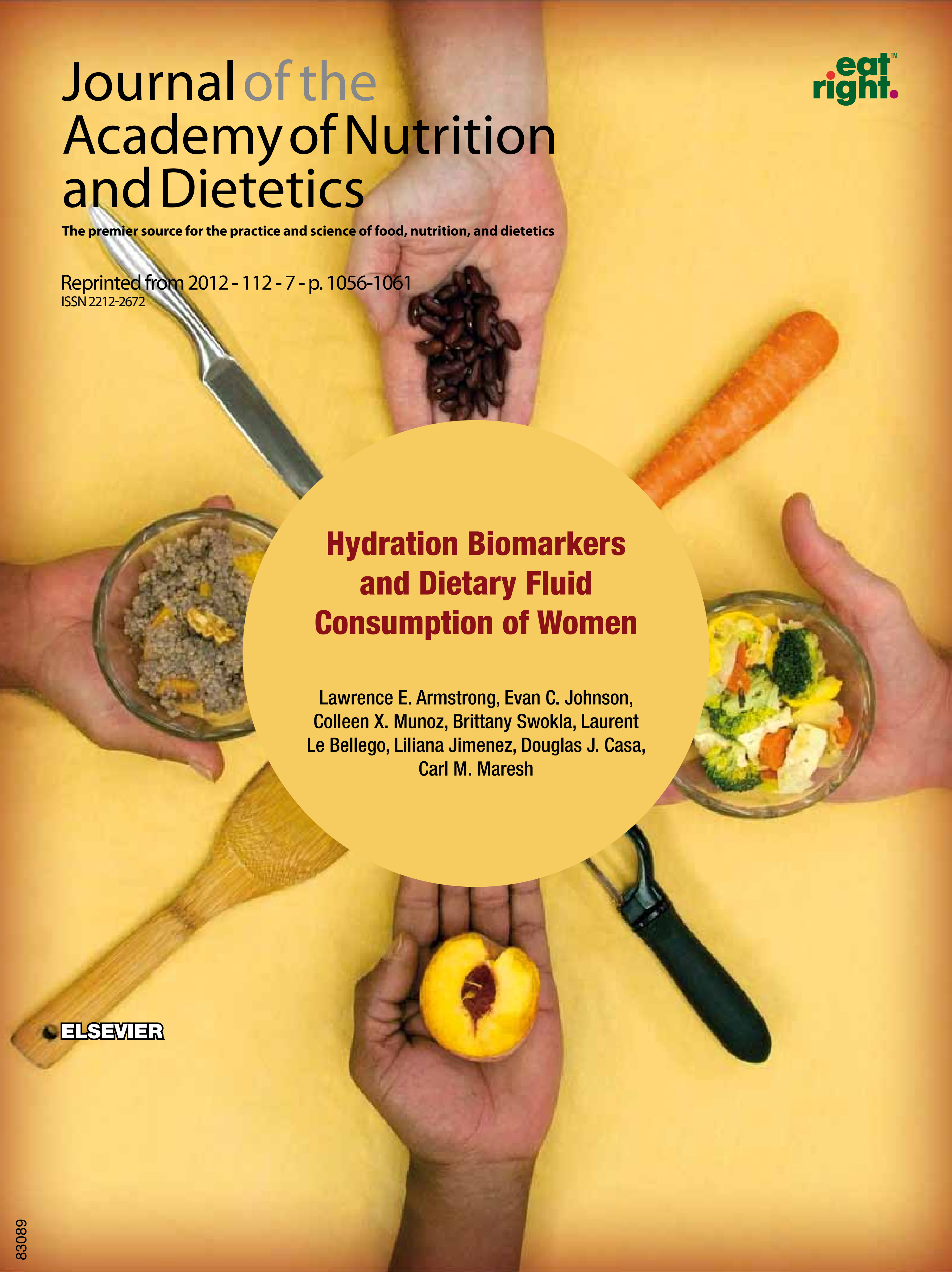
Journal of the Academy of Nutrition and Dietetics

The premier source for the practice and science of food, nutrition, and dietetics

eat right.

