

Dehydration is how you define it: comparison of 318 blood and urine athlete spot checks

Tamara D Hew-Butler, Christopher Eskin, Jordan Bickham, Mario Rusnak, Melissa VanderMeulen

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ABSTRACT

Clinical medicine defines dehydration using blood markers that confirm hypertonicity (serum sodium concentration ($[Na^+]$) >145 mmol/L) and intracellular dehydration. Sports medicine equates dehydration with a concentrated urine as defined by any urine osmolality (UOsm) \geq 700 mOsmol/kgH₂O or urine specific gravity (USG) \geq 1.020.

Objective To compare blood versus urine indices of dehydration in a cohort of athletes undergoing routine screenings.

Methods 318 collegiate athletes (193 female) provided blood and urine samples and asked to rate how thirsty they were on a 10-point visual analogue scale. Serum was analysed for $[Na^+]$, while serum and UOsm were measured using an osmometer. USG was measured using a Chemstrip. Data were categorised into dehydrated versus hydrated groupings based on these UOsm and USG thresholds.

Results Using UOsm \geq 700 mOsmol/kgH₂O to define dehydration, 55% of athletes were classified as dehydrated. Using any USG \geq 1.020 to define dehydration, 27% of these same athletes were classified as dehydrated. No athlete met the clinical definition for dehydration (hypertonicity; serum $[Na^+]$ >145 mmol/L). Normonatremia (serum $[Na^+]$ between 135 mmol/L and 145 mmol/L) was maintained in 99.7% of athletes despite wide variation in UOsm (110–1298 mOsmol/kgH₂O). A significant correlation was confirmed between serum $[Na^+]$ versus UOsm ($r=0.18$; $P<0.01$), although urine concentration extremes did not reflect derangement in serum markers or thirst rating.

Conclusion Urine concentration thresholds classified 27%–55% of collegiate athletes as dehydrated, while no athlete was dehydrated according to blood $[Na^+]$ measurement. Practitioners should caution against using urine indices to diagnose or monitor dehydration, because urinary output is a response rather than a reflection of (tightly regulated) blood tonicity.

INTRODUCTION

Dehydration is a term that generally reflects any loss of body water and linked to poor athlete performance, heat illness, skeletal muscle cramps and acute renal failure in athletes.¹

Given the potential for unfavourable health and performance consequences, a number of

What are the new findings?

- ▶ A urine osmolality above 700 mOsmol/kgH₂O would classify >50% of athletes as dehydrated, in a spot sample hydration check, despite normal blood sodium concentration, potassium concentration and osmolality values that would confirm the athletes were euhydrated (normonatremic) at rest.
- ▶ Urine concentration, either as urine osmolality or specific gravity, should not be used as a surrogate measure for blood hydration markers in a spot sample hydration check conducted at rest.
- ▶ A urine osmolality value above 700 mOsmol/kgH₂O, and/or urine specific gravity above 1.020, reflects urinary free water conservation and not clinically significant (intracellular) dehydration in a large cohort of collegiate athletes.

definitions have been used to define dehydration with an end goal of prevention. The American College of Sports Medicine uses non-invasive measures to define dehydration as a urine specific gravity (USG) \geq 1.020 and/or urine osmolality (UOsm) \geq 700 mOsmol/kgH₂O.¹ The advantage of using non-invasive measures is that they are quick, simple and easy to use in both laboratory and field settings. Studies using body weight and urine indices as outcome measures support the conclusion that athletes must drink beyond the physiological dictates of thirst to maintain adequate hydration levels.¹

In contrast to non-invasive measures of hydration status, clinical medicine is reliant on blood (serum or plasma) chemistry measurements to define and subdivide dehydration into two (often related) categories: intracellular and extracellular dehydration.^{2–3} Intracellular dehydration is defined by any loss of intracellular water that induces cellular desiccation and hypertonicity (ie, increases in blood sodium concentration ($[Na^+]$) or osmolality).^{2–3} Extracellular dehydration, which is also referred to as hypovolaemia or volume depletion, alternatively reflects any loss of



