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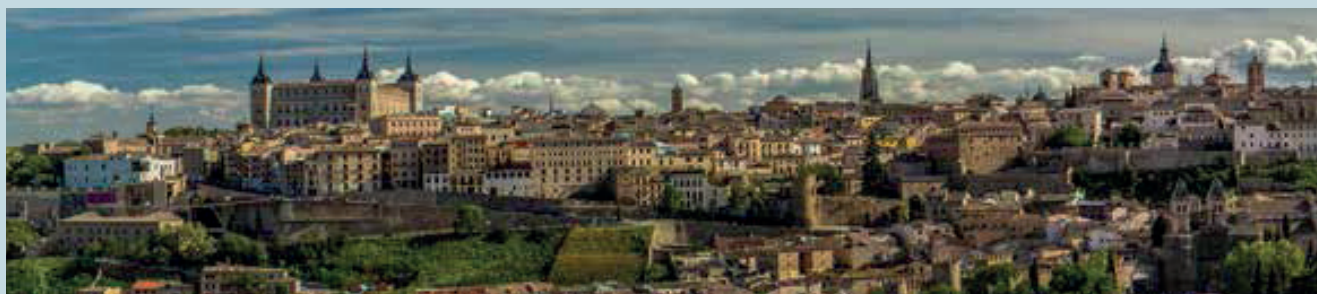
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II International and IV Spanish HYDRATION Congress

Toledo, December 2nd - 4th, 2015



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Conclusions of the II International and IV Spanish Hydration Congress. Toledo, Spain, 2nd-4th December, 2015

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Water is the major component of our organism representing about 60% of total body weight in adults and has to be obtained through the consumption of different foods and beverages as part of our diet. Water is an essential nutrient performing important functions, including transport of other nutrients, elimination of waste products, temperature regulation, lubrication and structural support. In this context, hydration through water has an essential role in health and wellness, which has been highly acknowledged in recent years among the health community experts such as nutritionists, dietitians, general practitioners, pharmacists, educators, as well as by physical activity and sport sciences experts and the general population.

- Water needs depend on individual aspects such as age, sex, physical activity, genomic profile and environmental conditions. Seasonality, climate (temperature and humidity), geographical and cultural environment influence the quantity of liquid required to maintain a proper hydration status.
- The daily water requirements set by the European Food Safety Authority (EFSA) are 2 and 2.5 liters per day for adult women and men, respectively, depending on their physiological status, physical activity and environmental conditions. However, the available scientific evidence suggests that most European populations do not meet the recommendations for adequate intakes.
- Hydration status depends on total body water, which is determined by the amount of liquid ingested from food and drinks

as well as the endogenous water production in balance with body losses due to either physiological or pathological status. There is no gold biomarker standard for hydration status but plasma osmolality, urine specific gravity and acute changes in body weight are frequently used but have limitations. There are specific questionnaires to evaluate liquid intakes and outcomes considering at the same time food and drink consumption, dietary habits, pharmaceutical drugs and pathological events, but these have not been scientifically validated yet.

- Common liquid intake measurement methods involve dietary and beverage questionnaires such as beverage frequency, 24-hour recall, diet history over a specific period, food and drink records, classical and photographic beverage record or specific beverage visual guides.
- Hydration status can be estimated among others by urine samples to determine volume (mL/d) and osmolality (mOsm/kg), blood samples, bioelectrical impedance analysis (BIA), equations to predict total body water (TBW) and extracellular water (ECW), and assessment of free water reserve (FWR), which measures urine volume minus the obligatory urine volume. These measures have severe limitations and there is a need for a valid easier and rapid measure of body hydration status.
- Bioelectrical impedance spectroscopy was found by some to predict individual values of total body water (TBW),

extracellular water (ECW), and intracellular water (ICW) more accurately than single frequency bioelectrical impedance analysis at 50 kHz, and may be possibly considered as a suitable methodological approach to estimate these water compartments in very active males and elite athletes, however, considerable more validation is needed.

- Other beverage composition factors should be considered in addition to simple volume of liquid ingested. Alcoholic and non-alcoholic drinks, apart from plain water, contribute to the daily energy intake. The consumer needs to be informed about the nutritional facts of the beverages they consume. Education on labeling interpretation and nutritional needs is required to improve dietary habits. Low- and no-calorie beverages alternatives are better choices for specific populations.
- Consumption preference patterns of different beverages vary considerably depending on age, socioeconomic status (SES), and culture levels. Whichever the factor, they all require an educational approach to spread proper liquid consumption recommendations.
- Education on hydration is an emerging research area that comprises nutritional, exercise, behavioral and biochemical sciences. Due to the demonstrated importance of a proper hydration status for health, life and socioeconomics, hydration educational programs should be included in the health care system as well as in schools and families.
- Special attention should be paid to groups of population at a major risk of dehydration as active athletes, children and adolescents, pregnant and breastfeeding women and the elderly.
- Some children and adolescents may fail to meet their hydration needs due to habitual dietary choices (e.g., low consumption of fruits, limited access to fluids during school day) or because of increased energy requirements from environmental living conditions or heavy physical activity, which may consequently influence mood as well as their performance in curricular and extracurricular activities.
- Pregnant women may be at greater risk of dehydration by not drinking water due to nausea and vomiting. Hydration during pregnancy plays a crucial role to maintain an adequate body water content and for the renewal of amniotic fluid. During breastfeeding liquids intake also influences milk osmolality.
- The normal ageing process is associated with several physiological changes that may affect thirst and drinking behavior, making them more susceptible to dehydration. Studies in hospitalized elderly people suffering from dehydration show an increase in morbidity-mortality of up to 40-70%, while proper hydration reveals considerable improvements in health and hospitalization quality/welfare. It has been shown that 95% of injuries such as pressure ulcers are preventable with proper nutrition and adequate water intake.
- Water consumption affects brain structure and functions and perhaps cognitive performance, particularly when involving motor skills. A better hydration status might help improve mood, attention and mental concentration scores.
- The variety of components (e.g., caffeine, sugar, protein, electrolytes) that are found in popular beverages results in considerable differences in post-consumption urine production. Recent development of a hydration index (HI) classification system estimates the expected fluid retention from 13 common beverages and may be useful in promoting euhydration in the general population. However, only young healthy male subjects have been observed.
- Adequate liquid intake also helps to achieve body weight loss as it has been suggested that it could suppress appetite before meals and be associated with adequate dietary habits (high consumption of fruits and vegetables). Thus, adequate liquid intake could be important for overweight/obese people as a means of weight management attenuating the risk of cardiovascular diseases or diabetes.
- Dehydration has been defined as the second most common comorbidity factor, occurring in 14% of all hospitalizations. In addition to its individual clinical impact, dehydration also represents an important public health issue imposing a significant economic burden: this represents a potential target for intervention to reduce healthcare expenditures and improve patients' quality of life.
- Physical activity has a direct impact on fluid intake. Water needs can increase substantially for more physically active individuals compared to those who have a sedentary lifestyle. Water and electrolyte losses during training and competition reduce the capacity for physical activity, make exercise feel more difficult, and adversely affect sports skills. For those reasons, there is a need for athletes to maintain a good level of hydration by consuming an adequate volume of fluids before, after and during exercise to support sports performance and avoid health risks linked to disruptions in fluid and electrolyte balance, particularly in warm environments, such as dehydration, hyperthermia, and hyponatremia.
- Dehydration accelerates the decline in cerebral blood flow during prolonged and incremental maximal exercise in the heat without affecting the cerebral metabolic rate for oxygen. The reason for this is that the concomitant reductions in cerebral oxygen supply are compensated by increases in oxygen extraction from the brain circulation. Thus, fatigue during prolonged and incremental maximal exercise in the heat is related to a reduction in cerebral blood flow rather than suppression in cerebral aerobic metabolism.
- Fluid ingestion maintains cerebral and extracranial perfusion throughout non-fatiguing prolonged exercise in the heat. During exhaustive exercise, however, maintenance of euhy-

dration via fluid ingestion during exercise delays but does not prevent the decline in cerebral perfusion.

- Although genomic studies on hydration and health are at an early stage and more research is needed, some studies have shown that certain genetic markers are associated with higher fluid intake needs; such is the case of mutations in genes involved in cystinuria and increased water intake for the prevention of kidney stone formation. New research results may provide more data about inter-individual variability in fluid

intake recommendations applied to the so-called precision medicine.

- The majority of food guidelines focused the recommendations of liquid intake on drinking 8 glasses (64 oz/day). These guidelines do not specify for fractioning liquids during a day, for hot environments periods and the life-course approach. Food and hydration guidelines need to be based on daily requirements, with more specificity to age, gender, and environment changes.



Impact of physical activity and sedentarism on hydration status and liquid intake in Spanish older adults. The PHYSMED study

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Abstract

Introduction: Data on hydration status in older adults are scarce and there are very few studies focusing on the impact of physical activity (PA) on drinking behavior.

Objective: To assess the impact of physical activity and sedentarism on fluid intake in Spanish elderly.

Methods: 433 non-institutionalized Spanish older adults (58% females), aged 55-88 years, volunteered for the PHYSMED study. PA data were obtained by means of the Minnesota and EXERNET questionnaires. Population was divided into four groups: ILS (inactive and low sedentary), IHS (inactive and high sedentary), ALS (active and low sedentary) and AHS (active and high sedentary). Serum from fasting blood samples was analysed for osmolality.

Results: The mean of total liquid intake was $1,751 \pm 628$ mL/d. Significant differences were observed for total liquid intake between ILS/ALS and IHS/ALS ($p < 0.001$). ALS subjects consumed a higher amount of beverages such as water, juice, milk, coffee, sport drink, beer, wine and distilled drinks than the other PA groups. There was a significant difference for water intake between PA groups ($p < 0.01$). Serum osmolality values were within reference ranges in all subjects, and there was a significant difference between PA groups ($p < 0.01$).

Conclusions: Spanish older adults meet the DACH recommendations set by the German, Austrian and Swiss nutrition societies' liquid intake recommendations in the mean independently of PA and sedentary level. All participants are within reference ranges of serum osmolality. Subjects in the active and low sedentary group consumed higher amounts of water and other beverages than in the other PA groups.

Key words: Beverages. Water intake. Physical activity. Elderly. Sedentary lifestyle.

INTRODUCTION

Quantification of water and beverage intake is an emerging topic in nutritional sciences as the optimal functioning of our body requires a proper hydration level (1,2). Likewise, water intake

and hydration status have recently gained attention as one of the many and potentially manageable factors associated with disease development and wellbeing (3). Water is an essential nutrient for human body and major key to survival (4).

However, the current lack of a hydration assessment gold standard greatly impedes attempts to link water intake and negative health outcomes as well as to make public dietary guidelines (5,6). The questions "what do you drink", "how much do you drink per day" and "how much fluids do you drink" are frequently omitted in the common dietary questionnaires, and recommendations for beverage intake are, or were until recently, missing in most "food pyramids" (7,8).

Maintaining an adequate fluid balance is an essential component of health at every stage of life, especially in elderly population since older adults are at higher risk of developing dehydration for various physiological reasons (7). Moreover, in elderly individuals, adequate fluid consumption has been associated with fewer falls, lower rates of constipation and laxative use, as well as better rehabilitation outcomes in orthopedic patients (9-11). Also, dehydration can precipitate emergency hospitalization and increases the risk of repeated hospitalizations (12).

Elderly may frequently meet difficulties in gaining access to beverages due to decreased mobility, visual problems, swallowing disorders, cognitive alterations, use of drugs and fear of incontinence (7). Numerous factors such as high ambient temperature and humidity levels, heat stress and physical activity can influence water needs (13). Therefore, adequate intake of fluids must be increased in relation to these conditions (1). High temperature and humidity also might provoke exacerbated dehydration, but there

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is limited information about the effects of seasonality and climate on water intake (14).

