Received: 22 May 2020 | Accepted: 19 October 2020

DOI: 10.1111/jvim.15952

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Prediction and measurement of diuretic responsiveness after oral administration of furosemide to healthy dogs and dogs with congestive heart failure

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Abstract

Background: In human patients, cumulative urine volume (uVol) and urine sodium (uNa) can be predicted using spot urine samples and these quantitative measures help detect low diuretic responsiveness (LDR).

Hypothesis/objectives: Formulas using spot urine samples predict cumulative uVol and uNa output after oral administration of furosemide to dogs.

Animals: Eight healthy dogs, 6 dogs with congestive heart failure (CHF).

Methods: Prospective interventional study. Spot urine samples at 180 and 270 minutes after furosemide (3 mg/kg PO) were used to predict cumulative uVol and uNa output over 7 hours. Differentiation of dogs fulfilling predefined criteria for LDR was examined using receiver operating characteristic (ROC) curves.

Results: Predicted uNa output at 180 minutes (r_s = 0.763, [95% confidence interval [CI], 0.375-0.923], P = .002) and 270 minutes (r = 0.816, [95% CI, 0.503-0.940], P < .001) was highly correlated to 7-hour uNa output. Predicted uVol at 180 minutes (r = 0.598, [95% CI, 0.098-0.857], P = .02) and 270 minutes (r = 0.791, [95% CI, 0.450-0.931], P < .001) was moderately correlated to 7-hour uVol. Predicted uNa using 180-minute (area under the curve [AUC], 0.933 [95% CI, 0.804-1.000]) and 270-minute (AUC, 0.911 [95% CI, 0.756-1.000]) samples identified dogs with LDR (n = 5) with high accuracy.

Conclusions and Clinical Importance: Urinary Na excretion and uVol are complementary but distinct aspects of diuretic responsiveness in dogs. Quantification of diuretic

Abbreviations: AUC, area under the curve; BUN, blood urea nitrogen; CHF, congestive heart failure; LDR, low diuretic responsiveness; EFWC, electrolyte free water clearance; eGFR, estimated glomerular filtration rate; Na, sodium; RAAS, renin angiotensin aldosterone system; ROC, receiver operating characteristic; [sNa], serum sodium concentration; [sCl], serum chloride concentration; [sCr], serum creatinine concentration; uNa, urine sodium; [uNa], urine sodium concentration; [uCr], urine creatinine concentration; [uK], urine potassium concentration; uVol, urine volume.

Kerry A. Loughran and Éva Larouche-Lebel contributed equally to this study.

responsiveness in the clinical setting opens new diagnostic, treatment, and monitoring strategies.

KEYWORDS
urine sodium, natriuresis, diuresis, urine volume

1 | INTRODUCTION

Congestive heart failure (CHF) stems from a series of hemodynamic, neurohormonal, and cardiorenal responses that promote retention of water and sodium (Na) in dogs and humans.[1] Increased total body water primarily accumulates in extracellular spaces, the bulk of which is interstitial with a lesser amount as plasma volume.[2] Total body Na content is increased secondary to activation of the renin angiotensin aldosterone system (RAAS) and increased Na avidity in the kidney. Sodium is particularly critical in the development of congestion as it represents the primary solute that regulates extracellular water balance.[3,4]

In both dogs and humans with CHF, diuretics, such as furosemide, are prescribed to counteract congestion and alleviate clinical signs.[5,6] In both species, dosing is guided by clinical tenets of "maintaining patient comfort"[6] and "relieving symptoms and reducing volume excess,"[7] however, there are substantial limitations to this subjective approach, and quantification of diuretic effect, termed diuretic responsiveness or efficiency, is the subject of considerable recent study in humans.[8-12] Measures of diuretic responsiveness include urine volume (uVol), net fluid loss, weight loss, fractional excretion of Na, and urinary sodium (uNa) excretion.[9] In purpose-bred dogs, diuretic responsiveness in the form of uVol per mg furosemide has been examined,[13-16] but data in dogs with spontaneous heart disease after oral administration of furosemide are lacking.

The ability to identify dogs with low diuretic responsiveness (LDR) would be helpful. Clinicians long have recognized that as CHF progresses, there often remains "persistent congestion despite adequate decongestive treatment" and this so-called diuretic resistance is associated with increased mortality and hospitalization.[9] Between 6 and 25% of dogs with severe chronic CHF had LDR according to various criteria adapted from human medicine.[17]

Measuring diuretic responsiveness is challenging. Cumulative uVol and uNa output over multiple hours after diuretic dosing have been assiduously collected and measured in humans[18-21] and experimental dogs[13,14] but is not practicable in most clinical settings. Instead, prediction of total uNa output and uVol using samples taken at a prescribed timepoint is an attractive alternative. In healthy dogs receiving constant rate infusion of furosemide, urinary Na concentration ([uNa]) to urinary potassium concentration ([uK]) ratio ([uNa]:[uK]) strongly correlated with cumulative 5-hour urine output.[13] In humans, a prediction equation based on renal physiological principles using spot [uNa], urine creatinine concentration ([uCr]), and serum creatinine concentration ([sCr]) taken 1 to 2 hours after administration of oral bumetanide was strongly correlated with 6-hour uNa output ($r = 0.91$, $P < .0001$) and accurately predicted poor natriuretic response.[18] In our study, we hypothesized that a similar approach could predict cumulative uNa output and uVol over the 7 hours after oral furosemide dosing in dogs and that these metrics could identify dogs with LDR. We put particular emphasis on uNa excretion because of the primacy of Na on extracellular water balance.[3] Our study aimed to correlate observed and predicted 7-hour uNa output and uVol using a modification of a previously developed prediction equation[18] in healthy and CHF dogs after oral administration of furosemide, as well as to explore detection of LDR using the predicted values.

2 | MATERIALS AND METHODS

A prospective trial was designed. The study protocol was approved by the University of Pennsylvania institutional animal care and use review committee and informed owner consent was obtained. Two cohorts of dogs were recruited from clients and staff of the Veterinary Hospital of the University of Pennsylvania, including healthy dogs without previous or current history of heart or systemic disease, murmur, or receipt of cardiac drugs, and a cohort of dogs with heart disease receiving ≥3 mg/kg/d oral administration of furosemide for treatment of chronic CHF. The study involved hospital admission and administration of furosemide (3mg/kg PO) followed by measurement of uVol at 180, 270, and 420 minutes after dosing and spot sampling of serum and urine at baseline within 30 minutes prior to diuretic dosing, and at 180 and 270 minutes after dosing. The 180 and 270-minute timepoints were established in a pilot study of 4 dogs as the best timepoints to predict the maximal rate of urine production (data not shown). In the current study, the dogs were taken outside at each timepoint and all voided urine was collected using a variety of containers, cups, and receptacles in order to eliminate or reduce any spillage or missed urine. If the dog did not voluntarily void or had urinated prior to the timepoint, cystocentesis was performed and 5 to 10 mL of urine was obtained to measure the timepoint-specific urine electrolytes. At each timepoint, ultrasound of the bladder was performed with dogs in lateral recumbency and bladder volume was calculated before and after voluntary voiding as mL of urine = $0.2 \times \pi \times L \times W \times (H_1 + H_2)/2$ where L = sagittal length, W = transverse width, H_1 = sagittal height, and H_2 = transverse height.[22,23] The uVol at each timepoint was calculated as the total volume of urine obtained by free catch and cystocentesis. If the dog urinated in the cage prior to the specific timepoint, the cage bedding was weighed, and for each gram greater than the dry weight of the bedding, 1 mL of urine was added to the timepoint's total. Water was provided ad libidum during the

