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ARTICLE

Novel hydration assessment techniques employing thirst and a water intake challenge in healthy men

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Abstract: Exploring novel hydration indices is important because no human biomarker has been shown to be incontrovertibly valid in all life situations. The present investigation was designed to identify inexpensive, nontechnical methods to use when self-assessing hydration status. This investigation evaluated the validity and efficacy of 2 novel techniques (i.e., thirst sensation and urine volume) to assess hydration state of 29 active men (mean ± SD; age, 23 ± 4 years; body mass, 76.02 ± 11.94 kg) at rest. Eight combinations of 4 water challenges (4.8, 9.3, 11.0, or 14 mL·kg^{-1}) and 2 hydration states (mildly hypohydrated (HY), –2.0%; euhydrated (EU), –0.2% body mass) were employed. First, thirst was linearly related to body water loss, and ratings of thirst distinguished HY from EU ($p < 0.001$) subsequent to 19 h of controlled food and fluid intake. Second, measurements of urine volume 60 min after consuming a water bolus (11.0 or 14 mL·kg^{-1}) were strongly and inversely correlated with entering hydration state, assessed by urine specific gravity ($r^2 = 0.76$, $p < 0.0001$) and urine osmolality ($r^2 = 0.77$, $p < 0.0001$). We concluded that healthy men can employ simple measurements of morning thirst sensation and urine volume to identify the presence of mild hypohydration and to guide fluid replacement. These 2 techniques are relevant because HY (–2% body mass) is the approximate threshold for the onset of thirst, reduced endurance exercise performance, and decrements of working memory and mood.

Key words: urine specific gravity, urine osmolality, hypohydration, thirst, hydration, body water.

Résumé : L'analyse des nouveaux indices d'hydratation est importante, car il n'y a aucun biomarqueur chez l'humain valide de façon incontestable dans toutes les situations de la vie. Cette étude est réalisée dans le but d'identifier une méthode peu coûteuse et non technique pour évaluer son propre degré d'hydratation. Cette recherche évalue la validité et l'efficacité de deux méthodes originales (sensation de soif et volume d'urine) d'évaluation au repos du degré d'hydratation chez 29 hommes actifs (moyenne é-t; 23 ± 4 ans, 76,02 ± 11,94 kg). On analyse huit combinaisons de quatre défis hydriques (4,8, 9,3, 11,0 ou 14 mL·kg^{-1}) et de deux degrés d'hydratation (hypohydratation légère (« HY »), –2,0%; euhydratation (« EU »), –0,2% de la masse corporelle). Tout d'abord, la soif est corrélée linéairement à la perte d'eau corporelle et la sensation de soif permet de faire la distinction entre HY et EU ($p < 0{,}001$) durant les 19 h subséquentes en condition d'apport hydrique et alimentaire contrôlé. Deuxièmement, la mesure du volume d'urine 60 min après avoir bu de l'eau (11,0 ou 14 mL·kg^{-1}) est fortement et inversement reliée au seuil d'hydratation déterminé par la masse volumique de l'urine ($r^2 = 0{,}76$, $p < 0{,}0001$) et l'osmolalité de l'urine ($r^2 = 0{,}77$, $p < 0{,}0001$). En conclusion, des hommes en bonne santé peuvent utiliser la méthode simple de la sensation de soif et du volume d'urine pour déterminer la présence d'une légère hypohydratation et pour indiquer la réhydratation adéquate. Ces deux méthodes sont pertinentes puisque HY (–2% de la masse corporelle) est le seuil approximatif de la sensation de soif, de la diminution de la performance d'endurance, de la dégradation de la mémoire pendant le travail et de l'humeur. [Traduit par la Rédaction]

Mots-clés : masse volumique de l'urine, osmolalité de l'urine, hypohydratation, soif, hydratation, eau corporelle.

Introduction

Water supports cellular metabolism, biochemical reactions, circulatory function, thermoregulation, and numerous other physiological functions. The importance of these processes to optimal physiological function and health emphasizes that active individuals should monitor hydration status daily. At least 13 hydration assessment techniques exist today (Armstrong 2007). These involve urinary, hematologic, whole-body, or sensory measurements and have been evaluated for use by athletes (Shirreffs and Maughan 1998; Oppliger and Bartok 2002), laborers, soldiers (Armstrong 2005), and patients (Mentes et al. 2006; Kavouras 2002). However, previous scientific and clinical publications offer no incontrovertible argument for the superiority of a single hydration index for use in all situations, populations, and activities (Armstrong 2005). This is true because the turnover of body water is complex (i.e., fluid moves between intracellular, interstitial, and extracellular compartments), dynamic (i.e., water and electrolytes are constantly lost from the skin, kidneys, intestines, and lungs; water and minerals are gained as food and fluids are consumed), and regulated by the brain (i.e., arginine vasopressin, aldosterone). Also, published (Armstrong 2007) and unpublished observations from our laboratory suggest that the accuracy of hydration markers differs, depending on whether experiments involve dehydration (acute, within-day) or hypohydration (chronic, across days).

