

## Original Research

# The Effect of Caffeinated, Non-Caffeinated, Caloric and Non-Caloric Beverages on Hydration

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**Key words:** hydration, caffeine, fluid balance, dehydration, 24-h urine volume, fluid requirements

**Objective:** To examine the effect of various combinations of beverages on hydration status in healthy free-living adult males.

**Methods:** In a counterbalanced, crossover manner, 18 healthy adult males ages 24 to 39, on four separate occasions, consumed water or water plus varying combinations of beverages. Clinical guidelines were used to determine the fluid allowance for each subject. The beverages were carbonated, caffeinated caloric and non-caloric colas and coffee. Ten of the 18 subjects consumed water and carbonated, non-caffeinated, citrus soft drink during a fifth trial. Body weight, urine and blood assays were measured before and after each treatment.

**Results:** Slight body weight loss was observed on all treatments, with an average of 0.30% for all treatments. No differences ( $p > 0.05$ ) among treatments were found for body weight changes or any of the biochemical assays. Biochemical assays conducted on first voids and 24-hour urines included electrolytes, creatine, osmolality and specific gravity. Blood samples were analyzed for hemoglobin, hematocrit, electrolytes, osmolality, urea nitrogen, creatinine and protein.

**Conclusions:** This preliminary study found no significant differences in the effect of various combinations of beverages on hydration status of healthy adult males. Advising people to disregard caffeinated beverages as part of the daily fluid intake is not substantiated by the results of this study. The across-treatment weight loss observed, when combined with data on fluid-disease relationships, suggests that optimal fluid intake may be higher than common recommendations. Further research is needed to confirm these results and to explore optimal fluid intake for healthy individuals.

## INTRODUCTION

The literature regarding hydration largely focuses on physically active [1–3] or ill individuals [4,5], while dietary consumption surveys have shed light on the types and quantity of beverages consumed [6,7]. But surprisingly little is published in the scientific literature about the hydration status of healthy, free-living adults or the effect of various beverages on hydration status. Despite the paucity of data, guidelines on the optimal amount and types of fluid to consume are widespread and considered common knowledge. Newspapers, magazines, the electronic media and even textbooks for health professionals [8] make statements about recommended fluid intake and types of beverages to consume. Although slight variations exist, the prevalent messages are that eight 8-ounce glasses

(1885 mL) will assure hydration and that caffeinated beverages should be excluded or the volume significantly reduced when assessing fluid intake.

Medical texts cover symptoms of and differential diagnoses and appropriate interventions for dehydration [4,5]. Likewise, the exercise physiology and sports nutrition literature is replete with studies documenting the consequences of dehydration on physical performance, determining methods of assessment and testing intervention strategies [1,2]. However, data on determining and maintaining euhydration of healthy, free-living, sedentary to moderately active individuals who are not under environmental or physiological stress are lacking. In this present study, we examined the effects of caffeinated caloric and non-caloric, and non-caffeinated caloric and non-caloric beverages on hydration status in free-living males. A second

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purpose was to determine if the measures of dehydration utilized in clinical conditions and in subjects under environmental and physiological stress are appropriate for assessing hydration status in healthy subjects under normal living conditions.

## MATERIALS AND METHODS

### Experimental Design

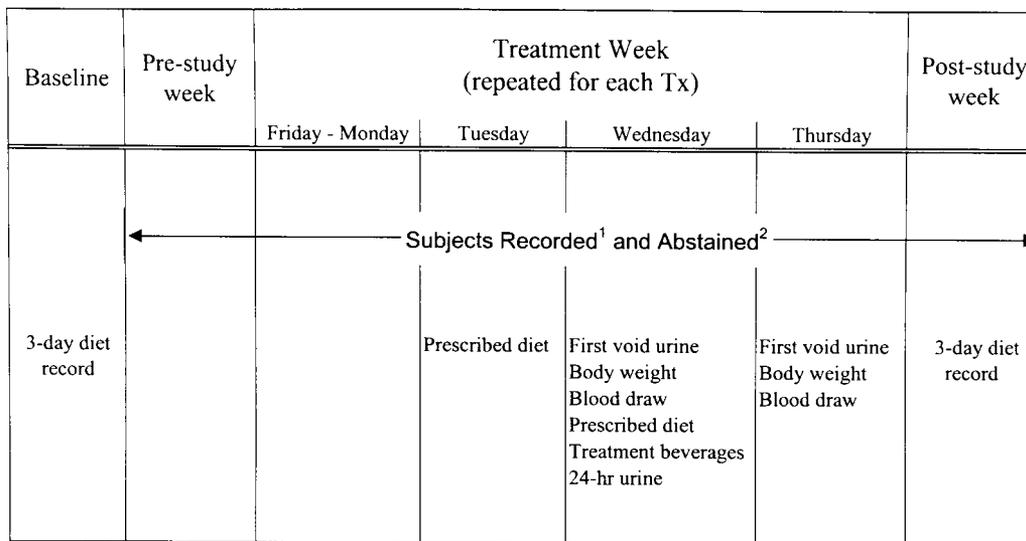
A within-subject design was used to evaluate the effect of five different treatments on the variables of body weight, urine and blood parameters. Holding each subject's total volume constant, the treatments (Tx) were water only (Tx A), equal amounts of water and caffeinated, carbonated cola (Tx B), equal amounts of water and caffeinated, carbonated non-caloric cola (Tx C), equal amounts of water, caffeinated, carbonated cola, caffeinated, carbonated non-caloric cola and instant coffee (Tx D) and half water and half carbonated citrus non-caffeinated soft drink (Tx E). Provision of treatments A-D was counterbalanced and randomized. Provision of Tx E was not randomized and was undertaken as an ancillary experiment by a subset of 10 volunteers after successful completion of Tx A-D. All aspects of the study were approved by the Institutional Review Board at the University of Nebraska Medical Center.

