International Journal of Sport Nutrition, 1994, 4, 265-279 Communications © 1994 Human Kinetics Publishers, Inc.

# Urinary Indices of Hydration Status

# Lawrence E. Armstrong, Carl M. Maresh, John W. Castellani, Michael F. Bergeron, Robert W. Kenefick, Kent E. LaGasse, and Deborah Riebe

Athletes and researchers could benefit from a simple and universally accepted technique to determine whether humans are well-hydrated, euhydrated, or hypohydrated. Two laboratory studies (A, B) and one field study (C) were conducted to determine if urine color  $(U_{col})$  indicates hydration status accurately and to clarify the interchangeability of  $U_{col}$ , urine osmolality  $(U_{osm})$ , and urine specific gravity  $(U_{sg})$  in research.  $U_{col}$ ,  $U_{osm}$ , and  $U_{sg}$  were not significantly correlated with plasma osmolality, plasma sodium, or hematocrit. This suggested that these hematologic measurements are not as sensitive to mild hypohydration (between days) as the selected urinary indices are. When the data from A, B, and C were combined,  $U_{col}$  was strongly correlated with  $U_{sg}$  and  $U_{osm}$ . It was concluded that (a)  $U_{col}$  may be used in athletic/ industrial settings or field studies, where close estimates of  $U_{sg}$  or  $U_{osm}$  are acceptable, but should not be utilized in laboratories where greater precision and accuracy are required, and (b)  $U_{osm}$  and  $U_{sg}$  may be used interchangeably to determine hydration status.

*Key Words:* urine osmolality, urine specific gravity, plasma osmolality, plasma sodium, hematocrit, dehydration

Daily renal maintenance of water and minerals, which are both fundamental nutrients, may be affected by three factors: circadian rhythms, osmoregulation, and volume regulation (e.g., circulatory homeostasis) (5). *Circadian rhythms* of electrolyte excretion (2) are inherent in the organs or tissues of the body. These rhythms may be altered by environmental cues (e.g., light–dark ratio) but are independent of posture, physical activity, environmental temperature, and water or salt deprivation (22). *Osmoregulation* is affected by alterations in water and electrolyte excretion, under the influence of arginine vasopressin. *Volume regulation* is associated with water and sodium balance and may be altered by changes in posture, physical activity, and the size of the extracellular space (20). Because the formation of urine involves these interacting processes, the interpretation of urinary measurements (i.e., specific gravity, osmolality, electrolyte concentrations, color, and volume) is difficult. In fact, no universally accepted method

∠annones o her som det den socke, ersamenes met her over set fikter som stad et i før de bener

The authors are with The University of Connecticut Human Performance Laboratory, 2095 Hillside Rd., Storrs, CT 06269-1110. exists for laboratories to determine whether test subjects are well hydrated, euhydrated, or hypohydrated (7).

Zambraski et al. (25) and Tipton (23) suggested that urinalysis might be used as a screening device to prevent excessively dehydrated individuals from participating in athletic contests. However, athletes rarely have their urinary variables measured because instruments are costly and require technical expertise to operate. Athletes could determine personal hydration by measuring 24-hr urine volume (1), but these collections are time-consuming, represent multiple fluid feedings, require several urine collections, and provide little information about any single time point during the day. Nevertheless, this information is of practical importance because hypohydration (in excess of 3-5% of body weight) decreases endurance and strength (4, 19) and is the primary cause of heat exhaustion (9).

In an attempt to simplify this process, experts have advised athletes to observe their urine color  $(U_{col})$  each day, as an index of hydration status (24), because urine specific gravity  $(U_{sg})$  and  $U_{col}$  usually are closely related (11). Many athletes follow this recommendation but do not realize that  $U_{col}$  also may be altered by medications, certain foods, vitamin supplements, illness, and exercise (8, 14, 18). We believe that the safety and performance of athletes would be enhanced if the validity of  $U_{col}$  as an index of hydration status was established. Therefore, this paper presents three experiments that utilized a novel  $U_{col}$  scale and examined corresponding urinary and hematologic measurements. The purposes of these experiments were (a) to determine if  $U_{col}$  indicates hydration status accurately, as verified by  $U_{sg}$ , urine osmolality ( $U_{osm}$ ), plasma osmolality ( $P_{osm}$ ), or hematocrit, and (b) to clarify the use of  $U_{col}$ ,  $U_{osm}$ , and  $U_{sg}$  measurements in research.

#### Methods

The three studies in this investigation involved different research designs, as described in Table 1. Study A involved measurements of total body water via deuterium oxide dilution and bioelectrical impedance. The purpose of Study B was to identify physiological and anthropometric predictors of physical performance during uniformed exercise in a hot environment. Study C was designed to measure the fluid–electrolyte balance of collegiate tennis players during three tennis matches, played over a 10-hr period, in a hot environment. Taken together, Studies A, B, and C represented a variety of factors (i.e., environmental conditions, gender, heat acclimatization status, bladder void order, rest, and exercise), This increased the variety of scenarios to which  $U_{col}$  might be applied as an index of hydration.

Temperature and relative humidity (%rh) were measured via a thermohygrometer (Model 3309-60, Cole-Parmer Instrument Co., Chicago, IL) in all studies. Prior to testing, each protocol was approved by the University Institutional Review Board; the subjects received a verbal and written explanation of all procedures, risks, and benefits and gave their voluntary consent to participate. The personal characteristics of test subjects are described in Table 2; they all were healthy and had no known renal or urinary tract disease. Calipers (Holtain Ltd., Crymych, UK) were used to measure skinfolds at seven sites; skinfolds were used to calculate the body fat percent of men and women according to the method of Pollock and Wilmore (17).

| and the second second states and the second seco | State of the second | and the second |            |
|---|--|--|------------|
| Design features   | Study A  | Study B  | Study C    |
| Laboratory study<br>Field study   | Х  | na se  | X          |
| Temperature (°C)  | $24 \pm 1$   | $32.2 \pm 0.4$   | 27.2–33.7ª |
| Relative humidity (%)   | $36 \pm 6$   | 57 ± 19  | 57–71ª     |
| Site  | New England  | New England  | Florida    |
| Measurements taken  |  |  |            |
| At rest (no exercise)   | X  |  | Xb         |
| Preexercise (resting)   |  | X  | X          |
| Postexercise  |  | X  | Xc         |
| Duration (hr)   | 4.5  | 4  | 72         |
|   |  |  |            |

