

Arterial Blood Pressure, Proteinuria, and Renal Histopathology in Clinically Healthy Retired Racing Greyhounds

S. Surman, C.G. Couto, S.P. DiBartola, and D.J. Chew

Background: Physiologic peculiarities of Greyhounds as compared to other dogs make interpretation of laboratory results in this breed challenging for veterinarians. Hypertension in retired racing Greyhounds (RRG) can contribute to microalbuminuria (MA), overt proteinuria, and renal histologic lesions.

Objectives: To evaluate clinicopathologic findings, hemodynamic status, and renal histology in a population of healthy RRG.

Animals: RRG presented to Ohio State University College of Veterinary Medicine for inclusion in a spay and neuter program.

Methods: Cross-sectional study. RRG were classified as normotensive (<160 mmHg) or hypertensive (>160 mmHg) based on blood pressure (BP) determinations using Doppler and oscillometric methods. Of the dogs evaluated, 62% (n = 29) were hypertensive and 38% (n = 18) were normotensive. Health status was evaluated using routine clinicopathologic tests (CBC, serum biochemistry, urinalysis) as well as evaluation of fractional excretion of electrolytes and MA determinations. Adequate renal biopsy specimens (n = 15) were evaluated using light, immunofluorescence, and electron microscopy.

Results: All serum biochemistry results were normal in 45/49 dogs, but MA was more common in hypertensive (84% positive for MA) as compared with normotensive (18% positive for MA) RRG. Observed renal lesions were mild and renal biopsy scores were low in this sample of RRG.

Conclusions: Hypertension is common in RRG and might be breed-related. It is associated with MA, but observed renal lesions are mild. Whether or not hypertension and MA in RRG leads to progressive renal damage requires longitudinal study.

Key words: Glomerular disease; Histopathology; Hypertension; Kidney; Microalbuminuria.

During the past 10 years, adoption of retired racing Greyhounds (RRG) has become increasingly popular. Approximately 20,000 retired Greyhounds are adopted each year, and currently as many as 120,000 Greyhounds are estimated to live as pets compared with 55,000 living on racetracks.¹ As a result of this popularity, veterinarians are likely to evaluate Greyhounds more frequently. Clinical evaluation is complicated by the presence of peculiarities that veterinarians must be aware of when evaluating clinicopathologic test results in Greyhounds. For example, Greyhounds have higher hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), sodium concentration, chloride concentration, total bilirubin concentration, and aspartate transaminase activity than do mixed breed dogs.^{2,3} They also have been shown to have lower platelet, white blood cell, and neutrophil counts as well as basal serum total and free thyroxine concentrations than mixed breed dogs.^{3–5}

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Submitted March 14, 2012; Revised June 12, 2012; Accepted August 16, 2012.

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10.1111/j.1939-1676.2012.01008.x

Abbreviations:

BP	blood pressure
bpm	beats per minute
CKD	chronic kidney disease
FE	fractional excretion
HCT	hematocrit
HR	heart rate
MA	microalbuminuria
MCHC	mean corpuscular hemoglobin concentration
PLN	protein-losing nephropathy
RRG	retired racing Greyhounds
UPC	urine protein-creatinine ratio
USG	urine specific gravity

Renal disease has been of particular interest in the Greyhound community as a consequence of a recent study that identified a high frequency of deaths related to renal disease in Greyhounds (8% of 747 Greyhounds).¹ Greyhounds have not been identified among breeds reported in studies of glomerulonephritis and other protein-losing nephropathies (PLN), but this might reflect the fact that, until 10–15 years ago, the breed was seen infrequently in private practice.^{6–8} An acute syndrome termed cutaneous and renal glomerular vasculopathy (also known as “Alabama rot”) occurs in young actively racing Greyhounds, and although this disorder can be associated with PLN, it generally is an acute illness that leads to multifocal ulceration of the skin accompanied by limb edema and sometimes acute renal failure.^{9–11}

Magnitude of proteinuria has been established as an important risk factor for progression of renal disease in both people^{12,13} and animals.^{14,15} Tubulointerstitial

damage induced by proteinuria is hypothesized to be responsible for this relationship.^{16,17} Progressive glomerular sclerosis can impair postglomerular blood flow and lead to additional tubulointerstitial damage and, in this setting, proteinuria also is a marker of ongoing renal injury.

Proteinuria has been linked to established hypertension and renal disease, and it can precede hypertension.^{18,19} In human patients, control of proteinuria has a positive impact on the rate of decline of renal function and survival.²⁰ Microalbuminuria (MA) has been identified as a reliable predictor of developing renal disease in people with diabetic nephropathy and in those with renal disease secondary to systemic hypertension.^{21–23} A similar association also has been identified in dogs with familial nephropathy, genetic predisposition for glomerulonephritis, and acquired immune complex glomerulonephritis.^{24–26}

Systemic hypertension has long been recognized as both a cause and consequence of chronic kidney disease (CKD). To complicate matters, hypertension in either setting can be self-perpetuating and lead to a progressive decline in renal function and progressive increase in blood pressure (BP). Several studies indicate that the prevalence of hypertension secondary to renal disease ranges from approximately 30% up to as high as 60 or 93% in dogs with CKD.^{27–29} The presence of systemic hypertension in people is associated with cardiovascular complications, progression of renal disease, and increased risk of death.^{30,31} In dogs, hypertension has been associated with more rapid progression of renal disease with a decreased time to uremic crisis and death in dogs with CKD and hypertension.²⁹

The objective of this study was to evaluate clinicopathologic findings, hemodynamic status, and renal histology in a population of clinically healthy RRG. We prospectively evaluated BP and putative evidence of early renal disease based on markers of renal damage including MA, overt proteinuria, fractional excretion of electrolytes, and histopathologic findings. The study was approved by the Institutional Laboratory Animal Care and Use Committee of the College of Veterinary Medicine of the Ohio State University.

