DETECTION OF EUHYDRATION IN HUMANS FROM THE DIURESIS RESPONSE TO A WATER LOAD

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ABSTRACT

Aim: to calculate the minimum amount of water to be ingested in order to find significant differences in one-hour urine volume between euhydrated and dehydrated humans.

Methods: Five participants (22.6±2.9 years old, 63.70±13.18 kg; mean±standard deviation) were evaluated following an overnight fast, on eight different non-consecutive days. For the euhydration condition (EuA, EuB, EuC and EuD), they remained seated for 45 minutes. For the dehydration condition (DeA, DeB, DeC and DeD), they exercised intermittently at 33±4° C, 65±6% relative humidity until they were dehydrated by 1% of body mass (BM). The order of treatments was randomized. Next, they ingested a water volume equivalent to 0.5% BM, 0.72% BM, 1.07% BM or 1.43% BM in 30 minutes, under both the euhydration and the dehydration conditions. Urine volumes were collected 0, 30, and 60 minutes after water ingestion.

Results: baseline values were consistent among conditions ($p>0.05$), and there was no difference in water intake volume between the euhydration and the dehydration conditions ($p>0.05$). There was a clear association between the volume of water intake and urine volume (R^2= 0.64, R^2_a= 0.58; p= 0.001); in addition, this tendency was different between euhydration and dehydration (interaction p=0.005). Finally, ingestion of water equivalent to 1.07% BM or more resulted in a 95% CI for the urine volume difference between euhydration and dehydration greater than 100 mL.

Conclusion: the minimum volume of water to be ingested to detect a 100 mL difference in one-hour urine volume between euhydrated and dehydrated humans is 1.07% BM.

Key words: water intake, urine, acute hydration status, euhydration, dehydration, fluid balance

Introduction.

Dehydration from sweat loss places athletes at a disadvantage against their competitors; as little as 2% of body mass loss may impair mental focus and some motor skills, and will increase heart rate and overall demand on the cardiovascular system which, in turn, may have a negative impact on sports performance (Shirreffs, 2003; Grandjean and Campbell, 2004; Armstrong et al 2007). It is therefore desirable to avoid dehydration during training and competition. Because previous, uncorrected dehydration can have a negative impact on performance as well (Armstrong, Costill and Fink, 1985), it is also important to assess acute hydration status before a training session or competition and to correct any existing hypohydration.

Normally, it is not easy to accurately and reliably measure acute hydration status, and it becomes more difficult with athletes who have larger fluid losses and frequently train twice a day (Cheuvront and Sawka, 2005). Some practical recommendations to assess acute hydration status with athletes include using urine color and/or urine specific gravity; it is also common to register body mass before and after exercise to estimate sweat loss (Cheuvront and Sawka, 2005; Oppliger and Bartok, 2002). The weight loss method can only give a relative amount of dehydration occurring over a period of time; the urine values are subject to wide variations under dynamic conditions, and the results can be misleading (Armstrong, 2007).

A recent study from Capitán and Aragón-Vargas (2009) proposed the evaluation of acute hydration status from the urine response to a standard water load equivalent to 1.43% of body mass (BM). Urine volume was monitored over 5 hours, in an attempt to verify whether urine elimination was different depending on pre-existing, controlled dehydration levels. Results from the study showed there is a clear difference in five-hour urine volume in response to the water load, between euhydration (1236.8 ± 489.4 mL), and dehydration (375.3 ± 170.2 mL, 235.9 ± 66.0 mL, and 261.7 ± 51.8 mL at 1, 2, and 3% BM, respectively, $p = 0.001$). In addition, the difference could be detected after only one hour of monitoring (Capitán Jiménez and Aragón

Vargas, 2009); based on these results, the authors proposed a test, which was later shown to be both valid and reliable (Capitán-Jiménez and Aragón-Vargas, 2010).

The urine volume differences reported are consistent with a normal renal response: reducing urine production during dehydration, but increasing it when a well hydrated person ingests water (Sawka et al. 2007; Valtin and Schafer,1995). The aforementioned method is promising, but in order for it to be practical, the volume of water ingested should be close to the urine volume eliminated in a reasonable time (i.e., one hour) in the euhydrated condition. This would allow for the person to initiate exercise without too much fluid excess in his/her body.