Therefore, the present investigation measured responses to consuming 4 different water volumes when test participants were in both euhydrated (EU) and mildly hypohydrated (HY; –2% body mass loss) states. Mild hypohydration (2% body mass loss) is relevant because this is the approximate threshold at which thirst is

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perceived (Greenleaf 1992), vigilance and working memory decline, and tension–anxiety and fatigue increase (Ganio et al. 2011). These cognitive and mood outcomes of mild hypohydration explain the value of avoiding a mild body water loss. Two novel approaches to the assessment of mild hypohydration were explored. Our goal was to identify valid, inexpensive techniques that could be used by healthy, active individuals (i.e., who have no technical expertise or access to laboratory instrumentation) to identify mild hypohydration. First, the sensation of thirst was evaluated. Although the water intake recommendations of the Institute of Medicine, National Academy of Sciences of the United States (Institute of Medicine 2005) state that thirst is involved in the complex physiological responses that maintain normal body water across days, thirst has been discounted as a hydration index because it is not perceived until 1%–2% body mass loss exists (Greenleaf 1992), and because it generally does not accurately track physiological biomarkers during acute dehydration experiments (Kavouras 2002; Oppliger and Bartok 2002; Greenleaf 1992). In addition, the efficacy of thirst has seldom been evaluated during gradual hypohydration (>8 h or overnight), which involves water restriction. Second, a water loading test is widely used by physicians to diagnose renal diseases (Chapman et al. 1990), but seldom have urinary markers of hydration state (e.g., osmolality, specific gravity, color) been studied via water challenge (Shirreffs 2003; Kavouras 2002; Oppliger and Bartok 2002). In the present investigation, the validity of these 2 novel techniques was evaluated by utilizing body mass change and urine biomarkers as the reference standards, in agreement with recent evidence that indicates that body mass change and urine specific gravity validly represent dehydration (Cheuvront et al. 2010). We hypothesized that the morning sensation of thirst, and urinary measurements following a water challenge, would distinguish HY (i.e., accomplished across 19 h) from the EU state, thereby demonstrating their utility as indicators of mild hypohydration. These variables were included because the sensation of thirst and renal responses are the primary regulatory components of total body water in non-exercising humans (Armstrong 2007). Further, we hypothesized that thirst would distinguish EU from HY, because the 19-h overnight water restriction period would be more likely to involve the intracellular space (i.e., intracellular dehydration) than a brief dehydration experiment (Gilman 1937; Greenleaf 1992).

Materials and methods

This protocol was approved by the University of Connecticut, Institutional Review Board for Human Subjects in Research. Prior to giving written informed consent, test subjects attended an informational meeting that described the methods, instruments, responsibilities, time requirements, risks, and benefits of participating. Subjects were excluded from participation if they were taking medications that affected body fluid retention, dehydration, or kidney function; or if they had gained or lost more than 2 kg of body mass during the previous 3 months. Characteristics (mean ± SD) of the 29 healthy male test subjects were age, 23 ± 4 years; height, 179.5 ± 6.8 cm; body mass, 76.02 ± 11.94 kg; body mass index, 24.9 ± 4.6. These men were active (i.e., performed physical training of various types at least 3 times per week) but not highly trained (i.e., not competitive athletes). They were instructed to maintain their typical pre-study exercise habits throughout the entire investigation, except that they did not exercise during the 24 h prior to each trial. Twenty-nine healthy males participated in various phases of this investigation. The 2 experimental days involved entering the laboratory in different hydration states: EU and HY. The order of experimental treatments (Table 1) was randomized and counter-balanced.

Preliminary visits

Subjects visited the laboratory in the morning for 5 min on 3 different occasions (visit 1, visit 2, visit 3), separated by at least 48 h, to have body mass measured (±100 g); this allowed calculation of a baseline body mass (Cheuvront et al. 2004). Subjects next visited the laboratory on the day prior to each EU and HY experiment for a body mass measurement (visit 4), which was the starting point for the 19-h period of controlled food and fluid intake. At this visit, subjects received instructions regarding the food and fluid regimen to follow, to reach the target body weight for each EU and HY experimental day. For example, the instructions given during visit 4 prepared subjects to arrive in the appropriate hydration state for visit 5 (EU trial) and the instructions given during visit 6 prepared subjects to arrive in the appropriate hydration state for visit 7 (HY trial).

The diets consumed prior to the EU and HY trials contained identical solid foods; thus, EU and HY differed only in the volume of fluid consumed during the 19 h before each experiment began. On the day of visit 4, subjects kept a record of all food and fluid that was consumed during the 24 h prior to the first experiment (visit 5); the solid food consumed prior to visit 5 was replicated prior to visit 7; however, the fluid consumed during these periods was considerably different (EU versus HY). Replication of each subject's diet was verified by an investigator prior to each experiment.

