The determination of GFR is usually accepted as the best overall estimate of kidney function in dogs and can be used to evaluate onset and progression of renal disease in dogs. However, because current criterion-referenced standard methods for GFR measurements that require the use of radioisotopes or nonradio-labeled markers are time consuming and expensive, they are rarely performed in most clinical settings.1 Concentrations of plasma creatinine or plasma urea have been used as endogenous markers of GFR for many decades; unfortunately, these provide only a crude estimate of GFR because concentrations in plasma might not change until nearly 75% of kidney function has already been lost.2 In dogs with chronic renal disease, abnormally high concentrations of plasma creatinine and plasma urea are often detected before clinical signs of renal disease become apparent.3 Furthermore, these concentrations provide no information about the functional capacity of the remaining functioning nephrons.4

Influence of kidney function on urinary excretion of albumin and retinol-binding protein in dogs with naturally occurring renal disease

Jens Raila, Dr med vet habil; Leo Brunnberg, Prof Dr med vet; Florian J. Schweigert, Prof Dr med vet; Barbara Kohn, Prof Dr med vet

Objective—To evaluate excretion of urinary albumin (UAib) and urinary retinol-binding protein (URBP) in dogs with naturally occurring renal disease.

Animals—64 client-owned dogs.

Procedures—Dogs were assigned to groups according to plasma creatinine concentration, urinary protein-to-urinary creatinine ratio (UP:UC), and exogenous plasma creatinine clearance (P-ClCr) rates: group A (n = 8), nonazotemic (plasma creatinine < 125 µmol/L) and nonproteinuric (UP:UC < 0.2) with P-ClCr rate > 90 mL/min/m²; group B (26), nonazotemic and nonproteinuric with P-ClCr rate 50 to 89 mL/min/m²; group C (7), nonazotemic but proteinuric with P-ClCr rate 53 to 98 mL/min/m²; group D (8), azotemic and borderline proteinuric with P-ClCr rate 22 to 45 mL/min/m²; and group E (15), azotemic and proteinuric (P-ClCr not evaluated). The UAib and URBP concentrations were measured via ELISA; UAib-to-urinary creatinine (UAib:UC) and URBP-to-urinary creatinine (URBP:UC) ratios were determined.

Results—UAib:UC and URBP:UC did not differ between groups A and B. Increased UAib:UCs and URBP:UCs were paralleled by increased UP:UCs in groups C, D, and E relative to values from groups A and B, independent of azotemia. There were significant positive correlations of UP:UC with UAib:UC and of UAib:UC with URBP:UC (r = 0.82 and 0.46, respectively). However, UP:UC, UAib:UC, and URBP:UC were not significantly correlated with P-ClCr rate.

Conclusions and Clinical Relevance—UAib and URBP concentrations were paralleled by urinary protein concentrations and may be useful in assessing renal management of plasma proteins. Determination of urinary protein, UAib, or URBP concentration was not sufficiently sensitive to detect reduced P-ClCr in nonazotemic dogs. (Am J Vet Res 2010;71:1387–1394)
are associated with renal fibrosis, which determines the progression and final outcome of the disease.\(^3\) Thus, improved diagnostic methods are needed because the detection of early stages of renal disease in dogs might shift the focus of medical intervention from treatment to prevention or could help to slow the progression of renal disease.\(^1,2\)

Evaluation of markers other than plasma creatinine or plasma urea, such as the excretion of UP, is of particular interest in clinical nephropathy. Persistent proteinuria is an indicator of renal injury and a possible contributor to the progression of renal disease; it is associated with increased mortality rates in dogs with chronic renal failure and decreased survival times in dogs with renal and nonrenal diseases, compared with those of nonproteinuric dogs.\(^5,3\) The composition of UP is determined by permeability of the glomerular barrier as well as by protein reuptake in renal proximal tubular cells and the capacity of these cells to degrade the filtered proteins.\(^6,9\) Detection of certain UPs may provide a new approach for the diagnosis of particular pathological processes in the glomeruli, proximal tubules, or both.\(^8\) Urinary albumin is the principal component of UP, and high concentrations of UAlb may be associated with progression of renal disease.\(^9,10\) Results of a preliminary investigation in dogs suggested that a slight increase in the excretion of UAlb in the range of microalbuminuria (ie, 1 to 30 mg of albumin/dL in 1.01 USG-normalized urine) is an early indicator of progressive renal disease, particularly for renal diseases that involve glomeruli.\(^8\) Additionally, other conditions have been associated with microalbuminuria in dogs, including inflammatory, infectious, neoplastic, metabolic, and cardiovascular diseases.\(^11,13,14\) Microalbuminuria was also reported\(^12\) to be an indicator of occult systemic disease in dogs that had negative results for UP when tested by use of urinalysis strips.