#### Table 1 Research Designs of Studies A, B, and C

<sup>a</sup>Range of outdoor measurements, 0800–1800 hr. <sup>b</sup>Day 4 only, <sup>c</sup>Urine electrolytes were measured in morning samples only.

| Study A         | Study B  | Study C   |   |
|-----------------|--|---|---|
|                 | <u>x</u>   |   | 21 <u>8. 8.</u>   |
| Male            | Male   | Female  | Male  |
| 23              | 11   | 8   | 12  |
| $23 \pm 2$      | $23 \pm 2$   | $20 \pm 2$  | $21 \pm 2$  |
| $179.2 \pm 7.5$ | 177.3 ± 6.7  | 166.8 ± 10.2  | $180.0 \pm 4.9$   |
| $77.4 \pm 12.4$ | 78.6 ± 13.7  | $60.3 \pm 6.3$  | $73.8 \pm 8.9$  |
| $15.3 \pm 6.2$  | $11.4 \pm 4.7$   | $21.3 \pm 4.6$  | $8.0 \pm 3.0$   |
| No              | No   | Yes   | Yes   |
|                 | Male<br>23<br>23 ± 2<br>179.2 ± 7.5<br>77.4 ± 12.4<br>15.3 ± 6.2 | MaleMale231123 $\pm 2$ 23 $\pm 2$ 179.2 $\pm 7.5$ 177.3 $\pm 6.7$ 77.4 $\pm 12.4$ 78.6 $\pm 13.7$ 15.3 $\pm 6.2$ 11.4 $\pm 4.7$ | Study AStudy BStudy CMaleMaleFemale2311823 $\pm 2$ 23 $\pm 2$ 20 $\pm 2$ 179.2 $\pm 7.5$ 177.3 $\pm 6.7$ 166.8 $\pm 10.2$ 77.4 $\pm 12.4$ 78.6 $\pm 13.7$ 60.3 $\pm 6.3$ 15.3 $\pm 6.2$ 11.4 $\pm 4.7$ 21.3 $\pm 4.6$ |

# Table 2 Personal Characteristics $(M \pm SD)$ of Subjects in Studies A, B, and C

# Study Protocols

Study A. The 23 males in Study A were college students who responded to a search for test subjects. A survey of their physical training habits and heat exposures, during the 30 days prior to testing, indicated that they ranged from unfit to very fit and were not heat acclimatized. They were instructed to drink two glasses of water ( $\geq$ 237 ml each) prior to going to sleep (approximately 9 hr before testing) and after waking on the day of testing, to decrease the likelihood that they would begin testing in a hypohydrated state. They also were instructed to consume no food on the morning of testing. A urine specimen collected in the laboratory, when subjects reported at 0730 hr, was the first bladder void in virtually all cases. This urine specimen was analyzed for U<sub>col</sub>, U<sub>osin</sub>, U<sub>sg</sub>, and U<sub>Na</sub>. Subsequently, body mass was measured ( $\pm$ 50 g) and a venous blood sample (10 ml) was collected via syringe, from an antecubital vein, and was analyzed for  $P_{osm}$ ,  $P_{Na}$ , and hematocrit. Subjects then consumed 115 ml of water and reclined in an air-conditioned room (22 °C) for 4 hr. Subjects provided a final urine sample by voiding the bladder, to allow measurement of 4-hr urine volume.

Study B. The 11 males in this study were college students who responded to a search for test subjects. A survey (see Study A) indicated that their physical fitness ranged from unfit to fit and that they were not heat acclimatized. Subjects were given instructions regarding their water and food intake that were identical to those described in Study A. They reported to the laboratory between 0800 and 1400 hr on the day of testing. Their pretest urine sample typically represented the second bladder void of the day. This specimen and the final urine specimen (5 min postexercise) were analyzed for  $U_{osm}$ ,  $U_{col}$ ,  $U_{sg}$ , and  $U_{Na}$ .

Prior to exercise, a cardiotachometer was placed on the chest and a rectal probe was inserted 10 cm beyond the anal sphincter, to allow continuous monitoring of heart rate and rectal temperature, respectively. Subjects next donned a three-layer clothing ensemble that consisted of sneakers, cotton socks, shorts, t-shirt, a thick hooded sweat suit (cotton pants and shirt), and a one-piece hooded Tyvek<sup>TM</sup> coverall. This ensemble allowed only the anterior surface of the face and the hands to be exposed to air. Subjects then entered a heated environment chamber ( $32.2 \pm 0.4$  °C,  $57 \pm 19$  %rh) and stood for 15 min to allow temperature and body fluid equilibration to occur. A 6-ml sample of blood was then drawn from an antecubital vein and was analyzed (along with the 10-min postexercise blood sample) for P<sub>osm</sub>, P<sub>Na</sub>, and hematocrit.

Exercise each hour involved 50 min of treadmill walking (4.0 km  $\cdot$  hr<sup>-1</sup>, 0% grade) followed by 10 min of standing rest. Although the duration of each trial was scheduled to be 3 hr, heart rate and rectal temperature safety limits were employed. If a subject's heart rate exceeded 180 beats  $\cdot$  min<sup>-1</sup> during 5 consecutive min, or if rectal temperature exceeded 39 °C, exercise was terminated prematurely, final measurements were taken, and the subject disrobed in a 22 °C environment and voided to allow measurements of postexercise urine volume. During exercise, subjects consumed only enough water to wet their mouths. Body mass was measured on a platform scale (±50 g; Model 700M, SR Instruments, Tonawanda, NY) immediately prior to dressing and after disrobing, to allow sweat rate to be calculated (corrected for water intake and urine output).

*Study C.* The 20 subjects (8 female, 12 male) in Study C were highly trained members of intercollegiate tennis teams (National Collegiate Athletic Association, Division I), who were heat acclimatized by virtue of their regular, strenuous training near the test site (Coral Gables, FL) for at least the 2 preceding months. These athletes played three strenuous tennis matches on each of 3 consecutive days, beginning at approximately 0800 hr (singles), 1200 hr (singles), and 1600 hr (doubles), in hot, humid conditions (Table 1). Subjects consumed food and fluids ad libitum during matches, between matches, and during non-testing hours (1800–0800 hr).

