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para evaluar el estado de
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transversal**
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the hydration status in a healthy
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Validated questionnaire to assess the hydration status in a healthy adult Spanish population: a cross sectional study

Validación de un cuestionario para evaluar el estado de hidratación de una población adulta sana española: un estudio transversal

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ABSTRACT

Introduction: adequate hydration status is crucial in most physiological functions; conversely, its assessment is hindered by the limited availability of research tools.

Objective: to develop and validate a novel questionnaire that evaluates the hydration status of a healthy adult Spanish population.

Method: a novel questionnaire was designed and validated relying on biochemical parameters related to blood, urine, and body water content. The study involved 39 healthy subjects aged between 18 and 39 years. Food and beverage consumption were assessed by the novel questionnaire and through a three-day dietary record. Physical

activity was assessed using both: accelerometers and the Short International Physical Activity Questionnaire (IPAQ). Validity was determined by correlation of the aforementioned parameters with the water balance and water intake obtained by the novel questionnaire. The questionnaire was administered twice over the course of 28 days to evaluate its reliability.

Results: water balance and total water intake were correlated with specific gravity, and urine color. Water intake obtained by the novel questionnaire was correlated with water intake results from the three-day dietary record. Intraclass correlation coefficient indicated moderate concordance between both recordings, and the Cronbach's alpha revealed high consistency. Finally, the Bland and Altman method indicated that the limits of agreement were acceptable to reveal the reliability of the estimated measures.

Conclusions: the questionnaire designed is a new valid and reliable screening tool to estimate hydration status of adult populations in dietary and nutritional assessment.

Key words: Hydration status. Water balance. Questionnaire. Validation.

RESUMEN

Introducción: un estado hídrico adecuado es crucial para la mayoría de funciones fisiológicas; sin embargo, su evaluación se encuentra obstaculizada por la escasez de herramientas de diagnóstico e investigación.

Objetivos: desarrollar y validar un cuestionario que permita evaluar el estado hídrico de población española adulta sana.

Métodos: un nuevo cuestionario ha sido diseñado y validado a través de parámetros bioquímicos en orina y sangre y contenido en agua corporal. El estudio incluyó finalmente a 39 sujetos sanos con edades comprendidas entre 18 y 39 años. El consumo de alimentos y bebidas se evaluó empleando el nuevo cuestionario y un diario dietéticos de

tres días. La actividad física fue estimada a través de acelerometría y con el Cuestionario Internacional de Actividad Física (IPAQ). La validez se determinó analizando la correlación de los parámetros citados, con el balance y con la ingesta hídrica. El cuestionario fue administrado por duplicado, con un transcurso de 28 días entre ambas cumplimentaciones para evaluar su reproducibilidad.

Resultados: el balance y la ingesta hídrica se correlacionaron con la gravedad específica y con el color de la orina. La ingesta hídrica se correlacionó con los resultados procedentes del diario dietético. Según el coeficiente de correlación intraclase, la concordancia entre ambas cumplimentaciones fue moderada y el alfa de Cronbach indicó consistencia elevada. El método Bland-Altman mostró que los límites de confianza eran aceptables para revelar la reproducibilidad de las medidas estimadas.

Conclusiones: el cuestionario diseñado constituye una herramienta de cribado válida y fiable para estimar el estado hídrico de población adulta sana.

Palabras clave: Estado de hidratación. Balance hídrico. Cuestionario. Validación.

INTRODUCTION

Water is a major component of human body and is involved in practically all functions of our organism playing a crucial role in life and health (1). It must be obtained externally through the consumption of foods and beverages because there is no real water storage in the body and the amount lost in metabolism exceeds the amount synthesized endogenously (1,2). Despite this, water consumption is often forgotten in dietary recommendations and the importance of adequate hydration is not usually mentioned (1).

