# Effect on urine specific gravity of the addition of glucose to urine samples of dogs and cats

**Ellen N. Behrend** VMD, PhD
**Annabel N. Botsford** DVM, MS
**Samuel A. Mueller** BA
**Erik H. Hofmeister** DVM, MA, MS
**Hollie P. Lee**

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From the Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL 36849. Dr. Botsford's present address is Atlantic Veterinary Internal Medicine and Oncology, 10000 Old Columbia Rd, Columbia, MD 21046.

Address correspondence to Dr. Behrend (behreen@auburn.edu).

**OBJECTIVE**
To evaluate effects of the addition of glucose to dog and cat urine on urine specific gravity (USG) and determine whether glucosuria affects assessment of renal concentrating ability.

**SAMPLE**
Urine samples from 102 dogs and 59 cats.

**PROCEDURES**
Urine for each species was pooled to create samples with various USGs. Glucose was added to an aliquot of each USG pool (final concentration, 2,400 mg/dL), and serial dilutions of the glucose-containing aliquot were created for each pool. The USG then was measured in all samples. The difference in USG attributable to addition of glucose was calculated by subtracting the USG of the unaltered sample from the USG of the sample after the addition of glucose. The relationship between the difference in USG and the USG of the unaltered, undiluted sample was evaluated by the use of linear regression analysis.

**RESULTS**
Addition of glucose to urine samples increased the USG. There was a significant relationship between USG of the undiluted sample and the difference in USG when glucose was added to obtain concentrations of 300, 600, 1,200, and 2,400 mg/dL in canine urine and concentrations of 600, 1,200, and 2,400 mg/dL in feline urine. The more concentrated the urine before the addition of glucose, the less change there was in the USG. Changes in USG attributable to addition of glucose were not clinically important.

**CONCLUSIONS AND CLINICAL RELEVANCE**
Substantial glucosuria resulted in minimal alterations in specific gravity of canine and feline urine samples. Thus, USG can be used to assess renal concentrating ability even in samples with glucosuria. (*Am J Vet Res* 2019;80:907–911)

Measurement of urine concentration is an important part of a urinalysis and crucial to differentiation of prerenal and renal azotemia. Generally, osmolality is considered a more exact measurement of urine concentration than is specific gravity. However, because of the ease of use, USG is most commonly used to evaluate renal concentrating ability. Osmolality is a measure of the number of dissolved particles in a fluid. In comparison, specific gravity represents the ratio of the weight of a solution to the weight of an equal volume of water.[1]

Refractometers typically are used in clinical settings for estimation of USG. Use of urine test strips to determine USG is not recommended because of poor reliability.[2–4] For refractometers, weight of a solution is not measured; instead, it is estimated on the basis of refraction of light.[5] The amount of refraction can be affected by solute concentration, chemical composition of the solute, and temperature. A strong linear correlation for human urine exists between urine osmolality and USG as measured by refractometry in samples that do not contain protein or glucose.[1,6]

**ABBREVIATIONS**
USG Urine specific gravity

The presence of glucose in urine has the potential to affect USG measurements. Conditions that commonly cause glucosuria are diabetes mellitus and primary renal glucosuria. The ability to accurately evaluate urine concentrating ability in diabetic patients is extremely important because many have concurrent disorders.[7] Because of the perception that urine glucose causes a false elevation in USG, it has been recommended to interpret USG in relation to the presence and amount of urine glucose.[8] The addition of 1 g of glucose/dL is expected to change the specific gravity of water by 0.003 to 0.005.[1] For example, a canine urine sample with a USG of 1.025 without glucose would have a USG of approximately 1.035 if 2,000 mg of glucose/dL was present, and interpretation of renal concentrating ability would differ substantially between these 2 results.

However, to our knowledge, expected changes in USG attributable to glucose in dog and cat urine have not been evaluated. Glucosuria has no effect on linearity of the relationship between urine osmolality and USG in dogs[9] and humans.[10] Therefore, USG may be an

accurate method for assessment of urine concentrating ability despite the presence of glucosuria. The purpose of the study reported here was to evaluate the effect on USG of the addition of glucose to urine samples of dogs and cats on USG and determine whether glucosuria affected the assessment of renal concentrating ability. We hypothesized that USG measured via refractometry would not be altered by the presence of glucose, and, accordingly, glucosuria would not affect the assessment of renal concentrating ability.

