

## Comparison between Urine Protein: Creatinine Ratios of Samples Obtained from Dogs in Home and Hospital Settings

M.E. Duffy, A. Specht, and R.C. Hill

**Background:** The urine protein:creatinine ratio (UPC) is used to quantify urine protein excretion and guide recommendations for monitoring and treatment of proteinuria.

**Hypothesis/Objectives:** Home urine samples will have lower UPCs than hospital samples. The objectives were to compare UPCs of samples collected in each setting and to determine whether environment of sample collection might affect staging, monitoring or treatment recommendations.

**Animals:** Twenty-four client-owned dogs.

**Methods:** Prospective, nonmasked study. Clients collected a urine sample from their dog at home and a second sample was collected at the hospital. Dogs receiving corticosteroids or angiotensin-converting enzyme inhibitors were excluded, as were those with urine samples of inadequate volume, no protein on dipstick analysis, or active urine sediment. Samples were refrigerated after collection, dipstick and sediment evaluations were completed and each sample was frozen at  $-80^{\circ}\text{C}$  within 12 hours. UPCs were performed on frozen samples within 2 months.

**Results:** From 81 paired samples, 57 were excluded. Of the remaining 24, 12/24 (50%) had higher hospital sample UPCs, 9/24 (38%) had identical UPCs, and 3/24 (12%) had lower hospital UPCs. The UPCs of hospital samples were higher than home samples for the total population ( $P = .005$ ) and the subset with  $\text{UPC} > 0.5$  ( $P = .001$ ).

**Conclusions:** Setting and related circumstances of urine collection in dogs is associated with UPC differences; results are usually higher in hospital than in home samples. This difference has the potential to affect clinical interpretation.

**Key words:** Canine; Kidney disease; Protein losing nephropathy; Proteinuria.

Early and accurate detection of persistent renal proteinuria is important because studies have demonstrated an association between proteinuria and morbidity and mortality in both dogs and cats.<sup>1,2</sup> Furthermore, risk of adverse outcomes increases as the magnitude of proteinuria increases.<sup>1,2</sup> Some interventions such as angiotensin-converting-enzyme inhibitors (ACEi) that decrease the severity of proteinuria also have reno-protective effects in markedly proteinuric dogs.<sup>3–7</sup> Change in magnitude of proteinuria is commonly used as a marker of response to these therapeutic interventions.

The urine protein: creatinine ratio (UPC) is among the most commonly used tests to quantify and monitor

### Abbreviations:

UPC	urine protein:creatinine ratio
ACVIM	American College of Veterinary Internal Medicine
IRIS	International Renal Interest Society
ACEi	angiotensin-converting enzyme inhibitor

proteinuria in dogs. An American College of Veterinary Internal Medicine (ACVIM) consensus statement recommended prospective monitoring, diagnostic investigation, and therapeutic intervention based on threshold UPC values for azotemic or nonazotemic dogs.<sup>8</sup> The International Renal Interest Society (IRIS) also has produced algorithmic guidelines for classification and treatment of chronic kidney disease in dogs based on similar threshold UPC values.<sup>9,10</sup>

Magnitude of proteinuria as determined by UPC may be affected by a number of factors such as nonrenal disease, endogenous or exogenous corticosteroids, dietary protein content, exercise, hypertension, and hyperthermia, among others.<sup>11–20</sup> Stress has been suggested as a cause or contributing factor for proteinuria in dogs in several reports.<sup>15,21,22</sup> One prior study found that caged animals had significantly higher UPC ratios compared to unconfined animals, and stress was suggested as a likely cause of the difference.<sup>23</sup> However, all urine samples were collected in an inpatient or outpatient hospital setting.<sup>23</sup>

One author has observed that UPC values from samples collected by clients at home were lower than UPC values from samples collected at a veterinary clinic in several individual patients, but in reviewing the medical literature, no study of the effect of urine collection in home or hospital environments was identified. Based upon these limited clinical observations, we hypothesized that there would be a significant difference

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between UPC measured in urine samples collected in a home environment and samples collected in a hospital setting.