study and water consumption recorded. Total uVol over the 7-hour study period (mL/kg/7 h) was calculated as the sum of uVol at 180, 270, and 420 minutes, minus the bladder volume by ultrasound at baseline and added to the bladder volume by ultrasound remaining at 420 minutes, divided by body weight. Total uNa output (mEq/kg/7 h) was calculated as the sum of uVol × [uNa] at each timepoint and divided by body weight. Electrolyte free water clearance (EFWC), a unitless value that represents renal free water excretion, was calculated as uVol × (1 − [[uNa] + [uK]/[sNa]]).[24] An EFWC value of 0 indicates production of urine that is isotonic to plasma, EFWC values >0 indicate increasingly solute-poor urine and EFWC values <0 indicate increasingly solute-rich urine. After the 7-hour study period, dogs were discharged, and if receiving furosemide for CHF, the evening dose was adjusted to achieve the regularly administered daily dose of furosemide. The total volume of water consumed by each dog over the study duration was measured. Net water and net Na balance were calculated as water intake minus cumulative uVol and dietary Na intake minus cumulative uNa output, respectively, whereby values <0 represented a net water or Na loss and values >0 indicated a net water or Na gain.

At baseline, 180 and 270-minute timepoints, up to 4 mL of blood was obtained by venipuncture and [sCr] and serum concentrations of blood urea nitrogen (BUN), Na ([sNa]), chloride ([sCl]), and potassium ([sK]) were measured by the in-house hospital clinical laboratory. Urine creatinine concentration as well as [uNa] and [uK] were measured at each timepoint. The ratio of [uNa]:[uK], which is a surrogate for mineralocorticoid-driven distal tubular Na retention in presence of furosemide,[13] was calculated. On the morning of the study, Doppler blood pressure (Model 811-B, Parks Medical, Aloha, Oregon), PCV, baseline urine and serum variables, and bladder volume were measured within 30 minutes prior to furosemide administration. The estimated glomerular filtration rate (eGFR) (mL/min/kg) was calculated as 2.6 × 1/[sCr].[25] The time of morning feeding and administration of any cardiac drugs other than furosemide, was recorded, and the type and amount of diet was used to calculate the morning dietary Na intake.

2.1 | Prediction of cumulative 7-hour uNa output and uVol

The 7-hour uNa output and uVol were predicted by modifying an equation that was developed to quantify 6-hour uVol and uNa output in humans with CHF after IV loop diuretic administration.[18] The equation was based on well-established basic renal physiological principles, namely that uVol is equal to GFR adjusted for the extent of urine concentration or dilution in the tubules.[26] Creatinine undergoes little reabsorption or secretion in the tubules, both in health and in CHF, and the ratio of sCr to uCr reflects the degree of tubular concentration or dilution. Thus, instantaneous rate of uVol formation can be derived as the product of GFR and the [sCr]:[uCr] ratio. The rate of uVol formation can be further multiplied by [uNa] to calculate the rate of uNa excretion. Cumulative uNa output is calculated by multiplying this rate with a time constant. In humans, a time constant of 3.25 hours and spot urine collected 1 to 2 hours after diuretic administration accurately predicted uNa output over a 6-hour collection period.[18] Estimated GFR in humans is calculated based on [sCr], sex, age, and race,[27] and along with body surface area (BSA) and spot urine sample, total 6-hour uNa output (mEq/subject) was predicted using Equation (1). Additional information regarding the specific units present during derivation of the equations is presented as Supplemental Information.

$$\text{Predicted uNa output}\,(\text{mEq}) = \text{eGFR} \times \left(\frac{\text{BSA}}{1.73}\right) \times \left(\frac{[\text{sCr}]}{[\text{uCr}]}\right) \times 60\frac{\text{min}}{\text{h}} \times 3.25\,\text{h} \times \left(\frac{[\text{uNa}]}{1000\,\text{mL/L}}\right) \quad (1)$$

In dogs,[25] GFR can be estimated using [sCr] (i.e., $\text{eGFR}\left(\frac{\text{ml}}{\text{min}}{\text{kg}}\right) = \frac{1}{[\text{sCr}]} \times 2.6$) which reduces the predicted uNa output equation to either of the following, the only difference being whether or not the constant is reduced to its simplest form:

$$\text{Predicted uNa output}\,(\text{mEq/kg}) = \frac{1}{[\text{uCr}]} \times 2.6 \times 60 \times 3.25 \times \left(\frac{[\text{uNa}]}{1000}\right) \quad (2)$$

$$\text{Predicted uNa output}\,(\text{mEq/kg}) = \frac{[\text{uNa}]}{[\text{uCr}]} \times 0.507 \quad (3)$$

Similarly, predicted uVol in dogs can be calculated as either of 2 simplified equations listed below, again the only difference being whether or not the constant is reduced to its simplest form:

$$\text{Predicted uVol}\,(\text{mL/kg}) = \frac{1}{[\text{uCr}]} \times 2.6 \times 60 \times 3.25 \quad (4)$$

$$\text{Predicted uVol}\,(\text{mL/kg}) = \frac{1}{[\text{uCr}]} \times 507 \quad (5)$$

Using these a priori calculations, we hypothesized that significant correlation would exist between observed and predicted uNa and uVol in dogs. The physiologic principles of urine formation are the same in the presence or absence of disease,[18] and we included both healthy and CHF dogs in our study in order to validate the equation over a wide range of uNa and uVol values. In addition, we also to sought to explore potential metrics for LDR. Accordingly, 5 different definitions of LDR, adapted from guidelines in humans[7,10,28-30] and studies in experimental dogs,[16] were constructed (Table 1). The number of criteria fulfilled by each dog was tabulated, and we arbitrarily defined LDR as dogs fulfilling ≥3 criteria. Based on this definition, we further hypothesized that predicted uNa and uVol would discriminate dogs with and without suspected LDR with high sensitivity and specificity. Pilot work in healthy dogs performed by the investigators revealed unexpectedly low uVol after furosemide dosing in some dogs (unpublished data), and we hypothesized that stress, reluctance to

TABLE 1 Five a priori criteria for low diuretic responsiveness in study dogs administered oral furosemide based on modifications of criteria or data in humans and purpose-bred dogs

