Indices of urine N-acetyl-β-D-glucosaminidase and γ-glutamyl transpeptidase activities in clinically normal adult dogs

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Objective—To establish reference ranges for indices of urine N-acetyl-β-D-glucosaminidase (NAG) and γ-glutamyl transpeptidase (GGT) activities in clinically normal adult dogs.

Animals—38 dogs.

Procedures—Each dog underwent a physical examination, CBC, serum biochemical analysis, urinalysis, and serologic testing for heartworm antigen and antibodies against *Ehrlichia canis* and *Borrelia burgdorferi*. Activities of NAG and GGT in urine were evaluated, and values of the respective indices were determined as urine NAG or GGT activity (U/L) divided by urine creatinine concentration (g/L).

Results—All dogs were considered clinically normal. A 90% prediction interval based on the 5th and 95th percentiles for GGT and NAG index values from both sexes was used to establish the reference ranges for dogs: 1.93 to 28.57 U/g and 0.02 to 3.63 U/g, respectively. Between males and females, urine NAG index differed significantly, whereas urine GGT index did not. When accounting for sex differences, reference ranges for the urine NAG index in males and females were 0.02 to 3.65 U/g and 0.02 to 2.31 U/g, respectively. Changes in urine pH significantly affected the urine GGT index but not the urine NAG index. Neither index changed significantly with changes in body surface area.

Conclusions and Clinical Relevance—Data suggest that increases in urine NAG and GGT indices allow for earlier detection of renal tubular damage in dogs. Such early detection would enable adjustment of the clinical management of affected dogs to decrease morbidity and death rates associated with acute tubular injury and acute tubular necrosis. (*Am J Vet Res* 2009;70:297–301)

Numerous renal tubular enzymes are excreted in the urine of mammals. Among those enzymes are NAG and GGT. These urinary enzymes are primarily located in the lysosomes and brush border, respectively, of the proximal convoluted tubule.[1] This portion of the nephron is a metabolically active segment that can be easily damaged. As a result of renal tubular injury, renal tubular enzymes are subsequently released into the lumen of the nephron or into the urine. Various infections, nephrotoxins, medications, or other causes can result in renal tubular damage[2–9] and may cause increases in renal enzyme activities. In studies of aminoglycoside-induced acute renal failure in dogs, increases in urine enzyme activities preceded clinically significant abnormalities in sCr concentration, USG, urine protein-to-creatinine ratio,[4] and 24-hour endogenous creatinine clearance.[10] In 6 dogs that were administered nephrotoxic doses of an aminoglycoside IM every 8 hours beginning day 1, urine GGT activity was significantly increased by day 5; this increase preceded a significant increase in sCr concentration detected on day 9.[10]

Abbreviations

GGT	γ-Glutamyl transpeptidase
NAG	N-acetyl-β-D-glucosaminidase
sCr	Serum creatinine
USG	Urine specific gravity

Routine analyses of blood and urine samples that are commonly performed in veterinary medicine detect renal dysfunction in animals only when 66% to 75% of the nephrons become nonfunctional. Several studies[4,10,11] have revealed that measurement of renal tubular enzyme activities (eg, NAG, GGT, alkaline phosphatase, and others) is more sensitive for detection of acute renal damage than the current standard veterinary diagnostic tests (ie, assessment of BUN and sCr concentrations and USG). Values of BUN and sCr concentrations greater than the upper reference limits can be insensitive and nonspecific for detection of acute renal damage[12] and are more likely to indicate a decrease in glomerular filtration rate rather than occurrence of renal tubular injury. Major renal tubular injury can occur without immediate alterations in glomerular filtration rate and may not be detected via commonly used screen-

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ing methods. The deposition of casts in urine occurs in approximately 30% to 40% of dogs with acute renal failure; thus, casts in urine are an inconsistent indicator of renal tubular injury.[13] Results of studies[4,10,11] have indicated that NAG and GGT activities in the urine of dogs with acute tubular damage increase prior to detectable increases in urine BUN and sCr concentrations. In several experimental studies,[4,10,14] evaluation of renal enzymuria (before and after renal tubular insult associated with gentamicin-induced nephrotoxicosis) has promise as a means of detection of renal tubular injury. Several studies[1,15–17] revealed significant differences in urine enzyme activities between the sexes of dogs, although another study[5] did not.

