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**Hydration marker diagnostic accuracy to identify mild intracellular and extracellular dehydration**

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**Running title:** Intra and extracellular dehydration markers

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**Abstract**

Identifying mild dehydration (≤2% of body mass) is important to prevent the negative effects of more severe dehydration on human health and performance. It is unknown whether a single hydration marker can identify both mild intracellular and extracellular dehydration with adequate diagnostic accuracy (≥0.7 receiver operating characteristic-area under the curve (ROC-AUC)). Thus, in 15 young healthy men, we determined the diagnostic accuracy of 15 hydration markers after three randomized 48-h trials; euhydration (EU, water 36 ml·kg·$d^{-1}$), intracellular dehydration caused by exercise and 48 h of fluid restriction (ID, water 2 ml·kg·$d^{-1}$), and extracellular dehydration caused by a 4 h diuretic-induced diuresis, begun at 44 h (ED, Furosemide 0.65 mg·$kg^{-1}$). Body mass was maintained on EU and dehydration was mild on ID and ED (1.9 (0.5)% and 2.0 (0.3)% of body mass, respectively). Urine color, urine specific gravity, plasma osmolality, saliva flow rate, saliva osmolality, heart rate variability and dry mouth identified ID (ROC-AUC; range 0.70-0.99) and postural heart rate change identified ED (ROC-AUC 0.82). Thirst 0-9 scale (ROC-AUC 0.97 and 0.78 for ID and ED) and urine osmolality (ROC-AUC 0.99 and 0.81 for ID and ED) identified both dehydration types. However, only thirst 0-9 scale had a common dehydration threshold (≥4; sensitivity and specificity of 100%, 87% and 71%, 87% for ID and ED). In conclusion, using a common dehydration threshold ≥4, the thirst 0-9 scale identified mild intracellular and extracellular dehydration with adequate diagnostic accuracy. In young healthy adults' thirst 0-9 scale is a valid and practical dehydration-screening tool.

**Keywords:** hypohydration**,** thirst, urine, plasma, saliva, tear, ROC curve.

## Introduction

No consensus currently exists on the best method to assess dehydration and prescribe fluid intake (Armstrong, 2007; Cheuvront & Kenefick, 2014; Cotter et al., 2014). This is in part because dehydration is a complex condition that manifests as different types. When fluid intake is inadequate, and the concentration of body fluids lost is hypoosmotic relative to plasma (e.g. exercise sweat loss), the body fluid redistribution that occurs results in a relatively larger loss of intracellular than extracellular fluid (Sawka, 1992). Consequently, this type of dehydration is referred to as intracellular dehydration and characterized by an increased plasma osmolality (hyperosmolality). In contrast, extracellular dehydration, is caused by iso-osmotic fluid loss and is characterized by volume depletion (hypovolemia) and the absence of hyperosmolality. Extracellular dehydration often occurs when people are ill, take medications (e.g. diuretics), are immersed in water, or exposed to cold and/or hypoxia (Cheuvront & Kenefick, 2014; Cotter et al., 2014). Whether hydration markers identify intracellular or extracellular dehydration is likely to depend on the relationship between the marker and the distinct physiological characteristics of each dehydration type.

Potential candidate markers to identify both types of dehydration are urine, saliva, ratings of thirst and cardiovascular parameters, including resting and postural changes in heart rate and blood pressure, and heart rate variability (HRV) (Cheuvront et al., 2012; Cotter et al., 2014; Fitzsimons, 1976; Oliver et al., 2008). These markers may respond directly to osmotic and volume stimuli, or indirectly to the subsequent alterations in autonomic tone (Charkoudian et al. 2005, Oliver et al. 2008, Sands & Layton 2009). While most of these hydration markers have shown promise to identify moderate and severe intracellular dehydration (>3% body mass; Armstrong et al. 1994, 2014, Walsh et al. 2004, Cheuvront et al. 2012), limited research has investigated the validity and diagnostic accuracy of these hydration markers to identify more mild extracellular or intracellular dehydration (≤2% of body mass). Mild dehydration is important to identify, as it is beyond this threshold that

human performance has been consistently shown to decline (Cheuvront & Kenefick, 2014; Goulet, 2012; Savoie et al., 2015).

The aim of this study was therefore to determine hydration marker diagnostic accuracy to identify mild intracellular and extracellular dehydration. Based on previous research examining hydration markers after moderate and severe dehydration (Cheuvront et al., 2012; Fortes et al., 2011; Oliver et al., 2008; Shirreffs et al., 2004), we hypothesized that urine, thirst, dry mouth, saliva and HRV markers would identify both types of mild dehydration with adequate diagnostic accuracy (ROC-AUC ≥0.7; Hooper et al. 2016). Based on this research we also hypothesized that plasma osmolality and tear osmolarity would identify mild intracellular dehydration, but not mild extracellular dehydration; and postural heart rate and blood pressure change would identify extracellular dehydration, but not intracellular dehydration.