Materials and Methods

RRG presented to The Ohio State University Veterinary Medical Center were eligible for inclusion in this study. Dogs that participated in a 3rd year veterinary student spay and neuter program were recruited for the study. Forty-eight dogs were enrolled in the study in a 4-month period. On initial presentation, all dogs underwent complete physical examinations. CBC^a and serum biochemistry profiles (Cobas C501 chemistry analyzer)^b also were performed. In total, 40 mL of blood was collected from each dog. Jugular venipuncture was performed using a Safety Lock Blood Collection Set^c and blood was collected into serum separator and 7.5% EDTA collection tubes.^d Dogs were included in the study based on unremarkable physical examination, normal results of CBC, and serum biochemistry profile based on breed standards, and lack of historical health concerns.

Blood Pressure Recording

All dogs were evaluated on day 1 after presentation to the hospital, and on day 3 after a 48-hour acclimation period, before any surgical procedures were performed. BP data were acquired by a single investigator (SS) using 2 standard techniques: ultrasonic^e and oscillometric.^f Dogs were placed in right lateral recumbency and allowed a 5-minute acclimation period with minimal restraint. The first several readings were discarded and 5 additional consecutive measurements were obtained. The arithmetic mean of the 5 measurements was used for data analysis. For the Doppler measurements, an inflatable cuff was placed directly around the left antebrachium without clipping the hair.³² The cuff size for both methods was chosen to match a width approximately 40% of the circumference of the antebrachium.³³ Measurements were obtained using standard techniques for each method; measurements obtained while the dog was moving were discarded. Systolic, diastolic, and mean arterial pressures, when available, and heart rate (HR) were recorded. A manual HR was determined by femoral pulse palpation to determine if the HR obtained using the oscillometric monitor was accurate. Any pressure measurement with a HR that did not match the manual HR was excluded.

Urine Collection and Evaluation

Urine was collected from all dogs on day 1, immediately after arrival, using routine ultrasonography-guided cystocentesis using a 22-gauge, 1.5-inch needle attached to a 6-mL syringe. All dogs had been fasted for 12 hours before arrival. Urine was divided into 5 aliquots for urinalysis, urine chemistry profile, urine culture, MA determination, and urine protein-creatinine ratio (UPC). Two mL were reserved for MA analysis, 0.5 mL for culture, and the remaining 3.75 mL were submitted for urinalysis, urine chemistry, and UPC. Samples for urinalysis, urine chemistry, urine culture, and UPC were submitted for immediate analysis. The standard procedure in the clinical pathology laboratory at The Ohio State University is to centrifuge 2 mL of urine for urine sediment examination. Urine was plated for aerobic culture on blood and MacConkey agar, and incubated for 3 days at 35°C. Urine electrolytes were measured (Cobas C501 chemistry analyzer)^b and fractional excretion (FE) (expressed as a percentage) was calculated using the following equation:

$$FE_e = [(U_e/P_e)/(U_{cr}/P_{cr})] \times 100$$

where

FE_e = Fractional excretion of electrolyte

U_e = Urine electrolyte concentration

P_e = Serum electrolyte concentration

U_{cr} = Urine creatinine concentration

P_{cr} = Serum creatinine concentration

Reference values for FE_e were based on results obtained in a study of clinically normal RRG in Australia using the same methodology.³⁴

Urinary protein and creatinine concentrations used to calculate UPC were measured on an automated chemistry analyzer^b using a turbidimetric method. Aliquots for semiquantitative analysis of MA were stored in individual airtight containers at –70°C until analysis. Urine can be stored at 4°C for 1 week without any degradation or urine albumin. Long-term storage of urine at –20°C can result in degradation of urine albumin, but human urine samples frozen at –70°C show minimal decreases in albumin or total protein concentration for up to 2.5 years.^{35,36}

Assessment of Microalbuminuria

Frozen aliquots of urine were allowed to thaw and equilibrate at room temperature. All samples from each group were processed by one of the investigators (SS) on the same day, within 14 days of collection. MA was determined using a semiquantitative point-of-care assay immunoassay (Canine ERD – Screen Urine Test)^g according to the manufacturer's instructions. Urine specific gravity (USG) for each sample was determined using a manual refractometer, and all samples were normalized to a USG of 1.010 by dilution with distilled water. The test device was inserted into the diluted sample for 5 minutes and the result determined by comparing the intensity of the 2 colored bands in the test window against the manufacturer-provided control results, corresponding to negative, low, medium, high, and very high for MA.