To ensure that they were not dehydrated at the beginning of EU experiments, participants were instructed to consume 568 mL (16 fluid ounces) of water prior to going to sleep (on the night before testing) and 568 mL of water when they awoke. Thus, the control condition involved a body mass that was nearly equal to the baseline body mass (e.g., which had been determined previously by measuring 3 morning body mass measurements; see above; Cheuvront et al. 2004), and was therefore declared to be a state of EU. The HY condition was accomplished by fluid restriction (i.e., no water or beverage intake) and consumption of dry foods (e.g., avoiding soups, watery fruits), during the 19-h period leading up to the experiment; neither exercise nor a hot environment were employed to enhance body water loss. The grand mean body mass change (during the 19 h prior to the water challenge) of all EU experiments was −0.2% ± 0.5%; this value was −2.0% ± 0.8% for all HY experiments, which represented mild hypohydration.

Experiments (1.3 h duration)

Each subject completed 1 EU and 1 HY experiment; the same volume of water was consumed *during* both experiments. EU and HY laboratory experiments lasted for 1.3 h each, began at the same time of day (0700–0800 h), and were separated by at least 3 days. Except for the 1.3-h test session, subjects went about their normal daily activities. When subjects arrived at the laboratory, they provided a 24-h diet record, provided a urine sample for analysis, voided the bladder, and had body mass measured. These data were used to determine that they had prepared themselves properly for each day's experiment. On the control (EU) day, prior to experiments, if urine specific gravity was >1.014 or if body mass exceeded ±1 kg of the baseline value, subjects were not allowed to participate.

Body mass was measured at 0 and 60 min (±100 g) during both EU and HY sessions. After the body mass measurement at minute 0, subjects sat quietly in an air conditioned room (23 °C) and consumed a bolus of water (Table 2) within 3 min, during both EU and HY experiments. Each subject consumed only 1 volume of water during EU and HY experiments: either 4.8, 9.3, 11.0, or 14.0 mL·kg body mass^{-1} (Table 2). Subsequently, participants quietly read or used a computer for 60 min; they did not listen to music, watch television, or view movies. All clocks in the testing area were removed to eliminate knowledge of elapsed time. Subjects were allowed to leave the laboratory voluntarily to collect all urine in a container if they reached a point that they felt a need to urinate, as they ordinarily would during daily activities; this occurred infrequently (3 out of 58 experiments; 5.1% of both EU and HY trials; Table 3).

Table 1. Measurements that occurred during each visit to the laboratory.

Experimental design factors	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Baseline body mass was determined	X	X	X				
Body mass was measured (kg)				X	X,X	X	X,X
24-h food and fluid intake began				X		X	
Solid food consumption was repeated, to match previous experiment						X	
1.3-h experiment (EU or HY; randomized order)					X		X
Urine sample				X	X,X,X	X	X,X,X
Thirst rating					X,X,X		X,X,X
Water intake challenge					X		X

Note: Euhydrated (EU) and hypohydrated (HY) experiments (1.3 h) occurred during visits 5 and 7.

Table 2. Participants performed 2 experiments (EU and HY; visits 5 and 7), and consumed all water (column 1) within 3 min.

Water challenge consumed		Mean (±SD) 19-h body mass change (%)[a]	
Amount consumed (mL·kg body mass^{-1})	Mean (mL)	EU[b,c]	HY[b,c]
4.8	365	−0.2±0.3	−2.0±1.1
9.3	707	−0.3±0.7	−1.6±0.9
11.0	836	−0.3±0.7	−2.0±1.1
14.0	1064	−0.3±0.4	−2.0±1.1

Note: EU, ehydrated; HY, hypohydrated.

[a]Measured at the beginning of testing (minute 0), before the water challenge.

[b]The number of test subjects was 6, 5, 9, and 9 for the 4.8-, 9.3-, 11.0-, and 14-mL·kg^{-1} water challenges, respectively.

[c]The grand mean (±SD) body mass change of all EU tests was −0.2% ± 0.5%, and was −2.0% ± 0.8% for all HY tests.

Urinalysis

Subjects provided urine samples in a clean, inert container at 3 min before, 30 min after, and 60 min after a water challenge. Urine specific gravity was measured with a hand-held refractometer (Atago A300CL, Japan) and urine osmolality was assessed in duplicate via freezing point-depression osmometer (Advanced Digimatic, Model 3DII, Needham Heights, Mass., USA). Urine volume was measured gravimetrically (±0.1 g) with a laboratory scale (Ohaus, Inc.). Resting baseline urine volume was defined as 88 mL·60 min^{-1}, as reported for healthy 23-year-old males by Parsons et al. (2007).