Subjects consumed treatment beverages on Wednesdays. On Tuesdays and Wednesdays of each week, subjects followed a prescribed diet. They reported to the laboratory each week on Wednesday and Thursday mornings for collection of blood,

urine and body weight measurements (Fig. 1). Subjects were free-living and allowed to carry on with their usual activities that were consistent with the protocol. After post-treatment data collection on Thursday morning, subjects followed their usual dietary habits, which included their normal caffeine beverage consumption. To monitor unusual variability, subjects recorded data on weight, urinary frequency, stool output and other routes of fluid loss during all days of the study period and for one week before and after the study. Any exceptions to the protocol, such as exertion (e.g., shoveling snow), diarrhea or other situations that could affect hydration were recorded. If subject experienced circumstances that could alter fluid balance, testing was postponed until the following week.

### Subjects

Males 19 to 39 years old were recruited through flyers, advertisements and mailings distributed throughout the university medical center campus. Potential subjects were screened via telephone interview to determine potential eligibility. To be considered, volunteers had to be of normal and stable weight, exercise less than four one-hour sessions per week, not participate in sports on a routine and competitive basis, be willing to abstain from alcohol on specified days of the testing period, have a usual, average caffeine consumption between 20 mg and 1000 mg/day, have normal gastrointestinal function, consume a diet with no extreme food, beverage, or dietary supplement intakes, be willing to abstain from supplements during the study, be free of medications that might influence weight or



<sup>1</sup> Subjects recorded:  
 Body weight am., pm.  
 Urinary frequency  
 Stool output  
 Other fluid losses

<sup>2</sup> Subjects abstained from:  
 Exercise  
 Dietary supplements  
 Caffeine-containing medications and herbs  
 Alcoholic beverages

Fig. 1. Schematic of study protocol.

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 day to day, including nocturnal sleep patterns. Subjects who met eligibility requirements participated in an orientation meeting and thereafter provided written informed consent to participate in the study.

### **Fluid Requirement**

Because no standard water requirement exists for free-living humans [3] and because fluid provision needed to be standardized for this study, clinical guidelines were selected to determine the total daily fluid allowance for each subject. The intent of fluid provision was to neither over nor under hydrate as both could mask treatment effect. Thirty-five mL per kg body weight per day, a guideline used in clinical practice [9,10] was selected. From this total, water from foods in the study diet and 300-mL for metabolic water was subtracted. The remainder was determined to be the beverage quantity for each subject. The mean volume of test beverages consumed by subjects was  $1745 \pm 408$  mL and ranged from 1202 to 2879 mL.

### **Beverage Allotment**

All beverages used in this study are commercially available and represent commonly used consumer products. All soft drinks were provided in unlabeled (except for a code number) glass bottles by a commercial bottler. Commercially bottled spring water was provided in unlabeled (except for a code number) plastic bottles as delivered from the bottler. Instant coffee was purchased by the investigator and was prepared the morning of the study using bottled water and provided in thermoses. Based on each subject's calculated fluid requirement, individual beverage allotments were weighed to the nearest gram on a digital bench scale (Model XL 6100, Denver Instruments, Arvada, CO). The amounts of the beverages to be consumed were marked on sequentially numbered bottles to guide the subject regarding the amount to drink in each time period. Except for Tx D, equal amounts of each beverage were consumed in four-hour periods between 6:00 a.m. and 10:00 p.m. In Tx D, the entire allotment of coffee (1/4 of the day's fluid) was consumed at the time of the subject's usual consumption, which for all subjects was between 6:00 and 10:00 a.m. The other three beverages were consumed in equal volumes in each of the three remaining periods of the day.

### **Diet**

Based on subjects' three-day food records and subject interviews, a registered dietitian developed a personalized one-day menu for each subject. Diets were designed to reflect normal eating habits. On a self-selection basis, in a free-living environment, subjects followed the diet for two days each week, the pre-treatment day (Tuesday) and the treatment day

(Wednesday). The same foods and quantity of fluid were consumed each Tuesday and Wednesday throughout the study period. Beverages consumed Tuesday were assigned based on the subjects' usual consumption and included juice, soft drinks, milk, coffee and tea. On Wednesday, the assigned beverage treatment replaced the usual beverages. Subjects were given printed copies of the daily menus on which they verified consumption and recorded any variations.

### **Procedures**

During the two weeks before treatment commenced, subjects received detailed instruction and training. Subjects received a portable scale accurate to 0.2% at 100 gm (Model SR241, SR Instruments, Inc, Tonawanda, NY) and procedures for collection of twice-daily body weight measurements at home for the duration of their participation in the study (seven to eight weeks). Subjects also received Home Data Booklets in which to record all daily output information, including urination frequency, stool frequency and type, sweating or vomiting. Any variations from the protocol were also recorded in the booklet. Subjects turned in and received booklets each week.

Tuesday of each week was a stabilization day, whereby subjects consumed the prescribed study diet. On Wednesday, treatment day, subjects collected the first urine void upon waking and reported to the laboratory in a fasted state. Upon arrival, subjects turned in first void samples and were interviewed regarding any variations from protocol. Subjects then rotated through stations to be weighed, have blood drawn, receive the day's beverage allotment and receive urine collection containers for the 24-hour urine collection. Subjects followed their study diet, consumed test beverages and collected all urine for the rest of the day.