Human or experimental criterion	Modified study criterion
1. Spot [uNa] <50-70 mEq/L in first 2 hours after diuretic administration[10]	1. Spot [uNa] <60 mEq/L in first 3 hours after diuretic administration
2. Spot [uNa]:[uK] ratio <1 during first 6 hours after diuretic administration[29]	2. Spot [uNa]:[uK] ratio <1 during first 7 hours after diuretic administration
3. uVol <100-150 mL/person/h (~1.5 mLs/kg/h) over any hour during the first 6 hours after diuretic administration[10]	3. uVol <1.5 mL/kg/h over any hour during first 7 hours after diuretic administration
4. Purpose-bred dogs had uNa output of 2-3.6 mEq/kg/24 h following furosemide 2 mg/kg PO qd[16]	4. Total uNa output/kg <1.0 mEq/kg over first 7 hours after diuretic administration
5. Successful decongestion involves net fluid loss[7,30,31]	5. Net positive water gain over first 7 hours after diuretic administration

Abbreviations: uNa, urinary sodium; [uNa], urinary sodium concentration; [uNa]:[uK], urinary sodium to urinary potassium concentration ratio; uVol, urine volume.

drink water, and sympathetic nervous system and RAAS activation while in-hospital could temporarily induce LDR[31] and that a wide range of uNa outputs and uVol might exist in both healthy and CHF dogs undergoing the study protocol.

2.2 | Statistical methods

Descriptive data were assessed for normality using Kologorov-Smirnov tests and reported as mean (SD) for parametric data or median (range) for nonparametric data. Baseline differences between healthy and CHF dogs were tabulated and compared using *t* tests, Mann-Whitney *U*, and Fisher's exact tests. Differences of longitudinal data across study timepoints were assessed using 1-way analysis of variance or Friedman's tests followed by Holm-Sidak's or Dunn's multiple comparison tests, respectively. Correlation between observed and predicted uNa using Equations (2) and (3) and observed and predicted uVol using Equations (4) and (5) was performed by calculation of Pearson's *r* or Spearman's r_s. Strength of correlation was described as very high, high, moderate, low, and negligible for *r* or r_s values of 0.9-1.0, 0.7-0.89, 0.5-0.69, 0.3-0.49, and 0-0.29, respectively.[32] The suitability of the a priori selection of equation constants was tested by post hoc calculation of constants that minimized the sum of squares between the observed and predicted uNa and uCr. Receiver operating characteristic (ROC) curves and their areas under the curve (AUC) were used to determine the sensitivity and specificity of the prediction equations to identify suspected DR dogs. Univariable linear or logistic regression was performed to explore the effect of dietary Na, presence or absence of heart disease, age, body weight, blood pressure, PCV, and baseline [sCr], BUN, [sNa], [sCl], and [sK] on total uNa output. Because of the small number of dogs, multivariable regression was not performed. Box and whisker plots display the median value (line), IQR (box), and 1.5 times the IQR above and below the 75th and 25th percentile values, respectively (whiskers). Statistical calculations were performed using software (Excel for Mac v16.28, Microsoft Corp., Redmond, Washington; Prism 8.3.0, GraphPad Software, La Jolla, California). Statistical significance was $P < .05$.

3 | RESULTS

3.1 | Study cohort

Fourteen dogs, including 8 healthy and 6 CHF dogs, were recruited. Baseline data, including comparisons between healthy and CHF dogs, is shown in Table 2. Dog breeds included 6 mixed breed, 2 Labrador Retrievers, 2 Cavalier King Charles Spaniels, and 1 Golden Retriever, American Pit Bull Terrier, Shiba Inu, and Boxer. Four dogs had degenerative mitral valve disease and 2 dogs had dilated cardiomyopathy.

3.2 | Furosemide natriuresis and diuresis

Furosemide induced natriuresis and diuresis in a time-dependent fashion in the study cohort (Figure 1). Hourly rate of uNa excretion was greatest from 0 to 180 minutes as compared to 180 to 270 minutes and 270 to 420 minutes (Figure 1A). Hourly uVol was greatest from 180 to 270 minutes as compared to 0 to 180 minutes and 270 to 420 minutes (Figure 1B). Over the 7-hour study duration, average total uNa output was 2.63 mEq/kg (SD, 1.44 mEq/kg) and average total uVol was 23.5 mL/kg (SD, 9.68 mL/kg). There was a significant and moderate linear correlation between observed uNa output versus observed uVol ($r = 0.643$, [95% confidence interval [CI], 0.170-0.875], $P = .01$) (Figure 2). Three CHF dogs noticeably departed from the relationship and were characterized as excreting relatively large cumulative uVol with low uNa output. There were no significant differences in 7-hour uNa output or uVol between healthy and CHF dogs (Figure 3). The eGFR was not significantly different between timepoints (baseline: 2.51 mL/min/kg [SD, 0.69 mL/min/kg]; 180 minutes: 2.40 mL/min/kg [SD, 0.62 mL/min/kg]; 270 minutes: 2.43 mL/min/kg [SD, 0.65 mL/min/kg], $P = .07$). Baseline [sCr] ($r = -0.59$, $P = .02$) was moderately correlated with cumulative uNa output. The remainder of the baseline characteristics were not significantly associated with total uNa output, including, BUN ($r = 0.52$, $P = .06$), presence or absence of CHF ($r = 0.30$, $P = .29$), age ($r = 0.37$, $P = .19$), body weight ($r = 0.17$, $P = .54$), blood pressure ($r = 0.14$, $P = .60$), [sNa] ($r = 0.17$,

TABLE 2 Characteristics of the study population

	Healthy (n = 8)	Heart disease (n = 6)	*P*
Age (years)	6.3 (2.5)	9.5 (1.7)	.02
Sex (M/F)	4/4	6/0	.08
Body weight (kg)	17.3 (6.0)	22.9 (15.7)	.41
Breeds	Mixed, n = 4	Mixed, n = 2	
	Pitbull Terrier	CKCS, n = 2	
	Shiba Inu	Boxer	
	Labrador Retriever	Labrador Retriever	
	Golden Retriever		
BCS	5 (5-5)	5.5 (5-6.25)	.06
Blood pressure (mm Hg)	136 (16)	121 (16)	.12
Dietary Na of morning meal (mg/kg)	15.3 (12.8-21.8)	10.0 (0-57.2)	.14
Time from meal to furosemide administration (mins)	130 (56)	105 (53)	.45
Baseline laboratory value			
PCV (%)	49 (4)	45 (5)	.16
BUN (mg/dL)	16 (3)	23 (8)	.04
Creatinine (mg/dL)	1.0 (0.2)	1.2 (0.4)	.22
eGFR (mLs/min/kg)	2.64 (0.59)	2.32 (0.83)	.41
Serum Na (mEq/L)	145 (2)	143 (2)	.20
Serum Cl (mEq/L)	115 (2)	108 (2)	<.0001
Serum K (mEq/L)	4.3 (0.3)	4.6 (0.4)	.10
USG	n = 6, 1.037 (1.016-1.040)	1.013 (1.006-1.025)	.009
Urine Na (mEq/L)	n = 6, 75 (79)	30 (25)	.22
Urine K (mEq/L)	n = 6, 117 (69-261)	37 (20-117)	.02
Urine creatinine (mg/dL)	n = 6, 248 (104-343)	47 (43-174)	.004
Cardiac medications			
Furosemide (mg/kg/d)	NA	n = 6, 3.7 (1.0)	
ACEI (mg/kg/d)			
Enalapril	NA	n = 5, 0.79 (0.21)	
Benazepril	NA	n = 1, 0.38	
Spironolactone (mg/kg/d)	NA	n = 6, 2.1 (0.8)	
Pimobendan (mg/kg/d)	NA	n = 6, 0.60 (0.09)	

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; BCS, body condition score; BUN, blood urea nitrogen; Cl, chloride; eGFR, estimated glomerular filtration rate; F, female; K, potassium; M, male; Na, sodium; NA, not applicable; USG, urine specific gravity.