## Materials and Methods

Urine samples from 102 dogs and 59 cats submitted between April and August 2014 and between May 2014 and March 2015, respectively, to the Clinical Pathology Laboratory of the Auburn University Veterinary Teaching Hospital for complete urinalysis were used. Signalment, method of urine collection, and reason for hospital visit were not considered. Because samples used were convenience samples obtained in the course of routine standard clinical care of patients and the urine used was the residual remaining after diagnostic testing was completed, specific client consent and approval by a clinical research review committee or institutional animal care and use committee were not required. Free catch samples were collected by medical personnel using a clean vessel.

Samples that had positive results for glucose on dipstick evaluation; that were visibly red, orange, or brown; or that were identified with > 5 RBCs/hpf or > 5 WBCs/hpf during evaluation of the urine sediment were excluded. Urine was centrifuged at 1,300 × *g* at room temperature (21°C) for 5 minutes. Supernatant was harvested and frozen at –20°C.

Urine supernatants were thawed at 21°C for 2 hours and used to make pools for each species. Supernatants were pooled on the basis of USG to obtain a final volume of ≥ 3 mL for urine pools with a USG within the following ranges: 1.005 to 1.009, 1.010 to 1.014, 1.015 to 1.019, 1.020 to 1.024, 1.025 to 1.029, 1.030 to 1.034, 1.035 to 1.039, 1.040 to 1.044, and 1.045 to 1.049. Only urine that had a USG within the specified range was used in creation of a pool (eg, for the range 1.005 to 1.009, all samples used to make the pool had a USG of 1.005 to 1.009). For canine samples, each range contained 5 urine pools. For feline urine samples, ranges contained 2, 4, 5, 2, 5, 5, 5, 3, and 4 urine pools, respectively. After urine pools were thoroughly mixed, they were refrozen at –20°C until use.

On the day of USG measurement, the pools were thawed at 21°C for 3 hours and allowed to equilibrate to room temperature. A stock solution of D-glucose[a] in water (100 g/L) was added to an aliquot of each urine pool to achieve a final glucose concentration of 2,400 mg/dL. The glucose-altered samples subsequently were serially diluted with the remainder of the urine for each pool to obtain final glucose concentrations of 1,200, 600, 300, 150, and 50 mg/dL for each USG. The USG of the altered samples as well as an unaltered sample was measured by use of an optical refractometer.[b] Calibration of the refractometer was confirmed by measurement of the specific gravity of distilled water. To minimize variation, all dog samples were evaluated by 1 investigator (SAM), and all cat samples were evaluated by 2 investigators (ANB and SAM). The same refractometer was used for all measurements.

Three pools of dog urine were randomly selected (numbers were drawn out of a hat) for verification of glucose concentration. Glucose concentration was determined in the glucose-altered samples (6 samples/pool; total, 18 samples) by use of an automated clinical chemistry analyzer[c] with the hexokinase method[d]; that concentration was considered to be the actual glucose concentration (criterion-referenced standard). Upper limit of quantification for glucose concentration with the clinical chemistry analyzer was 750 mg/dL. Samples with expected glucose concentrations of 1,200 and 2,400 mg/dL were diluted 1:10 and then analyzed to determine the actual glucose concentration.

### Statistical analysis

Statistical analysis was performed with a commercial program.[e] Normality of data distributions was determined by examination of histograms. The difference in USG attributable to the addition of glucose was calculated by subtracting the USG of the unaltered sample from the USG after the addition of glucose. The relationship between the difference in USG and the USG of the unaltered, undiluted sample was tested by use of linear regression analysis for each concentration of glucose. Multivariate linear regression analysis was performed with measured USG as the dependent variable and USG of the original unaltered sample and glucose concentration of the sample as independent variables. Significance was set at values of $P < 0.05$.

## Results

The altered glucose concentration was verified by use of the biochemical chemistry analyzer in 18 samples. Mean ± SD percentage difference from the expected concentration was –0.39 ± 0.14%.

Overall, the addition of glucose caused the USG to increase. There was a significant relationship between the USG of the undiluted sample and the difference in USG when glucose was added to canine urine for glucose concentrations of 300, 600, 1,200, and 2,400 mg/dL **(Figure 1)**. A significant relationship between the USG of the undiluted sample and the difference in USG when glucose was added was found in feline urine for glucose concentrations of 600, 1,200, and 2,400 mg/dL **(Figure 2)**. Multivariate analysis revealed that the USG of both the original unaltered samples and glucose concentration of the sample were significant ($P < 0.001$) predictors for measured a USG. The more concentrated the urine was before glucose was added, the less change there was in the a USG.