Thursday morning upon rising, subjects voided urine in a separate container from the 24-hour collection container, then returned to the lab. After turning in urine samples, the subjects rotated through stations to collect post treatment data and receive supplies for the following week. The process was repeated each week.

### **Body Weight**

Pre-treatment and post-treatment fasted early-morning body weights of each subject were measured by a trained investigator on a digital scale accurate to 0.1% at 100 gm (Model SR555, SR Instruments, Inc., Tonawanda, NY). If weight was not identical on the first two measurements, a third measurement was taken and replicated weight used. Subjects were instructed to void immediately before weighing. Subjects wore only shorts that were provided, with the weight of the shorts being subtracted to obtain true body weight.

To monitor daily weight changes beyond the two weekly laboratory weight measurements, subjects weighed at home on the portable scale provided. Subjects weighed twice each day,

upon rising and before retiring, in the nude, after voiding. Subjects recorded the weight in the Home Data Booklet.

### **Urine Collection and Analysis**

For each treatment, each subject collected three urine samples: pre, 24-hour and post. Pre was the first voided urine on test day, before treatment began. The 24-hour urine collection was the urine voided during the test day, beginning after weighing on Wednesday morning, through the time at which weight was measured on Thursday. The post urine was the first voided urine on Thursday morning. A 5-mL aliquot from this sample was kept separate for analysis and the remaining volume mixed with the 24-hour collection.

Aliquots were taken directly to the Nebraska Health System Clinical Laboratory, Omaha, NE for analysis. Pre and post urine samples were analyzed for chloride, sodium, potassium, creatinine, osmolality and specific gravity. The 24-hour urine was analyzed for creatinine, osmolality, specific gravity and volume. Urine chloride, sodium and potassium were determined by specific ion electrodes with *Vitros* dry, multilayered slides on the *Vitros* 950 AT automated chemistry analyzer used according to the manufacturer's directions (Johnson and Johnson Clinical Diagnostics, Inc. Rochester, NY). Creatinine was also determined on the *Vitros* by the multilayered film system converting creatinine to sarcosine and urea. The sarcosine is oxidized enzymatically, and the hydrogen peroxide formed reacts with a leuco dye to produce a colored product. Urine osmolality was determined by freezing point depression on The Advanced™ OSMOMETER, Model 3D3 (Advanced Instruments, Inc. Norwood, MA). Specific gravity was determined on a refractometer. To monitor compliance to Tx A (water), urine caffeine was analyzed using a 5-mL aliquot from the 24-hour urine. Twenty-mL aliquots of the 24-hour urine were frozen and stored at  $-70^{\circ}\text{C}$  until shipped on dry ice for caffeine analysis. Caffeine analysis was done at Toxicology Laboratories, Pathology and Laboratory Medicine Services, VA, North Texas Health Care Systems, Dallas, TX.

Caffeine was determined by gas chromatography mass spectrometry with 1-butyl-3,7-dimethylxanthine as the internal standard. Caffeine was extracted by the following method: to 5 mL of urine was added 100  $\mu\text{L}$  of a 20  $\mu\text{g}/\text{mL}$  solution of the internal standard and 4 mL of pH 5 acetate buffer. After the tubes were vortexed, the solution was transferred to ChemElut™ 1010 diatomaceous earth extraction columns (Varian Sample Preparation, Harbor City, CA). After a two-minute equilibration time, 12 mL of methylene chloride was added and the eluant collected. After another two-minute equilibration time, another 12 mL of methylene chloride was added and the eluant again collected. The collected eluant was reduced to about 7 mL by evaporation, transferred to a 10 mL screw capped tube and washed with 0.5 mL of 6N KOH. After centrifugation, the 6N KOH was removed by aspiration. Any excess aqueous solution was removed from the

methylene chloride layer with anhydrous sodium sulfate and subsequent centrifugation. The methylene chloride was transferred to a clean tube and evaporated to dryness. The extracts were reconstituted with 50  $\mu\text{L}$  of acetonitrile and transferred to the autosampler for analysis. The analysis was performed on a Hewlett Packard (Hewlett Packard Company, Wilmington, DE) Model 5890 Series II gas chromatograph coupled to a Hewlett Packard Model 5972 Mass Selective Detector. The GC column was a Hewlett Packard HP-1, 12.5m $\times$ 0.25 mm $\times$ 33  $\mu\text{m}$  film. Three microliters were injected into the gas chromatograph at an injection port temperature of 250°C and a heat pressure of 3.0 psi. The oven temperature was programmed from 180°C to 250°C at 25°C/min. The MDS was set to monitor ions M/Z 109 and 194 for caffeine and M/Z 236 and 219 for 1-butyl-3,7-dimethylxanthine. The respective ion ratios were required to be within 20% of the 1000 ng/mL caffeine calibrator for acceptability.

### **Blood Collection and Analysis**

Blood samples (approximately 10 mL) were drawn from a superficial vein, usually from the forearm, while subjects were seated or lying down (to prevent fainting). Blood was collected directly into three Vacutainer® collection tubes. Whole blood was collected into a tube with EDTA additive for hemoglobin and hematocrit analysis, a tube with no additives (clot tube) for serum osmolality, and a tube with PST gel with lithium heparin for chloride, sodium, potassium, urea nitrogen, creatinine and protein. Specimens were taken immediately to Nebraska Health Systems Laboratory, Omaha, NE for analysis. Hemoglobin and hematocrit were measured on a automated cell counter, CELL DYN® 4000 (Abbott Laboratories, Abbott Park, IL). Serum osmolality was determined by freezing point depression in The Advanced™ OSMOMETER, Model 3D3 (Advanced Instruments, Inc. Norwood, MA). Serum chloride, sodium and potassium were determined by specific ion electrodes with *Vitros* dry, multilayered slides on the *Vitros* 950 AT automated chemistry analyzer used according to the manufacturer's directions (Johnson and Johnson Clinical Diagnostics, Inc. Rochester NY). Urea nitrogen, creatinine and total protein were also determined on the *Vitros* with the instrument's multilayered film systems.