Biopsy Collection

Renal biopsy was performed on 20 female Greyhounds undergoing routine ovariohysterectomy. All dogs were induced by IV injection of propofol, and maintained under general anesthesia with isoflurane and oxygen. Samples were collected by 2nd and 3rd year surgery residents, supervised by a board-certified surgeon. Samples from the first 10 dogs were collected using an 18-gauge automated needle biopsy device with an 11-mm specimen notch (E-Z Core).^h An individual core did not provide sufficient tissue for all histopathologic examinations. Therefore, on each occasion, three 1–2 cm cores were obtained from each dog. Samples were immediately processed and divided by one of the investigators (SS). The 3 samples were placed on individual glass slides in a small pool of isotonic sterile, and the total mass divided into 3 aliquots designated for fixation in either 10% formalin (light microscopy), 3% glutaraldehyde (electron microscopy), or Michel's medium (immunofluorescence microscopy). All samples were placed in the appropriate media within 5 minutes of collection. All samples for light, electron, and immunofluorescence microscopy were refrigerated, and shipped on ice overnight to Texas A&M University Renal Pathology Center for processing. Because of poor quality samples from 5 of the first 10 dogs, the renal biopsy technique was changed for the next 10 dogs. Wedge biopsies were obtained from the left kidney of the remaining 10 dogs.

Tissues were fixed in 10% neutral buffered formalin, then routinely processed and embedded in paraffin. Thin (3 μ m) sections were cut and stained with hematoxylin and eosin, periodic acid-Schiff, and Masson's trichrome methods using standard procedures.

All light microscopy specimens were evaluated and scored by two of the investigators (SS, SPD). The biopsies were scored using a standardized scale (Appendix 1). Light microscopic samples were scored based on the distribution and severity of the glomerular lesions in 7 categories. Lesions were classified as absent, focal, multifocal, or diffuse in distribution and absent, mild, or moderate in severity, with a score of 0–3 assigned accordingly. For each type of lesion, the total number of points was determined by adding the points for distribution and severity, with a maximal score of 6 per variable assessed. The scores for all variables were added to give the total histopathologic score (Appendix 1).

Statistical Analysis

Bland-Altman plots were used to identify bias when evaluating 2 methods used on the same day, and the same method on different days. For comparing methods on individual days, each sys-

toxic Doppler pressure was compared with the corresponding oscillometric pressure. The mean difference was calculated by subtracting the oscillometric pressure from the Doppler pressure. For comparing the same method on different days, day 3 pressures were subtracted from day 1 pressures. Correlation was further assessed using the Pearson correlation coefficient (*r* value), intra-class correlation coefficient, and the concordance correlation coefficient.

On the basis of the average systolic Doppler measurements, dogs were classified as hypertensive (systolic BP > 160 mmHg) and normotensive (systolic BP < 160 mmHg). Dogs also were divided into quartiles based on their BP (Quartile 1 BP < 125 mmHg, Quartile 2 BP 126–150 mmHg, Quartile 3 BP 151–175 mmHg, and Quartile 4 BP > 176 mmHg). All BP measurements were assessed for normality using the Kolmogorov-Smirnov normality test, D'Agostino and Pearson omnibus normality test, and the Shapiro-Wilk normality test, and all data sets were determined to be normally distributed. Paired *t*-tests were used to assess for statistical significance between the overall average systolic BP measured using Doppler and oscillometric methods. An unpaired *t*-test was used to assess for statistical significance between the systolic pressures measured using same methods on different days, and between the systolic pressures measured using different methods on the same day. To assess for a relationship between age and BP status, a nonparametric test (Mann-Whitney *U*) was used. Chi-square analysis was used to evaluate the relationship between sex and BP status. On the basis of hypertension groupings, associations were evaluated for BP status and the presence or absence of MA, UPC, and urinary fractional excretion of electrolytes. Because of the low numbers associated with each category on the MA test, low, medium, and high values, all were considered positive for MA, and data were evaluated on the basis of either negative MA or positive MA. Chi-square analysis was used for the comparison between the BP status and MA. Nonparametric testing with the Wilcoxon Rank Sum test was used for the comparison between the BP groups and the fractional excretion of electrolytes.

Renal histopathology was assessed with respect to other variables. Pearson correlations were calculated to assess the relationship between overall biopsy score and fractional excretion of electrolytes and UPC. The Wilcoxon Rank Sum test was used to evaluate the association between overall biopsy score and BP, and overall biopsy score and MA. In addition, each histopathologic category was evaluated individually with regard to the presence or absence of MA using Fisher's exact test. For all testing, significance was defined as a *P* < .05.

Results

Serum biochemical and CBC data were available from 48 dogs. Of the 48 dogs, 28 (58%) were female and 20 (42%) were male; all dogs were intact at the time of evaluation. The mean \pm SD age of the dogs was 3.7 \pm 1.7 years (range, 1–9 years). Results of serum biochemistry profiles generally were unremarkable in all dogs. The mean \pm SD serum creatinine concentration was 1.57 \pm 0.97 mg/dL (range, 1.1–2.2 mg/dL), which was above the reference limit of 1.6 mg/dL in 17/49 (34.5%) of dogs. However, using 1.9 mg/dL as the upper limit of the reference range for healthy Greyhounds, only 4/49 (8.1%) were considered outside of the reference range for healthy dogs. The mean \pm SD BUN concentration was 15.7 mg/dL \pm 3.7 (range, 9–25 mg/dL). These results were above the reference limit for the clinical pathology laboratory in 4/49 (8%) of dogs.