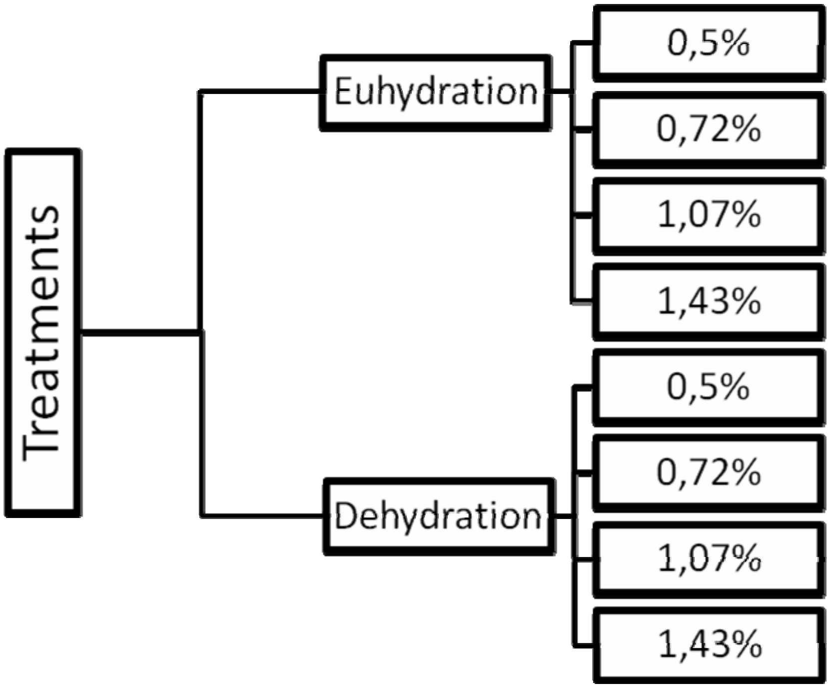
Therefore, the purpose of this study was to calculate the minimum water volume that must be administered to a person to identify significant differences in one-hour urine volume between a euhydrated and a dehydrated state.

Materials and methods

Participants. Four young females and a young male (22.6 ± 2.9 y.o.; mean ± standard deviation) agreed to participate in this study and signed an informed consent. They were healthy, physically active (exercised at least four times a week), had no known heart, renal or endocrine problems, had never suffered heat illnesses, and at the time of the study were not ingesting any diuretics. The study was approved by the University's Ethics and Science Committee.

Study design. This was an experimental study with two conditions: Euhydration (Eu) and Dehydration (De). In addition, four water ingestion volumes (water loads) were pre-determined (0.5% 0.72%, 1.07%, and 1.43% of body mass, equivalent to 350, 500, 750 and 1000 mL, respectively, for a 70-kg individual); these volumes were the same for both conditions. Each participant completed a total of eight treatments in a factorial block design with two conditions by four water loads (2x4) (each person is one block) (see Fig. 1). The order of treatments was randomized.

Figure 1. Factorial block design (2x4).



Procedures

Preparation. Each participant reported to the laboratory at 7 a.m. on eight different non-consecutive days, following an overnight fast (a minimum of 10 hours without solids or liquids). Upon arrival, they provided a urine sample which was analyzed for urine specific gravity (USG) with a manual refractometer (ATAGO®, model URC – Ne, d 1.000-1.050) to estimate initial hydration status. This urine sample was discarded.

After completely emptying their bladders, participants were weighed nude to the nearest 10 grams on a calibrated scale (e-Accura®, model DSB291). This fasting body mass (FastBM) was used to calculate the fluid volume to be ingested by each individual. Next, they ingested a standardized 750-kcal breakfast (24.6% fat, 20.7% protein, and 54.7% carbohydrate, including 250 mL of liquid and 1500 mg sodium), and proceeded to rest for 30 minutes.

Exercise. Following the rest period all participants were weighed nude and dry (baseline body mass, BBM); those whose protocol for the day called for dehydration started intermittent exercise: 15 minutes of stationary cycling, 15 minutes of jogging on a treadmill, and again 15 minutes of stationary cycling, as long as necessary to achieve a dehydration equivalent to 1% BBM; body mass was measured at the end of every 15 minutes with participants nude and dry. Participants exercised in a controlled environment chamber kept at 33 ± 4°C dry bulb and 65 ± 6% relative humidity, at a moderate-to-high intensity (75% to 80% of maximum heart rate) monitored with a Polar® heart rate monitor, model A1; maximum heart rate was estimated from 220 – age.

When their individual protocol did not require exercise (the four Euhydration sessions), participants remained at rest outside the chamber for 45 minutes.

Once the exercise or prolonged resting period was over, all participants took a cold shower. They were instructed not to drink any fluid, and to completely empty their bladders in a 750 mL plastic container. This urine was weighed on a food scale (OHAUS® Compact Scales, model CS2000), to the nearest 1 g. All participants were weighed again nude and dry at this point to obtain post-exercise body mass (PEBM).