The primary goal of this study was to compare UPC ratios of paired urine samples from individual dogs collected at home versus in a hospital setting. A secondary goal was to determine the percentage of cases in which a difference in UPC between 2 samples collected from the same individual in different settings would have the potential to change clinical decisions about either IRIS classification (nonproteinuric, borderline proteinuric, or proteinuric) or ACVIM consensus statement recommendations for assessment and management of proteinuria in dogs (no action, monitoring, investigation, or intervention).

## Materials and Methods

### *Design and Study Population*

This was a prospective, nonmasked study. Samples utilized were collected from all client-owned dogs presented to the small animal internal medicine service at the University of Florida Small Animal Hospital that conformed to study criteria. The research protocol was approved by the University of Florida Institutional Animal Care and Use Committee and University of Florida College of Veterinary Medicine's Hospital Research Review Committee.

During routine phone calls the day before each scheduled appointment, clients were informed about the study. If verbal consent for participation was obtained, clients were instructed to obtain a voided, midstream urine sample in a clean plastic or glass container on the morning of the appointment. Sterile urine cups were provided for patients that were not enrolled but would be returning for a recheck appointment. Only 1 pair of urine samples was collected from any individual dog and always included a morning sample from home and a sample collected <12 hours later during the same day in the hospital. Urine samples collected at home were immediately placed into the refrigerator at time of arrival to the hospital. Upon arrival at the hospital, each client was asked to sign a consent form and fill out a brief questionnaire about the dog's medical history, including current medications. Both voided and cystocentesis methods were used to obtain hospital samples. If urine collection was a part of the medical management plan for the patient, the method and timing of obtaining urine was left to the discretion of the primary clinician, but an additional 5 mL or more of urine was obtained for the study. If urine collection was not part of the medical management plan, a voided midstream urine sample was collected in a sterile urine cup at the first opportunity after examination of the patient and refrigerated immediately. All patients enrolled in the study were reported to have been fasted for  $\geq 8$  hours before any urine sample collection.

Paired urine samples from individual dogs were included in this study and UPC values were determined only if a signed consent form had been obtained, both samples contained  $\geq 5$  mL volume, there was no visible debris or pigment, there was  $\geq$  trace dipstick protein in at least 1 of the samples of each pair, the patient was not currently receiving corticosteroid or ACEi medication, and both home and hospital samples had inactive urine sediments. For this study, an inactive urine sediment was defined as  $< 5$  WBC/hpf,  $< 20$  RBC/hpf, and no visible bacteria.

### *Urinalysis and UPC measurement*

Urine specific gravity, dipstick chemistry, and microscopic sediment evaluations were performed within 12 hours of collection.<sup>24</sup>

Aliquots from each urine sample were frozen after no longer than 12 hours of refrigeration and stored at  $-80^{\circ}\text{C}$  until UPC analysis. When urinalysis was included in the diagnostic plan for the patient, a urine sample was submitted to the clinical pathology service and evaluated by a trained laboratory technician. The remainder of the urine samples were evaluated by 1 of the study authors (MD or AS). Standard operating procedures for the clinical pathology laboratory were followed in either case. Samples were assessed visually for evidence of debris or gross discoloration.<sup>25</sup> Urine specific gravity was measured using a refractometer.<sup>a</sup> Urine pH and concentrations of protein, heme protein, bilirubin, glucose, and ketones were measured semiquantitatively by assessing colour change at recommended times after urine was applied to a dipstick.<sup>b</sup> Concentrated and resuspended urine sediment was evaluated microscopically at low power (10 $\times$ ) for epithelial cells, casts, and mucous threads and at high-dry power (40 $\times$ ) for leukocytes, erythrocytes, bacteria, fungi, crystals, parasites, sperm, and lipid.

Urine protein concentrations (mg/dL) were measured in a thawed supernatant aliquot from each sample using a modified pyrogallol red-molybdate method on an automated chemical analyzer.<sup>c</sup> If urine protein concentrations were too high for the analyzer, the same sample was analyzed after a 1 : 2 or 1 : 3 dilution. Urine creatinine concentrations (mg/dL) were measured with the same automated chemical analyzer using a modified Jaffe procedure.

