

## Original Research

# The Effect on Hydration of Two Diets, One with and One without Plain Water

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**Key words:** hydration, dehydration, 24-hour urine volume, water-electrolyte balance, beverages, water requirements

**Objective:** To measure the effect on hydration of two regimens, one that included drinking water as part of the dietary beverages and one that did not.

**Methods:** In healthy, sedentary subjects, two different diets were evaluated for their effect on hydration. Trial A provided plain water to drink as part of the beverages served. Trial B omitted plain water from the beverages served. Twenty-seven males, during two three-day confinement periods, consumed one of two diets in a random, crossover and counterbalanced fashion, while diet, physical activity and environment were controlled and monitored. Body weight and 24-hour urine volumes were measured. Pre- and post-trial urine samples and 24-hour urines were assayed for osmolality, specific gravity, chloride, sodium and potassium, and sodium/potassium ratio was calculated. Twenty-four hour creatinine levels were determined.

**Results:** No differences ( $p > 0.05$ ) were found between trials for body weight or other indicators of hydration status measured.

**Conclusions:** Inclusion of plain drinking water compared to exclusion of plain drinking water in the diet did not affect the markers of hydration used in this study.

## INTRODUCTION

Fluid and electrolyte balance is essential for life; thus, the mechanisms for maintaining homeostasis are highly developed and tightly controlled. It is generally assumed that unless there is pathology or environmental stress, healthy adults maintain fluid and electrolyte balance. As such, very little scientific exploration of hydration status in normal healthy individuals has been conducted. Because of the paucity of objective data, numerous recommendations—recommendations not substantiated by the scientific process—are being given to the public regarding hydration.

Although fluid recommendations for healthy adults have not piqued a great deal of scientific interest, they have garnered considerable attention by the media and the lay public. Consumer health news abounds with advice to drink “water” to prevent dehydration [1–5]. Recommendations for water assume that the water can be from a variety of sources [6]; however,

consumers appear to take the recommendation literally—that the fluid has to be plain water [7–9].

Previous findings from our Center found no significant differences in the effect of various combinations of beverages on hydration status [10]. We found no significant differences in body weight, hematologic or urinary indices of hydration when subjects consumed no caffeine or when they consumed caffeine at the levels of 1.4 mg/kg BW or 3.13 mg/kg BW. Nor did hydration indicators differ when plain water composed 100%, 50% or 25% of the total beverage intake. However, in the previous study all trials included plain water. Additionally, in the prior study subjects consumed the controlled dietary regimen for only one day in a free-living environment. In the present study, the subjects followed the dietary regimen for three days, were confined to a research unit and one of the two trials did not include plain water as part of the diet. The objective of the current study was to measure the effect on hydration of two regimens, one that included plain drinking water as part of the dietary beverages and one that did not.

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Environmental factors, physical activity and food were held constant for three days in both regimens.

## SUBJECTS AND METHODS

### Subjects

Throughout this study, efforts were made to control confounding variables known to affect hydration status. The inclusion of only males was due to the uncertain effects of the menstrual cycle on hydration status of women. Other inclusion criteria were that volunteers be of normal and stable weight, exercise less than four one-hour sessions per week, abstain from exercise during the study, not participate in sports on a routine or competitive basis, abstain from alcohol throughout the testing period, consume coffee (or other caffeinated beverages) on a routine basis, have normal gastrointestinal function, consume a typical western diet with no extreme food, beverage or dietary-supplement intakes, abstain from supplements during the study, be free of medications that might influence weight or fluid and electrolyte balance, be free of any chronic illnesses, live and work in an environment of ambient temperature with no significant temperature and humidity variation and have a fairly routine schedule including nocturnal sleep patterns.

