

# Effects of a nutrient-enriched water on water intake and indices of hydration in healthy domestic cats fed a dry kibble diet

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## OBJECTIVE

To evaluate the effects of drinking nutrient-enriched water (NW) on water intake and indices of hydration in healthy domestic cats fed a dry kibble diet ad libitum.

## ANIMALS

18 domestic shorthair cats.

## PROCEDURES

Group-housed cats were assigned to tap water (TW; n = 9) or NW (9) groups. All cats received TW at baseline (days -7 to -1). No changes were made to the food-water regimen for the TW group. The NW group received NW instead of TW from days 0 through 10, then received TW and NW in separate bowls (days 11 through 56). Food intake was measured through day 10; liquid consumed by drinking was measured throughout the study. Blood and urine samples were collected at predetermined times for analyses; 48-hour urine collection (days 28 through 30 or 31 through 33) was performed to assess output volume and aid endogenous creatinine-based glomerular filtration rate (GFR) determination. Data were analyzed with linear mixed-effects models.

## RESULTS

Baseline TW and calorie intake were similar between groups. The NW treatment was significantly associated with increased liquid consumption during the treatment phase. Mean urine output was significantly higher in the NW group (15.2 mL/kg/d) than in the TW group (10.3 mL/kg/d). Mean GFR (1.75 vs 1.87 mL/min/kg, respectively) did not differ between groups. Effects of treatment and time were each significant for urine specific gravity and osmolality and urine creatinine, phosphate, and urea nitrogen concentrations, with lower values for the NW group.

## CONCLUSIONS AND CLINICAL RELEVANCE

Results suggested that consumption of the NW can increase liquid intake and improve measures of hydration in healthy cats. These effects may offer health benefits to some cats in need of greater water consumption. (*Am J Vet Res* 2018;79:733-744)

Estimates of daily water requirements have been reported for cats.<sup>1</sup> However, no consensus exists for how to define optimal hydration, optimal water intake volume in cats, or the overall impact of adequate hydration on health.<sup>2</sup> A basic and limited frame-

work of published research exists on water intake, water balance, and urine variables in cats, with data primarily collected for kennel-housed animals that necessitate citation to work conducted as long ago as the 1970s.<sup>1</sup> Daily water intake volume has been reported as milliliters per kilogram of body weight, milliliters per kilogram of dry matter ingested, and milliliters per kilocalorie of ME ingested (water-to-calorie intake ratio).<sup>1</sup> All these methods account for the intake of water from a combination of sources including food moisture, FW consumption (drinking), and MW. In general, the daily water-to-calorie intake ratio for water need has been estimated to be 0.6 to 0.7<sup>3,4</sup> and 0.9<sup>5</sup> for healthy cats consuming dry and wet food, respectively. Although cats can consume sufficient calories to meet their daily needs regardless of whether dry or wet food is given, this difference

## ABBREVIATIONS

FW	Free water
GFR	Glomerular filtration rate
LUTD	Lower urinary tract disease
ME	Metabolizable energy
MW	Metabolic water
NW	Nutrient-enriched water
QMR	Quantitative magnetic resonance
S <sub>osm</sub>	Serum osmolality
TBW	Total body water
TW	Tap water
U <sub>osm</sub>	Urine osmolality
USG	Urine specific gravity

in the daily water-to-calorie intake ratio is observed in healthy cats because they drink less water when fed dry food and thus do not consume as much water as they ingest through dietary moisture when eating wet food.<sup>1,3,6,a</sup>

The currently accepted understanding in regard to these differences in water intake in healthy cats is that the higher total water intake and higher water-to-calorie intake ratio result in greater diuresis in cats that eat wet food. In comparison, although cats that eat dry food have a lower daily water-to-calorie intake ratio, the total water intake is equally sufficient to meet their daily water requirements.<sup>1</sup> However, cats with various types of LUTDs appear to benefit from increased total water intake and urine output. Nutritional studies<sup>6-9,a,b</sup> to investigate health concerns related to LUTDs in cats have provided some evidence that increased water intake can be achieved through modification of dietary moisture content to increase water intake through food<sup>6,7,a</sup> or of dietary sodium content to stimulate drinking.<sup>8,9,b</sup>

Research with human patients has revealed that increased water intake leads to increased urine volume and dilution, thus supporting increased water intake as one of the typically recommended methods to help prevent urolith recurrence.<sup>10,11</sup> Voluntarily drinking more water is the most common means of increasing fluid intake in people, but this also includes greater consumption of other common beverages.<sup>10,12</sup> Although increased total water intake for cats has been achieved through dietary modification or stimulation of thirst mechanisms, to the authors' knowledge, there are no published reports of studies examining the effects of a water supplement on daily water intake, urine dilution, or hydration in this species. Results of 1 study<sup>13</sup> indicate increased palatability of water for cats (determined by preference testing) when a liquid nutritional supplement is added, compared with water alone.

A greater understanding of feline water intake patterns, water balance, and urine indices of hydration status is needed. The objective of the study reported here was to evaluate the effects of NW consumption on water intake and indices of hydration in healthy cats fed dry food (kibble). Goals included examination of voluntary TW consumption and characterization of multiple physiologic measures of hydration status prior to treatment, followed by testing for changes in liquid consumption, total water intake, and hydration variables after providing an NW (alone and as a supplement to TW provided separately) for 56 days. The nutrients in the prototype NW primarily included organic osmolytes, glycerol, and amino acids from whey protein and animal digest.

## Materials and Methods

### Animals

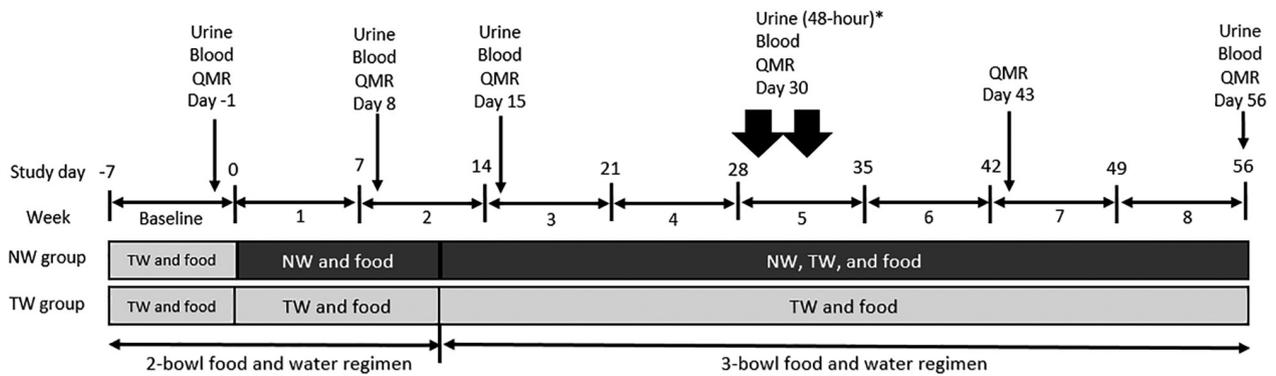
Adult domestic shorthair cats ( $n = 18$ ) owned by Nestlé Purina PetCare and housed at a pet facility were included in the study. All cats used were re-

quired to have overall good general health prior to beginning the study for pretrial selection and underwent evaluation by a veterinarian at the beginning of the trial, which also included routine serum biochemical analysis. At the start of the study, the cats had a mean  $\pm$  SD body weight of  $4.6 \pm 0.8$  kg and age of  $9.8 \pm 2.5$  years; body condition scores ranged from 5 to 7 on the basis of a 9-point scale.<sup>14</sup> Prior to the study, cats were group housed in 2 separate rooms (9 cats/room) on the basis of compatibility and sex. Cats were housed indoors with natural lighting and exposure to natural light cycles in environmentally controlled rooms ( $3.5 \times 4.3 \times 2.3$  m), with temperature ranging from  $21^\circ$  to  $24^\circ\text{C}$  and 50% humidity. Each room had 3 shelves on each wall running three-quarters of the length, with 1 set of wall shelves housing access to the electronic food-monitoring system. There was 1 litter pan/cat and several environmental enrichment items in each room (eg, plastic tables and chairs, various hiding spaces, and play items). Cats were provided environmental enrichment consisting of multiple perches, access to toys, and direct interaction with caretakers on a daily basis. The trial was conducted in accordance with approved animal care and use committee protocols at the pet-care facilities of Nestlé Purina PetCare.

