Fluid consumption, total water intake, and urine osmolality in Spanish adolescents: The HELENA study

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Conflict of interest

The authors declare no conflict of interests.

KEYWORDS: hydration, osmolatity, fluids, adolescents

ABSTRACT

Objective

To describe the hydration status and to assess the main food and/or fluid intake associated factors in healthy adolescents.

Methods: The Spanish sample participating in the cross-sectional "Healthy Lifestyle in Europe by Nutrition in Adolescence" (HELENA) study. 194 adolescents **aged 12.5 – 17.5 y** (99 males) were included. First morning urine was collected and osmolality was determined by freezing point depression osmometer. A self-reported computer-based 24-hour dietary recall was applied the same day of the urine collection. ANOVA, Kruskal-Wallis procedure or Pearson's $\chi 2$ were used to examine the group associations.

Results

71% of adolescents did not meet the European Food Safety Agency (EFSA) recommendations for total water intake (TWI), and **68%** had high first morning urine osmolality values. TWI and the proportion of those meeting EFSA reference values **significantly ($p<0.05$)** decrease while the osmolality increases. Males who met the EFSA recommendations consumed significantly **($p<0.05$)** more plain water **(1035.13 ml vs 582.68 ml)** and dairy drinks **(368.13 ml vs 226.68 ml)** than those who did not. In females, the consumption of water **(1359.41 ml vs 620.44 ml)** and sugar-sweetened beverages **(214.61 ml vs 127.42 ml)** was significantly higher in those meeting the EFSA recommendations than in those who did no. First morning urine osmolality was associated with vegetables (**unstandardized** β: -0.6; 95% CI): -1.02, -0.18) and fruits intakes (β: -

0.41; 95% CI: -0.63, -0.19) in males; and with dairy drinks (β: -0.39; 95% CI: -0.76, -0.02), and fruits (β: -0.41; 95% CI: -0.73, -0.10) in females.

Conclusions

There was a high prevalence of inadequate TWI and high urine osmolalities among these Spanish adolescents. The higher the consumption of vegetables in males; dairy drinks in females; and fruits in both, the lower the urine osmolalities were.

INTRODUCTION

Water is essential for all the functions of the body [1]. Total body water (TBW), as a percentage of body mass, varies as a function of body composition (from 50% to 70%) [2]. Water loss occurs constantly through the lungs, skin, kidneys and gastrointestinal tract [3], and these losses are compensated through metabolic water gain, food moisture, and fluid intake [4]. **The effect of lack of fluid intake is metabolic cell stress which in a long term perspective can cause shrinkage of brain tissue and an associated increase in ventricular volume, negative effects on cognitive performance [5], increased risk for urolithiasis, constipation or urinary tract infections, among others [6]**. Various international bodies have set dietary reference intakes (DRIs) for total water for young populations. However, not all DRIs have been set based upon the same considerations. For children between the ages of 4 and 13, the European Food Safety Authority (EFSA) bases its DRIs primarily upon caloric intake, while for adolescents aged 14 through adulthood, intake reference values are based upon population median consumption and the achievement of a desirable urine osmolality. **The current recommendations established by the EFSA are 2100 ml/day for males 9-13 years and 1900 ml/day for females. Adolescents from 14 years and older are considered as adults with 2.5 l/day for males and 2.0 l/day for females** [4]. In contrast, the Institute of Medicine (IOM) DRIs for the United States and Canada correspond to

median intakes observed in the National Health and Nutrition Examination Survey III (NHANES), for children ages 1-18 as well as for adults. **The current recommendations established by the IOM are 2400 ml/day for males 9-13 years and 2100 ml/day for females. Adolescents from 14 years and older have their adequate water intakes in 3300 ml/day for males and 2300 ml/day for females** [7]. However, all the health authorities highlight the necessity to establish the water intake recommendations based on the water balance [4].