Subjects were recruited via newspaper advertisement, signs posted in coffee shops and emails to previous study participants. Seventy-two individuals responded and were telephonically screened to verify whether they met age, weight, medical, lifestyle and diet criteria by self-report. The study was briefly described to those who met initial criteria, and they were invited to on-site screening. Forty-three individuals reported for the on-site screening conducted by two of the investigators, two registered dietitians, a registered nurse and a screening coordinator. The volunteers rotated through five stations. First, they read and listened to an audiotape of the informed consent form. After their questions were answered, they gave informed consent. At the second station, a medical history form was completed by a registered nurse who also measured pulse, respiration, temperature, blood pressure, height, (scale stadiometer) and weight (Model SR241, SR Instruments, Inc, Tonawanda, NY). The last three stations were one-on-one structured interviews conducted by the investigators to assess 1) volume and type of usual beverage consumption, 2) weight, dieting and physical activity history and 3) normal food intake and ability to consume the monotonous study diet. Serving trays depicting the actual study diet were shown to potential candidates, and they were asked about their willingness and ability to consume these same foods, during both trials, for four consecutive days (the stabilization day and the three trial days).

After the screening, the investigators reviewed the information. Thirty-five subjects met criteria and were invited to participate in the study. Three who were invited declined; thus, thirty-two subjects were enrolled.

Of the thirty-two subjects enrolled, two changed their minds and voluntarily withdrew on stabilization day. Additionally, one subject reported having diarrhea the day before stabilization day and thus was withdrawn by the investigators, and two more subjects were withdrawn due to weight instability between the two trials. Thus, 27 healthy males, 19 to 38 years of age, completed the study.

The Institutional Review Board at MDS Pharma Services, Lincoln, NE, approved the study protocol.

### Experimental Design

A within-subject design was used to evaluate the effect of two different diets, identical except for plain water, on hydration status, as determined by body weight change and urine analysis. During two consecutive weeks, subjects were confined for two three-day study periods. In a randomized, cross-over and counterbalanced fashion, the subjects were given one of two diets (see Table 1), one that included plain drinking water (Trial A) the other without (Trial B).

### Fluid Requirements

Thirty-five mL per kg body weight, a guideline used in clinical practice [11,12], was selected to estimate total daily fluid requirement. This volume, less water content of the foods in the study diet, was used to determine the volume of beverage for each subject. Beverages were weighed to the nearest gram on a digital bench scale (Model XL6100, Denver Instruments, Arvada, CO). All drinks were provided in glass bottles labeled with the subject's identification number, the date to be consumed, the meal or snack at which it was to be served and a bar code for scanning.

The mean total daily fluid requirement for subjects participating in this study was  $2638 \pm 313$  mL, with a range of 2104–3595 mL. The average amount of water provided by the food portion of the diet was  $550 \pm 32$  mL, with a range of 466–649 mL. Water content of the foods was determined using the Food Processor software, Version 7.6 (ESHA Research, Salem, OR) and data from the manufacturer. Beverage volume averaged  $2088 \pm 285$  mL with a range of 1638–2946 mL.

### Beverages

Beverages included in the study diets were selected to reflect beverages commonly consumed in the U.S. diet with the exception of milk and alcoholic beverages. Comprehensive data on beverage consumption patterns are limited. Water consumption in the United States from 1994–1996 was reported in a paper by Heller, *et al.* [13]. To describe water consumption patterns, Heller and colleagues analyzed data on 14,619 persons from the 1994–1996 Continuing Survey of Food Intakes by Individuals (CSFII), and the 1977–1978 Nationwide Food