Reprinted from 2012 - 112 - 7 - p. 1056-1061

ISSN 2212-2672



Hydration Biomarkers and Dietary Fluid Consumption of Women

Lawrence E. Armstrong, Evan C. Johnson, Colleen X. Munoz, Brittany Swokla, Laurent Le Bellego, Liliana Jimenez, Douglas J. Casa, Carl M. Maresh

ELSEVIER

83089

Hydration Biomarkers and Dietary Fluid Consumption of Women

Lawrence E. Armstrong, PhD; Evan C. Johnson, MA; Colleen X. Munoz, MS; Brittany Swokla; Laurent Le Bellego, PhD; Liliana Jimenez, PhD; Douglas J. Casa, PhD; Carl M. Maresh, PhD

ARTICLE INFORMATION

Article history:
Accepted 20 March 2012

Keywords:
Water
Beverage
Dehydration
Serum
Urine

2212-2672/$36.00
doi: 10.1016/j.jand.2012.03.036

ABSTRACT

Normative values and confidence intervals for the hydration indices of women do not exist. Also, few publications have precisely described the fluid types and volumes that women consume. This investigation computed seven numerical reference categories for widely used hydration biomarkers (eg, serum and urine osmolality) and the dietary fluid preferences of self-reported healthy, active women. Participants (n=32; age 20±1 years; body mass 59.6±8.5 kg; body mass index [calculated as kg/m^2] 21.1±2.4) were counseled in the methods to record daily food and fluid intake on 2 consecutive days. To reduce day-to-day body water fluctuations, participants were tested only during the placebo phase of the oral contraceptive pill pack. Euhydration was represented by the following ranges: serum osmolality=293 to 294 mOsm/kg; mean 24-hour total fluid intake=2,109 to 2,506 mL/24 hours; mean 24-hour total beverage intake=1,300 to 1,831 mL/24 hours; urine volume=951 to 1,239 mL/24 hours; urine specific gravity=1.016 to 1.020; urine osmolality=549 to 705 mOsm/kg; and urine color=5. However, only 3% of women experienced a urine specific gravity <1.005, and only 6% exhibited a urine color of 1 or 2. Water (representing 45.3% and 47.9% of 24-hour total fluid intake), tea, milk, coffee, and fruit juice were consumed in largest volumes. In conclusion, these data provide objective normative values for hyperhydration, euhydration, and dehydration that can be used by registered dietitians and clinicians to counsel women about their hydration status.
J Acad Nutr Diet. 2012;112:1056-1061.

THE PHYSIOLOGICAL REGULATION OF TOTAL BODY water responds to daily losses of sweat, urine, and respiratory water as well as fluid consumption secondary to the sensation of thirst; these factors interact with environmental conditions, exercise, and daily activities. Also, renal regulation of water balance via arginine vasopressin is distinct from regulation of sodium via aldosterone and atrial natriuretic peptide.[1] All hydration assessment techniques are best viewed as singular measures of a dynamic and complex fluid matrix.[2] However, it is difficult to assign numerical values to euhydration, dehydration, or hyperhydration because normative values do not exist.[3–5] Therefore, the primary purpose of this investigation was to discover reference values and 95% confidence limits for 24-hour fluid intake, serum osmolality, and four urinary indices (ie, volume, specific gravity, osmolality, and color). Secondly, this investigation also reported representative volumes and types of fluid that women consumed; few analyses of this type exist in the literature.[3–5] The authors hypothesized that this investigation would provide normally distributed reference values for hydration biomarkers, augmenting the few publications that present the volumes and varieties of fluids that women consume.[3,4]

METHODS

The Institutional Review Board for Human Studies of the University of Connecticut approved this protocol, and each subject provided written informed consent to participate during an informational meeting that described the risks, benefits, time commitment, and procedures of participation. Each woman completed a medical history questionnaire; this self-reported history was examined by a physician who verified that she met none of the following exclusionary criteria: tobacco use; history of a disease, illness, nutritional supplement, or medication that altered normal fluid-electrolyte balance, serum osmolality, or urinary osmolality; type 1 or type 2 diabetes; caffeine intake >500 mg/day; and engaged in aerobic endurance training for >7 hours/week. An activity questionnaire was administered to evaluate the frequency and duration of exercise during the 26 days before data collection. Subjects were instructed to maintain their usual exercise habits during this investigation, and this was verbally verified by an investigator at the start of each study day. Data were collected during the months of October and November.

During the initial visit to the laboratory, height was measured to the nearest 1 cm and body mass was measured on a floor scale (±100 g; Ohaus DS44L). Body mass index (BMI) was calculated from height (m) and body mass (kg) as kg/m^2.