The benefits of regular physical activity (PA) on health and disease prevention are well recognized (15). PA reduces both morbidity and mortality and can minimize the physiological effects of an otherwise sedentary lifestyle increasing the active lifespan (16,17). On the other hand, prolonged sitting has emerged as a risk factor for early mortality and has deleterious health effects. Nevertheless, sedentarism has not been included in the past in studies about physical activity (17). In spite of the powerful benefits of PA and optimal hydration status, there are only a few studies analysing together fluid intake, PA and sedentarism in elderly population. Hence, these parameters are a key point to define public health recommendations.

The aim of this study was to assess the impact of physical activity and sedentarism on fluid intake in Spanish older adults.

METHODS

STUDY DESIGN, SAMPLE AND ETHICS

The present study was based on a cross-sectional multicenter study aiming at identifying cardiovascular risk factors in sedentary and active elderly subjects. The survey was conducted from April 2013 to May 2014 in Madrid and Majorca (Spain). Participants were voluntaries recruited at health centers, sport federations, sport facilities and municipal clubs located at Madrid and Majorca. A total of 433 subjects, 186 male (43%) and 247 females (57%) aged 55 to 88 years old were included in this study. The exclusion criteria were age under 55, being institutionalized, suffering from a physical or mental illness that would have limited their participation in the physical fitness tests or their ability to respond to the questionnaires, or drug intake for clinical research.

All measurements at each institution were conducted according to the survey protocol. The study was performed according to the principles established in the Declaration of Helsinki and approved by the Ethical Committee of the Technical University of Madrid. Written informed consent was obtained from all participants.

HYDRATION QUESTIONNAIRE

A hydration questionnaire was developed by the Research Group based on food-frequency and eating habits questionnaires published in the literature (18,19), taking into account the modern beverage market.

The questionnaire is divided into two parts. The first part includes questions about the different types of fluids consumed one day before the questionnaire was filled out. The second part records the fluid intake during a normal week for each beverage type. The beverages included were: water, juice, soft drinks, diet drinks, milk, shake, coffee, tea or infusions, sport drinks, beer, wine and distilled drinks.

PHYSICAL ACTIVITY AND SEDENTARISM ASSESSMENT

PA was assessed by means of two validated self-reported questionnaires; the Minnesota Leisure Time Physical Activity Questionnaire measuring the activity during the previous year, and the EXERNET questionnaire, both of them validated for the Spanish population (20-22).

The Minnesota questionnaire includes diverse physical activities and participants are asked about PA duration, weekly and monthly frequencies. Time for each activity was expressed in minutes per day (min/d). On the other hand, the Exernet Questionnaire included four questions about regular physical activity duration, time spent walking, time spent doing housework and time spent sitting per day. All questions were bounded and answers included six options which were classified as less than 1 hour, between 1 and 2 hours, between 2 and 3 hours, between 3 and 4 hours, between 4 and 5 hours and more than 5 hours. The only exception was for regular physical activity duration. All activities were recorded in min/day.

In order to compare between different levels of PA and sedentarism, our population was classified in 4 groups: inactive and low sedentary (ILS), inactive and high sedentary (IHS), active and low sedentary (ALS) and active and high sedentary (AHS) taking into account cluster analyses set by Aparicio-Ugarriza et al. (23).

SERUM OSMOLARITY

Fasting blood samples were collected from each participant by standard venipuncture on vacuum Vacutainer® tubes in Madrid and Palma (Majorca), at the biochemical laboratory of the High Sports Council, Madrid, Son Espases Hospital and University of the Balearic Islands, Palma (Majorca), respectively. Serum was separated in 1 mL eppendorfs and was processed to analyse osmolality using an osmometer Osmo Station OM-6050 (Menarini Diagnostics, Florence, Italy, CV ≤ 1%).

STATISTICAL ANALYSIS

Descriptive values are shown as mean ± standard deviation. One-way ANOVA was performed to analyse the differences between serum osmolality, total liquid intake and beverages consumption and *post hoc* analyses were conducted with Bonferroni adjustment according to the PA and sedentarism groups (ALS, AHS, IHS and ILS).

All analysis were performed using the Statistical Package for Social Science software (SPSS, version 21.0; SPSS, Chicago, IL, USA) and values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Table I includes descriptive characteristics of the sample split by sex. Males were heavier and taller than females ($p < 0.01$) and

Table I. Descriptive characteristics of the study sample^{1,2}

	Male (n = 186)	Female (n = 247)	p
Age (years) *	65.4 ± 6.6	67.5 ± 6.6	< 0.01
Height (cm) *	170.2 ± 6.6	156.6 ± 5.6	< 0.01
Weight (kg) *	79.9 ± 10.8	65.4 ± 10.4	< 0.01
BMI (kg/cm ²) *	27.5 ± 3.1	26.7 ± 4.3	< 0.05
City†			
Madrid	78 (49.1)	122 (49.4)	N.S.
Mallorca	108 (58.1)	125 (50.6)	
Serum osmolarity (mOsm/L)*	289.25 ± 5.25	289.17 ± 4.46	N.S

¹Results are expressed as follows: *mean ± SD; †n (%).

²Significant differences between sex by one way ANOVA test. N.S.: non significant.

had a higher BMI ($p < 0.05$). No differences by sex were observed for mean serum osmolarity.

Figure 1 shows serum osmolarity according to PA groups. All subjects were within reference ranges of serum osmolarity and significant differences were found between PA groups ($p < 0.01$). After Bonferroni's adjustment, there was a significant difference between IHS and ALS ($p < 0.05$). The mean higher serum osmolarity was obtained for ALS group (290.97 mOsm/L).

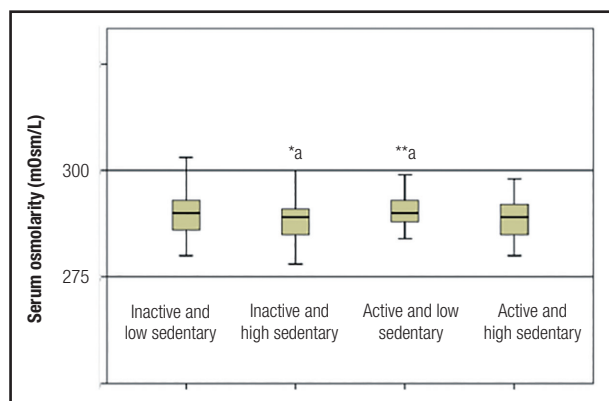
Figure 2 shows the mean (\pm SD) total liquid intake per day consumed according to PA groups. The ALS group and AHS group consumed 2,056.2 \pm 679.6 mL/d and 1,899.9 \pm 633.4 mL/d, respectively. On the other hand, the mean of total liquid intake was 1,647.8 \pm 569.4 mL/d for ILS group and 1,647.5 \pm 597.2 mL/d for IHS group. There were significant differences between ALS and ILS groups and also between ALS and IHS groups ($p < 0.001$).

Figure 3 shows the mean beverage consumptions per day divided by PA groups. Water was the beverage most consumed for all PA groups and there were significant differences between them ($p < 0.01$). ALS drank more beverages such as water, juice,

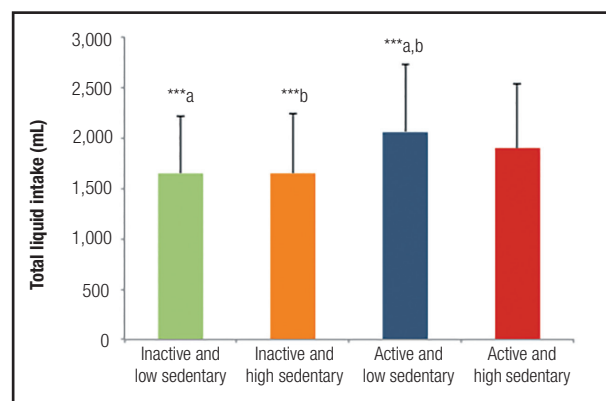
milk, coffee, sport drink, beer, wine and distilled drinks than the other PA groups. Significant difference was only observed for water between PA groups ($p < 0.01$). Moreover, AHS showed a trend to consume more soft and light soft drinks, shake and tea than the other PA groups (non-significant values).

DISCUSSION

The results from this cross-sectional study indicated that the mean of total liquid intake in our sample was high (1,751.3 mL/d, data are not shown) compared with the DACH recommendations set by the German, Austrian and Swiss nutrition societies (DACH) (1,310 mL/d) (24). The ALS group consumed significantly higher amounts of water ($p < 0.01$) and showed a trend of high consumption of other fluids (NS) than the other PA groups. There were differences ($p < 0.01$) in serum osmolarity between PA groups. However, despite these differences, all subjects were within serum osmolarity reference ranges.

**Figure 1.**

Serum osmolarity divided by PA groups. Horizontal lines represent serum osmolarity reference ranges limits. *a significant difference (level set at $p < 0.05$) between inactive and high sedentary and active and low sedentary after Bonferroni's adjustment.

**Figure 2.**

Mean (\pm SD) total liquid intake/day (mL/d) according to PA groups. ***a,b significant differences (level set at $p < 0.001$) between inactive and high sedentary and active and low sedentary.

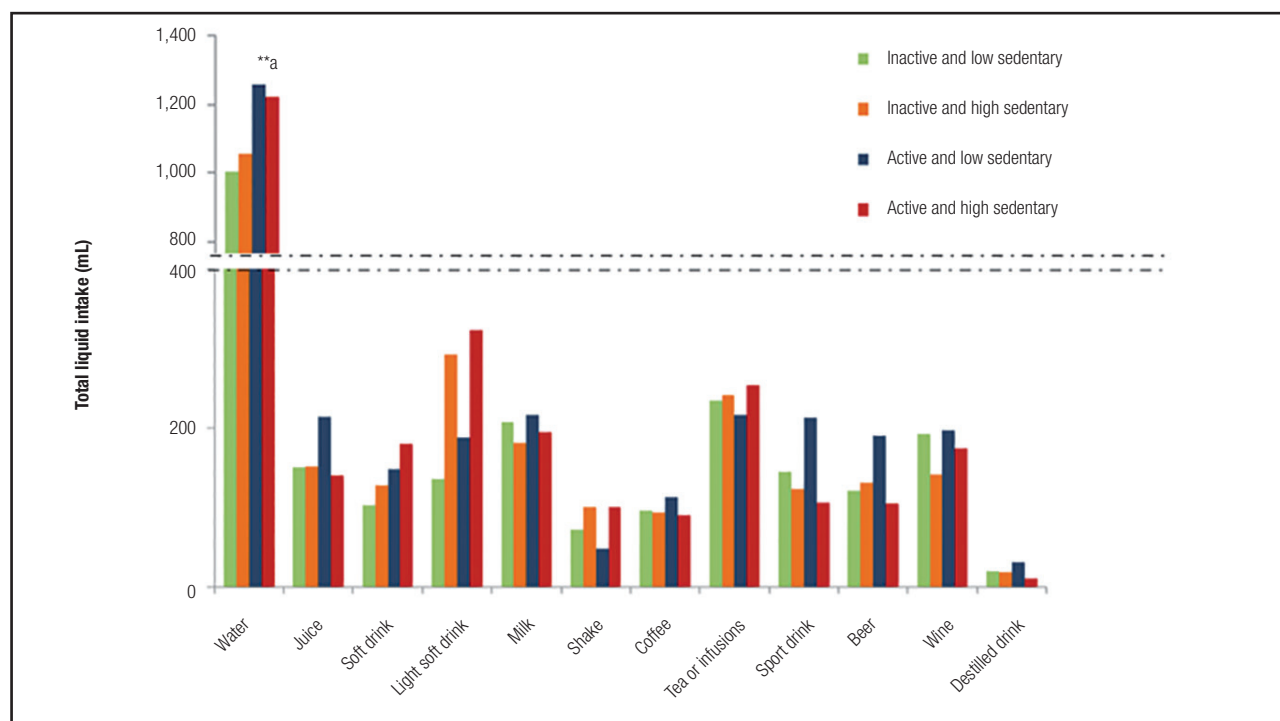


Figure 3.

Mean beverage consumptions/day divided by PA groups. **Significant differences ($p < 0.01$) between PA groups for water.