Thirst rating scale

A thirst rating scale (Engell et al. 1987) was presented to subjects on 28 × 21.5 cm printed posters, at 0, 30, and 60 min of all EU and HY experiments, and if subjects voluntarily left the room to urinate. This instrument included 5 written descriptors of thirst and required that subjects selecta number, from 1 to 9. Statements were associated with all odd numbers, serving as clarifying cues, as follows: 1, "not thirsty at all"; 3, "a little thirsty"; 5, "moderately thirsty"; 7, "very thirsty"; and 9, "very, very thirsty". Participants were instructed to focus on the sensations emanating from the mouth, tongue, throat, and lips, and to ignore other sensations they were feeling (e.g., headache). Subjects received standardized instructions during familiarization visits and prior to each EU and HY experiment.

Statistical analyses

Significant treatment effects were computed via repeated measures ANOVA. In the event of a significant F ratio ($p < 0.05$), pairwise differences were evaluated via a paired samples t test with Tukey's HSD corrections. Descriptive statistics (mean ± SD) were calculated for all variables. Regression analyses were performed to describe the relationships between urine volume and urine specific gravity, and also between urine volume and urine osmolality.

Results

The present experiments, involving 29 healthy young men who were active but were not highly trained, allowed us to evaluate 2 novel approaches to hydration assessment. These techniques involved a perceptual rating scale, and renal responses following consumption of a bolus of water within 3 min. Data are reported and discussed according to these 2 techniques.

Each subject participated in 1 EU and 1 HY experiment, both employing the same water challenge volume. The following mean (±SD) values for body mass (on the day prior to each EU and HY experiment) verified that subjects began the 19-h period of controlled food and fluid intake in a similar, EU state: 4.8 EU, 71.14 ± 8.59 compared with 4.8 HY, 71.18 ± 8.87 kg; 9.3 EU, 81.30 ± 11.74 versus 9.3 HY, 81.74 ± 11.86 kg; 11.0 EU, 84.60 ± 21.42 compared with 11.0 HY, 84.58 ± 21.33 kg; 14.0 EU, 84.16 ± 11.75 versus 14.0 HY, 84.10 ± 11.93 kg.

Thirst following water challenges

Figure 1 illustrates thirst ratings resulting from 4 different water challenges, measured when subject baseline hydration states were EU and HY (described in Table 2). A significant difference between treatments (EU versus HY trials, $p < 0.001$) occurred only during the first measurement, immediately prior to water challenges. All EU and HY thirst ratings were statistically similar at 30 and 60 min after the water challenge.

Urine output responses following water challenges

Table 3 presents the urine outputs of 29 males, observed 30 min and 60 min after consuming 4 different water volumes within 3 min. As expected, a greater mean percentage of each water challenge was excreted during EU than was excreted during HY: 4.8 EU, 47.5% ± 41.7% compared with 4.8 HY, 10.5% ± 2.2%; 9.3 EU, 53.2% ± 15.7% versus 9.3 HY, 14.4% ± 6.0%; 11.0 EU, 69.4% ± 15.6% compared with 11.0 HY, 10.2% ± 3.6%; and 14.0 EU, 58.4% ± 19.6% versus 14.0 HY, 9.7% ± 4.1%.

Figure 2 illustrates the relationship between initial (entering) urine specific gravity (at 0 min, prior to the water challenge) and the total urine volume produced at 60 min. All EU (rightmost data points) and HY (upper left quadrant) conditions, as well as all water challenges (4.8, 9.3, 11.0, and 14.0 mL·kg^{-1}), are included. Linear regression analysis of this strong inverse relationship ($p < 0.0001$), demonstrated that 76% of the variance of initial urine-specific gravity (y axis) is explained by the 60-min urine volume (x axis). Similarly, Fig. 3 depicts the relationship between initial (entering) urine osmolality and the total urine volume produced at 60 min. All EU and HY conditions, as well as all water challenges, are included. Linear regression analysis of this strong inverse relationship ($p < 0.0001$) demonstrates that 77% of the variance of initial urine osmolality (y axis) is explained by the 60-min urine volume (x axis).

Figure 4 illustrates the relationship between initial body mass change (from day −1 to 0 min, immediately prior to the water challenge) and thirst ratings, during controlled food and water

Table 3. Number of test subjects who produced urine during 29 euhydrated (EU) and 29 hypohydrated (HY) experiments, 30 and 60 min after the water challenge.