Little is known about water intake and hydration in childhood and adolescence. The available evidence suggests a high prevalence of insufficient water intake in some populations. A German **(3-18y)** longitudinal study of children and adolescents reported a mean total water intake of 1642 ml and 1457ml in 9-13 year old boys and girls, respectively [6]. This falls approximately 450 ml short of the corresponding EFSA reference values of 2100ml (boys) and 1900ml (girls) [8]. Similarly, intake data collected in two major American cities [9] revealed that among 9-11 year olds, median intake before arriving at school was only 260-270 ml of total water (from food and fluids), and that only 25% reported drinking water in the morning. These observations were confirmed by a high prevalence (63-66%) of elevated morning urine osmolality (>800 mOsm/kg) [9] suggestive of insufficient fluid intake. **Urinary hydration biomarkers have been shown to better reflect fluid intake in comparison with serum biomarkers (which better reflect acute dehydration) and better predict as well health outcomes such as chronic kidney disease [10].**

The 'Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) Cross-Sectional Study' is a large, multi-center study that obtained food and fluid intake data for adolescents across multiple European countries, using a standardized dietary recall. In a previous report, Duffey and colleagues [11] described mean fluid consumption and energy intake in adolescents from 8 European cities. As first morning urine samples were only collected in the adolescents from Zaragoza, we were able to assess their hydration status measuring urine osmolality. The purposes of the present study are to describe the hydration status (first morning urine osmolality) and fluid consumption in healthy

adolescents from the city of Zaragoza (Spain), and to investigate the association between first morning urine osmolality and food and beverages intakes.

METHODS

The cross-sectional HELENA Study obtained standardized, reliable and comparable data from European adolescents regarding nutrition and health-related parameters. From November 2006 to December 2007, field work took place in ten European cities (Vienna, Ghent, Lille, Dortmund, Athens, Heraklion, Pecs, Rome, Zaragoza and Stockholm) from nine countries. **A random cluster sampling (all pupils from a selection of classes from all schools in the selected cities) of 3000 adolescents aged 13.0–16.99 years, stratified for geographical location, age and socioeconomic status, was carried out. We obtained body composition data, laboratory information (blood, urine samples), dietary intake data, and also, we assessed physical activity.** The entire description of the HELENA Cross-Sectional Study was previously published [12].

Study sample

This analysis included only the sample from Zaragoza, Spain, one of the 10 centres involved in the HELENA study. Being the initial Zaragoza sample (n = 390; **age 12.5 – 17.5 y**) representative of the city **in terms of the health outcome studied-body mass index (BMI) [12],** only 288 participants provided a first morning urine sample. Of these subjects, 194 also provided food and fluid intake data corresponding to the day prior to the urine collection. Thus, the final number of included adolescents for the current analysis consisted of 99 males and 95 females, representing 49.7% of the original Zaragoza sample. **The protocol of the study followed the criteria from the Declaration of Helsinki and informed consent was obtained from all**

participants and their parents, and the protocol was approved by the Regional Human Research Review Committee of Aragón [13].

First Morning Urine Osmolality

The urine collected was the first of the morning **on a weekday during school period,** when adolescents were in a fasting state. Urine samples were collected by the adolescents at home upon waking and brought to the study center in the same morning. After preparing the aliquots needed for the originally planned analyses, remaining urine was frozen at -20ºC prior to being used for this study. Previous studies have shown that these procedures are valid to obtain reliable results [14]. Urine osmolality was determined by freezing point depression osmometer (Model 3300 Micro-Osmometer; Advanced Instruments, Inc., Norwood, MA, USA). In order to evaluate the possibility of hypohydration, a first morning urine osmolality cutoff of 800 mOsm/kg was applied. While no singular consensus exists on the most appropriate cutoff for elevated urine osmolality, values approaching 800 mOsm/kg have previously been reported as the upper limit for euhydration [14-16].

Dietary intake

In the present analyses only one 24-h dietary recall corresponding to food and fluid intake on the day prior to the urine collection was used. A self-administered, computer-based, validated [17] and culturally adapted [18] HELENA-dietary assessment tool (HELENA-DIAT) was used. The participants were guided by the software to introduce their food and beverage consumption on six meal occasions (breakfast, mid-morning snack, lunch, mid-afternoon snack, dinner, and after dinner snack). In addition, the software supported and enhanced the respondent's recall, by having a number of specific reminders to probe for beverages. The adolescents had the option of manually including any food or beverage not included in the software. Information on quantities was provided by use of household measurements or pictures of portion sizes. Information collected by