Mean±standard deviation of the physical characteristics of the 32 female test participants were: age=20±1 years; height=163±8 cm; body mass=59.6±8.5 kg; and BMI=21.1±2.4.

Controlled procedures were instituted to optimize the accuracy of self-reported diet records. Before the study, an investigator, trained by a registered dietitian and experienced in research using dietary records, met with subjects to describe diet record forms, serving sizes, food labels, and methods to record each item of food and fluid. On days 1 and 2, each woman recorded her 24-hour food and fluid intake on paper forms, immediately after each meal or snack, then reviewed all items with a diet counselor on the next morning. Portion sizes, method of preparation (ie, fried, baked), number of servings, manufacturers, and food labels were reviewed to optimize the accuracy of diet records. Diet records were analyzed with commercial software (Nutritionist Pro, version 1.2; N-Squared Computing). Participants were free to consume any type of fluid to drink, in any volume they wanted, with the exception of alcohol. They were instructed to change none of their ordinary eating and drinking habits. Water was considered to be a beverage.

Verification of Contraceptive Use and Timing of Observations

Body water and body weight of females can fluctuate 1 to 3 kg during the course of a 28-day menstrual cycle, due to the influences of progesterone and estradiol on fluid-electrolyte balance. To minimize variance in hydration measurements during this investigation, women were observed during the 4 to 7-day placebo phase of the oral contraceptive pill pack, when reproductive hormone levels were lower and relatively stable. During the first preliminary visit, all participants provided evidence that they had used oral ethinyl estradiol-progestin contraceptives for at least 3 months before this study. Dates of the placebo phase of the contraceptive pill pack allowed individualized onset of testing. In addition, experimental control included the time of day at which measurements were taken.

Body Fluid Analyses

Subjects collected all urine produced in 24 hour on days 1 and 2; technicians then measured urine volume gravimetrically, urine color,[6] and specific gravity in duplicate using a handheld refractometer (Atago A 300CL). Urine osmolality was analyzed in triplicate via freezing point-depression osmometer (Advanced Digimatic, Model 3DII). One 24-hour urine sample was inadvertently discarded, reducing the number of data points to 31. Also on these days, blood was obtained from an antecubital arm vein after participants (n=32) had been seated for 15 minutes. Serum osmolality (mOsm/kg) was measured in triplicate with the osmometer described here.

Statistics

Means, standard deviations, percentiles, skewness, kurtosis, normalcy, statistical correlations, regression analyses, and figure designs were computed with Microsoft Office Excel (version 12.0, 2007; Microsoft Inc) and Statistica (version 5.5A, 1999; StatSoft Inc) software. Significance was accepted at the 0.05 level. Sample size was calculated via 24-hour water intake (mL/day). Using data from a previous study (unpublished observations from this laboratory; mean daily intake of 3,168±1,167 mL), a two-tailed test, α=.05, and a desired minimum power of 0.8, calculations indicated that a sample of 12 subjects was required. Using a larger standard deviation (1,500 mL) indicated that a sample of 14 subjects was needed.

RESULTS AND DISCUSSION

Research evidence suggests that a single plasma osmolality measurement is the gold standard for hydration assessment.[7] This claim arises from laboratory tests involving controlled conditions (ie, when dietary, postural, exercise, and environmental factors are held constant across experimental trials). However, adult body fluid composition and water volume change dynamically throughout each day, in settings that are far different from controlled laboratory environments (ie, ranging from sedentary office workers to athletes). Adults require simple but valid ways to assess hydration status.[2] Fortunately, the basic principles of body fluid regulation support the use of various biomarkers (Table 1) to detect dehydration. However, norms and hydration categories do not exist for women; even the volume and types of fluids that women consume are not well understood.[3–5] Therefore, the present investigation focused on the following aims: to discover reference values and 95% confidence limits for 24-hour fluid intake, serum osmolality, and four urinary indices (ie, volume, specific gravity, osmolality, and color); and to report representative volumes and types of fluid that women consume.

To define the categories (Table 1), the methods of two previous publications were incorporated.[7,8] Specifically, the extreme categories represent the 10 percentile units farthest from the mean. The two categories bordering euhydrated represent 15 percentile units each, between the extremes. Euhydration, the central category, occupies the middle 20 percentile units of this distribution and was represented by the following ranges: serum osmolality=293 to 294 mOsm/kg; total 24-hour fluid intake from all sources=2,109 to 2,506 mL/24 hours; total 24-hour beverage intake=1,300 to 1,831 mL/24 hours; urine volume=951 to 1,239 mL/24 hours; urine specific gravity=1.016 to 1.020; urine osmolality=549 to 705 mOsm/kg; and urine color=5. The 95% confidence limits for each variable appear in Table 1. Statistical tests for skewness and kurtosis indicated that all variables in Table 1 are normally distributed.