### *Classification*

Each individual urine sample was given a classification based on the IRIS staging system for CKD patients.<sup>9</sup> Samples with UPC ratios  $< 0.2$  were classified as nonproteinuric, those with UPC ratios  $\geq 0.2$  and  $< 0.5$  were classified as borderline proteinuric, and those with UPC ratios  $\geq 0.5$  were classified as proteinuric. Each sample also was allotted a clinical response category based on the ACVIM consensus statement if a serum creatinine concentration obtained within 2 weeks of urine sample collection was available for review.<sup>8</sup> For nonazotemic dogs, categories included "no action" for samples with a UPC  $< 0.5$ , "monitor" for UPC  $\geq 0.5$  and  $< 1$ , "investigate" for UPC  $\geq 1$  and  $< 2$ , and "intervene" for UPC  $\geq 2$ . For azotemic dogs, there were only 2 categories: "nonintervention" for samples with UPC  $< 0.5$  and "intervene" for those with UPC  $\geq 0.5$ .

### *Statistical Methods*

Results were evaluated using a computerized statistics program<sup>d</sup> and presented as medians and ranges for nonparametric data. Normality of data was assessed visually and with the Shapiro-Wilk test. Urine pH, urine specific gravity, and UPCs of home and hospital sample pairs were compared using a Wilcoxon signed-rank test. Additionally, UPCs were compared between home and hospital samples in the subsets of patients with UPCs  $< 0.5$  and  $\geq 0.5$ . The power of the study to detect a difference in ratio of 0.2 was 95% if 21 dogs completed the study, the average ratio was 0.3 and the probability of type 1 error was  $< 5\%$ .

## Results

### *Population*

Urine samples were obtained from 81 dogs. Samples from 57 dogs were excluded: 15 had negative urine dipstick protein tests, 16 did not have paired samples of adequate volume, 10 had active urine sediment, 4 had initial samples obtained in an inappropriate location (eg, gas station, hotel), 4 had visible debris or pigment,

3 had missing laboratory results, 3 were receiving medical treatment that was not allowed (2, ACEi; 1, prednisone), and 2 had samples that were not evaluated within 12 hours. Of the 24 remaining dogs included in the study, there were 13 spayed females, 8 neutered males, 2 intact females, and 1 intact male. The median age was 9.25 years (range, 2–16 years). Several different breeds were represented.

### Urinalysis

All home samples were obtained by voiding. Fifteen of 24 (62%) hospital samples were obtained by voiding and 9/24 (38%) by cystocentesis. Twenty-three of 24 (96%) home samples were evaluated by 1 of the study authors and 1/24 (4%) was evaluated by a clinical pathology technician. Twelve of 24 (50%) hospital samples were evaluated by 1 of the study authors and the other 12 were evaluated by a clinical pathology technician. The urine pH from home and hospital samples had median values of 6.25 (range, 5–8.5) and 6.75 (range, 5–8.5) respectively, which were not statistically different. The urine specific gravities of home and hospital samples had median values of 1.028 (range, 1.008–1.050) and 1.024 (range, 1.008–1.050), respectively which were significantly different ( $P = .01$ ).

Dipstick protein results were reported based on the scale provided on the label (negative, trace, or 1–4+) and there was a range of values reported for home (negative to 3+) and hospital (negative to 4+) samples. Results from 24 home samples included 2 (8%) with 3+ protein, 4 (17%) with 2+, 5 (21%) with 1+, 8 (33%) with trace, and 5 (21%) with a negative dipstick test. Results from 24 hospital samples included 1 (4%) with 4+, 2 (8%) with 3+, 6 (25%) with 2+, 5 (21%) with 1+, 8 (33%) with trace, and 2 (8%) with a negative dipstick test. Overall, there was agreement between home and hospital urine protein evaluated by dipstick in 11/24 (46%) sample pairs. There were 2 sample pairs (8%) in which a higher value was recorded from the home sample. In these cases the home samples had values of trace and 1+, whereas both hospital samples had negative readings. The remaining 11 (46%) sample pairs had lower protein concentrations identified in the home sample than in the hospital samples, including 5 (21%) in which the home sample had a negative reading, whereas the hospital sample was positive, ranging from trace to 1+.