Department of Human Movement Science, Exercise Science Program, Oakland University, Rochester, Michigan, USA

Correspondence to

Dr Tamara D Hew-Butler; hew@oakland.edu

sodium from the extracellular space that compromises plasma volume.^{2 3} The distinction between intracellular and extracellular dehydration is diagnostically important when choosing the best therapeutic strategies for water lack (intracellular dehydration) and/or sodium lack (extracellular dehydration). This clinical definition also reflects the physiological tenets of fluid homeostasis, which strive to protect tonicity and tissue perfusion.^{2 4-6} Accordingly, the US Institute of Medicine states that the primary indicator of hydration status is plasma or serum osmolality^{7 8} with the maintenance of normonatremia (blood $[\text{Na}^+]$ between 135 mmol/L and 145 mmol/L) central to this evolutionary tenet.^{6 9} Accordingly, studies using plasma osmolality¹⁰⁻¹³ or plasma volume¹⁴ as indicators of hydration status support the conclusion that thirst is an adequate stimulus to prevent intracellular and extracellular dehydration.

Investigations assessing relationships between urine concentrations versus plasma osmolality (tonicity) in athletes fail to demonstrate statistical significance that would support the utility of using urine indices as a (more practical) surrogate measure of cellular hydration.¹⁵⁻²² The lack of diagnostic utility in using urine indices to predict intracellular dehydration has also been verified in a large sampling of 313 older people in a clinical setting.⁸ While plasma osmolality remains the hallmark measure of both tonicity and water balance,⁴⁻⁶ derangements in serum $[\text{Na}^+]$ represent extreme clinical manifestations of fluid imbalance that require emergent treatment.^{4 6} Accordingly, athlete deaths from underhydration have been associated with hypernatremia (serum $[\text{Na}^+] >145$ mmol/L),²³ while deaths from overhydration have been associated with hyponatremia (serum $[\text{Na}^+] <135$ mmol/L).²⁴

The primary aim of this study is to compare blood and urine indices using two common urine-based definitions of dehydration.^{1 21 25-27} Additionally, we chose serum $[\text{Na}^+]$ (instead of plasma osmolality) as our main outcome measure of blood tonicity to more critically reflect the clinical consequences documented in exercise-associated fatalities at the extremes of fluid imbalance.^{23 24} The practical importance of clarifying the sport (urine) versus clinical (blood) definition of dehydration distills down to one simple—yet hotly debated—question: how much should we drink? Our hypothesis is that dehydration—and utility of using thirst as a guide to prevent dehydration—is highly dependent on the criterion used as the main outcome measure.

METHODS

This project was approved by Oakland University's Institutional Review Board (IRBNET), and written informed consent was obtained from each participant prior to data collection. Data were obtained from collegiate athletes participating in two separate projects.

Project 1

In this project (IRBNET#888153), 40 male and female National Collegiate Athletic Association (NCAA)

Division 1 (D1) swimmers presented to the laboratory six times over the first 7 weeks of preseason (Fall) training. Testing occurred on Friday afternoons, between 14:00 and 16:00 as part of a larger project on rhabdomyolysis. Each swimmer provided a spot urine sample, and 5 mL of blood was withdrawn via venipuncture while in a supine position. All urine samples were analysed for USG using a URS-10 CHEMSTRIP inserted into an Uritek TC-101 urine reader (Tecodiagnosics, Anaheim, California, USA) and UOsm using a VAPRO Vapor Pressure Osmometer (Wescor, Logan, Utah, USA). Whole blood samples were analysed for serum $[\text{Na}^+]$ and potassium concentration ($[\text{K}^+]$) using ion selective electrodes at a nearby hospital laboratory (Crittenton Hospital). Serum osmolality was assessed using a VAPRO Pressure Osmometer.