Prior to and after match play each day, body weight was recorded ( $\pm 50$  g) and urine and blood samples were collected. Morning urine specimens, which represented the second bladder void, were analyzed for U<sub>sg</sub>, U<sub>col</sub>, and U<sub>Na</sub>. Blood samples (forearm vein, 10 ml, 15 min seated equilibration period prior to collection) were analyzed for hematocrit, serum osmolality (S<sub>osm</sub>), and P<sub>Na</sub>.

During nontesting periods (approximately 1800–0800 hr), subjects were given no specific instructions regarding water intake. All subjects also reported

on the morning subsequent to 3 days of match play (Day 4), for body weight, urine, and blood measurements. Other data from this study have been published as a doctoral dissertation (3).

#### **Body Fluid Measurements**

Analyses of body fluids were performed in duplicate, unless noted otherwise.  $P_{osm}$  (serum osmolality [ $S_{osm}$ ] in Study C) was analyzed with an osmometer (Model 3MO, Advanced Instruments, Needham Heights, MA). Plasma sodium ( $P_{Na}$ ) and urine sodium ( $U_{Na}$ ) were measured with selective ion-specific electrodes (Model 984-S, AVL Scientific Corp., Roswell, GA). A hand-held refractometer (Model A300CL, Spartan Refractometer, Japan) was used to visually appraise the specific gravity. In Studies A and B, hematocrit was measured in triplicate, by introducing samples into microcapillary tubes and centrifuging, using the microhematocrit technique. In Study C, hematocrit was assessed with a Coulter counter (Model STKR, Coulter Electronics, Hialeah, FL).

Urine analyses were organized in the following manner. Subjects placed a midstream urine specimen (at least 4 hr postprandial) into a 120-ml inert, polypropylene container. All specimens were analyzed for color and urine specific gravity within 30 min of collection. Urine specimens from subjects involved in Study C were obtained at the same time of day, on 4 separate days. Urine samples in Studies A and B were refrigerated in inert microcentrifuge tubes (4 °C, 1 ml) and analyzed for  $U_{Na}$  and osmolality, in triplicate, within 96 hr of collection;  $U_{osm}$  was not measured in Study C. Urine designated for sodium measurements was stored at -88 °C. Prior to analysis, these sealed samples were thawed, incubated in a 37 °C water bath for 15 min, and thoroughly mixed with a vortex shaker.

Urine color was determined by the same investigator in all studies. This was accomplished by holding each specimen container next to a color scale, in a well-lighted room. This novel urine color scale was developed in our laboratory and was based on observations of previous field and laboratory urine specimens. The eight-color scale included colors ranging from very pale yellow to brownish green. Because colors are difficult to standardize, the colors in our urine scale were compared to the classic compendium of colors published by Maerz and Paul (12) and matched the following standardized samples (plate/grid number): Color 1, 17/B1; Color 2, 9/H1; Color 3, 17/J1; Color 4, 17/L1; Color 5, 9/I3; Color 6, 9/L3; Color 7, 12/K6; Color 8, 23/L1.

Statistical Analyses. Studies A, B, and C incorporated calculations of linear regression and Pearson product correlation coefficients between all variables measured. Changes in urine and hematologic measurements during exercise in Study B were evaluated for significance by using Student's *t* test. In Study C, a two-way multivariate analysis of variance (MANOVA) was used to assess withinday (pre- vs. postexercise), between-day, and female versus male differences. Significance was determined at the .05 level of confidence. Tukey's post hoc test was used, when necessary. All terms were expressed as the mean  $\pm SD$ .

# Results

The same variables were measured in Studies A, B, and C, except that in Study C measurements of  $U_{osm}$  and urine volume were not taken, and  $S_{osm}$  was analyzed instead of  $P_{osm}$ . Measurements taken at the beginning of each testing period appear

#### 270 / Armstrong, Maresh, Castellani, et al.

in Tables 3 (Study A), 4 (Study B), 5 (Study C, females), and 6 (Study C, males). The postexercise values recorded in Studies B and C are also presented in Tables 4-6. Mean body weight loss did not exceed 3% per day in any study.

In Study B, neither final body weight nor change in body weight ( $\Delta BW$ ) was significantly correlated with any urine variable (U<sub>col</sub>, U<sub>sg</sub>, U<sub>Na</sub>, U<sub>osm</sub>, or urine volume). Similar comparisons were made for Study C, but only∆BW and hematocrit (r = -.61, p < .01) and  $\Delta BW$  and  $\Delta P_{Na}$  (r = -.52, p < .02) were significantly

Table 3 Measurements (M, SD, range) From Study A (23 Male College Students)<sup>a</sup>

| elements with the control of the control of the second second second second second second second second second  | ABORT Spanna References Freeza   |
|---|--|
| Measurement M   | <i>SD</i> Range  |
| and the second second second second   |  |
| $U_{col}$ , and the second se |  |
| $U_{osm} (mOsm \cdot kg^{-1})$  |  |
| $U_{sg}$ is the first distribution of the constraint $1.023$  | .006 1.005–1.033   |
| $U_{Na} (mEq \cdot L^{-1})$ 120.3   |  |
| Total urine volume (ml) 297   |  |
| $P_{osm}$ (mOsm · kg <sup>-1</sup> ) 289  |  |
| $P_{Na} (mEq \cdot L^{-1})$   | 1.8 141.3–150.2  |
| Hematocrit (%)  | other 2110 bas , Unit 44-50 this   |
|   | The second Alexandra |

Note.  $U_{col}$  = urine color;  $U_{osm}$  = urine osmolality;  $U_{sg}$  = urine specific gravity;  $U_{Na}$ urine sodium;  $P_{osm}$  = plasma osmolality;  $P_{Na}$  = plasma sodium. <sup>a</sup>Samples taken upon arrival at the laboratory at 0730 hr.