**Table 1.** Experimental Diets<sup>1</sup>

	Food	Beverages	
	Trial A & B	Trial A	Trial B
Breakfast	Plain bagel (57)		
	Peanut butter (42)		
	Banana (126)		
	Orange juice	128 mL	128 mL
	Instant coffee	343 mL	343 mL
Noon meal	White bread (42)		
	Sliced turkey breast (84)		
	American cheese (14)		
	Lettuce leaf (15)		
	Mayonnaise (12)		
	Potato chips (28)		
	Chocolate chip cookie (38)		
	Regular cola	343 mL	343 mL
	Caffeine-free diet cola	193 mL	0 mL
	Diet cola	0 mL	193 mL
Mid-afternoon snack	Water	492 mL	0 mL
	Diet cola	0 mL	492 mL
Dinner	Beef patty (71)		
	Hamburger bun (47)		
	Ketchup (11)		
	Mustard (5.5)		
	Macaroni salad (97)		
	Baby carrots (60)		
	Ranch dressing (24)		
	Applesauce (126)		
	Water	193 mL	0 mL
	Caffeine-free diet cola	150 mL	343 mL
Evening snack	Vanilla ice cream (66)		
	Orange juice	215 mL	215 mL
	Energy	10.1 MJ	10.1 MJ
		2421 kcal	2421 kcal
	Total fluid (mL)	2615	2615
	food (mL)	558	558
	beverage (mL)	2057	2057
	Plain water (mL)	685	0
	Protein (g)	68	68
	Carbohydrates (g)	301	301
	Fat (g)	112	112
	Potassium (mg/mEq)	2820/72	2855/73
	Sodium (mg/mEq)	3625/158	3616/157
	Caffeine (mg)	155	245

<sup>1</sup> Portion size (g) in parenthesis.

Consumption Survey (NFCS). Analysis showed that approximately one-third of the total beverage intake of persons aged 20 to 64 years of age was consumed as plain water.

Although not the purpose of a study by Michaud and colleagues, they also found that approximately 33% of the beverage volume was in the form of plain water [14]. Michaud and colleagues analyzed data on 47,909 males, 40 through 75 years of age from all 50 states, who participated in the prospective Health Professionals Follow-up Study.

To reflect the average consumption of plain water in the U.S., one-third of the total beverage volume in Trial A was

plain water. An equal amount of a non-caloric, caffeinated cola replaced the water in Trial B. The remaining volume was equally divided among: juice, coffee, caloric, caffeinated cola and non-caloric, non-caffeinated cola in both trials (see Table 1).

Energy and nutrient content of the diet was constant in both trials with the exception of potassium and sodium, which were minimally affected by the beverage substituted for the drinking water. Sodium in Trial A averaged  $156.8 \pm 15.9$  mEq ( $3606 \pm 365$  mg), and potassium averaged  $69.0 \pm 8.3$  mEq ( $2609 \pm 323$  mg). Sodium in Trial B averaged  $156.4 \pm 15.8$  mEq ( $3597 \pm 364$  mg) and potassium  $69.9 \pm 8.4$  mEq ( $2725 \pm 327$  mg). Caffeine content of Trial A and B averaged  $2.09 \pm 0.04$  and  $3.30 \pm 0.06$  mg/kg BW, respectively.

The variance in caffeine between the two trials was not considered a confounding factor. This was based, in part, on previous findings that consumption of 75% of the daily beverage intake as caffeinated beverages did not affect hydration compared to consuming 100% as plain water. Caffeine at levels of zero,  $1.4 \pm 0.15$  and  $3.13 \pm 0.35$  mg/kg BW had no effect on any of the indicators of hydration (body weight, urine or blood) measured in a previous study [10].

Beverages were consumed with the morning, noon and evening meals and during the mid-afternoon and evening snacks (Table 1). Coffee was consumed with breakfast. The mean volume of test beverages consumed by subjects was  $2088 \pm 285$  mL and ranged from 1638 to 2946 mL.

Beverages used in this study are commercially available and represent commonly used consumer products. While all attempts were made to blind both the subjects and research staff as to the contents of the beverages, it was impossible to do so for all beverages. While the research staff did not know the specific types of beverage, the subjects' discriminating taste limited the ability to blind totally.