P = .57), [sCl] (*r* = 0.15, *P* = .63), [sK] (*r* = 0.04, *P* = .87), and PCV (*r* = 0.24, *P* = .38).

3.3 | Prediction of cumulative uNa output and uVol using spot urine samples

Predicted 7-hour uNa output using spot samples at 180 minutes (r_s = 0.763, [95% CI, 0.375-0.923], *P* = .002) and 270 minutes (*r* = 0.816, [95% CI, 0.503-0.940], *P* < .001) were both significantly and highly correlated to observed uNa output (Figure 4). Predicted uVol using spot samples at 180 minutes (*r* = 0.598, [95% CI, 0.098-0.857], *P* = .02) and 270 minutes (*r* = 0.791, [95% CI, 0.450-0.931], *P* < .001) were significantly and moderately correlated to observed total uVol (Figure 5).

3.4 | Prediction of diuretic responsiveness

Five of 14 (36%) dogs met ≥3 criteria for LDR, including 2 healthy dogs and 3 CHF dogs. The numbers of dogs fulfilling each of the 5 LDR criteria are presented in Table 3.

Spot [uNa] at 180 minutes in the 5 suspected DR dogs was 61 mEq/L (SD, 38 mEq/L) and was significantly lower compared to that of the remaining 9 dogs (153 mEq/L [SD, 32 mEq/L], *P* < .001). The [uNa]:[uK] ratio was highly and significantly correlated with uVol

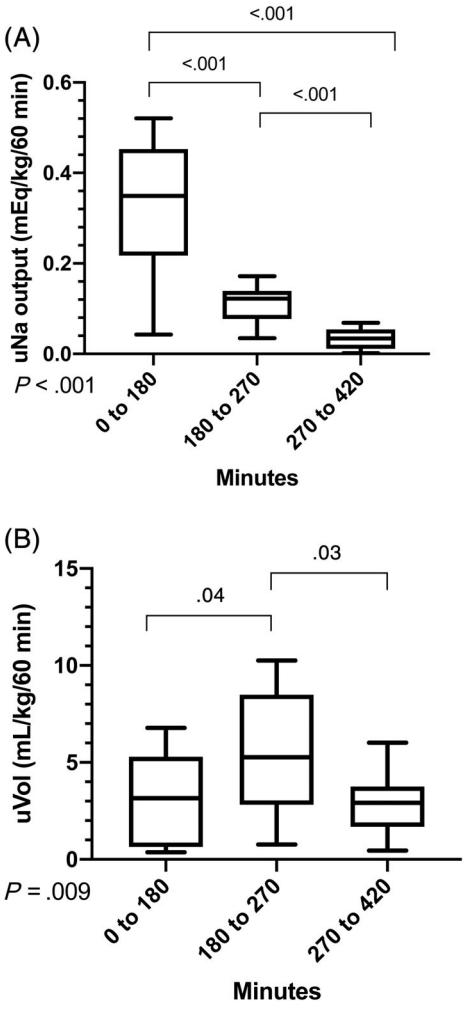

FIGURE 1 Hourly rate of A, urinary Na excretion (uNa) and B, urine volume (uVol) in 14 dogs after oral administration of furosemide (3 mg/kg)

(180 minutes: $r = 0.731$, $P = .003$; 270 minutes: $r = 0.747$, $P = .002$) and [uNa]:[uK] was lower in dogs with LDR (1.49 [SD, 0.70]) versus non-LDR dogs (3.36 [SD, 0.80], $P < .001$) over the 7-hour study duration. The uNa output of LDR dogs (0.99 mEq/kg [SD, 0.62 mEq/kg]) was significantly lower than non-LDR dogs (3.54 mEq/kg [SD, 0.74 mEq/kg], $P < .001$) (Figure 6A). The uVol in LDR dogs (17.5 mL/kg, [SD, 11.1 mL/kg]) was lower than non-LDR dogs (26.8 mL/kg [SD, 7.4 mL/kg]) but this difference was not significantly different ($P = .08$) (Figure 6B). Dogs with LDR excreted more free water as evidenced by significantly greater EFWC at 180 minutes (LDR dogs, 103.2 [SD, 174.3] versus non-LDR dogs, −58.0 [SD, 42.9], $P = .02$) and 270 minutes (LDR dogs, 70.3 [SD, 108.6] versus non-LDR dogs, −33.8 [SD, 28.5], $P = .02$). Net fluid balance was significantly and negatively correlated to uNa such that dogs with net fluid gain excreted less uNa ($r_s = -0.710$, $P = .006$) (Figure 7). Dogs drank an average of 21 mL water/kg (range, 1.8-66 mL/kg) during the study duration. Dogs with LDR experienced a net positive median water gain (9.0 mL/kg [range, −2.5 to 53.7 mL/kg]) versus remaining dogs

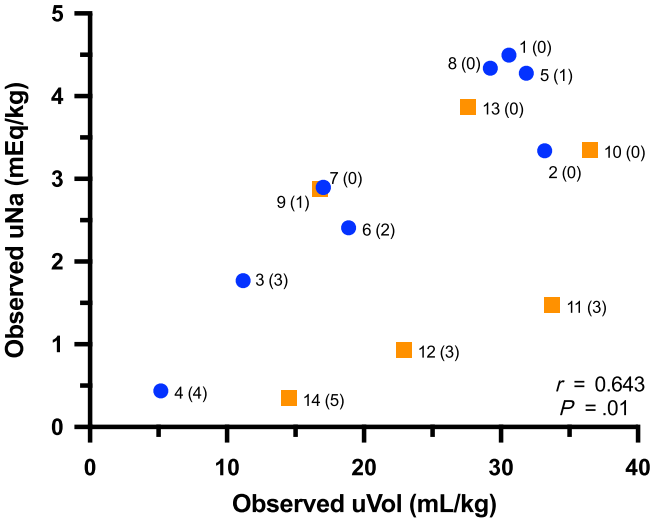

FIGURE 2 Observed cumulative urine volume (uVol) versus observed cumulative urinary Na (uNa) excretion over 7 hours after oral administration of furosemide (3 mg/kg) in 14 dogs, including 8 healthy dogs (blue circles) and 6 dogs with congestive heart failure (orange squares). The number of criteria for low diuretic responsiveness (out of 5) met by each dog is displayed in the parentheses