Table 1. Biochemical data in 48 clinically normal RRG.

Analyte (units)	Reference Range	Number of Dogs	Mean \pm SD	Range
Serum urea nitrogen (SUN) (mg/dL)	5–20	48	15.7 \pm 3.7	9–25
Creatinine (mg/dL)	0.6–1.6	48	1.6 \pm 0.2	1.1–2.2
Sodium (mEq/L)	143–153	48	149 \pm 4	144–167
Chloride (mEq/L)	109–120	48	112 \pm 4	108.5–129.4
Potassium (mEq/L)	4.2–5.4	48	3.9 \pm 0.3	3.4–4.76
Phosphorus (mg/dL)	3.2–8.1	48	3.7 \pm 1.0	1.8–6.3
Calcium (mg/dL)	9.3–11.6	48	10.6 \pm 0.6	9.5–12.8
Bicarbonate (mmol/L)	16–25	48	23.9 \pm 2.0	19.7–29
Alanine aminotransferase (IU/L)	10–55	48	69 \pm 34	27–184
Aspartate aminotransferase (IU/L)	12–40	48	48 \pm 23	19–167
Alkaline phosphatase (IU/L)	15–120	48	40 \pm 19	13–112
Cholesterol (mg/dL)	80–315	48	137 \pm 27	74–199
Total Bilirubin (mg/dL)	0.1–0.4	48	0.2 \pm 0.1	0.1–0.4
Total Protein (g/dL)	5.1–7.1	48	6.1 \pm 0.5	4.5–7.2
Albumin (g/dL)	2.9–4.2	48	3.8 \pm 0.3	2.8–4.7
Globulins (g/dL)	2.2–2.9	48	2.3 \pm 0.4	1.6–3.7
Glucose (mg/dL)	77–126	48	103 \pm 14	69–127

Serum biochemistry data are summarized in Table 1. CBC results were mostly within the reference range, but, on average, the Greyhounds had MCHC, HCT, and hemoglobin concentrations at the upper end of the reference range or even above the reference range, and platelet counts at the lower end or below the reference range.

Urine was available from 47 dogs. The mean \pm SD for urine specific gravity (USG) was 1.046 \pm 0.15 (range, 1.010–1.059). Although the range was wide, only 1 dog was classified as isosthenuric with a USG of 1.010, whereas 2 dogs had minimally concentrated urine with USG of 1.014 and 1.017. All others had USG $>$ 1.030. The 4 dogs with serum creatinine concentrations $>$ 1.9 mg/all had USG $>$ 1.040. Urine culture was negative in all dogs. The mean \pm SD for UPC was 0.11 \pm 0.16 (range, 0.03–1.14). Only 1 dog had a UPC $>$ 0.4. This dog's UPC was 1.14 and its urine was scored "high" on the MA semiquantitative test. Three other dogs had UPC of approximately 0.2, all 3 dogs were scored "medium" on the MA semiquantitative test. All other dogs, including those that were positive for MA, had UPC $<$ 0.2. Urine biochemistry data are summarized in Table 2.

MA was evaluated in 47 dogs. Because of low numbers in many of the groups, the data were collapsed into "negative" and "positive"; dogs measuring low, medium, and high were considered positive. Overall, 22/47 (47%) dogs were negative, and 25/47 (53%) dogs were positive. Of the dogs positive for MA, 13 (52%) and 12 (48%) were females and males, respectively. Results for MA are presented in Table 3.

BP data were collected from all 48 dogs on day 1 and day 3 of the study. The mean \pm SD systolic Doppler pressure was 166.9 \pm 17.1 mmHg (range, 134–210) and 167.7 \pm 14.9 mmHg (range, 142–204) on days 1 and 3, respectively. There was no significant difference between Doppler BP results on days 1 and 3 ($P = .53$). The mean \pm SD for systolic oscillometric pressure was 160.9 \pm 18.6 mmHg (range, 122–201) and 162.3 \pm 16.1 mmHg

Table 2. Urine profile results in 47 clinically normal RRG.

Analyte (units)	Number of Dogs	Mean \pm SD	Range
Urine Specific Gravity	47	1.05 \pm 0.02	1.010-1.059
Urine Urea (mg/dL)	47	2796 \pm 839	607-4168
Urine Creatinine (mg/dL)	47	427 \pm 151	97.9-938.3
Urine Sodium (mEq/L)	47	110 \pm 87	9-367
Urine Potassium (mEq/L)	47	111 \pm 59	12.1-399.2
Urine Chloride (mEq/L)	47	100 \pm 76	20-360
Urine Phosphorus (mEq/L)	47	126 \pm 107	0.6-526
Urine Calcium (mEq/L)	47	3.1 \pm 2.9	0.1-13.3
Urine Protein (mg/dL)	47	46 \pm 60	6-417
Urine Protein-Creatinine Ratio	47	0.11 \pm 0.16	0.03-1.14

Table 3. Frequency of microalbuminuria in 47 clinically normal RRG.