## Diet

Energy level of each subject's study menu was determined from his estimated daily energy requirement, less energy from the beverages. Daily energy requirement was estimated using the factor  $1.3 \times$  resting energy expenditure (REE). REE was calculated using the standard formulas for males 18 to 30 years of age [ $(15.3 \times \text{wt}) + 679$ ] or for males 30 to 60 years of age [ $(11.6 \times \text{wt}) + 879$ ] [15]. Serving sizes of menu items were adjusted accordingly. Energy intake averaged  $10.0 \pm 0.81$  MJ/day ( $2389 \pm 194$  kcal/day) with a range of 8.6 to 12.4 MJ/day (2055–2959 kcal/day).

The same one-day menu was served repeatedly to each subject for four days (the stabilization day and the three study days) during both trials (Table 1). The menu was designed to include foods in which water content was documented and was stable regardless of marginal preparation variability.

**Procedures**

A schematic of the study design and experimental protocol is shown in Table 2. To help prevent the subjects entering the study hypohydrated, they were required to abstain from exercise, alcoholic beverages, over the counter medications and dietary supplements for the three days (Monday–Wednesday) before stabilization day (Thursday). Additionally, subjects were given a list of events that could potentially affect fluid balance and/or weight, e.g., diarrhea, sweating, time in a hot environment constipation. The subjects were instructed to record the occurrence of such an event, which was then reviewed by an investigator. If it was determined that the event disrupted fluid and/or electrolyte balance or usual weight, the subject was disqualified as explained previously.

On Thursday, stabilization day, the subjects reported, in a fasted state, to the research unit. They were weighed, ate the study breakfast on site and were given their prescribed foods and beverages to be consumed off-site for lunch and afternoon snack. Between 5:00 and 6:00 p.m., the subjects checked in for the study, consumed dinner and an evening snack and were weighed. Beginning Thursday evening until the following Monday morning, they were confined and underwent controlled food and beverage intake, urine collection and weight monitoring as per study protocol.

**Body Weight**

A trained investigator weighed subjects, dressed in paper gowns, on a digital platform scale ( $\pm 100$  g, Model SR555, SR Instruments, Inc., Tonawanda, NY). If weight was not identical on the first two measurements, a third measurement was taken and the replicated weight recorded. The subjects were weighed in this manner twice each day, between 7:15 and 8:15 a.m. (after voiding), and again in the evening between 7:30 and 8:30 p.m. Only the morning weight data is included in the present analysis. The evening weight was collected as a quality assurance measure.

**Urine Collection and Analysis**

On Friday morning, upon rising, the pre-trial urine sample was collected from each subject. The first 24-hour urine collection commenced with the second void on Friday and was collected through the first morning void on Saturday. The 24-hour urine collections for Saturday and Sunday proceeded accordingly. An aliquot of the first morning void on Monday was designated as the post-trial sample. The void was then added to the pool to complete the third 24-hour urine collection.

The pre-trial and post-trial urine samples were assayed for chloride, sodium, potassium, osmolality and specific gravity. The sodium/potassium ratio was calculated. Laboratory analyses of the 24-hour urine collections included volume and creatinine as well as chloride, sodium, potassium, osmolality, specific gravity, and a calculated sodium/potassium ratio.

All assays except osmolality were conducted at the MDS Pharma Services Clinical Laboratory, Lincoln NE. Sodium, potassium and chloride concentrations were determined by ion-selective electrodes on a ROCHE/Hitachi 914. Urine creatinine was determined by an analysis based on the Jaffe reaction that was adapted to the ROCHE/Hitachi 914 analyzer. A manual refractometer was used for the specific gravity analysis. All methods have been validated by College of American Pathologists (CAP) standards. Quality control material was analyzed on all instruments each day of use. Quality results are further assured by participating in the CAP proficiency-testing program.

Aliquots were sent to Nebraska Health System Clinical Laboratory, Omaha, NE, for analysis of urine osmolality. The urine samples were shipped in capped containers at refrigerator temperature per Reference Lab Protocol. Urine osmolality was determined by freezing point depression on The Advanced™ OS-MOMETER, Model 3D3 (Advanced Instruments, Inc., Norwood, MA). Quality control material was analyzed each day of use.