Water challenge (mL·kg body mass^{-1})[d]	Test participants (no. of responders[a]/total no.)			
	EU[b]		HY[b,c]	
	0–30 min	0–60 min[d]	0–30 min	0–60 min
4.8	0/6 (0±0)	3/6 (169±148)	0/6 (0±0)	0/6 (37±8)
9.3	0/5 (0±0)	5/5 (377±125)[e]	0/5 (0±0)	3/5 (102±48)
11.0	4/9 (173±244)	9/9 (646±273)[e]	0/9 (0±0)	4/9 (89±28)
14.0	1/9 (47±140)	9/9 (687±250)[e]	0/9 (0±0)	6/9 (120±75)

[a]Responders are defined as test subjects whose rate of urine production exceeded the resting EU baseline urine production for 23-year-old men (44.1 and 88.2 mL·60 min^{-1}; Parsons et al. 2007).
[b]Numbers in parentheses (columns 2–5) represent the mean ± SD urine volume (mL) of all subjects within that cell.
[c]HY involved water restriction and consumption of a dry-food diet, during the 19 h before arrival at the laboratory.
[d]Consumed within 3 min.
[e]EU urine volume is significantly greater than HY at 60 min ($p < 0.01$ to 0.0001).

Fig. 1. Thirst ratings during 60 min of euhydrated (EU, solid lines) and hypohydrated (HY, dashed lines) conditions. The horizontal axis depicts time elapsed after water challenges (4.8, 9.3, 11.0, and 14.0 mL·kg^{-1}). A significant effect of treatment (EU versus HY trials, $p < 0.001$) existed only at 0 min, prior to water challenges.

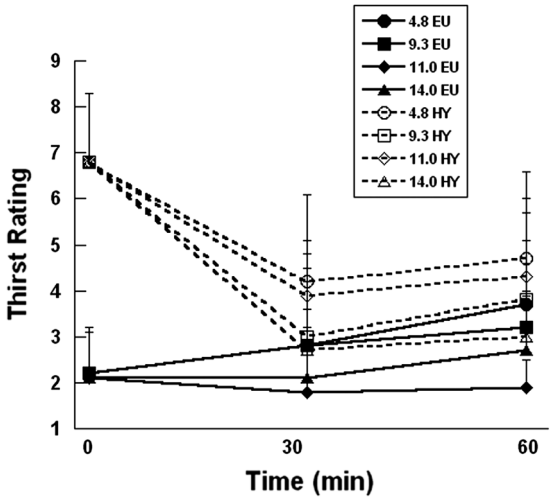


Fig. 2. Prediction ($n = 56$) of initial (0 min) urine specific gravity (y axis) from 60-min urine volume (x axis). EU, euhydrated; HY, hypohydrated.

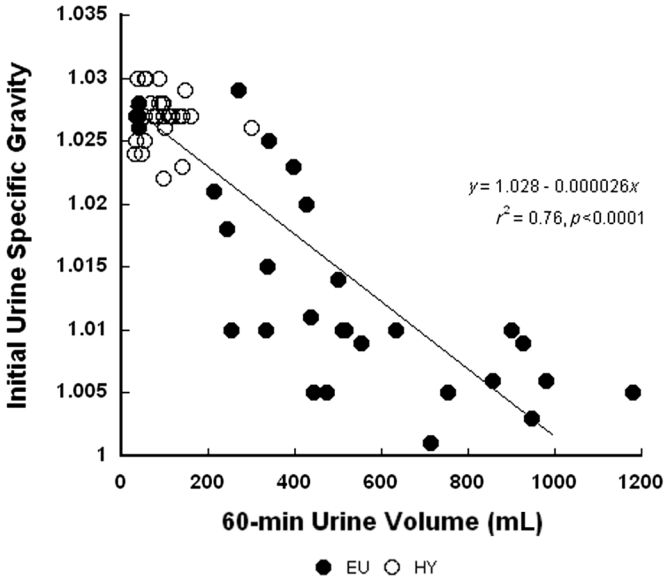
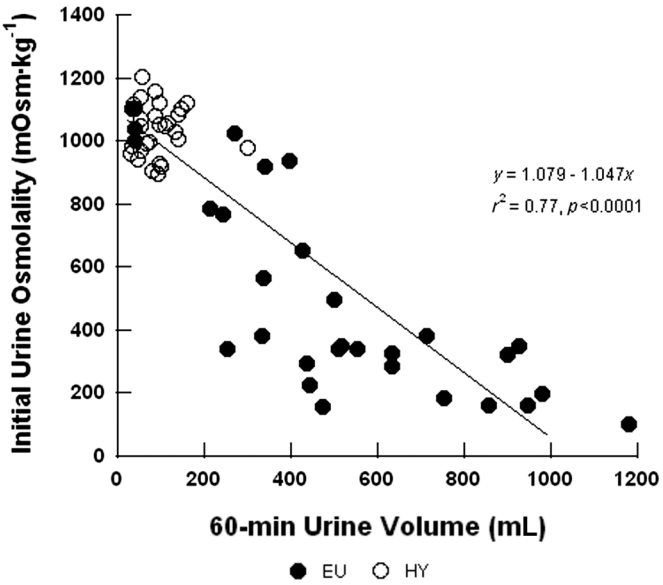


Fig. 3. Prediction ($n = 56$) of initial (0 min) urine osmolality (y axis) from 60-min urine volume (x axis). EU, euhydrated; HY, hypohydrated.