Variable	Level	Frequency (%)
Microalbuminuria	Negative	22 (47%)
	Low	13 (28%)
	Medium	10 (21%)
	High	2 (4%)
Microalbuminuria (collapsed)	Negative	22 (47%)
	Positive	25 (53%)

(range, 126–193) on days 1 and 3, respectively. There was no significant difference between oscillometric BP results on days 1 and 3 ($P = .07$). The overall mean systolic pressure was 167.3 \pm 15.5 mmHg (range, 139–207) and 161.6 \pm 17.1 mmHg (range, 124–197) for the Doppler and oscillometric methods, respectively. There was good correlation between the results obtained within methods

on different days, and between those obtained between methods on the same day. The highest correlations were seen between days using the same methods, with correlation slightly lower for different methods on the same day (see Table 4).

Of the 47 dogs evaluated, 29 were classified as hypertensive (systolic BP > 160 mmHg) and 18 were classified as normotensive (systolic BP < 160 mmHg). Of the hypertensive dogs, 15 (52%) and 14 (48%) were females and males, respectively. The mean \pm SD for BP were 178.4 ± 10.5 mmHg (range, 166–204) and 154.5 ± 6.4 mmHg (range, 142–164) respectively, which were significantly different ($P < 0.01$; Fig 1). The mean \pm SD HR was 84 ± 15 bpm (range, 59–124) and 83 ± 14 bpm (range, 63–113) for the hypertensive and normotensive groups, respectively. The mean HR between these 2 groups was not significantly different ($P = .25$).

The mean \pm SD for age was 3.7 ± 3.5 years (range, 1.5–8.5) and 3.8 ± 3.5 years (range, 1.0–9.0) for the hypertensive and normotensive groups, respectively. There was no significant difference in age between

Table 4. Pearson correlation coefficient, Intra-class correlation coefficient (ICC), and concordance correlation coefficient (CCC) for systolic blood pressure measurements between days and between methods.

Comparison	Pearson Correlation Coefficient	ICC	CCC
Day 1: Oscillometric versus Doppler	0.86 (0.77, 0.92)	0.80 (0.68, 0.89)	0.81 (0.72, 0.91)
Day 3: Oscillometric versus Doppler	0.77 (0.62, 0.86)	0.71 (0.56, 0.83)	0.73 (0.60, 0.86)
Oscillometric: Day 1 versus Day 3	0.95 (0.92, 0.97)	0.94 (0.90, 0.97)	0.94 (0.91, 0.98)
Doppler: Day 1 versus Day 3	0.86 (0.76, 0.92)	0.85 (0.76, 0.92)	0.86 (0.78, 0.93)

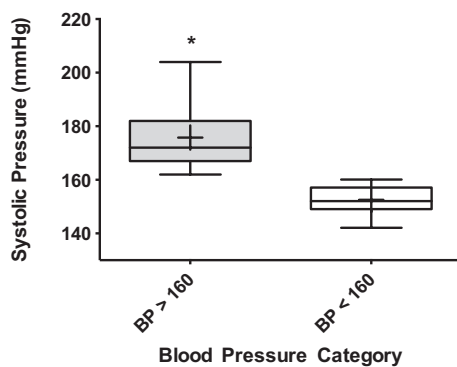


Fig 1. Box and whisker plots of mean systolic pressure in the hypertensive (BP > 160 mmHg) and normotensive (BP < 160 mmHg) groups. Boxes show the interquartile range (IQR), cross lines represent median values, + represents mean values. Whiskers represent $1.5 \times$ the IQR in the up and down direction range, and circles represent outlying values. The star (*) indicates statistical significance between the groups at $P < .001$.

these 2 groups ($P = .81$). In addition, no association was found between sex and BP status ($P = .16$).

When evaluated with respect to BP status, there was a highly significant association between the presence MA and BP status ($P < .0001$). In all, 83% of the normotensive dogs were negative for MA, whereas 76% of the hypertensive dogs were positive for MA. There were no significant associations between the presence of hypertension or BP and any other variables including urinary fractional excretion of electrolytes (sodium, chloride, potassium, calcium, and phosphorus) or UPC (Table 5).

Renal histopathology was available for 20 female dogs. The mean age \pm SD was 4.1 ± 2.3 years (range, 1–9.5). The first 10 dogs underwent needle biopsies of the left kidney at the time of exploratory laparotomy. In all, 5/10 dogs had adequate tissue, whereas in the remaining 5/10 dogs, the biopsies contained only renal medulla and no cortical tissue. Information from these 5 dogs was not included. The second 10 dogs had wedge biopsies of the left kidney performed, and all 10 samples contained sufficient tissue. Of the 20 dogs biopsied, 12 were MA negative, and 8 MA positive; 11 were normotensive and 9 hypertensive. After excluding the 5 dogs with inadequate biopsies, 10 were MA negative and 5 MA positive; 8 were normotensive and 7 hypertensive.

The mean \pm SD for histopathologic score was 11.5 ± 6.0 (range, 3–24). The light microscopic histopathologic score was evaluated for associations with hypertension, MA, UPC, and fractional excretion of electrolytes. There was no significant correlation between biopsy score and any of the variables evaluated, including MA, urinary fractional excretion of electrolytes, UPC, or BP status.