**Environment**

The Center for Human Nutrition’s facilities are not designed to accommodate overnight studies; thus, an off-site study

**Table 2.** Schematic of Study Design and Experimental Protocol

Run-In	Stabilization	Trial Period, Confined			Post-Study
Mon, Tues, Wed	Thurs	Fri	Sat	Sun	Mon
Alcohol, OTC medications, dietary supplements, exercise restricted	Diet and beverages provided Weight a.m., p.m.	Pre-trial urine (first a.m. void) Start 1 <sup>st</sup> 24-hour urine	Start 2 <sup>nd</sup> 24-hr urine after first a.m. void	Start 3 <sup>rd</sup> 24-hr urine after first a.m. void	Post-trial urine (first a.m. void) End 24-hour urine
Events which may disturb fluid/electrolyte balance self-recorded	6 p.m. Check-in	←←←	Food and beverage consumption controlled Weight measured a.m., p.m.	→→→	8 a.m. Discharge  Repeat protocol for second study period

setting was selected. Several environments were considered, including a metabolic research ward and hospital rooms. MDS Pharma Services (MDS) was selected because its capabilities, quality assurance and physical facilities provided the control requirements of the study. The facility provided the study requirements for 24-hour subject monitoring, monitored restrooms (unlocked only by request), quality assurance measures for data collection, environmental control and research quality food and beverage service.

Subjects were confined in the controlled research unit at MDS Pharma Services during both trials. Belongings were inspected before admission to ensure that the subjects did not carry in food or other prohibited items. Their living space consisted of two lounge areas for watching movies, playing pool, playing cards, studying, reading and other sedentary activities. All exercise was restricted. They slept in curtained bunk areas, two to a cubicle, with consistent, mandatory lights-out and wake-up times; thus, sleep/awake ratio was maintained between the two study periods.

Meals were prepared and served on site at designated times. Subjects lined up in the same order before each meal and were issued their trays and bottles from serving tables. Each subject wore a bar-coded armband that was scanned to ensure that it matched the bar code on the food tray and the beverage bottles he received. Subjects were observed throughout mealtime and were required to show their empty tray and bottles leaving the dining area. Restrooms were locked so that subjects had to summons a technician to receive their collection jug and use the restroom. Subjects were queried once each day about bowel habits and other symptoms. Any comments were recorded and reviewed with the investigators.

Temperature and humidity of rooms were controlled to the degree possible (via thermostat) and were determined every four hours with a RH80 Series Thermo-Hygrometer (Omega, Engineering, Inc. Stamford, CT).

### Statistical Procedures

ClinQuick and Oracle database systems were used to collect, store and clean data. System validation was conducted for the study-specific set up required. ClinQuick is a fully integrated remote data acquisition system, which immediately obtains data through an electronic process. Scheduled tests, meal acquisition, beverage consumption and other scheduled transactions are recorded by activating the system with a barcode that activated the appropriate subject records. Collection dates and times are automatically posted in the subjects' records. Automatic entry is used for all scheduled procedures. Information such as adverse events and urine collection were manually entered into ClinQuick by research technicians and visually verified. Field checks and logic checks were developed within ClinQuick to verify data accuracy. Upon completion of the study, the data was extracted from Oracle and converted into SAS® (Cary, North Carolina) datasets for analyses.

Data were then analyzed using paired *t*-tests to measure differences between each subjects Trial A and Trial B results (SPSS 9.0 for Windows, Chicago, IL.). Normality of the data was verified using Kolmogorov-Smirnov techniques. Data are presented as mean  $\pm$  SD unless otherwise noted.

## RESULTS

### Participants Characteristics

The characteristics of the subjects who completed both trials of this study are presented in Table 3. The subjects represent a wide variety of professions and vocations, as well as students.