The hydration categories in Table 1 were developed on the basis of the unique measurement method of each variable. Because instruments and techniques differ widely (ie, hematologic, urinary, fluid consumption methods), it is possible that an individual might be categorized somewhat differently, depending on which variable is used. However, the physiological inter-relationships among these hydration biomarkers (Table 1) often are strong (Figure) because fluid intake, blood concentration, and urinary losses all are part of the body's neuroendocrine regulation of fluid–electrolyte balance.[2]

It is not known whether a single measurement of hematocrit can be used as a valid marker for static assessment of hydration state.[2] Therefore, the morning hematocrit values of 32 young women (Table 1) were statistically compared to morning serum osmolality values via linear regression analysis; the resulting correlation coefficient (r^2=0.01; P=0.63) in-

Table 1. Categories of eight hydration variables for 59.6-kg young women based on the mean of 2 days in a study of hydration biomarkers and dietary fluid consumption

		Morning		24-Hour					
Hydration categories	Percentile[a] range	Serum osmolality (mOsm/kg)	Hematocrit (%)	Total fluid intake (mL)	Beverage intake (mL)	Urine volume (mL)	Urine specific gravity	Urine osmolality (mOsm/kg)	Urine color
	1–10	<290	<38.5	>3,407	>2,804	>2,070	<1.008	<320	<3
Increasing hyperhydration	11–25	290	38.5–39.4	2,946–3,407	2,471–2,804	1,828–2,070	1.008–1.011	320–382	3
	26–40	291–292	39.5–40.3	2,507–2,945	1,832–2,470	1,240–1,827	1.012–1.015	383–548	4
Euhydrated	41–60	293–294	40.4–41.7	2,109–2,506	1,300–1,831	951–1,239	1.016–1.020	549–705	5
	61–75	295–296	41.8–42.7	1,745–2,108	1,154–1,299	831–950	1.021–1.023	706–809	5
Increasing dehydration	76–90	297–299	42.8–44.4	1,507–1,744	954–1,153	531–830	1.024–1.026	810–863	6
	91–100	>299	>44.4	<1,507	<954	<531	>1.026	>863	>6
95% CI	2.5–97.5	287–301	36.0–46.2	658–4,152	135–3,445	5–2,599	1.004–1.030	118–1,091	2–7

[a]Percentiles were used to determine numerical values for each category.