### Experimental design

The study was designed to monitor daily liquid and food intake for cats in group housing (except as described for sample collection times). Liquid consumption was determined as the free liquid volume consumed by drinking; this differed from total daily water intake, which was calculated from all 3 water intake sources only during baseline and week 1 of the treatment phase as described herein. Each individual cat's daily food and liquid intake were recorded in the cat rooms by use of a 2-bowl automated monitoring system. The automated monitoring system consisted of each food bowl resting on a scale that weighed the bowls individually and repeatedly to track consumption over a 24-hour period. Each feeder read each cat's identification tag when the feeder was accessed to track individual consumption; only 1 cat at a time could access the food. Cat rooms each contained 12 to 15 feeders to ensure that there were always feeders available to all the cats in the room. Cats had ad libitum access to dry food during the entire study, except for the 12 hours preceding blood and urine sample collection. The food was a prototypical chicken-and-rice dry kibble food formulated to meet adult maintenance nutrient requirements.<sup>c</sup> Proximate analysis<sup>d</sup> of the diet indicated 7.6% moisture, 36.0% crude protein, 14.2% crude fat, 4.0% crude fiber, 7.2% ash, 5,509 ppm of sodium, 11,740 ppm of phosphorus, and 4,760 kcal/kg on an as-fed basis.

A timeline and breakdown of experimental procedures are provided (**Figure 1**). The 2 groups of cats ( $n = 9/\text{group}$ ) received TW only during the baseline assessment phase (7 days prior to the start of experimental treatment) and were arbitrarily assigned to



**Figure 1**—Timeline depicting feeding and watering protocols and sample collection times in a study to evaluate the effects of an NW on water intake and indices of hydration in healthy domestic cats fed a dry kibble diet ad libitum. Group-housed cats were assigned to TW ( $n = 9$ ) or NW ( $9$ ) groups; all cats received TW for drinking and had food and TW intake measured during the week before the treatment period (ie, baseline). No changes were made to the food and water regimen for cats of the TW group. Cats of the NW group received NW instead of TW from days 0 through 10 and were offered TW and NW in separate bowls for determination of water preferences from days 11 through 56. Measurements were made with a 2-bowl automated system; therefore, to allow for measurements of both water types, food was provided in a third bowl for the NW group beginning on day 11, and food intake monitoring ceased. \*Cats were individually housed for 48 hours on days 28 through 30 or 31 through 33 (nominal day 30) for measurement of total urine output volume and collection of a pooled urine sample for GFR analysis.

receive either TW only (TW group) or NW only followed by a choice of NW or TW (NW group) during the 56-day treatment phase. The NW content was summarized (**Appendix**).

Daily liquid (TW) and food ingestion were recorded during the baseline phase for the NW and TW groups. The treatment phase was subdivided to evaluate the effects of a 2-bowl or 3-bowl food and water regimen (Figure 1) on water consumption. On days 0 through 10 of the treatment phase, the 2-bowl food and water regimen was continued, and daily ingestion of liquid (in bowl 1) and food (in bowl 2) was recorded for both groups. During this 11-day period, cats of the NW group had ad libitum access to NW and food through the automated feeding system, and cats of the TW group had ad libitum access to TW and food through the same automated system. The positioning (left vs right) of bowls containing liquid and food was alternated daily at each feeder. Daily calorie intake from food consumption was calculated for each cat in accordance with the proximate analysis of the kibble.

Starting on day 11 until the end of the trial, a 3-bowl food and water regimen was implemented with food available ad libitum outside of the automated feeding system (in bowl 1, with no monitoring of food intake) for both groups of cats. Cats of the NW group had ad libitum access to NW (in bowl 2) and TW (in bowl 3) via the automated feeding system, whereas cats in the TW group received TW in both bowls through the automated system.

### Calculation of total daily water intake

Total water intake was calculated for each day and included FW, MW, and food moisture only during baseline and week 1, when food intake was recorded. The FW (grams) was either TW or the water-only component of the NW (with grams of dry-matter

content removed from the calculation). For MW, the calculation was based on a conservative estimate of 10 mL of water/100 kcal of ME.<sup>15</sup> In addition, MW content was calculated for the nutrient substrate oxidation of the protein component ingested from NW (41 g of water/100 g of protein oxidized<sup>1</sup>) as determined by proximate analysis. Each individual animal's total daily water intake was calculated as total volume (mL) of water ingested relative to the total calories (ME in kcal) ingested on a daily basis (water-to-calorie intake ratio) and as total daily volume of water adjusted for body weight.

### Sample collection and analysis

To evaluate selected physiologic variables associated with hydration, blood and cystocentesis-collected urine samples were obtained. Samples were collected on days -1, 8, 15, 30, and 56 (Figure 1). In addition, cats were individually housed for 48 hours on days 28 through 30 or 31 through 33 for measurement of total urine output volume and collection of a pooled urine sample. Urine was collected repeatedly into a clean collection vessel containing no preservative and pooled with previous urine while stored at 4°C. Urine and blood collected on day 33 for the second group of cats were designated day 30 in the summary tables for simplicity. This pooled urine sample was used for the determination of GFR on the basis of endogenous creatinine clearance.

On sample collection days, blood (3 mL) was collected from the jugular vein, transferred to blood tubes,<sup>c</sup> and allowed to clot for 10 minutes at room temperature (approx 20°C). Serum was collected after centrifugation of clotted blood samples and stored at -80°C until aliquoted samples were analyzed to obtain  $S_{osm}$  with an osmometer<sup>f</sup> and biochemical data by means of an automated biochemical assay system<sup>g</sup> used in accordance with the manufacturer's instruc-

tions. In addition to measurement of creatinine concentrations in serum and urine as described,<sup>g</sup> additional measurement was performed with a kinetic colorimetric assay<sup>h</sup> according to the Jaffe method with a pseudocreatinine chromogen correction of -0.3 mg/dL applied to the data output.

Urine samples were analyzed on the day of collection for determination of specific gravity with a refractometer,<sup>i</sup> color by comparison with a urine color chart,<sup>j</sup>  $U_{osm}$  with an osmometer,<sup>f</sup> and pH with a handheld microprocessor-based meter.<sup>k</sup> For urine color, an 8-color scale developed for assessment of human urine samples was used, with colors ranging from very pale yellow (1) to brownish green (8).<sup>16</sup> Remaining urine from each sample was stored at -80°C until aliquoted samples were analyzed for urea nitrogen and creatinine concentrations by means of an automated biochemical assay system<sup>g,h</sup> previously validated for feline samples. Stored urine samples were also analyzed for sodium, potassium, calcium, and magnesium content (as  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$ , respectively) by inductively coupled plasma optical emission spectroscopy<sup>l</sup> and for phosphate ( $PO_4^{3-}$ ) and chloride ( $Cl^-$ ) content by ion chromatography.<sup>m</sup>

## GFR measurement

For each cat, GFR adjusted for body weight (mL/min/kg) was calculated by measurement of endogenous creatinine<sup>17</sup> concentrations (mg/dL) in serum and urine and the mean 24-hour urine output volume (mL) determined from the 48-hour collection period. Serum for creatinine measurement was collected on the last day of the 48-hour urine collection period (ie, day 30 or 33). Calculation of GFR was performed by use of the following equation:

$$GFR \text{ (mL/24 h/cat)} = (UCr \cdot UOV) / SCr$$

where UCr is the endogenous urine creatinine concentration, UOV is the mean 24-hour urine output volume, and SCr is the endogenous serum creatinine concentration. The result was then reduced to a numeric equivalent of milliliters per minute per cat, which was then adjusted for body weight to yield a final result as milliliters per minute per kilogram.

