

# ELIMINATION OF URINE IN RESPONSE TO WATER INTAKE IS CONSISTENT IN WELL-HYDRATED INDIVIDUALS

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## ABSTRACT

A simple method has been recently proposed to assess acute hydration status in humans; however, several questions remain regarding its reliability, validity, and practicality. **Objective:** Establish reliability of a simple method to assess euhydration, that is, to analyze whether this method can be used as a consistent indicator of a person's hydration status. In addition, the study sought to assess the effect exercise has on urine volume when euhydration is maintained and a standardized volume of water is ingested. **Methods:** Five healthy physically active men and five healthy physically active women,  $22.5 \pm 2.3$  years of age (mean  $\pm$  standard deviation) reported to the laboratory after fasting for 10 hours or more on three occasions, each one week apart. During the two identical resting euhydration conditions (EuA and EuB), participants remained seated for 45 minutes. During the exercise condition (EuExer), participants exercised intermittently in an environmental chamber (average temperature and relative humidity =  $32 \pm 3^\circ\text{C}$  and  $65 \pm 7\%$ , respectively) for a period of 45 minutes and drank water to offset loss due to sweating. The order of treatments was randomized. Upon finishing the treatment period, they ingested a volume of water equivalent to 1.43% body mass (BM) within 30 minutes. Urine was collected and measured henceforth every 30 minutes for 3 hours. **Results:** Urine volume eliminated during EuExer ( $1205 \pm 399.5$  ml) was not different from EuB ( $1072.2 \pm 413.1$  ml) or EuA ( $1068 \pm 382.87$  ml) ( $p$ -value = 0.44). Both resting conditions were practically identical ( $p$ -value = 0.98) and presented a strong intraclass correlation ( $r = 0.849$ ,  $p$ -value = 0.001). **Conclusions:** This method, besides simple, proved to be consistent in all conditions; therefore, it can be used with the certainty that measurements are valid and reliable.

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**KEY WORDS:** Reliability, rehydration, exercise, diuresis.

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## INTRODUCTION

Controlling and eliminating factors that may impair performance is extremely important for athletes, even more so now that sports are reaching such a competitive level that every detail can make a difference between winning and losing. Continued loss of sweat during training and competitions can cause dehydration in athletes. Performance may decrease if this happens; consequently, trying to offset loss due to sweating during exercise with adequate fluid intake is of great importance. However, it is difficult for trainers and athletes to see if a person is dehydrated with the naked eye.

There are several methods to determine whether a person is well hydrated or not ranging from the simple to the most advanced, each with advantages and disadvantages. Some of these methods have been summarized by Shirreffs (2003): change in body weight before and after exercise (body mass; this method does not assess the acute state, but only changes in hydration); color of urine, urine osmolality, and urine specific gravity (USG) (all of these are indirect indicators of acute hydration status). Other methods that can be used are the change in blood plasma volume (another change indicator) and the bioelectrical impedance analysis, which gives an estimate of total water volume

in the body. Also, the urine volume response to a water load was recently studied (Capitán & Aragon, 2009a).

Although there are different techniques to assess hydration status, not all of them are useful in the world of athletics because they may be very expensive, may require specialized equipment or personnel, or are just not very practical since they have to be conducted at specific times and under specific conditions. The importance of being able to assess the hydration status of athletes has resulted in many evaluations (Cheuvront & Sawka, 2005; Kovacs, Senden, & Brouns, 1999; Oppliger & Bartok, 2002; Shirreffs, 2003), which are in search of a technique that allows both trainers and researchers to obtain reliable data on the state of hydration.