## Materials and Methods

### *Participants*

Fifteen healthy males volunteered to complete the study (age 22.8 (5.4) years, height 180.4 (5.0) cm, mass 78.9 (8.6) kg, BMI 24.2 (1.8) $kg \cdot m^{-2}$, $\dot{V}O_2max$ 52.3 (6.9) $ml \cdot kg^{-1} \cdot min^{-1}$). Participants were excluded if they were, smokers, had abnormal blood chemistry or renal function, suffered from diabetes, asthma, bronchitis, epilepsy, hypertension, dental or oral disease or were receiving any medication or treatment. Informed written consent was obtained from each participant. The study was approved by the Institutional Ethics Committee and adhered to the Declaration of Helsinki.

### *Preliminary measures*

As body mass loss during the 48-h trials was the reference standard in this study, we standardized energy intake and physical activity 24 h before and during trials. Energy intake was calculated as the product of resting metabolic rate and an estimated physical activity factor. Resting metabolic rate

97 was estimated from anthropometry (Harris & Benedict, 1918) and adjusted by a general daily
98 physical activity and diet induced thermogenesis factor coefficient of 1.6, which was determined
99 from the activities completed on trials (Todorovic & Micklewright 2004). Participants were also
100 habituated with the hydration assessment techniques and completed a graded cycle exercise test to
101 determine their peak power output, which was used to prescribe the workload for the experimental
102 trial cycling exercise (Excalibur Sport, Lode, Netherlands).
103
104 ***Study protocol***
105 The study followed a crossover design. Separated by seven days, participants completed three trials
106 in a random order including a euhydrated control trial (EU), an intracellular dehydration (ID) trial,
107 and an extracellular dehydration (ED) trial. Each trial consisted of a baseline hydration assessment,
108 an exercise bout, one of the three 48-h interventions, and a second hydration assessment (Figure 1).
109 Hydration assessments and exercise was performed in an air-conditioned laboratory, temperature
110 and humidity, 19.4 (1.0) °C and 42 (6)%, respectively.
111
112 The day before each experimental trial participants abstained from alcohol, caffeine or strenuous
113 physical activity and consumed a standardized individually prescribed diet (energy and sodium
114 intake 3034 (245) kcal and 2.2 (0.1) g; 62%, 25%, 13% carbohydrate, fats and protein, respectively).
115 Daily energy intake was the same for the duration of the trials except on day one participants
116 consumed additional food (391 (193) kcal) to replace energy expended during the cycling exercise.
117 This was calculated from indirect calorimetry during the habituation visit cycling exercise test (Cortex
118 MetaLyzer 3B, Germany).
119
120 On day one of each trial participants woke at 07:00 h and drank water equal to 6 ml·kg$^{-1}$of body
121 mass (471 (52) ml). On arrival to the laboratory at 08:00 h participants received a further bolus of
122 water equal to 6 ml·kg$^{-1}$of body mass and a standardized breakfast (690 kcal, sodium 0.8 (0.1) g;

62%, 23% and 15% carbohydrate, fat and protein, respectively). To monitor and standardize physical activity on the trial's participants were fitted with pedometers and provided with step count targets (Digi-Walker SW200, Yamax, Japan). At 12:00 h participants returned to the laboratory for the baseline hydration assessment. Immediately after, dehydration was induced via cycling exercise at 70% peak power output until exhaustion. After the cycling exercise, the participants began one of three 48-h trials. The calculated sweat loss from the cycling exercise was replaced with water on EU and ED but not on ID. Drinking water was restricted on ID to 2 ml·kg$^{-1}$ of body mass per day (total 314 (35) ml). In contrast, on EU and EH participants drank water equal to 36 ml·kg$^{-1}$ of body mass per day (total for 48 h 5728 (600) ml). This fluid intake strategy was adapted from those previously used in our laboratory to maintain euhydration (Oliver et al., 2007; 2008; Walsh et al., 2004). On day three, participants reported to the laboratory at 07:30 h. At 08:00 h, and after a standardized breakfast, on EH participants consumed the diuretic Furosemide as a liquid equal to 0.65 mg·kg$^{-1}$ (51 (6) mg Frusol, Rosemount Pharma, UK). All urine voided between 08:00 h and 12:00 h was collected to measure total urine volume. At 12:00 h on all trial's participants began the hydration assessment 2.

