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Urine creatinine concentration and urine protein-to-creatinine ratios in healthy non-racing Greyhounds

Running header: Urine creatinine concentration and UPCs in Greyhounds

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Abstract

Background: Serum creatinine concentrations are higher in Greyhounds when compared with non-sighthound breeds. Greyhounds might also have higher urine creatinine concentrations compared with other breeds, which could affect urine protein-to-creatinine ratio (UPC) references.

Objectives: We aimed to determine the UPC reference interval (RI) in healthy non-racing Greyhounds and compare this with UPC values in a group of healthy non-sighthounds and with the current International Renal Interest Society (IRIS) guidelines.

Methods: The study used an observational cross-sectional design, involving clinically healthy, non-racing Greyhounds (n=98) and non-sighthound dogs of similar weight, age, and sex (n=24). Packed cell volume, total solids, and urine protein concentrations, serum and urine creatinine concentrations, urine specific gravity (USG) measurements, and UPCs were determined. Linear regression was used to compare urine creatinine and urine protein concentrations, relative to USG, between Greyhound and non-sighthound groups. Greyhound UPC RIs were determined using non-parametric methods and compared with UPC values in non-sighthounds and current IRIS guidelines.

Results: Mean urine creatinine concentration, adjusted for USG, was approximately 22% higher in Greyhounds when compared with non-sighthounds ($P=0.002$). Mean urine protein concentration ($P=0.46$) and UPC ($P=0.1$) were not significantly different between Greyhounds and non-sighthounds. The upper limit of the Greyhound UPC RI was 0.20 or 0.42, depending on whether strict or moderate exclusion criteria were applied, respectively.

Conclusions: Greyhounds have higher urine creatinine concentrations than non-sighthounds. Although the suggested RI for UPC in Greyhounds is slightly lower than cut-offs recommended in generic canine IRIS guidelines, this difference is not likely to be clinically significant.

Keywords: Greyhound, urine concentration, urine creatinine, UPC.

Introduction

Serum creatinine concentration and the urine protein-to-creatinine ratio (UPC) are frequently analyzed when assessing renal health in dogs. Serum creatinine concentration is higher in Greyhounds compared with non-sighthounds, and Greyhound-specific reference intervals (RIs) for serum creatinine concentration are now in common use.¹⁻⁵ Since serum creatinine is predominantly eliminated by the kidneys,⁶ Greyhounds might be expected to have higher urine creatinine concentrations when adjusted for urine concentration. However, this has not yet been determined. It is also unknown whether higher serum creatinine concentrations in this breed influence the UPC RI.

Compared with other dog breeds, Greyhounds appear to have a higher prevalence of renal disease, hypertension, and microalbuminuria.^{3,7-11} Currently, International Renal Interest Society (IRIS) guidelines are used to classify proteinuria in Greyhounds because there is not a breed-specific RI for UPC.¹² However, generic canine recommendations might not be appropriate if urine creatinine concentrations are significantly higher in Greyhounds compared with other breeds. Establishment of an appropriate RI for UPC might, therefore, improve the accuracy in the diagnosis of renal dysfunction, clinical decision making, and prognostication in Greyhounds.

The objectives of this study were to compare urine creatinine concentration, relative to urine concentration as assessed by urine specific gravity (USG), in healthy non-racing Greyhounds and healthy non-sighthounds, and to establish a RI for UPC in healthy non-racing Greyhounds. We hypothesized that urine creatinine concentration, relative to urine concentration (as assessed by USG), would be higher in Greyhounds compared with non-sighthounds and that this could lead to lower UPC ratios in this breed.

Materials and Methods

This study was approved by The University of Melbourne's Animal Ethics Committee (ID 1613906), and all owners signed a consent form prior to participation.

Inclusion and exclusion criteria

Healthy dogs, aged 1-12 years, of any gender or neutering status, were included. Enrolled Greyhounds had not actively raced or trained within the 7 days prior to sampling. The non-sighthound (control) group included dogs of any breed except sighthounds, weighing between 24 and 42 kg. Health status was assessed based on history, physical examination findings, measurement of packed cell volume (PCV), total solids (TS), serum creatinine concentrations, and urinalysis.