Hydration status (HS) is defined as the body's fluid level and is determined by water balance (WB) (net difference between water input and output) (2). It is influenced by dietary intake, physical

activity level, age, and environmental conditions amongst others, and its regulation is very precise, as loss of 1% of body water is usually compensated within 24 hours. Under conditions of temperate ambient temperature (18-20 °C) and with a moderate activity level, WB remains relatively constant; nevertheless, water deficit as well as water excess can occur (2). In this context, it is important to mention that even small losses of body water can have negative effects in health: reductions in the subjective perception of alertness, ability to concentrate, disruption in mood, cognitive functioning, increase of tiredness and headache, as well as a decrease in performance capacity, between others (3). To keep an adequate WB, individuals are recommended to comply with reference values of total water intake (WI) (3,4). These reference values are largely based on observational studies of total WI conducted in healthy individuals and the estimation of WB. However, the established reference values vary considerably, which can be partly explained by differences in the methodology used to estimate fluid intake and/or losses (5). In addition, in recent years it has been shown that an important portion of the population does not hydrate properly, which potentially could lead to higher hypohydration prevalence (6). In Spain, results from the nationwide representative ANIBES study (n = 2,285) showed that more than 75% of individuals were not meeting the European Food Safety Authority (EFSA) WI recommendations (7).

The lack of suitable research tools designed to estimate the HS (8,9), coupled with the lack of consensus about the best method to evaluate WI (10), constitutes one of the most important limitations in the field of hydration. This is mainly due to the complexity involved in its assessment: there is no “gold standard” and techniques and methods available are expensive, invasive and/or complicated, making it impossible to apply them at the population level (8,9).

For all the aforementioned, developing suitable methodologies which evaluate HS is of key interest. Given its simplicity and cost-effectiveness, questionnaires could be useful both, in the field of

research and in the clinical and community practices. The objective of the present study was to develop a novel questionnaire to evaluate the HS of a healthy adult Spanish population.

MATERIAL AND METHODS

In this cross sectional study, a novel questionnaire entitled The Hydration Status Questionnaire (HSQ) (Supplementary file 1) was developed and validated. Ethical approval was granted by the Clinical Research Ethics Committee of the CEU San Pablo University (Madrid). The study has been performed in accordance with the ethical standards laid down in the Declaration of Helsinki of 1964 and its later amendments. Participants signed an informed consent prior to their inclusion in the study. All personal data are confidential and only investigators assigned to the project have access to them. In any case, it complies with the Law 15/1999 of 13th December, of Personal Data Protection.

Design of the questionnaire

After thorough research (2,4,10-12), the main factors that affect HS were included in the questionnaire and compiled into five items: a) personal information; b) medical history; c) hydration habits and knowledge; d) water, beverages and food frequency questionnaire (WBFFQ); and e) water elimination (WE). This information allows for the assessment of the profile of the interviewee and the estimation of WB.

Water input was recorded through the WBFFQ. For its development, beverages and foods with water content higher than 75% were selected from the Spanish Food Composition Tables (13) and classified into 12 groups: (d.1) water; (d.2) juices; (d.3) milk and dairy products; (d.4) sodas; (d.5) coffees; (d.6) tea and infusions; (d.7) alcoholic beverages; (d.8) other beverages (alcohol free beer, energy drinks, sorbets, jellies and sports drinks); (d.9) others (plant-based beverages, *horchata* and meal replacement drinks); (d.10) fruits;

(d.11) vegetables; and (d.12) cooked dishes. The amount, frequency and time of consumption were recorded, and the seasonality of foods and beverages, which are predominant at a specific time of the year, were taken into account. To assess serving sizes, different household measures were used. The frequency of consumption was evaluated using four categories: a) never; b) per month; c) per week; and (d) daily. Times of consumption were recorded as “before breakfast”, “at breakfast”, “in the morning”, “at lunch”, “in the afternoon”, “at dinner” and “at night”.

To estimate water output three elimination pathways were taken into account (skin, kidneys and digestive system). Urination and defecation were recorded on the basis of frequency (urination options: once/day, two-four times/day, five-seven times/day, eight-ten times/day and more than ten times/day; defecation options: once/day, five-six time/week, three-four times/week, one-two times/week or less than one time/each ten days) (14). To calculate WE from sweating, a ten-point scale was used for both, physical activity and sedentary conditions (14). To assess physical activity, two different approaches were used: the short International Physical Activity Questionnaire (IPAQ-S) (15) and accelerometry by ActiGraph GT3X™ model accelerometer.