**Hydration assessments**

Hydration markers were obtained in the same order on each trial and at each hydration assessment. First, participants completed subjective ratings of thirst and dry mouth on 100 mm visual analogue scale (VAS), and the 0-9 thirst sensation scale (0 = "not-at-all" to 9 = "severe"; Engell et al. 1987). Participants were instructed to respond to the scale based on how they felt at that moment. Second, a urine sample was collected in a container and immediately analyzed for urine color by an 8-point chart (Armstrong et al., 1994), urine specific gravity (USG) was measured in duplicate using a handheld refractometer (Atago, Japan) and urine osmolality was measured in triplicate by a freezing point depression osmometer (Model 3300, Advanced Instruments, USA). Third, nude body mass was determined to the nearest 50 g using a digital platform scale (Model 705 Seca, Germany). Fourth,

participants were fitted with a heart rate monitor (Polar RS800, Finland), after 2 min of seated rest, beat-to-beat heart rate was recorded for 10 min for the determination of HRV (Marek, 1996). All R–R series were extracted with a processing program (Polar Precision Performance, Polar Electro, Finland) and analyzed in the time and frequency-domain after automatic removal of occasional ectopic beats (Kubios, BSAMIG, Finland). Fifth, the participants sat quietly for 5 min before a tear fluid sample was analyzed for tear osmolarity from the right eye as previously described (Fortes et al. 2011, TearLab™ Osmolarity System, USA). Sixth, after 5 min supine rest, blood pressure and heart rate were recorded (Tango, SunTech Medical Ltd, USA). These measures were then repeated after exactly 1 min of standing for the determination of postural change measures of blood pressure and heart rate calculated as the difference between lying and standing measures. Seventh, a seated 5 min unstimulated saliva sample was collected for the determination of saliva flow rate and osmolality as previously described (Oliver et al., 2008). Finally, after 10 min seated rest, a venous blood sample was collected by venipuncture without venestasis into a vacutainer tube containing lithium heparin (Becton Dickinson, UK). This blood was immediately used to determine, in triplicate, hematocrit (packed cell volume) by microcentrifugation (Hawksley and Sons Ltd., Sussex, UK) and hemoglobin by automated analyzer (B-Hemoglobin, Hemocue, Sweden). Plasma volume change was then estimated from the change in hemoglobin and hematocrit values between hydration assessment 1 and 2 (Dill & Costill, 1974; Strauss et al., 1951). The remaining blood was centrifuged at 1500 g for 10 min at 5 °C and plasma was analyzed for osmolality in triplicate. If any of the intra-sample osmolalities differed by more than 1% a further sample was measured and the mean of the four samples was used.

**Statistical analysis**

Hydration marker diagnostic accuracy to identify mild ID and ED was determined from hydration assessment 2 data by ROC-AUC with 95% CIs (MedCalc Software bvba, Belgium) as recommended (Zweig & Campbell, 1993). Body mass change was used as the mild dehydration reference standard

as it is a precise measure of body fluid change in controlled laboratory studies (Cheuvront et al., 2010; Oliver et al., 2008). Body mass loss was calculated on all trials to ensure euhydration was maintained on EU and mild dehydration was achieved on ID and ED. A 1% threshold was used as this has previously been reported as the typical day-to-day variability of body mass in active men (Cheuvront et al., 2010). Hydration markers were also given a qualitative ROC-AUC descriptor that relates to the quantitative diagnostic accuracy statistic as poor (0.6), adequate (0.7), moderate (0.8), high (0.9), near perfect (0.95) and perfect (1.0) (Obuchowski et al., 2004). For hydration markers to be considered to have adequate diagnostic accuracy it has also previously been specified that ROC-AUC should be ≥0.7 (Hooper et al., 2016). A value of 0.5 indicates that a hydration marker has no better ability than chance to discriminate between euhydration and dehydration whereas 1.0 indicates that the marker has perfect discrimination (Zweig & Campbell, 1993). A sample size of 15 was selected, to allow for drop-out, and based on a balanced design (i.e. equal numbers of participants with and without dehydration) that indicated a sample size of 14 was sufficient to enable a marker with a diagnostic accuracy of ≥0.7 to be statistically discriminated from 0.5, i.e. no better than chance. For hydration markers with adequate diagnostic accuracy (≥0.7) a secondary analysis was performed where the Youden Index was used to generate an objective mild dehydration threshold (Schisterman et al., 2005). Hydration markers at the hydration assessments were also compared between trials by one-way analysis of variance (ANOVA) with planned multiple comparisons by Tukeys (GraphPad Prism version 6.0, USA). Unless stated all values are mean (SD) and statistical significance was accepted at $P<0.05$.