### **Beverage Analysis**

Random samples of all beverages except the bottled water were sent to the previously mentioned Toxicology Laboratories in Dallas, TX for caffeine analysis. Beverages were diluted with water at the time of analysis. The coffee was diluted 1 in 500 and the colas 1 in 150. The method for determining caffeine was described earlier in the section on urine collection and analysis.

### **Statistical Procedures**

Data were analyzed (SPSS 9.0 for Windows, SPSS Corp., Chicago, IL.) using nonparametric methods since the data was

**Table 1.** Subject Characteristics<sup>1</sup>

|                                     | $\bar{x} \pm SD$ | Range       |
|-------------------------------------|------------------|-------------|
| Age (years)                         | 28.7 $\pm$ 4.0   | 23.4–38.5   |
| Height (cm)                         | 181.7 $\pm$ 8.2  | 162.6–193.0 |
| Weight (kg)                         | 80.1 $\pm$ 12.2  | 62.7–113.5  |
| Percent Body Fat                    | 16.6 $\pm$ 5.1   | 8.5–24.2    |
| Body Surface Area (m <sup>2</sup> ) | 2.0 $\pm$ 0.2    | 1.8–2.4     |

<sup>1</sup> n = 18

not normal (tested using Kolmogorov-Smirnov methods). To determine differences between all treatments the Friedman test was used, and for selected pairs of treatments the Wilcoxon Signed Rank test was used. It did not become necessary to adjust for multiple comparisons since no p values were found to be <0.05. To determine which lab measurements were most predictive of dehydration (as measured by weight loss), stepwise regression was used (entry criteria  $F < .05$  and removal criteria  $F > .100$ ). The 0.05 level of significance was selected for this study. Data are presented as mean  $\pm$  SD unless otherwise noted.

The intent of this pilot study was to evaluate multiple measures used to document hydration to determine any treatment effect and to assess the sensitivity of the assays. As is the nature of a pilot study, no single hypothesis was proposed for formal evaluation, and thus neither sample size nor power were calculated prospectively.

## RESULTS

### Participants Characteristics

The characteristics of the study population, which consisted of eighteen males, are shown in Table 1. The majority of subjects were graduate students in the health professions, primarily medical students.

### Body Weights

Pre and post body weights by treatment are presented in Table 2. The  $\% \Delta$  BW averaged  $-0.17$ ,  $-0.43$ ,  $-0.33$ ,  $-0.37$  and  $-0.15$  on treatments A, B, C, D and E respectively, equating to 150, 340, 280, 290 and 120 grams respectively. Eighteen subjects participated in a total of 82 treatments. Of those 82 treatments, subjects lost weight on 64 treatments (78%). Although slight trends were observed, no significant

**Table 2.** Pre and Post-Treatment Body Weights<sup>1,2,3</sup>

|                                 | Tx A<br>(n=18)    | Tx B<br>(n=18)    | Tx C<br>(n=18)    | Tx D<br>(n=18)    | Tx E<br>(n=10)    |
|---------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Pre-Treatment Body Weight (kg)  | 80.01 $\pm$ 12.34 | 80.29 $\pm$ 12.19 | 80.18 $\pm$ 12.42 | 80.23 $\pm$ 12.32 | 85.00 $\pm$ 12.29 |
| Post-Treatment Body Weight (kg) | 79.86 $\pm$ 12.19 | 79.95 $\pm$ 12.20 | 79.89 $\pm$ 12.27 | 79.93 $\pm$ 12.27 | 84.88 $\pm$ 12.34 |

<sup>1</sup>  $\bar{x} \pm SD$ .<sup>2</sup> Friedman's test was performed to detect any differences between the groups. The results were insignificant.<sup>3</sup> Paired comparisons of Tx A with Tx B, C, D and E were made using Wilcoxon Signed Rank Test. All results were insignificant.

differences were found between the five treatments. Pairwise comparisons of Tx A to the other treatments were made; none was significant.

### Urinary Variables

Twenty-four hour urine volumes are presented in Table 3. Individual 24-hour urine volumes ranged from 748 to 2602 mL. Mean volumes were equal for Tx A and B, slightly less for Tx C, and 10.6% more (151 mL) for Tx D. The ten subjects participating in Tx E had an average 24-hour volume of 1421 mL compared to 1404 mL for the same subjects on Tx A. No significant differences were found for urinary output between the five treatments or in pairwise comparisons of Tx B, C, D or E compared to Tx A.

Twenty-four hour urinary creatinine, osmolality and specific gravity are presented in Table 3. 24-hour creatinine levels reflected compliance in urine collection. No significant differences were found between the five treatments or in pairwise comparisons of Tx B, C, D and E compared to Tx A for osmolality or specific gravity.

Urinary chloride, sodium, potassium, sodium/potassium ratio, creatinine, osmolality and specific gravity on pre and post treatment morning voids are shown in Table 4. All pre and post treatment group means were within reference ranges. No significant differences for any indices were found between the five treatments or in pairwise comparisons of Tx B, C, D or E compared to Tx A.

Urinary chloride, sodium, potassium and sodium/potassium ratio decreased on all treatments with the exception of potassium on Tx D, a circumstance which most likely reflects the potassium content of coffee. Creatinine increased on all treatments except for an 8.9% decrease in the ten subjects on Tx E. During Tx A, those same ten subjects had a decrease of 0.4%.