The body has several mechanisms to maintain water balance. For instance, it is known that the kidneys play an important role in maintaining fluid balance in the body (Ulate, 2007), which is done through urination. If a person is dehydrated the kidneys reduce the production of urine in order to retain fluid, whereas if the person ingests liquid being euhydrated urine output increases to remove excess liquid. This could also occur when rehydration is very aggressive (Sawka et al., 2007). Based on these concepts, a method that has been recently investigated (Capitán & Aragón, 2009a) is the measurement of urine output in response to a standardized intake of water (1.43% of body mass, BM), just the same way there is a test to measure blood glucose response to an oral glucose load. The study revealed that this method clearly distinguishes between euhydrated and dehydrated individuals to 1%, 2%, or 3% of body mass (BM). However, this method, like many others mentioned in this paper, has weaknesses. For instance, reliability of the study is unknown (it has not been determined how consistent measurements are in this new method), nor has it been determined whether differences in urine production were really due to hydration status or to the effect exercise has on urine production (Capitán & Aragón, 2009b).

Therefore, the purpose of this study was to establish the reliability of Capitán and Aragón's method, in order to assess whether it can be used as a consistent indicator of a person's hydration status. Another aspect measured was if exercise in euhydrated individuals had any effect on urine output.

## METHODOLOGY

**Participants.** A convenience sample was selected from a group of university students who responded to a public announcement and met previously established requirements. Five men and five women ( $22.5 \pm 2.3$  years, mean  $\pm$  standard deviation) consented to participate in this study. Subjects had the following characteristics: healthy, physically active (performed physical activity at least 4 times a week), had no kidney, endocrine or heart problems, had not suffered from heat illness, and at the time of the study were not taking diuretic drugs. This study was approved by the University's Scientific Ethics Committee.

**Instruments.** An e-Accura® scale, model DSB291, was used with an accuracy of 0.01 kg (10 g) to measure body weight.

Urine samples were collected in plastic containers with a capacity of 750 ml and the amount of urine was measured with a nutritionist scale (precision scale) (Model CS2000 OHAUS® Compact Scale), with an accuracy of 0.001 kg (1 g).

A hand-held Atago® refractometer, model URC-NE, d 1.000-1.050, was used to analyze urine specific gravity and a Polar® heart rate monitor, model A1, was used to control exercise intensity during dehydration.

## PROCEDURES

**Prehydration.** Each participant reported to the laboratory at 7 am on 3 separate occasions having fasted from food and drink for at least 10 hours.

In the laboratory each participant provided a urine sample and urine specific gravity was measured to estimate the initial hydration status (Initial USG). This urine sample was discarded.

After completely emptying their bladders, subjects were weighed nude (weight after fasting, WAF) and that weight was used to determine the volume of liquid to ingest. Once they provided the urine sample and were weighed, participants ate a standardized breakfast of 750 kcal (corresponding to 24.6% lipid, 20.7% protein, and 54.7% carbohydrates including 250 ml natural orange juice), and rested for 30 minutes. This liquid helped ensure euhydration.

**Exercise.** After having breakfast, participants were weighed nude and dry (baseline body weight, BBW) and later exercised intermittently for 45 minutes. The cycle included 15 minutes in the stationary bicycle and 15 minutes on the treadmill, stopping every 15 minutes to be weighed. In order to stay euhydrated, participants ingested the amount of water equivalent to the weight difference due to sweat loss with respect to the baseline body weight (BBW). Only one exercise session was performed (EuEXER).

Exercise was conducted in a controlled environment chamber. Average temperature was  $32 \pm 3^{\circ}\text{C}$  and relative humidity was  $65 \pm 7\%$ ; exercise intensity was moderate (75% - 80% of the maximum heart rate, calculated by the formula  $220 - \text{age}$ ).

**Rest sessions.** Two rest sessions were performed (EuA and EuB), for which the protocol did not require exercise (i.e. 0%). After eating breakfast, participants were weighed nude and dry (baseline body weight) and were later asked to remain sitting outside the controlled environment room for 45 minutes, where they were again weighed nude and dry every 15 minutes. In order to stay euhydrated, participants ingested the amount of water equivalent to the weight difference due to sweat loss with respect to the baseline body weight.

Average room temperature was  $25 \pm 1^{\circ}\text{C}$  and relative humidity was  $60 \pm 2\%$ . Heart rate remained between 35% - 40% of the maximum heart rate, calculated by the formula  $\text{HR}_{\text{max}} = 220 - \text{age}$ .