## RESULTS

### Hydration assessment 1 and trial physical activity

Standardization of pre-trial fluid and energy intake was successful as indicated by consistent euhydrated hydration status at hydration assessment 1 (CON, ID and ED: plasma osmolality 287 (4), 289 (5), 287 (3) mOsm·kg$^{-1}$, $P=0.10$; urine specific gravity 1.009 (0.004), 1.009 (0.004), 1.007 (0.003)

g·ml$^{-1}$, *P*=0.34; body mass 78.4 (8.4), 78.3 (8.3), 78.4 (8.7) kg, *P*=0.89; coefficient of variation for plasma osmolality, urine specific gravity and body mass were 1.0%, 0.3% and 0.6%, respectively). Also similar on all trials was the cycling exercise time and sweat loss (CON, ID and ED: time to exhaustion 1200 (377), 1339 (415), 1323 (431) s, *P*=0.15; sweat loss 470 (200), 540 (150), 590 (200) ml, *P*=0.10) and trial physical activity (CON, ID and ED: 15299 (4172), 17182 (5106), 17982 (4625) steps·trial$^{-1}$, *P*=0.08).

**Hydration assessment 2**

Body mass, plasma osmolality and volume were stable during EU confirming euhydration and supporting that the decreased body mass on ID and ED represents mild dehydration and not an energy deficit (Table 1, *P*<0.001). Intracellular dehydration was confirmed on ID by increased plasma osmolality (Table 1). Extracellular dehydration was confirmed on ED by decreased plasma volume without a change in plasma osmolality (Table 1). Further, after the diuretic on ED urine production was increased compared to EU and ID as expected (1677 (338) vs. 772 (311) and 138 (54) ml, *P*<0.001). Increased urine production on ED ceased before hydration assessment 2 as indicated by a similar urine volume on all trials at hydration assessment 2 (Mean (SD) CON, ID and ED: 143 (110), 97 (57), 189 (120) ml, *P*=0.13). Compared to EU, the HRV index LF/HF ratio was increased after ID but not ED (Table 1). Further cardiovascular and renal differences between ID and ED, and the descriptive statistics for other hydration markers studied for diagnostic accuracy are outlined in Table 2.

**Hydration marker diagnostic accuracy**

Thirst 0-9 and urine osmolality had adequate diagnostic accuracy to identify both mild intracellular and extracellular dehydration (Table 3). The diagnostic accuracy of these markers was near perfect to identify mild intracellular dehydration and moderate for mild extracellular dehydration. For thirst 0-9, the Youden index derived the same threshold for both mild intracellular and extracellular

dehydration (≥4). The sensitivity and specificity of this threshold was 100% and 87% for ID and 71% and 87% for ED (Table 3). For urine osmolality, the Youden index derived two different thresholds depending on the type of dehydration (Table 4).

Several other hydration markers identified mild intracellular dehydration with adequate diagnostic accuracy (ROC-AUC ≥0.7, Table 3). The discriminatory accuracy was perfect for urine markers (color and specific gravity), near perfect for plasma osmolality, high for thirst (VAS) and dry mouth (VAS) and adequate for heart rate variability, saliva flow rate and osmolality. The mild intracellular dehydration thresholds for these hydration markers and their sensitivity and specificity to identify mild intracellular dehydration are shown in Table 4. In addition to thirst 0-9 scale and urine osmolality, postural change in heart rate was the only other hydration marker to identify mild extracellular dehydration with adequate diagnostic accuracy (ROC-AUC ≥0.7).

**DISCUSSION**

This study extends current hydration marker understanding by using diagnostic accuracy statistics to evaluate several markers' validity to identify mild intracellular and extracellular dehydration. A particular strength of this study is the standardization of energy intake and physical activity during the experimental trials, which alongside the maintenance of body mass within typical day-to-day variation (Cheuvront et al., 2010) on the euhydrated control trial, provides confidence that individual participant body mass losses on ID and ED represent mild fluid rather than energy deficits. The primary finding of this study is that thirst 0-9 and urine osmolality were the only hydration markers with adequate diagnostic accuracy to identify both mild intracellular and extracellular dehydration, caused by exercise and 48 h of fluid restriction and a 4 h diuretic-induced diuresis, respectively. However, thirst 0-9 was the only marker with a common dehydration threshold to identify mild intracellular and extracellular dehydration (≥4 for ID and ED, Table 4).