Urinary osmolality increased (pre to post) an average of 4.7% on Tx A and 5.3% on Tx D. Osmolality decreased on Tx B, C and E. Urinary specific gravity remained unchanged for Tx A, B and C and increased 0.001 on Tx D. For the 10 subjects participating in Tx E, specific gravity decreased 0.001 on both Tx A and Tx E.

### Circulatory Variables

Data for hemoglobin and other circulatory variables are illustrated in Table 5. All circulatory indices measured remained stable across all treatments. The largest pre/post differences were in

**Table 3.** Twenty-Four Hour Urine Volumes and Select Indices by Treatment<sup>1,2,3</sup>

|                              | Tx A<br>(n=18) | Tx B<br>(n=18) | Tx C<br>(n=18) | Tx D<br>(n=18) | Tx E<br>(n=10) |
|------------------------------|----------------|----------------|----------------|----------------|----------------|
| Urine Volume (mL)            | 1424 ± 395     | 1424 ± 410     | 1403 ± 431     | 1575 ± 524     | 1421 ± 437     |
| Urinary Creatinine (mg/24 h) | 1996.7 ± 285.3 | 1982.3 ± 401.6 | 1937.7 ± 270.7 | 1954.1 ± 248.6 | 1935.8 ± 532.6 |
| Urinary Osmolality (mOsm/kg) | 664.9 ± 200.4  | 666.4 ± 159.7  | 676.0 ± 181.8  | 644.9 ± 200.4  | 663.8 ± 196.5  |
| Urinary Specific Gravity     | 1.018 ± 0.005  | 1.018 ± 0.004  | 1.018 ± 0.004  | 1.017 ± 0.005  | 1.018 ± 0.005  |

<sup>1</sup>  $\bar{x}$  ± SD.

<sup>2</sup> Friedman’s test was performed to detect any differences between the groups. All results were insignificant.

<sup>3</sup> Paired comparisons of Tx A with treatments B, C, D and E were made using Wilcoxon Signed Rank Test. All results were insignificant.

UN/Creat on treatments A, B, C and D, with the mean percentage decrease ranging from 2.6 on Tx C to 5.8 on Tx D. The change on Tx E averaged +0.6%. No significant differences were found between the five treatments or in pairwise comparisons of Tx B, C, D or E compared to Tx A for any measures.

**Caffeine Intake**

Some of the beverages used in treatments B, C and D contained caffeine. Average caffeine intake while on treatment ranged from 114 mg/d (±26) for both Tx B and Tx C to 253 mg/d (±59 mg/d) for Tx D. Calculated as grams per kilogram body weight (bw), the mean intake ranged from 1.4 mg/kg (±0.15) on Tx B and Tx C to 3.13 mg/kg (±0.35) on Tx D.

Based on three-day diet records collected prior to the study, an estimated usual intake was calculated. Usual caffeine intake ranged from 61 mg/d to 464 mg/d or 0.8 mg/kg bw to 6.17 mg/kg bw, with an average intake of 180 mg/d (±113) or 2.3 mg/kg bw (±1.54).

**DISCUSSION**

**Defining Dehydration**

A challenge of this study was selecting indicators of hydration status in healthy males. As defined in a commonly used introductory nutrition textbook, dehydration is the condition in which body water output exceeds water intake [11]. Such a definition takes into consideration all sources of water intake (beverages, food and metabolic) as well as all losses (urine, feces, sweat and the like) Hence, this definition of dehydration is impossible to assess in conditions other than a metabolic ward.

In a report on evaluation and management of dehydration in older adults issued by the American Medical Association’s Council on Scientific Affairs, the authors stated that no absolute definition of dehydration exists. The authors went on to say that a useful definition is the rapid weight loss of greater than 3% of body weight [12]. When using percent decrease in body weight for the clinical assessment of fluid deficit in children, mild dehydration is defined as equal to or less than 5% [13].

**Table 4.** Urinary Indices on Pre- and Post-Treatment Morning Voids<sup>1,2,3</sup>

|                           | Tx A<br>(n=18) | Tx B<br>(n=18) | Tx C<br>(n=18) | Tx D<br>(n=18) | Tx E<br>(n=10) |
|---------------------------|----------------|----------------|----------------|----------------|----------------|
| Pre Cl (mmol/L)           | 70.9 ± 44.6    | 85.9 ± 38.5    | 77.9 ± 48.0    | 85.0 ± 46.0    | 88.0 ± 40.0    |
| Post Cl (mmol/L)          | 69.2 ± 37.4    | 75.8 ± 34.6    | 75.1 ± 34.6    | 78.6 ± 42.2    | 80.9 ± 41.5    |
| Pre Na (mmol/L)           | 99.7 ± 51.2    | 114.3 ± 39.8   | 111.4 ± 38.0   | 124.6 ± 53.7   | 114.0 ± 39.8   |
| Post Na (mmol/L)          | 98.3 ± 41.3    | 106.2 ± 39.4   | 103.5 ± 39.3   | 112.3 ± 58.4   | 110.3 ± 57.0   |
| Pre K (mmol/L)            | 30.17 ± 15.57  | 34.78 ± 20.79  | 35.28 ± 20.70  | 29.50 ± 16.49  | 38.70 ± 19.91  |
| Post K (mmol/L)           | 29.33 ± 13.81  | 31.22 ± 12.35  | 30.28 ± 13.39  | 33.22 ± 16.82  | 28.60 ± 12.09  |
| Pre Na/K                  | 3.954 ± 2.831  | 3.855 ± 1.754  | 4.245 ± 2.442  | 5.335 ± 3.601  | 4.074 ± 3.195  |
| Post Na/K                 | 3.794 ± 1.953  | 3.791 ± 1.642  | 4.003 ± 2.067  | 4.307 ± 2.598  | 4.028 ± 1.566  |
| Pre Creatinine (mg/dL)    | 166.67 ± 62.49 | 164.72 ± 71.19 | 172.28 ± 75.75 | 152.00 ± 66.36 | 170.40 ± 71.72 |
| Post Creatinine (mg/dL)   | 177.78 ± 61.60 | 170.61 ± 52.89 | 176.94 ± 62.86 | 186.22 ± 75.48 | 155.20 ± 37.60 |
| Pre Osmolality (mOsm/kg)  | 652.2 ± 212.4  | 713.8 ± 234.0  | 725.5 ± 263.7  | 681.4 ± 242.9  | 704.2 ± 158.2  |
| Post Osmolality (mOsm/kg) | 682.6 ± 221.1  | 692.8 ± 187.6  | 712.3 ± 221.4  | 717.8 ± 231.4  | 661.8 ± 178.2  |
| Pre Specific Gravity      | 1.019 ± 0.006  | 1.019 ± .007   | 1.020 ± .009   | 1.018 ± .007   | 1.019 ± .005   |
| Post Specific Gravity     | 1.019 ± 0.006  | 1.019 ± .005   | 1.020 ± .006   | 1.019 ± .007   | 1.018 ± .004   |