## Body composition measurements

Body weight was recorded by 8:00 AM on body composition analysis days. Body composition scans of awake cats were run in triplicate by use of QMR, with cats placed in a polymethylmethacrylate crate for imaging as previously described.<sup>18,n</sup> Total body water, lean body mass, and fat mass were determined.<sup>18</sup> Scans were performed on days -1, 8, 15, 30, 43, and 56 of the treatment phase (Figure 1).

## Statistical analysis

For variables other than GFR and urine output volume, a linear mixed-effects model was used to account for the nonindependence of the data in comparison between groups with a commercially avail-

able software package.<sup>19,o</sup> Cat identification was used as a random effect, and the intercept was allowed to vary by cat. Treatment (TW vs NW), time, and the interaction between treatment and time were entered as fixed effects. Satterthwaite approximation of degrees of freedom was used to calculate the *P* values. Tukey post hoc tests were then conducted. For GFR and urine volume, the Welch 2-sample *t* test was conducted to determine differences between treatments. Values of  $P \leq 0.05$  were considered significant.

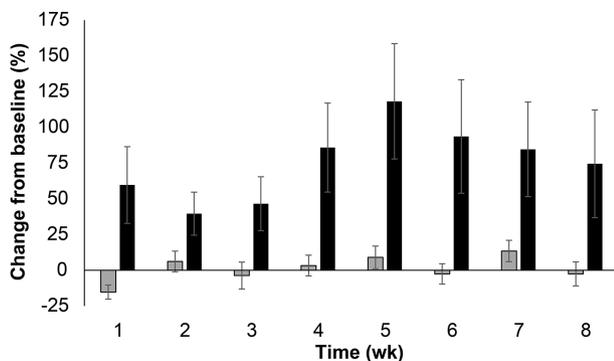
## Results

### Calorie intake from food consumption and liquid intake

In general, cats' calorie intake from food consumption and the amount of liquid consumed by drinking from bowls during the baseline (pretreatment) period varied only slightly from day to day with the exception of days -2 and -1 (data not shown), with TW provided to both groups throughout and food being withheld for 12 hours (beginning the previous evening) before sample collection on day -1. Mean daily liquid and calorie intake amounts, reported for 5 days (days -7 to -3) of the baseline period and for all days of week 1 (days 0 through 7), were compared within and between groups. Replacement of TW with NW beginning on day 0 was associated with a marked increase in liquid consumption for the NW group, and a significant ( $P = 0.010$ ) time-by-treatment interaction was detected for this variable. The mean  $\pm$  SE amount of liquid consumed was similar between the TW ( $107 \pm 7$  g/d) and NW ( $93 \pm 9$  g/d) groups at baseline ( $P = 0.90$ ), and results for this variable did not differ ( $P = 0.72$ ) between baseline and week 1 ( $89 \pm 5$  g/d) for the TW group. However, liquid intake increased significantly ( $P = 0.01$ ) from the baseline value for cats in the NW group during week 1 (mean  $\pm$  SE,  $148 \pm 26$  g/d), and the amount of liquid consumed during week 1 was significantly ( $P = 0.03$ ) greater for this group than for the TW group.

No significant ( $P = 0.41$ ) time-by-treatment interaction was found for calorie intake from food or for the main effect of treatment on this variable ( $P = 0.63$ ), whereas the main effect of time was significant ( $P < 0.001$ ) between baseline and week 1. Mean  $\pm$  SE week 1 calorie intake was  $186 \pm 15$  kcal/d and  $187 \pm 13$  kcal/d for the TW and NW, groups, respectively, compared with  $137 \pm 12$  kcal/d and  $153 \pm 12$  kcal/d, respectively, at baseline (a decrease of approx 22% overall).

Mean weekly liquid intake (calculated from daily intake data) for each group throughout the treatment phase was compared with the mean baseline value for the same group, and percentage change was calculated (Figure 2). The main effects of treatment ( $P = 0.020$ ) and time ( $P < 0.001$ ) were significant. The time-by-treatment interaction was not significant ( $P = 0.84$ ). Although only the individual main effects were significant, the data were reported on a weekly ba-



**Figure 2**—Comparison of mean  $\pm$  SE percentage change in the weekly amount of liquid consumed by drinking relative to baseline for the same 18 cats as in Figure 1. Gray and black bars represent data for the TW and NW groups, respectively. See Figure 1 for remainder of key.

sis to demonstrate the relative differences between treatment groups over the course of the trial. For the NW group, mean free liquid intake increased approximately 60% during week 1 (when only NW was provided for drinking), compared with that at baseline. During the remainder of the study (with transition from the 2-bowl to 3-bowl regimen to include access to TW beginning on day 11), the NW group continued to have significantly greater liquid intake, with differences from baseline ranging from approximately 40% to 118%. The TW group had minimal variation over the 8-week treatment phase, with differences ranging from approximately -15% to 14%.

Although liquid consumption by cats in the NW group was significantly greater than that for cats in the TW group during the treatment phase (mean  $\pm$  SE, 153  $\pm$  26 g/d vs 104  $\pm$  5 g/d, respectively), individual responses varied substantially. To examine this variability, the overall mean percentage change for all 8 weeks of the treatment phase was calculated. The variability was largely driven by 3 cats that had particularly high intake of the NW. In summary, 3 cats in the NW group had low response rates (< 25% increase), 3 cats had moderate response rates (25% to 75% increase), and 3 cats had high response rates (> 75% increase), with the 2 highest differences calculated as 175% and 270% increases relative to baseline. To account for this variance, all 18 cats in the study were categorized as responders or nonresponders. Responders had an overall mean increase in liquid consumption > 25%, and nonresponders had an increase  $\leq$  25%. The TW group had 8 nonresponders and 1 responder, whereas the NW group had 3 nonresponders and 6 responders. A  $\chi^2$  test revealed a significant ( $P = 0.05$ ) relationship between treatment (NW vs TW) and response (responder vs nonresponder).

To generally assess the preferences for NW or TW within the NW group, the proportion of NW versus TW (percentage of daily intake) consumed by cats of the NW group was calculated for weeks 2 through 8. Daily results calculated for all individual cats, not the group mean, ranged from a minimum of 58% NW

(42% TW) to a maximum of 100% NW (0.0% TW; data not shown) and was not influenced by whether NW was provided in the left or right bowl. When each cat's weekly intake was calculated from the daily data, results ranged from a minimum of 90.3% NW (9.7% TW) up to a maximum of 99.8% NW (0.2% TW; data not shown). The overall daily mean  $\pm$  SD percentage of total daily liquid ingested as NW was 96.6  $\pm$  3.0%. This indicated that even cats in the NW group categorized as nonresponders for daily liquid consumption volume primarily ingested the NW versus TW, but at volumes that were generally similar to baseline.

### Total water intake and water-to-calorie intake ratios

Calculated daily total water intake (the sum of FW consumed by drinking [TW and the water-only component of NW], MW [conversion from food and NW nutrients], and food moisture components) was determined and was used to calculate mean daily water-to-calorie intake ratios (as mL per kcal of ME) for the same 5 days at baseline and days 0 through 7 of week 1 only, owing to the previously described changes in feeding protocols that prevented analysis for longer periods (Figure 1). A significant ( $P = 0.040$ ) time-by-treatment interaction was identified for the water-to-calorie intake ratio. At baseline, the mean  $\pm$  SE daily water-to-calorie intake ratio was similar between the TW (0.71  $\pm$  0.03) and NW (0.63  $\pm$  0.03) groups, and the week 1 value for the TW group (0.85  $\pm$  0.05) was similar to that at baseline. In contrast, this ratio was significantly ( $P < 0.001$ ) increased for the NW group during week 1 (1.12  $\pm$  0.14) relative to baseline for the same group; however, the difference between the NW and TW groups during week 1 (with the mean approx 32% greater for NW than for TW) was nonsignificant ( $P = 0.06$ ).