253 Notably, the present study is the first to determine the validity of thirst ratings using diagnostic
254 accuracy statistics (Table 3). As hypothesized, thirst had adequate diagnostic accuracy to identify
255 both types of mild dehydration, which may be expected as it is the major homeostatic effector
256 mechanism for restoring euhydration. Further, that thirst identified both intracellular and
257 extracellular dehydration, is in agreement with known physiological regulators whereby thirst is
258 sensitive to changes in both osmotic and volume stimuli (Fitzsimons, 1976). Osmolality is the
259 principal thirst regulator (Cheuvront & Kenefick, 2014) and this may explain the better diagnostic
260 accuracy of thirst to identify intracellular dehydration than extracellular dehydration in this study
261 (Table 3). Indeed, plasma osmolality was increased by 3.5% after intracellular dehydration, which
262 exceeds the reported 2% osmotic threshold of thirst (Table 1, Zerbe & Robertson 1983). The blood
263 volume reduction is the most likely stimuli for the increase in thirst after mild extracellular
264 dehydration as other thirst regulators plasma osmolality, dry mouth and saliva flow rate were similar
265 after the ED and EU control trials.
266
267 In agreement with our hypothesis, plasma osmolality, saliva flow rate and osmolality, dry mouth,
268 urine markers and HRV showed adequate diagnostic accuracy to identify mild intracellular
269 dehydration, whilst postural change in heart rate showed adequate diagnostic accuracy to identify
270 mild extracellular dehydration (Table 3). The diagnostic accuracy of these markers compares
271 favorably to that previously reported after more severe dehydration (ROC-AUC range, 0.89-0.98;
272 Bartok et al. 2004, Cheuvront et al. 2010, 2012, Armstrong et al. 2014). Identifying milder
273 dehydration with similar diagnostic accuracy is practically advantageous. Contrary to our hypothesis,
274 tear osmolarity did not identify intracellular dehydration and saliva osmolality, HRV and postural
275 blood pressure change did not identify extracellular dehydration with adequate diagnostic accuracy.
276 The reason for the poorer than anticipated diagnostic accuracy in these markers compared to
277 previous studies (equivalent to ≥3% of body mass; Oliver et al. 2008, Fortes et al. 2011, Ely et al.
278 2014) may relate to the smaller fluid-deficit and osmotic, volume and autonomic nervous system

(ANS) alterations. In addition, our HRV results highlight that ANS alterations, when compared with euhydration, may be greater after intracellular than extracellular dehydration of the same magnitude (Table 1; *P*=0.04 CON vs ID; *P*=0.14 CON vs ED). Given the postulated role of ANS system in saliva control (Oliver et al. 2008) this may explain why saliva parameters' diagnostic accuracy was adequate to identify ID but not ED.

As thirst 0-9 and urine osmolality were the only markers to identify mild intracellular and extracellular dehydration with adequate diagnostic accuracy, they might be considered the most suitable to identify persons that require simple oral rehydration to prevent the negative consequences of more severe dehydration to performance. Practically, thirst 0-9 has some additional advantages to urine osmolality. This includes a common threshold to identify mild dehydration regardless of the dehydration type. Further, thirst can be assessed instantly, and is easy to assess repeatedly, which could be particularly useful to help guide daily fluid intake, and rehydration from exercise, with persons aiming to achieve thirst ratings below or equal to 4. Urine osmolality in contrast has a lengthy collection and analysis process that requires the collection of a urine sample, which is not always possible, and specialist laboratory analysis. We therefore recommend that the thirst 0-9 scale is used as the initial screening tool to identify mild dehydration, and where determining the type of dehydration is important, plasma osmolality and postural change in heart rate are used to confirm if the dehydration is intracellular or extracellular, respectively.

Our hydration marker findings should be considered carefully within the context they were obtained, i.e. dehydration methods used, environmental conditions and population studied. Urine volume at the second hydration assessment was similar and suggests overall fluid balance was stable at the time when hydration marker diagnostic accuracy was determined. However, the time to mild dehydration was much longer on ID than ED (48 h ID and 4 h ED), and consequently, fluid redistribution between body fluid compartments may have been more complete after ID than ED

(Sawka, 1992). As extracellular dehydration is typically acute, e.g. when people are ill, take medications (e.g. diuretics), are immersed in water, or exposed to cold and/or hypoxia, it is a practical strength of this study that we determined hydration marker diagnostic accuracy after acute rather than chronic extracellular dehydration. In contrast, intracellular dehydration may occur chronically, as in this study, or acutely, e.g. sweating from passive heating and/or exercise sweat. As these different dehydration methods may influence fluid regulation and redistribution (Sawka, 1992), and hydration marker diagnostic accuracy, future studies are warranted comparing the diagnostic accuracy of hydration markers to identify different dehydration methods, particularly that occur across different time courses. As in the present study, these future studies would benefit from measuring fluid compartments to confirm fluid redistribution by isotope or dye tracer techniques (e.g. bromide, Evans blue). Given the potential of thirst as a practical hydration marker, studies are needed to compare the diagnostic accuracy of thirst to identify acute and chronic mild intracellular dehydration. These studies are important as causes of acute intracellular dehydration including exercise, and exposure to hot and dry environments may alter thirst independently of dehydration due to direct effects of high ventilation, heat and drying of the oral cavity. Future studies should also determine the diagnostic accuracy of thirst in other populations e.g. females, children and the elderly. In the elderly, the diagnostic accuracy of thirst may be poorer than in young healthy adults as ageing and disease impair kidney and saliva gland function; in addition, the elderly are more likely to take medications that induce dry mouth which may alter thirst independently of dehydration (Kenney & Chiu, 2001; Scully, 2003). Further, elderly persons with dementia and young children may not interpret the thirst scale as young healthy adults.