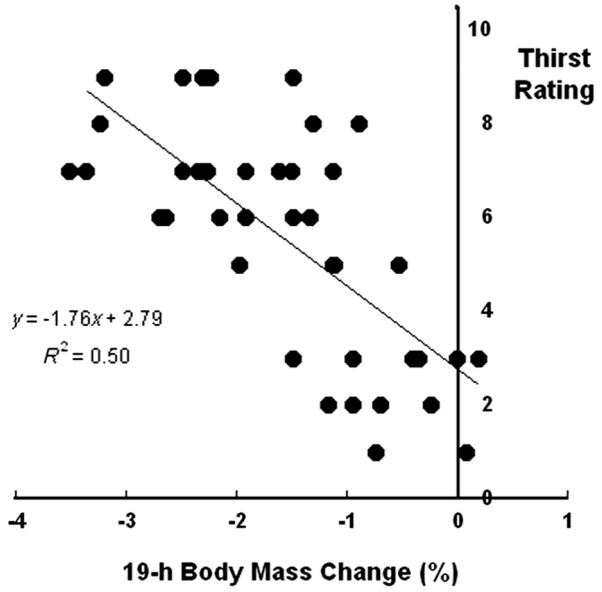
consumption; EU and HY data have been combined. The number of data points in this figure is 40, because some thirst ratings were inadvertently not recorded at 0 min of the 4.8 mL·kg^{-1} EU and 9.3 mL·kg^{-1} EU experiments.

Discussion

This investigation (Tables 1 and 2) evaluated the efficacy of 2 previously unexplored hydration assessment methods in an attempt to discover techniques that could be employed at rest by healthy, active individuals, to evaluate mild hypohydration at a point in time. Body mass change (±100 g) and urine hydration variables (e.g., urine specific gravity) during HY and EU trials were the reference standards used to evaluate the validity of these methods. Because body mass change during HY experiments averaged –2.0% ± 0.8% (Table 2), these findings describe (*i*) responses to a mild hypohydration that many humans experience or exceed during the course of a typical week, and (*ii*) the point at which hypohydration influences endurance performance and behavior (Shirreffs et al. 2004; Ganio et al. 2011).

The major findings of this investigation involved both thirst ratings and urine volume measurements as hydration indices. First, the rating of thirst distinguished EU from HY ($p < 0.001$, Fig. 2) after 19 h of dehydration, accomplished by controlled fluid and food intake. Second, 2 initial morning urinary biomarkers

Fig. 4. Relationship between 19-h body mass change and thirst rating, immediately before the water challenge (0 min; $n = 40$, $p < 0.0001$).



(i.e., specific gravity and urine osmolality) represented a change of hydration state and predicted the 60-min urine volume, following a water challenge.

Thirst ratings

The sensation of thirst is the primary reason one seeks water when dehydrated (i.e., it drives drinking behavior) (Engell et al. 1987; Greenleaf 1992). As such, it is an integral part of total body water regulation by the central nervous system (Armstrong 2007). However, thirst often is discounted as a hydration index because it does not accurately reflect dehydration during acute experiments (Kavouras 2002; Oppliger and Bartok 2002), and because thirst is not perceived until 1%–2% body mass loss exists (Greenleaf 1992). Indeed, thirst ratings at 30 and 60 min after the water challenge (Fig. 1) were statistically similar for all treatments. However, the statistically significant difference of mean thirst ratings (EU, 7 ± 2; HY, 2 ± 1; $p < 0.001$; Fig. 1) before the water challenge (0 min) demonstrated that thirst distinguished 19 h of water restriction (HY) from EU, in which men consumed 568 mL of water twice (night and morning), in addition to their usual ad libitum fluid intake. Although blood samples were not collected in the present investigation, previous studies provide insights that explain why thirst indicated mild hypohydration at –2% body mass during our prolonged control of fluid intake from beverages and solid foods. As a chronic, slow-acting mechanism for maintenance of fluid balance, thirst is influenced by changes of plasma osmolality and plasma volume (Kavouras 2002), as well as intracellular dehydration (Gilman 1937; Greenleaf 1992). When plasma osmolality increases or plasma volume decreases, perception of thirst rises (Fitzsimons 1976). Of these 2 signals, the former is the main regulator at an increase of approximately 2% in plasma osmolality, which induces a strong increase in the sensation of thirst (Zerbe and Robertson 1983), whereas plasma volume must decrease by approximately 10% to stimulate thirst (Sagawa et al. 1992).