Project 2

In this screening project (IRBNET#778936), 79 NCAA D1 athletes participating in Fall sports (women's soccer, men and women's cross country and basketball) presented to the laboratory once, preseason, for a single blood draw at a time (between 09:00 and 15:00) suitable for each team. As part of a larger project, each student-athlete provided a spot urine sample, and 5 mL of blood was withdrawn via venipuncture while in a supine position. All urine samples were analysed for USG using a URS-10 CHEMSTRIP inserted into an Uritek TC-101 urine reader and UOsm using a VAPRO Vapor Pressure Osmometer. Whole blood samples were analysed for $[\text{Na}^+]$ and $[\text{K}^+]$ using a Roche 9180AVL electrolyte analyser (Roche Diagnostics, Indianapolis, Indiana, USA). Serum osmolality was assessed using a VAPRO Pressure Osmometer.

Fluid intake was neither monitored nor measured in either project. In general, our student-athletes were not given standardised guidance on the topic of hydration and free to drink any beverage at any time. Our training room provided water bottles plus free access to a sports beverage (Gatorade), milk (plain, chocolate, strawberry and soy) and water to all student-athletes.

In both projects, all urine and serum biochemical hydration indices were measured within 2 hours of collection. Duplicate measurements were conducted in serum samples only when they appeared out of the normal reference range, with the value closest to the normal physiological range used. Blood and urine sampling occurred without restrictions on diet, hydration or activity as 'spot checks' obtained in a field setting.¹⁸ Thirst was assessed using a 10-point rating scale, with '0' representing 'not thirsty at all' and '10' representing 'extremely thirsty', either when entering the lab (project 1) or at the time of venipuncture (project 2). This rating scale was adapted from Engell¹² and validated as a 9-point^{28 29} and 11-point rating scale³⁰ as well as a 10 cm (unmarked) visual analogue scale.^{10 13}

Table 1 Demographics of male and female participants from project 1 and project 2

Athlete	Participants	Height (m) mean±SD	Weight (kg) mean±SD	BMI (kg/m ²) mean±SD
Project 1 (tested six times)	Males (n=17)	1.8±0.1*	77.6±6.1*	23.4±1.9
	Females (n=23)	1.7±0.1	66.3±6.3	22.9±2.2
Project 2 (tested once)	Males (n=23)	1.9±0.1*	85.7±15.5*	24.0±2.6
	Females (n=56)	1.7±0.1	66.9±11.8	23.0±2.8

*P<0.05 between project 1 versus project 2 males. BMI, body mass index.

Definitions of dehydration

Combined data were artificially categorised into two groups, dehydrated versus hydrated athletes, based on the following two commonly used urine-based definitions of dehydration: (1) UOsm \geq 700 mOsmol/kgH₂O¹ and (2) USG \geq 1.020.^{1,31} The following variables were then compared between dehydrated versus hydrated athletes: serum [Na⁺], [K⁺] and osmolality; UOsm and USG; and thirst rating. Normonatremia was defined as any serum [Na⁺] between 135 mmol/L and 145 mmol/L, with clinical (cellular) dehydration defined as any serum [Na⁺] >145 mmol/L (hypernatremia).^{4,6} Relative overhydration was defined as any serum [Na⁺] <135 mmol/L (hyponatremia).^{4,6,24}

Statistics

All data analysed using Statistica V.13 (Dell, Round Rock, Texas, USA). T-tests with unequal variance were used to analyse differences between urine markers, serum markers and thirst rating using the two urine-based thresholds that categorised athletes into dehydrated versus hydrated athlete groups. Relationships between variables were assessed using Pearson's product-moment correlations. Data reported as means±SD with statistical significance set a priori at P<0.05.

RESULTS

For project 1, of the 40 swimmers (23 female) that were tested six times, only one individual had missing data

at one testing time point (thus n=239, not 240). For project 2, there were 23 males and 56 females who were tested once. Demographic data from both projects detailed in [table 1](#). Data from both projects were analysed collectively, as each data point represented 318 individual athlete 'spot checks', tested at random in an ecologically valid setting.

[Table 2](#) depicts the hydration variables of interest for the entire cohort of spot checks (n=318), divided into male (n=125) and female (n=193) spot checks, along with the normal range of values. On average, all biochemical variables of interest were within the normal reference range. The only statistically significant difference between male versus female spot check values was noted in USG, with females demonstrating a (mathematically) higher USG compared with males.