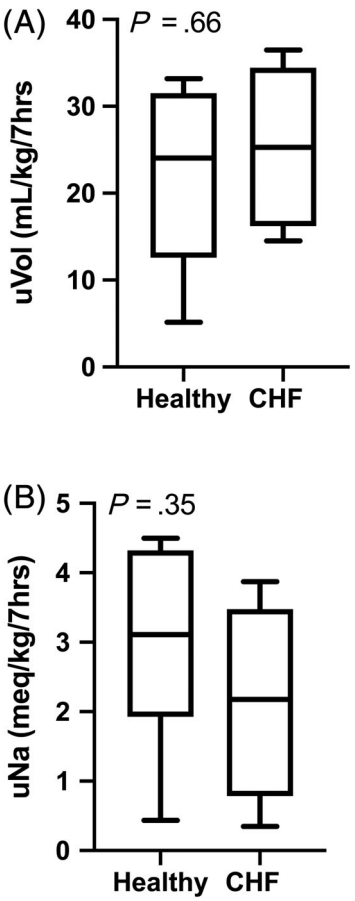

FIGURE 3 Urinary Na (uNa) excretion and urine volume (uVol) over 7 hours in healthy dogs and dogs with congestive heart failure (CHF) after oral administration of furosemide (3 mg/kg)

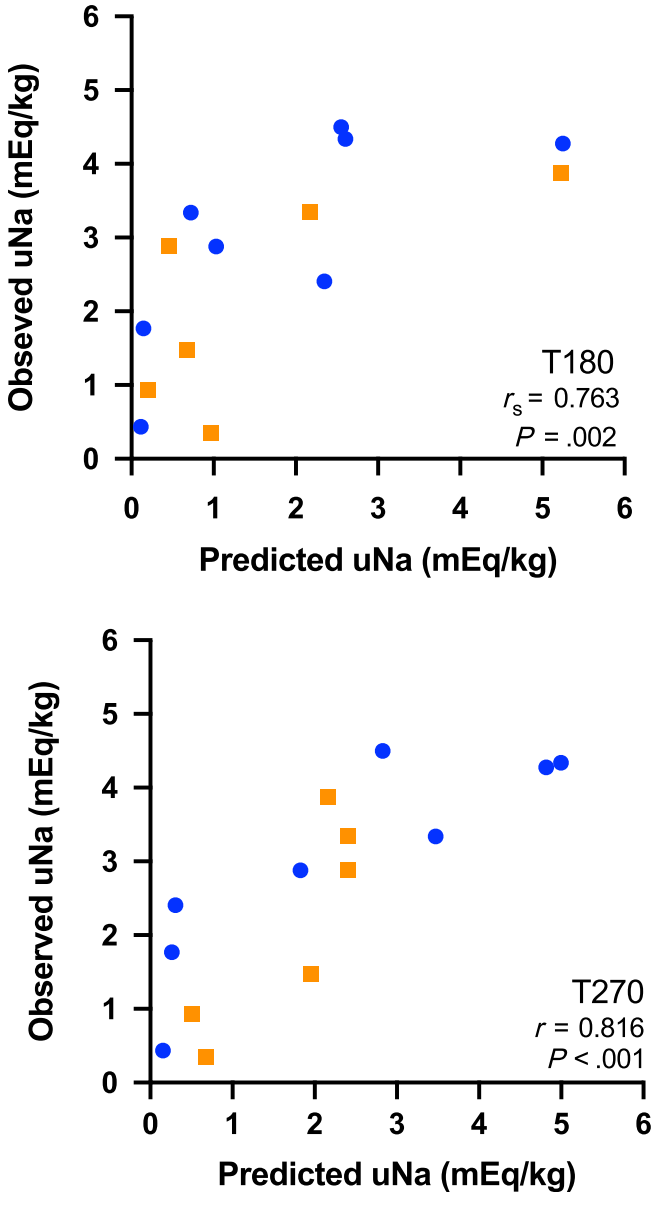

FIGURE 4 Predicted versus observed 7-hour urinary Na excretion (uNa) in 14 dogs after oral administration of furosemide (3 mg/kg) including 8 healthy dogs (blue circles) and 6 dogs with congestive heart failure (orange squares) using a prediction equation that utilized spot urine samples obtained 180 minutes (T180) or 270 minutes (T270) after dosing

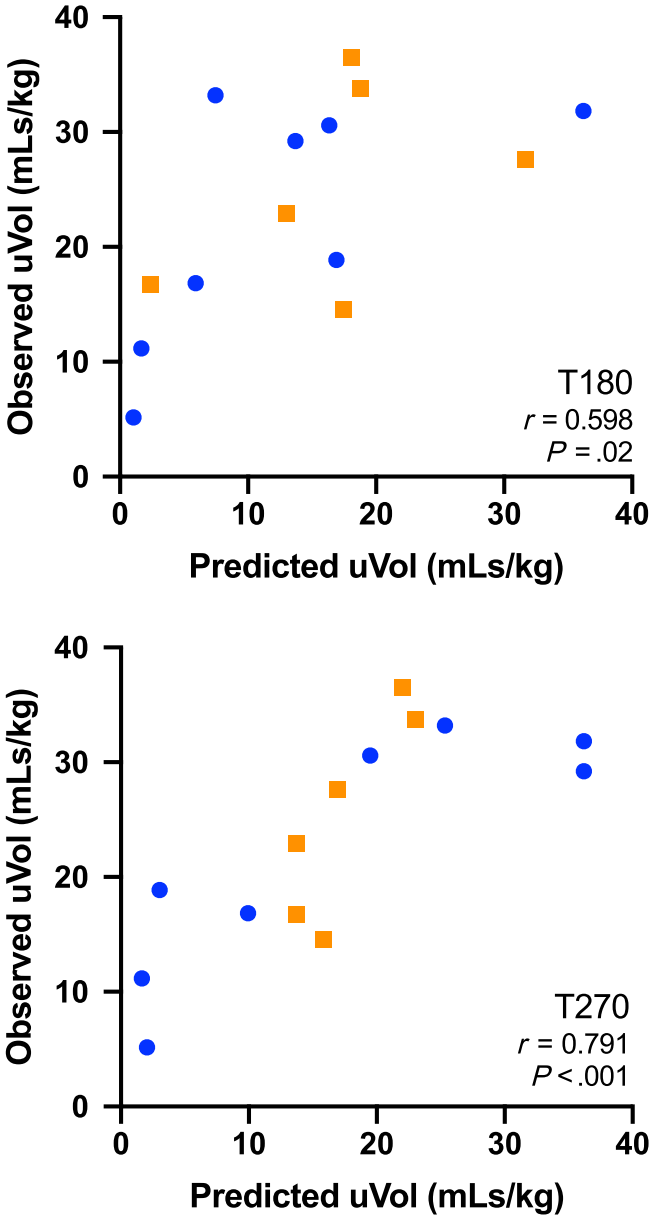

FIGURE 5 Predicted versus observed 7-hour urine volume (uVol) in 14 dogs after oral administration of furosemide (3 mg/kg) including 8 healthy dogs (blue circles) and 6 dogs with congestive heart failure (orange squares) using a prediction equation that utilized spot urine samples obtained 180 minutes (T180) or 270 minutes (T270) after dosing