Although it is presently impossible to quantify acute dehydration and hypohydration on the basis of thirst rating alone, Fig. 1 demonstrates that thirst ratings have discriminative value during slowly developing, prolonged hypohydration. Also, Fig. 4 depicts a statistically significant relationship between whole-body hypohydration and thirst. This suggests that future studies will find thirst to be a useful hydration biomarker in long-term scenarios, because of intracellular dehydration and (or) changes in plasma osmolality (Greenleaf 1992).

The sensation of thirst originates in brain cells that function as individual osmometers, responding to water and solute movements between intracellular and extracellular compartments (Fitzsimons 1976). Acknowledging that many factors interact to influence thirst (Farrell et al. 2011; Greenleaf 1992; McCauley et al. 2012), the following interpretation of Fig. 1 is plausible. First, the 19-h overnight dehydration period resulted in intracellular dehydration and a higher plasma osmolality during HY experiments; upon waking in the morning, thirst sensation was considerably greater in HY than in EU (at 0 min; Fig. 1). Second, after the water challenge, all HY and EU thirst ratings were statistically similar at 30 and 60 min (Fig. 1). Indeed, drinking any volume of water (i.e., 365 to 1064 mL, Table 2) reduced thirst markedly and similarly in HY experiments; this was likely due to a rapid osmolality change, thirst-related factors such as gastric distention and oropharyngeal reflexes (Greenleaf 1992), as well as a rapid decrease of plasma arginine vasopressin (Geelen et al. 1984, 1996). This exemplifies the multiple and redundant mechanisms that protect water, the quintessential nutrient (Greenleaf 1992).

Figure 4 illustrates the effect of body water loss on ratings of thirst after a 19-h period of controlled food and fluid intake; it combines both EU and HY experiments. The thirst scale (Engell et al. 1987) included statements that have applied value. Specifically, a rating of 1 meant "not thirsty at all" and a rating of 3 meant "a little thirsty". Thus, all ratings between 1–3 represented no or mild thirst sensations, and supported previous observations (Greenleaf 1992; Armstrong 2007) that the sensation of thirst occurs between –1% and –2% body mass loss. At the other extreme, a rating of 7 referred to "very thirsty" and a rating of 9 meant "very, very thirsty". However, these ratings of 7–9 represented a wide range (–0.9% to –3.5%) of body mass change.

Sixty-minute urine volume

In both Figs. 2 and 3, a large 60-min urine volume (lower right quadrant) represents hyperhydration, and a small 60-min urine volume (upper left quadrant) represents a water deficit. We hypothesized that EU (i.e., lower urine specific gravity and urine osmolality) would result in a different excreted urine volume than HY but we did not know if a significant relationship would exist at this mild level of hypohydration (–2% of body mass). Indeed, a greater mean percentage of the water consumed was excreted during EU (range, 47.5%–69.4%) than during HY (range, 9.7%–14.4%); and the 60-min urine volume during EU (range, 377–677 mL) was significantly greater than during HY (range, 89–120 mL), when 9.3, 11.0, and 14.0 mL·kg^{-1} of water was consumed (Table 3).

Urine osmolality and urine specific gravity are widely accepted hydration biomarkers (Kavouras 2002; Oppliger and Bartok 2002; Shirreffs 2003), which are strongly predictive ($r^2 = 0.81$; Armstrong et al. 2010; $r^2 = 0.94$; Armstrong et al. 1994). Thus, not surprisingly, both measures provided similar information when correlated with 60-min urine volume. Specifically, the prediction equation shown in Fig. 2 ($p < 0.0001$) demonstrates that the 60-min urine volume accounted for 76% of the variability of initial urine-specific gravity (range: 1.002–1.030). In Fig. 3, the 60-min urine volume accounted for 77% of the variability in entering urine osmolality ($p < 0.0001$). Based on these relationships, we conclude that initial hydration state can be predicted validly by voiding the bladder, consuming a water bolus within 3 min, collecting excreted urine for 60 min, and measuring the resulting urine volume. Capitan-Jimenez and Aragon-Vargas (2010) measured urine volume at 30-min intervals for 3 h, after subjects consumed a standardized 1.03-L volume of fluid (i.e., very similar to the 14.0-mL·kg^{-1} volume in the present study; see Table 2). They reported that the test–retest reliability of repeated urine volume measurements, on separate days, was high (intraclass correlation, $r = 0.849$, $p = 0.001$).

Characteristics of the water challenge

With regard to the timing of urine sampling (Table 3), a 60-min duration was deemed superior to a 30-min duration because urine was excreted above a resting level during only 17.2% of experiments (5/29 EU tests) at the 30-min mark. Interestingly, previous research measured urine volume at 30-min intervals, for 3 h after young men consumed a 1.03-L bolus of water. The maximal 30-min urine output occurred at 60 min (Capitan-Jimenez and Aragon-Vargas 2010). Thereafter, urine volume decreased during successive 30-min periods.