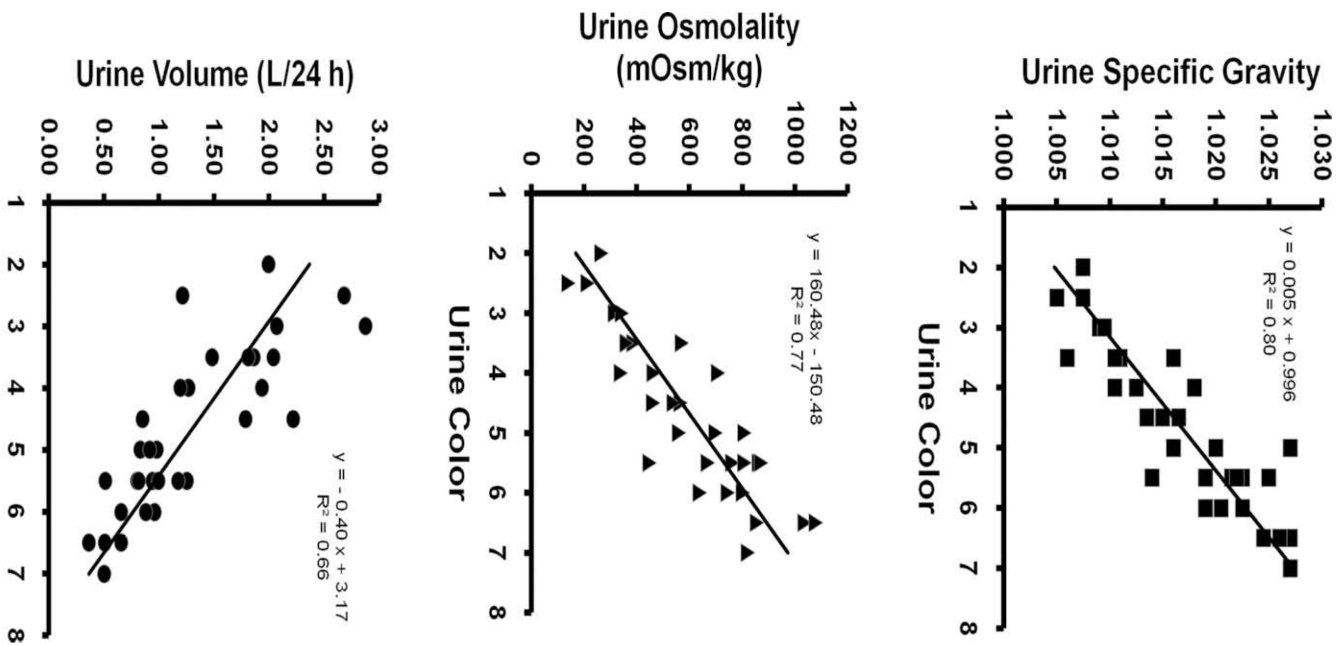


Figure. Relationships between urine color and three other urine variables: urine specific gravity (top panel; r^2=0.80; P<0.000001), urine osmolality (middle panel; r^2=0.77; P<0.000001), and urine volume (bottom panel; r^2=0.66; P<0.000001), as measured in 24-hour urine collections. Values (n=31) represent the mean of 2 consecutive days.

dicated a weak relationship. Similar weak relationships (all $r^2<0.02$) were determined between morning hematocrit values and four other hydration biomarkers: urine osmolality, urine volume, total 24-hour fluid intake, and total 24-hour beverage intake. Although these findings suggest that a single hematocrit measurement should not be used as a static biomarker of hydration status, changes of hematocrit and hemoglobin are widely used to evaluate plasma volume shifts.[9]

Only 3% of urine specific gravity values in Table 1 were <1.005, supporting the findings of two previous publications.[6,8] A urine specific gravity <1.007 and a urine color of 1 are extreme for a 24-hour hydration regimen, and suggest an individual tendency to overconsume fluids. This is an important consideration because most cases of symptomatic exertional hyponatremia occur in women.[10] Although athletes and recreational enthusiasts have been advised to maintain urine specific gravity <1.010,[11] the authors do not recommend excessive 24-hour fluid consumption, resulting in a urine specific gravity <1.007 and urine color of 1 because it predisposes women to fluid overload and potential water intoxication.[10]

The Figure illustrates the range of three different hydration biomarkers that were measured during this study and their unique relationships with urine color. Urine specific gravity and urine osmolality are used routinely as hydration biomarkers in clinical settings. These three plots include prediction equations and r^2 values derived from linear regression analyses. The high r^2 values in the top and middle graphs show that 80% and 77% of the variance in urine color can be explained by urine specific gravity and urine osmolality, respectively. Urine color is widely recognized as a useful hydration assessment technique for a variety of individuals (eg, recreational exercise enthusiasts, sedentary adults, senior adults[2,6,8,11–13]) and the Figure supports it as a field-expedient technique.[6]

Fluid Volumes and Varieties

The second goal of this investigation was to precisely assess fluid volumes and fluid varieties. The Institute of Medicine, National Academy of Sciences[3] reports that the adequate intake of all fluids (ie, from beverages, water, and foods) for 57-kg women is 2.7 L/24 hours. The European Food Safety Authority[14] recommends that women consume 2.0 L/24 hours. These recommendations are, respectively, slightly above and below the mean 2.4 L fluid (total 24-hour fluid intake) consumed by 59.6-kg test participants each day (Table 1). However, the range of total 24-hour fluid intake volumes was large, spanning 0.89 to 4.40 L/day.