| Table 4Measurements (M, SD, | range) From S | Study B, Pre- and | l Postexercise |
|-----------------------------|---------------|-------------------|----------------|
| in a Hot Environment        |               | n horn charachadh | gas boyant ann |

|                                      | o sodojikaj<br>Ge <u>ropo</u> j | Preexer |             | Postexercise |                   |                 |  |
|--------------------------------------|---------------------------------|---------|-------------|--------------|-------------------|-----------------|--|
| Measurement                          | M SD                            |         | Range       | М            | SD                | Range           |  |
| TT                                   |                                 |         |             |              | <u>- 1916-5 5</u> | hals - 2, 2016) |  |
|                                      | 3                               |         | 1–6         | 5*           | 2                 | 2–7             |  |
| $U_{osm} (mOsm \cdot kg^{-1})$       | 510                             | 332     | 76-1,172    | 683          | 268               | 209-1,024       |  |
| U <sub>sg</sub>                      | 1.013                           | 0.009   | 1.003-1.028 | 1.019        | 0.008             | 1.005-1.034     |  |
| $U_{Na} (mEq \cdot L^{-1})$          | 83.0                            | 77.7    | 15.1-263.2  | 64.2         | 61.4              | 17.6–199.6      |  |
| Total urine volume (ml)              |                                 |         |             | 120          | 243               | 0–689           |  |
| $P_{osm}$ (mOsm · kg <sup>-1</sup> ) | 286                             | 0.31    | 283-292     | 293**        | 3                 | 287-301         |  |
| $P_{Na} (mEq \cdot L^{-1})$          | 145.3                           | 1.4     | 143.1-148.1 | 148.1***     | 2.2               | 144.5–151.5     |  |
| Hematocrit (%)                       | 46                              | 2       | 42–49       | 48           | 2                 | 43–51           |  |

Note. Subjects were 11 male college students wearing an insulated clothing ensemble. \*p < .025, pre- versus postexercise. \*\*p < .0001, pre- versus postexercise. \*\*\*p < .0001.005, pre- versus postexercise.

| Measurement                                |      | $M \pm SD$         |                 |                    | Range              |                                       |        |        |        |
|--|------|--------------------|-----------------|--------------------|--------------------|---------------------------------------|--------|--------|--------|
|  | Time | Day 1              | Day 2           | Day 3              | Day 4 <sup>a</sup> | Day 1                                 | Day 2  | Day 3  | Day 4ª |
| U <sub>col</sub>                           | Pre  | 5                  | 6               | 5                  | 5                  | 3–6                                   | 3–7    | 2–6    | 2–7    |
|  |      | ±1                 | ±1              | ±2                 | ±3                 |                                       |        |        |        |
|  | Post | 6                  | 6               | 7                  |                    | 6–7                                   | 6–7    | 5-7    |        |
|  |      | ±1                 | ±1              | ±1                 |                    |                                       |        |        |        |
| U <sub>sg</sub>                            | Pre  | 1.018              | 1.021           | 1.017 <sup>b</sup> | 1.022              | 1.003-                                | 1.005- | 1.004- | 1.003- |
|  |      | ±0.008             | ±0.008          | ±0.010             | ±0.010             | 1.026                                 | 1.028  | 1.029  | 1.030  |
|  | Post | 1.024 <sup>b</sup> | 1.028           | 1.029              |                    | 1.015-                                | 1.026- | 1.024- |        |
|  |      | ±0.007             | ±0.002          | ±0.004             |                    | 1.034                                 | 1.031  | 1.034  |        |
| $U_{Na} (mEq \cdot L^{-1})$                | Pre  | 122.3              | 77.0            | 81.5               | 112.9              | 32.3-                                 | 4.9-   | 0.0-   | 0.0-   |
|  |      | ±80.0              | ±39.2           | ±75.0              | ±101.9             | 194.3                                 | 110.5  | 191.8  | 227.1  |
|  | Post |                    |                 |                    |                    | · · · · · · · · · · · · · · · · · · · |        |        | ·      |
| $S_{osm}$ (mOsm $\cdot$ kg <sup>-1</sup> ) | Pre  | 284                | 285             | 282                | 283                | 282-                                  | 281-   | 281-   | 279-   |
|  |      | ±2                 | ±3              | ±3                 | ±4                 | 288                                   | 289    | 289    | 290    |
|  | Post | 285                | 286             | 288                |                    | 280-                                  | 282-   | 284-   |        |
|  |      | ±4                 | ±5              | ±4                 |                    | 291                                   | 290    | 294    |        |
| $P_{Na} (mEq \cdot L^{-1})$                | Pre  | 145.2              | 144.0           | 142.9              | 143.3              | 142.9-                                | 141.3- | 141.7- | 140.2- |
|  |      | ±1.5               | $\pm 1.8$       | ±1.0               | ±1.8               | 147.3                                 | 146.9  | 143.9  | 144.8  |
|  | Post | 144.9              | 145.3           | 145.3              |                    | 142.9-                                | 140.7- | 145.6- |        |
|  |      | ±1.7               | ±3.2            | ±1.0               |                    | 148.1                                 | 149.1  | 146.0  |        |
| Hematocrit (%)                             | Pre  | 38 <sup>b</sup>    | 37 <sup>b</sup> | 36 <sup>b</sup>    | 36 <sup>b</sup>    | 35-42                                 | 35-39  | 33-40  | 33-38  |
|  |      | ±2                 | ±2              | ±3                 | ±2                 |                                       |        |        |        |
|  | Post | 38 <sup>b</sup>    | 37 <sup>b</sup> | 36 <sup>b</sup>    |                    | 35-42                                 | 35-41  | 34-38  |        |
|  |      | ±2                 | ±3              | ±2                 |                    |                                       |        |        |        |

 Table 5 Measurements (M, SD, range) From Study C (8 Female Collegiate Tennis Players), Taken Pre- and Post- Outdoor Match Play in a Hot Climate

*Note.* No significant within-day or between-day differences were observed.  $S_{osm}$  = serum osmolality. <sup>a</sup>Morning measurements only; no matches were played. <sup>b</sup>Significantly different (p < .05 to .0001) from the male subjects in Table 6, at the corresponding day and time.