<sup>1</sup>  $\bar{x}$  ± SD.

<sup>2</sup> Friedman’s test was performed to detect any differences between the groups. All results were insignificant.

<sup>3</sup> Paired comparisons of Tx A with Tx B, C, D and E were made using Wilcoxon Signed Rank Test. All results were insignificant.

**Table 5.** Pre and Post-Treatment Circulatory Indices<sup>1,2,3</sup>

|                                 | Tx A<br>(n=18) | Tx B<br>(n=18) | Tx C<br>(n=18) | Tx D<br>(n=18) | Tx E<br>(n=10) |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
| Pre Hemoglobin (g/dL)           | 14.8 ± 0.9     | 14.8 ± 1.1     | 14.7 ± 0.9     | 14.7 ± 0.9     | 14.6 ± 0.9     |
| Post Hemoglobin (g/dL)          | 14.8 ± 1.0     | 14.9 ± 0.9     | 14.7 ± 0.9     | 15.0 ± 1.0     | 14.8 ± 0.9     |
| Pre Hematocrit (%)              | 43.8 ± 2.5     | 43.4 ± 2.6     | 43.4 ± 2.3     | 43.4 ± 2.7     | 42.9 ± 2.4     |
| Post Hematocrit (%)             | 43.4 ± 2.5     | 43.5 ± 2.6     | 43.5 ± 2.3     | 43.9 ± 2.3     | 43.6 ± 2.2     |
| Pre Serum Osmolality (mOsm/kg)  | 291.9 ± 2.2    | 291.1 ± 2.9    | 291.6 ± 3.7    | 290.6 ± 3.0    | 289.5 ± 2.1    |
| Post Serum Osmolality (mOsm/kg) | 291.7 ± 2.7    | 290.8 ± 2.8    | 291.8 ± 2.8    | 290.3 ± 2.5    | 290.9 ± 2.8    |
| Pre Plasma Cl (mmol/L)          | 102.6 ± 1.7    | 102.4 ± 1.8    | 102.1 ± 1.8    | 101.9 ± 1.5    | 102.1 ± 1.2    |
| Post Plasma Cl (mmol/L)         | 102.8 ± 1.8    | 102.6 ± 2.1    | 102.9 ± 1.2    | 102.7 ± 1.6    | 103.1 ± 1.5    |
| Pre Plasma Na (mmol/L)          | 141.3 ± 1.3    | 141.1 ± 2.0    | 141.1 ± 1.5    | 141.4 ± 1.4    | 140.1 ± 1.8    |
| Post Plasma Na (mmol/L)         | 141.6 ± 1.8    | 141.8 ± 1.4    | 142.0 ± 1.6    | 141.6 ± 1.6    | 141.3 ± 1.5    |
| Pre Plasma K (mmol/L)           | 3.91 ± 0.19    | 3.89 ± 0.24    | 3.85 ± 0.23    | 3.84 ± 0.25    | 3.91 ± 0.27    |
| Post Plasma K (mmol/L)          | 3.84 ± 0.24    | 3.84 ± 0.20    | 3.84 ± 0.16    | 3.86 ± 0.15    | 3.95 ± 0.35    |
| Pre Na/K Ratio                  | 36.203 ± 1.753 | 36.408 ± 2.167 | 36.780 ± 2.295 | 37.000 ± 2.546 | 35.996 ± 2.669 |
| Post Na/K Ratio                 | 37.016 ± 2.423 | 36.986 ± 1.914 | 37.051 ± 1.547 | 36.733 ± 1.589 | 36.013 ± 3.041 |
| Pre Urea Nitrogen (mg/dL)       | 15.5 ± 2.7     | 15.4 ± 2.9     | 15.4 ± 3.7     | 15.2 ± 2.5     | 16.0 ± 3.3     |
| Post Urea Nitrogen (mg/dL)      | 15.1 ± 2.7     | 15.0 ± 3.5     | 15.3 ± 3.6     | 15.1 ± 2.8     | 16.1 ± 2.6     |
| Pre Creatinine (mg/dL)          | 0.99 ± 0.08    | 0.99 ± 0.09    | 0.99 ± 1.01    | 0.98 ± 0.10    | 0.97 ± 0.13    |
| Post Creatinine (mg/dL)         | 1.00 ± 0.11    | 1.01 ± 0.10    | 1.01 ± 0.096   | 1.03 ± 0.12    | 0.97 ± 0.11    |
| Pre UN/Creatinine               | 15.6 ± 2.3     | 15.6 ± 2.7     | 15.5 ± 3.2     | 15.5 ± 2.1     | 16.5 ± 2.0     |
| Post UN/Creatinine              | 15.1 ± 2.4     | 14.9 ± 3.1     | 15.1 ± 2.8     | 14.6 ± 2.1     | 16.6 ± 2.0     |
| Pre Protein (g/dL)              | 7.8 ± 0.3      | 7.7 ± 0.3      | 7.7 ± 0.2      | 7.7 ± 0.4      | 7.5 ± 0.4      |
| Post Protein (g/dL)             | 7.8 ± 0.2      | 7.8 ± 0.3      | 7.8 ± 0.3      | 7.8 ± 0.3      | 7.6 ± 0.3      |