The purpose of the study reported here was to establish reference ranges for indices of urine NAG and GGT activities in clinically normal adult dogs (both male and female). It was anticipated that the reference ranges could be used to later identify dogs with suspected acute renal tubular damage. Such reference ranges are important to establish because, for most affected dogs examined in a clinical setting, values of those urine enzymes will not have been determined previously for comparison. Furthermore, as assessment of enzymuria in research settings increases, determination of reference ranges for urine enzymes in a representative population of dogs would also be useful.

Materials and Methods

Dogs—The study protocol was approved by the Oklahoma State University Institutional Animal Care and Use Committee. Owners of all dogs were provided with, and signed, an informed consent form. All dogs were regarded by their owners as free of clinical signs of illness. Thirty-eight clinically normal client-owned dogs (1 to 5 years old) were included in the study. There was 1 sexually intact female, 2 sexually intact males, 15 spayed females, and 20 neutered males. The dogs weighed 6 to 72 kg. Breeds included mixed (n = 11), Shetland Sheepdog (2), Great Dane (3), Australian Cattle Dog (2), German Shepherd Dog (2), German Shorthaired Pointer (2), Labrador Retriever (2), Beagle (1), Bichon Frise (1), Border Collie (1), Bulldog (1), Catahoula leopard dog (1), Dalmatian (1), English Setter (1), English Shepherd (1), Golden Retriever (1), Parson's terrier (1), Schnauzer (1), Staffordshire Bull Terrier (1), Standard Poodle (1), and Weimaraner (1). To determine overall health status, a CBC, serum biochemical analyses, urinalysis (including bacterial culture and antimicrobial susceptibility testing), and serologic testing for heartworm antigen and antibodies against *Ehrlichia canis* and *Borrelia burgdorferi*[a] were performed for each dog. Urine samples were collected and screened for the presence of microalbuminuria[b] and creatinine concentration, and GGT and NAG activities were assessed.

Hematologic evaluations—A CBC[c] (with differential cell assessment) and serum biochemical analyses[d] were performed for each dog. The serum biochemical analyses included evaluations of sodium, potassium, chloride, total carbon dioxide, BUN, creatinine, phosphorus, glucose, calcium, total protein, albumin, globulin, cholesterol, and total bilirubin concentrations; alkaline phosphatase, alanine transaminase, γ-glutamyltransferase, and amylase activities; and osmolality. All samples were processed within 4 hours after collection.

Urologic evaluations—Samples of urine were collected aseptically from each dog via antepubic cystocentesis. Ultrasound-guided placement of the needle into the urinary bladder was used if the bladder was small or difficult to palpate. Urine was subsequently analyzed by use of a urine dipstick,[e] and USG was assessed by use of a refractometer; within 4 hours after collection, some of the sample was centrifuged, and the urine sediment underwent microscopic examination. Another portion of the urine sample was used to measure creatinine concentration via the Jaffé method and to evaluate for microalbuminuria via immunoassay. A urine sample also was submitted for bacterial culture and antimicrobial susceptibility testing.

Urine enzyme indices—Urine activity of GGT was determined by use of an automatic analyzer[d] (L-γ-glutamyl-p-nitroanilide method) within 8 hours after sample collection. Colorimetric methods were used to measure urine NAG activity by means of the sodium salt of sodium-3-cresolsulphonphthaleinyl-N-acetyl-β-D-glucosaminide, which is hydrolyzed by NAG, allowing the release of 3-cresolsulphonphthalein, sodium salt (3-cresol purple). This product was then measured photometrically[d] at 580 nm. The freshly collected urine supernatant was stored at –20°C, and NAG activity was measured within 30 days of urine sample collection. The NAG index (U/g) and GGT index (U/g) were determined as urine NAG or GGT activity (U/L) divided by urine creatinine concentration (g/L).

Statistical analysis—All analyses were conducted by use of computer software.[f] The results of the CBC and biochemical analyses were analyzed by use of parametric statistical methods. For all parametric analyses, a value of $P < 0.05$ was considered significant. Indices of urine GGT and NAG activities were analyzed for an effect of sex by use of 2 population *t* tests. If no significant evidence of a sex effect was detected, the data were pooled. Studentized values (value minus mean divided by SD) were calculated for each response variable for GGT and NAG indices. Outliers were determined to be those observations for which the absolute value of the studentized value was > 3 (ie, more than a difference of 3 SD from the mean). Those values were culled from the original data set, and the mean, median, and relevant percentiles were calculated from this trimmed data set to determine the reference ranges for urine GGT and NAG indices. For all nonparametric analyses, a value of $P < 0.05$ was considered significant. Data are reported as mean ± SD and interquartile range (5th to 95th percentile).