In conclusion, thirst 0-9 scale was the only hydration marker, with a common dehydration threshold, to identify both mild intracellular and extracellular dehydration with adequate diagnostic accuracy in young healthy males, residing in a thermoneutral environment. The practical utility of thirst is

reinforced because it is a free and simple to use hydration marker that could also guide fluid intake to maintain euhydration.

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**Table 1.** Characterization of experimental hydration status after mild intracellular and extracellular dehydration

| | Euhydration (EU) | Intracellular dehydration (ID) | Extracellular dehydration (ED) |
|---|---|---|---|
| Body mass change (%) | 0.0 (0.6) | -1.9 (0.5) ** | -2.0 (0.3) ** |
| Body mass change range (%) | +0.9 to -0.7 | -1.2 to -2.9 | -1.5 to -2.5 |
| Body mass change (kg) | 0.0 (0.5) | -1.5 (0.5) ** | -1.6 (0.3) ** |
| Blood volume change (%) | 0.8 (4.7) | 0.0 (4.3) | -3.5 (2.8) ‡ |
| Plasma volume change (%) | 1.7 (6.2) | -0.3 (5.7) | -6.6 (4.0) ‡‡ |
| Plasma osmolality ($mOsm \cdot kg^{-1}$) | 287 (4) | 297 (7) †† | 286 (5) |
| HRV (LF/HF ratio) | 1.8 (1.1) | 3.4 (2.2) * | 2.9 (2.1) |

**Note:** HRV, Heart rate variability; LF/HF ratio, low-to-high frequency heart rate variability power ratio. Values represent mean (SD). Post hoc test differences indicated by * $P < 0.05$ vs. EU, ** $P < 0.01$ vs. EU, ††$P < 0.01$ vs. EU and ED, ‡ $P < 0.05$ vs. EU and ID, ‡‡ $P < 0.01$ vs. EU and ID.

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**Table 2.** Hydration markers after mild intracellular and extracellular dehydration

| | Euhydration (EU) | Intracellular dehydration (ID) | Extracellular dehydration (ED) |
|---|---|---|---|
| Thirst (0-9) | 3 (1) | 6 (1) †† | 4 (1) ** |
| Thirst (VAS) | 33 (19) | 69 (17) † | 43 (17) |
| Dry mouth (VAS) | 27 (17) | 60 (21) †† | 36 (12) |
| Urine osmolality ($mOsm \cdot kg^{-1}$) | 267 (138) | 1054 (127) †† | 402 (110) ‡ |
| Urine specific gravity ($g \cdot ml^{-1}$) | 1.008 (0.004) | 1.028 (0.005) †† | 1.010 (0.004) |
| Urine colour (1-8) | 2 (1) | 6 (1) †† | 2 (1) |
| Saliva flow rate ($\mu L \cdot min^{-1}$) | 365 (241) | 196 (165) † | 425 (321) |
| Saliva osmolality ($mOsm \cdot kg^{-1}$) | 56 (12) | 64 (13) † | 55 (12) |
| Tear osmolality ($mOsm \cdot l^{-1}$) | 296 (12) | 300 (11) | 292 (12) |
| Postural change in HR ($b \cdot min^{-1}$) | 14 (8) | 19 (10) | 26 (12) ‡ |
| Postural change in SBP (mmHg) | 8 (12) | 4 (14) | 0 (9) |
| Supine HR ($b \cdot min^{-1}$) | 56 (10) | 56 (12) | 57 (15) |
| Supine SBP (mmHg) | 112 (8) | 111 (10) | 108 (10) |

**Note:** HR, heart rate; SBP, systolic blood pressure. Values represent mean (SD). Post hoc test differences indicated by * $P < 0.05$ vs. EU, ** $P < 0.01$ vs. EU, † $P < 0.05$ vs. EU and ED, †† $P < 0.01$ vs. EU and ED, ‡ $P < 0.05$ vs. EU and ID.