(−11 mL/kg [range, −27.3 to 20.5 mL/kg], P = .001). Median dietary Na intake the morning of the study was 0.59 mEq/kg (range, 0-2.49 mEq/kg) and there was moderate and significant correlation between dietary Na intake and cumulative uNa output (r = 0.68, P = .01). Median net Na balance was −2.02 mEq/kg (range, −3.56 to 0.31 mEq/kg) with 1 dog experiencing a net gain. Dogs suspected as having LDR had significantly less net Na loss than other dogs (LDR: −0.63 mEq/kg [SD, 0.68 mEq/kg] versus with no LDR: −2.66 mEq/kg [SD, 0.78 mEq/kg], P < .001). The baseline sNa, sCl, and sK between dogs with and without LDR were not significantly different (data not shown).

3.5 | Identification of dogs with and without LDR

Receiver-operating characteristic curves were used to assess the ability of predicted uNa and uVol to differentiate the 5 LDR dogs (Figure 8). Predicted uNa at 180 and 270 minutes possessed AUC of 0.933 (95% CI, 0.804-1.000, P = .01) and 0.911 (95% CI, 0.756-1.000, P = .01), respectively. Predicted uVol at 180 and 270 minutes

Criterion	Time (minute)	# Dogs fulfilling criteria	Central tendency of entire cohort
1	180	3/14 (21%)	120 (56) mEq/L
2	180	3/14 (21%)	2.83 (1.84)
	270	3/14 (21%)	3.28 (1.96)
	420	1/14 (7%)	1.98 (1.11)
3	0-180	4/21 (29%)	3.1 (2.2) mL/kg/h
	180-270	3/14 (21%)	5.4 (3.3) mL/kg/h
	270-420	3/14 (21%)	2.8 (1.5) mL/kg/h
4	0-420	3/14 (21%)	2.63 (1.44) mEq/kg
5	0-420	4/21 (29%)	−7.7 (−27.3 to 53.7) mL/kg
≥3 criteria met	5/14 (36%)		

TABLE 3 Number of dogs fulfilling each of the 5 a priori criteria for low diuretic responsiveness. Central tendency of the entire study cohort is listed as average (SD) or median (range)

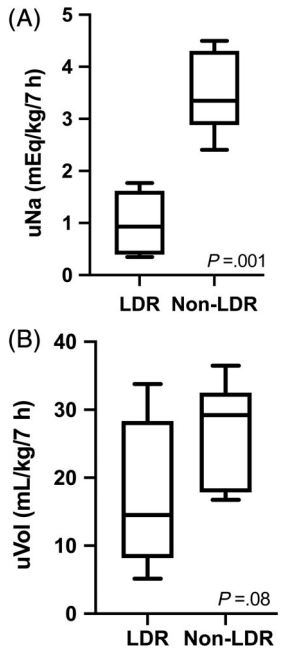

FIGURE 6 A, Urinary Na (uNa) output and B, urine volume (uVol) over 7 hours after oral dosing of furosemide in 5 dogs with low diuretic responsiveness (LDR) versus 9 dogs without LDR

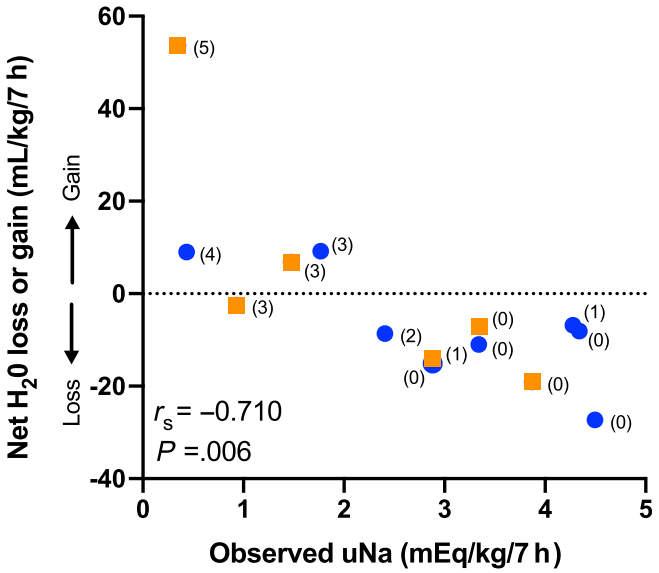

FIGURE 7 Urinary sodium (uNa) output versus net H_2O loss or gain in 14 dogs over 7 hours after oral furosemide dosing, including 8 healthy dogs (blue circles) and 6 dogs with congestive heart failure (orange squares). The number of criteria for low diuretic responsiveness (out of 5) met by each dog is displayed in the parentheses

possessed AUC of 0.644 (95% CI, 0.315-0.975, P = .39) and 0.744 (95% CI, 0.466-1.000, P = .14), respectively. Post hoc constants for uNa output and uVol equations were calculated as 0.55 and 599, respectively, compared favorably to a priori values, and did not significantly change the AUCs of the ROC analysis (data not shown).

4 | DISCUSSION

The main result of our study was the validation of equations based on spot urine sampling at 180 and 270 minutes to predict 7-hour cumulative uNa output and uVol in response to oral furosemide administration. The methodology and results of this study closely mirrored-results in humans with CHF[18] and support the validity of the underlying basic physiological principles upon which the equations were based. In both studies, the correlation between the observed and predicted uNa was high and strong enough to identify subjects with poor diuretic response versus those with more robust responses. The findings of our study have important clinical implications. It has long been known that dogs with CHF possess greatly increased total body Na and extracellular water content.[33] Thus, clinical assessment of diuretic responsiveness, particularly uNa output, could help individualize CHF therapy beyond what is currently performed.[6,10,19,34]

An important feature of our study was the focus on uNa output, which, as opposed to uVol or water or weight loss, has been increasingly recognized as the best index of diuretic responsiveness and predictor of important clinical outcomes.[8,10,18,35-41] Natriuresis and diuresis, while closely intertwined, are distinct aspects of diuretic responsiveness, and play related but different roles in the clinical assessment of efficacy. The varied relationship between cumulative