Results

Physical examination revealed no clinically important abnormalities in any dog. Among the urine samples collected from the 38 dogs, there was no evidence of gross hematuria. Urine specific gravity was ≥ 1.030 in 25 dogs, 1.015 to 1.029 in 12 dogs, and < 1.015 (ie,

1.010 to 1.013) in 3 dogs. One dog (a 12-month-old sexually intact male Staffordshire Bull Terrier) had proteinuria (10 to 30 mg/dL) as determined by use of a sulfosalicylic acid turbidity test and low numbers of WBCs and bacteria in the urine, but results of bacterial culture of urine were negative. Seven dogs had trace hematuria (≤ 3 RBCs/hpf). Additionally, 6 dogs had microscopic hematuria (5 to 75 RBCs/hpf) and low numbers of WBCs (< 5 WBCs/hpf); results of bacterial culture of urine were negative for these dogs. In 13 dogs, results of bacterial culture of urine were negative and there was no crystalluria, but mild microscopic hematuria with or without low numbers of WBCs was detected. Crystalluria was detected in 10 dogs (8 had struvite crystals, 1 had oxalate-struvite crystals, and 1 had urate crystals). Bacterial culture of urine yielded positive results for 4 dogs; urine samples from these dogs contained trace evidence of *Staphylococcus* spp (n = 1), *Escherichia coli* (3), β *E coli* (1), *Streptococcus* spp (1), and *Enterococcus* spp (2). One dog in which *E coli* and β *E coli* were detected and 1 dog in which *E coli* and *Enterococcus* spp were detected had no hematuria or pyuria. Heartworm antigen and antibodies against *E canis* and *B burgdorferi* were not detected in serum samples from any dog. Additionally, no dogs had microalbuminuria (urine albumin concentration ≤ 1 mg/dL in all dogs).

Urine enzyme indices—The urine GGT and NAG indices were calculated for each dog. Among all dogs, the mean ± SD urine GGT index was 13.49 ± 7.03 U/g, and the mean urine NAG index was 1.10 ± 0.97 U/g. One dog had a GGT index of 60.34 U/g and an NAG index of 7.04 U/g, and another dog had an NAG index of 7.15 U/g; these values were eliminated from the reference range determinations. The reference ranges (ie, 90% prediction intervals based on the 5th and 95th percentiles following removal of extreme observations) were 1.93 to 28.57 U/g for the urine GGT index and 0.02 to 3.63 U/g for the urine NAG index. In the 4 dogs for which bacterial culture of urine yielded positive results, urine GGT and NAG indices were within the reference ranges.

Data excluding outliers were examined on the basis of sex. For male dogs, the mean urine GGT index was 12.61 ± 6.33 U/g, and mean urine NAG index was 1.29 ± 1.01 U/g. Among the neutered male dogs, the GGT and NAG indices ranged from 4.73 to 60.34 U/g and 0.01 to 7.04 U/g, respectively. In the 2 sexually intact male dogs, urine GGT index was 14.28 and 10.23 U/g, respectively, and urine NAG index was 7.15 and 2.35 U/g, respectively. For female dogs, the mean urine GGT index was 14.65 ± 7.91 U/g, and mean urine NAG index was 0.85 ± 0.89 U/g. Among the spayed female dogs, the GGT and NAG indices ranged from 1.93 to 34.81 U/g and 0.03 to 2.31 U/g, respectively. In the 1 sexually intact female dog, urine GGT index was 7.87, and urine NAG index was 0.32 U/g.

Between sexes, urine GGT index values did not differ significantly ($P = 0.967$). However, urine NAG index values were significantly ($P = 0.049$) different between male and female dogs. Given that latter finding, reference ranges for urine NAG index for male and female dogs were calculated. With regard to male dogs, the NAG index reference range was 0.02 to 3.65 U/g; with regard to female dogs, the NAG index reference range was 0.02 to 2.31 U/g.