Table 2 describes beverage types and volumes that were consumed on days 1 and 2 (mean±standard deviation and ranges). Water was, by far, the most popular beverage item; it accounted for 47.9%±25.7% (day 1) and 45.3%±26.4% (day 2) of total 24-hour fluid intake, and 63.2%±30.5% (day 1) and 62.7%±34.3% (day 2) of the total 24-hour beverage intake. In addition, total 24-hour fluid intake was strongly and positively correlated with total 24-hour beverage intake ($r^2=0.92$; $P<0.000001$) and with 24-hour water intake ($r^2=0.77$; $P<0.000001$). The authors offer the following hypothetical explanations: water was readily available and inexpensive, young women perceive water as a healthy beverage, the social environment of a university encourages water consumption, and water contains 0 kilocalories energy. Tea, milk, coffee, and fruit juice ranked second through fifth among beverages, respectively, but the amounts consumed (range of means=75 to 126 mL/24 hours; Table 2) were <10% of the volume of water. These daily beverage choices are somewhat different from the 1994 to 1996 Continuing Survey of Food Intakes by Individuals.[15] In that database, water was consumed in the greatest volume (approximately 32% of the total beverage volume), followed by coffee, carbonated-sweetened soft drinks, milk, and fruit juices.

Strengths and Limitations

Three primary strengths exist in this investigation. Firstly, carefully controlled procedures were instituted to optimize the accuracy of self-reported diet records. For example, a nutritionist personally met with each woman to describe diet record forms, serving sizes, food labels, and recording methods; participants were instructed to record food and fluid intake immediately after each meal or snack; and each woman met on the following morning with a diet counselor to review this practice as well as portion sizes, method of preparation (ie, fried, baked), number of servings, and food labels. Secondly, commercial software was used to analyze dietary macro- and micronutrients and data entry personnel were thoroughly trained in food item selection. Thirdly, to minimize variance in body mass and hydration measurements, all women were observed during the 4- to 7-day placebo phase of their oral contraceptive pill pack.

Three limitations are acknowledged in this investigation. Firstly, women consumed food in a university dining facility. Although free to select their own meals, the social and institutional framework might have biased participant food and fluid selections. A second limitation arises from the sample of women selected for this investigation. Because the participant sample was homogeneous, the external validity of these findings might be limited to healthy, young women. Thirdly, test participants were instructed to maintain their normal exercise and activity schedules during this investigation. This procedure was verified during a daily conversation with an investigator (ie, that no extraordinary exercise events occurred), but no written records were kept.

CONCLUSIONS

Although daily hydration assessment is widely recommended,[2,6,11–13] biomarkers are difficult to interpret and extremes are difficult to identify because reference norms do not exist and regulation of human water balance is complex and dynamic. These findings enable registered dietitians and sports medicine professionals to provide women with objective numerical values for eight commonly used hydration biomarkers. A well-hydrated woman (59.6 kg; Table 2) consumes >1.8 L of beverages, >2.5 L of fluid per day, and produces >1.3 L of urine per day. She also exhibits a urine specific gravity <1.016 and a urine color of ≤4. However, it is rare (<3% of all samples) for healthy, active women to achieve a urine specific gravity <1.005 and a urine color of 1 or 2. Regarding the fluid types that healthy young women consume, the mean daily water intake accounted for 45.3% and 47.9% of total 24-hour fluid intake and 62.7% and 63.2% of total 24-hour beverage intake (Figure). Because total 24-hour fluid intake was

Table 2. Beverages consumed by 32 women on 2 consecutive days in a study of hydration biomarkers and dietary fluid consumption

Day	Water (mL)	Milk (mL)	Tea (mL)	Coffee (mL)	Fruit juice (mL)	Soft drink[a] (mL)	Diet soft drink[b] (mL)	Sport drink (mL)	Energy drink (mL)	Volume (mL) of all liquid beverages ($B_{24\ hours}$)[c]	Portion of $TF_{24\ hours}$[d] consumed as liquid beverages (%)
	mean±SD[e]										
1	1,298±1,037	123±197	126±241	112±185	75±158	48±136	0±0	48±138	0±0	1,829±975	72.9±14.1
	range										
	0–4,022	0–591	0–946	0–589	0–591	0–473	0–0	0–532	0–0	739–4,259	40.4–94.5
	mean±SD										
2	1,206±979	95±202	109±285	81±159	117±240	93±198	22±87	11±49	15±84	1,750±803	71.6±14.7
	range										
	0–3,549	0–887	0–1,006	0–473	0–804	0–769	0–355	0–266	0–473	237–3,549	38.8–96.3

[a]Carbonated, contains sugar.
[b]Carbonated, contains no sugar (0 kcal).
[c]$B_{24\ hours}$=total 24-hour beverage intake; sum of nine beverages in columns 2 to 10.
[d]$TF_{24\ hours}$=total 24-hour fluid intake from beverages and solid food.
[e]SD=standard deviation.

strongly and positively correlated with total 24-hour beverage intake (r^2=0.92; P<0.000001) and with 24-hour water intake (r^2=0.77; P<0.000001), water intake was the difference between women who consume a small vs large total fluid volume (total 24-hour fluid intake). If active individuals anticipate a large sweat loss or fluid deficit on a given day, these numerical values can serve as daily hydration reference points.

