/hpf), or RBC (>50/hpf) on microscopic urine sediment analysis. Dogs also were excluded postenrollment if they had an increased UCCR in combination with proteinuria, HTG, or both.

A 12-lead ECG recording was obtained as previously described[23,24] for the initial 17 dogs enrolled in the study, but was discontinued because of ECG machine failure midway through the enrollment time period.

Follow-up Analysis of HTGP Dogs

Follow-up medical records were reviewed for dogs with HTGP. Specific values recorded included UPC and serum triglyceride, BUN, creatinine, and albumin concentrations. Because only 2 dogs with HTGP enrolled in the primary study had follow-up data, we also acquired follow-up data on 6 Miniature Schnauzers with HTGP that had participated in a previous study.[5]

Statistical Analysis

A priori sample size calculations were performed by a freeware program[c] to determine the number of dogs needed to detect differences in BP, ATIII, and cTnI between dogs without HTG or proteinuria (referred to as the normotriglyceridemic, nonproteinuric [NTGNP] group) and those with both HTG and proteinuria (referred to as the HTG-associated proteinuria [HTGP] group). The BP difference of clinical interest was set to 30 mmHg with a standard deviation of 15 mmHg, antithrombin III activity was set to 30% with a standard deviation of 15%, and cTnI was set to 100 ng/L with a standard deviation of 50 ng/L. These effect sizes were selected based on the belief that they could impact clinical recommendations for a disease condition, because there is currently no standard for what constitutes a clinically relevant difference in any of these 3 variables. For all calculations, power was set at 0.8 and the type I error rate at 0.05. The sample size required to detect differences of this magnitude or greater between groups was determined to be 10 dogs total (ie, 5 per group).

Statistical analyses were performed by R software for statistical computing.[d] A Student's *t*-test was used to compare the mean age between the NTGNP and HTGP groups, and a Fisher's exact test was used to compare sex proportions. Wilcoxon rank sum tests were used to compare BP, ATIII, cTn1, platelet count, urine specific gravity (USG), and serum concentrations of BUN, creatinine, albumin, cholesterol, and glucose, and alkaline phosphatase (ALP) activity between the NTGNP and HTGP groups. Multivariate regressions also were performed with triglyceride concentration and UPC as predictors and the following outcome variables for all dogs included in the study: cholesterol, glucose, BUN, creatinine, albumin, ALP, platelet count, ATIII, cTn1, UCCR, and BP. Cholesterol, BUN, ALP, and BP did not follow a normal distribution (determined by inspection of Q–Q plots and the Shapiro-Wilk test) and were log-transformed for analysis. Raw *P* values were corrected for multiple comparisons by the Bonferroni correction with statistical significance defined as an adjusted *P* value <0.05. For the follow-up analysis, clinicopathologic data from the initial study visit at which HTGP was diagnosed were compared with data from the last follow-up visit available. Wilcoxon signed rank tests for paired samples were used to determine whether serum creatinine or albumin concentrations changed over time in dogs with HTGP, and a *P* value <0.05 was considered significant. Median (range) is reported for all clinicopathologic data, regardless of normality, because the range was considered to be more clinically relevant for data interpretation in this relatively small group of dogs. Age is reported as mean ± standard deviation.

Results

Study Population

Thirty-eight Miniature Schnauzers were recruited for the study. Of these, 6 dogs were excluded. Four dogs were excluded for concurrent disease, 2 with bacterial cystitis, 1 with a stump pyometra, and 1 with a total serum T4 concentration <0.7 μg/dL. A fifth dog was excluded for a mildly increased UCCR (5.4 nmol/mmol; reference range, 0–5.0 nmol/mmol); although the sample had been collected in hospital for this dog and no abnormal clinical signs were reported, the dog had mild proteinuria (UPC = 0.5) and hypertriglyceridemia (228 mg/dL). Hyperadrenocorticism could not be ruled out as the underlying cause of these abnormalities, and no follow-up adrenal axis testing was performed. The final

excluded dog had severe obesity (9 of 9 body condition score, 16.5 kg), severe proteinuria (UPC = 6.3), and a fasting serum triglyceride concentration of 96 mg/dL. The dog was negative for *B. burgdorferi* antibodies,[a] and a urine culture yielded no microbial growth. It was treated with a weight loss diet and an ACE inhibitor. At 6-week follow-up, the dog had lost 2 kg (7 of 9 BCS) and had a UPC of 1.4. At 4-month follow-up, it had lost 4.4 kg total (6 of 9 BCS), and the UPC was normal (0.4). The ACE inhibitor was discontinued at that time. The dog's weight remained stable, and 2 months later, the UPC was still 0.4 without medication.