Regarding the amount of water required for a valid assessment of hydration status, a 4.8 mL·kg^{-1} water challenge did not successfully distinguish between EU and HY (Table 3); however, the 9.3, 11.0, and 14.0 mL·kg^{-1} water challenges successfully identified a difference between the EU and HY treatments ($p < 0.01$ to 0.0001) at 60 min. Further, considering that relatively few subjects (50% EU and 0% HY) responded by excreting more urine than a resting baseline level (88.2 mL urine·60 min^{-1}) after consuming the 4.8 mL·kg^{-1} of water challenge, this volume (mean of 365 mL) was deemed inferior to the 9.3-, 11.0-, and 14.0-mL·kg^{-1} challenge volumes. We next considered the 2 largest water challenges. After reviewing urine volumes (Table 3), percent of the ingested water that was excreted in 60 min (Results section), and the number of subjects who produced urine (Table 3), we concluded that the 11.0 and 14.0 mL·kg^{-1} of water challenges were similar and could be used interchangeably.

One precaution is necessary when using a water challenge. During exercise, excessive fluid retention with a plasma sodium (Na^+) concentration below the adult normal range of 135–145 mEq·L^{-1} is known as exertional hyponatremia. A plasma Na^+ concentration below 129 mEq·L^{-1} is clinically significant (i.e., symptomatic) and a plasma level below 125 mEq·L^{-1} requires immediate medical treatment, to avoid pulmonary and cerebral edema (Ayus et al. 2000). Interestingly, the volume of excess fluid (i.e., fluid consumed in excess of sweat volume) required to dilute plasma Na^+ to 120 mEq·L^{-1} is relatively small. In a 50-kg athlete, it may amount to only 200 mL of excess fluid per hour, when consumed during a 9-h ultra-endurance event (Montain et al. 2006). Therefore, when fluid overload is suspected for any reason (i.e., weight gain during an endurance event), we recommend that a water challenge *not* be used. Instead, the athlete should monitor other hydration indices (e.g., urine color, urine specific gravity, urine conductivity; Shirreffs and Maughan 1998; Shirreffs 2003), evaluate signs and symptoms of hyponatremia (Ayus et al. 2000), and follow the advice of a physician. Using this approach, a large urine output (>2.25 L·24 h^{-1}) with no fluid intake verifies a state of extreme hyperhydration (Armstrong et al. 2010). Further, it is important that healthy individuals not attempt to achieve a hyper-hydrated state constantly. A urine specific gravity less than 1.010 or a urine osmolality less than 300 mOsm·kg^{-1} are unusual, in young males during daily activities, and are ordinarily observed only after consuming a large volume of water or hypotonic fluid (Armstrong et al. 2010). In the present investigation, test participants entered EU trials slightly hyperhydrated. Men consumed 568 mL twice, during the evening and morning before testing, in addition to their usual ad libitum fluid intake. This explains why all data points with a urine volume > 300 mL in Figs. 2 and 3 represent values measured during EU experiments. This finding also suggests that a water challenge should be administered no more than once per 24-h period.

Limitations

Three limitations are acknowledged in this investigation. First, the EU condition was prescribed and HY was produced across 19 h. Although a 2% level of dehydration is not uncommon during the daily activities of most adults (Armstrong 2007; Ganio et al. 2011; Mentes et al. 2006), the validity of these findings for free-living adults, undertaking daily activities, remains to be determined. Second, the influence of test participant characteristics on thirst and on the responses to a water challenge deserves additional study; it is possible that the present findings may not be relevant to children, women, and obese or senior adults. Third, the definition of thirst was carefully described to test participants in the present investigation (see Materials and methods section above). However, the efficacy of thirst as a hydration biomarker without this specific definition is unknown. In addition, large inter-individual differences exist in ratings of thirst, and the plasma osmolality threshold at which thirst is sensed (Zerbe et al. 1991).

In summary, 2 user-friendly hydration assessment methods, involving the sensation of thirst and the measurement of urine volume, were validated in healthy young men. We recommend that both techniques be employed upon waking in the morning, before exercising, or consuming food and fluids. The first requires the user to rate thirst. If he or she is "very thirsty", this strong sensation signals mild hypohydration of approximately –2% (Fig. 2). The second technique involves the following steps: void the bladder, consume 11.0 mL·kg^{-1} of water within 3 min, rest for 60 min in a mild environment, void the bladder, and measure the volume of urine produced. Similar to urine color (Armstrong et al. 1994), we recommend that these 2 techniques be used when an inexpensive method, which requires little technical expertise, is desired. Because the test participants in this investigation were young active males, we also recommend that these methods be evaluated in different scenarios (e.g., during and after exercise), populations, and stressful environments.

Conflict of interest statement

This research was supported with internal funds; no external grants were involved. The authors declare no conflicts of interest.

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