### Body Weights

Pre- and post-trial body weights are presented in Table 4. Most of the subjects lost a small amount of weight on both trials. Of the 54 pre/post weight change measurements, 49 (91%) showed weight loss, three showed no change and one showed weight gain. Mean  $\Delta$ BW on Trial A was  $-0.5$  kg and on Trial B was  $-0.6$  kg. No significant difference was found between mean weight changes on Trial A compared to Trial B ( $p = 0.146$ ).

### Urinary Variables

Twenty-four hour urine volumes are presented in Table 5. Data are presented for only 22 subjects because of sample collection errors. One sample was lost into the toilet, and two samples were erroneously poured into the wrong collection jugs, necessitating excluding 24-hour volume data for five subjects. Individual 24-hour urine volumes ranged from 1413 mL to 2853 mL. Mean volumes were 2009 mL on Trial A and 1994 mL on Trial B. No significant differences were found for urinary output between the two trials.

Twenty-four hour urinary creatinine, osmolality, specific gravity, chloride, sodium, potassium, and sodium/potassium ratio are presented in Table 5. No significant differences were found between the two trials for any of the measures. All group means were within reference ranges.

Pre- and post-trial chloride, sodium, potassium, sodium/potassium ratio, creatinine, osmolality and specific gravity are

**Table 3.** Subject Characteristics<sup>1,2</sup>

	Mean $\pm$ SD	Range
Age (years)	26.6 $\pm$ 5.0	19–38
Height (cm)	178.4 $\pm$ 5.5	168–191
Weight (kg)	75.0 $\pm$ 8.6	59.4–101.7
BMI	23.7 $\pm$ 2.3	19–29
Percent body fat	13.4 $\pm$ 5.1	6.6–26.7
Body surface area (m <sup>2</sup> )	1.93 $\pm$ 0.12	1.67–2.28

<sup>1</sup>  $\bar{x} \pm$  standard deviation.

<sup>2</sup> n = 27.

**Table 4.** Pre- and Post-Trial Body Weights<sup>1,2</sup>

	Trial A (n = 27)	Trial B (n = 27)
Pre-trial body wt (kg)	74.9 ± 8.5	74.9 ± 8.5
Post-trial body wt (kg)	74.4 ± 8.5	74.3 ± 8.5

<sup>1</sup>  $\bar{x} \pm$  standard deviation.

<sup>2</sup> Paired *t*-test comparisons of Trial A and Trial B were made. All results were NS (*p* > 0.05).

shown in Table 6. All pre and post treatment group means were within reference ranges and indicative of normal hydration. No significant differences for any indices were found between Trial A and Trial B in pairwise comparisons.

### Heat and Humidity

Heat and humidity were controlled to the degree possible and monitored to help ensure a relatively consistent environment during both testing periods. The average temperature and humidity during the day (8 a.m. to 10 p.m.) was 22.6° C and 45.8% for the first testing period and 22.8° C and 41.3% for the second. Temperatures and humidity at night (11:00 p.m. to 7:00 a.m.) were 21.7° C and 48.8% for the first period and 21.8° C and 44.9% for the second.

## DISCUSSION

The purpose of this study was to measure, in healthy, sedentary individuals, the effect on hydration of two regimens, one that included drinking water as part of the dietary beverages and one that did not. Three key decisions in designing the study were determination of fluid intake, caloric intake and indices of hydration. Regarding fluid intake, we chose a commonly used clinical recommendation, 35 mL/kg. In the end, total water content of the study diet (food and beverage) averaged 2638 ± 313 mL with a range of 2104–3595 mL. Beverage volume averaged 2087 ± 285 mL (range 1638–2946 mL), which equates to 70.6 fl oz, or 8.8 eight-ounce servings.