Water load. After showering and weighing out, each participant ingested a volume of water equivalent to 0.5%, 0.72%, 1.07% or 1.43% of FastBM, according to his or her particular protocol for the day. This water load was divided into three equal volumes for ingestion, with a 10-minute break after the first and second aliquot.

Urine collection. Participants were instructed to completely empty their bladders into labeled plastic containers immediately upon completion of water ingestion (time 0) and after 30 and 60 minutes; they remained at rest for this urine collection period. The containers were weighed to the nearest 1 g, and the volume was recorded assuming 1 g is equivalent to 1 mL.

Statistical analysis. Descriptive statistics (mean and standard deviation) were calculated for age, body mass, and height in order to characterize the participants. These and the other variables were checked for normality.

Inferential statistics were calculated using JMP version 7. To verify that baseline values were the same, a one-way, repeated measures analysis of variance (ANOVA) was performed for each of two reference variables: USG and FastBM. In addition, a two-way, two-factor repeated-measures ANOVA (2 conditions X 4 water loads) was performed on the actual volume of water ingested for each water load.

A multiple regression analysis was performed in order to understand the main effects of the condition (acute hydration status: euhydration or dehydration), as well as acute hydration status interacting with water loads (interaction), on urine volume. For this regression, one-hour urine volume was used as the dependent variable; predictor variables were water load and acute hydration status (0 for dehydration and 1 for euhydration). In this model, fasting body mass (FastBM) and initial USG were used as covariates since they are pre-existing conditions which may influence urine volume. Individuals were included as blocks to minimize the effect of the error due to natural biological variability among people. This is the conceptually correct, full model which must be evaluated.

Another multiple regression analysis was performed but this time without including the individuals' blocks as a predictor variable (the simplified model). This was done in order to obtain operational results, that is, to obtain a specific water load (as a %BM) where differences could be identified between euhydration and dehydration. With the regression equation obtained from this analysis, 95% confidence intervals (95%CI) were calculated for urine volumes for both the euhydrated and the dehydrated conditions.

To establish the minimum difference in one-hour urine volume between conditions, the upper limit of the 95%CI for dehydration was subtracted from the lower limit of the 95%CI for euhydration, according to the formula:

[Eq. 1] $$Expected\ Minimum\ Difference = Eu\ 95\%CI\ LowerLimit - De\ 95\%CI\ UpperLimit$$

Finally, a basic multiple regression analysis was performed using only water load, condition, and their interaction, for comparison purposes (the basic model).

Results

All participants completed the study. Their age = 22.6 ± 2.9 y.o.; height = 1.63 ± 0.06 m; body weight = 63.7 ± 13.18 kg (mean ± standard deviation).

Baseline conditions. No differences in body mass or urine specific gravity were found among treatments (see *Table 1*). Actual water intake volumes for each of the water loads were not different when comparing euhydration and dehydration (see *Table 2*).

Table 1. Baseline values.

Treatment	FastBM (kg) * ($\bar{X}$ ± SD)	USG+ ($\bar{X}$ ± SD)
EuA (0.5%)	**63.62 ± 14.60**	**1.02 ± 0.01**
EuB (0.72%)	**63.65 ± 14.46**	**1.02 ± 0.01**
EuC (1.07%)	**63.59 ± 14.51**	**1.02 ± 0.01**
EuD (1.43%)	**63.78 ± 14.68**	**1.02 ± 0.01**
DeA (0.5%)	**63.73 ± 14.47**	**1.02 ± 0.01**
DeB (0.72%)	**63.62 ± 14.57**	**1.02 ± 0.01**
DeC (1.07%)	**63.97 ± 14.77**	**1.02 ± 0.01**
DeD (1.43%)	**63.65 ± 14.39**	**1.02 ± 0.01**

FastBM: fasting body mass. USG: urine specific gravity.

* *p = 0.98 among treatments*

+ *p = 0.99 among treatments*

Table 2. Actual water intake for each treatment.

Water load (%FastBM)	Euhydration (mL) $\overline{X}$ ± SD	Dehydration (mL) $\overline{X}$ ± SD
0.50%	**318.11 ± 73.11**	**318.63 ± 72.33**
0.72%	**458.29 ± 104.09**	**458.08 ± 104.88**
1.07%	**680.37 ± 155.27**	**684.00 ± 158.03**
1.43%	**912.05 ± 209.90**	**910.20 ± 205.80**

For all water loads, $p > 0.05$ euhydration vs. dehydration.