Questionnaire analysis

The water content from beverages and foods was calculated using the Spanish Food Composition Tables by Moreiras et al. (13). The amount of water from drinking water, beverages and foods were calculated separately and expressed as milliliters of WI per day. Water provided by each beverage was calculated according to the following formula: milliliters of beverage consumed per day * duration of its seasonality in days/365 * water content/100. Water from foods was calculated in the same way but also taking into account the edible portion. To calculate WE from sweating in sedentary conditions, the ten-point scale described before was used. The duration in hours per day of this

condition was multiplied with a factor to quantify WE; this factor depends of the score that the participant gave for sweating in the scale (i.e., point 1 corresponded to 0.01 ml water/h and point 10 to 0.02 ml/h), in-between values varying in a proportional manner. The WE from sweating during exercise was also estimated using the ten-point scale. The duration and intensity level of the physical activity performed during three consecutive days were estimated by accelerometers. The duration of physical activity in hours was multiplied with a factor that depends on the score given in the scale and on the activity intensity level: for intense exercise, point 1 corresponded to 1,000 ml water/h and point 2 to 2,000 ml/h; for moderate exercise, point 1 corresponded to 400 ml/h and point 10 to 700 ml/h; and for mild exercise, point 1 corresponded to 200 ml/h and point 10 to 400 ml/h (16,17). In-between values varied in a proportional manner. To estimate WE from urination and defecation, participants had five frequency options in both cases. These options were transformed into a five-point scale, in which the first option (once/day) corresponded to point 1 and the last one (more than ten times/day or one time/ten days) corresponded to point 5. For urination, point 1 corresponded to 750 m water/day and point 5 to 2,500 ml water/day (4,17). For defecation, point 1 corresponded to 150 ml water/day and point 5 to 75 ml water/day (17,18). In-between values varied in an analogous manner. WB was defined as the difference between total WI and total WE.

Questionnaire validation

The study took place from October to December 2015 in the Montepríncipe Campus of CEU San Pablo University (Madrid, Spain). Participants were recruited at the University premises by informative talks and posters. The inclusion criteria were: individuals who were a) mentally and physically healthy; and b) aged 18-39 years. Exclusion criteria were suffering from diseases related to HS, and/or women who were menstruating during the study. Finally, 40 healthy volunteers

participated in the validation process. This sample size keeps to the Nunnally criterion (19), which recommends a ratio of minimum five participants for each item of the questionnaire.

The validity study took place through the use of several biomarkers, most of them acknowledged as important biological indicators of HS (20-22): urine specific gravity (USG) and urine color (UC), plasma hemoglobin and hematocrit in blood and total body water (TBW). To validate the WBFFQ included in the questionnaire, results derived from it were compared with water consumption from a three-day dietary record (3DR). In addition, hemodynamic data (pulse, systolic blood pressure [SBP] and diastolic blood pressure [DBP]) were collected (23). For the reliability study, participants completed the questionnaire twice over the course of 28 days. The validation process was performed under similar weather conditions in the same laboratory of the University.

Validation protocol

Each volunteer's first visit was preceded by a short explanation on the procedures involved in the validation process and its protocol (Fig. 1). Participants completed a 3DR over the course of three consecutive days (one weekend and two weekdays) in which they were asked to give detailed descriptions of each food and beverage item consumed, providing them previously with clear instructions on how to fill in it. Subjects were also instructed to follow their usual diet. The DIAL™ software (24) was used to process the information of the 3DR. During these three days, individuals also wore an accelerometer that estimated their physical activity. The fourth day of the study, participants completed the HSQ and the following laboratory tests and measurements were performed under fasting conditions:

- Hematological variables: hemoglobin, hematocrit and erythrocyte were determined by capillary finger-stick whole blood with Calligari™ Analyzer.
- Body composition: TBW and water percentage was estimated by bioelectrical bioimpedance analysis (BIA) with Bioscan

Spectrum™ Multifrequency. Individuals were weighed using a digital scale with an accuracy of 200 g (Seca™ 877). Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca™ 213). The anthropometric measurements were made according to the recommendations of the International Standards for Anthropometric Assessment (ISAK) (25) by level I and II accredited anthropometrists.

- Urine variables: volunteers provided a first morning urine sample in which urine pH and USG was determined through the use of urine stick test Spinreact™ and UC via the urine color chart (26). Results were compared with reference values of hydration biomarkers in first urine morning spot established by Lawrence E et al. (27) (euhydration: specific gravity = 1.023-1.025, urine color = 4-5).
- Hemodynamic variables: pulse, SBP and DBP were determined using a digital sphygmomanometer (Omron™, M3 model).