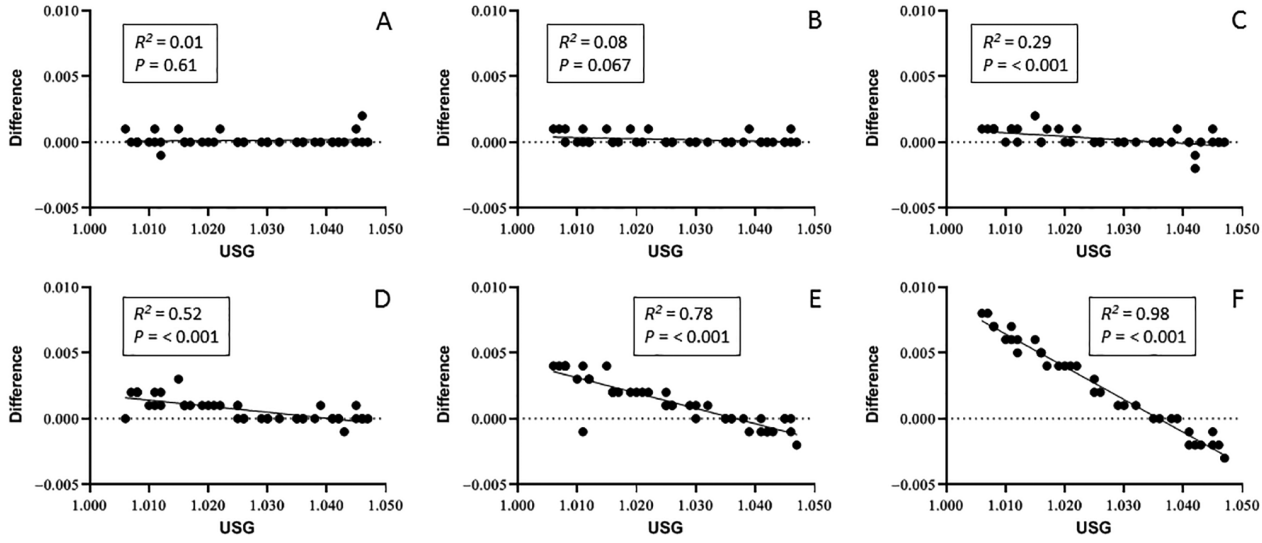


**Figure 1**—Graphs of the relationship between the USG of unaltered pooled dog urine and the difference in USG attributable to the addition of glucose to create urine samples with glucose concentrations of 50 (A), 150 (B), 300 (C), 600 (D), 1,200 (E), and 2,400 (F) mg/dL. Each circle represents results for a single sample, and the line represents the linear regression line. The line of values with no difference is indicated (dotted line). A relationship was considered significant at a value of $P < 0.05$ as determined by use of linear regression analysis.