[Table 3](#) depicts the analyses of hydration variables when dehydration was defined as any UOsm value \geq 700 mOsmol/kgH₂O.¹ Using this definition, 174 (55%) of the athlete spot checks would classify these athletes as dehydrated on arrival into the laboratory. When compared with athletes classified as 'hydrated' using this definition, the 'dehydrated' athletes had statistically significant elevations in UOsm (by design), USG, serum [Na⁺] and thirst ratings.

[Table 4](#) depicts the analyses of hydration variables when dehydration was defined as any USG value \geq 1.020.¹ Using this definition, 85 (27%) of the athlete spot checks would classify these athletes as dehydrated on arrival into the laboratory. When compared with athletes classified as 'hydrated' using this definition, the 'dehydrated' athletes had statistically significant elevations in USG (by design) and UOsm. There were no differences in any serum variable or thirst rating.

[Figure 1](#) demonstrates the statistically significant positive relationship (r=0.18; P<0.01) between serum [Na⁺] versus UOsm. Of note, despite the wide range of UOsm values (110–1298 mOsmol/kgH₂O), only one serum [Na⁺] value (134 mmol/L) was outside of the normal reference range of 135–145 mmol/L.²⁴ Significant correlations were also noted between serum [Na⁺] versus thirst rating (r=0.12; P<0.05), serum [Na⁺] versus serum osmolality (r=0.24; P<0.001), UOsm versus

Table 2 Markers of hydration status for entire cohort (n=318) and subdivided into females and males

Variable	Combined mean±SD (min–max)	Females mean±SD n=193	Males mean±SD n=125	Normal range
Serum [Na ⁺] (mmol/L)	139.9±2.1 (134–145)	139.7±2.0	140.1±2.2	135–145
Serum [K ⁺] (mmol/L)	4.4±0.3 (3.6–6.4)	4.4±0.3	4.4±0.4	3.5–5
Serum osmolality (mOsmol/kgH ₂ O)	280.6±8.7 (261–307)	280.8±8.9	280.2±8.3	275–295
Urine osmolality (mOsmol/kgH ₂ O)	682.7±302 (110–1298)	671.6±298.2	699.7±308.3	300–900
Urine specific gravity	1.014±0.006 (1.005–1.030)	1.014±0.006	1.015±0.006*	1.005–1.030
Thirst rating (0–10)	4.4±1.8 (0–10)	4.5±1.8	4.2±1.9	

*P<0.05 between females versus males.

The normal physiological range for each biochemical variable is listed in the last column. serum [K⁺], potassium concentration; serum [Na⁺], sodium concentration.

Table 3 Dehydration defined by urine osmolality (UOsm) whereas dehydration is any UOsm ≥ 700 mOsmol/kg/H₂O, while adequately hydrated is any UOsm < 700 mOsmol/kg/H₂O

Variable	Dehydrated mean \pm SD n=174	Hydrated mean \pm SD n=144
Serum [Na ⁺] (mmol/L)	140.3 \pm 2.0	139.4 \pm 2.1***
Serum [K ⁺] (mmol/L)	4.4 \pm 0.3	4.4 \pm 0.4
Serum osmolality (mOsmol/kgH ₂ O)	280.7 \pm 9.1	280.4 \pm 8.0
Urine osmolality (mOsmol/kgH ₂ O)	918.7 \pm 127.9	397.4 \pm 181.0***
Urine specific gravity	1.017 \pm 0.005	1.011 \pm 0.005***
Thirst rating (0–10)	4.6 \pm 1.7	4.0 \pm 2.0**

By this definition, 55% of the athletes were dehydrated.

*P<0.05; **P<0.01; ***P<0.001 between dehydrated versus hydrated.

serum [K⁺], potassium concentration; serum [Na⁺], sodium concentration.

thirst rating (r=0.20; P<0.001) and UOsm versus USG (r=0.64; P<0.001).