There was a positive glucose result (3+) in 1 hospital sample in the absence of concurrent hyperglycemia. There were 7 positive (1+) heme protein readings; 2 patients had heme protein in both home and hospital samples, whereas 3 patients had heme protein in the hospital sample only. There were no positive dipstick results for bilirubin or ketones.

On microscopic evaluation, 20/24 (83%) and 18/24 (75%) of home and hospital samples respectively had 0 WBC/hpf, 2/24 (8%) and 4/24 (17%) respectively had 0–1 WBC/hpf, and 2/24 (8%) from each group had 1–3 WBC/hpf. On evaluation for microscopic hematuria, 13/24 (54%) samples from both home and hospital

groups had 0 RBC/hpf, 10/24 (42%) and 8/24 (33%) respectively had  $\leq 5$  RBC/hpf, and 1/24 (4%) and 3/24 (13%) had 5–20 RBC/hpf. Microscopic crystalluria was identified in 8/24 (33%) of home samples and 7/24 (29%) of hospital samples. Rare or  $\leq 1$  epithelial cells/lpf were identified in 3/24 (13%) home and 7/24 (29%) hospital samples, whereas  $\leq 5$ /lpf were identified in an additional 3/24 (13%) hospital samples. Lipid was identified in 4/24 (17%) home and 5/24 (21%) hospital samples. Rare hyaline or granular casts were observed in 2/24 (8%) of hospital samples.

There was no evidence that collection method (voided versus cystocentesis) or evaluator (laboratory personnel versus study authors) had any effect on USG, dipstick, or most sediment parameters for hospital samples. The only parameter in which there was a potential difference between samples based on these variables was the percentage of samples with epithelial cells. Epithelial cells were noted in a higher percentage of cystocentesis hospital samples compared to voided hospital samples (6/9 and 4/15, respectively), but this was not statistically significant ( $P = .054$ ) and 2 patients with epithelial cells noted in the cystocentesis sample also had epithelial cells noted on their home voided sample. Epithelial cells also were noted in a higher percentage of laboratory-reviewed samples compared to author-reviewed samples (9/12 and 1/12 respectively), and this difference was significant ( $P = .0006$ ), but 2 patients with epithelial cells noted in the laboratory-reviewed sample also had epithelial cells noted on their author-reviewed home sample. All of the cystocentesis samples were evaluated by a laboratory technician.

### UPC Results

The UPC results are presented in Figure 1. Home urine samples had UPCs ranging from 0.17 to 3.3 and for hospital samples the range was 0.15 to 7. The percentage difference between home and hospital samples for individual patients ranged from 0% to 96%. The UPC values were significantly higher in hospital samples compared to home samples ( $P = .005$ ). Proportionally, 9/24 (38%) had identical UPCs in home and hospital samples, 3/24 (13%) had higher UPC measurements in the home sample, and 12/24 (50%) individuals had a higher UPC in the hospital sample.

Fifty percent of patients had a UPC  $>0.5$  for both samples and 50% had a UPC  $<0.5$  for both samples. When comparing samples from patients with UPCs  $>0.5$ , UPCs of hospital samples were significantly higher than home samples ( $P = .001$ ). There was no evidence of a difference when comparing home and hospital samples from patients with UPCs  $<0.5$  ( $P = .25$ ).

When comparing the 2 relevant urine solute concentrations involved in calculating UPC ratios, urine protein concentrations from home and hospital samples were significantly different ( $P = .004$ ) and urine creatinine concentrations were not significantly different ( $P = .6$ ). For the subpopulation of patients with a UPC  $>0.5$ , urine protein concentration was significantly dif-



or frozen at  $-20^{\circ}\text{C}$  for up to 3 months.<sup>24</sup> Based on these previously established points and the exclusion criteria utilized in this study, variables of collection methods (voided versus cystocentesis), collection at different times of day, and potential for additional time at a higher temperature between collection of the home sample and analysis would not be expected to cause the significant differences in UPC measurement observed in this study.