There is no consensus in the literature regarding water and beverage intake recommendations. We compared to the DACH recommendations because they split recommendations into water from beverages and from food specifically for people aged over 65 years. In contrast, the World Health Organization in 2005 (25) recommended 2.2 L/d and 2.9 L/d for females and males, respectively. The European Food Safety Authority in 2010 (26) established 2 L/d for females and 2.5 L/d for males as the total water intake without differentiating into water from food and beverages. Other authors consider that the minimum water intake for drinking water and beverages must range from 1.5-1.8 L/d, although some adaptations are established depending on age, sex and medications (7).

Likewise, the best way of measuring hydration in humans is still a general controversy (27) because there are different methods such as estimates of water balance (thirst rating, total water intake and output or body weight changes), hydration markers (plasma or urine osmolality) and total body water measurements by bioelectrical impedance or isotope dilution (28). In our study, we measured serum osmolality as it is closely controlled and rarely varies by more than 2% around a set point of 280-290 mOsm/L (1). According to Jequier et al. (1), a basal mean value of 287 mOsm/L is maintained in well-hydrated individuals. In our sample, the mean of serum osmolality levels was 289 mOsm/L for both sexes, though a significant difference was obtained between PA groups ($p < 0.01$).

On the other hand, in our study, water was the beverage most consumption in all PA groups and a significant difference was

obtained between them ($p < 0.01$) followed by light soft drinks. In their study, Zizza et al. (29) found that water was the most consumption, followed by coffee. De Francisco et al. (30) observed that people with high PA levels drank more fluids compared with those with low PA levels. We found the same patterns in our study since both active groups (ALS and AHS) had higher intakes than both inactive groups.

Furthermore, we found that ALS subjects drank higher amounts of water, juice, milk, coffee, sport drinks, beer, wine and distilled drinks than the other PA groups. Additionally, AHS consumed more soft and light soft drinks, milk shakes and tea than the other PA groups.

STRENGTHS AND LIMITATIONS

This study has several strengths. One of them was the use of clusters. Clustering of activities produces an alternative approach to summarizing physical activity participation and may provide a helpful methodological development when questionnaires are used to assess physical activity. An additional strength was the use of a specific hydration questionnaire in order to obtain reliable data on water and beverage intake. Moreover, the sampling procedure and the strict standardization of the field work among the cities involved in the study avoided to a great extent the kind of confounding bias.

On the other hand, this study has also several limitations. First, it is directly related to the intrinsic nature of the Exernet Ques-

tionnaire, in which for several questions only closed answers were available and also for the subjectiveness of the physical activity and sedentary questionnaires. Secondly, our study has a cross-sectional design, therefore impeding the determination of cause-effect relationships.

CONCLUSIONS

The mean of Spanish older adults meets DACH liquid intake recommendations independently of physical activity and sedentary level; furthermore, all participants are within reference ranges of serum osmolarity. Subjects in the active and low sedentary group consumed higher amounts of water and other beverages than in the other PA groups. Physical activity and sedentarism should be considered to obtain a holistic approach to beverage intake and hydration status in future researches. Additionally, longitudinal studies are needed in order to establish behavior patterns.

ACKNOWLEDGEMENTS

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Nutrición Hospitalaria



Nutritional differences in malnourished patients according to their liquid-intake habits after hospital discharge

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Abstract

Introduction: Malnutrition is a serious and relatively common problem among hospitalized patients; moreover, it is known that a good hydration state contributes to health and wellbeing.

Objective: The aim of this study was to determine the relationship between nutritional status, functional dependency, quality of life and liquid-intake habits in malnourished patients after hospital discharge.

Methods: Cross-sectional descriptive study in 91 patients (45 males) who presented malnutrition at hospital discharge. The patients were grouped according to their liquid intake estimated through the Mini Nutritional Assessment questionnaire: 3-5 glasses (n = 42), and > 5 glasses (n = 46); removing from analysis < 3 glasses of liquid intake (n = 3). The body mass index, weight, Malnutrition Universal Screening Tool (MUST), functional dependency (Barthel questionnaire), and quality of life (Short Form 12 Health Survey [SF-12]) were assessed 2-months after discharge.

Results: The > 5 glasses liquid intake group showed better nutritional status than the 3-5 glasses intake group, for weight (p < 0.001), body mass index (p = 0.001), and MUST scale (p = 0.020). Additionally, the > 5 glasses liquid intake group significantly scored higher values in the total SF-12 questionnaire (p = 0.013), presenting better self-reported quality of life, and higher functional independency in the Barthel index (p = 0.037) than the 3-5 glasses liquid intake group (p = 0.013).

Conclusions: Although further research is needed to elucidate the characteristics of this relationship, descriptive comparisons between groups showed favorable nutritional status, functional independency and quality of life for the > 5 glasses of liquid intake compared with the 3-5 glasses of liquid intake group during a 2-months follow-up.

Key words: Dependency. Quality of life. Nutritional status. Malnutrition. Hydration.

ly in Spain (2). Therefore, a considerable number of well-nourished patients at the hospital admission can return to the community with malnutrition, making necessary its detection and home follow-up to improve their health. In this line, the health related quality of life of malnourished patients at hospital discharge can be impaired, as it has been reported in a group of Spanish patients (3). This population showed an increased risk of morbidity and mortality compared to their peers (4,5), requiring prolonged hospital stays and a higher number of readmissions (6); thereby increasing even more their malnutrition risk.

Although the interest on these facts has increased in recent years, and consensus on the approach to hospital malnutrition in Spain has been published (7), there is a lack of studies assessing the hydration status in malnourished patients. It is known and generally accepted that a good hydration state contributes to health and wellbeing; hence, the effect of hydration habits on health of malnourished patients can be useful information for healthcare practice. Specially, if we take into account that the liquid intake of the Spanish population is below the recommended levels (8).

Thus, assessing the nutritional status according to liquid-intake habits in malnourished patients after hospital discharge, and its impact on quality of life and dependency, could be interesting factors for the health clinic practice and follow-up.

INTRODUCTION

Malnutrition is a serious and relatively common problem among hospitalized patients; moreover, this health risk increases during hospitalization (1), a fact that has been widely reported, specifical-

OBJECTIVES

The aim of the present study was to assess differences in body mass index (BMI), weight, malnutrition status, dependency and quality of life according to liquid-intake habits of malnourished

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patients after hospital discharge during a 2-month home follow-up.

METHODS

DESIGN

This was a cross-sectional descriptive study, conducted at the healthcare district of Málaga-Guadalorce, Spain. The nutritional status, dependency and self-reported quality of life levels were assessed in malnourished patients 2 months after hospital discharge, and compared by their liquid-intake habits (2 groups). The present study was approved by the Research Ethics Committee of the Málaga Healthcare District and was in accordance with the Declaration of Helsinki.

PARTICIPANTS

A total of 88 patients (44 males and 44 females), aged 72.3 ± 11.84 years old, were included in the study. After the participants were informed about the procedure and possible risks involved, written informed consent was obtained from all participants. The inclusion criteria were the following: a) hospitalization; b) medium-high risk of malnutrition on the MUST scale; c) older than 18 years; d) willingness to participate in the study and signing of the informed consent form; and e) resident of the geographical area corresponding to the participating health center. The exclusion criteria consisted of having undergone any of the following during hospitalization: a) treatment with oral food supplements, enteral or parenteral nutrition; b) treatment with chemotherapy or radiation therapy; and c) malabsorption syndrome.

PROCEDURES

Screening was conducted to determine the patient eligibility during hospitalization by using the Nutritional Filter (Filtro Nutricional, FILNUT) (9) computer program. If the presence of a significant risk was detected, we proceeded with the assessment of each patient using the Malnutrition Universal Screening Tool (MUST) (10). If a medium-high risk of malnutrition was detected through the MUST, the patient was offered the opportunity to join the study provided they were not undergoing treatment with dietary supplements or enteral or parenteral nutrition. The patient was then informed of the study and asked for his/her informed consent.

After a 2-month follow-up, the 91 patients filled out the Mini Nutritional Assessment (MNA) questionnaire, in Spanish language (11). One item of the MNA is able to classify 3 categories according to the number of glasses of liquid intake: a) < 3 glasses; b) 3-5 glasses; and c) > 5 glasses of liquid per day. Of the 91 patients, only 3 were included in the < 3 glasses of liquid intake group; as the sample size does not provide enough statistic power, the 3 mentioned participants were excluded of the analysis. Therefore,

88 patients were finally included in the study classified into two categories: the 3-5 glasses of liquid intake group consisted of 42 patients, while the > 5 glasses intake group consisted of 46 patients.

The nutritional state was again assessed through the MUST; additionally, weight and BMI, calculated as $\text{weight (kg)}/\text{height(m)}^2$, were recorded as nutritional state indicators. The participants' height and weight were recorded both in the morning, between 8 am and 10 am. The degree of functional independency in daily life activities was assessed by using the Barthel index in its Spanish version (12). The patient perceived quality of life was assessed using the Spanish version of the Short Form 12 Health Survey (SF-12) (13), which provides 3 scores: physical component, mental component, and total score (the sum of both components).

STATISTICS

Data are expressed as mean \pm standard deviation. After the normality of distribution was proven through the Kolmogorov-Smirnov test, dependent variables were compared by the liquid-intake habits (> 5 glasses or 3-5 glasses) by using the Student's *t* test. The level of significance was set at $p < 0.05$. All statistical analysis were performed using the SPSS package.

RESULTS

The > 5 glasses liquid intake group showed better nutritional status than the 3-5 glasses intake group, with statistically significant differences for weight, BMI, and MUST scale. The group with higher liquid intake habits also presented a higher functional independency than the group who drunk from 3 to 5 glasses per day, assessed through the Barthel index. Additionally, the > 5 glasses liquid intake group significantly scored higher values in the total, physical and mental SF-12 questionnaire components, presenting better self-reported quality of life than the 3-5 glasses liquid intake group (Table I). The groups did not present differences by sex or age.

DISCUSSION

The main results of this study are the statistically significant differences between groups for their nutritional status (BMI and MUST), dependency (Barthel index) and quality of life (SF-12) levels in favor of the patients who drunk a higher number of glasses of liquids. Patients presented protein malnutrition at hospital discharge and the assessment was performed 2 months later. Therefore, these results stand out the relevance of adequate patient follow-up, the role of case manager nurses, and the communication and collaboration of the hospital and Primary Care, since patients' habits during follow-up were associated with health differences. In this sense, some malnutrition approaches have been previously reported; for example, dietary counseling seems to be

Table I. Differences in nutritional status, dependency and quality of life according to the liquid intake habits of patients who presented malnutrition at hospital discharge

Liquid intake habits	3-5 glasses	> 5 glasses	p values
Weight (kg)	60.8 (2.08)	70.3 (1.6)	< 0.001
Body mass index (kg/m ²)	23.9 (0.58)	26.5 (0.54)	0.001
MUST scale	0.88 (0.16)	0.39 (0.11)	0.020
Barthel index	72.4 (4.09)	82.1 (3.12)	0.037
SF-12 total score	43.7 (2.39)	52.8 (2.55)	0.013
SF-12 physical component	39.4 (2.29)	48.1 (2.55)	0.013
SF-12 mental component	48.1 (2.61)	57.5 (2.61)	0.013
N	42	46	

Data are expressed as mean (standard deviation). MUST: Malnutrition Universal Screening Tool; SF-12: Short Form 12 Health Survey.

an effective strategy for improving nutritional status, quality of life and functional dependency of malnourished patients (14,15). Nevertheless, the impact of hydration counseling on health has not been properly analyzed yet, and little information is available regarding malnourished patients (16).

In our study, health related quality of life was positively associated with hydration; however, to the best of our knowledge, information about this possible relationship in malnourished patients has not been published yet. The patients' SF-12 score mean was similar to the Spanish mean in the > 5 glasses of liquid intake group, whereas the 3-5 glasses of liquid intake group showed a SF-12 mean significantly lower than reference values (13). The SF-12 is useful in describing overall community health status and testing clinical improvement during home follow-up (17). Accordingly, 2 months after hospital discharge, patients with liquid-intake habits from 3 to 5 glasses a day showed an impaired health-related quality of life and their assessment when returning to the community is convenient.