<sup>1</sup>  $\bar{x} \pm SD$ .<sup>2</sup> Friedman's test was performed to detect any differences between the groups. All results were insignificant.<sup>3</sup> Paired comparisons of Tx A with Tx B, C, D and E were made using Wilcoxon Signed Rank Test. All results were insignificant.

Percent body weight is commonly used in athletics and research exploring effect, prevention and treatment of dehydration under physiological and environmental stress. For example, dehydration of more than 2% of body weight is known to impair exercise performance [14–16]. While researchers and clinicians utilize a variety of variables to determine dehydration, body weight is the most practical index.

### Body Weight

Weight as an indicator of hydration status of subjects in the current study would suggest that very slight dehydration occurred in all treatments. The average percent of body weight lost was 0.30% (0.39 SD, range –1.52 to +0.90) for all treatments. The large standard deviation may be due in part to the small sample size. However, it should be noted that this weight loss (0.30%) is not clinically significant. One possible explanation for the mean weight loss seen on all treatments may be insufficient fluid intake. The conservative method utilized to determine the treatment volumes would lend credence to this assumption. Part of the weight loss could also be explained by normal divergence. In healthy individuals body water has been shown to vary  $\pm 0.22\%$  ( $\pm 165$  mL) of body weight under normal, temperate conditions [17].

### Twenty-four Hour Urine Volume

Few human studies have been published reporting 24-hour urine volumes of healthy subjects [18–20]. Textbooks and

reference monographs often cite amounts ranging from 1000 to 1600 mL as the approximate daily adult urine output under normal conditions [8,11,15,21]. Gender specific reference ranges are 600–1600 mL/d for adult females and 800–1800 mL/d for adult males [22].

For the five treatments reported here, the average 24-hour urinary output ranged from 1403 mL to 1575 mL/d. While these volumes approximate the 1400 mL/d frequently cited, wide variation on constant volume of intake was observed. Of the eighty-two 24-hour urine samples collected, volumes ranged from 748 to 2602 mL. These results are consistent with the wide inter and intra individual difference reported by others [18,20].

In healthy sedentary men, it appears that 24-hour urinary output varies greatly even with consistent intake and thus is of limited value as an isolated indicator of hydration status. This is not to suggest that urine volume is a useless parameter. For example, in clinical conditions, when collected sequentially, urinary output may serve to collaborate with other determinants of hydration status.

Pairwise comparisons of Tx B, C, D and E compared to Tx A found no statistically significant differences in 24-hour urine volumes. This is not consistent with studies reporting caffeine-induced diuresis. However, studies reporting an acute diuresis in response to caffeine have, in most cases, used caffeine-naïve subjects and/or have collected urine for only a few hours [23–27]. It has been shown that the amount of urine produced in a specific time period cannot be used to predict output over

a longer time span [20]. Additionally, caffeine tolerance develops after habitual consumption [18,28,29], and individual response is highly variable [20,25].

Neuhäuser-Berthold, *et al.* [19] reported a 41% increase in 24-hour urine excretion when subjects were given six cups of coffee (642 mg of caffeine). However, subjects abstained from all foods and beverages containing methylxanthines for five days prior to treatment. The half-life of caffeine in plasma is three to seven hours [30]. Only four days of abstinence is needed for subjects to become caffeine naïve [31]. Thus subjects in the Neuhäuser-Berthold [19] study would be classified as caffeine naïve. Habitual caffeine users acquire a tolerance to caffeine which reduces its effect [28,32–35]. The subjects in the study presented here were habitual consumers of caffeine. As such, caffeine tolerance can partially explain the lack of treatment effect on urinary output, as half of the fluid consumed on Tx B and Tx C and 75% of the fluid consumed on Tx D was from caffeine containing beverages.

### Laboratory Measurements

Scientists and clinicians use a variety of circulatory and urinary indices to determine or diagnose dehydration. We assessed several such parameters. Analysis of data by treatment and pairwise comparisons found no significant differences. Stepwise regression was performed to evaluate which of the biochemical indices included in this study were most predictive of weight loss (the criteria used were  $F \leq 0.05$  to enter and  $F > 0.100$  to remove). The pre/post differences in the biochemical indices, as well as the 24-hour biochemical indices, were entered into the model. The following variables remained in the model: differences in pre and post blood creatinine, pre and post urine chloride and pre and post total protein. For this model,  $r^2 = 0.26$ , which indicates that only 26% of the variability in weight loss is explained by the variables available for analysis. Further study is needed to determine what assays, if any, are sensitive to small fluctuations in hydration status.