Previous investigations\(^13,14\) conducted by our laboratory group suggested that a significant increase in the excretion of URPB in dogs that had urolithiasis or chronic renal disease, compared with concentrations detected in healthy dogs, was associated with kidney injury. The 21-kDa RBP circulates in plasma, where it serves to transport and deliver vitamin A.\(^15\) The unbound fraction of RBP is freely filtered through the glomeruli and is catabolized after reabsorption in the proximal tubules.\(^16\) Because of this process, only trace amounts of RBP should be excreted in the urine of healthy dogs, whereas urinary loss of RBP is expected to increase in dogs with proximal tubule disorders. Thus, RBP has been suggested as a sensitive marker of proximal tubule dysfunction in dogs.\(^17\)

Although proteinuria is associated with renal disease progression in dogs, the clinical relevance of UAlb and URPB in renal disease of dogs is not fully understood. The objective of the study reported here was to evaluate differences in the excretion of UAlb and URPB among 5 groups of dogs (healthy control dogs; nonazotemic, nonproteinuric dogs with reduced P-Cl\(_{\text{cr}}\) rates; nonazotemic dogs with proteinuria and reduced or normal P-Cl\(_{\text{cr}}\) rates; borderline proteinuric dogs with azotemia and reduced P-Cl\(_{\text{cr}}\) rates; and azotemic, proteinuric dogs that were not tested for P-Cl\(_{\text{cr}}\)).

### Materials and Methods

**Animals**—Sixty-four dogs of various breeds (40 males and 24 females; median age, 6.2 years [range, 1 to 14]) were included in the study. All dogs were client- or student-owned animals and were brought to the Small Animal Clinic of the Freie Universität Berlin for blood donation and annual physical examination or to receive clinical treatment for renal disease. Dogs with pathological conditions other than renal diseases (eg, infectious or inflammatory disease, neoplasia, endocrinopathy, or lower urinary tract disease) were excluded from the study. Because samples were collected from dogs as part of a routine clinical evaluation at the hospital, approval of the university’s animal care and use committee was not required. Owner permission was obtained for use of the samples.

**Initial sample collection**—Food was withheld from each dog for 12 hours prior to sample collection. The first voided urine sample in the morning was collected via free catch in the home environment for each dog; blood was collected in the clinic from a cephalic vein in 1.3-mL lithium-heparin tubes.\(^\text{a}\) Plasma was prepared by centrifugation of blood samples (1,500 \(\times\) g, 10 minutes at 4°C) within 1 hour after collection. Urine was centrifuged for 2 minutes at 300 \(\times\) g for sediment analysis and removal of cells and particulate matter. Urine supernatant and plasma samples were kept frozen at –80°C until use; all assays were performed ≤4 months after collection.

**Plasma biochemical analysis and urinalysis**—Plasma creatinine, urea, total protein, and albumin concentrations were measured by the use of an automated analysis system.\(^\text{e}\) Qualitative urinalysis included use of urinalysis test strips\(^\text{f}\) and sediment analysis via light microscopy. The USG of the urine sample supernatant was determined by use of a refractometer\(^\text{g}\) and concentrations of UP were assessed via Bradford colorimetric assay.\(^\text{h}\) UC concentration was determined via a standard Jaffe reaction.\(^\text{i}\) The UP/UC was calculated to estimate the degree of proteinuria.