There was a significant ( $P = 0.010$ ) time-by-treatment interaction for total water intake adjusted for body weight of cats. The NW treatment resulted in significantly ( $P = 0.010$ ) higher mean  $\pm$  SE total water intake during week 1 (37.5  $\pm$  7.0 mL/kg), compared with results for the same group at baseline (24.3  $\pm$  2.5 mL/kg), whereas no significant difference was observed for this variable during week 1 (25.1  $\pm$  2.9 mL/kg) relative to the baseline value (29.7  $\pm$  3.0 mL/kg) for the TW group.

### Urine characteristics

The USG,  $U_{osm}$ , and color data were summarized (Table 1). The main effects of time and treatment were significant ( $P < 0.001$  for both) for USG, without a significant time-by-treatment interaction. Cats of the TW group had a higher mean USG (1.054  $\pm$  0.001 g/mL) that was generally stable throughout the study, with the group mean varying among time points by  $\leq$  0.006 g/mL. Cats of the NW group had a lower mean USG (1.040  $\pm$  0.002 g/mL). In addition, the NW group had a slightly lower mean USG at baseline, compared with that for TW-group cats (1.053 vs

**Table 1**—Comparisons of mean ± SE urine variables measured in a study to evaluate the effects of an NW on water intake and indices of hydration in healthy domestic cats fed a dry kibble diet ad libitum.

Variable	Day					P value*		
	-1	8	15	30†	56	Time	Treatment	Time by treatment
USG (g/mL)						< 0.001	< 0.001	0.14
TW	1.058 ± 0.002	1.052 ± 0.002	1.053 ± 0.003	1.055 ± 0.002	1.052 ± 0.002			
NW	1.053 ± 0.004	1.031 ± 0.004	1.038 ± 0.005	1.037 ± 0.004	1.041 ± 0.005			
U <sub>osm</sub> (mOsm/kg)						0.002	0.01	0.003
TW	2,434 ± 86 <sup>a,A</sup>	2,180 ± 81 <sup>a,A</sup>	2,191 ± 50 <sup>a,A</sup>	2,402 ± 116 <sup>a,A</sup>	2,114 ± 98 <sup>a,A</sup>			
NW	2,178 ± 134 <sup>a,A</sup>	1,337 ± 182 <sup>b,B</sup>	1,651 ± 201 <sup>b,B</sup>	1,683 ± 187 <sup>a,b,B</sup>	1,744 ± 185 <sup>a,b,A</sup>			
pH						0.57	0.80	0.003
TW	6.55 ± 0.10 <sup>a,A</sup>	6.35 ± 0.09 <sup>a,A</sup>	6.37 ± 0.10 <sup>a,A</sup>	6.35 ± 0.05 <sup>a,A</sup>	6.28 ± 0.10 <sup>a,A</sup>			
NW	6.19 ± 0.08 <sup>a,A</sup>	6.33 ± 0.06 <sup>a,b,A</sup>	6.28 ± 0.08 <sup>a,b,A</sup>	6.55 ± 0.07 <sup>b,A</sup>	6.46 ± 0.08 <sup>a,A</sup>			
Color‡						< 0.001	0.02	0.04
TW	4.4 ± 0.2 <sup>a,A</sup>	4.1 ± 0.2 <sup>a,A</sup>	3.7 ± 0.2 <sup>a,A</sup>	5.2 ± 0.1 <sup>a,A</sup>	4.2 ± 0.4 <sup>a,A</sup>			
NW	4.9 ± 0.3 <sup>a,A</sup>	3.1 ± 0.3 <sup>b,A</sup>	3.1 ± 0.4 <sup>b,A</sup>	4.0 ± 0.2 <sup>a,A</sup>	3.1 ± 0.3 <sup>b,A</sup>			
Sodium (mmol/L)						0.14	0.55	0.67
TW	147 ± 23	154 ± 22	116 ± 19	ND	126 ± 32			
NW	143 ± 24	119 ± 17	120 ± 19	ND	107 ± 16			
Calcium (mmol/L)						0.001	0.90	0.41
TW	1.68 ± 0.38	0.96 ± 0.21	0.60 ± 0.11	ND	0.83 ± 0.14			
NW	1.49 ± 0.33	0.68 ± 0.10	1.04 ± 0.54	ND	0.75 ± 0.23			
Potassium (mmol/L)						0.01	< 0.001	0.62
TW	246 ± 19	215 ± 13	190 ± 16	ND	207 ± 9			
NW	195 ± 11	138 ± 17	144 ± 18	ND	169 ± 22			
Magnesium (mg/dL)						0.08	0.14	0.98
TW	5.07 ± 1.48	3.89 ± 0.78	3.25 ± 0.63	ND	3.60 ± 0.82			
NW	3.64 ± 0.84	2.62 ± 0.68	1.83 ± 0.59	ND	2.50 ± 0.38			
Phosphate (mmol/L)						0.009	0.003	0.02
TW	94 ± 9 <sup>a,A</sup>	98 ± 9 <sup>a,A</sup>	80 ± 6 <sup>a,A</sup>	ND	87 ± 9 <sup>a,A</sup>			
NW	85 ± 6 <sup>a,A</sup>	47 ± 8 <sup>b,B</sup>	50 ± 10 <sup>b,B</sup>	ND	55 ± 10 <sup>b,A</sup>			
Creatinine (mg/dL)						< 0.001	< 0.001	0.02
TW	506 ± 16 <sup>a,A</sup>	489 ± 30 <sup>a,A</sup>	430 ± 33 <sup>a,A</sup>	366 ± 21 <sup>a,A</sup>	442 ± 30 <sup>a,A</sup>			
NW	453 ± 31 <sup>a,A</sup>	288 ± 39 <sup>b,B</sup>	308 ± 44 <sup>b,B</sup>	239 ± 32 <sup>b,A</sup>	337 ± 39 <sup>b,B</sup>			
Urea nitrogen (g/dL)						< 0.001	0.008	0.007
TW	4.6 ± 1.4 <sup>a,A</sup>	4.1 ± 1.6 <sup>a,A</sup>	4.2 ± 1.6 <sup>a,A</sup>	4.4 ± 1.8 <sup>a,A</sup>	4.2 ± 1.8 <sup>a,A</sup>			
NW	4.0 ± 2.5 <sup>a,A</sup>	2.3 ± 3.3 <sup>b,B</sup>	3.0 ± 3.9 <sup>b,A</sup>	3.0 ± 3.4 <sup>b,B</sup>	3.3 ± 4.0 <sup>a,A</sup>			

Cats of the TW (n = 9) and NW (9) groups received TW and dry kibble during the week before the treatment period (ie, baseline; days -7 to -1). No changes were made to the food and water regimen for cats of the TW group. On days 0 to 10, cats of the NW group received NW instead of TW, and from days 11 to 56, they were offered TW and NW in separate bowls.

\*P values were generated from a linear mixed model. †Urine was collected over a 48-hour period (days 28 to 30 or 31 to 33) for the time point designated as day 30. ‡Color was assessed with a scale<sup>161</sup> from very pale yellow (1) to brownish green (8).

<sup>a,b</sup>Within a row, values with different lowercase superscript letters differ significantly (pairwise comparisons) within a treatment group. <sup>A,B</sup>Within a column, values with different uppercase superscript letters differ significantly (pairwise comparisons) between the 2 treatment groups. Comparisons are indicated with superscript letters only for variables with significant time-by-treatment interactions. For all comparisons, values of *P* < 0.05 were considered significant.

1.058 g/mL), and had a notable decrease of 0.022 g/mL for this variable by day 8 of the treatment phase. The USG remained lower for the NW group than for the TW group on all subsequent sampling days during the 8-week treatment phase.