For the most part, researchers measuring hydration status and corresponding changes in blood and urine indices have done so in subjects losing (acutely or chronically) more than 1% bw from water loss [36–38]. Bergeron and colleagues [36] found that collegiate tennis players participating in a three-day tournament in a hot environment maintained overall fluid and electrolyte balance, as indicated by serum osmolality and plasma Na, even though weight loss was 1.3% ( $\pm 0.8$ ) for men and 0.7% ( $\pm 0.8$ ) for women.

As part of a large-scale field study, Francesconi *et al.* [38] assessed urinary and hematologic indices of hydration on 230 male and female members of U.S. Army units. Their results indicated that mild dehydration, associated with elevated urinary specific gravity (USG) and creatinine (first-void samples), was not reflected in the common circulatory indices used, i.e., hematocrit and osmolality. They concluded that urea nitrogen-to-creatinine ratio (UN/Creat) may be a sensitive circulatory

index of imminent hypohydration. When stratified by weight loss and USG  $> 1.03$ , UN/Creat ranged from a mean of 14.7 ( $\pm 3.91$ ) in subjects with weight loss  $< 3\%$ , to a mean UN/Creat of 18.2 ( $\pm 3.95$ ) in subjects with weight loss  $> 3\%$ . Mean UN/Creat in the study presented here ranged from 14.6 ( $\pm 2.1$ ) for post values on Tx D to 16.6 ( $\pm 2.0$ ) for post values on Tx E. Considering the insignificant differences in weight changes and the average 24-hour USG of 1.020 or less, it is not surprising that biochemical laboratory parameters were not indicative of hypohydration. Their cutoff for moderate dehydration however was less than 3% weight loss, significantly greater than the mean 0.3% weight loss in this study. Laboratory tests appear to be most useful with weight changes of greater magnitude than those observed in this study.

### Fluid Intake Recommendations

As explained previously, the beverages used in this study were measured as though all beverages were equal in water content. Of interest is the fact that while the bottled water is 100% water, the cola and citrus drink are only 89 percent water. Thus, available water varied because beverages were not corrected for the volume of water provided. Had adjustments for available water been made, the volumes consumed by subjects would have been increased by 5.5% for Tx B and E, and 2.8% for Tx D. This could explain some of the variability observed between Treatments A, B, D and E. As described in the methods section, the subjects in this study consumed a conservative amount of fluid. Consuming the prescribed fluid the day before treatment day may have left subjects slightly hypohydrated at the onset of all treatments. A study utilizing the same treatments but providing plentiful amounts of fluid would further elucidate the ability of various beverages to maintain hydration status.

Using one day of dietary intake data for 190 males aged 20 to 29 who participated in the 1994 portion of the United States Department of Agriculture Continuing Survey of Food Intakes by Individuals, Stookey [39] proposed a method for water intake adjustment. The formula utilized by Stookey applied quantitative estimates of the effects of alcohol and caffeine to determine the retention, or “bioavailability,” of the water consumed. Assuming subjects are in water balance, Stookey estimated water losses of 1.17 mL/mg caffeine and 10 mL/g alcohol, for an average of an 8% decrease in water bioavailability when caffeine and alcohol are consumed at amounts reported in the 1994 CSFII. It must be kept in mind that the post caffeine challenge urinary excretion estimates used in Stookey’s formula were data on caffeine naïve subjects. Additionally, numerous assumptions were made.

The 1994–1996 Continuing Survey of Food Intake by Individuals [6] found that the average daily intake of beverages for males 20 to 29 years of age was 1,482g and 1,399g for males 30 to 39 years of age. The subjects in this study consumed slightly higher than that, an average of 1745 mL, which

converts to 7.4 8-oz servings. In 64 of the total 82 treatments, or 78% of the time, subjects lost weight. This finding combined with research on fluid-disease relationships suggests that optimal fluid intake for healthy free-living individuals might be higher than common recommendations.

Michaud and colleagues [40] examined the relation between total fluid intake and the risk of bladder cancer over a period of 10 years among 47,909 participants in the prospective Health Professionals Follow-Up Study and determined that participants consuming more than 2391 mL/d had a 49% lower incidence of bladder cancer than those consuming less than 1398 mL. The fluid intake for participants in the fifth quintile (intake >2531 mL/d; or 10.7 8-oz servings) was higher across all beverage types (milk, fruit juice, soda, water, alcoholic beverages, coffee and tea) when compared to all other quintiles. These results are of increased interest when considered in light of the frequently advocated eight 8-oz, or 1885 mL, per day consumption recommendation.

## CONCLUSIONS

Acknowledging the limitations of the study, results indicate that consuming caffeinated beverages did not significantly alter hydration status as measured by weight change. It is further concluded that blood and urine indices are insensitive to slight fluctuations in hydration status.

Additional research is needed to verify the findings of this study. Without question, a metabolic ward setting would have added a greater degree of control, but the intent was to ascertain results applicable to free-living individuals. In future research on the effect of beverages on hydration status, testing other commonly consumed beverages would have merit. Because the biochemical variables used in this study were not sensitive enough to mark small changes in hydration status as measured by body weight, deliberate dehydration may be necessary to assess biochemical indicators of hydration status.

Determining optimal fluid intake was not an objective of this study, but the results have implications therein. For example, the mean fluid intake of subjects nearly met the generally recommended eight 8-oz servings daily, but was inadequate to prevent weight loss, suggesting that adequate fluid intake may be greater than 64 oz. per day for many people. The importance of better defining optimal fluid intake is reinforced by the research showing an inverse relationship between fluid intake and cancer risk [40–42]. Defining optimal fluid intake in the free-living population may have important disease prevention implications.

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