The pH of the urine has been reported to influence urine GGT and NAG activities.[18] Therefore, the data were analyzed on the basis of urine pH. Among the 38 study dogs, urine pH ranged from 5.0 (7 dogs) to 8.0 (1 dog); values were < 7.0 in 20 dogs and ≥ 7.0 in 18 dogs. The mean urine GGT index was 10.80 ± 1.39 U/g among dogs with urine pH < 7.0 and 19.03 ± 2.90 U/g among dogs with urine pH ≥ 7.0; after accounting for unequal variances, this difference was significant ($P = 0.017$). The mean urine NAG index was 1.72 ± 0.19 U/g among dogs with urine pH < 7.0 and 1.07 ± 0.52 U/g among dogs with urine pH ≥ 7.0; this difference was not significant ($P = 0.258$).

The data were also evaluated on the basis of body surface area (m^2). The urine GGT or NAG indices in dogs that weighed ≤ 25 kg (body surface area ≤ 0.85 m^2) and in dogs that weighed > 25 kg (body surface area > 0.85 m^2) did not differ significantly.

Discussion

In human and veterinary medicine, BUN and sCr concentrations are commonly measured to assess renal function. Excluding nonrenal causes of azotemia, increases in BUN and sCr concentrations occur when approximately 75% of the nephrons become nonfunctional.[19] In dogs and cats, large changes in glomerular filtration rate early in the course of renal disease cause relatively small increases in BUN and sCr concentrations.[19] Activities of renal tubular enzymes in the urine increase prior to detection of increases in BUN and sCr concentrations; thus, assessment of the former is considered more sensitive for identification of early renal tubular injury.[4,10,11] Because these enzymes are located in the brush border of the renal tubules or in renal tubular epithelial lysosomes, they can be useful as markers for localization of the renal tubular injury or necrosis and may be more specific for renal tubular damage.[1,11] The large molecular weights of the urine enzymes prevent their filtration through the glomeruli.[1,20] Studies[2,3,7] in dogs have revealed no correlation between proteinuria and urine enzyme activities. Urine NAG excretion is commonly used as a marker of tubular and glomerular injury in human medicine.[21,22]

In the present study, GGT index in acidic and basic urine samples differed significantly, whereas NAG index values did not. Inactivation of GGT in urine occurs at pH 4.7.[18] Conversely, inactivation of NAG in urine occurs at higher pH values (typically > 8.0).[18,23] Among the 20 dogs in which the urine pH was < 7.0, the lowest pH value was 5.0 (7 dogs). Among the 18 dogs in which the urine pH was ≥ 7.0, the highest pH value was ≥ 8.0 (1 dog). Simultaneous measurement of urine pH should be performed when assessing urine enzyme activities to avoid misinterpretation of urine index values.

In 1 study[5] of urinary excretion of NAG in healthy dogs and dogs with urinary diseases, urine NAG index in 4 dogs with lower urinary tract infections without evidence of pyelonephritis was not increased, compared with findings in healthy dogs, whereas 2 dogs with low-

er urinary tract infections and pyelonephritis did have an increased NAG index, which was suggestive of tubular lesions in those 2 dogs. In a study[2] involving 55 dogs with pyometra, urine enzyme concentrations in the groups of dogs with and without bacteriuria were highly similar. Epithelial cells of the urogenital tract contain a low amount of the enzymes of interest and are unlikely to contribute to detectable increases in urine enzyme activities.[1] In the present study, bacterial culture of urine yielded positive results in 4 dogs. Of these 4 positive results, 2 were suspected to be the result of contamination because of the type of bacterial species cultured and the fact that the 2 dogs from which the samples were collected had no hematuria or pyuria. *Escherichia coli* and β *E coli* were detected in urine from 1 of the 2 dogs, and *E coli* and *Enterococcus* spp were detected in urine from the other. In all 4 dogs, both urine enzyme indices were within the calculated reference ranges. If urine enzyme indices are investigated in a dog, a complete urinalysis is warranted because hematuria or pyuria may influence the urine enzyme-to-creatinine ratio.[1,14] Hematuria was not grossly evident in urine samples collected from any of the dogs in the present study. However, microscopic hematuria (5 to 75 RBCs/hpf) and low numbers of WBCs (< 5 WBCs/hpf) were detected in 6 dogs although bacterial culture of urine from those dogs yielded negative results. The underlying etiology of the microscopic hematuria was not apparent to the authors and was not investigated further. Trace hematuria (≤ 3 RBCs/hpf) was detected in 7 dogs and could have been a result of blood contamination during cystocentesis. The hematuria did not appear to influence the urine enzyme indices because the values were within the calculated reference ranges in all but 1 of the 7 dogs.