Overall, biopsy scores tended to be fairly low, consistent with the mild renal lesions observed. Considering the 5 dogs with the highest renal biopsy scores, 3/5 had mild mesangial glomerulopathy characterized by an increase in mesangial matrix and mesangial cell proliferation, and the other 2 had mild glomerulosclerosis. The majority of dogs (12/15) had some degree of thickening of Bowman's capsule and 12/15 had expansion of the mesangial matrix. Renal biopsy score results according to extent of MA and BP are presented in Table 6.

Immunofluorescence was performed for IgG, IgM, IgA, and C3. Thirteen of the 15 dogs had diffuse, segmental to global, mesangial staining for IgM. One dog also was positive for IgG and C3. All other dogs were negative for IgG, IgA, and C3. Ultrastructural changes were mild and nonspecific. All 15 dogs evaluated had focal, mild podocyte effacement. Mesangial interposition was seen in 7/15 dogs, but this change was extremely mild. Ultrastructural evaluation confirmed very mild mesangial matrix expansion in those dogs positive for this lesion on light microscopy. With regard to the glomerular basement membrane, 2/15 dogs had mild, focal electron dense deposits, but thickening or splitting of the glomerular basement membrane was not observed in any dog. Overall, the findings on immuno-

Table 5. Summary data for age, sex, MA, fractional excretion of electrolytes, and UPC for hypertensive and normotensive RRG.

Variable	Level	Not Hypertensive (n = 18)	Hypertensive (n = 29)
Sex	Female	13 (72%)	15 (52%)
	Male	5 (28%)	14 (48%)
Age	Mean ± SD (range)	3.8 ± 3.5 (1, 9)	3.7 ± 3.5 (1.5, 8.5)
Microalbuminuria	Negative	15 (83%)	7 (24%)
	Low	2 (11%)	11 (38%)
	Medium	0 (0%)	10 (34%)
	High	1 (6%)	1 (3%)
Microalbuminuria (collapsed)	Negative	14 (83%)	7 (24%)
	Positive	3 (17%)	22 (76%)
Calcium	Mean ± SD (range)	0.1% ± 0% (0%, 0.2%)	0.1% ± 0.1% (0%, 0.3%)
Chloride	Mean ± SD (range)	0.4% ± 0.3% (0.1%, 1.1%)	0.3% ± 0.3% (0.1%, 1.4%)
Phosphorus	Mean ± SD (range)	9.8% ± 10.6% (0.2%, 28.7%)	12.5% ± 12.8% (0.3%, 26.2%)
Potassium	Mean ± SD (range)	10.1% ± 9.9% (2.9%, 7.5%)	10.8% ± 10.4% (3.5%, 20.5%)
Sodium	Mean ± SD (range)	0.3% ± 0.2% (0%, 1%)	0.3% ± 0.2% (0%, 1%)
Urine Protein/Creatinine Ratio	Mean ± SD (range)	0.1 ± 0.1 (0, 0.2)	0.1 ± 0.1 (0, 1.1)

Table 6. Mean biopsy scores by hypertension and microalbuminuria group for RRG.

Group	N	Mean	SD	(min, max)	P-Value
No microalbuminuria	7	11.4	7.1	(3, 24)	.95
Positive microalbuminuria	8	11.6	5.3	(3, 19)	
Not hypertensive	8	13.0	6.0	(3, 24)	.33
Hypertensive	7	9.9	6.0	(3, 19)	
1: BP 125–150 mmHg	3	17.3	5.9	(13, 24)	.16
2: BP 150–175 mmHg	8	9.5	4.8	(3, 16)	
3: BP 175–200 mmHg	4	11.3	6.8	(3, 19)	
4: BP > 200 mmHg	0	–	–	–	

fluorescence and electron microscopy were nonspecific and remain unremarkable.

Discussion

Results of this study identified a high prevalence of hypertension in a population of otherwise healthy RRG. More than 60% of the dogs were classified as hypertensive, with systolic BP > 160 mmHg. Most had been shipped a fairly long distance before arriving at the site of evaluation. We failed to identify a significant white coat effect as there were no significant differences in BP or HR between days 1 and 3. In veterinary studies that have identified white coat hypertension, HR also was increased during periods of stress.³⁷ In contrast with untrained laboratory Beagles, the Greyhounds in this study were from racetracks and accustomed to travel, routine veterinary care, and housing in a kennel environment. Given the husbandry of these dogs, the lack of effect of stress is not entirely surprising. In addition, Greyhounds as a breed have significantly higher BP compared with other breeds.^{38–40} Increased BP in Greyhounds has even been proposed as a model for essential hypertension in people. What is still unclear, however, is whether this higher BP in Grey-

hounds leads to pathologic changes that are clinically relevant. Despite the association between MA and hypertension, no significant correlation was found between MA or hypertension and renal biopsy score.

In clinical practice, measurement of BP is becoming increasingly important as the consequences of hypertension are becoming better appreciated. The gold standard of direct arterial BP measurement is time-consuming, labor-intensive, expensive, requires substantial technical skill, is invasive, and carries a higher risk of complications than other methods. Indirect methods are inexpensive and fairly simple to perform with appropriate training, but their reliability compared with the gold standard has been questioned. Numerous studies in both human and veterinary medicine have reported that, although imperfect, indirect techniques correlate well with results obtained by direct arterial puncture, and with each other.^{37,41,42} Although direct arterial measurements were not used in this study, 2 other methods of BP measurement, with known reliability, were used on each dog on 2 separate days to assess BP. The results obtained indicated good agreement between the 2 methods. Overall, however, there was a higher correlation between the Doppler measurements compared with the oscillometric methods. This observation might indicate that the Doppler measurements were more reliable, and consequently the average Doppler pressure was used to classify individuals as hypertensive or normotensive.