| Measurement                          | Time | $M \pm SD$ |        |        | Range              |        |        |        |        |
|--------------------------------------|------|------------|--------|--------|--------------------|--------|--------|--------|--------|
|                                      |      | Day 1      | Day 2  | Day 3  | Day 4 <sup>a</sup> | Day 1  | Day 2  | Day 3  | Day 4ª |
| U <sub>col</sub>                     | Pre  | 6          | 6      | 6      | 5                  | 4–7    | 4–7    | 3–7    | 2–7    |
|                                      |      | ±1         | ±1     | ±1     | ±1                 |        |        |        |        |
|                                      | Post | 7          | 6      | 6      |                    | 6–7    | 5-7    | 3–7    |        |
|                                      |      | ±1         | ±1     | ±2     |                    |        |        |        |        |
| U <sub>sg</sub>                      | Pre  | 1.023      | 1.027  | 1.027  | 1.022              | 1.012- | 1.018- | 1.018- | 1.013- |
|                                      |      | ±0.006     | ±0.005 | ±0.005 | ±0.005             | 1.031  | 1.034  | 1.033  | 1.031  |
|                                      | Post | 1.029      | 1.031  | 1.028  |                    | 1.020- | 1.025- | 1.018- |        |
|                                      |      | ±0.004     | ±0.004 | ±0.005 |                    | 1.032  | 1.037  | 1.034  |        |
| $U_{Na}$ (mEq · L <sup>-1</sup> )    | Pre  | 106.8      | 79.2   | 59.0   | 89.3               | 21.7-  | 0.0-   | 0.0-   | 0.0-   |
|                                      |      | ±45.0      | ±58.5  | ±43.0  | ±59.6              | 173.7  | 158.8  | 124.5  | 146.4  |
|                                      | Post |            |        |        |                    |        |        |        |        |
| $S_{osm}$ (mOsm · kg <sup>-1</sup> ) | Pre  | 287        | 287    | 286    | 285                | 283-   | 282-   | 281-   | 282-   |
| Ç,                                   |      | ±3         | ±3     | ±3     | ±3                 | 291    | 294    | 290    | 290    |
|                                      | Post | 288        | 288    | 289    |                    | 283-   | 282-   | 280-   |        |
|                                      |      | ±5         | ±4     | ±5     |                    | 296    | 295    | 296    |        |
| $P_{Na} (mEq \cdot L^{-1})$          | Pre  | 145.6      | 144.7  | 143.9  | 141.9 <sup>a</sup> | 140.9- | 139.8- | 141.2- | 139.5- |
|                                      |      | ±2.4       | ±3.3   | ±1.7   | ±2.1               | 150.1  | 150.6  | 145.5  | 146.8  |
|                                      | Post | 145.7      | 144.6  | 144.5  |                    | 143.3- | 137.2- | 141.1- |        |
|                                      |      | ±2.0       | ±3.4   | ±2.5   |                    | 150.0  | 150.2  | 149.4  |        |
| Hematocrit (%)                       | Pre  | 43         | 43     | 44     | 44                 | 40-47  | 40-48  | 41-50  | 41-47  |
|                                      |      | ±3         | ±3     | ±2     | ±2                 |        |        |        |        |
|                                      | Post | 42         | 43     | 42     |                    | 40-46  | 40-50  | 38-46  |        |
|                                      |      | ±2         | ±3     | ±2     |                    |        |        |        |        |

Table 6 Measurements (M, SD, range) From Study C (12 Male Collegiate Tennis Players) (Protocol Identical to That in Table 5)

Note. No significant within-day differences were observed. aSignificantly different from the preexercise values on Day 1 and Day 2, p < .05.

correlated. In addition, no urinary variable ( $U_{col}$ ,  $U_{sg}$ ,  $U_{Na}$ , or  $U_{osm}$ ) was significantly correlated with any hematologic variable ( $P_{osm}$ ,  $S_{osm}$ ,  $P_{Na}$ , or hematocrit) in any of the studies. Because there is no standard for fluid–electrolyte assessment that is used in all clinical and research settings (7),  $U_{osm}$  and  $U_{sg}$  (widely recognized as hydration indices; see References 1, 2, 6, 7, 12, 14, 16, 20, 23) were used as the standards for comparisons with  $U_{col}$ , to determine if  $U_{col}$  could be used as an acceptable index of body hydration status.

### Study A

A matrix of correlation coefficients between all variables in Table 3 identified the following significant relationships (r, p level):  $U_{col}$  and  $U_{sg}$ , +.54, p < .01;  $U_{col}$  and  $U_{osm}$ , +.62, p < .01; and  $U_{osm}$  and  $U_{sg}$ , +.78, p < .000001.

#### Study B

A correlation matrix of all preexercise variables in Table 4 identified the following significant relationships (r, p level): U<sub>col</sub> and U<sub>sg</sub>, +.93, p < .000001; U<sub>col</sub> and U<sub>osm</sub>, +.92, p < .0001; U<sub>osm</sub> and U<sub>sg</sub>, +.98, p < .000001; and U<sub>sg</sub> and U<sub>Na</sub>, +.79, p < .01.

The mean preexercise heart rate and rectal temperature were  $79 \pm 15$  beats  $\cdot \min^{-1}$  and  $37.1 \pm 0.4$  °C. The corresponding postexercise values were 144  $\pm 24$  beats  $\cdot \min^{-1}$  and  $38.8 \pm 0.3$  °C. Only 4 subjects completed the entire 3-hr exercise protocol before reaching preestablished physiological termination limits. The mean exercise duration was  $149 \pm 38$  min. The mean sweat rate was 0.88  $\pm 0.56$  L  $\cdot$  hr<sup>-1</sup>. Subjects consumed an average of  $4 \pm 4$  ml water during this protocol; 6 individuals consumed no water.

Of all fluid-electrolyte variables presented in Table 4, only  $U_{col}$ ,  $P_{osm}$ , and  $P_{Na}$  were altered by exercise (p < .025 to .0001). A correlation matrix of postexercise measurements in Table 4 identified these significant relationships (r, p level):  $U_{col}$  and  $U_{sg}$ , +.88, p < .0005;  $U_{col}$  and  $U_{osm}$ , +.89, p < .0005; and  $U_{osm}$  and  $U_{sg}$ , +.96, p < .00005.