Of practical interest, this study shows that 35 mL/kg water can translate to a fluid intake that is similar to the common “eight, eight ounce glasses” per day. One must bear in mind the relatively low water content of the study diet. While the foods composing the diet were selected because of their constant water content regardless of holding time and preparation, they were, however, not atypical of the diet of many Americans. Diets that contain more fruits and vegetables, pasta, rice, soup, yogurt and so forth would have a higher water content. Alternately, fluid requirements would increase in the free living environment where there is more physical activity and sweating. Also of practical interest is comparison of the subject’s fluid intake to the current RDA for water, 1.0–1.5 mL/kcal [6]. Converting the total fluid intake from food and beverages to a volume per kcal equates to 1.1 mL/kcal. This study was not

designed to determine fluid requirements, but these results are comparable to the Food and Nutrition Board’s allowance for adults under average conditions of energy expenditure and environmental exposure [6].

The second major consideration was estimating energy expenditure. Weight loss, although not statistically or clinically significant, occurred during both trials. In view of the fact that urinary data are not consistent with dehydration, the most plausible explanation for the small weight loss includes glycogen, muscle and/or fat loss that would be expected in non-obese males in negative energy balance. The equation used to determine energy expenditure was from the 1989 RDA, using REE multiplied by a factor of 1.3 (very light activity) [15]. Average intake was 2389 kcal/day. Using the current Estimated Energy Requirement (not published at the time of this study), the average estimated energy expenditure for sedentary activity would have been higher, 2564 kcal/day. Although this still may be low for this group, it appears to be more valid than the previous RDA estimation.

A third key design decision was selection of feasible and sensitive indices for measuring small changes in hydration. Body weight change was selected as the primary measure of hydration based on previous findings [10], and the principle that, in subjects who are eating adequately, an acute (up to 72 hours) change in body weight will be due almost solely to a change in total body water [16]. The acceptance and substantiation of body weight as a sensitive, accurate, straightforward and affordable indicator of hydration status crosses disciplines, including medicine, physiology and exercise science [16–30]. Valtin and Schafer, in their textbook on renal function, state that body weight is more accurate and simple and can be determined more cheaply than other methods of assessing fluid balance and that its usefulness for measuring body weight in the field of fluid and solute balance cannot be overemphasized [16].

Identification of an accurate, simple, uncomplicated, gold-standard measure that is sensitive to small changes in hydration status has been the goal of several investigators [10,17,18,23–31]. Urinary measures such as color, specific gravity and osmolality have been reported to be more sensitive at indicating moderate levels of hypohydration than are blood measurements of hematocrit, serum osmolality and sodium concentrations [26,29]. However, urine color and osmolality may not correlate to hydration status during acute rehydration [32]. The sensitivity of urinary measures during hypohydration was substantiated by Francesconi *et al.* [26]. They assessed urinary and circulatory indices of hydration in male and female military personnel and found that mild dehydration, determined by weight loss was associated with elevated urinary specific gravity (USG) and creatinine (first-void samples), but was not reflected in hematocrit or serum osmolality. However, contrary findings were reported by Popowski and colleagues [30]. They concluded 1) that plasma osmolality is the best measure of euhydration, 2) that it accurately

**Table 5.** Twenty-Four Hour Urine Volumes and Select Indices<sup>1,2</sup>

	Trial A (n = 22)	Trial B (n = 22)	Reference Range <sup>3</sup>
Urine volume (mL)	2009 ± 341	1994 ± 320	NE
Urinary creatinine (mg/24 hours)	2018.2 ± 607.9	2220.67 ± 563.5	NE
Urinary osmolality (mOsm/kg)	439.8 ± 71.6	467.5 ± 80.1	50–1000
Urinary specific gravity	1.013 ± 0.003	1.014 ± 0.004	1.005–1.030
Urinary chloride (mEq/L)	75.68 ± 0.49	77.23 ± 8.50	NE
Urinary sodium (mEq/L)	83.58 ± 11.38	86.88 ± 10.92	40–220
Urinary potassium (mEq/L)	32.34 ± 6.67	34.24 ± 8.25	25–120
Sodium/Potassium ratio	2.76 ± 0.68	2.76 ± 0.71	NE