Two studies[15,16] in dogs have revealed a significant difference in urine NAG index between sexes. In another study[17] of sexually intact male Beagles, an admixture of secreted fluid from the gonadal system in the urinary bladder resulted in high urine NAG index values. Such high index values were not evident in Beagles after bilateral vasectomy or castration.[17] Although the urine GGT index for male and female dogs in the present study did not differ significantly, urine NAG index values did differ between the sexes. In our study, there were only 2 sexually intact male dogs; in 1 of those dogs, the urine NAG index was greater than the upper limit of the calculated reference range, but the GGT index was not abnormal. Other studies[5,24] in which urine enzyme indices were investigated in dogs revealed no significant differences between males and females.

The clinical relevance of increases in urine enzyme activity in dogs that have minor degrees of renal damage may be difficult to determine because some urine enzymes are associated with wide biological variation.[10,15,25] Analysis of spot urine samples has revealed a correlation between 24-hour urinary GGT excretion and urine GGT-to-creatinine ratio.[14,24,25] Serial measurements of the activity of more than 1 type of enzyme in urine would minimize the effects of variability in urine enzyme excretion.[1,12] Also, serial measurements of urine enzyme activities can be useful for determining resolution of renal injury.[1] The usefulness of urine enzymes as markers with which to monitor chronic renal disease needs further evaluation. Results of 1 study[5] indicated that urine NAG activity was greater in dogs with chronic renal failure, compared with findings in healthy dogs, but results of another study[20] did not corroborate such a difference. Both GGT and NAG are fairly stable in canine urine samples kept at room temperature (approx 20°C) and at 4°C for several days and may be measured in urine supernatant without prior removal of enzyme inhibitors.[15,25,26] Circadian variation in urine GGT and NAG indices was evaluated in 22 adult dogs, and there were no significant differences in the values for the 24-hour urine sample and the values for any of the urine samples collected at 4-hour intervals preceding the 24-hour time point.[24] This suggests that the enzyme activity in a single randomly collected urine sample is an accurate representation of an animal's urine enzyme status.

Because the present study was performed in 1 laboratory, the data must be interpreted in light of certain inherent limitations. Among laboratories, instrumentation, personnel, and assays used vary. Although ideal, it can be difficult to establish reference intervals for each variable in each laboratory. Assays for measurements of urine creatinine concentration and urine GGT activity are available in most commercial laboratories and are not cost prohibitive. Currently, kits for measurement of urine NAG activity are also available through commercial laboratories and are not cost prohibitive but are typically purchased in bulk.

Evaluation of urine enzyme activities could be used for earlier detection of renal tubular damage. As a result of early detection, changes in the clinical management of affected patients could decrease morbidity and mortality rates associated with acute tubular injury and acute tubular necrosis. With the establishment of reference ranges for urine NAG and GGT indices, further studies are needed to determine the sensitivity and specificity of urine enzyme indices for identification of various causes of acute renal tubular damage in dogs.

a. Snap 3Dx test, IDEXX Laboratories Inc, Westbrook, Me.
b. HealthScreen, Heska Corp ERD, Loveland, Colo.
c. Cell-Dyne, model 3500, Abbott Laboratories, Abbott Park, Ill.
d. Vitros 250, Ortho-Clinical Diagnostics, Johnson and Johnson Co, Piscataway, NJ.
e. Bayer, Pittsburgh, Pa.
f. SAS, version 9, SAS Institute Inc, Cary, NC.

References

1. Clemo FAS. Urinary enzyme evaluation of nephrotoxicity in the dog. *Toxicol Pathol* 1998;26:29–32.
2. Heiene R, Moe L, Mølmen G. Calculation of urinary enzyme excretion, with renal structure and function in dogs with pyometra. *Res Vet Sci* 2001;70:129–137.
3. De Schepper J, De Cock I, Capiau E. Urinary γ-glutamyl transferase and the degree of renal dysfunction in 75 bitches with pyometra. *Res Vet Sci* 1989;46:396–400.
4. Rivers BJ, Walter PA, O'Brien TD, et al. Evaluation of urine gamma-glutamyl transpeptidase-to-creatinine ratio as a diagnostic tool in an experimental model of aminoglycoside-induced acute renal failure in the dog. *J Am Anim Hosp Assoc* 1996;32:323–336.
5. Sato R, Soeta S, Miyazaki M, et al. Clinical availability of urinary N-acetyl-β-D-glucosaminidase index in dogs with urinary diseases. *J Vet Med Sci* 2002;64:361–365.
6. Uechi M, Nogami Y, Terui H, et al. Evaluation of urinary enzymes