Of the remaining 32 dogs included in the study, 14 were spayed females, 16 were neutered males, 1 was an intact female, and 1 was an intact male. The mean age of dogs included in the study was 10.1 ± 1.7 years. The median BCS was 6 (range, 3–7) of 9. All included dogs had a serum total T4 concentration within the laboratory reference range. Two dogs had mild hyperglycemia (132 and 133 mg/dL; reference range, 75–117 mg/dL); the remaining dogs had serum glucose concentration within the laboratory reference range. One included dog had a UCCR slightly above the reference range (5.1 nmol/mmol); this dog was permitted in the study because the sample had been collected in hospital, the dog had no clinical signs of hyperadrenocorticism, and both the fasting triglyceride concentration and UPC were normal (75 mg/dL and 0.1, respectively).[25] Three additional dogs had insufficient urine sample volumes for determination of UCCR; none had HTG and all 3 had UPCs of 0.1.

Comparison of NTGNP and HTGP Groups

Fourteen of 32 dogs (44%) were normotriglyceridemic. Of these, 2 dogs had a UPC of 0.5. The remaining 12 dogs comprised the NTGNP group and had a median serum triglyceride concentration of 65 (range, 35–85) mg/dL and a median UPC of 0.1 (range, 0.1–0.4). This NTGNP group included 4 spayed females and 8 neutered males. The mean age of this group was 9.6 ± 1.7 years.

Eighteen of 32 dogs (56%) were diagnosed with primary HTG, including 12 dogs with mild, 2 with moderate, and 4 with severe HTG. Of these dogs with HTG, 10 had no clinical proteinuria (UPC <0.5); 9 of the 10 without proteinuria had mild HTG (median, 153 mg/dL; range, 110–360 mg/dL) and 1 had moderate HTG (578 mg/dL). The remaining 8 dogs had proteinuria (3 mild, 2 moderate, and 5 severe). These 8 dogs comprised the HTGP group and had a median serum triglyceride concentration of 463 (range, 271–1,164) mg/dL. The median UPC of the HTGP group was 1.9 (range, 0.5–4.6). The HTGP group included 3 spayed females and 5 neutered males. The mean age of this group was 11.1 ± 2.2 years. There were no significant differences in the age and sex of the HTGP and NTGNP groups ($P = 0.12$ and 1.0, respectively). Six of the 8 dogs in the HTGP group were evaluated for antibodies to *B. burgderfori*.[a] Two dogs were evaluated at the time of admission to the study. Two dogs were evaluated 4 months after admission to the study, at their annual wellness examinations. Two dogs were evaluated 6 months before study admission at their wellness examinations, and then also 3–6 months after admission to the study. All tests for *B. burgderfori*,[a] at all time points, were negative.

The BP and clinicopathologic results for the NTGNP and HTGP dogs are shown in Table 1. There was no significant difference in BP, BUN, creatinine, glucose, or platelet count between the 2 groups. No dog was azotemic. Serum albumin concentration was found to be significantly higher in the HTGP dogs compared to the NTGNP dogs (Fig 1, $P_{adjusted} = 0.035$); no dog was