Statistical analysis

Results are presented as mean (95% confidence interval) for the normally distributed variables (weight, height, body water percentage, TBW, hematocrit, erythrocyte, hemoglobin, SBP, DBP and pulse) and as median (interquartile range) for non-parametric ones (USG, pH and UC). WI, WB and WE were treated as non-parametric data. Variables were tested for normality using the Shapiro-Wilk test. Differences between normally distributed variables were assessed with the Student's paired t-test and the Mann-Whitney U test was applied for non-parametric ones. Differences were considered as significant at $p < 0.05$. The validity of the questionnaire was evaluated through the use of Spearman's (ρ) coefficient to estimate the correlation between WB and quantitative discrete variables (hemoglobin, hematocrit and TBW) and Kendall's tau-b (τ) for ordinal qualitative ones (UC). Test-retest reliability was assessed using the intraclass correlation coefficient (CCI) to demonstrate that results were consistent over time. The Bland-Altman plot was used to

represent graphically the agreement between measurements in both completions of the questionnaire. Moreover, Spearman's coefficient between the difference and the average of the variables estimated was calculated to assess potential bias (significant values of Spearman's coefficient indicate divergence in the variable between the two completions). Wilcoxon signed-rank was applied to further evaluate the differences between the two completions. Cronbach's alpha (α) was also applied to assess the internal consistency of the HSQ. All statistical analyses were performed using SPSS 24.0 Software (IBM Corp., Armonk, NY, USA).

RESULTS

Sample characteristics

A total of 40 healthy volunteers, 22 males (55%) with a mean of 24.4 (22.3-26.5) years of age and 18 females (45%) with a mean age of 21.6 (20.1-23.2) years participated in the validation process. One female was eliminated for completing the questionnaire incorrectly. Their anthropometric characteristics, body water content, urine and blood markers, and hemodynamic data are presented in table I.

As it can be observed in table I, average values for TBW were lower in females than in males, there were no significant differences in urinary indices and hematological indices were higher in males than in females. Lastly, SPBs of males were higher than in females.

Results of WI, WE and WB estimated by the HSQ and sorted by gender are presented in table II. As it can be observed, except in WE, there were no significant differences in any variable between both genders. The main reason for this difference was the lower WE from sweating in females in comparison to men, given that no differences were found in WE from urine and feces. Results using accelerometer information also show that males eliminated 1,405.5 (1,135.3-1,864.1) ml per day by sweat while females eliminated 963.7 (680.1-1,311.5) ml/day ($p = 0.004$) (this difference is mainly due to the intensity and duration of the physical activity practiced by each

gender).

According to results from 3DR, total WI of the sample was 2,459.0 (2,009.0-3,084.0) ml/day. Sorting results by gender, total WI of males was 2,867.5 (2,278.2-3,507.2) ml/day and 2,261.0 (1,835.5-2,818.0) ml/day in females.

Validity of the questionnaire

WB, as estimated by the HSQ, was further correlated with urine indices to assess the validity of the tool. Moderate agreement between the WB and the respective biomarkers was evident for UC ($\tau = -0.392$, $p = 0.001$) and USG ($\rho = -0.524$, $p = 0.001$). Total WI was correlated with the same biomarkers ($\tau = -0.346$, $p = 0.004$, $\rho = -0.551$, $p = 0.000$). Drinking water and total WI from HSQ correlated moderately with WI data from 3DR ($\rho = 0.465$, $p = 0.001$; $\rho = 0.432$, $p = 0.006$). No correlation was obtained for the rest of parameters.

Results analyzed by gender showed that the WB was correlated with USG and UC among females ($\tau = -0.438$, $p = 0.021$; $\rho = -0.672$, $p = 0.003$, respectively) and males ($\tau = -0.402$, $p = 0.016$; $\rho = -0.451$, $p = 0.035$, respectively). Total WI was correlated with USG in both females and males ($\rho = -0.516$, $p = 0.034$; $\rho = -0.570$, $p = 0.006$, respectively), but UC only among males ($\tau = -0.392$, $p = 0.018$). Drinking water and total WI correlated with WI data from 3DR ($\rho = 0.668$, $p = 0.001$; $\rho = 0.660$, $p = 0.01$, respectively) in males, but not in females.