175 the HELENA-DIAT was linked afterwards with the German Food Code and Nutrient Data Base
176 (BundeslebensmittelschluSsel (BLS), version II.3.1. [18].
177
178 *Beverage and food groups*
179 1723 different food and beverages groups obtained in the computerized 24-h recall were
180 recoded into 26 food and 10 beverage categories. Some food groups were further aggregated, such
181 as alcoholic beverages (e.g., beer, wine, liquor), complex carbohydrates (e.g., pasta, rice, flour),
182 added sugars and other caloric sweeteners (e.g., honey, sugar), oily fruits (e.g., nuts & seeds,
183 avocado & olives), and some milk products (e.g., desserts, creams, **ice-creams**). Some other groups
184 were not included in the analysis due to very low or non-existent consumption (e.g., products for
185 special nutritional use, puddings or margarines and lipids of mixed origins).
186 **Total water intake (TWI) was calculated as the total amount of water provided by**
187 **fluids and foods. To calculate this, the German Food Code and Nutrient Data Base was used**
188 **to obtain the percentage of water contained in each specific food item. On the other hand,**
189 **Total fluid intake (TFI) was calculated as the sum of liquid provided by all types of fluids or**
190 **beverages, including plain water.** Beverages were regrouped into five broad categories: plain
191 water (still or sparkling), alcohol (wine, beer, cider, and liquors), **beverages containing sugars or**
192 **sugar-sweetened beverages (SSBs)** (soft drinks, fruit juices, infusions with added sugar, milk-fruit
193 combined products), milk & milk products, and other drinks (infusions without added sugar,
194 **vegetable juices**, and diet soft drinks) [19].
195
196 *Covariates*
197 Body mass and height were measured with an electronic scale (SECA 861) and with a
198 telescopic stadiometer (SECA 225) to the nearest 0.1 kg and to the nearest 0.1 cm, respectively
199 [20], and were used to calculate body mass index (BMI). **These measurements were taken the**
200 **same day that the urine collection and the 24-hour dietary recall was administered.** Protein

intake was also taken into consideration as a statistical covariate as it determines the obligatory amount of water needed for the urinary excretion of solutes [4].

Statistical analyses

PASW 17.0 for Windows (SPSS, Inc.) was used for all analyses. Characteristics of the study sample are presented as means (standard deviation), unless otherwise stated. Baseline characteristics between the sexes were compared using a Student's t test (for normally distributed variables) or Mann–Whitney U test (if the normality assumption was violated) for continuous variables. For group comparisons, ANOVA, Kruskal-Wallis procedure (for non-normal variables) or Pearson's $\chi 2$ (for categorical variables) were used. All the analyses were stratified by sex. Tests for normality were performed using the Kolmogorov–Smirnov test. Associations between first morning urine osmolality and its potential determinants (age, body mass index, protein intake and food and fluid intakes) were tested with linear regression (**unstandardized β coefficients and 95% Confidence Intervals)**. For this analysis, non-normal variables were logarithmically transformed. Two-sided significance levels are quoted at 0.05.

RESULTS

Sample characteristics

A total of 99 males **(age: 14.4±1.2 y;** BMI 21.2±3.5 kg/m^2) and 95 females (**age: 14.7±1.1 y;** BMI 21.4±3.2 kg/m^2) were included in the analysis. TWI was similar between males and females (1977±692 and 1801±784 ml/day, respectively). 72% of males and 69% of females did not achieve the EFSA recommendations for TWI. TFI was also similar (1286±479 and 1236±602 ml/day). Males consumed significantly more protein than females (113±57 vs. 79±46 g/day; $p<.001$). Mean first morning urine osmolality was above 800 mOsm/kg in both groups (882±230

and 840±232 mOsm/kg for males and females, respectively). The prevalence of elevated first morning urine osmolality (> 800 mOsmol/kg) was 71% in males and 65% in females.

Table 1 describes trends in fluid and protein intake across quintiles of first morning urine osmolality. Among males and females, as first morning urine osmolality increased across quintiles, TWI and the proportion of males meeting EFSA recommendations tend to decrease. This was also the case for TFI, and intake of plain water in females.

Reported beverage consumption by level of first morning urine osmolality

Figures 1a and b describe the contribution (in ml) of each beverage group to TFI of the participants by urine osmolality quintile in males and females, respectively. Plain water was the single largest contributor to TFI, followed by milk and milk products in most urine osmolality quintiles for both males and females. The exceptions were the fifth quintile in males, and the third and fourth quintiles in females, in which SSB consumption out-weighed milk and milk product consumption. In addition, it is possible to appreciate a significant difference (p-value<0.05) in the mean consumption of plain water in females belonging to the quintile 3rd (Mean: 1214.49) in comparison to those belonging to the 4th (Mean: 692.63), and 5th (Mean: 679.61).