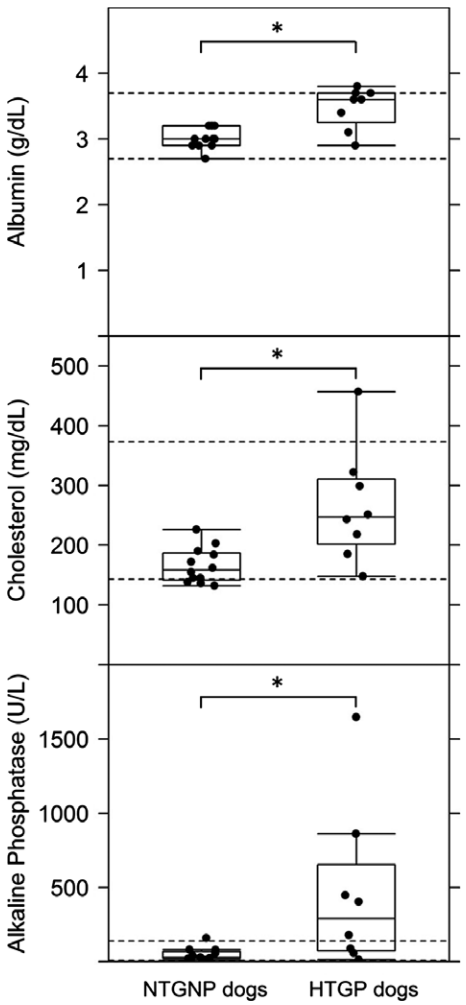

Fig 1. Box and whisker plots of serum albumin (top panel), cholesterol (middle panel), and alkaline phosphatase (bottom panel) in 12 normotriglyceridemic, nonproteinuric (NTGNP) Miniature Schnauzers and 8 with hypertriglyceridemia-associated proteinuria (HTGP). The boxes represent the interquartile range (25th–75th percentile), the solid horizontal line within the boxes represents the median, and the whisker bars extend to 1.5 times the interquartile range. Dots represent individual dog measurements; those that fall above or below the whisker bars are outliers. The horizontal dashed lines represent the upper and lower limits of the laboratory reference range. *Denotes a significant difference between groups with $P < 0.05$ after correction for multiple testing.

hypoalbuminemic. The HTGP dogs had significantly higher median cholesterol concentration compared to the NTGNP dogs (Fig 1, $P_{adjusted} = 0.035$), but only 1 dog in the HTGP group had a serum cholesterol concentration above the laboratory reference range. Five of 8 HTGP dogs and 1 of 12 normal dogs had increased ALP activity, and the median ALP activity was significantly higher for the HTGP dogs (Fig 1, $P_{adjusted} = 0.035$). None of the dogs had ATIII activity below the reference range, and ATIII activity did not differ significantly between groups. A few dogs in each group had increased cTnI concentrations, but the medians did not significantly differ between groups. Urine specific gravity ranged widely, and there was no significant difference between groups.

Full Population Regressions

In addition to comparing the 12 NTGNP and 8 HTGP dogs, data from all 32 dogs were analyzed. A significant positive correlation was found between fasting serum triglyceride concentration and UPC ($P < 0.001$, Fig 2). Triglyceride concentration and UPC were evaluated in multivariate regressions for association with variables of interest. Albumin, cholesterol, and ALP had significant positive associations with serum triglyceride concentration (Table 2). None of the other variables tested (BP, BUN, creatinine, glucose, platelet count, cTn1, ATIII, or USG) were significantly associated with serum triglyceride concentration and UPC in the regressions.

Cardiac Variables

Electrocardiography was performed in 17 dogs, including 6 of the 8 HTGP dogs. All dogs had normal sinus rhythm on 12-lead ECG, but 3 of 11 non-HTGP dogs had other abnormalities detected. One of the dogs from the NTGNP group had a right axis shift in the mean electrical axis (MEA) suggestive of right ventricular hypertrophy (MEA, −150). One dog with mild HTG and a normal UPC had a marginal left axis shift (MEA, +30). Another dog with mild HTG and a normal UPC had evidence of myocardial infarction characterized by the presence of large Q waves (0.9 mV) and ST segment deflection (−0.2 mV deflection on lead 2, +0.2 mV deflection on aVR, significant positive slope on V1). This dog also went on to have an echocardiogram performed which showed diffuse wall thickening involving the interventricular septum and both ventricular free walls. The dog's cTnI was increased at 513 ng/L. Of the 6 HTGP dogs, 5 had normal ECG results, and a single dog in this group had borderline ST deflection (−0.15 mV deflection in leads 2 and 3) and occasional junctional escape beats. This dog's cTnI was mildly increased at 94 ng/L.

Follow-up Analysis of HTGP Dogs

Eight Miniature Schnauzers that previously had been diagnosed with HTGP were followed for a median of 18 (range, 3–31) months. At diagnosis, median fasting serum triglyceride concentration was 417 (range, 198–1,895) mg/dL, and median UPC was 2.7 (range, 0.5–4.8). All dogs had serum creatinine and albumin concentrations within reference ranges at the time of diagnosis of HTGP. Five dogs received no treatment for HTGP, 1 dog was treated with enalapril, 1 with benazepril and a therapeutic low-fat diet,[e] and 1 with bezafibrate and a therapeutic low-fat diet.[f] At the time of last follow-up, neither creatinine nor albumin had changed compared with concentrations at the time of diagnosis (creatinine change = 0.0 ± 0.2 mg/dL, $P = 0.75$, and albumin change = −0.2 ± 0.4 g/dL, $P = 1.0$; Fig 3), and these results remained within reference ranges. Two dogs were euthanized at the time of last follow-up, 1 for suspected metastatic neoplasia (pulmonary nodule and cranial abdominal mass) at 29 months post-HTGP diagnosis and 1 for an anal gland adenocarcinoma at 26 months post-HTGP diagnosis. Another dog was diagnosed with diabetes mellitus at 3 months post-HTGP diagnosis. The remaining 5 dogs were doing well at the time of last follow-up. None of the 8 HTGP dogs followed had resolution of their HTG, and their proteinuria was persistent.