**Evaluation of renal function and group assignment of dogs**—The GFR was determined by use of a modified exogenous P-Cl\(_{\text{cr}}\) test.\(^\text{\text{3,10}}\) Of 64 dogs in the study, 49 that did not have clinical signs of uremia (ie, inappetence, lethargy, and vomiting) or indications of proteinuria with azotemia were selected for P-Cl\(_{\text{cr}}\) testing. For the selection of these dogs, azotemia was defined as an increase in values above the laboratory reference ranges for concentrations of plasma creatinine (53 to 125 mmol/L [0.6 to 1.4 mg/dL]) and plasma urea (3.5 to 9.9 mmol/L [0.6 to 1.4 mg/dL]); proteinuria was defined as a UP/UC > 0.5 mg/mg. Fifteen dogs that were proteinuric and azotemic were excluded from P-Cl\(_{\text{cr}}\) testing.

Food was withheld from the 49 dogs for 12 hours before the exogenous P-Cl\(_{\text{cr}}\) test but water was available ad libitum before and during the procedure. After hair clipping and disinfection of the skin, an indwelling catheter was placed in the left cephalic vein; a blood sample (1 mL) was collected via venipuncture of the right cephalic vein for use in determining endogenous
plasma creatinine concentrations. A single bolus of a 5% solution of creatinine was administered via the IV catheter at a dose of 2.4 g/m² of BSA. The catheter was flushed with sterile saline (0.9% NaCl) solution immediately after creatinine administration. Blood samples (1 mL) were obtained from the right cephalic vein 3 times during the period from 4 to 9 hours after injection. Plasma creatinine was measured as previously described. The measured values for plasma creatinine were standardized on the basis of BSA because, in mammals, the number of nephrons corresponds closely to BSA. The BSA was calculated according to the following formula: BSA (m²) = body mass (kg)0.454 × 0.1. The P-ClCr rate was estimated as the amount of creatinine injected divided by the area under the curve calculated via the trapezoidal method by use of a noncompartmental model. Calculations were performed by use of a commercially available computer software program.

For the group assignment of dogs after P-ClCr testing, reference values for plasma creatinine and UP concentrations were based on guidelines established by the International Renal Interest Society for dogs with chronic renal disease. Azotemia was defined as plasma creatinine concentration > 125 µmol/L; dogs were considered nonproteinuric for UP:UC < 0.2, borderline for UP:UC 0.2 to 0.5, and proteinuric for UP:UC > 0.5 mg/mg.

Dogs were assigned to 1 of 5 groups on the basis of results of plasma creatinine concentration, UP:UC, and P-ClCr rate analysis. Group A (healthy control dogs; n = 8) comprised clinically normal, nonazotemic, and nonproteinuric dogs with P-ClCr rates > 90 mL/min/m²; group B (26) consisted of nonazotemic and nonproteinuric dogs with mildly reduced P-ClCr rates (50 to 89 mL/min/m²); group C (7) included nonazotemic but proteinuric dogs (P-ClCr rates for this group ranged from 53 to 98 mL/min/m²); the rates for all but 1 dog were mildly reduced; group D (8) consisted of azotemic, borderline proteinuric dogs with moderately reduced P-ClCr rates (22 to 45 mL/min/m²); and group E (15) included dogs with azotemia and proteinuria that were not tested for P-ClCr because of their preexisting conditions. Samples were obtained for analyses prior to the administration of fluids or angiotensin-converting enzyme inhibitors for dogs that required such treatment.

Determination of plasma RBP and URBP—Concentrations of RBP in plasma and urine were determined by use of a 2-site ELISA with a commercially available RBP antibody as described in another study. Intra-assay CVs for plasma and urine samples were 2.9% and 5.1%, respectively. Interassay CVs for plasma and urine samples were 4.2% and 8.9%, respectively.