Significant time-by-treatment interactions were observed for U<sub>osm</sub> (*P* = 0.003) and urine color (*P* = 0.040; Table 1). For both variables, mean baseline values were similar between treatment groups. For cats in the TW group, no difference was observed among sampling times for either variable. For the NW group, mean U<sub>osm</sub> was significantly (*P* < 0.05) decreased during the first 2 weeks of the treatment phase, compared with the baseline value. Although U<sub>osm</sub> was numerically lower on days 30 and 56 than at baseline for this group, these differences were nonsignificant. Urine color analysis revealed similar findings, with significantly (*P* < 0.05) lower mean color scores on days 8, 15, and 56 relative to baseline for the NW group. A significant (*P* = 0.003) time-by-treatment interaction was observed for urine pH; however, individual effects

of treatment and time were nonsignificant. Mean urine pH did not differ between groups at any time point. For the TW group, the value was highest at baseline but did not differ significantly among time points. Urine pH was lowest at baseline for the NW group, with a significant (*P* < 0.05) difference observed only on day 30 relative to baseline.

Time-by-treatment interactions were significant (*P* ≤ 0.020) for urine phosphate, creatinine, and urea nitrogen, but not potassium, concentrations (Table 1). For phosphate, urea nitrogen, and creatinine, mean urine concentrations were similar between groups at baseline. These values were significantly (*P* < 0.05) lower than the baseline value by day 8 of the treatment phase for the NW group, and these differences were generally maintained until the end of the study. In addition, a significant (*P* < 0.001) main effect of treatment was identified for potassium. This was a result of the NW group having a significantly (*P* < 0.05) lower mean urine potassium concentration at all sampling days, compared with those for the TW group, with this difference also evident at baseline.

## Urine output volume and GFR

Because some cats did not urinate during the first 24 hours of the 48-hour urine collection period (Figure 1), the volume of urine collected on the second day was greater for those cats than for cats that voided urine on both days (data not shown). Regardless, the daily mean urine volume was calculated for all cats and subsequently used to determine the daily mean urine output adjusted for body weight. The mean  $\pm$  SE result was significantly ( $P = 0.010$ ) higher for cats of the NW group ( $15.2 \pm 1.8$  mL/kg/d) than for cats of the TW group ( $10.3 \pm 0.7$  mL/kg/d). The mean  $\pm$  SE GFR measured during this period did not differ significantly ( $P = 0.51$ ) between the NW ( $1.75 \pm 0.11$  mL/min/kg) and TW ( $1.87 \pm 0.18$  mL/min/kg) groups.

## Serum characteristics

The serum analyte concentration data were summarized (Table 2). Time-by-treatment interactions

were not significant for any of these variables except for creatinine ( $P < 0.01$ ),  $S_{osm}$  ( $P < 0.001$ ), and phosphorus ( $P = 0.010$ ). Serum creatinine concentration and  $S_{osm}$  differed significantly ( $P < 0.05$ ) between treatment groups at baseline, but did not differ between groups at any sampling time during the treatment phase except on day 56, with  $S_{osm}$  greater for the NW than the TW group. Serum phosphorus concentration was similar between groups at all time points, but differed between baseline and week 1 for the TW group. The main effect of time, but not treatment, was significant ( $P < 0.010$ ) for serum albumin, glucose, sodium, and urea nitrogen concentrations.

## Body composition

The main effect of treatment was nonsignificant for measures of body weight and all body composition variables (Table 3). Time-by-treatment interactions were also nonsignificant. The effect of time was

**Table 2**—Comparisons of mean  $\pm$  SE serum variables for the same 18 cats as in Table 1.

Variable	Day					P value*		
	-1	8	15	30	56	Time	Treatment	Time by treatment
Albumin (g/dL)								
TW	3.29 $\pm$ 0.13	3.30 $\pm$ 0.11	3.26 $\pm$ 0.12	3.28 $\pm$ 0.09	3.14 $\pm$ 0.10	0.01	0.99	0.76
NW	3.28 $\pm$ 0.08	3.24 $\pm$ 0.06	3.26 $\pm$ 0.10	3.32 $\pm$ 0.07	3.18 $\pm$ 0.08			
Creatine kinase (U/L)								
TW	208 $\pm$ 33	203 $\pm$ 49	183 $\pm$ 37	193 $\pm$ 29	138 $\pm$ 19	0.31	0.17	0.78
NW	176 $\pm$ 21	149 $\pm$ 16	127 $\pm$ 10	169 $\pm$ 31	142 $\pm$ 22			
Creatinine (mg/dL)								
TW	1.37 $\pm$ 0.05 <sup>a,A</sup>	1.51 $\pm$ 0.06 <sup>a,A</sup>	1.41 $\pm$ 0.07 <sup>a,A</sup>	1.52 $\pm$ 0.07 <sup>a,A</sup>	1.43 $\pm$ 0.05 <sup>a,A</sup>	0.19	0.05	0.004
NW	1.70 $\pm$ 0.08 <sup>a,B</sup>	1.66 $\pm$ 0.09 <sup>a,A</sup>	1.60 $\pm$ 0.09 <sup>a,A</sup>	1.57 $\pm$ 0.07 <sup>a,A</sup>	1.61 $\pm$ 0.05 <sup>a,A</sup>			
Glucose (mg/dL)								
TW	89 $\pm$ 4	83 $\pm$ 4	84 $\pm$ 4	92 $\pm$ 5	84 $\pm$ 4	0.01	0.80	0.71
NW	88 $\pm$ 9	88 $\pm$ 7	81 $\pm$ 5	93 $\pm$ 3	89 $\pm$ 7			
$S_{osm}$ (mOsm/kg)								
TW	328 $\pm$ 6 <sup>a,A</sup>	324 $\pm$ 2 <sup>a,A</sup>	328 $\pm$ 3 <sup>a,A</sup>	320 $\pm$ 2 <sup>a,A</sup>	320 $\pm$ 3 <sup>a,A</sup>	0.01	0.79	< 0.001
NW	312 $\pm$ 2 <sup>a,B</sup>	319 $\pm$ 1 <sup>a,A</sup>	330 $\pm$ 2 <sup>b,A</sup>	328 $\pm$ 3 <sup>b,A</sup>	335 $\pm$ 2 <sup>b,B</sup>			
Phosphorus (mg/dL)								
TW	3.58 $\pm$ 0.14 <sup>a,A</sup>	4.16 $\pm$ 0.11 <sup>b,A</sup>	4.12 $\pm$ 0.19 <sup>a,A</sup>	3.93 $\pm$ 0.10 <sup>a,A</sup>	3.77 $\pm$ 0.14 <sup>a,A</sup>	0.04	0.27	0.01
NW	4.16 $\pm$ 0.13 <sup>a,A</sup>	4.20 $\pm$ 0.13 <sup>a,A</sup>	4.07 $\pm$ 0.12 <sup>a,A</sup>	3.82 $\pm$ 0.13 <sup>a,A</sup>	4.26 $\pm$ 0.21 <sup>a,A</sup>			
Sodium (mmol/L)								
TW	154 $\pm$ 1	155 $\pm$ 1	154 $\pm$ 1	153 $\pm$ 1	151 $\pm$ 1	0.002	0.42	0.41
NW	155 $\pm$ 1	155 $\pm$ 1	155 $\pm$ 1	155 $\pm$ 1	149 $\pm$ 3			
SUN (mg/dL)								
TW	25 $\pm$ 1	24 $\pm$ 1	26 $\pm$ 2	27 $\pm$ 2	26 $\pm$ 1	< 0.001	0.18	0.26
NW	26 $\pm$ 1	24 $\pm$ 1	27 $\pm$ 1	27 $\pm$ 1	28 $\pm$ 1			

See Table 1 for key.

**Table 3**—Comparisons of mean  $\pm$  SE body weight and body composition variables for the same 18 cats as in Table 1.