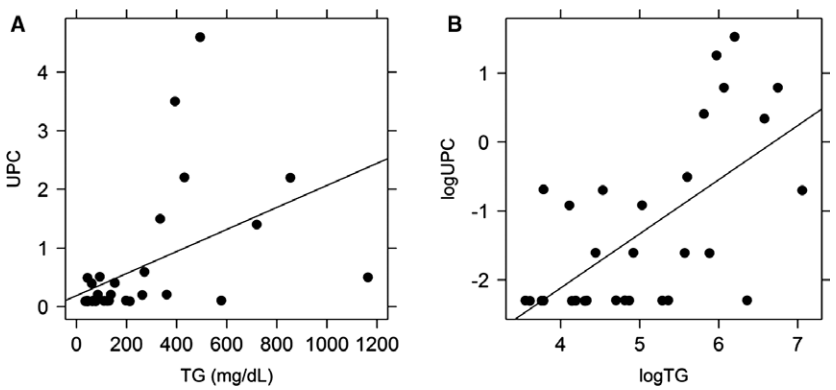

Fig 2. Plots of the relationship between fasting serum triglyceride concentration (TG) and urine protein-to-creatinine ratio (UPC) in Miniature Schnauzer dogs. (**A**) Untransformed data. (**B**) Log-transformed data (logTG and logUPC). A significant positive correlation was present ($r = 0.64$, $P < 0.001$). *r*, Pearson correlation coefficient.

Table 2. Multivariable regression for effects of triglyceride concentration and urine protein-to-creatinine ratio (UPC) on albumin, cholesterol, and alkaline phosphatase (ALP).

				P Value	
Parameter	Estimate	SE	*T* Value	Raw	Adjusted
Albumin				<0.001	0.0038
LogUPC	−0.03	0.04	−0.7	0.47	
LogTG	0.22	0.05	4.0	<0.001	
Cholesterol				<0.001	0.0018
LogUPC	−0.01	0.05	−0.1	0.92	
LogTG	0.22	0.06	3.9	<0.001	
ALP				<0.001	0.0062
LogUPC	0.29	0.18	1.6	0.12	
LogTG	0.49	0.22	2.2	0.039	

LogUPC, log-transformed UPC; LogTG, log-transformed TG.

Discussion

Our results confirm that proteinuria is common in Miniature Schnauzers affected by primary HTG and correlates with the severity of HTG. Furthermore, our data suggest that dogs with HTGP do not have other laboratory abnormalities commonly seen with protein-losing nephropathy. The dogs in our study maintained normal serum albumin, BUN, and creatinine concentrations, had normal ATIII activity, and were not more likely to have hypertension than healthy dogs. Long-term follow-up in a small subset of dogs with HTGP did not identify any other indicators of renal disease (as assessed by serum creatinine and albumin concentrations), although a few dogs were being treated with ACE inhibitors or treatment to manage HTG.

Serum albumin concentrations in the dogs with HTGP were significantly higher than in the NTGNP dogs. It is unknown whether this finding is an artifact related to sample lipemia or a true biologic effect of lipemia on albumin, because albumin concentrations were not confirmed with serum protein electrophoresis. Marked sample lipemia can result in increased albumin measurements when analyzed by spectrophotometric methods, although this interference is analyzer-specific.[26,27] The chemistry analyzer used for albumin determination in our study (Beckman Coulter AU480[g]) has no known statistically significant interference (>10% change from baseline) of lipemia on albumin quantification when human serum samples are spiked with Intralipid[h] solution up to a concentration of 800 mg/dL. Intralipid[h] is a 20% IV fat emulsion used to mimic samples with turbidity associated with lipemia. The analyzer also determines a lipemia index for all samples with a grade from 1 to 5+. A lipemia index of 5+ is consistent with lipid concentrations >500 mg/dL. Therefore, all samples graded up to 5+ lipemic are not expected to have substantial albumin interference whereas interference of samples graded as 5+ would be dependent on the concentration of lipid present in the sample (substantial interference possible at >800 mg/dL of lipid). These analyzer specifications were determined using human samples, and to our knowledge, no published validation studies are available for dogs preventing determination of whether or not canine samples are subject to interference at lower levels of lipemia.