Quantitation of UAlb—The concentration of UAlb was determined by use of an ELISA with a double-antibody sandwich technique. Wells of microtiter plates were coated with an IgG fraction of affinity-purified goat anti-dog albumin (1 mg/mL in 50 mM carbonate buffer, pH 9.6; 50 µL/well) and incubated for 2 hours at 37°C. Wells were washed 4 times with 200-µL aliquots of a washing buffer (pH, 7.4) that consisted of PBS and polysorbate 20® (ie, PBST [10 mM PBSS, 150 mM NaCl, and 0.05% polysorbate 20]). Wells were emptied; plates were allowed to dry and were stored overnight at 4°C. Nonspecific binding was blocked by incubation for 5 minutes at 37°C with blocking buffer® in Tris-buffered saline solution (200 µL/well). The dog albumin standard was prepared by serial dilution in PBSS (range, 10 to 0.156 µg/dL). After 4 washes with PBST, wells (in triplicate) were filled with 50 µL of diluted dog albumin standard® or diluted urine samples (1:2,000 to 1:4,000 as needed) and incubated for 1 hour at 37°C with constant shaking. After 3 washes with PBST, 50 µL of peroxidase-conjugated IgG fraction of goat anti-dog albumin® (1:8,000 dilution) was added to each well and incubated for 1 hour at 37°C. After 4 final washes with PBST, the substrate reaction was developed by the addition of 0.137% o-phenylenediamine dihydrochloride solution® (100 µL/well) and incubation in the dark for 30 minutes at 23°C. The reaction was stopped by the addition of 1 M H2SO4 (50 µL/well). Optical densities were determined at 490 nm by use of a dual wavelength mode microtiter plate reader® that automatically subtracted the background optical density of the substrate reaction blank. The standard curve obtained for each plate was used to calculate albumin concentrations in the samples on that plate. The detection limit was 0.15 µg/dL of assay solution, which was defined as the minimum concentration of albumin that yielded an absorbance > 10 SDs of the mean absorbance for blank samples. Intra-assay and interassay CVs for urine samples were 8.0% and 9.2%, respectively.

Fractional excretion rates—To evaluate the capacity for reabsorption of albumin and RBP by proximal tubular cells, fractional excretion was calculated according to the following equation: Fractional clearance = (UX/UC) × (PC/PX) × 100, where UX is the concentration of a specific protein (ie, albumin or RBP) in a spot urine sample (a single-voided sample), PX is the plasma concentration of that specific protein, UC is the urine concentration of creatinine in the spot urine sample, and PC is the plasma concentration of creatinine.

Statistical analysis—Results were expressed as medians and ranges. Statistical analysis was accomplished by use of nonparametric procedures. A Kruskal-Wallis test was used to determine significant differences in variables among groups. When a significant effect was detected, a Mann-Whitney U test was performed to determine differences in proportions among groups. Spearman rank correlation coefficients were used to test the association between variables used to evaluate renal function. To identify independent determinants of P-ClCr rate and UP concentration, linear regression analysis was performed. Values of P < 0.05 were considered significant.

Results

Association with age—Nonazotemic dogs with proteinuria (group C) and azotemic dogs with overt proteinuria (group E) were significantly older than dogs of groups A, B, and D. Age was significantly correlated with plasma creatinine concentration (r = 0.35;
rates for dogs in groups A through C. Azotemic and borderline proteinuria (group D), compared with 

---

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of dogs</th>
<th>Age (y)</th>
<th>No. of males</th>
<th>No. of females</th>
<th>P-ClCr rate (mL/min/m²)</th>
<th>Creatinine (µmol/L)</th>
<th>UP:UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8</td>
<td>3.5–1 (1–7)</td>
<td>4</td>
<td>4</td>
<td>&gt; 90</td>
<td>&lt; 125</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>B</td>
<td>26</td>
<td>4³ (1–14)</td>
<td>17</td>
<td>9</td>
<td>50–89</td>
<td>&lt; 125</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>9³ (7–12)</td>
<td>5</td>
<td>2</td>
<td>53–98</td>
<td>&lt; 125</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>6³ (2–12)</td>
<td>3</td>
<td>5</td>
<td>22–45</td>
<td>&gt; 125</td>
<td>0.2–0.5</td>
</tr>
<tr>
<td>E</td>
<td>15</td>
<td>8³ (1–14)</td>
<td>11</td>
<td>4</td>
<td>ND</td>
<td>&gt; 125</td>
<td>&gt; 0.5</td>
</tr>
</tbody>
</table>