Multiple regression analysis. *Table 3* shows the results of the multiple regression models at each one of three stages of complexity. There is a significant interaction between the two predictor variables (water load and condition) regardless of the model. There is also a significant main effect of water load on urine volume.

The regression equation from *Table 3* (simplified model), which is able to explain 58% of the variance ($R^2_a = 0.58$), is as follows:

[Eq. 2]

$$Vol = 8659.31 + (233.99 * WL) - (60.66 * cond) + (0.51 * FastBM)$$
$$- (8559.26 * USG_i) + [(155.88) * (WL) * (cond)]$$

where

Vol is the one-hour urine volume;

WL, the water load, is the water intake volume expressed as a percentage of body mass (%FastBM);

cond is the numerical value for each condition: use 0 for dehydration or 1 for euhydration;

FastBM is the fasting body mass; and

***USG**$_i$* is the urine specific gravity for the initial sample obtained upon arrival.

Table 3. Secuence of regression models.

	Model 1 (Basic)		Model 2 (Simplified) (with covariates)		Model 3 (Complete)	
Parameters	**Estimate**	**Probability**	**Estimate**	**Probability**	**Estimate**	**Probability**
Intercept	-24.47	0.6702	8659.31	0.0111	-1531.49	0.7843
Individual	-	-	-	-	1588.54	0.1130
Water load	226,82	0.0003	233.99	0.0001	222.09	0.0001
Condition(0)	-60.84	0.2927	-60.66	0.2428	-51.62	0.2941
FastBM	-	-	0.51	0.7868	104.63	0.0350
Initial USG	-	-	-8559.27	0.0124	-5057.64	0.2826
Water load * Condition	153,29	0.0111	155.88	0.0046	149.63	0.0043

Both USG and FastBM were included as covariates in this regression equation in order to reduce error, considering that these variables were not controlled for experimentally.

Figure 2 represents the simplified regression and shows how the slopes corresponding to the euhydration and dehydration conditions are different from each other (urine volume changes in response to different water loads are different).