Reported beverage consumption in those meeting the EFSA recommendations.

Males meeting the EFSA recommendations for TWI consumed significantly more plain water (1035 ml vs. 583 ml; p <0.00) and milk and milk products (368 vs. 227 ml; p = 0.01) than those not meeting the recommendations (Figure 2a). In females, the consumption of plain water (1359 vs. 620 ml; p <0.00) and SSBs (215 vs. 127 ml; p= 0.03) were significantly higher in those meeting the EFSA recommendations than in those not meeting the recommendations (Figure 2b).

First morning urine osmolality and EFSA recommendations

A significant difference in first morning urine osmolality was observed between adolescents' females meeting (Mean: 770.03) and not meeting (Mean: 876.24) the EFSA recommendations for total water intake (p-value 0.034). **For males, there were also differences but not with statistical significance (Means: 829.20 for those who meet the recommendations vs 905.28 for those who did not).**

Associations between first morning urine osmolality and food and beverage consumption

Table 2 describes the associations between first morning urine osmolality and different contributors of total water intake (beverages and some water-rich foods), adjusted by, age, protein intake, and BMI, in males and in females. Significant inverse relationships were found between first morning urine osmolality and vegetables (excluding potatoes), and fruits in males; and with dairy drinks, and fruits in females. The lower the first morning urine osmolality, the higher was the consumption of these foods and beverages.

DISCUSSION

The study was motivated by recent reports [9, 21] of high prevalence of elevated urine osmolality and hyperosmotic stress among children and adolescents. The results from the current analysis confirm previous findings, as a high proportion of the adolescents in the HELENA-Zaragoza sample consumed less than the EFSA recommendations for total water (71% fell below the age- and sex-specific dietary reference value), and 68% had a low hydration status (first morning urine osmolality above 800 mOsm/kg).

Compared to the HELENA sample as a whole [11], adolescents in the Zaragoza subsample reported lower total fluid intake (1455 ml/d vs 1262 ml/d) than those adolescents belonging to the other cities. From all sources of fluid intake, plain water was the largest contributor to total fluid intake among adolescents, representing 54% and 65% of total fluid intake in males and females,

277 respectively. Slightly lower values were observed in the entire HELENA sample [11], where girls
278 also reported a higher percentage of plain water than boys (45% vs. 55% in males and females,
279 respectively). One difference between the HELENA sample as a whole and the Zaragoza
280 subsample was that the second largest contributor to total fluid intake in Zaragoza was milk and
281 milk products. This is in contrast with the entire HELENA sample, in which fruit juices were the
282 second largest contributor to TFI [11]. This suggests an important cultural component to fluid
283 intake among adolescents, which can be observed in previous studies. In France **(12-19y)**, plain
284 water and dairy drinks were also recently reported as the first and second main contributors to TFI,
285 although total fluid intake was lower than in our sample [22]. In a longitudinal study of German
286 adolescents (the DONALD study) [23], when considering a period of 5-years, regular soft drinks
287 and fruit juices accounted for the second highest proportion of TFI after plain water. Similarly, in a
288 Brazilian study **measuring youths from 3-17y** [24], plain water intake was followed by soft drinks,
289 and dairy drinks in the third position.
290
291 There was a high prevalence of adolescents not meeting EFSA recommendations (72% of
292 males and 69% of females), that was substantiated by a high prevalence of elevated first morning
293 urine osmolality: 71% of males and 65% of females had first morning urine osmolality values
294 higher than 800 mosmol/kg. Based on the American recommendations [7], the corresponding
295 percentages of adolescents not meeting the recommendations would be even higher (96% of males
296 and 85% of females). This finding seems to support the EFSA recommendations for TWI. **In any**
297 **case, further research is needed to support these recommendations, as measuring hydration**
298 **status based on first morning urine osmolalities is not the best approach, so these conclusions**
299 **should be interpreted cautiously. Nevertheless, the results suggest that between 65 and 71% of**
300 **these adolescents experienced hyperosmotic stress on cells during the morning hours, with**
301 **undesirable consequences such as DNA damage [25], or reduction in cognitive performance**
302 **[4, 14], among others.**