# Study C

Tables 5 and 6 present the mean group values for females and males, respectively, during the field study. Two correlation matrices were compiled for preexercise measurements on Days 1 and 4 (females and males combined). The matrix for Day 1 identified two significant relationships, (r, p level):  $U_{col}$  and  $U_{sg}$ , +.78, p < .0001; and  $U_{sg}$  and  $U_{Na}$ , +.42, p < .05. The matrix for Day 4 identified three significant correlation coefficients:  $U_{col}$  and  $U_{sg}$ , +.78, p < .0005;  $U_{sg}$  and  $U_{Na}$ , +.58, p < .05; and  $S_{osm}$  and  $P_{Na}$ , +.53, p < .05.

The duration of exercise (two singles matches and one doubles match) each day was approximately 4.5 hr. The mean sweat rate during match play (Days 1–3) ranged from 0.9 to 1.0 L  $\cdot$  hr<sup>-1</sup> for females and from 1.6 to 1.9 L  $\cdot$  hr<sup>-1</sup> for males. The range of the mean volume of water consumed during each match (Days 1–3) was 1.1 to 1.5 L  $\cdot$  match<sup>-1</sup> for females and 1.5 to 1.8 L  $\cdot$  match<sup>-1</sup> for males. The mean body weight loss, between 0800 and 1800 hr, was 0.71 ± 0.15 kg for females and 1.37 ± 0.27 kg for males. The two-way MANOVA of the variables in Tables 5 and 6 identified a few significant differences (p < .05 to .0001) between females and males (hematocrit, all days; U<sub>sg</sub>, Day 1 and Day 3). None of the fluid–electrolyte variables in Tables 5 and 6 were altered within-day (pre- vs. postexercise); this was attributed to the rest, food intake, and consumption of water between matches. Only one measurement (P<sub>Na</sub>, Day 4, males only) exhibited a between-day change (p < .05).

#### Studies A, B, and C Combined

The measurements of all studies (female and male) were combined to produce two figures. Figure 1 presents frequency distributions of  $U_{osm}$  (n = 45),  $U_{sg}$  (n = 85), and  $U_{col}$  (n = 84) values (pre- and postexercise, females and males) in Studies A, B, and C. The means  $\pm SD$  (and 95% confidence limits) of these frequency distributions were as follows:  $U_{col}$ ,  $5 \pm 2$  (1–8);  $U_{sg}$ , 1.021  $\pm$  .008 (1.005–1.037); and  $U_{osm}$ , 747  $\pm$  305 (137–1,357).

Figure 2 presents selected relationships between  $U_{col}$ ,  $U_{sg}$ , and  $U_{osm}$  for nonexercise measurements. The correlation coefficients determined for these measurements were as follows (r, p level, n):  $U_{osm}$  and  $U_{sg}$ , +.97, p < .0001, n = 45;  $U_{col}$  and  $U_{sg}$ , +.80, p < .0001, n = 54; and  $U_{col}$  and  $U_{osm}$ , +.82, p < .0001, n = 53.

#### Discussion

Results presented in this paper support previous observations made by Francesconi and colleagues (7) during a longitudinal field study which reported that hematocrit and P<sub>osm</sub> were not significantly different when test subjects were categorized on the basis of low  $U_{sg}$  (<1.030) versus high  $U_{sg}$  ( $\geq$ 1.030). Specifically, we found that neither U<sub>col</sub> nor any other urinary variable was significantly correlated with Posm, PNa, or hematocrit (Tables 3-6). These findings support the two hypotheses of Francesconi et al. (7): (a) Plasma volume is defended by the body to maintain cardiovascular stability, and plasma variables are unaffected until a threshold level of body water loss (i.e., >3% of body weight) has been achieved, and (b)  $U_{col}$ ,  $U_{sg}$ , and  $U_{osm}$  are more sensitive indices of moderate hypohydration than are blood measurements. The fact that Table 4 shows significant increases in  $P_{osm}$ ,  $P_{Na}$ , and  $U_{col}$  without significant changes in either  $U_{sg}$  or  $U_{osm}$  may be reconciled by recognizing that Study B involved within-day responses to exercise, whereas Francesconi et al. (7) recorded resting measurements on Days 1, 20, and 44 of a field study. Therefore, these two hypotheses may not apply to acute, exercise-induced changes.

Urinary indices have been used to assess hydration status in many subject populations, including collegiate wrestlers (23), triathletes (6), soldiers (1, 7, 10), and children (2, 17).  $U_{sg}$  is the most commonly measured index of urine concentration in these studies, but  $U_{osm}$  is physiologically more accurate than  $U_{sg}$  because it is unaffected by solutes such as glucose, protein, and urea (18). The presence of these solutes may explain why some authors have reported that  $U_{sg}$  and  $U_{osm}$  are not strongly correlated (2), whereas others have reported that  $U_{sg}$  and  $U_{osm}$  are closely related (18). In the current studies,  $U_{osm}$  and  $U_{sg}$  were linearly related (r = +.97, p < .0001). Similarly,  $U_{col}$  was strongly correlated with both  $U_{osm}$  and  $U_{sg}$  (r = +.82 and +.80, both p < .0001). Figure 2 suggests that  $U_{col}$  may

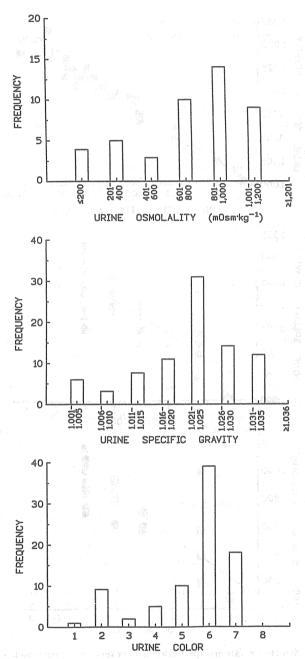


Figure 1 — Frequency distributions for all  $U_{osm}$  (n = 45),  $U_{sg}$  (n = 85), and  $U_{col}$  (n = 84) values in Studies A, B, and C. Pre- and postexercise, female, and male values have been included.