Variable	Day						P value*		
	-1	8	15	30	43	56	Time	Treatment	Time by treatment
BW (kg)									
TW	4.47 $\pm$ 0.25	4.38 $\pm$ 0.25	4.43 $\pm$ 0.27	4.44 $\pm$ 0.29	4.46 $\pm$ 0.29	4.47 $\pm$ 0.32	0.77	0.47	0.64
NW	4.78 $\pm$ 0.27	4.74 $\pm$ 0.28	4.79 $\pm$ 0.28	4.75 $\pm$ 0.28	4.81 $\pm$ 0.29	4.77 $\pm$ 0.29			
LBM (kg [% BW])									
TW	3.25 $\pm$ 0.13 (73.6)	3.31 $\pm$ 0.14 (73.9)	3.29 $\pm$ 0.14 (75.0)	3.26 $\pm$ 0.15 (74.4)	3.28 $\pm$ 0.15 (74.5)	3.28 $\pm$ 0.15 (74.6)	< 0.001	0.60	0.83
NW	3.36 $\pm$ 0.17 (70.5)	3.31 $\pm$ 0.16 (70.3)	3.41 $\pm$ 0.17 (71.7)	3.38 $\pm$ 0.17 (71.5)	3.43 $\pm$ 0.18 (71.8)	3.40 $\pm$ 0.17 (71.7)			
TBW (kg [% BW])									
TW	2.42 $\pm$ 0.11 (56.0)	2.32 $\pm$ 0.12 (54.7)	2.38 $\pm$ 0.12 (55.6)	2.36 $\pm$ 0.12 (55.3)	2.36 $\pm$ 0.11 (55.2)	2.37 $\pm$ 0.12 (55.4)	0.03	0.69	0.16
NW	2.41 $\pm$ 0.12 (50.6)	2.39 $\pm$ 0.11 (50.8)	2.47 $\pm$ 0.12 (51.9)	2.44 $\pm$ 0.11 (51.8)	2.48 $\pm$ 0.12 (51.9)	2.46 $\pm$ 0.12 (51.9)			
Body fat (kg [% BW])									
TW	0.85 $\pm$ 0.15 (18.2)	0.80 $\pm$ 0.15 (17.5)	0.80 $\pm$ 0.15 (17.1)	0.82 $\pm$ 0.17 (17.3)	0.83 $\pm$ 0.18 (17.4)	0.83 $\pm$ 0.19 (17.1)	0.72	0.39	0.33
NW	1.07 $\pm$ 0.13 (21.9)	1.05 $\pm$ 0.13 (21.6)	1.02 $\pm$ 0.13 (20.8)	1.02 $\pm$ 0.14 (20.9)	1.03 $\pm$ 0.14 (20.8)	1.03 $\pm$ 0.14 (20.9)			

BW = Body weight. LBM = Lean body mass.

See Table 1 for remainder of key.

significant for lean body mass ( $P < 0.001$ ) and TBW ( $P = 0.030$ ). However, mean TBW at baseline and day 56 did not differ within groups, and the time effect was identified as resulting from low values on day 8 for both groups. Lean body mass was slightly higher on day 56 for both groups, compared with the respective baseline values.

### Relationships among measures of hydration and liquid intake

In the linear mixed-effects model (with data from all sample collection periods), USG, and  $U_{osm}$  were positively and significantly ( $P < 0.001$ ) associated with each other ( $\beta = 38,363.65$ ; **Figure 3**). In contrast,  $U_{osm}$  and  $S_{osm}$  had no significant ( $P = 0.94$ ) relationship ( $\beta = 0.33$ ). Visual evaluation of the data revealed some notable  $U_{osm}$  clustering among samples from the NW group during the treatment phase. Further evaluation indicated that although data were widely distributed across  $S_{osm}$  measurements, most (11/16 samples)  $U_{osm}$  measurements  $< 1,500$  mOsm/kg were from cats that had increased their mean weekly liquid consumption (that obtained through drinking) by  $> 25\%$  (range, 27% to 361%), compared with baseline consumption. Other clustering of samples with  $U_{osm} > 2,000$  mOsm/kg was largely (7/11 samples) from cats of the NW group that had increased their liquid drinking by  $< 25\%$  (range, -21% to 19%).

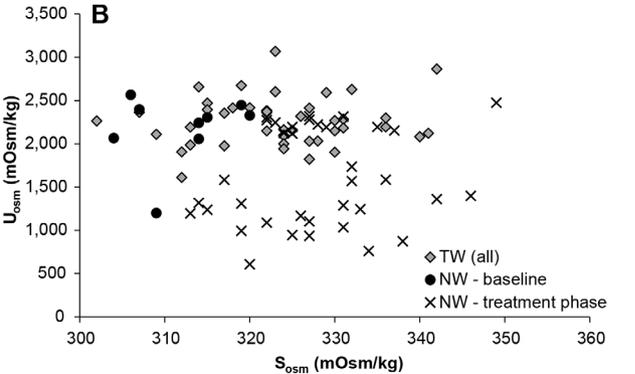
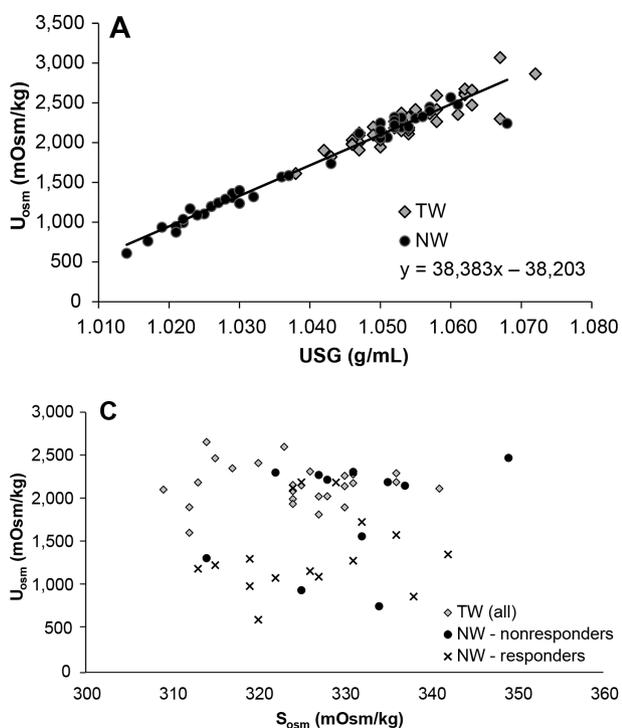
Finally, to evaluate how water consumption related to changes in  $U_{osm}$ , the weekly water-to-calorie intake ratio data from baseline and week 1 were compared with  $U_{osm}$  data from day -1 (end of baseline) and day 8 (end of week 1) by mixed-effects model analysis. A significant inverse linear relationship ( $\beta =$

$-1,168.05$ ;  $P < 0.001$ ) was observed (**Figure 4**). There was a negligible improvement in the association when a second-order polynomial model was used ( $P = 0.40$ ; data not shown).

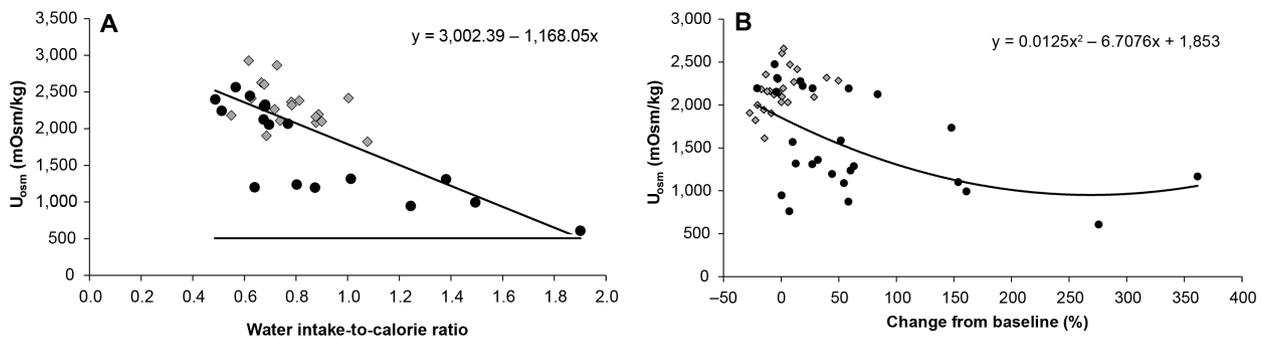
An inverse linear relationship ( $\beta = -14.61$ ;  $P < 0.01$ ) was also found between total water intake adjusted for body weight and  $U_{osm}$  (data not shown). Because the relative increase in liquid drinking appeared to result in clustering of  $U_{osm}$  values (Figure 3), the relationship between the percentage change in weekly liquid intake from drinking during weeks 1, 2, and 8 was plotted against  $U_{osm}$  measured on days 8, 15, and 56 (which excluded the 48-hour urine collection sample on nominal day 30). A second-order polynomial model fit the data better than a linear model ( $P < 0.02$ ; Figure 4); a maximum dilution of urine (ie, minimum  $U_{osm}$ ) with increasing amounts of liquid ingestion appeared to occur at approximately 800 mOsm/kg.