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**Table 3.** Diagnostic accuracy of hydration markers to identify mild intracellular and extracellular dehydration

| Hydration marker | Intracellular dehydration (ID) | | | Extracellular dehydration (ED) | | |
|---|---|---|---|---|---|---|
| | ROC-AUC | 95% CI | SE | ROC-AUC | 95% CI | SE |
| 1. Urine osmolality (mOsm·kg$^{-1}$) | 0.99* | 0.88-0.99 | 0.01 | 0.81* | 0.63-0.93 | 0.09 |
| 2. Thirst (0-9) | 0.97* | 0.84-0.99 | 0.02 | 0.78* | 0.59-0.90 | 0.08 |
| 3. Urine specific gravity (g·ml$^{-1}$) | 0.99* | 0.88-0.99 | 0.01 | 0.68 | 0.48-0.83 | 0.10 |
| 4. Thirst (VAS) | 0.92* | 0.76-0.98 | 0.04 | 0.66 | 0.47-0.83 | 0.10 |
| 5. Dry mouth (VAS) | 0.88* | 0.69-0.97 | 0.06 | 0.66 | 0.47-0.83 | 0.10 |
| 6. Urine colour (1-8) | 0.99* | 0.88-0.99 | 0.01 | 0.52 | 0.33-0.70 | 0.11 |
| 7. Plasma osmolality (mOsm·kg$^{-1}$) | 0.96* | 0.82-0.99 | 0.03 | 0.53 | 0.34-0.71 | 0.11 |
| 8. Postural change in HR (b·min$^{-1}$) | 0.66 | 0.47-0.82 | 0.10 | 0.82* | 0.64-0.93 | 0.08 |
| 9. HRV (LF/HF ratio) | 0.72* | 0.52-0.87 | 0.09 | 0.64 | 0.45-0.81 | 0.11 |
| 10. Saliva osmolality (mOsm·kg$^{-1}$) | 0.70* | 0.51-0.85 | 0.09 | 0.55 | 0.36-0.73 | 0.11 |
| 11. Saliva flow rate (µl·min$^{-1}$) | 0.70* | 0.51-0.85 | 0.09 | 0.55 | 0.36-0.73 | 0.11 |
| 12. Tear osmolality (mOsm·l$^{-1}$) | 0.61 | 0.41-0.78 | 0.11 | 0.61 | 0.42-0.82 | 0.11 |
| 13. Postural change in SBP (mmHg) | 0.56 | 0.37-0.74 | 0.11 | 0.65 | 0.46-0.82 | 0.10 |
| 14. Supine SBP (mmHg) | 0.56 | 0.37-0.74 | 0.11 | 0.64 | 0.44-0.80 | 0.11 |
| 15. Supine HR (b·min$^{-1}$) | 0.53 | 0.34-0.72 | 0.11 | 0.52 | 0.33-0.70 | 0.11 |

**Note:** HRV, Heart rate variability; LF/HF ratio, low-to-high frequency heart rate variability power ratio; ROC, receiver operating characteristic; ROC AUC, area under the ROC curve; CI, binomial exact confidence interval for AUC; SE, standard error (Hanley & McNeil, 1982); * indicates that the hydration biomarker identifies dehydration type better than chance. Note: hydration markers are ranked by combined diagnostic accuracy.

**Table 4.** Sensitivity and specificity of Youden derived mild dehydration thresholds for hydration markers

| Hydration marker | Intracellular dehydration (ID) | | | Extracellular dehydration (ED) | | |
|---|---|---|---|---|---|---|
| | Mild Dehydration Threshold [b] | Sensitivity (%) | Specificity (%) | Mild Dehydration Threshold [b] | Sensitivity (%) | Specificity (%) |
| Urine Osmolality (mOsm·kg$^{-1}$) | >595 | 99 | 99 | >341 | 80 | 87 |
| **Thirst (0-9)** | **≥4** | **99** | **87** | **≥4** | **71** | **87** |
| Urine specific gravity (g·ml$^{-1}$) | >1.016 | 99 | 99 | No | - | - |
| Thirst (VAS) | >47 | 93 | 80 | No | - | - |
| Dry mouth (VAS) | >40 | 79 | 80 | No | - | - |
| Urine colour (1-8) | ≥4 | 99 | 99 | No | - | - |
| Plasma osmolality (mOsm·kg$^{-1}$) | ≥291 | 93 | 87 | No | - | - |
| Postural change in HR (b·min$^{-1}$) | No | - | - | >14 | 93 | 60 |
| Saliva osmolality (mOsm·kg$^{-1}$) | ≥57 | 73 | 67 | No | - | - |
| Saliva flow rate (µl·min$^{-1}$) | ≤137 | 67 | 67 | No | - | - |
| HRV (LF/HF ratio) | >2.8 | 57 | 93 | No | - | - |
| Tear osmolality (mOsm·l$^{-1}$) | No | - | - | No | - | - |
| Postural change in SBP (mmHg) | No | - | - | No | - | - |
| Supine HR (b·min$^{-1}$) | No | - | - | No | - | - |
| Supine SBP (mmHg) | No | - | - | No | - | - |

**Note:** HR, heart rate; SBP, systolic blood pressure; HRV, Heart rate variability; LF/HF ratio, low-to-high frequency heart rate variability power ratio. [b]Youden derived mild dehydration threshold, where ROC-AUC ≥0.70.