Dogs were excluded if they had received topical or oral corticosteroids, stilboestrol, or antibiotics within the previous 14 days, had eaten in the 8 hours prior to sampling, or if a free catch urine sample could not be collected. Based on published USG values for healthy Greyhounds,¹³ dogs from either group were excluded if they had a USG <1.025 regardless of the serum creatinine concentration. Greyhounds were also excluded if they had values outside of Greyhound-specific RIs established at the University of Melbourne's U-Vet Animal Hospital laboratory for the following: serum creatinine >170 $\mu\text{mol/L}$ with a USG <1.030; PCV <0.36 L/L; or TS <48 g/L. Similarly, non-sighthounds were excluded if they had values outside the following canine RIs established at the U-Vet Animal Hospital laboratory: serum creatinine >140 $\mu\text{mol/L}$ with USG <1.030; PCV < 0.37 L/L; or TS < 60 g/L. Dogs were also excluded if urine samples exhibited gross hematuria, or if there was cytologic evidence of inflammation (≥ 5 WBC /high power field) or bacteriuria.

Sample collection

Sampling took place where the animals were housed, in public spaces during dog walking events, or at the U-Vet Animal Hospital. Free access to water was permitted unless it was withheld prior to a planned medical or surgical procedure (eg, neutering surgery). Each dog was leash-walked, and a voluntary midstream urine sample was collected into a clean container. If urine collection was successful, 3 mL of blood was collected from either the jugular vein using a

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21G needle (NIPRO Corporation, Osaka, Japan) and 3 mL syringe (Becton Dickinson, Singapore) or during the placement of a cephalic intravenous catheter (22G, Smith's Medical, Kent, UK) if an elective medical or surgical procedure had been planned following collection. Whole blood samples were placed into a 2.5 mL serum separation tube (Vacuette tube, Greiner Bio-One Frickenhausen, Germany) and 0.5 mL EDTA microtube immediately (MiniCollect, Greiner Bio-One, Frickenhausen, Germany) and kept chilled at 4°C until analysis.

Analytical methods

Serum tubes were centrifuged at 1,450g for 3 minutes (Spintron GT-10S, Spintron Australia), within 4 hours of sample collection, and approximately 0.5-1mL of serum was placed into an Eppendorf tube (Eppendorf AG, Hamburg, Germany) that was then either analyzed immediately or refrigerated and analyzed within 36 hours of collection at the U-Vet Animal Hospital's laboratory. Serum creatinine concentrations were measured using the COBAS INTEGRA 400 plus (Roche Diagnostics Ltd, Rotkreuz, Switzerland) analyzer with a kinetic colorimetric assay based on the Jaffé method (CREJ2, Roche Diagnostics Ltd, Rotkreuz, Switzerland) and calibrated as directed by the manufacturer. PCVs were determined by centrifuging a plain microhematocrit tube (Frontline PTY Ltd., NSW, Australia) filled with EDTA anticoagulated whole blood at 14,800g for 5 minutes (Orbital 260 centrifuge; Clements NSW, Australia). Total solids were determined by refractometry (Atago, Tokyo, Japan) using plasma from the centrifuged microhematocrit tubes. The refractometer was calibrated daily with distilled water. Urinalysis was performed by the principal investigator (RL) within 6 hours of collection; 5 mL of urine was centrifuged at 2,100g for 3 minutes, and 3 mL of the supernatant was divided into two separate Eppendorf tubes equally. The remaining sediment was re-suspended with 0.5 mL of supernatant and was examined microscopically to determine the number of red blood cells, white blood cells, and epithelial cells per ×40 objective field. The presence of casts, crystals, bacteria, and spermatozoa was recorded. An air-dried sediment smear was also examined at the time of urinalysis after staining with a Wright Giemsa stain to confirm findings noted on the wet preparation.