Of the dogs included in our study, a single dog was found to have a lipemia index of 5+. This dog was clinically affected with hypertriglyceridemia and proteinuria. Thus, the finding of normal to higher serum albumin concentrations in dogs with HTGP compared to those without HTGP is not easily explained by artifact but also not consistent with physiologic expectations for proteinuric renal disease. The renal pathology associated with HTGP is believed to be glomerular lipid thromboembolism, as a previous study documented this unusual lesion in Miniature Schnauzers with HTG that underwent renal biopsy for evaluation of proteinuria.[6] One of the 7 dogs with confirmed glomerular lipid thromboemboli was reported to have hypoalbuminemia. In people, glomerular lipid thrombi are a hallmark of lipoprotein glomerulopathy, and the disease can progress to nephrotic syndrome if hyperlipidemia is not controlled.[28] However, we found no evidence of nephrotic syndrome in 8 dogs with HTGP, and a previous study similarly reported normal albumin concentrations

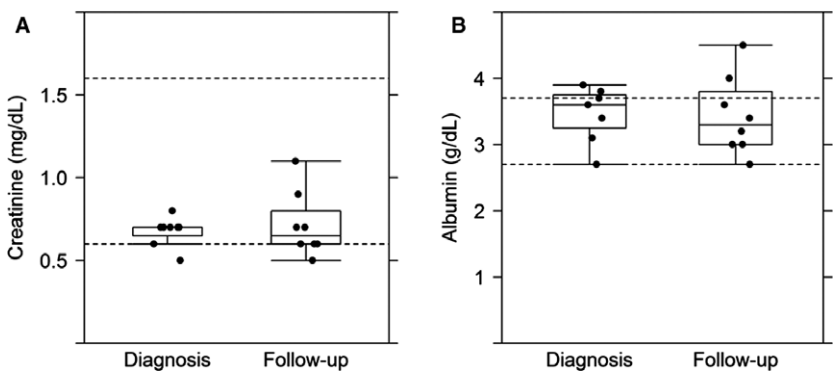

Fig 3. Box and whisker plots of (**A**) serum creatinine concentration and (**B**) serum albumin concentration in 8 Miniature Schnauzer dogs at the time of diagnosis compared to time of last follow-up. The horizontal dashed lines represent the upper and lower limits of the laboratory reference range. No significant change was observed between diagnosis and follow-up for either parameter ($P > 0.05$).

in 18 dogs with HTGP.[5] Therefore, dogs with HTG, proteinuria, and hypoalbuminemia should not be assumed to have HTGP and should be evaluated for alternative causes of proteinuria.

No significant differences in BUN or creatinine were found between the dogs with and without HTGP. These findings are consistent with previous reports of this condition. None of the 18 Miniature Schnauzers with HTGP in a previous study were azotemic.[5] In the study that documented glomerular lipid thromboemboli in Miniature Schnauzers with HTG, 1 of 7 dogs was reported to have an increased serum creatinine concentration, but that dog had concurrent arterionephrosclerosis.[6] The absence of azotemia in the HTGP group contrasts with data evaluated in 1 study of 156 dogs with non-nephrotic glomerular disease (UPC >1.0), in which 52% were azotemic at the time of diagnosis.[10] As such, azotemia in a Miniature Schnauzer with proteinuria and HTG should prompt further investigation of renal disease.

No overt evidence of cardiac disease was seen in our population of dogs. There was no difference in cTnI between the groups. Increases in cTnI have been documented previously in dogs with renal disease,[29,30] although these dogs were azotemic. In people, microalbuminuria is associated with ECG changes,[16] but microalbuminuria was not identified in our dogs. Hyperlipidemia in humans is also a risk factor for the development of atherosclerosis, and can lead to the onset and progression of primary cardiac disease. We may have been limited in our assessment of dogs with cardiac disease, because of failure of the ECG machine. However, given the data collected, the risk of cardiac disease overall in our dogs appears to be low. Blood pressure measurements also were normal between groups.

In a clinical setting, it is important to differentiate primary HTG from hyperadrenocorticism in Miniature Schnauzers, because both can present with similar laboratory abnormalities. Specifically, the HTGP dogs in our study had significantly higher ALP activity compared with dogs without proteinuria or HTG, and ALP had a positive correlation with serum triglyceride concentrations in the population as a whole. An association between serum triglyceride concentrations and liver enzyme activity has been noted previously in Miniature Schnauzers.[1] Platelet counts were not significantly different between dogs with and without HTGP. However, half of the dogs with HTGP had thrombocytosis. Rabbits with congenital hyperlipidemia have altered megakaryocyte transit, but this is not currently known in dogs.[31] None of the HTGP dogs were reported to have polyuria or polydipsia, and none had physical examination abnormalities consistent with hyperadrenocorticism. Therefore, hyperadrenocorticism screening tests, such as a UCCR or an adrenocorticotropic hormone stimulation test, should be considered in Miniature Schnauzers with HTG, proteinuria, and increased ALP activity to rule out this disease. All of the HTGP dogs in our study had normal UCCRs.