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Food was withheld from dogs for 12 hours prior to sample collection and P-ClCr testing but dogs had free access to water. All urine values were determined from analysis of the first voided urine sample in the morning, which was collected by free catch in the home environment for each dog. Exogenous P-ClCr rates and creatinine values were determined by analysis of plasma samples obtained 4 to 9 hours after IV administration of a 5% solution of creatinine at a dose of 2.4 g/m² of BSA. Dogs in group A were considered nonazotemic, nonproteinuric healthy control dogs; dogs in group B were nonazotemic and nonproteinuric but had mildly reduced P-ClCr rates; dogs in group C were nonazotemic but proteinuric, and P-ClCr rates for all but 1 dog were significantly (P < 0.01) reduced in dogs with azotemia and borderline proteinuria (group D), compared with rates for dogs in groups A through C. Azotemic and proteinuric dogs (group E) were excluded from P-ClCr testing; plasma concentrations of creatinine and urea were significantly (P < 0.01) increased, whereas plasma albumin concentrations were significantly decreased, compared with concentrations for dogs in groups A through D. Plasma concentrations of RBP were similar among dogs in groups A through D but were significantly increased in dogs in group E relative to values for dogs in all other groups.

---

**Table 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference value</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-ClCr rate (mL/min/m²)</td>
<td>&gt; 90</td>
<td>110³</td>
<td>96³</td>
<td>80³</td>
<td>43³</td>
<td>ND</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>53–125</td>
<td>81.7³</td>
<td>98.1³</td>
<td>69.0³</td>
<td>152³</td>
<td>781³</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>3.5–9.9</td>
<td>4.7³</td>
<td>8.3⁴</td>
<td>3.4³</td>
<td>4.9³</td>
<td>12.8³</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>54–75</td>
<td>61.0</td>
<td>62.5</td>
<td>60.7</td>
<td>65.4</td>
<td>61.1</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>25–44</td>
<td>31.4³</td>
<td>31.5³</td>
<td>25.3³</td>
<td>23.1³</td>
<td>26.3³</td>
</tr>
<tr>
<td>RBP (mg/L)</td>
<td>—</td>
<td>31.5³</td>
<td>31.5³</td>
<td>28.7³</td>
<td>26.8³</td>
<td>35.0³</td>
</tr>
</tbody>
</table>

---

**Table 3**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference value</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-ClCr rate (mL/min/m²)</td>
<td>&gt; 90</td>
<td>110³</td>
<td>96³</td>
<td>80³</td>
<td>43³</td>
<td>ND</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>53–125</td>
<td>81.7³</td>
<td>98.1³</td>
<td>69.0³</td>
<td>152³</td>
<td>781³</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>3.5–9.9</td>
<td>4.7³</td>
<td>8.3⁴</td>
<td>3.4³</td>
<td>4.9³</td>
<td>12.8³</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>54–75</td>
<td>61.0</td>
<td>62.5</td>
<td>60.7</td>
<td>65.4</td>
<td>61.1</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>25–44</td>
<td>31.4³</td>
<td>31.5³</td>
<td>25.3³</td>
<td>23.1³</td>
<td>26.3³</td>
</tr>
<tr>
<td>RBP (mg/L)</td>
<td>—</td>
<td>31.5³</td>
<td>31.5³</td>
<td>28.7³</td>
<td>26.8³</td>
<td>35.0³</td>
</tr>
</tbody>
</table>

---

Variables were assessed via analysis of plasma obtained from blood samples collected after food was withheld for 12 hours; samples were obtained prior to exogenous P-ClCr testing.

— = Not applicable.

**Values in the same column with different superscript letters differ significantly (P < 0.05).**

See Table 1 for remainder of key.
all other groups; URBP:UC did not differ significantly between groups C and E. Because glomerular-filtered proteins are rapidly cleared by proximal tubular cells, the function of these cells was tested by determination of the fractional clearance rates of albumin and RBP. Increased excretion of UAlb:UC and URBP:UC in dogs of groups C through E was associated with increased fractional clearance rates of these proteins. The fractional clearance rates of UAlb and URBP were significantly (P < 0.001) higher in group E dogs, compared with values for dogs in groups A through D.

Relationships between proteinuria and variables used to assess renal function—Multivariate linear regression analysis of plasma creatinine concentration, USG, UP:UC, UAlb:UC, and URBP:UC as predictors for P-ClCr rate in groups A through D revealed that plasma creatinine concentration is the single strongest determinant of P-ClCr rate (Table 4). In addition, plasma urea concentration (P < 0.01) but not age (P = 0.07), sex (P = 0.68), or body weight (P = 0.18) was significantly independent of P-ClCr rate. The evaluation of plasma creatinine concentration, USG, UAlb:UC, and URBP:UC as dependent variables of UP:UC revealed that UAlb:UC and URBP:UC were the best predictors of UP:UC.