The supernatant was used for dipstick analysis, USG measurement, and UPC determination. Dipstick (Multistix, Siemens Healthcare Diagnostics Inc. NY, USA) analysis was performed

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manually according to the manufacturer's instructions. USG was measured within 4 hours of collection by placing a drop of supernatant onto a hand-held refractometer (Atago, Tokyo, Japan) to obtain a single measurement.

Urine protein and creatinine concentrations were analyzed immediately, or the supernatants were refrigerated after centrifugation and then analyzed at the U-Vet Animal Hospital laboratory within 50 hours of collection. Urine protein and creatinine concentrations were determined using the same analyzer as described for serum analyses. Urine protein concentrations were measured using the turbidimetric method (TPUC3, Roche Diagnostics Ltd, Rotkreuz, Switzerland), and urine creatinine concentrations were measured using the modified Jaffé method (CREJ2, Roche Diagnostics Ltd, Rotkreuz, Switzerland) after diluting urine samples 1:10 with distilled water.

Urine creatinine concentration was converted from $\mu\text{mol/L}$ to g/L by multiplying by 0.113 and then dividing by 1,000 so that both urine creatinine and urine protein concentrations were in the same units.¹⁴ UPC was then calculated using the following formula:

$$\text{UPC} = \frac{\text{urine protein concentration}}{\text{urine creatinine concentration}}$$

Statistical Analyses

Study data were collated and managed using the REDCap electronic data capture tools hosted at the University of Melbourne.¹⁵ Statistical analysis was carried out with Minitab 17 Statistical Software (State College, PA, USA) and Microsoft Excel 2013 for Windows (Microsoft Corp., Redmond, WA, USA) with the Reference Value Advisor v2.1 add-in (freeware v2.1: <http://www.biostat.envt.fr/reference-value-advisor>).¹⁶

The number of Greyhounds enrolled in this study was based on guidelines from the American Society for Veterinary Clinical Pathology and the Clinical and Laboratory Standards Institute, which recommend at least 120 samples to determine RIs using non-parametric methods with 90% confidence limits.^{17,18} The number of animals enrolled in the non-sighthound group was based on power calculations to detect a difference between Greyhounds and non-sighthounds with a power and alpha-error of 80% and 5%, respectively, based on previously published measurements of urine creatinine in healthy Greyhounds and non-Greyhound dogs.^{3,19} The

power calculations suggested that 7 animals were necessary for each group; however, to increase precision and control for the confounding factors apparent in previous studies^{3,19}, the study aimed to enroll 35 animals into the non-sighthound group.

Greyhounds and non-sighthound dogs were compared for mean age and weight using two-sample *t*-tests and were compared for gender and neutering status using a chi-square test.

Laboratory data (PCV, TS, USG, urine creatinine, urine protein, UPC) were assessed for conformity to a normal distribution using the Anderson-Darling test and frequency histograms. Skewed data were log-transformed, and normality was re-assessed using the Anderson-Darling test. The two-sample *t*-test was then used to compare mean laboratory parameters between Greyhounds and non-sighthounds. Regression lines were fitted to compare the relationships between groups for USG vs urine protein and USG versus urine creatinine concentrations. The RI for UPC in Greyhounds was determined non-parametrically and comprised the central 95% of the fitted distribution with 90% confidence intervals calculated around the lower (2.5%) and upper (97.5%) limits.¹⁸ The Dixon method was used to detect outliers.¹⁸ Statistical significance was set at $P < 0.05$ for all analyses.