Reliability of the questionnaire

Results from both HSQ completions are presented for the total sample in table III. To analyze the test-retest reliability, the ICC was calculated obtaining a value of 0.501. As it can be observed in table III, there were no differences in any variables between the two recordings. According to the Bland-Altman method (Fig. 2), the mean differences of the estimated variables did not differ from zero (Wilcoxon signed-

rank test). The limits of agreement were quite narrow; all six-scatter plots were predominantly distributed within the 95% limits of agreement and were considered as acceptable to reveal the reliability of the estimated measures. No bias was evident regarding the two recordings in all studied cases (drinking water: $\rho = -0.026$, $p = 0.875$; water from beverage: $\rho = -0.121$, $p = 0.400$; water from food: $\rho = 0.005$, $p = 0.978$; WI: $\rho = -0.048$, $p = 0.772$; WE: $\rho = -0.061$, $p = 0.710$; WB: $\rho = -0.14$, $p = 0.388$). To test the internal consistency of the questionnaire, the Cronbach's α of each recording was calculated; 0.832 and 0.852, respectively, were obtained as a result.

DISCUSSION

The HSQ has been designed with the objective of creating and validating a new tool, which can allow for the estimation of WB of the population at a community level. The simplicity, quickness and low-cost of questionnaires make this technique appropriate to achieve the targeted goal. From our knowledge, the Water Balance Questionnaire (WBQ) (11) is the only questionnaire that evaluates WI and WE. It evaluates WI throughout a food frequency questionnaire (FFQ) and has been designed and validated in the Greek population, which belongs to a Mediterranean region but with differences among eating patterns in contrast to the Spanish population (28,29). Moreover, FFQ should be designed specifically for each population of study, because the ethnic, cultural and socio-economic level as well as the food preferences could influence food and beverage intake (30).

An accurate estimation of WI and WE is a key factor in the assessment of WB. Water inputs come from beverage and food ingestion and normal metabolic processes, while skin, kidneys, lungs and digestive system are the sources of water output (2). The HSQ takes into account most of these pathways: WI from food and beverages and WE from skin, kidneys and digestive system. Because the amount of water lost from lungs is similar to the amount of

endogenous water formation (2), neither of these two aspects were considered. The assessment of WE was performed through the use of point scales for self-estimation (14,16-18). Score ranges provided in the point scale corresponded to the range of physiological WE through the equivalent route. It should be noted that the estimation of WE through sweat has an added difficulty: it depends on several factors among which physical activity is determinant, because inter and intra-individual variation can be very large. To overcome this limitation and assess the quantity, intensity and typology of the physical activity from each participant, the information was acquired by accelerometers, which is, at the present, the most refined method to quantify physical activity (31). WE data provided by the questionnaire were in accordance with current literature, which is 1,500-3,100 ml/day for adults (3,4), in moderate climates such as the Spanish Mediterranean.

The water consumption obtained by the HSQ was similar to other studies that evaluate WI through specific questionnaires (7); nevertheless, it was higher than results of hydration studies that are based on general questionnaires (6). Ordinarily, fluid specific records report higher fluid intake compared to tools that are not specifically designed to record WI, mainly due to the fact that general questionnaires only record eating occasions around meals and snacks, but not all drinking occasions (11,32). There were no differences in WI between genders, being these results in accordance with known literature (33). In addition, results from different epidemiological studies have shown that foods may provide 20-32% of total WI (6,32), being results of the present study (28%) consistent with these data.

To date, different biomarkers of HS have been proposed (20,33,34), but no single method appears to be ideal for all situations, therefore, the combination of different hydration indices seems to be the most appropriate method to evaluate HS. The validity evaluation of the present study has been based in several of them (USG, UC, TBW, hemoglobin and hematocrit). Recent investigations have