Quality of life and functional independency are closely related; specifically, the Barthel index evaluates the capacity to carry out basic daily activities. The participants' index means corresponded with a moderate dependency (18), although the 10 points of difference between groups might affect independency for drinking. This difference can be explained by a malnutrition-induced muscle loss since groups differed by nutritional state. Thereby, the possible relationship between functional dependency, quality of life and hydration in hospitalized patients could be a new research focus with impact on the clinic practice and the patient's wellbeing.

Additionally, hospital care should include the use of MUST to screen nutritional status of hospitalized patients, since it provides great reliability, reproducibility and simplicity (19). According to our data, 32 patients presented moderate risk of malnutrition in the MUST scale (score > 1), which means that 34.6% of malnourished patients at hospital discharge keeps still under malnutrition risk after 2 months of their returning to the community. Hence, if malnutrition is detected during hospitalization, the use of MUST in Primary Care follow-up is recommended, especially in more vulnerable patients, such as old-people (e.g., over 75 years). In

this population, the risk of malnutrition is positively related to the number of chronic diseases and the social risk (20), and, additionally, they are under dehydration risk (21), making it difficult to discriminate between protein/caloric malnutrition and dehydration impacts on health. Thus, a significant limitation of our study is the fact that we cannot establish a cause-effect relationship between liquid intake habits and nutritional status, dependency and quality of life; we can just establish certain associations. Therefore, it is necessary to obtain further scientific evidence about the impact of different hydration strategies on the patients' health from randomized clinical trials, after these modest results from cross-sectional design have been presented and the lack of relevant scientific information has been highlighted.

To sum up, liquid-intake habits are related to nutritional status, quality of life and dependency in malnourished patients at hospital discharge during a 2 months follow-up, although further research is needed to elucidate the characteristics of this relationship. In our sample, most patients drunk more than 3 glasses a day (96.70%), while a similar percentage of patients were in the MNA questionnaire category corresponding to the > 5 glasses (46 patients) or in the 3-5 glasses (42 patients) of liquid intake a day. Comparisons between groups showed favorable nutritional status, functional independency and quality of life for the > 5 glasses of liquid intake group, reporting preliminary findings that can boost the development of clinical trials on this topic.

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Nutrición Hospitalaria



Urinary hydration biomarkers and water sources in free-living elderly

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Abstract

Introduction: Accurate estimates of water intake and hydration status in populations are essential to identify populations at risk of dehydration and define strategies to improve standards of water intake.

Objective: To evaluate the hydration status and the contribution of food and beverages to the total water intake in a sample of free-living physically active Portuguese elderly.

Methods: A sample of 74 individuals (28 men), aged 60 to 83 years, were included in this study. A 24 h urine sample was collected; 24 h urine volume and osmolality were quantified in order to estimate the free water reserve (FWR) used to assess the hydration status. A 24 h food recall corresponding to the day of urine collection was obtained. Food and beverages were grouped according to their nutritional composition, namely water content. The contribution of those groups to total water intake and its association with the hydration status were estimated. Urinary markers and food groups' contribution to total water intake were compared between sexes and according to the median FWR, using the t-test and Mann Whitney test.

Results: Less than 10% of the participants were classified as hypohydrated/at hypohydration risk. Water from food was nearly half of the total water intake (47% in females and 48% in males, $p = 0.757$). "Water" (22%) and "foods with reduced water content" (19%) were the groups that contributed the most to the total water intake in women and men, respectively. In men, the contribution of "alcoholic beverages" was significantly higher than that of women (10.5% vs 1.7%, $p < 0.001$).

Conclusions: Even though most of the study participants were classified as euhydrated, the contribution of water-rich and nutritionally dense food, and non-alcoholic beverages, particularly in men, should be promoted.

Key words: Hydration status. Water sources. Elderly. Free water reserve.

INTRODUCTION

Water is an essential element for the functioning of the body (1), thus maintaining an adequate state of hydration is an important determinant of human health (2). Water is required for a wide range of physiological functions, such as metabolic transport, temperature regulation, maintenance of circulatory volume, cellular waste disposal, and as a solvent in organic reactions (3).

The human body has several mechanisms to regulate water content, which operate simultaneously to maintain the balance between gains and losses. This adjustment depends on hypothalamic mechanisms to control thirst, antidiuretic hormones, the kidneys ability to retain or excrete water as well as water loss by respiration and perspiration (4).

Although the elderly present fluid intake needs similar to those described in young adults, older individuals are exposed to a higher risk of dehydration when compared to younger adults. In fact, the aging process is associated with various physiological changes, including the decrease in perception of thirst and consequent insufficient water intake, loss of muscle mass and changes in renal function (5-9). Older individuals may also show an increased loss of liquids via infection, dementia, diuretics, etc. (2,10). Furthermore, according to Godfrey et al. (2012), some factors can contribute to reducing water intake in older people, such as fatigue, lack of pleasure associated with eating and the fear associated with urine incontinence or the need to urinate frequently (11).

Even mild dehydration is slight, defined by a loss of 1% to 2% of body weight, it affects physical performance and cognition, particularly in the elderly and children (12,13). For the elderly, dehydration is the most common electrolyte disorder and is a common cause of hospitalization (7,8).

The consequences of acute dehydration are well described. It evolves over a short period and can trigger low blood pressure, loss of consciousness, seizures, coma and even death if water loss reaches about 8% (14). In contrast, although associated with an increased risk of diseases such as urolithiasis, constipation, urinary tract infections, headaches and kidney dysfunction (15), mild chronic dehydration has not been sufficiently studied from a long-term perspective.

Although there is no universally accepted method for measuring hydration status (HS), various procedures, such as the

evaluation of fluid losses by variation of body weight, blood markers, urinary markers, electrical bioimpedance (16) and physical signs, have been used (17). Some authors argue, however, that the free water reserve (FWR) is the most appropriate marker to characterize hydration of individuals in a 24 hour period (18,19).

Analyzing water intake is a complex process and is often omitted in nutritional intake evaluation studies. However, accurate estimates of water intake and HS are essential to identifying populations at risk of dehydration (20) and in defining strategies to improve patterns of water intake.

OBJECTIVES

The aim of the present study is to evaluate the hydration status and the contribution of food and beverages to the total water intake in a sample of free-living physically active elderly.

METHODS

STUDY DESIGN

This cross-sectional study was approved by the Ethics Committee of the University of Porto and the National Data Protection Commission. At the first meeting, all participants received a full explanation on the purpose of the study and all related procedures. Informed consent was obtained from all participants and confidentiality was guaranteed. The study took place between November 2012 and April 2013.

PARTICIPANTS

The study sample included individuals taking part in a physical activity program at the Faculty of Sport, University of Porto (FADEUP), and users of a day center. The sample was selected based on convenience. We invited, by telephone and in-person, 148 people with a minimum age of 60, in stable posture and functional autonomy, who frequented those institutions. None of the participants were institutionalized at the time of data collection.

From the 148 invited elderly, 113 (78%) agreed to participate (38 men and 75 women), but 39 (10 men) were excluded due to daily diuretic therapy, cognitive impairment or incomplete urine collection, according to a creatinine ratio (described in detail below).

For practical reasons throughout this work, the term "elderly" was adopted to describe participants that were at least 60 years of age, in contrast to the more common meaning used to nominate individuals from the age of 65 (21).

A sample of 74 individuals (28 men), between 60 and 83 years of age, were included in this study.

DATA COLLECTION

A structured questionnaire was used to collect socio-demographic (age, sex and education), and clinical (medical history and current medication) data. The IPAQ - International Physical Activity Questionnaire, validated in Portuguese adults (22) was used to assess physical activity and to evaluate the cognitive state, the MMSE - Mini-Mental State Examination (23) was applied.

The collection of anthropometric data (weight and height) was performed according to standard procedures (24). Weight was measured using a digital balance (SECA®; range 0.1-150 kg; precision 100 g) and height was obtained using a stadiometer (SECA®; range 70-205 cm; precision 1 mm). Body mass index (BMI) was calculated using the formula: weight/height^2 . Participants were categorized according to the reference values of the World Health Organization (WHO) (25) as underweight ($< 18.5 \text{ kg/m}^2$), normal weight ($18.5\text{-}24.9 \text{ kg/m}^2$), overweight ($25.0\text{-}29.9 \text{ kg/m}^2$) or obese ($\geq 30 \text{ kg/m}^2$). Due to the small number of participants classified as "underweight" ($n = 2$) in this study, the decision was made to aggregate the two lower BMI classes, for statistical analysis.

A 24-hour urine sample was collected, after having distributed flyers illustrating the instructions inherent in the process of collecting urine and then reinforced the explanation orally. In addition, telephone contact was offered at any time of the day, in order to clarify questions regarding urine collection. Thus, all participants were instructed to discard the first urine in the morning and to collect all the urine over the following 24 hours, including the first urine of the next morning. The 24-hour urine collection was stored in individual containers with preserver, and participants were asked to keep the container refrigerated until delivery time on the day that urine collection was concluded. All samples were analyzed at a certified laboratory (LabMED) and the following urinary markers were quantified: urine volume (ml), urinary creatinine (mg/day) and urine osmolality (mOsm/kg) for 24 hours. Urinary creatinine was measured by the Jaffe method. The completeness of the samples was verified by analysis of creatinine excretion in relation to body weight, using the formula: $\text{coefficient of creatinine} = \text{creatinine (mg/day)}/\text{body weight (kg)}$. The presence of coefficients between 14.4 and 33.6 in men and between 10.8 and 25.2 in women was considered as an acceptable window of 24 urine samples (26).

The HS was evaluated based on the parameter FWR (ml/24 h) ($15,18,27\text{-}30$), calculated by subtracting 24 hour urine volume to obligatory urine volume (solute in urine $24 \text{ h [mOsm/day]}/[830\text{-}3.4] \times [\text{age}-20]$) and allows for the classification of the 24 hour hydration status (euhydrated vs hypohydrated subjects or at risk of hypohydration [27]).

Additionally, a 24-hour food recall was applied, corresponding to the urine collection day. The Portuguese Food guide was used for the estimation of ingested portions (31). For the conversion of food into nutrients, including the contribution of water from food, we used the Food Processor Plus® (ESHA Research, USA). Although this software uses the Table of Food Composition of the

United States Department of Agriculture (32), containing raw and/or processed foods, for this work the nutritional content of food or typical Portuguese culinary dishes consumed by the sample in this study has been added to that database, according to the table of Portuguese food composition (33). For industrial products, when it was referenced food brands, nutritional information described on the label of the package was used.

Food and beverage groups were created (Table I) to estimate the contribution of food groups to total water intake and its association with the hydration status.

STATISTICAL ANALYSIS

Data was analyzed using the statistical program IBM SPSS® Inc. (version 21.0) for Microsoft Windows®. The Kolmogorov-Smirnov test was used to test the normality of continuous variables. Descriptive statistics was used to characterize the sample. Categorical variables were expressed as absolute and relative frequencies, and continuous variables as mean and standard deviations (mean \pm standard deviation). The Student's t-test for independent samples and the Mann-Whitney test were used to compare cardinal variables according to their normality. Chi-squared test was also used to compare proportions. It was considered as statistically significant at $p < 0.05$.