Results

Study Population

Non-racing Greyhounds (n=149) and non-sighthounds (n=35) were enrolled from September 2016 to July 2017. Fifty-one Greyhounds and 11 non-sighthounds were excluded based on defined study criteria, leaving 98 Greyhounds and 24 non-sighthounds in the final analysis (Figure 1). All dogs lived in Victoria, Australia. None of the dogs included in the study received any medications other than routine anthelmintics. Greyhounds were sourced from 32 different owners, including a program that rehomes retired racing Greyhounds (n=38), 3 different racing trainers/breeders (n=25), an animal shelter (n=2), and private dog owners (n=33). Five dogs from the non-sighthound group were sourced from a shelter; the remainder were owned by staff, students, or clients of the University of Melbourne. The non-sighthound group included the following breeds; mixed breed (n=8), Labrador Retriever (n=8), Koolie (n=1), Wirehaired Pointer (1), Kelpie (n=1), German Shepherd dog (n=1), Belgian Shepherd dog (n=1), Mastiff

(n=1), Golden Retriever (n=1), and Setter (n=1). There was no significant difference in gender proportions ($P=0.65$), neutering status ($P=0.72$), age ($P=0.47$), or weight ($P=0.73$) between the Greyhound and non-sighthound groups (**Table 1**).

Laboratory Evaluation

Mean PCV was significantly higher ($P < 0.001$), and TS concentration was significantly lower ($P < 0.001$) in Greyhounds when compared with non-sighthounds (**Table 2**). Serum creatinine concentrations were significantly higher ($P < 0.001$) in Greyhounds compared with non-sighthounds while there was no significant difference in the mean USG between the two groups ($P = 0.21$; **Table 2**).

Information regarding urine creatinine and protein concentrations without adjustment for USG is shown in **Table 2**. Concentrations of both analytes were skewed, and data were therefore, log-transformed for analysis. Urine creatinine concentration in Greyhounds was not significantly different compared with non-sighthounds when USG was not corrected for ($P = 0.07$). Further, there was no significant difference in urine protein concentration between the groups ($P = 0.46$).

To evaluate the effect of USG on urine creatinine concentrations, urine creatinine concentration was plotted against USG. Urine creatinine concentration was significantly and positively correlated with USG in Greyhounds ($r = 0.64$; $P = 0.001$) and non-sighthounds ($r = 0.41$; $P = 0.047$). Initial assessment of scatterplots revealed that urine creatinine values were skewed at a higher USG (data not shown); thus, a log transformation was performed, and regression lines were fitted (Figure 2). There was no statistically significant difference between the slopes for Greyhounds and non-sighthounds ($P = 0.11$), and so a single slope was fitted to all the data. There was then a significant difference between the intercepts ($P = 0.002$). The fitted equations were:

Greyhounds: $\log(\text{UCr}) = -21.9 + 30.9 \text{ USG}$

Non-sighthounds: $\log(\text{UCr}) = -22.1 + 30.9 \text{ USG}$

The estimated difference in mean urine creatinine between the Greyhound and non-sighthound group was 0.199 on the log scale, or a ratio of $\exp(0.199) = 1.22$ on the original scale, indicating

that on average, Greyhounds have 22% higher urine creatinine relative to USG than non-sighthounds.

In contrast, urine protein concentration was not significantly correlated with USG in either Greyhounds ($r = 0.08$, $P = 0.46$) or non-sighthounds ($r = 0.21$, $P = 0.33$). When the association of urine protein with urine concentration was evaluated by graphing urine protein against USG, there was no statistically significant difference between groups ($P = 0.63$) or between slopes ($P = 0.94$).

Data for UPC in Greyhounds and non-sighthounds were clearly skewed (Figure 3), and a log transformation did not obtain an approximately Gaussian distribution for either group (Anderson-Darling test, both $P < 0.05$). To establish a RI for UPC, the lower 2.5% and upper 97.5% limits were, therefore, determined using non-parametric methods.¹⁸ The RI for UPC in Greyhounds was 0.037–0.42; there were too few non-sighthounds to calculate an accurate RI for this group. Three Greyhounds and two non-sighthounds were identified as outliers by the Dixon method; two of the excluded Greyhounds had trace or 1+ positive hemoglobin results on dipstick analysis, and three excluded dogs were intact. The removal of these outliers had little or no effect on the median UPC in either group. With exclusion of outliers, the RI for UPC in Greyhounds was 0.036–0.23 (**Table 3**).