demonstrated that both USG and UC are strongly correlated with urine osmolality, which has been proposed as the most promising HS marker available nowadays (35,36). Indeed, during acute progressive dehydration, USG, osmolality and UC may be used interchangeably (9,33-35). Recent studies have demonstrated USG utility not only in acute water loss, but also in real-life conditions (8,22), being an accurate and rapid indicator of HS. UC is more subjective, but it can be used as a marker in combination with a more quantifiable method. Although refractometry seems to provide the most accurate measurement of USG (37), in this study it was measured through reagent strips, whose suitability as screening method has also been supported by several studies (38,39). WB and total WI obtained by the HSQ correlated with USG and UC, being both correlations very similar. It is mainly due to the fact that in sedentary to moderately active adults, and with moderate weather conditions, WB is largely determined by the adequacy of fluid intake (22). Nevertheless, it is important to highlight that a low daily WI and a low WE, which could result in an adequate WB, are not equivalent to an adequate HS (22). Body water content has also been recognized as a marker of HS. In this study, it was estimated through BIA. It is known that the most accurate methodology available to estimate TBW is mass spectrometry through tritium or deuterium dilution, but it cannot be applied at community level due to ethical implications. Given the limitations of BIA, it must be performed under controlled conditions and the information acquired must be interpreted with caution (40). Nevertheless, even when these conditions are achieved, BIA is not able to identify small changes in TBW. In the current study no correlation was obtained for this parameter.

Due to their potential as HS indicators, the correlation between blood parameters and WB was analyzed (20). However, there is an important limitation: changes in their concentration represent changes in plasma volume and not in TBW. To estimate changes in plasma volume the baseline values of those parameters have to be

known. In this study, WB was not correlated with blood parameters. Finally, HS also affects hemodynamic parameters (23), even though researchers are not able to identify fluid imbalances independently of other indices. For this reason, SBP, DBP, and pulse were measured in the present study and used as additional information in the assessment of HS.

In order to evaluate results of water consumption from the new questionnaire, a well-known dietary intake estimation method was used; in particular, a 3DR. Drinking water data and WI from the questionnaire were compared with data from the 3DR showing the existence of a moderate correlation between both ($\rho = 0.465$, $p = 0.001$; $\rho = 0.432$, $p = 0.006$, respectively). Nevertheless, results of WI from 3DR were lower than from HSQ, being this results in accordance with known literature (6,11,32).

With regard to the reproducibility of the questionnaire, the Cronbach's α revealed a high and similar consistency in both recordings (values equal or higher than 0.7 are considered as adequate). The ICC revealed the existence of moderate concordance between both administrations of the questionnaire. The Bland-Altman method allows assessing the agreement between the methods across the range of WI and losses and can determine if there was any systematic difference between the administrations of the questionnaire, and to what extent the two administrations agree (limits of agreement). Accordingly, the HSQ was repeatable for all the components studied. The validity and reliability shown by the HSQ in both genders, the combination of different biomarkers in the validation process, coupled with the simplicity and low-cost of the tool designed are the main advantages of the present study. However, the most important limitation refers to the non-availability of a gold standard against which to validate it, as well as the measurement of USG through reagent strips instead of refractometry and the non-availability of 24 hours urine samples.

CONCLUSIONS

These findings show that the HSQ is a reliable and valid tool, which could be used as an affordable, rapid screening method to estimate WB of healthy adults. Its application at community level would allow a deeper knowledge of the HS as part of the nutritional status and, consequently, the possibility of establishing recommendations based in real hydration needs. Developing future studies that confirm these results and allow the use of this tool in other population groups are of further interest.

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Nutrición
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Table I. Anthropometric characteristics, body water content, urine and blood markers and hemodynamic data of participants (n = 39)

	Males (n = 22)	Females (n = 17)	p values*
Weight (kg)	74.0 (69.2-78.7)	56.9 (54.0-59.8)	0.000
Height (cm)	180.9 (178.2-183.6)	165.5 (163.3-167.7)	0.000
Body water (%)	57.1 (55.0-59.2)	52.5 (50.8-54.1)	0.002
Body water (l)	41.8 (40.4-43.1)	29.8 (28.6-31.0)	0.000
USG	1.025 (1.020-1.030)	1.025 (1.020-1.030)	0.123
pH	5 (5-6)	5 (5-5)	0.952
UC	4.0 (3.0-5.0)	4.0 (3.0-4.5)	0.579
Hematocrit (%)	44.9 (43.1-46.6)	40.5 (39.2-41.9)	0.000
Erythrocyte (mill/ μ l)	4.8 (4.7-5.0)	4.3 (4.2-4.5)	0.000
Hemoglobin (g/dl)	15.7 (15.1-16.3)	13.5 (12.7-14.3)	0.000
SBP (mmHg)	126.9 (121.9-132.0)	111.7 (106.6-117.2)	0.000
DBP (mmHg)	69.4 (65.6-73.1)	65.5 (61.8-69.1)	0.138
Pulse (beats/minute)	64.6 (60.7-69.0)	70.6 (64.1-77.1)	0.097

USG: urine specific gravity; UC: urine color; SBP: systolic blood pressure; DBP: diastolic blood pressure. Results are presented as mean (confidence interval) for the normally distributed variables and as median (interquartile range) for the non-parametric ones. *p values derived through Student's t test for the normally distributed variables and Mann-Whitney U test for the non-parametric ones after controlling for the normality of the characteristics distribution.