in dogs with early renal disorder. *J Vet Med Sci* 1994;56:555–556.
7. Palacio J, Liste F, Gascón M. Enzymuria as an index of renal damage in canine leishmaniasis. *Vet Rec* 1997;140:477–480.
8. Narita T, Sato R, Tomizawa N, et al. Safety of reduced-dosage ketoprofen for long-term oral administration in healthy dogs. *Am J Vet Res* 2006;67:1115–1120.
9. Narita T, Tomizawa N, Sato R, et al. Effects of long-term oral administration of ketoprofen in clinically healthy beagle dogs. *J Vet Med Sci* 2005;67:847–853.
10. Greco DS, Turnwald GH, Adams R, et al. Urinary γ-glutamyl transpeptidase activity in dogs with gentamicin-induced nephrotoxicity. *Am J Vet Res* 1985;46:2332–2335.
11. Cowgill LD, Francey T. Acute uremia. In: Ettinger SJ, Feldman EC, eds. *Textbook of veterinary internal medicine*. 6th ed. St Louis: Elsevier Saunders Co, 2005;1731–1751.
12. Loeb WF. The measurement of renal injury. *Toxicol Pathol* 1998;26:26–28.
13. Vaden SL, Levine J, Breitschwerdt EB. A retrospective case-control of acute renal failure in 99 dogs. *J Vet Intern Med* 1997;11:58–64.
14. Grauer GF, Greco DS, Behrend EN, et al. Estimation of quantitative enzymuria in dogs with gentamicin-induced nephrotoxicosis using urine enzyme/creatinine ratios from spot urine samples. *J Vet Intern Med* 1995;9:324–327.
15. Reusch C, Vochezer R, Weschta E. Enzyme activities of urinary alanine aminopeptidase (AAP) and N-acetyl-β-D-glucosaminidase (NAG) in healthy dogs. *Zentralbl Veterinarmed [A]* 1991;38:90–98.
16. Nakamura M, Itoh T, Miyata K, et al. Difference in urinary N-acetyl-β-*D*-glucosaminidase activity between male and female beagle dogs. *Ren Physiol* 1983;6:130–133.
17. Higashiyama N, Nishiyama S, Itoh T, et al. Effect of castration on urinary N-acetyl-β-*D*-glucosaminidase levels in male beagles. *Ren Physiol* 1983;6:226–231.
18. Jung K, Pergande M, Schreiber G, et al. Stability of enzymes in urine at 37°C. *Clin Chim Acta* 1983;131:185–191.
19. DiBartola SP. Renal disease: clinical approach and laboratory evaluation. In: Ettinger SJ, Feldman EC, eds. *Textbook of veterinary internal medicine*. 6th ed. St Louis: Elsevier Saunders Co, 2005;1716–1730.
20. Heiene R, Biewenga WJ, Koeman JP. Urinary alkaline phosphatase and γ-glutamyltransferase as indicators of acute renal damage in dogs. *J Small Anim Pract* 1991;32:521–524.
21. Skálová S. The diagnostic role of urinary N-acetyl-β-D-glucosaminidase (NAG) activity in the detection of renal tubular impairment. *Acta Medica Cordoba* 2005;48:75–80.
22. Bazzi C, Petrini C, Rizza V, et al. Urinary *N*-acetyl-β-glucosaminidase excretion is a marker of tubular cell dysfunction and a predictor of outcome in primary glomerulonephritis. *Nephrol Dial Transplant* 2002;17:1890–1896.
23. Mandić LM, Aćimović JM, Jovanović VB. The possibility of determining *N*-acetyl-β-D-glucosaminidase isoenzymes under alkaline conditions. *Clin Biochem* 2005;38:384–389.
24. Uechi M, Terui H, Nakayama T, et al. Circadian variation of urinary enzymes in the dog. *J Vet Med Sci* 1994;56:849–854.
25. Gossett KA, Turnwald GH, Kearney MT, et al. Evaluation of γ-glutamyl transpeptidase-to-creatinine ratio from spot samples of urine supernatant, as an indicator of urinary enzyme excretion in dogs. *Am J Vet Res* 1987;48:455–457.
26. Matteucci E, Giampietro O. To store urinary enzymes: how and how long? *Kidney Int Suppl* 1994;47:58–59.