A dog excluded from our study had marked obesity, which was associated with severe proteinuria. During the dog's follow-up period, there was improvement in the hyperlipidemia and resolution of proteinuria which coincided with weight loss, but concurrent treatment with an ACE inhibitor complicates interpretation of the association of proteinuria with obesity. Obesity-related glomerulopathy has been described in people and is characterized by focal segmental glomerulosclerosis, glomerulomegaly, or both resulting in proteinuria; it has a relatively low incidence of nephrotic syndrome compared with other glomerulopathies and often follows an indolent course.[32] Glomerular lesions have been reported with experimentally induced obesity in dogs,[33] but urinary protein concentration was not quantified for the dogs. In a study that included 6 obese (BCS, 8–9 of 9) client-owned dogs, none had an increased UPC.[34] Further studies evaluating the relationship between obesity and HTG should be performed to determine whether improved BCS influences the severity of proteinuria seen in markedly obese dogs.

In our study, no dog with HTGP had decreased ATIII activity. However, ATIII activity may not be a sufficiently sensitive marker to rule out hypercoagulability in dogs with protein-losing nephropathies.[11,12] A recent study found that 89% of dogs with protein-losing nephropathies were hypercoagulable based on thromboelastography data, whereas only 26% had decreased ATIII activity.[12] Therefore, more research is needed to better determine whether HTGP dogs are hypercoagulable and predisposed to thromboembolic complications.

The sample size calculation performed before data collection indicates that the study was appropriately powered to detect clinically relevant differences between groups of dogs with and without HTGP. However, the sample size may not have been adequate for detecting if a more severe clinical course of the disease is only present in a small proportion of dogs with HTGP. Another limitation of the study was that none of the dogs had renal histopathology performed. Therefore, a subset could have had HTG with proteinuria caused by a different glomerular pathology or even as a consequence of systemic disease rather than from glomerular lipid thromboemboli. Additionally, our study used creatinine as a biomarker of renal disease in the HTGP dogs, but symmetric dimethylarginine is a superior biomarker of early renal dysfunction.[35] At the time of study enrollment (2015), the symmetric dimethylarginine assay was not available as a routine test in dogs.

Our study used the 2004 ACVIM Forum consensus statement definition of proteinuria as a UPC $\geq$0.5.[22] In 2015, the International Renal Interest Society (IRIS) provided modified definitions that state that UPC results of 0.2–0.5 represent borderline proteinuria and a UPC of >0.5 represents proteinuria.[36] When re-evaluating our patients, 4 dogs in the NTGNP group had proteinuria within the borderline range based on this new definition. The current IRIS guidelines recommend re-evaluation of the UPC in 2 weeks to 2 months in dogs with this range of proteinuria, as part of substaging for dogs with chronic kidney disease. In clinically healthy dogs, similar to the dogs in our study, there is no clear

recommendation for a course of action. Furthermore, a recent study found that borderline proteinuria is a common finding in apparently healthy geriatric dogs, again similar to those used in our study.[37]

The majority of dogs in our study had a single urine sample analyzed, rather than pooled samples as is considered ideal for UPC determination.[35] This approach could have resulted in less accurate UPC results for an individual dog. One study has suggested that collection of urine samples in hospital versus at home can significantly affect UPC results and clinical interpretation,[38] but a larger more recent study found a strong correlation between the 2 collection methods with an average difference of only 0.02.[39] All dogs in our study had at least 1 urine sample collected in hospital for UPC determination, but for a subset of dogs the in-hospital sample was pooled with a sample collected at home.

Conclusions

Miniature Schnauzers with HTGP may have a good prognosis. The dogs in our study with HTGP were neither hypoalbuminemic nor azotemic; these abnormalities should be considered likely indicators of other diseases. Dietary treatment, medical treatment, or both for moderate to severe HTG is indicated based on associations with other complications such as pancreatitis[2] and gall bladder mucoceles,[4] but it is unknown whether specific treatment for proteinuria (eg, ACE inhibitors) or anticoagulant medications is beneficial. Cardiac disease is not a hallmark of this condition. The HTGP condition in Miniature Schnauzers can mimic laboratory abnormalities observed with hyperadrenocorticism, and endocrine testing should be considered to rule out this disease. Additional research on a larger population of dogs with HTGP is needed to determine whether a subset suffer from complications that were not detected in our study.