Discussion

The precise role of proteinuria in the pathogenesis of renal disease in dogs is uncertain at present. Therefore, the validation of urinary proteins as diagnostic markers is needed to improve detection and investigation of the site and progression of renal injury. In addition to evaluation of UAlb excretion, several studies have addressed the pathophysiological role of urinary proteins in dogs with various diseases associated with renal injury. The assessment of specific types of proteinuria may be useful to distinguish between glomerular and tubular disorders. Such information as well as the degree of damage in the glomerular and tubulointerstitial compartments of the nephron might be of predictive value for functional outcome of renal disease and may be useful in regard to implementation of therapeutic strategies for individual patients.

Table 3—Median (range) urine values of selected variables used as indicators of kidney function in the 64 dogs in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference value A</th>
<th>Group B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>USG (mL/dL)</td>
<td>1.001–1.065</td>
<td>1.045a</td>
<td>1.039a</td>
<td>1.012a</td>
<td>1.019a</td>
</tr>
<tr>
<td>UP-UC (mg/mg)</td>
<td>&lt; 0.2</td>
<td>0.02a</td>
<td>0.04a</td>
<td>2.56a</td>
<td>0.30a</td>
</tr>
<tr>
<td>UAlb:UC (µg/mg)</td>
<td>NA</td>
<td>2.18a</td>
<td>2.31a</td>
<td>186c</td>
<td>27.6c</td>
</tr>
<tr>
<td>URBP:UC (µg/mg)</td>
<td>NA</td>
<td>0.01a</td>
<td>0.03a</td>
<td>0.86c</td>
<td>0.10b</td>
</tr>
<tr>
<td>FAlb (%)</td>
<td>0a</td>
<td>0a</td>
<td>0.06b</td>
<td>0.01b</td>
<td>0.90c</td>
</tr>
<tr>
<td>FRBP (%)</td>
<td>0a</td>
<td>0.02a</td>
<td>0.07a</td>
<td>0.55a</td>
<td>0.88b</td>
</tr>
</tbody>
</table>

Variables were assessed via analysis of voided urine samples obtained prior to P-ClCr testing. FAlb = Fractional albumin excretion. FRBP = Fractional RBP excretion. NA = Not applicable. Values in the same row with different superscript letters differ significantly (P < 0.05).

See Table 1 for remainder of key.

Table 4—Results of linear regression analyses of renal functional variables as predictors of P-ClCr rate and UP:UC in the 64 dogs in Table 1.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Correlation (r)</th>
<th>Standardized β</th>
<th>Correlation X standardized β X 100 (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-ClCr rate</td>
<td>Creatinine</td>
<td>−0.56</td>
<td>−0.480</td>
<td>27.4</td>
<td>0.004</td>
</tr>
<tr>
<td>USG</td>
<td>0.28</td>
<td>0.179</td>
<td>5.01</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>UP-UC</td>
<td>0.12</td>
<td>0.085</td>
<td>1.02</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>UAlb:UC</td>
<td>0.05</td>
<td>0.005</td>
<td>0.02</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>URBP:UC</td>
<td>0.04</td>
<td>0.088</td>
<td>0.35</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

| UP:UC              | Creatinine           | −0.09          | −0.016        | 0.14                                   | 0.91   |
| USG                | −0.04                | −0.061         | 0.24          | 0.06                                   |
| UAlb:UC            | 0.82                 | 0.766          | 62.8          | < 0.001                                |
| URBP:UC            | 0.46                 | 0.309          | 13.9          | 0.02                                   |
| Total              | —                    | —              | —             | —                                      |

See Tables 1 and 2 for key.
tein reuptake in the proximal tubules. We evaluated the concentrations of these 2 proteins in healthy control dogs (group A); apparently healthy, nonazotemic, and nonproteinuric dogs with reduced P-ClCr rate (group B); and dogs with naturally occurring renal disease (groups C through E).

An important discovery in the present study was that UP:UC and UAlb:UC were not significantly associated with P-ClCr rates in dogs that had plasma creatinine values within the laboratory reference range. Because P-ClCr rate is directly related to the number of functioning nephrons in the kidneys, the lack of this association indicates that the determination of UP:UC or UAlb:UC is not diagnostically useful in detecting a mildly decreased P-ClCr rate at an early stage of renal disease in dogs.