**Postexercise.** Once the rest and exercise periods were finished, subjects showered with cold water, ingested no liquid and completely emptied their bladders in a container, if necessary. This urine sample was weighed and taken as loss of fluid due to exercise. After showering, they were weighed nude and dry (rehydration weight, RHW).

**Rehydration.** Once bathed and weighed, participants ingested a volume of bottled water (Crystal® brand, sodium = 7.0 mg/L) equivalent to 1.43% of the weight they had when they arrived at the laboratory (WAF), as stipulated by Capitán and Aragon's method. This percentage of liquid was the same in all conditions.

The total volume of fluid was ingested in three equal aliquots, separated by 10 minutes each.

**Urine collection.** Once the rehydration protocol was finished, urine was collected every 30 minutes for 3 hours. Each urine sample collected was weighed and specific gravity was measured before properly disposing of the sample.

**Statistical analysis.** Data was analyzed using descriptive statistics (mean and standard deviation) for age, baseline body weight, and volume of water ingested. Inferential statistics were applied with the SPSS statistical package, version 16, to analyze variance and *post hoc* tests.

Three one-way analyses of variance (ANOVA) were conducted, one for each of the following variables: urine specific gravity, baseline body weight and volume of water ingested, to determine whether participants began the study in the same conditions in all treatments.

A two-way ANOVA was performed with repeated measures on both factors (3 treatments x 7 measurements) to determine whether there were differences between treatments.

In addition, a two-way ANOVA was performed with repeated measures (3 treatments x 2 measurements) to determine whether there were differences in weight before and after exercise in order to verify if participants remained euhydrated during the exercise and rest periods.

An intraclass correlation and a paired t-test were conducted between the two resting conditions to determine reliability.

A statistical power analysis was used to determine whether the sample size could give an acceptable result.

## RESULTS

**Initial conditions.** There were no significant differences in the initial hydration status (p-value = 0.429), which was measured with the specific gravity of the first urine of the day, or in body weight of participants after fasting (p-value = 0.179), nor were significant differences found in the volume of water ingested during the different conditions (p-value = 0.179) (see Table 1). On average, subjects were euhydrated at the beginning of the session in all conditions; there were only two individual clear cases of dehydration (USG = 1.030).

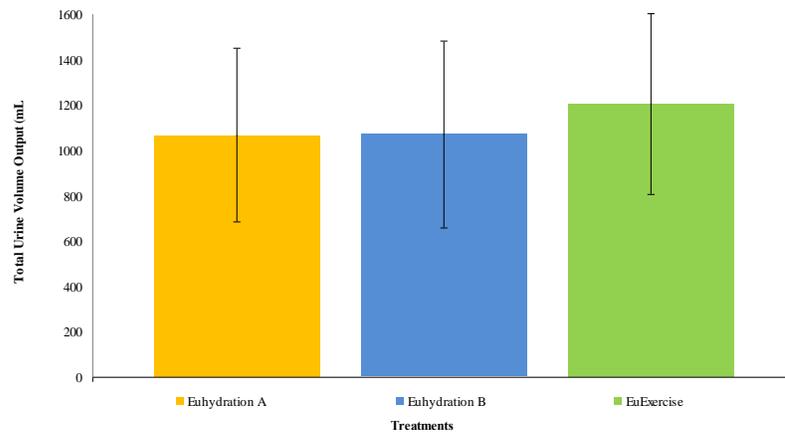
**Total volume of urine eliminated.** Figure 1 shows average volumes of urine eliminated in the different conditions. Since no statistically significant differences were observed between men and women in the total volume of urine eliminated in any of the conditions ( $p > 0.05$ ), data is presented together. This graph shows that there is no significant difference (p-value = 0.44) among conditions (EuA, EuB and EuExer).

**Exercise.** Table 2 shows body weight of participants before starting the 45 minutes of rest or exercise and the end of those periods between the conditions of 0% BM (with and without exercise).