Containers used for collection and storage of home urine samples were not standardized. For the convenience of obtaining home samples from new patients, we were not able to provide sterile urine cups for most clients. Thus, a large variety of containers were used for collecting home samples, although most were glass or Tupperware<sup>®</sup>-type plastic. The sterile urine cups used for the samples collected in hospital were made of polypropylene. Other plastic containers vary in composition, however, many plastic Tupperware<sup>®</sup>-type containers also are made of polypropylene. Because decreased protein binding is reported with hydrophilic surfaces, some urine containers are coated with a nonionic detergent.<sup>32,33</sup> This type of protein binding seems to be most important with very low concentrations of albumin (in the microalbuminuric range) or very high surface area-to-fluid ratios.<sup>33,34</sup> The effect decreases proportionally as protein concentration increases such that it would be unlikely to significantly affect measured protein concentration in the patients in this study.<sup>33,34</sup> Nonetheless, some degree of binding or denaturing of protein could have occurred in containers used for home samples resulting in falsely decreased urine protein concentrations measurements.

Establishing persistence of proteinuria is strongly recommended even in cases that have been appropriately localized as having renal proteinuria because of substantial day-to-day variation in urine protein concentration.<sup>8,27</sup> The IRIS algorithm for substage classification recommends that unless the UPC is  $>2$ , decisions should not be based on only 1 sample.<sup>9</sup> Similarly, the ACVIM consensus and IRIS consensus clinical practice guidelines recommend repeat sample collection to document persistence and establish a baseline for the individual.<sup>8,9,27</sup> There was no attempt to document persistence of urine protein in the patient population for this study or to determine if the pattern of higher hospital UPC values observed at this single time point was consistent over time. If differences in UPC were persistent over time, 6/24 dogs (25%) from this study would either have been assigned a different IRIS substage (1 dog) or a different clinical response based on ACVIM guidelines (5 dogs). Furthermore, even without this information UPC results similar to those reported in this study have the potential to lead to different interpretations regarding the clinical importance of the magnitude of proteinuria in some patients. Four of 24 (17%) dogs had UPCs  $<2.0$  in the home samples, but  $>2.0$  in the hospital samples. These dogs might receive treatment with an ACEi medication if urine samples were only collected while the patient was at the hospital, but would likely not receive medications without further investigation if

samples were only collected at home.<sup>8,10</sup> Because of the potential to affect clinical interpretation and decision making, the difference between UPCs of home and hospital samples warrants further investigation.

Localizing the source of protein loss to pre-renal, renal, and postrenal causes also is important when considering diagnostic test choices, prognosis, and treatment in clinical patients. The IRIS and ACVIM guidelines listed above apply only to cases of pathologic renal proteinuria. Localization of proteinuria was only minimally addressed in the patient population in this study by limiting inclusion to patients without hematuria, pyuria, or those receiving corticosteroid or ACEi medications. Sediment evaluation likely provides enough information to enable comparison of urine protein measurements between samples without further evaluation of possible postrenal causes of proteinuria because most of those would be unlikely to change significantly between home and hospital urine collection times without a change in sediment.<sup>25,31</sup> Many pre-renal causes of proteinuria were not evaluated in this population and therefore cannot be excluded as possible causes or contributing factors for either the proteinuria or difference in magnitude of proteinuria between collection environments. Further investigation for pre-renal causes of proteinuria also could affect assignment of IRIS substage or clinical response recommendations for some individuals.

There are several ways in which this study population may not be representative of either a general population or a subpopulation of dogs for which UPC testing typically would be recommended. Patients were included in this study if urine protein concentrations in any sample were evaluated as trace or higher using a dipstick. It is possible that a UPC may not have been clinically indicated if some dogs had negative sulfasalicylic acid turbidometric, species-specific point-of-care, or quantitative albumin ELISA tests.<sup>8,27</sup> Even patients for which a urine protein assessment was indicated may have travelled farther or remained at the hospital for longer than they would for a specific urine collection appointment at a primary care facility.