Table I. Food and beverage groups created to estimate the contribution of food groups to total water intake

Food groups	Food included
Dairy	Milk and yogurt
Vegetables	Soup and vegetables: raw, cooked, canned and frozen
Fruits	Fresh fruit and canned (no syrup)
Coffee, barley	Coffee and barley
Other drinks	Soft drinks including light versions/diet/zero (carbonated and non-carbonated), drinks with sugar (lemonade, iced tea), flavored drinks, sports drinks, juices and soy beverages
Tea, infusions	Tea and infusions
Water	Mineral or spring water, with or without gas, bottled or not, and tap water
Alcoholic beverages	Wine, sangria, beer, spirits
Other foods	Meat, fish, eggs, cereal, pastries, potatoes, legumes, fats and cheese

RESULTS

The general characteristics of the final sample are given in table II. Participants were mainly female (62.2%) and 70.2 ± 5.99 years of age on average. About half of the sample (56.5% women and 46.4% of men) reported having 4 years, at most, of schooling and 47.8% of women and 53.6% of men had BMI values between 25.0 - 29.9 kg/m^2 . The majority of the sample reported moderate (30.4% of women and 39.3% of men) and high (54.3% of women and 39.3% of men) levels of physical activity. Approximately half of the participants reported having hypertension (57.8% female and 48.1% male) and hypercholesterolemia (51.1% female and 51.9% male). Kidney failure was reported by a 5.5% of the sample.

Most of the participants were classified as being euhydrated (91.9%). It was found that urinary parameters analyzed did not differ significantly between sexes, except for the obligatory urine volume, which was higher in men (1251.0 ml vs 1013.2 ml in women, $p = 0.001$).

Table II also describes the characteristics of the participants according to HS (below and above the median FWR), by sex. In men, age, education, level of physical activity and weight status were not significantly associated with HS. In turn, women above the median FWR showed, on average, higher age (72.8 vs 67.9 years, $p = 0.010$). All urinary parameters were significantly associated with hydration status, except for obligatory urine volume, in both sexes. Osmolality was lower in women and men below the median FWR (292.6 ml vs 512.9 ml , $p < 0.001 \text{ ml}$ vs 573.3 and 334.7 ml , $p < 0.001$, respectively, in women and men). On the other hand, the total urine volume was significantly higher in "better hydrated" participants ($2,299.1$ vs $1,364.8 \text{ ml}$, $p < 0.001$ vs $1,457.9$ and $2,507.1 \text{ ml}$, $p < 0.001$, respectively, in women and men), similar to FWR ($1,296.8 \text{ ml}$ vs 340.7 ml , $p < 0.001$ and $1,231.1 \text{ ml}$ vs 231.9 ml , $p < 0.001$, respectively, in women and men).

Total water intake was significantly higher in women who were above the median FWR compared to those who were below the median ($2,353.3$ vs $1,884.3 \text{ ml}$, $p = 0.018$), with no significant difference in men ($1,999.4$ vs $2,417.1 \text{ ml}$, $p = 0.198$). The contribution of different groups of food and beverages for total water intake did not differ according to the HS.

Total water intake, considering food and beverages, was $2,153 \text{ ml}$ on average, with no differences between sexes. The contribution of water from various groups of food and beverages did not differ between sexes either, except for "alcoholic beverages", with a significantly higher contribution in men (10.5% vs 1.7% , $p < 0.001$) (Table II).

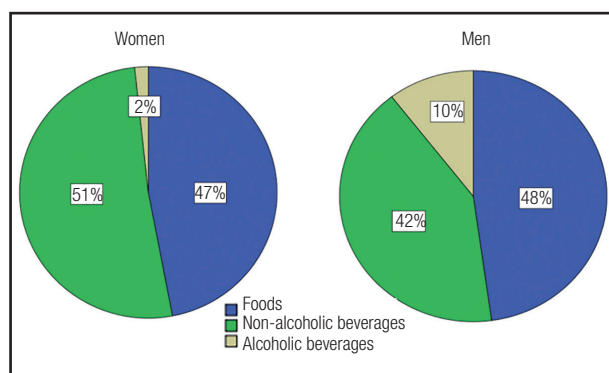
As shown in figure 1, the contribution of water from food was approximately half of the total intake of water (47% in females and 48% in males, $p = 0.757$). In women, the contribution of "non-alcoholic beverages" was significantly higher than that of men (51% vs 42% , $p = 0.029$).

On figure 2 we can observe the contribution of water intake by groups of food and beverages, by sex. "Water" (22%) and "foods with reduced water content" (19%) were the groups that contributed most to the total water intake in women and men, respectively.

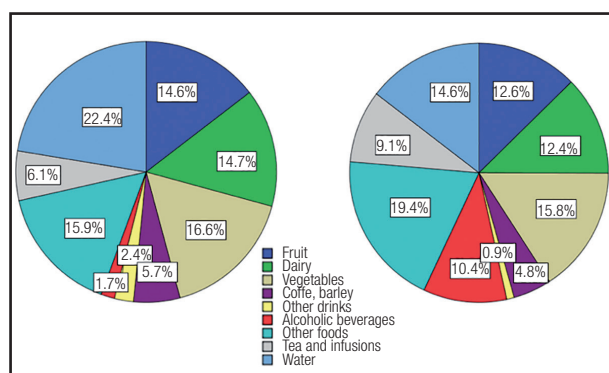
Table II. Characteristics of participants according to sex and hydration status

		Women (n = 46)	Men (n = 28)	p	Women (n = 46)			Men (n = 28)		
					Below the median of FWR (n = 23)	Above the median of FWR (n = 23)	p	Below the median of FWR (n = 14)	Above the median of FWR (n = 14)	p
Age, mean ± SD	years	70.3 ± 6.63	70.0 ± 4.87	0.803	67.9 ± 5.81	72.8 ± 6.59	0.010	70.4 ± 4.60	69.5 ± 5.24	0.623
Education, n (%)										
0-4	years	26 (56.5)	13 (46.4)	0.255	12 (52.2)	14 (60.9)	0.913	7 (50.0)	6 (42.9)	0.790
5-9		14 (30.4)	7 (25.0)		8 (34.8)	6 (26.1)		4 (28.6)	3 (21.4)	
> 9		6 (13.0)	8 (28.6)		3 (13.0)	3 (13.0)		3 (21.4)	5 (35.7)	
BMI, n (%)										
< 25	kg/m ²	14 (30.4)	6 (21.4)	0.698	6 (26.1)	8 (34.8)	0.710	3 (21.4)	3 (21.4)	0.476
25-29.9		22 (47.8)	15 (53.6)		11 (47.8)	11 (47.8)		9 (64.3)	6 (42.9)	
> 30		10 (21.7)	7 (25.0)		6 (26.1)	4 (17.4)		2 (14.3)	5 (35.7)	
Level of physical activity, n (%)										
Low		7 (15.2)	6 (21.4)	0.485	3 (13.0)	4 (17.4)	0.715	5 (35.7)	1 (7.1)	0.091
Moderate		14 (30.4)	11 (39.3)		6 (26.1)	8 (34.8)		6 (42.9)	5 (35.7)	
High		25 (54.3)	11 (39.3)		14 (60.9)	11 (47.8)		3 (21.4)	8 (57.1)	
Total water, mean ± SD	ml	2,118.8 ± 679.05	2,208.2 ± 848.49	0.619	1,884.3 ± 476.51	2,353.3 ± 775.34	0.018	1,999.4 ± 658.22	2,417.1 ± 983.91	0.198
Contribution of water from foods and beverages, mean ± SD										
Fruits	%	14.6 ± 9.95	13.0 ± 6.46	0.328	12.7 ± 10.51	16.5 ± 9.18	0.197	12.2 ± 7.05	13.2 ± 6.04	0.680
Dairy		14.7 ± 11.60	12.4 ± 10.06	0.403	14.2 ± 10.05	15.1 ± 13.20	0.801	10.6 ± 9.77	14.3 ± 10.35	0.329
Horticulture		16.6 ± 12.82	15.8 ± 14.87	0.828	16.1 ± 14.18	17.0 ± 11.60	0.814	17.8 ± 15.30	13.9 ± 14.73	0.500
Coffee, barley*		5.7 ± 6.97	4.8 ± 7.10	0.508	5.3 ± 5.63	6.1 ± 8.22	0.438	6.0 ± 7.80	3.6 ± 6.39	0.213
Other drinks*		2.4 ± 4.71	0.9 ± 4.92	0.70	1.4 ± 3.96	3.3 ± 5.27	0.097	1.9 ± 6.96	0.0 ± 0.00	0.150
Tea, infusions*		6.1 ± 9.40	9.1 ± 14.13	0.519	7.4 ± 9.83	4.9 ± 8.98	0.341	11.6 ± 17.71	6.5 ± 9.32	0.684
Water		22.4 ± 16.72	14.6 ± 19.00	0.070	23.0 ± 19.92	21.8 ± 13.20	0.813	9.3 ± 12.14	20.0 ± 23.25	0.143
Alcoholic beverages*		1.7 ± 4.04	10.5 ± 12.81	≤ 0.001	2.1 ± 3.90	1.3 ± 4.21	0.166	10.2 ± 7.13	10.8 ± 17.02	0.243
Other foods		15.9 ± 9.46	19.4 ± 10.23	0.130	17.7 ± 10.46	14.0 ± 8.14	0.184	20.5 ± 10.00	18.4 ± 10.74	0.597
Urinary parameters, mean ± SD										
Urine osmolality 24 h	mOsm/kg	402.7 ± 149.40	454.0 ± 158.46	0.166	512.9 ± 118.62	292.6 ± 78.76	< 0.001	573.3 ± 132.59	334.7 ± 62.65	< 0.001
Urinary volume 24 h	ml/day	1,832.0 ± 655.79	1,982.5 ± 654.24	0.341	1,364.8 ± 352.68	2,299.1 ± 546.77	< 0.001	1,457.9 ± 342.35	2,507.1 ± 423.02	< 0.001
Obligatory urine volume	ml/day	1013.2 ± 273.66	1251.0 ± 305.38	0.001	1,024.1 ± 270.72	1,002.4 ± 282.22	0.791	1,225.9 ± 257.21	1,276.0 ± 355.22	0.673
FWR 24 h	ml/day	818.7 ± 655.99	731.5 ± 582.35	0.565	340.7 ± 278.50	1,296.8 ± 569.94	< 0.001	231.9 ± 299.49	1,231.1 ± 277.60	< 0.001

* The distribution of these groups of food/beverages in the sample was different from the normal distribution, so in these cases we used the Mann Whitney test.

**Figure 1.**

Contribution of food, alcoholic and non-alcoholic beverages, by sex.

**Figure 2.**

Contribution of water intake by groups of food and beverages, by sex

DISCUSSION

In this study, total water intake was 2,208 ml in males and 2,118 ml in females. Approximately half of the participants did not reach the reference values of the European Food Safety Authority - EFSA, although the difference between mean and reference values was not statistically significant. When focusing on FWR values, most of the participants were classified as euhydrated. FWR appears to be a suitable method for the characterization of HS, given the inclusion of the maximum capacity of kidney concentration and a margin to ensure adequate intake of water in almost all healthy individuals (97%) of a population (27). A limitation of this study was the single recording period of 24 hours for food and urine collection, which may not represent an individual's normal behavior.

The total water intake was 2,153 ml/day on average; in the same range as the French (2,017 ml/day) (34) and German (2,334 ml/day) senior population (27), but less than the observed in the United States of America (2,650 ml/day) (35). However, it is important to consider the methodological differences in data collection across countries (36). It is also important to note that we have a convenience sample, avoiding the extrapolation of results

for all non-institutionalized elderly. The study sample appears to be a special group with regard to water intake, possibly because it is a group of seniors who voluntarily attend an exercise program. That, by itself, can be understood as a group of people with specific characteristics regarding physical, cognitive and social factors. A study by Chidester et al. assumes that the non-institutionalized elderly are generally healthy and have easy access to a variety of drinks. The same study showed that institutionalized and dependent elderly are generally weaker and have a more limited access to beverages, hence at greater risk of dehydration (37).

The contribution of water from various groups of food and beverages did not differ between sexes, except for "alcoholic beverages". This contribution was significantly higher in men; almost half exceed alcoholic drinking recommendations, so, in part, the contribution of alcoholic beverages should revert to non-alcoholic drinks or water rich-foods, because, although the regular consumption of alcohol can reduce diuretic effects, a high consumption can be harmful to health (38). In women, the contribution of "non-alcoholic beverages" was significantly higher than in men. The same trend was observed in a study conducted in 2009 on a representative sample of the Portuguese adult population, in which women reported a higher intake of water, dairy products, tea and coffee, while men reported consuming a higher amount of alcohol (39).