Footnotes

[a] SNAP 4Dx Plus, IDEXX Laboratories, Westbrook, ME
[b] Marshfield Laboratories, Marshfield, WI
[c] PS: Power and Sample Size Calculation Program, version 3.1.2
[d] R, version 3.3.1. www.r-project.org
[e] Hill's Prescription Diet, i/d Low Fat Canine, Hill's Pet Nutrition Inc, Topeka, KS
[f] Royal Canin Veterinary Diet, Canine Gastrointestinal Low Fat, Waltham Centre for Pet Nutrition, Leicestershire, UK
[g] Beckam Coulter Inc, Brea, CA
[h] Intralipid, Fresenius Kabi AB, Uppsala, Sweden

Acknowledgments

The authors thank the technicians at the University of Minnesota College of Veterinary Medicine Center for Investigative Studies for their assistance recruiting dogs, scheduling appointments, and collecting samples for the study.

Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Xenoulis PG, Suchodolski JS, Levinski MD, Steiner JM. Investigation of hypertriglyceridemia in healthy Miniature Schnauzers. J Vet Intern Med 2007;21:1224–1230.

2. Xenoulis PG, Suchodolski JS, Ruaux CG, Steiner JM. Association between serum triglyceride and canine pancreatic lipase immunoreactivity concentrations in miniature schnauzers. J Am Anim Hosp Assoc 2010;46:229–234.

3. Xenoulis PG, Suchodolski JS, Levinski MD, Steiner JM. Serum liver enzyme activities in healthy Miniature Schnauzer dogs with and without hypertriglyceridemia. J Am Vet Med Assoc 2008;232:63–67.

4. Kutsunai M, Kanemoto H, Fukushima K, et al. The association between gall bladder mucoceles and hyperlipidaemia in dogs: A retrospective case control study. Vet J 2014;199:76–79.

5. Furrow E, Jaeger JQ, Parker VJ, et al. Proteinuria and lipoprotein lipase activity in Miniature Schnauzer dogs with and without hypertriglyceridemia. Vet J 2016;212:83–89.

6. Furrow E, Lees GE, Brown CA, Cianciolo RE. Glomerular lesions in proteinuric miniature Schnauzer dogs. Vet Pathol 2016;54:484–489.

7. Mazzi A, Fracassi F, Dondi F, et al. Ratio of urinary protein to creatinine and albumin to creatinine in dogs with diabetes mellitus and hyperadrenocorticism. Vet Res Commun 2008;32:299.

8. Herring IP, Panciera DL, Were SR. Longitudinal prevalence of hypertension, proteinuria, and retinopathy in dogs with spontaneous diabetes mellitus. J Vet Intern Med 2014;28:488–495.

9. Marynissen SJ, Smets PM, Ghys LF, et al. Long term follow up of renal function assessing serum cystatin C in dogs with diabetes mellitus or hyperadrenocorticism. Vet Clin Pathol 2016;45:320–329.

10. Klosterman ES, Moore GE, de Brito Galvao JF, et al. Comparison of signalment, clinicopathologic findings, histologic diagnosis, and prognosis in dogs with glomerular disease with or without nephrotic syndrome. J Vet Intern Med 2011;25:206–214.

11. Lennon EM, Hanel RM, Walker JM, et al. Hypercoagulability in dogs with protein-losing nephropathy as assessed by thromboelastography. J Vet Intern Med 2013;27:462–468.

12. White CR, Langston C, Hohenhaus AE, et al. Evaluation of the relationship between clinical variables and thromboelastographic findings in dogs with protein-losing nephropathy. J Vet Emerg Crit Care 2016;26:74–79.

13. Cook AK, Cowgill LD. Clinical and pathological features of protein-losing glomerular disease in the dog: A review of 137 cases (1985–1992). J Am Anim Hosp Assoc 1996;32:313–322.

14. IRIS Canine GN Study Group Standard Therapy Subgroup, Brown S, Elliott J, et al. Consensus recommendations for standard therapy of glomerular disease in dogs. J Vet Intern Med 2013;27(Suppl 1):S27–S43.

15. Syme H. Hypertension in small animal kidney disease. Vet Clin North Am Small Anim Pract 2011;41:63–89.

16. Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease: A statement from the American Heart Association Councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention. Hypertension 2003;42:1050–1065.