### Discussion

Daily water needs in healthy cats are not well-defined, and little is known regarding how incremental changes in water intake translate into changes in blood or urine measures associated with hydration. Differences in total daily water intake exist for cats eating only dry food, or even a combination of wet and dry foods, compared with that for cats fed wet food alone.<sup>6,a</sup> This suggests that cats fed dry food may benefit from having a greater daily water intake through increased drinking. The 2 objectives of the present study were achieved, first by further characterizing how daily water drinking corresponds to changes in several variables associated with urine



**Figure 3**—Results of linear mixed-effects regression analysis for  $U_{osm}$  versus USG (A) or  $S_{osm}$  (B and C) for the same 18 cats as in Figure 1. Panel A includes data points that represent each cat at each sample collection time (days -1, 8, 15, 30, and 56). In panels B and C, data points represent samples collected during the treatment phase only (days 8, 15, and 56, which excluded the 48-hour urine collection) for each cat. Data for the TW group are presented in each panel as a whole; data for the NW group are presented as a whole (A) or further subdivided into baseline and treatment phases (B) or responders and nonresponders (C; cats with  $> 25\%$  or  $\leq 25\%$  increase in liquid consumption by drinking, respectively [on the basis of percentage change in intake during weeks 1, 2, and 8 relative to baseline]).



**Figure 4**—Results of mixed-effects regression analysis for relationships between  $U_{osem}$  and water intake variables for the same 18 cats as in Figure 1. A—The  $U_{osem}$  versus weekly water-to-calorie intake ratio (determined on the basis of calculated total water intake, which included FW [TW and the water-only component of NW], MW [conversion from food and NW nutrients], and food moisture components) at the end of baseline (day -1) and week 1 (day 8). B—The  $U_{osem}$  (days 8, 15, and 56) versus percentage change in weekly liquid consumption (by drinking) relative to baseline for weeks 1, 2, and 8. Data for cats of the TW and NW groups are represented as gray diamonds and black circles, respectively.

indices of hydration in healthy cats eating dry food. Second, the study results indicated that the NW treatment was significantly associated with changes in the amount of water consumed by drinking and that consumption of the NW resulted in increased total water intake and dilution of urine. This study generated several unique findings, which included a significantly greater preference for consumption of the NW by the cats when free access to TW was also available and that high liquid intake helped maintain a more dilute urine over a 2-month study period. The study also generated evidence suggestive of a water intake threshold that appears necessary to dilute urine concentration for healthy cats eating dry food, although further research is needed to confirm this.

Mean daily TW and dry food ingestion for cats in the TW and NW groups during baseline were similar, with TW intake varying only slightly on a daily basis (mean  $\pm$  SE,  $107 \pm 7$  g/d and  $93 \pm 9$  g/d for the TW and NW groups, respectively). The mean water-to-calorie intake ratio of 0.8 (determined on the basis of total water intake [ie, FW consumed by drinking, MW, and food moisture components]) during baseline and week 1 of the study for the TW group was consistent with the previously reported mean of approximately 0.7 mL:1 kcal of ME for cats drinking TW and fed dry food.<sup>3,4</sup> However, cats drinking the NW had a significant ( $P < 0.001$ ) increase in mean water-to-calorie intake ratio from 0.6 mL:1 kcal of ME during baseline to 1.1 mL:1 kcal of ME during week 1.

Because food intake was observed to be lower for both groups of cats during week 1 of the treatment phase, compared with that at baseline, it was possible that lower calorie intake would have influenced the water-to-calorie intake ratio. Therefore, total water intake adjusted for body weight was independently calculated and reported. For cats of the TW group, our observations of approximately 25 to 30 mL/kg of body weight were slightly higher, compared with previously group means of 21 to 23 mL/kg.<sup>5,6</sup> Because body weight was relatively unchanged (mean difference,  $< 3\%$  for both groups) between baseline and

week 1 of the study, these calculations revealed that cats of the NW group had increased total water intake on a milligram-per-kilogram basis during week 1 (a 54% increase from baseline;  $P = 0.010$ ), whereas cats of the TW group had a slightly (15%) lower water intake during week 1 that did not differ significantly from the baseline value.

Because the water-to-calorie intake ratio could not be calculated beyond day 11 (when food intake measurements ceased owing to the addition of a TW option for cats of the NW group), the daily amount of water consumed by drinking from bowls was converted to weekly free liquid intake values for the entire study, and data for the treatment period were reported as the percentage change from baseline. Interestingly, mean free liquid consumption for the NW group appeared greater in weeks 4 through 8 than in weeks 1, 2, and 3, although these data were not evaluated statistically. Variability of liquid consumption for the NW group during the last 5 weeks of the study was also generally higher than that observed in the first 3 weeks of the treatment period, revealing variations in water type preference among cats in this group. The 3-bowl regimen was implemented to examine the cats' preference for NW versus TW, and the results indicated that the proportional increase in total free liquid drinking was a direct result of the cats consuming large amounts of NW. We found that even cats that had a low response rate ( $< 25\%$  increase in weekly liquid consumption during the treatment period, compared with that at baseline;  $n = 3$ ) consumed  $> 90\%$  of their daily free liquid as NW, and the proportion ranged as high as 99.8% for some cats. Similar to self-moderation of food consumption when fed ad libitum, the presence of some low response rates for the NW group indicated that some cats self-regulated their total liquid intake, whereas others substantially increased their consumption, particularly of the NW when it was made available. It is important to note that because the small number of cats in the NW group that had moderate or high response rates ( $n = 6$ ) represented a substantial limitation to

the present study, the data should be interpreted cautiously, as additional research is needed to expand on this initial body of evidence.

The NW used in the present study contained various nutrients that are considered osmolytes, mostly amino acids, glycerol, and electrolytes. Total solids concentration in the NW was calculated to be approximately 2.3% by summation of the proximate analysis results on an as-fed basis, which included 1% glycerol not measured by proximate analysis. Because the solids portion of the NW formula was largely composed of whey protein concentrate and a hydrolyzed poultry digest in addition to glycerol, it was not surprising that the proximate analysis included 1.7% crude protein. The NW was also determined to contain 0.0283% sodium and 0.016% phosphorus. On the basis of mean NW consumption measured in week 1 (approx 150 g/cat/d), cats in the NW group consumed a mean of approximately 42.5 mg of sodium and 24 mg of phosphorus/d. Although electrolytes were present in the formula, they were a much smaller proportion of osmolytes (0.028%) when compared with amino acids (1.7%) and glycerol (1%). Thus, it was likely that the significant increase in liquid drinking by cats of the NW group was primarily attributable to the whey and glycerol, as well as poultry flavor, and not the low sodium content. Additional research is necessary to isolate the effects of these separate nutritional components on liquid intake.