DISCUSSION

Urine-based definitions identified 27%–55% of our collegiate athletes as ‘dehydrated’ at the time of testing. Those athletes classified as dehydrated using urine criteria (UOsm ≥ 700 mOsmol/kgH₂O or USG > 1.020) would have subsequently been instructed to drink more fluids (above the dictates of thirst) to achieve ‘adequate’ hydration levels.^{1 21 25–27} Conversely, none of our athletes were identified as dehydrated according to serum [Na⁺] measurement (figure 1). The maintenance

Table 4 Dehydration defined by urine specific gravity (USG) whereas dehydration is any USG ≥ 1.020 , while adequately hydrated is any USG < 1.020

Variable	Dehydrated mean \pm SD n=85	Hydrated mean \pm SD n=233
Serum [Na ⁺] (mmol/L)	139.9 \pm 2.2	139.9 \pm 2.1
Serum [K ⁺] (mmol/L)	4.4 \pm 0.4	4.4 \pm 0.3
Serum osmolality (mOsmol/kgH ₂ O)	280.6 \pm 8.3	280.6 \pm 8.8
Urine osmolality (mOsmol/kgH ₂ O)	893.4 \pm 189.7	605.8 \pm 299.0***
Urine specific gravity	1.022 \pm 0.003	1.011 \pm 0.004***
Thirst rating (0–10)	4.3 \pm 1.8	4.4 \pm 1.8

By this definition, 27% of the athletes were dehydrated.

*P<0.05; **P<0.01; ***P<0.001 between dehydrated versus hydrated.

serum [K⁺], potassium concentration; serum [Na⁺], sodium concentration.

of normonatremia—despite wide fluctuations in urine concentration—suggests that these athletes were drinking adequate amounts of fluid in response to osmotic thirst stimulation.^{4 10 11} The lack of clinical sensitivity for urine indices to detect intracellular dehydration supports previous results obtained from smaller studies involving athletes/exercise^{15–22} and larger studies conducted in older patients⁸ and young children.³² The popularity of using urine indices to define ‘inadequate hydration’,^{33 34} despite a growing body of contradictory evidence, thereby raises critical concern over the apparent medicalisation of a normal physiological response (kidney water conservation).^{35 36}

The maintenance of normonatremia has been documented previously in 80% of 2135 endurance athletes, completing a variety of races ranging from standard (42.2 km) marathons through Ironman Triathlons across four countries.³⁷ With dehydration and water turnover expected to be exceedingly high immediately following prolonged endurance races, only 13% of this large cohort were hypernatremic, while 7% were hyponatremic on race finish.³⁷ This low incidence of dysnatremia thereby underscores the strength of the osmoregulatory system, even under conditions of heightened physiological and psychological stress.

In contrast to blood indices, UOsm definitions categorised 55% (UOsm ≥ 700 mOsmol/kgH₂O)¹ of our student-athletes as ‘dehydrated’ at the time of measurement (table 3). By design, both urine values (UOsm and USG) were significantly higher in the dehydrated versus hydrated groups. Serum [Na⁺] and thirst ratings also demonstrated statistically significant increases in the dehydrated versus hydrated groups. However, the mathematical difference between groups for these regulated variables (140.3 mmol/L vs 139.3 mmol/L for serum [Na⁺] and 4.6 vs 4.2 for thirst) were not clinically meaningful. The high incidence of dehydration (55%) based on UOsm criteria in the present study concurs with a study performed on 46 (26 male, 20 female) adolescent swimmers, using UOsm ≥ 700 mOsmol/kgH₂O to define dehydration.²⁵ Those authors found that 67% of their swimming cohort were dehydrated on rising (first morning urine sample), 78% were dehydrated immediately prior to training and thirst rating was not significantly different before (4.4/10) versus after (5.5/10) training.²⁵ Thus, despite UOsm concentrations being twice as high in the dehydrated versus hydrated categories in the present study, serum biomarkers ([Na⁺], [K⁺] and osmolality) and thirst perception remained remarkably stable.