The transformed UPC data were then compared between Greyhounds and non-sighthounds. The mean of the UPC was -2.62 in Greyhounds and -2.36 in non-sighthounds. The difference between these means was 0.26 on the log scale (or $e^{0.26} = 1.29$ on the original scale). Therefore, the mean UPC in Greyhounds was estimated to be 29% lower than in non-sighthounds, but this did not reach statistical significance (two-sample t -test; $P = 0.10$).

Discussion

This study aimed to compare urine creatinine concentrations in Greyhounds and non-sighthounds and found that urine creatinine concentration was approximately 22% higher in Greyhounds compared with non-sighthounds when adjusted for urine concentration. Because creatinine is completely excreted by the kidneys and not reabsorbed, the most likely cause for higher urine creatinine in Greyhounds is the filtration of higher concentrations of serum creatinine in

Greyhounds compared with non-sighthounds. Other potential causes or contributing factors for the higher urine creatinine in Greyhounds include active renal tubular secretion and a high glomerular filtration rate (GFR). Tubular secretion is an unlikely cause because previous studies have found this to be of negligible importance in dogs.^{6,19} The GFR in the Greyhound has been assessed previously; one study reported a higher GFR in Greyhounds compared with non-Greyhounds, but this was not supported by two other studies.^{20,21} Thus, the relationship between GFR and urine creatinine concentration in Greyhounds requires further study.

The second objective of this study was to establish a RI for UPC in non-racing Greyhounds. To achieve this, urine protein concentrations were compared between Greyhounds and non-sighthounds. As expected, no statistically significant difference was found between groups since the excretion of urine protein depends very little on urine concentration and GFR, and unlike creatinine, is not affected by muscle mass. Instead, urine protein excretion depends on pre-renal, renal, and post renal factors.⁵ The UPC was then calculated, and it was estimated that mean UPC in Greyhounds was 29% lower than mean UPC in non-sighthounds; however, the differences in mean UPCs were not statistically significant between groups. It is interesting to note that the magnitude of the difference in UPCs is similar to the difference in urine creatinine concentrations between groups (22%). Given that urine protein concentration was not significantly different between groups, it is likely that the trend toward a lower UPC in Greyhounds is due to higher urine creatinine concentrations in this breed.

A UPC RI was established for Greyhounds, but the result obtained depended on whether outliers were included or excluded. When outliers were excluded, the upper reference limit was 0.23, whereas when the outliers were included, the upper reference limit was 0.42. Identification and elimination of outliers are important in the evaluation of reference data, particularly in the accurate determination of RIs. However, the outliers identified in this study were not automatically excluded for the following reasons: 1) According to the American Society for Veterinary Clinical Pathology RI guidelines, if “individuals are selected randomly from well-defined populations and health is confidently established, retention of all reference values is favored”²²; 2) The dogs identified as outliers were not evidently dehydrated or unwell on physical examination; and 3) The outliers sat in the tails of the heavily skewed data, and the most

extreme data points had two dogs with similar values, which makes them less likely to be anomalies. For these reasons, these outliers were not excluded when determining the upper limit for UPC in healthy Greyhounds but were taken into account when determining ‘borderline proteinuria’ or dogs that may need ongoing monitoring.

Dogs identified as outliers were examined for potential causes for the increased UPC. Two Greyhounds had trace or 1+ positive hemoglobin results on dipstick analysis; however, urine samples with grossly indistinguishable blood contamination were not excluded because several studies have shown that mild blood contamination (that is not evident grossly) had no significant effect on UPC.^{23,24} Other abnormalities that could potentially cause a high UPC, such as pyuria,^{25,26} were not observed in the outlier samples.