**Table 1.** Average urine specific gravity and body weight at the beginning of each condition.

Condition	Initial urine specific gravity (USG)* (X ± SD)	Baseline body weight (kg)+ (X ± SD)	Volume of water ingested (ml) ∞ (X ± SD)
EuA	1.015 ± 0.010	72.1 ± 19.6	1031 ± 280.5
EuB	1.018 ± 0.007	72.6 ± 20.3	1037 ± 290.8
EuEXER	1.015 ± 0.009	72.4 ± 20.3	1036 ± 290.5

\*p-value = 0.429    +p-value = 0.179    ∞p-value = 0.179



**Figure 1.** Average volume of urine eliminated under the different conditions.

p-value = 0.44 between EuExer and rest (EuA and EuB) and p-value = 0.98 between EuA and EuB

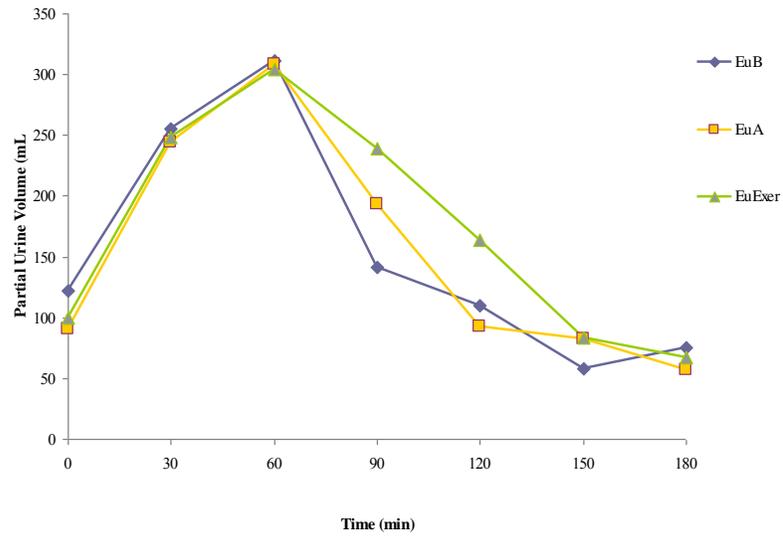
**Table 2.** Average reference and rehydration weights in the different conditions.

Condition	Baseline body weight	Rehydration weight
EuA	72.11± 19.62	72.47±19.61
EuB	73.04±20.22	73.03±20.20
EuEXER	72.92±20.28	72.75±20.17

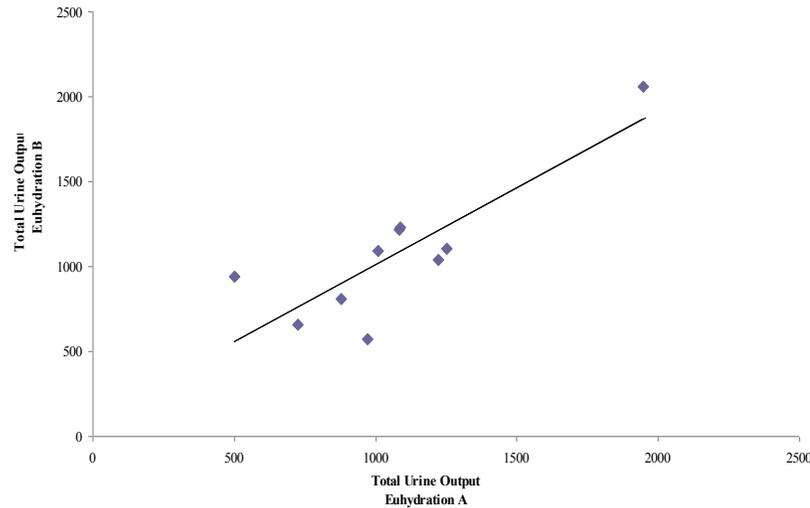
Comparison between baseline body weight and rehydration weight: p > 0.05

**Time of urine collection.** In order to test whether there were any differences in the volume of urine eliminated over time, volumes eliminated from minute 0 to minute 180 were analyzed. Figure 2 shows the differences in the volume of urine eliminated by time in each condition (EuA, EuB, and EuEXER).