We collected 5 samples over the 2-month study period for measurement of urine and blood variables associated with hydration. These periodic assessments revealed that the mean concentrations of urine analytes and urine density remained relatively unchanged for cats of the TW group; all these cats had USG measurements within a fairly narrow range throughout the study, and the mean overall USG of 1.055 g/mL was similar to values (1.050 to 1.056) reported for cats fed dry food in other studies.<sup>4,6,8</sup>

The NW in this study was significantly associated with changes in urine variables that reflected greater hydration status of cats, including decreased USG, decreased  $U_{osm}$ , lower color scores, and lower concentrations of some minerals relative to the baseline data. During the 48-hour urine collection, the mean urine output volume for cats of the NW group was significantly ( $P = 0.010$ ) greater than that for cats of the TW group (15.2 vs 10.3 mL/kg/d), and corresponding differences of lower mean USG (33%) and  $U_{osm}$  (30%) were observed for the NW group, compared with the TW group. It is also notable that the cat receiving NW had significant reductions in mean urine concentrations of phosphate, creatinine, and urea nitrogen, which were primarily attributable to more dilute urine, during the treatment period.

The mean GFR (determined on the basis of endogenous creatinine concentrations and mean 24-hour urine output volume<sup>17</sup>) did not differ between treatment groups in the present study. Current veterinary research literature<sup>20</sup> indicates that other methods are

considered superior to use of endogenous creatinine measurements for determining GFR in cats, and endogenous creatinine was determined in the present study by use of the modified Jaffe method, which may have resulted in an underestimation of GFR. However, considering that all the necessary data to calculate GFR by this method were obtained independently per the experimental design, the results provide useful information regarding kidney function in healthy cats, even with the known limitations. The GFRs for cats of the present study were lower than previously reported values for healthy cats in studies that used the same method.<sup>17,21</sup> These data indicated that the cats of the NW group had healthy kidney function in the presence of higher urine volume, lower urine concentration, and greater water intake that effectively diluted urine phosphate while still maintaining serum phosphorus, creatinine, and urea nitrogen concentrations within reference ranges for healthy cats throughout the study.

Mean TBW content as determined by QMR remained relatively consistent throughout the study, with a maximum change (on a percentage body weight basis) of 1.3% between the start and end of the study for both groups. Importantly, the ease and safe repetitive use of QMR for measuring TBW and body composition in healthy, awake cats enabled documentation of TBW stability even with increased water intake, increased water-to-calorie intake ratio, and high urine output volume for the NW group during the treatment phase.

Urine osmolality is routinely reported in studies related to hydration status and urine concentration in cats and has also been shown to correlate highly with USG,<sup>22</sup> similar to the findings in our study. Our results also supported that healthy cats can have a very wide range of  $S_{osm}$  that does not relate to the degree of  $U_{osm}$  or relative amount of water intake. Kidneys in healthy cats can produce highly concentrated urine, presumably because of an evolutionary need for greater water retention during times of low water intake. However, even when liquid intake for cats of the NW group was significantly greater than at baseline,  $S_{osm}$  was similar to that of cats in the TW group, and subjectively, the values for all individual cats were within the same broad range. Thus, when cats drink larger volumes of water or liquid, the water reabsorption process in the kidneys appears to become saturated, leading to dilution of urine. Thus,  $S_{osm}$  in cats may play less of a role in predicting general hydration status than in other species because it reveals little in regard to urine concentration or water intake.

Examining the relationship between percentage change in liquid intake and  $U_{osm}$  confirmed that healthy cats have a weak drive to drink TW; visual evaluation of plots ( $U_{osm}$  vs  $S_{osm}$ ) also suggested that even when cats have high  $S_{osm}$  ( $> 330$  mOsm/kg), the vasopressin hormone system appears to be minimally sensitive to triggering a thirst response, as cats of the TW group in this category also had high  $U_{osm}$  ( $> 2,100$

mOsm/kg). Additionally, several NW-group cats were also observed to maintain high  $S_{osm}$  ( $> 330$  mOsm/kg) in the presence of high (responders) or normal (nonresponders) liquid intake relative to their TW ingestion at baseline in this study. This suggested that the cats were not drinking large amounts of NW because of thirst, but because of preference for the NW liquid. Therefore, cats in need of greater water intake would likely benefit from drinking a highly palatable NW to overcome the lack of thirst response or drive to consume TW, particularly when being generally hydrated would otherwise result in limited additional water intake. This could be particularly crucial for older cats with lower motivation to eat and drink as well as cats with a history of urolith formation that would benefit from greater water intake and diluted urine output.

Although some data regarding water intake, plasma osmolality, and plasma vasopressin concentrations have been generated for dogs,<sup>23</sup> vasopressin and thirst-trigger relationship data do not appear to be established in cats. Although it has been reported that vasopressin secretion occurs with increases in plasma osmolality from approximately 310 to 330 mOsm/kg in cats,<sup>24</sup> several cats of the NW group in the present study had  $S_{osm}$  values between 330 and 350 mOsm/kg with concurrently highly concentrated urine, which also corresponded to free liquid intake that increased  $< 25\%$  from baseline (and was considered within the range of normal variation for daily liquid intake rather than a response to the treatment). Thus, although higher  $S_{osm}$  may trigger vasopressin secretion, this did not appear to be associated with greater water drinking, compared with that of cats with lower  $S_{osm}$ . Further investigations of relationships among blood vasopressin concentration,  $S_{osm}$ , and water intake may provide better understanding of thirst stimulation in cats.

The linear mixed-effects model analysis of total water-to-calorie intake ratios and  $U_{osm}$  determined at baseline and during week 1 of the treatment phase for all 18 cats revealed a significant ( $P < 0.001$ ) inverse relationship between these variables. On the basis of the described linear regression model, targeting a  $U_{osm}$  of 1,500 mOsm/kg would yield an estimate for water-to-calorie intake ratio of approximately 1.3 mL:1 kcal of ME. Alternatively, evaluation of the percentage change in liquid consumed by drinking during weeks 1, 2, and 8, compared with baseline, revealed a significant ( $P < 0.02$ ) second-order polynomial model and indicated that a given cat may have to increase its liquid (water) consumption by approximately 100% to dilute  $U_{osm}$  to 1,500 mOsm/kg. Although the selection of 1,500 mOsm/kg was arbitrary, this value represents a target of diluted urine that is still concentrated enough that it does not mimic findings associated with nephron dysfunction, as 1,500 mOsm/kg is approximately equivalent to a USG of 1.035 g/mL. However, the number of cats in

the study was small, with only 9 cats receiving the NW, and further research is needed to confirm these findings.

Many situations or health conditions can arise that cause hypohydration in pets. The present study provided a basis for greater understanding of water needs in healthy cats and of how the amount of daily water ingestion impacts various measures of hydration and physiologic variables. More research is needed to determine whether cats eating dry food may benefit from greater daily water intake for general hydration improvement and, if so, how to achieve this goal beyond a generic recommendation of suggesting that owners always have fresh water available. Furthermore, alternative strategies should be developed to improve hydration, increase water intake, or both for cats that need additional veterinary support to lower the risk of urolith formation or require management for renal insufficiency, LUTD, or hypohydration resulting from age, injury, or surgery. Our findings indicated that healthy cats can have greater total water intake and improved indices associated with hydration when a palatable NW is supplied for drinking. A water supplement may serve as an alternative method or complement to dietary modification with a high-moisture food or sodium enrichment to increase water ingestion in cats, and research to investigate specific benefits in sick or injured cats is necessary and warranted on the basis of these findings in healthy cats.

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## Footnotes

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- i. HSK-VET veterinary refractometer, Heska, Loveland, Colo.
- j. Human Hydration LLC, Hampton, Va.
- k. pH 11 meter, Oakton Instruments, Vernon Hills, Ill.

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## Appendix

Ingredient composition and proximate analysis of an NW used in a study to evaluate the effects of the product on water intake and indices of hydration in healthy domestic cats fed a dry kibble diet ad libitum.

Component	Concentration
<b>Ingredients</b>	
Whey protein (%)	1.2
Glycerol (%)	1.0
Potassium chloride (%)	0.100
Hydrocolloids (%)*	0.110
Poultry digest (%)	1.0
<b>Proximate analysis</b>	
Moisture (%)	97.5
Crude protein (% as fed)	1.7
Crude fat (% as fed)	0.35
Crude fiber (% as fed)	< 0.2
Phosphorus (% as fed)	0.016
Sodium (% as fed)	0.0283

\*Mix of guar gum and xanthan gum.