"Water" and "foods with reduced water content" were the groups that contributed most to the total water intake in women and men, respectively. In this way, it is important to promote the consumption of non-alcoholic drinks and foods with a high water content, such as fruit and vegetables, to ensure adequate HS, in addition to other nutritional gains.

Even within the elderly, there appears to be differences in hydration parameters. Vivanti et al. reported that the elderly, aged 85 to 99, were six times more likely to be admitted to a hospital with dehydration in comparison to those aged 65-69 (40); this finding can be explained by the perception and response to stimulation of thirst (41). In addition, it was reported that from the age of 65, there is a decrease in water intake among the elderly (42). Although the importance of age in hydration studies is well known, we did not obtain data stratified by age, given the limited sample size. We found, however, that women classified above the median FWR were older than others.

CONCLUSIONS

Although most of the study participants were classified as euhydrated, the contribution of water-rich and nutritionally dense food, and non-alcoholic beverages, particularly in men, should be promoted.

DECLARATION OF INTEREST

P.P. was a member of the Scientific Board of the Institute of Hydration and Health between 2008 and 2015.

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The association between water intake, body composition and cardiometabolic factors among children - The Cuenca study

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Abstract

Introduction: Beverage consumption and its possible association with current obesity epidemic and metabolic syndrome is under investigation in recent years, however water intake is probably the most underestimated of all beverages and could play an important role.

Objective: The aim of this study was to examine the association between water intake, body composition and cardiometabolic factors in a sample of Spanish children.

Methods: A cross-sectional study was conducted in 366 schoolchildren (53.5% girls) aged 9-11 years from the province of Cuenca in Spain. Data of anthropometrics, body composition, cardiometabolic risk factors and cardiorespiratory fitness variables were collected. Beverage consumption was assessed using two non-consecutive 24 h dietary recalls.

Results: We found an inverse association between the consumption of water (ml)/kg per weight with BMI, body fat, fat-free mass, waist circumference, insulin levels, HOMA-IR ($p < 0.001$), and with arterial pressure parameters, systolic ($p < 0.010$) and diastolic blood pressure ($p < 0.028$), and mean arterial pressure ($p < 0.012$), as well as direct associations with HDL cholesterol ($p < 0.001$). In ANCOVA analyses, children who drank less water (ml)/kg per weight, had higher levels of LDL cholesterol ($p < 0.050$) and lower levels of HDL cholesterol ($p < 0.042$), and overweight-obesity subjects drank less water (ml)/kg per weight than normal peers ($p < 0.011$). Besides, children with lower levels of HDL cholesterol and higher levels of triglycerides and blood pressure had less water intake as a beverage. Finally, children who drank less water from beverages had high levels of LDL cholesterol.

Conclusions: Higher consumption of water (ml)/kg per weight was negatively associated with BMI, body fat, fat-free mass, waist circumference, insulin levels, HOMA-IR, and positively with HDL cholesterol in children independently of age, sex and cardiorespiratory fitness. In addition, overweight-obese children drank less water (ml)/kg per weight than normoweight ones. Therefore, water consumption is associated with numerous health benefits and its adequate intake could contribute to prevent obesity and metabolic syndrome in childhood.

Key words: Water consumption. Serum lipids. Insulin resistance. Body composition.

INTRODUCTION

The majority of the main risk factors associated with cardiovascular disease are preventable and modifiable by lifestyle changes (1). Evidence has shown that overweight, eating behaviors and physical activity affect most cardiometabolic variables associated with cardiovascular risk factors, such as serum lipids, blood pressure, waist circumference and insulin resistance (2).

Regarding these modifiable factors, beverage consumption and their possible association with the current obesity epidemic and metabolic syndrome is under investigation in recent years (3). Most studies have focused on sugar sweetened beverages consumption (4-7), although there are also studies that analyzed diet beverages (8,9). On the other hand, there are studies focused on dairy consumption and its possible positive association with cardiovascular health (10,11), as well as on natural juices intake (12). Preliminary analysis of a recent study has shown that a *Healthy Beverage Index*, a measure of the overall healthfulness of an individual's beverage intake, was associated with reduced cardiometabolic risk in adults (13). Other authors provide additional evidence suggesting a potential protective effect of higher total water intake (particularly plain water) on kidney but not on cardiovascular risk (14).

With regard to these hydration habits, one question generally forgotten has been water consumption (15). Regulation of water balance is essential for the maintenance of health and life, and some authors have attempted to elucidate its possible link with obesity (16).

Drinking more water has been proposed as a method to reduced weight gain since water consumption could replace other caloric beverages and thereby reduce total calories consumed (16). However, evidence about water intake and its association with cardiovascular health is scarce (17), and in most studies, they are related to water hardness (18). To our knowledge, there is no research focused on children regarding this topic. Therefore, the aim of this study was to examine the association between water intake, body composition and cardiometabolic factors in a sample of Spanish children.

MATERIAL AND METHODS

STUDY DESIGN AND PARTICIPANTS

This study was a cross-sectional analysis of baseline measurement data from a cluster-randomized trial aimed to assess the effectiveness of a physical activity program for preventing excess weight in schoolchildren (19). For this report, we used data from a sub-sample of 366 children (196 girls) aged 9 to 11 years, in fifth grade of Primary Education from 20 public primary schools in the Spanish province of Cuenca. The Clinical Research Ethics Committee of the Virgen de la Luz Hospital, in Cuenca, approved the study protocol.

ANTHROPOMETRIC AND BODY COMPOSITION ASSESSMENTS

All measurements were obtained at the schools by trained nurses. Height and weight were measured twice with a five-minute interval between measurements with the children lightly dressed. Weight was measured to the nearest 0.1 kg using a calibrated digital scale (SECA Model 861; Vogel & Halke, Hamburg, Germany). Height was measured to the nearest millimeter using a wall-mounted stadiometer, with the children standing straight against the wall without shoes, to align the spine with the stadiometer. The head was positioned so that the chin was parallel to the floor. The mean of the two weight and height measurements was used to calculate body mass index (BMI) as weight in kilograms divided by the square of the body height in meters (kg/m^2). Waist circumference (WC) was calculated as the average of two measurements taken with flexible tape at the natural waist (the midpoint between the last rib and the iliac crest). Body fat percentage and fat-free mass percentage were estimated using a BC-418 bioimpedance analysis system (Tanita Corp., Tokyo, Japan). The mean of two readings taken in the morning under controlled temperature and humidity conditions, after urination and a 15-minute rest, with the child being shoeless and fasting was used.

RESTING BLOOD PRESSURE MEASUREMENT

Diastolic and systolic blood pressure (DBP; SBP) were determined as the average of two measurements separated by a five-minute interval, with the child resting for at least five minutes

before the first measurement. The child was seated in a quiet, calm environment, with the right arm in a semi-flexed position at the heart level. Blood pressure was measured automatically using the OMRON M5-I monitor (Omron Healthcare Europe BV, Hoofddorp, Netherlands). Mean arterial pressure (MAP) was calculated using the following formula: $\text{DBP} + (0.333 \times [\text{SBP} - \text{DBP}])$.

BIOCHEMICAL ASSESSMENTS

Blood samples were taken in standardized conditions between 8:15 and 9:00 a.m. after at least 12 hours fasting by puncturing the cubital vein. Before the extraction, fasting was confirmed by the child and his parents. The samples were processed using a Roche Diagnostics COBAS C711. The following parameters were determined: triglycerides (GPO-PAP enzymatic method), HDL-cholesterol and LDL-cholesterol (2nd generation method without de-proteinization).

The homeostasis model of assessment (HOMA-IR) was used to determine insulin resistance (IR) and its individual components. Fasting glucose and insulin were determined using standard protocols (chemiluminescent microparticle immunoassay).

CARDIORESPIRATORY FITNESS

Cardiorespiratory fitness was assessed by the 20-m shuttle run test (20). Participants were required to run between two lines 20 m apart, while keeping pace with audio signals emitted from a pre-recorded compact disc. The running speed started at 8.5 km/h and increased 0.5 km/h each minute. The children were stopped when they could not follow the signal any more. We noted the last half-stage completed as an indicator of their cardiorespiratory fitness (CRF).

ASSESSMENT OF WATER AND OTHER BEVERAGE INTAKES

Beverage and water intake of each participant were estimated using a self-administered computerized 24 h dietary recall validated for European adolescents called the Young Adolescents' Nutrition Assessment on Computer (YANA-C) (21). The Spanish YANA-C questionnaire was administered twice in a week: one day asking about a weekday and another one about a weekend day. The YANA-C program was installed in the computer room of each school, where pupils completed the questionnaire in groups. A staff member instructed the children and, then, pupils completed the program autonomously, although two or three staff members were present to provide assistance as required. Interviewers were previously trained.

STATISTICAL ANALYSIS

The distribution of continuous variables was checked for normality before the analysis. Due to their skewed distribution,

variables were log-transformed prior to analyses. Continuous variables were expressed as the mean \pm SD for normally distributed continuous data. Categorical variables were expressed as n (%). To aid interpretation, data were back-transformed from the log scale for presentation in the results.

Normal weight and overweight-obese were defined according to the BMI cut-offs published for children and adolescents (22).

Categorization of blood pressure (BP) was done using sex, age and height specific cut points informed by the Fourth Report on the Diagnosis, Evaluation and Treatment of High Blood Pressure in Children and Adolescents by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents (23). High BP (stage 1) was defined as a systolic or diastolic BP at 95th percentile or higher but lower than the 99th percentile; high BP (stage 2) was defined as a systolic or diastolic BP at 99th percentile or higher; and borderline BP was defined as a systolic or diastolic BP at the 90th percentile or higher but lower than the 95th percentile. To obtain more accurate results we simplified the four criteria obtained in two categories, as follows, normotensive ($p < 90$) and hypertension (stages 1 and 2) ($p \geq 90$).

Adverse lipid concentrations were defined as follows: total cholesterol concentrations ≥ 200 mg/dl or higher; HDL < 40 mg/dl; LDL concentrations ≥ 130 mg/dl; and triglycerides concentrations ≥ 130 mg/dl (24). Insulin resistance was defined as follows: fasting glucose concentrations ≥ 126 mg/dl (25); insulin concentrations > 15.05 μ U/ml (25); and HOMA-IR > 3.43 (26).

Multiple linear regression analyses were made to explore possible relationships between water intake, body composition and cardiometabolic factors adjusted by age, sex, and cardiorespiratory fitness. ANCOVA models were estimated to test the differences in water intake variables by categories of BMI (normoweight vs overweight-obese children) and cardiometabolic risk (non-risk vs at risk children).

RESULTS

The sample characteristics are presented in table I. There were statistically significant gender differences in the mean of height (higher in girls, $p = 0.008$), body fat percentage (higher in girls, $p < 0.001$), and CRF (higher in boys ($p < 0.001$), but not for BMI. The prevalence of overweight-obesity was 36.6% for the total sample. Regarding cardiometabolic factors, boys had higher levels of HDL cholesterol ($p = 0.002$), fasting plasma glucose ($p < 0.001$) and SBP ($p = 0.008$) than girls. Girls had higher insulin levels ($p < 0.001$) and HOMA-IR ($p < 0.001$) than boys. Concerning lipids parameters, 7.4% of children had higher levels of triglycerides, 15% total cholesterol elevated, 3.8% HDL cholesterol low, and 9.6% had adverse LDL cholesterol. All children had normal amounts of fasting plasma glucose (≥ 126 mg/dl). Finally, we found a 9.6% of children with hypertension. Concerning water intake, for most of water variables, except for water from food variable, girls took more quantities than boys, although the differences were non-significant.

Table II shows the ANCOVA analysis. We found that children who drank fewer water as a beverage had higher values of triglycerides ($p = 0.009$), worst levels of HDL cholesterol ($p < 0.001$) and hypertension ($p = 0.023$). On the other hand, children who drank less water from all beverages had adverse levels of LDL cholesterol ($p = 0.022$). Moreover, those who ingested less water (ml)/kg per weight, had lower levels of HDL cholesterol ($p = 0.042$) and higher levels of LDL cholesterol ($p = 0.050$). Finally, overweight-obese children drank less water (ml)/kg per weight than normal peers did ($p = 0.011$) and had more consumption of water/kcal day ($p = 0.023$).