17. Hess RS, Kass PH, Van Winkle TJ. Association between atherosclerosis and glomerulopathy in dogs. Intern J Appl Res Vet Med 2006;4:224–231.

18. Brown S, Atkins C, Bagley R, et al. Guidelines for the identification, evaluation and management of systemic hypertension in dogs and cats. J Vet Intern Med 2007;21:542–558.

19. Laflamme DP. Development and validation of a body condition score system for dogs: A clinical tool. Canine Pract 1997; 22:10–15.

20. Kawasumi K, Kashiwado N, Okada Y, et al. Age effects on plasma cholesterol and triglyceride profiles and metabolite concentrations in dogs. BMV Vet Res 2014;10:57.

21. Reimers TJ, Lawler DF, Sutaria PM, Correa MT. Erb HN Effects of age, sex, and body size on serum concentrations of thyroid and adrenocortical hormones in dogs. Am J Vet Res 1990;51:454–457.

22. Lees GE, Brown SA, Elliott J, et al. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM Forum Consensus Statement (small animal). J Vet Intern Med 2005;19: 377–385.

23. Wagner GS, Bond RR, Finlay DD, et al. Chapter 2: Recording the Electrocardiogram. In: Wagner G, Strauss D, eds. Marriott's Practical Electrocardiography, 12th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2014:23–41.

24. Larry Tilley P. Essentials of Canine and Feline Electrocardiography, 3rd ed. Philadelphia, PA: Lea & Febiger; 1992:21–39.

25. van Vonderen IK, Kooistra HS, Rijnberk A. Influence of veterinary care on the urinary corticoid: Creatinine ratio in dogs. J Vet Intern Med 1998;12:431–435.

26. Alleman AR. The effects of hemolysis and lipemia on serum biochemical constituents. Vet Med 1990;85:1272–1284.

27. Jacobs RM, Lumsden JH, Grift E. Effects of bilirubinemia, hemolysis and lipemia on clinical chemistry analytes in bovine, canine, equine and feline sera. Can Vet J 1992;33:605–608.

28. Hu Z, Huang S, Wu Y, et al. Hereditary features, treatment, and prognosis of lipoprotein glomerulopathy in patients with the APOE Kyoto mutation. Kidney Int 2014;85:416–424.

29. Porciello F, Rishniw M, Herndon WE, et al. Cardiac troponin I is elevated in dogs and cats with azotaemia renal failure and in dogs with non-cardiac systemic disease. Aust Vet J 2008;86:390–394.

30. Sharkey LC, Berzina I, Ferasin L, et al. Evaluation of serum cardiac troponin I concentration in dogs with renal failure. J Am Vet Med Assoc 2009;234:767–770.

31. Ebbe S, Dalal K, Forte T, Tablin F. Microcytic thrombocytosis, small megakaryocytes, platelet lipids and hyperreactivity to collagen, lymphocytopenia, eosinophilia and low blood volume in genetically hyperlipidemic rabbits. Exp Hematol 1992;20:486–493.

32. Kambham N, Markowitz GS, Valeri AM, et al. Obesity-related glomerulopathy: An emerging epidemic. Kidney Int 2001;59:1498–1509.

33. Henegar JR, Bigler SA, Henegar LK, et al. Functional and structural changes in the kidney in the early stages of obesity. J Am Soc Nephrol 2001;12:1211–1217.

34. Tefft KM, Shaw DH, Ihle SL, et al. Association between excess body weight and urine protein concentration in healthy dogs. Vet Clin Pathol 2014;43:255–260.

35. Hall JA, Yerramilli M, Obare E, et al. Serum concentrations of symmetric dimethylarginine and creatinine in dogs with naturally occurring chronic kidney disease. J Vet Intern Med 2016;30:794–802.

36. IRIS Staging of CKD (modified 2015) – including algorithms (PDF). Available at: http://www.iris-kidney.com/pdf/staging-of-ckd.pdf. Accessed March 9, 2017.

37. Marynissen SJ, Williams AL, Paepe D, et al. Proteinuria in apparently healthy elderly dogs: Persistency and comparison between free catch and cystocentesis urine. J Vet Int Med 2017;31:93–101.

38. LeVine DN, Zhang D, Harris T, et al. The use of pooled vs serial urine samples to measure urine protein: Creatinine ratios. Vet Clin Pathol 2010;39:53–56.

39. Duffy ME, Specht A, Hill RC. Comparison between urine protein: Creatinine ratios of samples obtained from dogs in home and hospital settings. J Vet Int Med 2015;29:1029–1035.