Table II. Water intakes from all the sources, water elimination and water balance obtained from the novel questionnaire, sorted by gender (n = 39)

	Males (n = 22)	Females (n = 17)	p values*
Drinking water (ml/day)	1,230.3	1,335.7	0.922
Water from beverages (ml/day)	(1,000-1,800) 2,334.4	(875.0-2,000.0) 2,395.4	0.475
Water from food (ml/day)	(1,930.6-2,727.0) 694.7	(1,940.8-3,003.1) 785.6	0.181
Total water intake (ml/day)	(360.7-934.5) 3,123.7	(523.0-1,212.8) 3,277.6	0.528
Water loss (ml/day)	(2,276.4-3,753.3) 3,114.7	(2,753.6-4,161.6) 2,460.3	0.036
Water balance (ml/day)	(2,551.5-3,360.3) 102.1	(2,309.1-3,086.5) 521.4	0.067
	(-617.4-738.4)	(-51.2-1,441.3)	

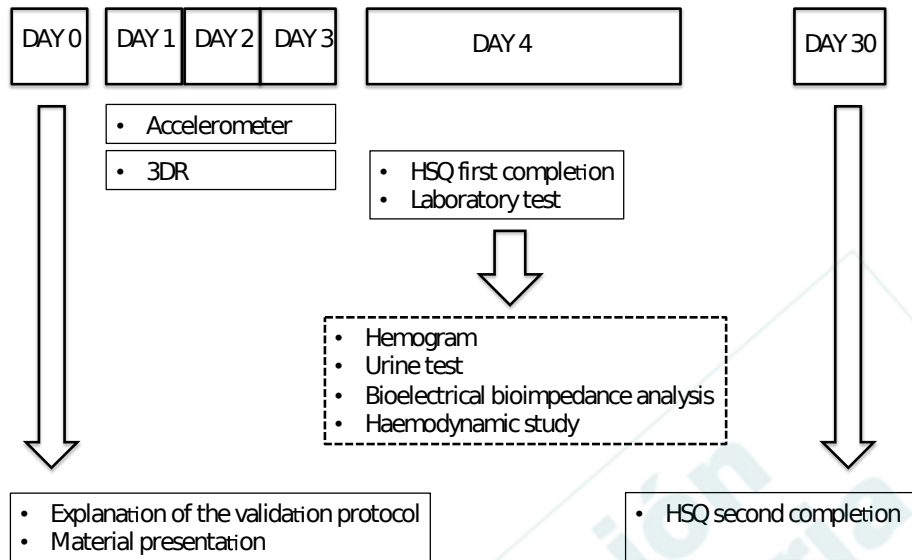
Results are presented as median (interquartile range). *p values derived through Mann-Whitney U test.

Table III. Results of the reliability procedure for the HSQ

	1 st completion	2 nd completion	Mean difference	p values*	Limits of agreement
Drinking water (ml/day)	1,235.7 (1,000.0-1,800.0)	1,371.4 (1,000.0-2,000.0)	-51.9	0.569	-1,183.4-1,079.6
Water from beverage (ml/day)	2,352.8 (1,964.7-2,865.2)	2,343.2 (2,009.4-3,028.5)	-98.8	0.665	-1,729.8-1,532.1
Water from food (ml/day)	736.6 (460.8-1,038.3)	750.0 (475.9-1,154.0)	-27.9	0.812	-743.4-687.4
Total water intake (ml/day)	3,240.4 (2,573.4-4,026.3)	3,082.1 (2,704.2-4,195.1)	-126.8	0.686	-2,124.0-1,870.4
Water loss (ml/day)	2,826.3 (2,398.0-3,270.0)	2,826.3 (2,409.6-3,261.6)	-2.6	0.982	-451.4-446.3
Water balance (ml/day)	430.9 (-289.7-979.0)	514.3 (-270.2-1,150.5)	-124.2	0.727	-2,167.9-1,919.4

HSQ: hydration status questionnaire. Results are presented as median (interquartile range). *p values derived through Mann-Whitney U test.