Table III presents the relationship between water intake variables, BMI and cardiometabolic risk factors adjusted by age, sex and cardiorespiratory fitness. We found a positive association between water as a beverage with BMI ($p < 0.001$), body fat ($p < 0.004$), fat free mass ($p < 0.001$) and waist circumference ($p < 0.001$). A positive association also appears between water/kcal day and BMI ($p = 0.009$), body fat ($p < 0.001$) and waist circumference ($p = 0.011$). On the other hand, there was a negative association between water from caloric beverages with BMI ($p = 0.005$), body fat ($p = 0.010$), fat free mass ($p = 0.010$) and waist circumference ($p = 0.003$). Regarding water (ml)/kg per weight, we showed an inverse relationship with BMI, body fat, fat free mass, waist circumference, insulin levels, HOMA-IR ($p < 0.001$), and arterial pressure parameters (SBP, $p = 0.010$; DBP, $p = 0.028$; and mean arterial pressure, $p = 0.012$). Finally, we found direct associations between water (ml)/kg per weight and HDL cholesterol ($p = 0.001$).

DISCUSSION

The aim of the study was to examine the association between water intake, body composition and cardiometabolic risk factors in a sample of Spanish children. Overall, our results showed an inverse association between water (ml)/kg per weight and several body composition variables, lipid profile and insulin resistance parameters, independently of age, sex and cardiorespiratory fitness.

Water consumption is probably the most underestimated of all beverages intakes (15). Water is essential for life and plain water, instead of other caloric beverages, is one approached for decreasing energy intake (27), and therefore may play an important role to fight against obesity and metabolic syndrome. However, the mechanism remains unclear. Some studies have suggested that water intake elicited acute changes in human physiology because it provides a sympathetic stimulus, increasing the metabolic rate, which in turn augments the daily energy expenditure (28,29). Other authors reported that water drinking activates the autonomic nervous system and induces acute hemodynamic changes (30). The actual stimulus for these effects is undetermined but might be related to either gastric distension or osmotic factors (30). In our sample, water (ml)/kg per weight was inversely associated with BMI, body fat, fat free mass and waist circumference, as well as, overweight-obesity children drank less water(ml)/kg per weight than normal weight peers.

Table I. Baseline characteristics of the sample

	Total (n = 366)		Boys (n = 170)		Girls (n = 196)		<i>p</i> [*] Boys vs girls
	Mean	SD or %	Mean	SD or %	Mean	SD or %	
Age	10	0.5	10.0	0.5	9.9	0.4	0.093
Body composition							
Height, cm	141.8	6.9	140.8	6.7	142.7	6.9	0.008
Weight, cm	39.2	9.1	38.9	8.6	39.5	9.4	0.497
BMI, kg/m ²	19.4	3.6	19.5	3.5	19.3	3.7	0.556
Normoweight, n (%)	232	66.3	103	60.6	129	65.8	0.303
Overweight-Obese, n (%)	134	36.6	67	39.4	67	34.2	
Body fat, %	25.7	6.4	24.2	6.9	26.9	5.7	< 0.001
Fat-free mass, %	28.7	4.7	28.9	4.3	28.5	5.1	0.304
Waist circumference, cm	68.8	9.2	69.2	9.2	68.5	9.3	0.454
Cardiorespiratory fitness CRF, n	3.8	1.7	4.4	1.9	3.2	1.3	< 0.001
Triglycerides, mg/dl	72	37	69	33	75	39	0.089
Normal, n (%)	339	92.6	158	92.9	181	92.3	0.828
High, n (%)	27	7.4	12	7.1	15	7.7	
Total cholesterol mg/dl	172	29	172	25	171	32	0.801
Normal, n (%)	311	85	149	87.6	162	82.7	0.179
High, n (%)	55	15	21	12.4	34	17.3	
HDL cholesterol, mg/dl	59.8	13.2	62.1	13.1	57.8	13.1	0.002
Normal, n (%)	352	96.2	167	98.2	185	94.4	0.048
High, n (%)	14	3.8	3	1.8	11	5.6	
LDL cholesterol, mg/dl	99	24	98	20	100	27	0.462
Normal, n (%)	331	90.4	162	95.3	169	86.2	0.002
High, n (%)	35	9.6	8	4.7	27	13.8	
Glucose, mg/dl	84	6	85	6	82	6	< 0.001
Normal, n (%)	366	100	170	100	196	100	
High, n (%)	0	0	0	0	0	0	
Insulin, µU/ml	8.2	4.6	7.1	3.6	9.2	5.1	< 0.001
Normal, n (%)	342	93.4	164	96.5	178	90.8	0.025
High, n (%)	24	6.6	6	3.5	18	9.2	
HOMA - IR	1.7	0.9	1.5	0.7	1.9	1.1	< 0.001
Normal, n (%)	344	94	165	97.1	179	91.3	0.017
High, n (%)	22	6	5	2.9	17	8.7	
Systolic blood pressure, mmHg	101.9	9.2	103.3	8.9	100.7	9.2	0.008
Diastolic blood pressure, mmHg	63.2	7.5	63.4	7.3	63.2	7.6	0.789
Mean arterial pressure, mmHg	76.1	7.4	76.7	7.2	75.7	7.6	0.202
Normotensive, n (%)	331	90.4	155	91.2	176	89.8	0.654
Hypertension, n (%)	35	9.6	15	8.8	20	10.2	
Water as a beverage, ml/day	504.5	381.6	457.1	352.4	545.5	401.6	0.027
Water from food and beverages, ml/day	1484.3	508	1443.4	491.4	1519.9	521.4	0.151
Water from beverages, ml/day	959.3	389.3	911.5	382.4	1002.2	391.8	0.026
Water from food, ml/day	524.2	237.3	531.8	239.5	517.7	235.8	0.572
Water from caloric beverages, ml/day	427.6	228.2	418.1	249.1	435.9	208.7	0.455
Water/kg weight, ml/kg	13.3	10.3	12.0	9.1	14.4	11.1	0.024
Water/kcal/day, ml/kcal	0.4	0.4	0.4	0.4	0.4	0.4	0.531

Values are means (standard deviations \pm SD) and number and proportions (%) for categorical data. BMI: body mass index; CRF: cardiorespiratory fitness measured by 20-m shuttle run test (stage); HDL: high density lipoprotein; LDL: low density lipoprotein; HOMA-IR: homeostatic model assessment insulin resistance. $p < 0.05$

Table II. Differences in the frequency of each cardiometabolic risk category and water intake variables

		BMI p ≥ 90		Triglycerides ≥ 130 mg/dl		Total cholesterol ≥ 200 mg/dl		HDL < 40 mg/dl		LDL ≥ 130 mg/dl		Fasting insulin > 15.05 µU/ml		HOMA-IR > 3.43		Hypertension p ≥ 90°	
		Normal	Ob	Normal	High	Normal	High	Normal	Low	Normal	High	Normal	High	Normal	High	Normal	High
Water as a beverage, ml/day	Mean	499.3	513.3	513.5	390.1	501.9	518.4	512.7	296.9	512.7	426.4	507.2	464.2	505.7	483.6	514.2	411.5
	SD	372.1	398.6	379.7	392.8	384.4	368.1	381.5	330.1	388.4	303.1	381.7	384.2	381.3	392.9	386.2	324.6
	F	0.103		6.841		0.202		12.03		3.653		0.001		0.274		5.215	
	p	0.479		0.009		0.653		0.001		0.057		0.969		0.601		0.023	
Water from food and beverages, ml/day	Mean	1471.8	1505.9	1491.8	1389.6	1485.7	1475.9	1489.9	1343.7	1492.1	1410.4	1485.5	1466.6	1481.3	1531.1	1493.9	1392.8
	SD	514.7	498.5	503.4	569.3	509.8	505.1	512.9	362.5	512.1	474.1	505.5	559.1	507.5	531.9	518.1	401.8
	F	0.533		2.220		0.178		1.644		1.895		0.453		0.068		1.197	
	p	0.466		0.137		0.673		0.201		0.169		0.501		0.795		0.275	
Water from beverages, ml/day	Mean	956.8	965.7	965.1	895.9	961.8	949.8	963.3	878.3	971.5	852.0	963.9	905.5	960.8	948.7	965.8	905.1
	SD	386.3	396.4	388.2	407.4	386.4	410.3	392.2	314.2	392.9	342.5	389.2	398.9	390.5	382.8	398.1	297.2
	F	0.219		1.326		0.466		1.077		5.283		1.545		0.999		0.474	
	p	0.640		0.250		0.495		0.300		0.022		0.215		0.753		0.492	
Water from food, ml/day	Mean	514.9	540.2	526.6	493.6	523.9	526.1	526.5	465.4	520.6	558.4	521.6	561.1	520.5	582.3	528.1	487.8
	SD	236.8	238.1	236.1	253.9	244.4	193.8	238.9	187.1	240.9	199.2	234.6	274.3	234.5	276.3	242.5	178.2
	F	0.205		1.310		0.315		1.024		1.454		0.312		1.023		0.511	
	p	0.651		0.253		0.575		0.312		0.229		0.577		0.313		0.475	
Water from caloric beverages, ml/day	Mean	430.53	422.6	423.9	473.7	429.9	414.4	422.1	567.5	428.7	417.1	427.8	425.1	426.3	447.9	423.4	467.8
	SD	221.8	239.4	229.6	206.8	233.5	196.4	224.8	273.9	231.5	196.7	228.8	223.9	228.9	219.5	227.4	234.9
	F	0.070		1.595		0.000		3.340		0.035		0.030		0.180		0.971	
	p	0.791		0.207		0.983		0.068		0.853		0.863		0.672		0.325	
Water/kg weight, ml/kg	Mean	14.7	10.8	13.6	9.1	13.2	13.8	13.6	7.1	13.6	10.3	13.6	8.9	13.6	9.4	13.6	10.4
	SD	11.0	8.4	10.4	8.3	10.4	9.7	10.3	8.1	10.6	6.7	10.4	7.4	10.4	7.6	10.4	8.3
	F	6.568		3.181		0.099		4.162		3.881		3.506		2.653		2.772	
	p	0.011		0.075		0.753		0.042		0.050		0.062		0.104		0.097	
Water/kcal/day, ml/kcal	Mean	0.3	0.4	0.4	0.3	0.4	0.4	0.4	0.2	0.4	0.3	0.38	0.3	0.4	0.4	0.4	0.3
	SD	0.3	0.6	0.4	0.2	0.4	0.3	0.4	0.3	0.4	0.2	0.41	0.3	0.4	0.3	0.4	0.2
	F	5.227		2.346		0.084		1.893		1.046		0.185		0.056		1.503	
	p	0.023		0.126		0.773		0.170		0.307		0.667		0.813		0.221	

Adjusted by sex, age and cardiorespiratory fitness. *According to criteria of Fourth Report on the Diagnosis, Evaluation and Treatment of High Blood Pressure in Children and Adolescents (23).