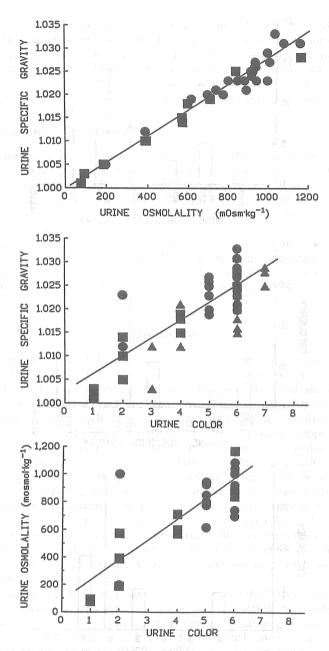


Figure 2 — Selected relationships between preexercise measurements of  $U_{osm}$ ,  $U_{col}$ , and  $U_{sg}$ . Text provides correlation coefficients and statistical significance of each graph.  $\Phi$  = Study A;  $\blacksquare$  = Study B;  $\blacktriangle$  = Study C.

be used in athletic or industrial settings, where close estimates of  $U_{sg}$  or  $U_{osm}$  are acceptable, but should not be utilized in laboratories where greater precision and accuracy are required.

#### **Exercise and Gender Effects**

Although Studies B and C involved pre- and postexercise data, Study B was unique because subjects consumed water only to wet their mouths  $(4 \pm 4 \text{ ml})$ . This protocol resulted in the only significant exercise-induced changes observed in any fluid–electrolyte variables ( $U_{col}$ ,  $P_{Na}$ , and  $P_{osm}$  in Table 4). The fact that  $U_{col}$  changed during exercise suggests that it was a sensitive index of dehydration and that it reflects reductions in renal plasma flow and glomerular filtration rate which accompany treadmill walking in a hot environment (21).

Even though Study C involved 4.5 hr of strenuous tennis match play in hot, humid conditions, no exercise-induced changes were observed (Tables 5 and 6) in any variable. This probably resulted from the consumption of fluids during matches, and food or fluids between matches. Similarly, it has been shown that most endurance athletes competing in a 9- to 13-hr triathlon in a mild environment maintained normal hemoglobin, hematocrit,  $P_{Na}$ , and glucose throughout the contest and developed only mild dehydration. This was attributed to their highly trained state and free access to food and fluids (6).

The female (n = 8) and male (n = 12) tennis players in Study C responded similarly during the 3 days of competitive match play. Gender-related differences occurred only in hematocrit and  $U_{sg}$  (Tables 5 and 6). It is well-known that normal hematocrit of females  $(42 \pm 5\%)$  is lower than that of males  $(47 \pm 7\%)$ (13). The significantly lower  $U_{sg}$  of female tennis players on Day 1 (postexercise) and Day 3 (preexercise) suggested that they, on occasion, maintained total body water more effectively than their male counterparts. Further support for this finding was derived from the observation that none of the 48 male urine specimens collected on Days 1 and 2 of Study C had a  $U_{col} < 4$  or a  $U_{sg} < 1.012$ .

### **Practical Applications**

Considering these findings, we recommend that urinary indices of hydration be utilized in the following ways:

1.  $U_{col}$  may be utilized as a meaningful index of hydration status when measurements of  $U_{osm}$  and  $U_{sg}$  are not possible or practical (e.g., athletic teams, laborers, field studies). Recognizing the limitations of color names\_(11), we recommend that athletes seek to produce urine that is "very pale yellow," "pale yellow," or "straw colored." These descriptive terms correspond to our  $U_{col}$  numbers 1, 2, and 3, respectively. Our data (Figure 2) indicate that the mean  $U_{osm}$  and  $U_{sg}$  will be respectively less than 520 mOsm  $\cdot$  kg<sup>-1</sup> and 1.014, if this is accomplished.

2. In research settings similar to Studies A, B, and C,  $U_{osm}$  and  $U_{sg}$  may be used interchangeably (r = +.97) to assess euhydration versus hypohydration at rest. The linearity of this relationship is slightly diminished at  $U_{osm} >$ 900 mOsm  $\cdot$  kg<sup>-1</sup> and  $U_{sg} > 1.024$  (Figure 2).

3. Considering the frequency distributions of  $U_{col}$ ,  $U_{osm}$ , and  $U_{sg}$  in Figure 1, we recommend that the term *well-hydrated* be defined as at least 1 SD less

#### 278 / Armstrong, Maresh, Castellani, et al.

than the mean ( $U_{col} < 3$ ,  $U_{osm} < 442$  mOsm  $\cdot$  kg<sup>-1</sup>,  $U_{sg} < 1.013$ ), that *euhydrated* be defined as the range from -1 to +1 *SD* ( $U_{col}$  3–7;  $U_{osm}$  442–1,052 mOsm  $\cdot$  kg<sup>-1</sup>;  $U_{sg}$  1.013–1.029), and that *hypohydrated* be defined as at least 1 *SD* greater than the mean ( $U_{col} > 7$ ,  $U_{osm} > 1,052$  mOsm  $\cdot$  kg<sup>-1</sup>,  $U_{sg} > 1.029$ ). These statistical demarcations of hydration status were derived in a manner analogous to the method used to determine the U.S. Recommended Daily Allowances for food nutrients (15). To our knowledge, these hydration terms have not been quantified previously (7).

4. The ranges of  $U_{osm}$ ,  $U_{sg}$ , and  $U_{col}$  in Tables 3 and 4 suggest that some individuals may require more than verbal instructions (i.e., feedback from objective measurements of  $U_{osm}$  and  $U_{sg}$ ) before learning how much fluid must be drunk to produce a dilute urine. Despite instructions to drink additional water in Studies A and B, some subjects arrived at the laboratory in a hypohydrated state  $(U_{osm} > 1,052 \text{ mOsm} \cdot \text{kg}^{-1}, U_{sg} > 1.029$ , see Item 3 above).

5. The results of Study C (Tables 5 and 6) suggest that day-to-day differences in mean urine measurements will be insignificant if meals, fluid consumption during exercise, and training are consistent. Athletes may reduce betweenday variability of urine measurements by standardizing these factors and the time at which urine specimens are collected. Also, athletes should recognize that firstvoid urine specimens tend to be slightly more concentrated than subsequent voids (14). The results of the current investigation suggest that the  $U_{col}$  of the second daily void, although not specifically tested, provides a stronger correlate of  $U_{osm}$ and  $U_{sg}$  than the  $U_{col}$  of the first void.