Figure 2. Water load by condition interaction

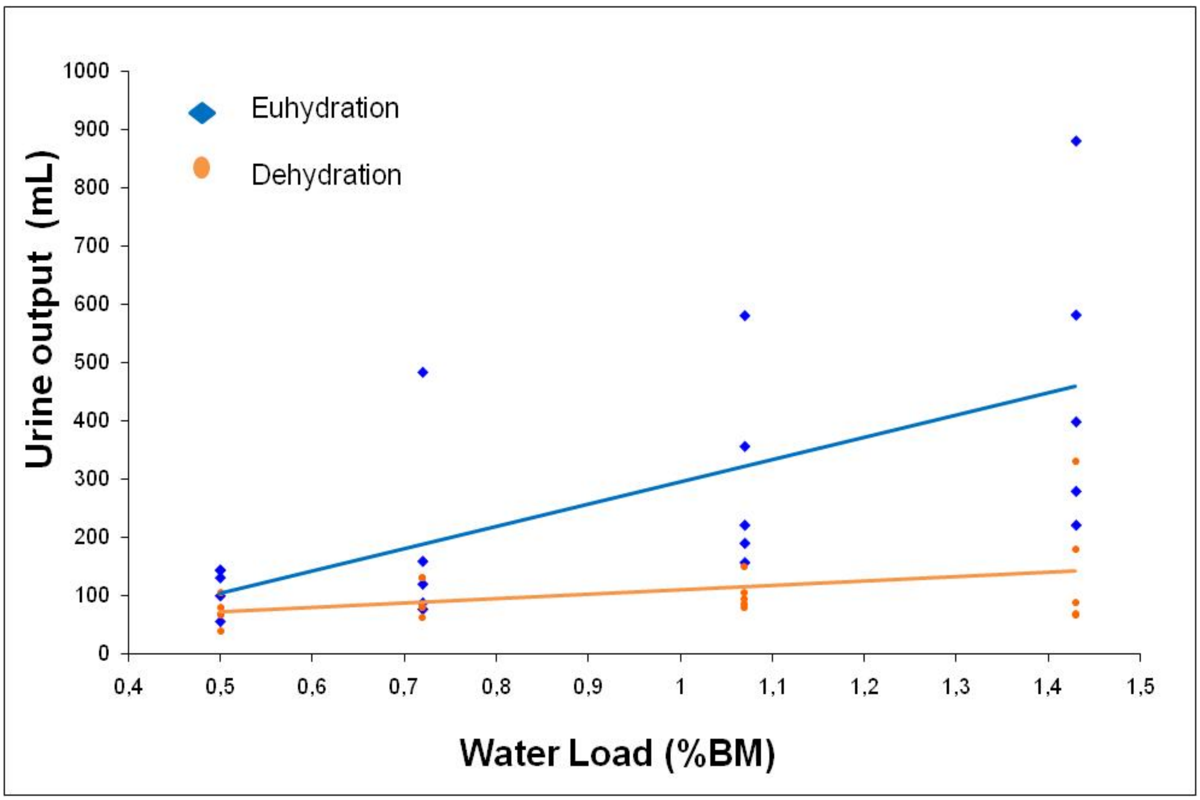


Table 4 shows one-hour urine volume 95%CIs for the euhydration and dehydration conditions, corresponding to various arbitrary water loads, as calculated from the simplified multiple regression model (Eq. 2). Expected minimum differences are also shown, as estimated using Equation 1. According to *Table 4*, the water load should be at least 1.07%FastBM to obtain a minimum difference of 100 mL between a euhydrated and a dehydrated condition.

Table 4. Ninety-five percent confidence intervals for one-hour urine volume.

Water load (%FastBM)	Dehydration		Euhydration		Minimum expected difference (mL)
	Urine volume 95%CI (mL)		Urine volume 95%CI (mL)		
	Lower limit	Upper limit	Lower limit	Upper limit	
0.50%	0.00	78.07	93.76	246.50	15.69
0.85%	42.75	149.41	187.27	295.89	37.86
0.93%	50.09	154.23	218.81	322.95	64.58
1.05%	56.48	166.26	261.91	371.79	95.65
1.07%	57.15	169.35	270.03	382.37	100.68
1.20%	57.65	189.93	312.52	445.04	122.59
1.43%	51.13	230.81	374.45	554.51	143.64

Discussion

The purpose of this study was to determine the minimum amount of water to be ingested by an individual in order to find significant differences between euhydration and dehydration in the urine volume excreted in one hour. With an R^2= 0.64 and R^2_a = 0.58 (p = 0.001), a strong association has been identified between urine volume and predictor variables (acute hydration status and water load). In addition, if a water load equivalent to 1.07% of body mass is administered to an individual, a difference of at least 100 mL will be found with 95% certainty.

A previous study by Capitán Jiménez and Aragón Vargas (2009) showed a clear difference in urine volume between individuals in a euhydrated and a dehydrated state, using a water load equivalent to 1.43%BM. Nevertheless, they stated the need to identify a smaller water load for the test, in order to reduce the amount of extra fluid remaining in the body after one hour in the case of euhydrated individuals. By reducing the water load to 1.07%BM, the remaining fluid would be substantially reduced: for instance, a 70-kg individual would ingest 750 mL (as opposed to 1 liter), of which approximately 438 mL would be eliminated in one hour.

To estimate the lowest possible water load in this study, a multiple regression analysis was used. The complete model from *Table 3* included all possible variables which could influence one-hour urine volume in response to a water load. For this analysis, individuals were included as blocks, expecting significant differences among participants. This, however, did not happen, as the global effect of individuals was not significant (*Table 3*). A second, simplified model was tested where individuals were not included as blocks, a model which enables the authors to generalize the present results.

It was with this second multiple regression analysis that a water load could be estimated to result in a minimum expected difference of 100 mL of urine between euhydration and dehydration, as calculated from the 95% confidence intervals. This 100 mL difference between conditions had been defined *a priori* taking into consideration the fact that normal urine production in one hour is approximately 60 mL; if a water load is ingested, this volume would be expected to increase (Valtin and Schafer, 1995).

The simplified regression equation (Eq. 2) dictates mathematically that for each additional percentage point that the water load is increased, urine volume would increase by (233.99 + 155.88 - 60.66 mL) = 329.21 mL for a euhydrated individual, or 233.99 mL for a dehydrated one. In practice, however, the present results should not be extrapolated beyond the extreme water loads tested here, namely, 0.50% and 1.43% BM, as urine volume behavior outside these limits is currently unknown.

The results from this study give quantitative support to the intuitive statement that urine volume is significantly different between individuals in a euhydrated and a dehydrated state, as already confirmed in a previous study (Capitán and Aragón-Vargas, 2009); furthermore, the minimum water load to be ingested to detect those differences with collection of urine volume for one hour is identified.

In conclusion, the minimum water load that must be ingested by an individual to detect a 100 mL difference in one-hour urine volume between euhydration and dehydration is the equivalent of 1.07% of body mass (750 mL for a 70-kg person).

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