This result is in agreement with a few studies in humans. In a clinical trial in patients with type 2 diabetes, an uncoupling of the relationship between GFR and UAlb excretion was revealed because decreased GFR detected in the same patients over time was not accompanied by increased UAlb concentrations. Another study showed that UAlb excretion was not significantly related to decreased P-ClCr rates (< 60 mL/min × 1.73 m² of BSA) in nonhypertensive human patients with coronary heart disease, thereby limiting the importance of UAlb excretion as a marker for increased risk of cardiovascular disease. The relationship between proteinuria and P-ClCr rate was also investigated in dogs with various renal and nonrenal diseases. In contrast to the results of the study reported here, the investigators in that study reported a weak but significant inverse correlation (r = −0.28; P < 0.05) between UP:UC and exogenous P-ClCr rate, although dogs with P-ClCr rates < 1.99 mL/min/kg excreted more UP than did dogs with a P-ClCr rate > 2.0 mL/min/kg.

Several dogs in the present study had plasma creatinine concentrations within the reference range but had UP:UCs deemed pathological (values > 0.5; group C). Proteinuria despite normal renal function has been described in some dogs with nonrenal diseases such as neoplasia, endocrinopathies, or chronic infections. Such causes seemed unlikely but could not be completely excluded in dogs of the present study. Alternatively, proteinuria can be associated with early glomerular damage, despite a physiologically normal GFR. The present investigation also revealed that some dogs with azotemia were not proteinuric, a condition that has also been described in humans with type 1 and type 2 diabetes mellitus. The underlying mechanisms for this result are unclear, and because renal ultrastructural information was not obtained, only limited conclusions can be drawn regarding the possible pathogenesis of this condition in dogs.

In the present study, the increased fractional excretion of UAlb in dogs with renal disease, compared with that of healthy control dogs and apparently healthy, nonazotemic, and nonproteinuric dogs with reduced P-ClCr rates, was associated with increased excretion of UP. Increased UAlb excretion may result from an increase in the glomerular passage of albumin or from a decrease in the protein reabsorption capacity of the proximal tubules. In this context, it was suggested that UAlb excretion in the range of microalbuminuria (ie, 1 to 30 mg of albumin/dl in 1.010 USG-normalized urine) is the mildest detectable form of abnormal renal protein management.

Microalbuminuria is commonly detected in dogs, but it is most often assessed by semiquantitative testing, and few data are available on the reference ranges of UAlb:UC in healthy and diseased dogs. Investigators of a study in cats and dogs in 2008 determined UAlb concentration by use of an immunoturbidimetric assay with a polyclonal anti-human albumin antibody and reported UAlb:UCs of 1 to 819 (no units were reported). Those authors included dogs with chronic renal disease, diabetes mellitus, or hypercortisolism in the study but did not include healthy control dogs. A modified immunoturbidimetric assay designed for use with human samples was also used to investigate the effect of hydrocortisone on UAlb excretion in dogs; results of that study revealed that both UP:UC and UAlb:UC significantly increased with hydrocortisone treatment. Cutoff values for UAlb:UC (100 and 200 µg/mg) were established by other investigators but were not of diagnostic value for use in screening of dogs to detect systemic diseases. In the present study, we determined UAlb excretion by use of a sensitive ELISA test and found UAlb:UC values from 1.55 to 3.97 µg/mg for healthy control dogs. This is lower than a set of previously reported UAlb:UC values in healthy dogs, which ranged from 3 to 8.2 µg/mg, and is a smaller range than that of 0 to 340 µg/mg reported in another study. One potential explanation for this discrepancy is that it is a method-dependent result because those investigators used an adapted human immunoturbidimetric assay to measure UAlb concentrations. Additional possibilities include potential circadian variations in UAlb excretion or poor comparability between UAlb:UC of the spot samples analyzed in the study reported here and 24-hour UAlb excretion in dogs. Results of those studies and of the present study also suggest that under certain physiologic or pathological conditions in dogs, glomerular filtered proteins such as albumin are not completely removed from the renal tubular fluid, which emphasizes the importance of proximal tubule integrity for protein reabsorption.