There are few studies evaluating white coat effect in veterinary medicine, one of which evaluated dogs in their home environment and in the hospital using oscillometric methods. In that study, the investigators failed to demonstrate white coat effect.⁴³ Another study identified white coat effect in untrained laboratory dogs, and documented resolution with acclimation. A maximal decrease in BP occurred after 14 days, with stabilization of BP for 161 days.⁴⁴

Ideally, BP measurements in our dogs would have been followed over a period of 2–3 weeks to insure

that the BP was persistently high. The risks of white coat hypertension dictate that mild-to-moderate hypertension without definitive evidence of target organ damage should not be treated based on a single measurement. In addition, a gold standard, such as direct arterial BP measurement, would have been valuable to confirm the results of the indirect methods. Indirect methods tend to underestimate BP, and so it is likely that direct methods would have confirmed hypertension, possibly even of higher magnitude.

Even mild hypertension is a well-known risk factor for development of MA, proteinuria, and kidney disease as well as progression of preexisting renal disease. The Greyhounds in this study had no clinical evidence of preexisting kidney disease. Four of the Greyhounds did have increased serum creatinine concentrations, but all 4 had USG > 1.040. Three dogs had had isosthenuria or minimally concentrated urine, but none of these was azotemic. Many of the dogs had renal histopathologic lesions, but these changes generally were mild and, based on laboratory test results, did not appear to affect renal function. Glomerular filtration rate was not measured in these Greyhounds, and such measurements could have provided more definitive evidence of normal renal function.

Identification of renal disease early in its course before the onset of azotemia allows more opportunity for potentially valuable therapeutic interventions. Markers of early renal disease may provide insight into disease status and risk of progression. Overt renal proteinuria is a well-known marker of renal dysfunction and in human and veterinary medicine has been shown to be a prognostic indicator, and therapeutic target.¹²⁻¹⁷ Unfortunately, overt proteinuria as identified by increased UPC suggests that clinically relevant renal disease already might be present. This concern has led to investigation of markers of earlier renal dysfunction (such as MA) that may precede the onset of overt proteinuria. There has been considerable interest in this phenomenon in people, especially in diabetics and patients with primary hypertension, in whom MA has been shown to precede the onset of overt proteinuria, predict progression of renal disease, and predict other complications, such as cardiovascular disease.^{21,23} Similar associations have been shown in dogs with X-linked nephropathy, soft-coated Wheaten Terriers with glomerular disease, and dogs with heartworm disease.²⁴⁻²⁶ Therapeutic resolution of MA in people decreases the risk of cardiovascular and renal complications. Whether or not early identification of MA would affect treatment and prognosis in animals remains to be determined. One limitation to the measurement of MA is the potential effect of lower urinary tract disease and nonrenal systemic illness both of which can lead to MA that can be transient. Several systemic inflammatory, infectious, metabolic, and neoplastic diseases, as well as extreme exercise (ie, swimming), can be associated with MA or overt proteinuria in dogs.⁴⁵⁻⁴⁸ In addition, healthy dogs can have MA. As a result, interpretation MA requires that both prerenal and postrenal causes be ruled out, and that MA be repeatable.

None of the dogs in the current study had hematuria or pyuria, which makes postrenal proteinuria unlikely. The effect of hematuria and pyuria on MA assessment is limited. Hematuria does not increase urine albumin concentration above 1 mg/dL until the urine is visibly pink or red, generally corresponding to > 250 RBC/hpf. The same study found that pyuria (up to 50 WBC/hpf) had little effect on the urine albumin concentration, with 67% of pyuric samples having negligible urine albumin concentrations, and no association was found between magnitude of pyuria and urine albumin concentration. The only factor that significantly increased urine albumin concentration was the presence of concurrent pyuria and hematuria, or pyuria and bacteriuria.⁴⁹

Because of the study design, we were unable to evaluate these RRG over time to document persistent MA. Ideally, conclusions regarding the clinical relevance of proteinuria should be drawn only after it has been determined to be persistent (ie, present on repeated testing on 3 or more occasions, 2 or more weeks apart).⁵⁰ Because we were unable to follow these RRG over a prolonged period of time, it is impossible to know whether or not MA would have been persistent or progressive. However, given the lack of other systemic illness and the association with hypertension, MA might have been persistent. The method used for assessment of MA in the current study was semiquantitative. This test was chosen because it is a point-of-care assay used in veterinary practice to identify MA. This technique is less time-consuming, and is a reliable indicator of the presence of MA with good correlation to quantitative assessment of MA.⁵¹ A study comparing semiquantitative test strips and quantitative albumin assessment indicated that 29% of dogs with measurable MA of > 1.4 mg/L were negative using the semiquantitative test strips, whereas 71% were positive. Using 10 mg/L as a cut-off for MA, the test strips had false-negative and false-positive rates of 9 and 8%, respectively.⁵¹