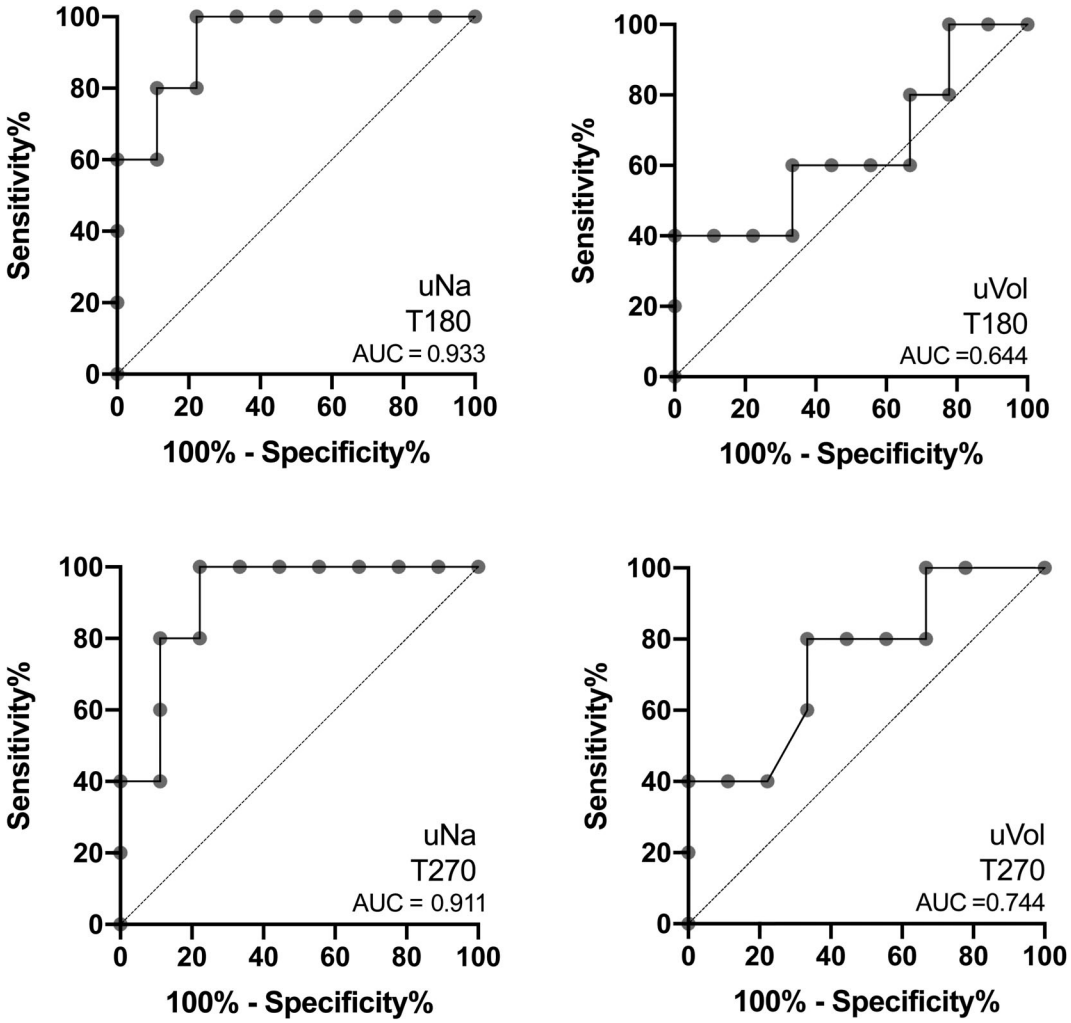

FIGURE 8 Receiver-operating characteristic curves for the detection of 5 dogs with low diuretic responsiveness (LDR) based on urinary Na (uNa) output and urine volume (uVol) predicted from equations using spot urine sample at 180 minutes (T180) and 270 minutes (T270) after oral furosemide dosing. AUC, area under the curve

uNa and uVol as shown in Figure 2 highlights the fact that these measures are not interchangeable. It does not necessarily follow that dogs excreting high uVol also excrete high uNa. In humans, clinical assessment after diuretics traditionally involves relief of symptoms, such as dyspnea, net water or weight loss, and evidence of volume depletion such as hemoconcentration,[7,10] however, substantial congestion can persist despite meeting these criteria.[42,43] In dogs, diuretic decongestion also is primarily guided by fluid loss and symptomatic relief[6] yet, in both species, it is extremely difficult to disentangle decongestion (i.e., removal of water and Na from the extracellular space) from dehydration (i.e., removal of water from the intravascular or intracellular space) when basing response on uVol or net water loss alone. As previously mentioned, water balance in extracellular spaces, such as the pulmonary interstitium, is primarily controlled by extracellular NaCl, which makes up over 90% of the extracellular solute.[3] Water freely diffuses between the extracellular and intracellular spaces according to the osmotic gradient. The most efficient decongestion ideally involves urine that is isotonic (i.e., ~140 mEq/mL) or hypertonic to plasma so that extracellular fluid is removed without depleting intracellular fluid.[44] Excessive loss of water with low solute (i.e., excessive free water excretion) without concomitant net loss of Na can ultimately result in incomplete decongestion, recurrent edema, and increased mortality.[34,38,45] The futility of long-term low-solute diuresis is evidenced by the inability of vasopressin antagonists, which promote free water excretion, to reduce mortality.[46] Thus, while uVol and net fluid loss are important components of the diuretic response, the ultimate measure of diuretic responsiveness is maintenance of Na balance in the extracellular space and forced natriuresis in the face of increased renal Na avidity.[41] The direct line from extracellular Na to extracellular water gives uNa output more "biologic plausibility" as a measure of diuretic responsiveness than fluid loss.[9]

Diuretic responsiveness from the standpoint of diuretic-induced activation of RAAS, uNa excretion, and prediction of uVol has been studied.[13,47] In healthy dogs receiving constant rate infusion of furosemide, hourly uNa output and [uNa]:[uK] were strongly correlated with hourly uVol (uNa versus uVol: r = 0.996, P < .0001; [uNa]:[uK]

versus uVol: $r = 0.976$, $P < .001$) highlighting the close relationship between Na and uVol, under what might be considered optimal conditions.[17] In our study, the correlation between cumulative uNa output and uVol was comparatively less ($r = 0.643$) and highlighted how uNa and uVol can become disconnected, particularly in dogs with CHF. Specifically, 3 dogs noticeably departed from the main uNa to uVol relationship, all excreting relatively large volumes of Na-poor urine. To clinicians and owners, the large uVol after dosing might be interpreted as a sign of good diuretic response, however, primarily because of a combination of low uNa and poor net water loss—and in some instances net water *gain* (Figure 7)—all 3 of these dogs met criteria for LDR. The low uNa:uVol ratio might be caused by kidney phenomena such as medullary washout or compulsive water consumption, which 1 of the 3 dogs appeared to exhibit during the study period. Thus, results of our study expand existing knowledge by demonstrating how consideration of uNa output and uVol in tandem can help better identify individuals with LDR.