<sup>1</sup>  $\bar{x} \pm$  standard deviation.<sup>2</sup> Paired *t*-test comparisons of Trial A and Trial B were made. All results were NS ( $p > 0.05$ ).<sup>3</sup> NE indicates no normal range established for this clinical lab.**Table 6.** Pre- and Post-Trial Urinary Indices<sup>1,2</sup>

	Trial A (n = 27)	Trial B (n = 27)	Reference Range <sup>3</sup>
Pre chloride (mEq/L)	82.5 ± 31.67	82.1 ± 34.4	NE
Post chloride (mEq/L)	81.2 ± 19.5	78.8 ± 22.2	NE
Pre sodium (mEq/L)	130.7 ± 136.1	110.0 ± 41.7	40–220
Post sodium (mEq/L)	91.0 ± 18.3	89.3 ± 21.0	40–220
Pre potassium (mEq/L)	35.67 ± 17.14	36.70 ± 15.71	25–120
Post potassium (mEq/L)	38.38 ± 13.49	41.40 ± 14.50	25–120
Pre sodium/potassium ratio	4.729 ± 7.875	3.410 ± 1.621	NE
Post sodium/potassium ratio	2.621 ± 0.918	2.320 ± 0.654	NE
Pre osmolality (mOsm/kg)	681.3 ± 216.2	726.7 ± 187.6	50–1000
Post osmolality (mOsm/kg)	585.6 ± 200.8	557.3 ± 192.5	50–1000
Pre specific gravity	1.021 ± 0.006	1.023 ± 0.005	1.005–1.030
Post specific gravity	1.017 ± 0.006	1.017 ± 0.006	1.005–1.030

<sup>1</sup>  $\bar{x} \pm$  standard deviation.<sup>2</sup> Paired *t*-test comparisons of Trial A and Trial B were made. All results were NS ( $p > 0.05$ ).<sup>3</sup> NE indicates no normal range established for this clinical lab.

measures modest changes in hydration status during acute dehydration and rehydration and that 3) urine specific gravity and osmolality, while sensitive to small changes in hydration status, lag behind plasma osmolality during acute dehydration.

Two of several differences between the Francesconi and Popowski studies were that the subjects (n = 230) in Francesconi's study were male and female members of U.S. Army units undergoing routine training with evaluation occurring over a period of 44 days, whereas Popowski's study included 12 male athletes in which dehydration (5%) was induced in a little less than three hours (168 ± 50 minutes). Thus, it appears that the changes in plasma osmolality observed during acute dehydration dissipate over time, even in a state of mild dehydration as measured by weight loss. This most likely reflects renal adaptations to maintain plasma volume.

In view of the fact that this study was designed to evaluate hydration status over a period of days, because the intent was not to induce acute dehydration, and in view of results from a previous study similar in nature to the one reported here [10], urinary indices were chosen to corroborate body weight data. We also selected 24-hour volumes, which are assumed more reflective of hydration status. To our knowledge, this is only

the second study to date to collect 24-hour urine while assessing hydration status [10,33]. This is significant in view of the fact that single urine samples are not representative of 24-hour samples due to circadian rhythmicity [34–38].

In summary, the results herein are preliminary, but suggest that inclusion of plain drinking water compared to exclusion of plain drinking water in the diet for three days did not affect the measures of hydration used in this study. This information is useful to those in crisis situations when no safe water supply is available, to those who travel to destinations with questionable water quality, to those who experience unneeded worry if they do not have access to plain water each day and even to those whose personal preference does not include plain water.

It is not the intent of the authors to imply that one need not drink water as such. Plain water, as well as other beverages, will support hydration. Additionally, one should not overlook the benefits of water beyond hydration. For example, the public water supply is a major source of fluoride [13,39]. The benefits of fluoridated water are proven. Other potential benefits of fluid intake remain elusive and are deserving of study, for example, the potential role of fluids in disease prevention [14,40–44]. Both the types of fluids consumed, as well as total daily

volume, and the association not only with hydration but also with health and disease is a pertinent and deserving area of future study. It is our hope that the data reported here serves as a catalyst for future research.

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