**Reliability.** To determine reliability a paired t-test was used with the urine volume in the two resting conditions where no exercise was performed (EuA and EuB): no difference was observed between conditions (t = -0.58, p-value = 0.95). In addition, an intraclass correlation coefficient was calculated, which yielded a high association between tests (r = 0.849, p-value = 0.001). A 95% confidence interval was obtained for the intraclass correlation coefficient (0.496 to 0.961). Figure 3 shows the relationship between conditions.



**Figure 2.** Volume of urine eliminated by time and by condition.  $p > 0.05$



**Figure 3.** Correlation of urine volumes.  $r = 0.849$ ,  $p\text{-value} = 0.001$

**Statistical power.** A statistical power test was used to rule out the possibility that no differences were found between resting conditions due to the sample size. It was determined that the power of the test is 95% with a sample equal to  $N=10$  to detect a 200 ml difference in the total volume of urine eliminated.

## DISCUSSION

The objective of this study was to determine the reliability of a simple method to assess euhydration. With a high correlation between EuA and EuB, measurements are consistent in this method, which makes it reliable.

The study also aimed to determine whether exercise has any effect on urine production of euhydrated participants in response to a standardized amount of water. No differences were found for either total urine volumes eliminated or partial urine measurements between resting conditions and exercise (EuA and EuB vs. EuExer), considering that participants started under the same conditions each time.

During physical activity body temperature rises and the body attempts to dissipate heat by sweating. Fluid loss through sweating causes a decrease in total body water. To avoid unnecessary water loss during exercise the body increases the secretion of antidiuretic hormone or vasopressin, which is responsible for reducing the production of urine during exercise (Wilmore & Costill, 2004).

A change in plasma osmolality is the primary regulator of vasopressin secretion. Therefore, if osmolality is below the threshold (280 mOsm/kg) secretion will be practically nil (Ulate, 2007), which means that urine output is not altered. On the other hand, if this value increases (e.g. due to dehydration), the secretion of vasopressin also increases, thereby reducing urine production.

Therefore, as stated in the theory, the body eliminates through urine any excess fluid ingested (Wilmore & Costill, 2004). This means that when the body is euhydrated and is given a volume of

water, it would become extra fluid in the body and be eliminated by the organism, thus increasing urination. Consequently, if a balance is maintained during exercise between what is lost through sweating and what is ingested, the mechanisms that stimulate the decrease of urine volume, such as the secretion of the antidiuretic hormone, should remain unchanged. The volumes of urine eliminated, both in resting conditions (EuA and EuB) and exercise were very similar, indicating that the production of urine during euhydration was not related to whether or not exercise was performed. This suggests that urine output is more dependent on the hydration status than on having exercised or not.

When urine volumes were analyzed by time, no differences were found between resting conditions (EuA and EuB) at 60 minutes into the urine collection. The analysis was performed with the urine eliminated after 60 minutes since this is a reasonable time to determine differences (Capitán & Aragón, 2009b). This reinforces the reliability test because not only are there no differences in total urine volume, but also the partial urine volumes are the same.

Although this study was able to find that the difference between conditions is due to the water volume ingested and not to exercise, there is still a problem to be solved: even though the method is sensitive and reliable, it loses a lot of its practicality since after 60 minutes of urine collection most individuals would still have in their bodies between 67% and 13% (Capitán & Aragón, 2009b) of the water ingested. Therefore, it is necessary to determine the amount of water that would allow detecting differences, but at the same time be a smaller volume of extra water remaining in the body at the end of 60 minutes. If this is achieved, this method will be sensitive, reliable, practical, and suitable to evaluate the acute stage of hydration, with the advantage of being inexpensive, easy to use and applicable to many individuals or an entire team simultaneously.

In conclusion, this study found that Capitán and Aragón's method is reliable and also showed how exercise, under the conditions of this experiment, did not cause a decrease in urine output when compared with urine produced at rest.

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