### References

- 1. Adolph, E.F. Urinary excretion of water and solutes. In *Physiology of Man in the Desert*, E.F. Adolph (Ed.), New York: Interscience, 1947, pp. 96-109.
- Ballauff, A., W. Rascher, H.G. Tolle, T. Wember, and F. Manz. Circadian rhythms of urine osmolality and renal excretion rates of solutes influencing water metabolism in 21 healthy children. *Miner. Electrolyte Metab.* 17:377-382, 1991.
- 3. Bergeron, M.F. *Evaluation of Fluid–Electrolyte Balance Associated With Tennis Match Play in a Hot Environment*. Storrs, CT: The University of Connecticut, Doctoral dissertation, 1993, pp. 1-110.
  - Coyle, E.F., and M. Hamilton. Fluid replacement during exercise: Effects on physiological homeostasis and performance. In *Fluid Homeostasis During Exercise* (Vol. 3), C.V. Gisolfi and D.R. Lamb (Eds.), Carmel, IN: Benchmark Press, 1990, pp. 281-308.
  - 5. de Vries, L.A., S.P. Ten Holt, J.J. van Daatselaar, A. Mulder, and J.G.G. Borst. Characteristic renal excretion patterns in response to physiological, pathological, and pharmacological stimuli. *Clin. Chim. Acta* 5:915-937, 1960.
- Farber, H., J. Arbetter, E. Schaefer, S. Hill, G. Dallal, R. Grimaldi, and N. Hill. Acute metabolic effects of an endurance triathlon. *Ann. Sports Med.* 3:131-138, 1987.
- Francesconi, R.P., R.W. Hubbard, P.C. Szlyk, D. Schnakenberg, D. Carlson, N. Leva, I. Sils, L. Hubbard, V. Pease, J. Young, and D. Moore. Urinary and hematologic indexes of hypohydration. *J. Appl. Physiol.* 62:1271-1276, 1987.
  - 8. L. Graff. A Handbook of Routine Urinalysis. Philadelphia: Lippincott, 1983, pp. 2-19.
- 9. Hubbard, R.W., and L.E. Armstrong. The heat illnesses: Biochemical, ultrastructural, and fluid–electrolyte considerations. In *Human Performance Physiology and Environ*-

mental Medicine at Terrestrial Extremes, K.B. Pandolf, M.N. Sawka, and R.R. Gonzalez (Eds.), Indianapolis, IN: Benchmark Press, 1988, pp. 305-360.

- 10. Katz, A.I., S. Massry, J. Agmon, and M. Toor. Concentration and dilution of urine in permanent inhabitants of hot regions. *Isr. J. Med. Sci.* 1:968-978, 1965.
- 11. Lippman, R.W. Urine and Urinary Sediment. Springfield, IL: Charles C Thomas, 1957, pp. 94-103.
- 12. Maerz, A., and M.R. Paul. *Dictionary of Color* (2nd ed.). New York: McGraw-Hill, 1950, pp. 41-69.
- 13. *Medical Technology Board Examination Review* (10th ed.). Anaheim, CA: Berkeley Scientific, 1984, pp. 1-707.
- 14. Modern Urine Chemistry. Elkhart, IN: Miles Laboratories, 1986, pp. 1-104.
- 15. National Research Council. *Recommended Dietary Allowances* (10th ed.). Washington, DC: National Academy Press, 1989, pp. 10-13.
- 16. Phillip, M., C. Chaimovitz, A. Singer, and D. Golinsky. Urine osmolality in nursery school children in a hot climate. *Isr. J. Med. Sci.* 29:104-106, 1993.
- 17. Pollock, M.L., and J.H. Wilmore. *Exercise in Health and Disease: Evaluation and Prescription for Prevention and Rehabilitation* (2nd ed.). Philadelphia: Saunders, 1990, pp. 329-346.
- Ross, D.L., and A.E. Neely. *Textbook of Urinalysis and Body Fluids*. Norwalk, CT: Appleton-Century-Crofts, 1983, pp. 46-73.
- Sawka, M.N., and K.B. Pandolf. Effects of body water loss on physiological function and exercise performance. In *Fluid Homeostasis During Exercise* (Vol. 3). C.V. Gisolfi and D.R. Lamb (Eds.), Carmel, IN: Benchmark Press, 1990, pp. 1-38.
- 20. Share, L. Vasopressin and regulation of water homeostasis and cardiovascular function.
- See In Endocrinology, People and Ideas, S.M. McCann (Ed.), Bethesda, MD: American Physiological Society, 1988, pp. 1-22.
- 21. Smith, J.H., S. Robinson, and M. Pearcy. Renal responses to exercise, heat, and dehydration. J. Appl. Physiol. 4:659-665, 1952.
- 22. Stanley, S.W., and A.E. Thomson. Diurnal variations in electrolyte excretion. *Clin. Sci.* 10:267-293, 1951.
- Tipton, C. Consequences of rapid weight loss. In *Nutrition and Athletic Performance*, W. Haskell, J. Scala, and J. Whittam (Eds.), Palo Alto, CA: Bull, 1982, pp. 176-197.
- 24. Wadler, G.I. Putting the crimp on cramps. Tennis 8:79-80, 1990.
- 25. Zambraski, E.J., C.M. Tipton, H.R. Jordon, W.K. Palmer, and T.K. Tcheng. Iowa wrestling study: Urinary profiles of state finalists prior to competition. *Med. Sci. Sports* 6:129-132, 1974.

- conference of approximation configuration of the second s

#### Acknowledgments

The authors gratefully acknowledge the technical assistance of the following individuals: Marcos Echegaray, Ralph Matott, Jodi Senk, Neema Shakibai, and Renate Staedel. The cooperation and perseverance of the coaches and varsity team members at the University of Miami and Florida International University were essential to the completion of Study C.

A copy of the urine color chart used in this study is available from Professor Armstrong, University of Connecticut, 2095 Hillside Rd., Box U-110, Storrs, CT 06269-1110.