Another commonly used definition to assess ‘dehydration’ is USG, which takes into account both urine solute mass as well as concentration.³⁸ Using the threshold of any USG value ≥ 1.020 to define dehydration,¹ 27% of our athletes were classified as ‘dehydrated’ at the time of measurement (table 4). This incidence is much lower than previous rates demonstrated in other athletic cohorts such as 90% of 107 male adolescent soccer players measured before practice³⁹ and 66% of 263 (138 male,

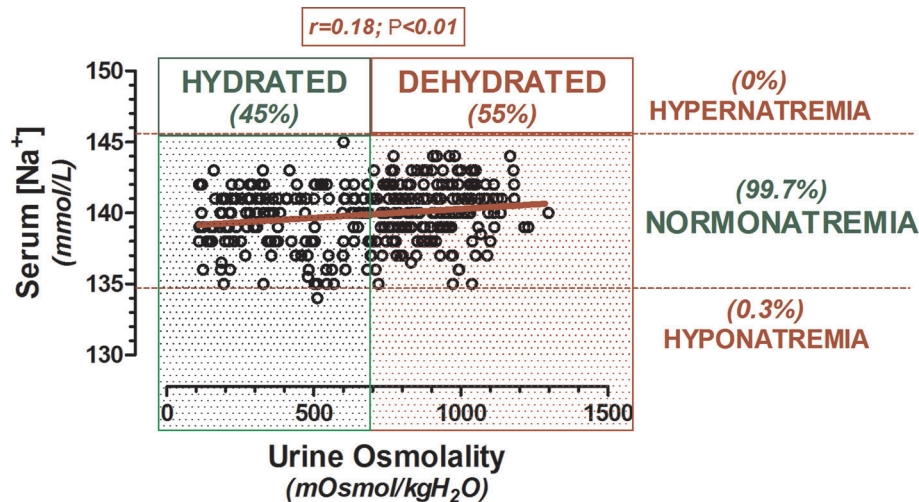


Figure 1 Graph of serum $[Na^+]$ versus urine osmolality, when all data points ($n=318$) were combined. Across the y-axis, no athlete demonstrated intracellular dehydration (hypernatremia; serum $[Na^+] > 145$ mmol/L), 99.7% demonstrated normonatremia (serum $[Na^+]$ between 135 mmol/L and 145 mmol/L), while one athlete was hyponatremic (serum $[Na^+]=134$ mmol/L). Across the x-axis, 55% of athlete spot checks fell into the dehydrated range ($UOsm \geq 700$ mOsmol/kg H_2O), while 45% fell into the hydrated range ($UOsm < 700$ mOsmol/kg H_2O). The correlation coefficient (r) was statistically significant ($P < 0.01$), despite serum $[Na^+]$ explaining only 3% of the variance in urine osmolality. serum $[Na^+]$, sodium concentration; $UOsm$, urine osmolality.

125 female) NCAA D1 athletes who provided random urine samples.⁴⁰ Similar to the $UOsm$ findings, there were no mathematical or clinically relevant differences in either serum markers ($[Na^+]$, $[K^+]$ and osmolality) or thirst rating, since blood tonicity is a physiologically regulated variable.

The previous literature has been consistent with our blood versus urine findings, demonstrating significant relationships between urine markers (such as USG vs $UOsm$)^{22 38} but not between urine versus blood markers of hydration status ($[Na^+]$ and osmolality).^{8 21 22} The confounding effects of diet, the timing of fluid intake and the renal response to exercise likely contribute to the poor prognostic utility of using urine indices as surrogate markers for water and sodium homeostasis (plasma tonicity or volume status).⁴¹ In contrast to osmoregulatory thirst and arginine vasopressin (AVP) stimulation, urine concentration is not a regulated physiological variable associated with fluid homeostasis.^{4-6 10 42 43} Urine volume and solute concentration are renal effector responses that are largely subservient to circulating plasma AVP levels.^{4 5 43} Copious urinary free water excretion is reflective of either: (1) AVP suppression, which largely occurs when fluid intake is in excess of osmoregulatory need,⁴ or (2) AVP antagonism at the V_2 receptor, triggering dilute urine with cellular dehydration.⁴⁴ Clinically speaking, AVP suppression and antagonism characterise central and nephrogenic diabetes insipidus, both of which are successfully compensated by osmotically driven thirst stimulation to maintain tonicity balance.⁴

Drinking according to the dictates of thirst will thereby prevent cellular dehydration. Drinking to keep urine clear or maintain body weight may lead to overhydration.²⁴ Accordingly, drinking above thirst has been associated with a 33%–57% incidence of hyponatremia in

professional rugby players tested after match play, field and gym training.⁴⁵ Thus, although urine concentration may be a useful measurement tool, caution is advised against the potential for overzealous adherence to fluid intake guidelines based on urine or any other hypotonic fluid secretion, which may overshoot osmoregulatory need or renal excretion capabilities.