Other potential causes of variation in UPC were considered, including collection method, gender, and neutering status. Samples were collected via free catch rather than cystocentesis due to welfare and ethical considerations; previous studies have shown no significant difference in UPC measurements in samples collected via free catch or cystocentesis.²⁵⁻²⁸ Collection method, gender, and neutering status are unlikely to be the cause of lower UPCs in Greyhounds in this study because there was no significant difference in these variables between Greyhounds and non-sighthounds. However, all three dogs in this study with a UPC greater than 0.5 were intact. UPCs have been reported to be higher in intact male dogs, although not greater than 0.5.²⁹ Blood pressure was not measured in this study, but hypertension was considered as a potential cause for high UPCs. Hypertensive Greyhounds have been found to have a greater degree of microalbuminuria than non-hypertensive Greyhounds, but there was no difference in UPC between these groups.³

There were several limitations to this study. Attempts to exclude animals with disease were made based on history, physical examination, and laboratory findings alone; GFR, blood pressure assessment, renal biopsies, and full hematology and biochemical testing were not performed. It is, therefore, possible that some dogs had subclinical disease, especially given that Greyhounds are prone to renal disease and hypertension.^{3,8} Efforts to minimize the effect of this limitation were made by using the American Society for Veterinary Clinical Pathology guidelines to calculate a de novo RI.¹⁸

At least 120 subjects are recommended for the establishment of a RI; this was also the aim of this study, and 149 Greyhounds were initially screened. Samples were collected from Greyhounds that were sourced from several locations, with a wide age range and different neutering status, to assemble a sample group that was characteristic of non-racing Greyhounds in Australia. A previously published USG RI was used to determine exclusion criteria.¹³ Subsequently, it was found that many Greyhounds (26%) and non-sighthounds (20%) that appeared clinically normal had a USG less than 1.025, and this led to many dogs being excluded from the study, resulting in a final study population of 98 Greyhounds and 24 non-sighthounds. The reason why so many dogs were excluded for insufficient urine concentration is unclear and requires further study. It is also possible that many healthy dogs were unnecessarily excluded due to strict criteria. The authors felt it was important to apply strict criteria to avoid the inadvertent inclusion of dogs with subclinical renal disease. Financial and ethical constraints prohibited the inclusion of more dogs, and unfortunately, this meant that the study could have been underpowered, therefore potentially resulting in type II errors, for example when comparing urine creatinine and UPC between groups. However, while only 98 Greyhounds were included in the final analysis, this number compares favorably to many other veterinary studies that have established RIs.³⁰⁻³²

Analytes are potentially influenced by storage, but this is unlikely to have affected the result of this study. Although urine samples were refrigerated at 4°C for up to 72 hours before UPC analysis; urine protein and urine creatinine have previously been shown to be stable at these conditions.²⁵ Serum creatinine is stable for up to 4 days at room temperature³³ and is likely also to be stable when stored at 4°C. Storage protocols and durations were the same for the Greyhound and non-sighthound samples, so that sample storage was not a confounding factor in any differences observed between groups.

Actively racing Greyhounds were excluded from this study because exercise can affect GFR and urine protein excretion.³⁴⁻³⁷ Other sighthound breeds were excluded from this study. Sighthounds show some similarities and some significant differences in hematologic and biochemical results between breeds.^{2,38,39} Therefore, the RIs established for Greyhounds in this study should not be extrapolated to Greyhounds in active race training or other sighthound breeds until further studies are performed to establish whether these RIs are applicable to these specific groups.

The results from this study suggest that Greyhounds with UPCs greater than 0.42 should be considered proteinuric, and Greyhounds with a UPC of 0.23–0.42 could benefit from close monitoring. Interestingly, this recommendation aligns well with the algorithm for sub-staging canine chronic kidney disease by proteinuria proposed by IRIS.¹² We conclude that differences in urine creatinine and UPC between non-racing Greyhounds and non-sighthounds are unlikely to be of clinical significance and; therefore, the generic IRIS UPC interpretation guidelines can be used in Greyhounds.