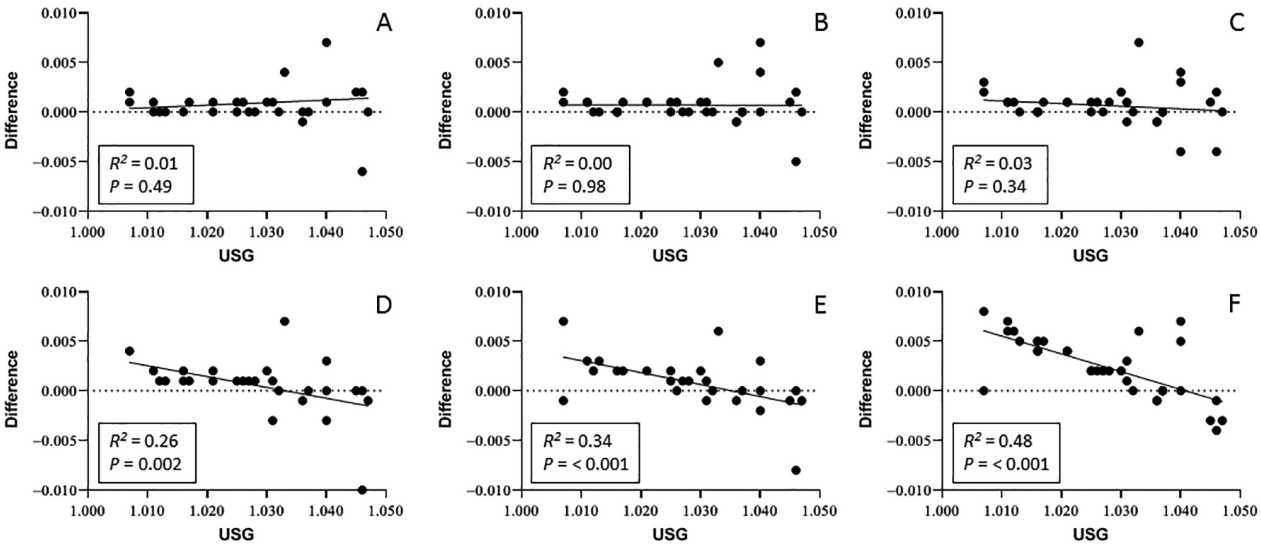


**Figure 2**—Graphs of the relationship between the USG of unaltered pooled cat urine and the difference in USG attributable to the addition of glucose to create urine samples with glucose concentrations of 50 (A), 150 (B), 300 (C), 600 (D), 1,200 (E), and 2,400 (F) mg/dL. ***See*** Figure 1 for remainder of key.

The USG was graphed as a function of glucose concentration for each pool **(Figure 3)**. Graphs were then examined to assess whether glucosuria in dogs or cats would affect the determination made on the basis of USG as to whether azotemia was prerenal or postrenal. A USG ≥ 1.030 can be used to distinguish prerenal and postrenal azotemia in dogs,[11] and a USG ≥ 1.040 can be used to distinguish prerenal and postrenal azotemia in cats.[11] Addition of glucose did not cause USG < 1.030 to increase to > 1.030 in dog urine and USG < 1.040 to increase to > 1.040 in cat urine.

## Discussion

Urine specific gravity is an important measure of urine concentrating ability. Despite the widespread common belief that glucosuria substantially alters USG, results of the present study indicated that the presence of glucosuria in samples from dogs and cats did not prevent accurate assessment of urine concentrating ability. To the authors' knowledge, this was the first study conducted to directly assess the effect of glucosuria on USG of samples from dogs and cats.

The change in USG caused by the presence of glucose was dependent on both the USG without

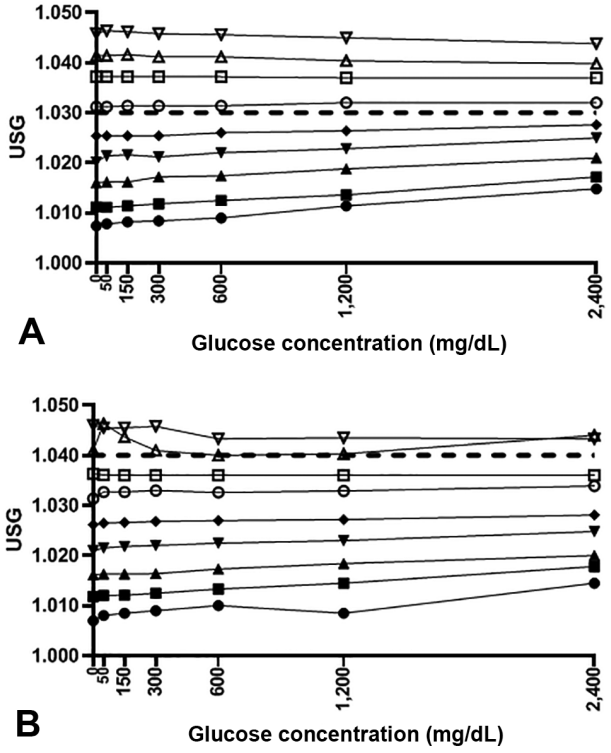


**Figure 3**—Graphs of the relationship between the glucose concentration and mean USG for pooled dog (A) and cat (B) urine samples with various USGs. Urine within a specified USG range was pooled (5 pools/range for dogs and 2 to 5 pools/range for cats). Pools for each species were created for USG within the range of 1.005 to 1.009 (black circles), 1.010 to 1.014 (black squares), 1.015 to 1.019 (black triangles), 1.020 to 1.024 (inverted black triangles), 1.025 to 1.029 (black diamonds), 1.030 to 1.034 (white circles), 1.035 to 1.039 (white squares), 1.040 to 1.044 (white triangles), and 1.045 to 1.049 (inverted white triangles). Each symbol represents mean results for the unaltered, undiluted urine pool and the pooled urine with various concentrations of glucose (50, 150, 300, 600, 1,200, and 2,400 mg/dL). The USG that distinguishes prerenal and renal azotemia in dogs (USG = 1.030) and cats (USG = 1.040) is indicated for each species (dashed line).