Limitations of our study include an inability to control fluid intake or standardise exercise prior to testing. Our inability to control fluid intake or timing may have contributed to delays in the adjustment of urine indices to plasma changes from fluid absorption, as proposed elsewhere.^{15 18} Previous research has also shown that exercised-induced fluid losses—without clinically significant dehydration—may have significant effects on other important physiological variables such as heart rate, core temperature, sweat loss, rating of perceived exertion and skeletal muscle metabolism, which may hinder physical and mental performance.^{1 46 47} However, despite these clear limitations and need for further study, we believe that these data provide a robust ‘snapshot’ of the typical collegiate athlete who is able to preserve cellular size (normonatremia) despite a wide range of urine concentrations, exercise and hydration habits.

In summary, normonatremia was maintained in 99.7% of this random sample of hydration spot checks performed at rest. $UOsm \geq 700$ mOsmol/kg H_2O classified 55%, while $USG \geq 1.020$ classified 27% of athletes as dehydrated at the time of testing. This discrepancy between serum versus urine indices likely reflect the differences between using a physiologically regulated versus non-physiologically regulated variables to define dehydration. Since thirst is a physiologically regulated variable of fluid homeostasis, drinking to thirst would be an appropriate fluid intake strategy using serum or plasma

DEHYDRATION

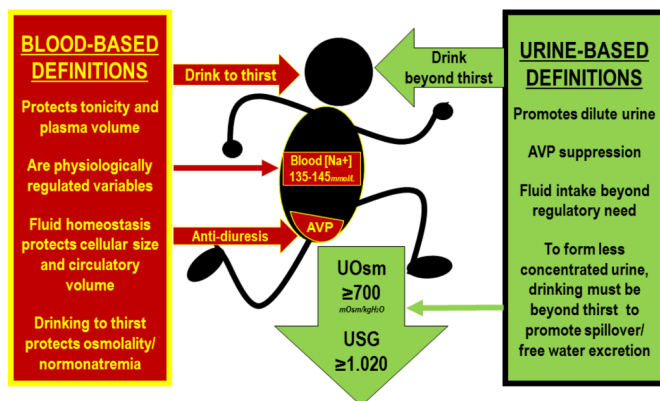


Figure 2 Infographic representing an athlete and summarising how the definition of dehydration that is used (blood vs urine indices) affects whether thirst is (or is not) an appropriate hydration strategy to prevent dehydration in a rested state (pre-exercise), according to the threshold used. UOsm, urine osmolality; USG, urine specific gravity.

criterion to prevent hypernatremia or hyponatremia, at least during resting conditions (pre-exercise). However, according to urine output based definitions of dehydration, drinking above the dictates of thirst is required to suppress AVP and promote a clear and copious free water excretion (aquaresis). Thus, the definition of dehydration varies greatly within various study populations, with subsequent hydration advice subservient to the definition that is used. **Figure 2** summarises how the different definitions may yield differential hydration advice from a physiological perspective, at least during a rested state. It is important to emphasise that these data do *not* question the potential deleterious effects of dehydration—nor drinking to thirst—on *performance* but rather questions the utility of using urine concentration as a surrogate marker for clinical dehydration in routine student-athlete urine spot checks.

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Contributors TDH-B participated in study design, data acquisition and analyses and drafting of the manuscript. CE participated in data acquisition, analyses and drafting of the manuscript. JB, MR and MVM participated in data acquisition. All authors participated in revising the manuscript, and all authors gave final approval on the paper.

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