Diuretic responsiveness is complex and a result of many pharmacokinetic, pharmacodynamic, physiological, and dietary variables, including oral bioavailability, tubular secretion, kidney function, neurohormonal activation, and dietary Na intake. Poor responsiveness from any or all of these reasons could be considered forms of LDR. In dogs, specific criteria for LDR are lacking, and further study is warranted. In humans, criteria vary, and no standard definition exists.[10,36] The most intriguing criteria are associated with important clinical outcomes, such as increased mortality or hospital admission.[9] For instance, in humans with acute CHF, uNa <60 mEq/L is associated with a significantly increased in risk of death, rehospitalization, need for mechanical circulatory support, or home-based IV inotropic therapy compared to those with greater [uNa].[39,45] In our study, similar to those in humans,[28] we supposed LDR based on fulfillment of multiple criteria involving a combination of uNa output, uVol, and net water balance, rather than a single criteria. In our study, our chief aim was to validate a method to quantify responsiveness using spot samples. Our study was neither designed to validate a definition of LDR nor to differentiate specific causes, however, several of our findings related to LDR bear mention. Firstly, the changes in uVol and uNa that accompany LDR are mediated through GFR and the tubular concentration or dilution. While GFR and sCr:uCR might quantitatively change, the governing principles of urine formation expressed in the prediction methods do not, which permits detection of low responsiveness by our methods independent of underlying cause and in both health and disease. Secondly and relatedly, 2 healthy dogs met criteria for LDR, which was consistent with our pilot experience with healthy dogs. Low diuretic responsiveness also has described in healthy humans consuming a low salt diet.[48] In our study, we hypothesized that stress and increased sympathetic and RAAS activity related to hospitalization and the study procedures blunted the diuretic response.[31] In the current study, 2 healthy dogs were suspected with LDR and were the only dogs in the overall cohort that demonstrated [uNa]:[uK] <1 over multiple timepoints, suggesting high RAAS activity.[13] Thirdly, the specific relationship between uNa and uVol in subjects with low LDR might offer clues as to underlying etiology that can be further studied. For instance, low uNa coupled with low uVol is more suggestive of low bioavailability, low Na intake, and high RAAS activity, whereas low uNa coupled with high uVol suggests medullary washout, distal tubular hypertrophy, excessive vasopressin release, or high amounts of free water intake. A fourth important consideration is that selection of specific criteria to define LDR affects the predictive ability of related metrics. In our study, we employed a mixture of LDR criteria involving both uNa and uVol to try and avoid biasing the ROC analysis towards either metric, yet, the predictive ability of uNa was substantially greater than that of uVol, supporting the important role of Na in diuretic responsiveness. A fifth important consideration relevant to LDR is dietary Na, which has the potential to profoundly affect the natriuretic response to diuretics.[48,49] In our study, dietary Na intake was moderately correlated to 7-hour uNa output, and dogs had consumed a wide range of dietary Na the morning of the study. In humans, dietary Na can be standardized, which better allows for better definition and detection of LDR based solely on uNa output.[18] In circumstances where dietary Na is not standardized, LDR based on net Na balance (i.e., uNa output minus dietary Na intake) might be a better indicator of responsiveness.

There are important potential limitations of our study, some of which have been previously mentioned. The subject number was small and confidence intervals around estimates were wide, reflecting the omnipresent challenge of cohort size in veterinary trials, especially those that involve laborious methodology, such as in our study. We might not have had sufficient power to detect important differences between groups. We used eGFR rather than measuring GFR using iohexol. Our privately owned dogs did not undergo bladder catheterization as has been done in purpose-bred dogs and hourly estimations of uNa output and uVol represented the average value within the timeframes represented by each particular sampling period. The absence of bladder catheterization also increased the risk of spilled or missed uVol, however, we attempted to minimize this to the extent possible. Our study has important practical implications insofar as our spot urine sample obtained at 180 minutes represents urine produced from 0 to 180 minutes, with the dog's bladder as empty as possible prior to dosing. Similarly, when obtaining a spot urine sample representing 270 minutes, the dog's bladder at 180 minutes should be as empty as possible. Urine and Na loss from a dog voiding at times other than at 0 and 180 could affect spot results if the urine composition substantially changes from the start of the window of accumulation to the end of the window. Despite these limitations, the proposed methodology using spot samples is far easier and practical to perform in a clinical setting than attempting collection of urine produced over 7 hours. The criteria for LDR used in our study are best viewed as a starting point for further study and validation. For instance, the fact that cumulative uNa <2 mEq/kg was sensitive and specific to our LDR dogs could help formulate LDR hypotheses for future testing. Our study identified dogs with LDR but did not differentiate any of the potential pharmacologic, pharmacodynamic, renal or neurohormonal causes of LDR. As currently regarded in humans, the pathophysiology of and criteria for LDR tilt in favor of uNa output

as the metric of prime importance with less emphasis on uVol. In our study, the greater strength of correlation between predicted and observed uNa, as compared to predicted and observed uVol, facilitates consideration of uNa output. The potential effect of dietary Na on diuretic responsiveness has already been mentioned. An additional limitation involving dietary Na was the fact that time between feeding and diuretic dosing was not consistent amongst dogs. Water consumption amongst dogs varied considerably and might have contributed to the lag in peak uVol formation versus uNa excretion, and specific studies of water consumption habits in dogs receiving diuretics are of interest. Our study examined dogs with chronic CHF rather than acute CHF, and diuretic responsiveness in humans with chronic CHF is less understood than in acute CHF.[19,30,37] However, as previously stated, the basic renal principles governing urine formation and uNa output used in the prediction equations are the same in chronic CHF as in acute CHF, as well as in the healthy subject. One uNa measurement specific to chronic CHF that warrants further investigation is using spot [uNa] to predict impending decompensation. In a study[37] of humans with chronic CHF, [uNa] from first morning void was persistently lower in the patient cohort that experienced future decompensation, and spot [uNa] further dropped by another 35% in the week prior to decompensation. Finally, the prediction equations validated in our study are specific to 3 mg/kg PO furosemide and urine sampling at the prescribed timepoints. Diuretic responsiveness to parenteral furosemide dosing or treatment with other diuretic drugs requires additional study.

In conclusion, we validated a priori physiological equations to predict cumulative uNa output (uNa [mEq/kg] = [uNa/uCr] × 0.507) and uVol (uVol [mL/kg] = 1/[uCr] × 507) over the 7 hours after 3 mg/kg PO furosemide administration in healthy and CHF dogs. Urinary Na excretion and uVol are complementary but distinct aspects of diuretic responsiveness and predicted uNa output demonstrated better correlation to observed uNa as well as greater ability to identify dogs with LDR than did predicted uVol. The ability to quantify diuretic responsiveness opens doors to many different avenues of further inquiry, including understanding of the pathophysiology of DR, validation of dog-specific criteria for LDR, individualization of diuretic dosing based on dietary Na and net Na balance, detection of impending decompensation in apparently stable dogs, comparative effectiveness of different loop diuretics and dosing regimens, as well as strategies to improve or restore effectiveness in dogs with LDR.

ACKNOWLEDGMENT

No funding was received for this study. The authors thank the owners who volunteered their dogs for this study.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

University of Pennsylvania IACUC approval, #806527.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Loughran KA, Larouche-Lebel É, Huh T, Testani JM, Rao VS, Oyama MA. Prediction and measurement of diuretic responsiveness after oral administration of furosemide to healthy dogs and dogs with congestive heart failure. *J Vet Intern Med*. 2020;34:2253–2264. https://doi.org/10.1111/jvim.15952