Fig. 1. Protocol of the validation process.



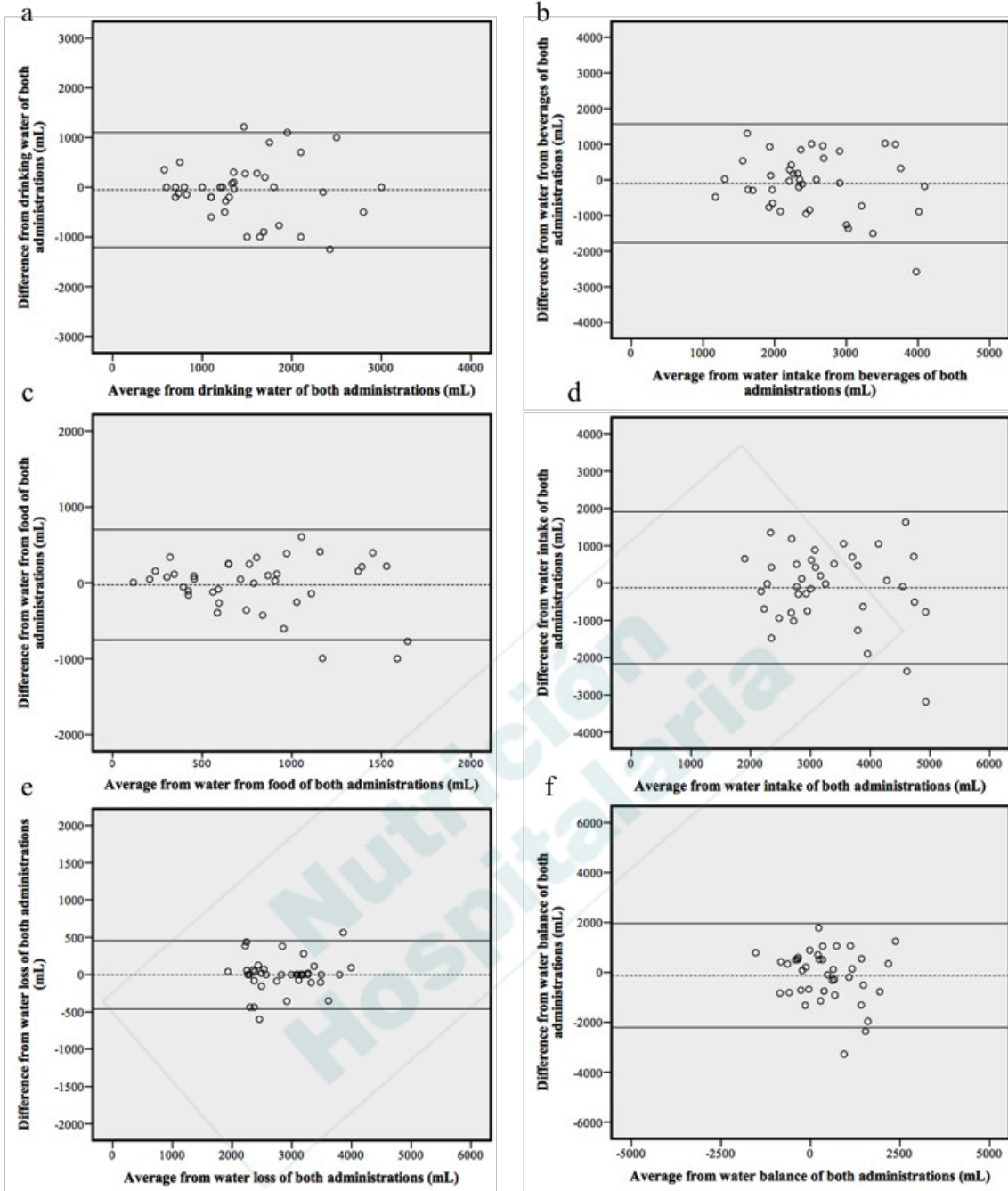


Fig. 2. Bland-Altman plots of differences *versus* means for the variables: a) drinking water; b) water from beverages; c) water from food; d) total water intake; e) water loss; and f) water balance.