glucose and the amount of glucose. Glucose caused larger increases in USG in both more dilute urine and in urine to which more glucose was added (Figures 1 and 2). The effect was greater in dog urine than in cat urine. The addition of glucose to urine significantly increased the USG for higher concentrations of glucose. However, the differences were small and would not be clinically important (ie, the greatest difference in USG was only 0.008 and occurred with addition of 2,400 mg/dL glucose to the urine sample with the lowest USG [approx 1.005]). The higher the initial USG, the smaller the difference attributable to the addition of glucose.

Many patients with diabetes mellitus have concurrent disorders, and up to 22% and 27% of these patients have increases in BUN and creatinine concentrations, respectively.[7] Urine specific gravity is an inexpensive and readily available means for the assessment of urine concentrating ability and differentiation of prerenal and renal azotemia. On the basis of results for the study reported here, USG can be used in the same manner to assess renal concentrating ability, regardless of whether glucose is present in a urine sample. However, it must be remembered that USG must always be interpreted in light of an animal's hydration status, electrolyte concentrations, and medication history, among other factors.

Interestingly, the effect of glucose appeared to be greater in dog urine. Regression coefficients for dog and cat urine were not compared because of low power of the test. The reason for the difference between species may have been related to the higher refractivity of cat urine.[12] Refractometers do not measure USG directly; instead, they measure the angle of light refraction as light passes between an aqueous solution and air. Feline urine may contain a substance that interferes with light passage.[13]

The study reported here had some limitations. First, USG was used as a measure of urine concentration, rather than use of the criterion-referenced standard of freezing point osmometry. However, it has been reported that glucosuria has no effect on the correlation between USG as measured by use of refractometry and osmolality in dogs[9] and humans.[10] Second, sample contents were not standardized. To eliminate the potential effects of blood,[14] only samples that were yellow were used. Although urine samples were centrifuged to remove potential effects attributable to urine sediment on results, samples were not excluded on the basis of the presence of ketones, protein, bilirubin, or hemoglobin. However, the correlation between USG measured by use of refractometry and osmolality in dog urine is unaffected by proteinuria, bilirubinuria, and hemoglobinuria, and the effect of ketones on the correlation is considered to be clinically unimportant.[9] Third, only a single refractometer was used; differences can exist among refractometers.[13,15,16] When albumin, sodium chloride, and glucose are added to water, the magnitude of their effect depends on the refractometer used for measurements.[15] In studies[13,16] that involved the use of clinical samples, the difference in measurements obtained with 2 refractometers was considered to be clinically unimportant. Thus, we think it unlikely that the effect of glucose on USG would differ among refractometers. Finally, 2 investigators measured USG in cats; thus, interobserver variability for refractometer measurements may have influenced the results.

For the study reported here, a significant difference in USG was detected when glucose was added to urine and resulted in concentrations of 300, 600, 1,200, and 2,400 mg/dL in dog urine and concentrations of 600, 1,200, and 2,400 mg/dL in cat urine. However, these changes were not likely to be of clinical importance. The more concentrated the urine was before the addition of glucose, the less change there was in the USG. Thus, clinicians should evaluate USG and renal concentrating ability in the same manner, regardless of the glucose con-

centration in urine samples. In addition, USG must always be interpreted in light of the hydration status, electrolyte concentrations, and medication history of each patient.

## Acknowledgments

The authors declare that there were no conflicts of interest.

## Footnotes

a. Thermo Fisher Scientific, Waltham, Mass.
b. TS-400, Reichert Technologies, Buffalo, NY.
c. Hitachi 911, Boehringer Mannheim, Indianapolis, Ind.
d. Roche/Hitachi reagents, Roche Diagnostics, Indianapolis, Ind.
e. GraphPad Prism, version 8, GraphPad Software Inc, San Diego, Calif.

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