To our knowledge, investigation of the URBP:UC in relation to P-ClCr rate has not been reported prior to the present study. Because URBP is a low–molecular-weight protein, it has been suggested that this is a useful indicator for the detection of minor changes in proximal tubule function long before the concentrations of other indicators such as UP or plasma creatinine are increased above reference ranges. However, the results of the study reported here indicate that analysis of URBP values cannot detect a reduced P-ClCr rate (30 to 89 mL/min/m² of BSA) in apparently healthy dogs with plasma creatinine concentrations within the reference range. In the present study, URBP concentration was measured by use of an ELISA in 8 healthy control dogs and URBP:UC values ranged from 0.007 to 0.03 µg/mg. Whether these values might be considered as a reference range should be evaluated in further investigations. Although dogs with azotemia and proteinuria (group E) had increases in URBP:UC and fractional RBP excre-
tion, compared with the values in all other groups, the plasma concentrations of RBP in these dogs were also significantly increased. This might be attributable to the decreased ability of the kidneys to filter RBP, which is a 21-kDa protein.43,44 A loss of filtering capacity could potentially lead to impaired catabolism and abnormal retention of RBP and thus result in increased plasma concentrations of the protein.24,40

Linear regression analysis revealed that both UAlb and URBP concentrations might be significant predictors (approx 63% [P < 0.001] and 14% [P = 0.02], respectively) of UP excretion. Particularly in dogs with proteinuria (groups C and E), UAlb:UC and URBP:UC values were increased, compared with ratios for dogs from the other groups. Although the proximal tubules have a large capacity for protein endocytosis,23,45 increased fractional clearance rates of albumin and RBP in dogs reflect that, under proteinuric conditions, concentrations of filtered albumin and RBP in the remaining functional nephrons exceed maximal reabsorptive capacity of the proximal tubular cells, resulting in urinary excretion of large amounts of both proteins.44 Moreover, extreme proteinuria, indicated by tubular albumin concentrations that affect the absorptive function of the proximal tubular cells, contributes to competitive receptor binding, which in turn may contribute to excretion of URBP.42,45 Conversely, if proteinuria is attributable to proximal tubule malfunction in which the reabsorption of filtered proteins is impaired, this could also contribute to URBP excretion. The concentration of filtered RBP in the remaining functional nephrons exceeds the reabsorptive capacity of the proximal tubules, resulting in urinary excretion of large amounts of RBP. Because the reabsorption of albumin and reuptake of RBP by proximal tubular cells depend on megalin activity, it is also possible that defective megalin function or megalin deficiency might be involved in the development of UAlb and URBP excretion.15

The study reported here had several limitations. First, most dogs were examined only once in our study. Therefore, it cannot be determined if the abnormal values were attributable to acute events or to chronic renal disease. Second, UAlb and URBP concentrations were assessed only on a single urine specimen; thus, the possibility of intra-individual variability cannot be excluded. Nonetheless, the use of spot urine specimens for UP:UC determination can be recommended because the test can be easily performed in a clinical setting and good correlation (r = 0.975; P < 0.01) between the UP:UC and the 24-hour UP excretion has been reported.46 However, such correlations have not been evaluated for UAlb:UC and URBP:UC. Finally, we did not measure proteins, such as Tamm–Horsfall protein, that are physiologically secreted into the renal tubular lumen and might also contribute to proteinuria.16,17 This determination can be achieved by use of gel electrophoresis, although the method is limited for the discrimination of physiologic and pathological proteinuric elements. To circumvent this problem in future studies, the detection of specific marker proteins in urine by use of immunologic methods such as western blotting or ELISA might be of diagnostic value.

Analysis of the results of the present study suggest that the increased excretion of UAlb and URBP were associated with increased excretion of UP. The excretion of UP, UAlb, and URBP was not affected by P–Cl_\text{2}^- rate and therefore UAlb and URBP have no diagnostic value as markers for the early detection of reduced P–Cl_\text{2}^- rate in nonazotemic dogs. Because urine is readily accessible for noninvasive sample collection, further studies should be undertaken to determine whether serial measurements of albumin, RBP, or other marker proteins found in urine of affected dogs might be useful in early recognition of deteriorating renal function.

References

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