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Tables

Table 1. Summary of gender, neutering status, age, and weight in Greyhound and non-sighthound groups

Category	Greyhound n = 98	Non-sighthound n = 24	P-value
Male intact, n (% of group)	11 (11.2%)	2 (8.3%)	0.65 ^a
Male neutered, n (% of group)	43 (43.9%)	10 (41.7%)	0.72 ^b

Female intact, n (% of group)	14 (14.3%)	5 (20.8%)	
Female neutered, n (% of group)	30 (30.6%)	7 (29.2%)	
Age (years) mean (SD)	4.1 (2.3)	4.5 (2.4)	0.47
Range (years)	1–10	1–8	
Weight (kg) mean (SD)	31.6 (3.8)	31.2 (5.2)	0.73
Range (kg)	24 - 42	24 - 42	

Note: ^a *P*-value (chi-square test) for comparing gender proportions (male/female) between groups and ^b *P*-value (chi-square test) for comparing neutering status (intact/neutered) between groups

Table 2. Summary of selected laboratory parameters in Greyhound and non-sighthound groups

Category	Greyhound (n=98)	Non-sighthound (n=24)	<i>P</i> -value
PCV (L/L), mean (SD)	52.0 (5.8)	44.4 (4.8)	<0.001
TS (g/L), mean (SD)	6.1 (0.5)	6.7 (0.6)	<0.001
SCr (μmol/L), mean (SD)	125.6 (13.8)	87.5 (19.2)	<0.001
Range	86.0–161.0	48.0–119.0	
USG, mean (SD)	1.039 (0.010)	1.041 (0.007)	0.21
Range	1.025–1.050	1.028–1.051	
UCr (μmol/L), mean (SD)	27,562 (10,903)	23,773 (8,237)	0.07 ^a
Median	25,377	23,945	
Q1-Q3	21,303–32,018	17,404–26,302	
Range	11,510–86,200	13,276–49,740	

UPr (g/L), mean (SD)	0.29 (0.32)	0.31 (0.30)	0.46 ^a
Median	0.20	0.21	
Q1–Q3	0.13–0.31	0.16–0.33	
Range	0.05–2.49	0.10–1.28	

PCV: Packed cell volume; TS: Total solids; SCr: Serum creatinine; USG: Urine specific gravity; UCr: Urine creatinine; UPr: Urine protein; SD: standard deviation; Q1-Q3: Interquartile range; ^a data were skewed; hence a two-sample t-test was performed on log-transformed data

Table 3. Urine protein:creatinine ratios in Greyhounds and non-sighthounds

Category	Greyhound (n=98)	Greyhounds (outliers removed, n= 95)	Non-sighthound (n= 24)	Non-sighthounds (outliers removed, n=22)
UPC mean (SD)	0.095 (0.11) ^a	0.079 (0.049) ^a	0.13 (0.17) ^a	0.087 (0.038) ^a
UPC median	0.061 ^a	0.060 ^a	0.081 ^a	0.081 ^a
Q1 to Q3	0.05 to 0.09 ^a	0.05 to 0.08 ^a	0.06 to 0.12 ^a	0.08 to 0.11 ^a
Range	0.02–0.96 ^a	0.023–0.25 ^a	0.04–0.85 ^a	0.038–0.20 ^a
Reference Interval	0.037–0.42	0.036–0.23	N/A	N/A
90% CI for lower limit	0.023–0.038	0.023–0.039	N/A	N/A
90% CI for upper limit	0.23–0.96	0.21–0.25	N/A	N/A

Note: UPC: Urine protein-to-creatinine ratio; N/A: not applicable, too few dogs to establish a reference interval. ^a data was not normally distributed; mean, median, interquartile ranges Q1-Q3, and ranges reflect non-transformed values.

Figures

Figure 1. Flow chart demonstrating exclusions in this study. Note: n, total number of dogs; g, Greyhounds; c, non-sighthounds; SCr, serum creatinine; USG, urine specific gravity

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471 Figure 2. Scatterplot with regression lines for the association between the log-transformed urine
472 creatinine and urine specific gravity for Greyhounds and non-sighthounds.

473 Figure 3. Histogram of urine protein-to-creatinine ratio for Greyhounds and non-sighthounds
474 with a normal distribution overlaid. Note the skewing to the right. The green dashed line
475 represents the International Renal Interest Society (IRIS) cut-off for borderline proteinuria, and
476 the orange dashed line represents the IRIS cut-off for overt proteinuria.

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