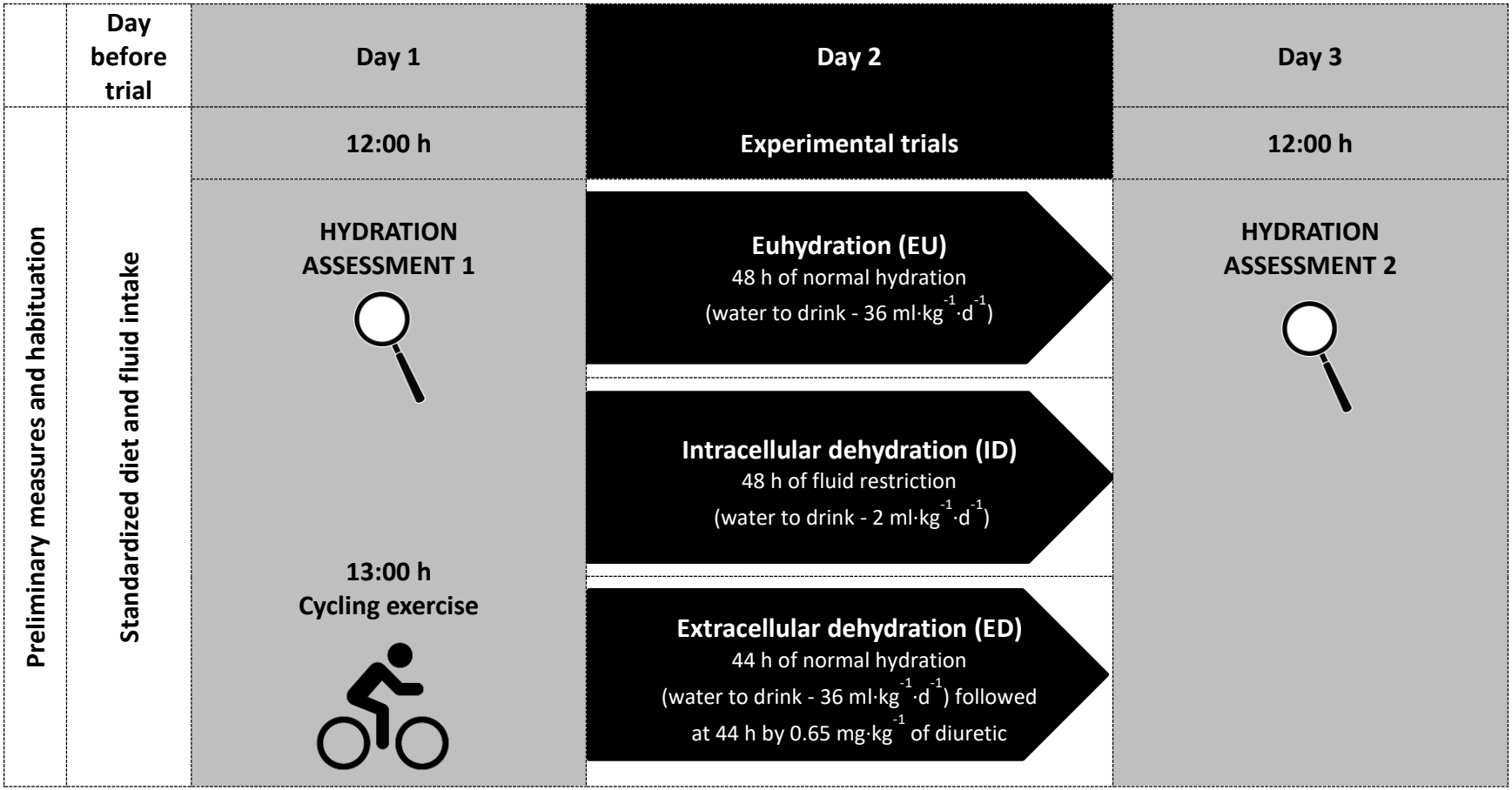


**Figure 1.** Schematic representation of experimental trial. The cycling exercise intensity was 70% of peak power output until exhaustion. Hydration assessments and exercise was performed in an air-conditioned laboratory, temperature and humidity, 19.4 (1.0) °C and 42 (6)%, respectively.