The specific underlying cause of the difference in UPCs between home and hospital samples remains unknown. The dogs' stress level is certainly 1 factor that may have changed between the 2 sample collection environments. Changes in epinephrine, cortisol, blood pressure, or other physiologic variables related to stress could temporarily affect glomerular filtration in a hospital setting, but these factors were not measured. Prior studies did not identify changes in UPC in normal dogs receiving pharmacologic doses of prednisone or hydrocortisone before 5–7 days.<sup>12,13</sup> However, endogenous corticosteroids could exert a greater effect in patients that are already proteinuric. The potential relationship between blood pressure and urine protein concentration appears to be complicated with some studies showing a positive correlation and others reporting no direct association.<sup>16–19</sup> Because of inconsistent reporting, it was not possible to statistically evaluate the effect of travel time to the hospital, travel distance to the hospital, or

time between arrival at the hospital and sample collection. Subjective evaluation of these variables did not identify any obvious trends, and it is unlikely that these variables were significantly different for the patients included in this study than for typical clinical patients at our hospital. Because we do not have information about home and hospital values for certain physical examination variables that are commonly associated with stress or excitement such as heart rate, respiratory rate, and body temperature, we were not able to evaluate whether there might be a relatively greater difference in these variables between the home and hospital environments in those patients that also had an increase in UPC between home and hospital samples. However, there was no evidence of a difference in the recorded in-hospital values for these variables between the population of dogs with an increase in UPC from home to hospital and those without an increase in UPC. All dogs were fasted for a longer period of time before collection of the hospital sample than the home sample and we cannot eliminate an effect of this longer period of fasting. Finally, it also is possible that a nonphysiologic, preanalytic variable such as the time the urine was in contact with its container before refrigeration or testing, lack of container standardization, or other factors may have caused preanalytical changes.

Although the results of this study showed that for proteinuric patients, urine samples collected in our hospital environment had significantly higher UPCs compared to samples collected in a home setting, they do not indicate whether 1 of these environments is preferable. Existing recommendations for classification and clinical responses do not specifically address location of sample collection. However, because of the potential for changes in clinical interpretation of UPCs based on differences resulting from sample collection environment, this variable should not be ignored. Although it would be inappropriate to change current guidelines based on information from this study, consistency in location of sample collection should be considered.

Within this population of dogs, there was a statistically significant difference between home and hospital urine specific gravities. The mean difference was 0.005 (standard deviation, 0.009). This difference may have been partly a result of a difference in water consumption during the time period between the most recent urination and the collection of each sample because dogs are likely to drink less overnight than during the morning, however, the urine creatinine concentration was not significantly different as would be expected if this was the only explanation for the change in urine concentration. Nonetheless, the difference in USG is unlikely to be the primary source of the difference in UPC values because there was a statistically significant change in urine protein concentration, but not urine creatinine concentration between the home and hospital samples. Epithelial cells also seemed to be more common in hospital-acquired samples, appearing in 10 samples compared with just 3 samples collected at home. This did not seem to be a function of collection method (cystocentesis versus voiding), but in 6 of these cases "rare" or

"occasional" epithelial cells were reported by a laboratory technician evaluating the hospital-collected sample, whereas none were reported by the study authors (MD or AS) evaluating the home sample. This probably is not a clinically relevant difference. There was no apparent difference in presence or number of WBC, RBC, crystals, or lipid between home and hospital samples and no apparent correlation between the presence or absence of these findings and method of sample collection, sample evaluator, or urine protein results.

In summary, this study identified higher UPC values in canine urine samples collected in a hospital setting compared to samples collected at home. When the population was subdivided into groups of dogs with UPC <0.5 and those with UPC >0.5, this difference was only identified in the latter group. The degree of difference identified in some of these patients could affect clinical interpretation of UPC results and clinical decisions about diagnostic, monitoring, or therapeutic plans. Thus, the location of sample collection should be considered when interpreting UPCs, and urine samples probably should be obtained from a consistent location when evaluating response to an intervention. Nevertheless, how this difference should be integrated into current guidelines requires further consideration and study.

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## Footnotes

- <sup>a</sup> NIC-500ATC Portable Clinical Refractometer, National Industrial Supply, Temecula, CA
  - <sup>b</sup> Multistix 10SG reagent strip, Siemens Medical Solutions, Malvern, PA
  - <sup>c</sup> Dimensions RXL Chemistry Analyzer, Siemens Medical Solutions and Diamond Diagnostics, Holliston, MA
  - <sup>d</sup> SAS Institute Inc, Cary, NC
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*Conflict of Interest Declaration:* Authors disclose no conflict of interest.

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

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