References

1. Shore AC, Markandu ND, Sagnella GA, et al. Endocrine and renal response to water loading and water restriction in normal man. *Clin Sci*. 1988;75(2):171-177.
2. Armstrong LE. Assessing hydration status: The elusive gold standard. *J Am Coll Nutr*. 2007;26(suppl 5):575S-584S.
3. Food and Nutrition Board, Institute of Medicine. *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. Washington, DC: National Academies Press; 2004.
4. Centers for Disease Control and Prevention. *The Third National Health and Nutrition Examination Survey (NHANES III 1988-94) Reference Manuals and Reports [CD-ROM]*. Bethesda, MD: National Center for Health Statistics; 1996.
5. Academy of Nutrition and Dietetics Evidence Analysis Library. Hydration. http://www.adaevidencelibrary.com/search.cfm?keywords=hydration. Accessed March 10, 2012.
6. Armstrong LE, Maresh CM, Castellani J, et al. Urinary indices of hydration status. *Int J Sport Nutr*. 1994;4(3):265-279.
7. Cheuvront SN, Ely BR, Kenefick RW, Sawka MN. Biological variation and diagnostic accuracy of dehydration assessment markers. *Am J Clin Nutr*. 2010;92(3):565-573.
8. Armstrong LE, Pumerantz AC, Fiala KA, et al. Human hydration indices: Acute and longitudinal reference values. *Int J Sport Nutr Exerc Metab*. 2010;20(2):145-153.
9. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol*. 1974;37(2):247-248.
10. Hew TD. Women hydrate more than men during a marathon race. Hyponatremia in the Houston Marathon: A report on 60 cases. *Clin J Sports Med*. 2005;15(4):148-153.
11. Casa DJ, Armstrong LE, Hillman SK, et al. National Athletic Training Association position statement: Fluid replacement for Athletes. *J Athl Train*. 2000;35(2):212-224.
12. Oppliger RA, Bartok C. Hydration testing of athletes. *Sports Med*. 2002;32(15):959-971.
13. Mentes J, Wakefield B, Culp K. Use of a urine color chart to monitor hydration status in nursing home residents. *Biol Res Nurs*. 2006;7(3):197-203.
14. European Food Safety Authority. Scientific Opinion on Dietary Reference Values for water. *EFSA J*. 2010;8(3):1459-1507.
15. Heller KE, Sohn W, Burt BA, Eklund SA. Water consumption in the United States in 1994-96 and implications for water fluoridation policy. *J Public Health Dent*. 1999;59(1):3-11.

AUTHOR INFORMATION

L. E. Armstrong is a professor, E. C. Johnson and C. X. Munoz are doctoral students, B. Swokla is a student investigator, D. J. Casa is a professor, and C. M. Maresh is department head, Human Performance Laboratory, Department of Kinesiology, University of Connecticut, Storrs. L. Le Bellego is head of Hydration & Health Affairs and L. Jimenez is director of Hydration & Health Department, Danone Research, R&D Waters, Palaiseau, France.

Address correspondence to: Lawrence E. Armstrong, PhD, Human Performance Laboratory, Department of Kinesiology, University of Connecticut, U-1110, Storrs, CT 06269-1110. E-mail: lawrence.armstrong@uconn.edu

STATEMENT OF POTENTIAL CONFLICT OF INTEREST

L. Le Bellego and L. Jimenez are employed as scientists by Danone Research. L. E. Armstrong serves as a Scientific Advisory Board member and consultant to Danone Research. No potential conflict of interest was reported by E. C. Johnson, C. X. Munoz, B. Swokla, D. J. Casa, and C. M. Maresh.

FUNDING/SUPPORT

This research was funded by Danone Research, R & D Waters, Palaiseau, France.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the intellectual and technical contributions of Michael Judd.

ELSEVIER
MASSON

Elsevier Masson SAS
62, rue Camille-Desmoulins, 92442 Issy-les-Moulineaux cedex

Tel.: (33) 1 71 16 55 00
Fax: (33) 1 71 72 51 99

www.em-consulte.com

This article reprint is distributed with the support of Danone.