With regard to other markers of renal dysfunction, no consistent histopathologic changes were seen, fractional excretion of electrolytes was normal, and most UPC results were within the reference range. Many dogs did have mild changes on light microscopy, immunofluorescence, and electron microscopy. These changes however generally were mild and nonspecific, and no association was identified between overall biopsy score and the presence of hypertension or MA. Several of the histopathologic changes were present in a large proportion of dogs (eg, 15/15 had podocyte effacement and 13/15 had diffuse staining for IgM). The presence of such lesions in both affected and unaffected dogs suggests they are not clinically relevant. These findings might have been a result of the population of animals evaluated. Most of these Greyhounds were young, with an average age of 3.7 years, whereas clinical experience with hypertensive proteinuric Greyhounds suggests that older dogs are more often affected. The lack of other abnormal findings in this study could indicate that renal dysfunction is slow to develop and might not

become evident until later in life. Long-term longitudinal studies of hypertensive Greyhounds would be required to evaluate this possibility.

Although nearly equal numbers of MA-positive and MA-negative dogs initially were biopsied, after removal of 5 dogs because of inadequate samples, there was disparity in the breakdown of dogs biopsied. Ten of the 15 dogs with adequate biopsy samples were MA negative and only 5 were MA positive. This disparity in sample size could have affected the relationship between biopsy score and the presence of MA.

In this study, we evaluated healthy RRG and identified a high percentage of dogs with hypertension. Greyhounds have been proposed as a model for primary hypertension because many are hypertensive on routine testing, often in the absence of known secondary causes of hypertension. Currently, this finding is thought to be a breed-related trait in Greyhounds, and its clinical relevance remains unknown.⁴²⁻⁴⁴ We identified a significant relationship between MA and hypertension in our dogs, which supports the clinical relevance of MA in Greyhounds. We were unable to document any additional evidence of underlying renal dysfunction or abnormal renal histology in this population of dogs. Microalbuminuria is known to be one of the earliest findings in hypertensive nephropathy and other primary glomerular diseases, preceding overt proteinuria, tubular dysfunction, and histopathologic changes in the kidney.

One limitation of this study is that only female dogs were evaluated using renal biopsy. Biopsies were obtained from female dogs during routine laparotomy for ovariohysterectomy, and performing a laparotomy on healthy male dogs for the sole purpose of obtaining renal biopsies was not justifiable. No published information is available on the prevalence of renal lesions in male as compared to female Greyhounds. Although the histopathologic findings in our study were obtained from female dogs, there was no sex predilection for hypertension or MA in the Greyhounds evaluated. These observations support a lack of a sex predilection, but future studies involving larger numbers of dogs of both sexes are warranted.

In the future, a similar study performed in a cohort of RRG using long-term follow-up would allow for more accurate characterization of BP status, MA, overt proteinuria, and long-term changes in renal function. Such a study also would allow for better characterization of the renal histopathologic lesions that may develop in proteinuric Greyhounds, because the decision to biopsy could be based on progression to overt proteinuria rather than the presence of MA. Microalbuminuria in this study did not correlate well with renal histopathologic changes. Treatment of MA and hypertension also could be evaluated in a long-term longitudinal study because individuals documented to have MA and hypertension could be randomized into different treatment groups (eg, dietary modification, angiotensin-converting enzyme inhibitors, calcium channel blockers, no treatment) to see if various treatments affect the rate of progression of MA to overt

proteinuria or decrements in renal function (eg, decreased glomerular filtration rate).

Footnotes

- ^a IDEXX LaserCyte; Westbrook, ME
 - ^b Cobas C501, Roche Diagnostics; Indianapolis, IN
 - ^c Becton Dickinson; Sparks, MA
 - ^d Monoject; Mansfield, MA
 - ^e Model 811 Ultrasonic Doppler Flow Detector; Parks Medical Electronics, Aloha, OR
 - ^f Cardell Veterinary Monitor 9402; Sharn Veterinary Inc, Tampa, FL
 - ^g Canine ERD – Screen Urine Test, Heska; Loveland, CO
 - ^h E-Z Core Biopsy Needle, Products Group International; Lyons CO
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Acknowledgments

The authors thank Dr George Lees, College of Veterinary Medicine, Texas A&M University for processing and interpretation of renal biopsy samples and the Department of Biostatistics at Ohio State University for statistical analysis.

Conflict of Interest Declaration: Dr DiBartola is a Co-Editor-in-Chief at the *Journal of Veterinary Internal Medicine*.

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Appendix 1

Histopathologic scoring system used for light microscopic evaluation of renal biopsies

Lesion	Lesion Severity ^A	Lesion Distribution ^B	Total Score ^C
Glomerular obsolescence	0–3	0–3	0–6
Glomerulosclerosis	0–3	0–3	0–6
Periglomerular fibrosis	0–3	0–3	0–6
Bowman capsule thickening	0–3	0–3	0–6
Glomerular basement membrane thickening	0–3	0–3	0–6
Mesangial matrix expansion	0–3	0–3	0–6
Mesangial cell proliferation	0–3	0–3	0–6

A: 0 – absent; 1 – mild; 2 – moderate; 3 – severe; B: 0 – absent; 1 – focal; 2 – multifocal; 3 – diffuse; C: Severity score (0–3) + distribution score (0–3).