In addition, the fact that, there is a significant difference (at least in females) in mean first morning urine osmolalities between those who met and those who didn´t the EFSA recommendations for TWI, stress the importance of drinking fluids, preferably plain water. This is consistent with previous studies conducted in Israel **(10-12.4y)** and the United States **(9-11y)** [9, 21], and suggests that elevated urine osmolality in adolescents is prevalent across multiple countries with different drinking habits. It is interesting to note that in our study, adolescents not meeting the EFSA recommendations for TWI had a low consumption of plain water. Plain water was also significantly lower in the higher urine osmolality quintiles in the females in our study. This is in line with results from Stookey et al. [9], who showed that the likelihood of elevated urine osmolality was twice as high in those not drinking plain water in the morning, even if those children **(9-11y)** consumed other beverages.

It is remarkable that higher values of protein intakes can be observed in the groups with lower values of osmolalities based on quintiles. However, the differences in protein intakes were not statistically significant among quintiles. Moreover, statistically significant differences were found for TWI in both males and females and for TFI and water intake only in females. Also, another plausible explanation could be done by differences in fruits, vegetables and dairy products intakes, as they have shown to be better associated with hydration status than other beverages consumption, including water. Nevertheless, we have only found statistically significant differences for fruits in males, consuming higher amounts of fruits those included in the quintile 1 of osmolalities in comparison with those included in quintiles 2, 4, 5 (p=0.001).

Studies on the effect of foods and beverages intakes on hydration status are scarced. To our knowledge, this is one of the first studies quantifying the association between food and beverages intakes and hydration status using first morning urinary osmolality in healthy adolescents. In this study, fruit intake was inversely associated with urine osmolality in both males and females, as well

as dairy drinks for females, and vegetables for males, which is in line with a recent published study [26] based on the DONALD ongoing open-cohort in children from 4 to 10 years old, among others [27, 28]. As occurs with the previous cited study [26], our results are opposite to the hypothesis of the decrease in water intake from other sources when the consumption of fruits and vegetables are high for compensation. The very few studies [6, 26, 29] available relating diet and urinary markers to assess hydration, suggested that hydration biomarkers were determined by the quality of the diet, as was showed by ours.

One disadvantage of our study was that only first morning urine samples, and not 24 hour samples, were available for analysis. As sleeping essentially reflects an overnight water restriction, the relation between first morning urine osmolality and TFI is weak [30]. This is also reflected in our study, in which a strong relation between total fluid intake and urine osmolality was not established. **However, a study conducted in southern Israel [14] with school-aged children (10-12.4y), observed that children who were classified as dehydrated in the morning (based on first morning urine osmolality), 81% remained dehydrated at noon-time. Thus, in spite of a single urine morning sample is not the best biomarker to reflect the 'usual' day-to-day status of an individual, at the population level, it could be considered representative of the 'usual' prevalence of elevated urine osmolality at that time of the day [9].** Another limitation of this study is that the lack of representiveness of the city of Zaragoza, lost in the selection of adolescents with data in all the variables included in this study. Due to its cross-sectional nature, we can not draw conclusions about cause-and-effect chains.

A major advantage of this study is that procedures and measurements were validated, standardized, and reliable [12].

Conclusions

The main result of this analysis was the observation of low total fluid intake of this sample of Spanish adolescents (72% of males and 69% of females did not achieve the EFSA recommendations), and the correspondingly high urine osmolality values (71% of males and 65% of females had higher urine osmolalities values than 800 mosmol/kg) which lend support to the intakes reported using the dietary recall. In this sample of Spanish adolescents, the largest amount of per capita fluid came from water, followed by dairy drinks and SSBs.

Another important contribution of this study, is that for the second time in literature [26], positive associations between hydration level and vegetables and fruits consumption were described. Although more research, and, ideally, 24 hour urine osmolality, is needed to better evaluate these relationships. Future research should focus on the study of the fluid intake-hydration status-health outcomes relationship to be able to prevent some consequences of chronic dehydration such as impaired physical performance, sleepiness, difficulties in concentration, or cognitive impairment functions, among others.

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Conflict of interest

The authors declare no conflict of interests.

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Figure legends

Figure 1a and b. Absolute contribution of each beverage group to total fluid intake based on osmolality quintiles in males and females.

Figure 2a and b. Consumption of beverages according to EFSA recommendations for total fluid intake in males and females.