Table III. Multiple regression analysis between intake variables, body composition and cardiometabolic parameters

	Water as a beverage, ml/day	Water from food and beverages, ml/day	Water from beverages, ml/day	Water from food, ml/day	Water from caloric beverages, ml/day	Water/kg weight, ml/kg	Water/kcal/day/ml/kcal
	β	β	β	β	β	β	β
BMI, kg/m ²	0.293***	0.006	0.385	0.049	-0.215**	-0.817***	0.178**
Body fat %	0.225**	0.194	0.192	-0.029	-0.188*	-0.714***	0.225**
Fat-free mass %	0.471**	0.200	0.334	-0.027	-0.213*	-0.596***	0.099
Waist circumference, cm	0.264**	-0.022	0.437	0.122	-0.224**	-0.817***	0.171*
Triglycerides, mg/dl	0.016	0.486	-0.253	-0.236	0.030	-0.104	-0.031
Total cholesterol, mg/dl	0.032	-0.667	0.208	0.375	0.139	0.213	0.111
HDL-c, mg/dl	0.056	-0.256	-0.054	0.126	0.132	0.340**	-0.030
LDL-c, mg/dl	0.046	-0.711	0.312	0.415	0.084	0.063	0.156
Glucose, mg/dl	0.010	0.081	0.179	-0.004	-0.135	-0.134	0.002
Insulin, μ U/ml	0.081	0.409	-0.15	-0.130	-0.127	-0.457***	0.088
HOMAR-IR	0.079	0.407	0.009	-0.126	-0.140	-0.460***	-0.281
Systolic blood pressure, mmHg	0.031	-0.414	0.561	0.243	-0.065	-0.286*	1.466
Diastolic blood pressure, mmHg	0.058	0.228	0.047	-0.126	-0.024	-0.241*	-0.003
Mean arterial pressure, mmHg	0.051	-0.026	0.267	0.020	0.042	-0.278*	0.054

Adjusted by age, sex and cardiorespiratory fitness. BMI: body mass index; HOMA-IR: homeostatic model assessment insulin resistance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Healthy eating is one of the keys for obesity prevention (31) and knowledge of the energy contribution from beverages is particularly important, since consumption of healthy fluids is part of a balanced diet (32). Drinking plain water instead of caloric beverages may reduce dietary energy density and help in body weight management (33). Among children, several surveys have checked that promotion and provision of drinking water could effectively reduce the risk of overweight (34) and that an increase of water intake has been associated as well with weight loss in overweight children (35). However, in adults, some review studies showed that whereas encouraging water consumption may facilitate weight management, the evidence was very limited (16,36,37). Moreover, we found an inverse relationship between water from caloric beverages (sugar sweetened beverages, dairy consumption and fruit juices) with BMI, body fat, fat free mass, and waist circumference. This is controversial because most reviews link sugar sweetened beverages consumption with greater risk of obesity and metabolic syndrome (6,38). In this sense, it is important to say that, in our study, water from all types of liquids that provide calories, including dairy products and fruit juices, were the drinks most consumed by our children (39), which could justify our results, as some authors present a possible positive relationship with body composition and cardiovascular health (12,40).

Otherwise, there is little evidence about beverage consumption and its association with blood lipids. We observed that children who drank less plain water had higher blood triglycerides values and lower HDL cholesterol values. Consistent with this, children

who drank less water (ml)/kg per weight had lower HDL cholesterol levels and higher LDL cholesterol levels. Finally, we have shown a direct association between HDL cholesterol values and water(ml)/kg per weight. These two categories refer to plain water intake and plain water intake per kg of weight. In this sense, a review reports that drinking water results in greater rates of fat oxidation because fat oxidation is maximal when blood insulin levels are low (41). The reason for this is that insulin inhibits or decreases the ability of rate limiting enzymes that breakdown triglyceride fats into free fatty acids (41). Since water does not contain calories or carbohydrates like other beverages, it does not trigger an insulin response (41). Moreover, one study in women showed that drinking one liter or more water per day was associated with decreases in triglycerides, total cholesterol and LDL cholesterol over 12 months (42).

Besides, we have observed an inverse relationship between water (ml)/kg per weight and fasting insulin and HOMA-IR. There is limited evidence regarding this issue. Some authors had drawn attention to a low water intake as a possible new risk factor for impaired glycemia, suggesting that an increase in water intake (an easy and costless intervention) could prevent or delay the onset of hyperglycemia and subsequent diabetes (43). In addition, the same study in women reported that a higher consumption of water per day was associated with significant decreases in fasting insulin and HOMA-IR (42).

Lastly, information about the relationship between blood pressure and water intake is scarce. There is some evidence for the

relationship between drinking water content of magnesium and calcium and the risk for cardiovascular disease, because these captions regulate muscular contractility, and a lack of magnesium leads to an increase in vascular tension and a lower muscular contraction threshold (18). A recent case-control study in children and adolescents has shown that the magnesium and calcium levels content of drinking water may have a protective role against early stages of atherosclerosis (44). In adults, a randomized controlled trial reported an improvement in SBP replacing caloric beverages with non-caloric alternatives as water (45). In addition, another study showed a significant decrease in blood pressure in women that drunk one liter or more of water per day (42). Our findings showed a negative association between the consumption of water (ml)/kg per weight and SBP, DBP and mean arterial pressure, and in accordance with this, children who drank less plain water had more prevalence of hypertension ($p \geq 90$) (23). We could speculate that water content in minerals could be the reason of these relationships; however, we have not quantified the amount of sodium and potassium content in our drinking water.

This study is not without limitations. First, our study was a cross-sectional design, thus observational findings do not allow us to evaluate whether water intake has a possible causal relationship with body composition and cardiometabolic factors over time. Additionally, we have found little evidence between these associations, and most of them are in adults, although, in general, the physiological differences between children and adolescents compared to adults are related to water content in the body, insensible water loss, sweating and index of renal function in the case of children aged less than two years. In spite of physiological differences, thermoregulatory capacity of children and adolescents is comparable to that of adults (46). Moreover, the assessment of diet in children, either directly or by adult proxy, has some methodological challenges. It has been suggested that 10-year-old children are not able to give valid responses to food frequency questionnaires covering periods longer than one day. In our study, the use of pictures in the YANA-C software helped children to remember not only the food eaten during the last 24 hours, but also the portion size. Finally, we have not been able to quantify the mineral content in children's water intake, so results cannot be easily compared to some literature reports.

CONCLUSIONS

In conclusion, our data show an inverse association between water (ml)/kg per weight and BMI, body fat, fat free mass, waist circumference, fasting insulin, HOMA-IR, and blood pressure parameters in children. We observed as well that children who drink less plain water and less quantity of plain water/kg per weight had higher levels of triglycerides and LDL cholesterol, and lower values of HDL cholesterol. Finally, overweight-obese children's intake of plain water/kg per weight is lower than that of normal counterparts. Hopefully, our study could serve as a benchmark to design appropriate randomized clinical trials testing

the efficacy of water intake, instead of other beverages, to prevent obesity and cardiometabolic diseases in children.

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Nutrición Hospitalaria



Comparison of beverage consumption in adult populations from three different countries: do the international reference values allow establishing the adequacy of water and beverage intakes?

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Abstract

Introduction: Recommendations of adequate total water intake (aTWI) have been proposed by the European Food Safety Agency (EFSA) and the Institute of Medicine (IOM) of the United States of America. However, there are differences in the approach used to support them: IOM recommendation is based on average intakes observed in NHANES III (Third National Health and Nutrition Examination Survey) and EFSA recommendation on a combination of observed intakes from 13 different European countries. Despite these recommendations of aTWI, the currently available scientific evidence is not sufficient to establish a cut-off value that would prevent disease, reduce the risk for chronic diseases or improve health status.

Objective: To compare the average daily consumption of fluids (water and other beverages) in selective samples of population from Mexico, US and Spain, evaluating the quantity of fluid intake and understanding the contribution of each fluid type to the total fluid intake. We also aim to determine if they reached adequate intake (AI) values, as defined by three different criteria: IOM, EFSA and water density.

Methods: Three studies were compared: from Mexico, the National Health and Nutrition Survey conducted in 2012 (NHNS 2012); from US, the NHANES III 2005-2010 and from Spain the ANIBES study leaded in 2013. Different categories of beverages were used to establish the pattern of energy intake for each country. Only adult population was selected. TWI of each study was compared with EFSA and IOM AI recommendations, as well as applying the criterion of water density (mL/kcal).

Results: The American study obtained the higher value of total kcal/day from food and beverages ($2,437 \pm 13$). Furthermore, the percentage of daily energy intake coming from beverages was, for American adults, 21%. Mexico was slightly behind with 19% and Spain ANIBES study registered only 12%. ANIBES showed significantly low AI values for the overall population, but even more alarming in the case of males. Only 12% of men, in contrast with 21% of women, do satisfy the EFSA criterion. The IOM criterion reaches even less with higher recommended values for daily intake. In contrast, 60% of the American population reached the recommended intake of the IOM criterion. However, available data did not allow calculating the percentage reached by the EFSA criterion. Data from the Mexican study did not permit conducting comparisons with IOM or with EFSA. However, the water density criteria (mL/kcal) was higher than 1.

Conclusion: There is a notable difference between all three populations in terms of TWI. Furthermore, within the same population, values of adequacy of

TWI changed significantly when they were assessed using different criteria. More scientific evidence is required for the production of better defined water intake recommendations in the future as well as more studies focusing on beverage consumption patterns in different settings.

Key words: Adequate total water intake. ANIBES. Energy intake. Beverages. Adults.

INTRODUCTION

Adequate total water intake (aTWI) has been proposed by specific age and gender by some international authorities. However, those amounts vary widely. In 2010, the European Food Safety Agency (EFSA) (1) set the aTWI in 2.5 l/day for men and 2.0 l/day for women older than 14 years of age. In contrast, the Institute of Medicine (IOM) (2) of the United States of America set the recommended aTWI in 3.7 l/day for men and 2.7 l/day for women. In Mexico, a panel of experts convened by the Ministry of Health recommended the consumption of 0.75-2.0 l (6 to 8 average size glasses) of water per day. As for other fluids, the panel classified beverages in 6 levels, from level 1 (those that should be consumed as a major beverage, i.e., water) to level 6, the least preferred beverages that should be consumed in limited quantities. In summary, the current recommendations of the IOM, EFSA and the panel of experts agree on basic aspects. They recognize that the value of "adequate intake" (AI) is a variable event in which differences are in part due to the inter-individual variation for water needs in response to different health status, metabolism and environmental factors such as ambient temperature and humidity, as well as

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individual factors such as age, body size and level of physical activity (3). Furthermore, the water needs also depend partially on overall diet and the water contained in food. Both, the EFSA and the IOM established their recommendations on the same criteria of adequate hydration that include at least 1.0 l per 1,000 kcal and theoretical calculations.

However, there are slight differences in the approach used to support them: IOM set AI based on average intakes observed in NHANES III (Third National Health and Nutrition Examination Survey). They clarify that physical activity and ambient temperature increase water loss and require compensation drinking. Therefore, it uses an osmotic equilibrium criterion to formulate the recommended amounts. In contrast, EFSA based their AI recommendations on a combination of observed intakes in population groups with desirable osmolality values of urine of 500 mOsmol/l and desirable water volumes per energy unit consumed. The intake data were obtained from observational national surveys in healthy populations in 13 different European countries. They recommended that the AIs only apply in moderate environmental temperatures and at moderate physical activity levels (4).

In 2004, the IOM published the daily reference values of nutrients, including the amount of "water, potassium, sodium, chloride, and sulfate" (2). The suggested intake profile was for an adult male with an average intake of 2,200 kcal/day. All beverages represent 12.5% of the total daily energy intake. Fluid intake was 3.9 l, from which 48% of them would be water, 20% unsweetened drinks (coffee and tea) and 32% milk, juice and other calorie beverages.

In Mexico, the obesity epidemic and the observation of the high increase of sugar sweet beverages intake led to establish an expert panel (5) which, in 2008, developed a set of recommendations on beverage intake for a healthy life including portion size recommendations for each beverage category and healthy consumption patterns for men and women: the Beverage Guidance System. The main conclusion of this panel was that water is the best option and first recommendation for ideal beverage. They also support the World Health Organization recommendation that no more than 10% of the daily calorie intake should come from beverages (6,7).

Even though a TWI has been established, methods to estimate daily ingestion and adequacy of water and other fluids at a population level remain insufficient in the literature. The scientific evidence available is not enough to establish a level of TWI that would prevent disease, reduce the risk for chronic diseases or even improve health status. As a result, there is no clear association between maximum or minimum limits of water consumption that might produce a health benefit by diminishing a specific risk. A proper assessment of water and other beverages intake is important in relation to public health for many reasons, including the need to promote the adoption of healthy life styles in order to avoid the growing epidemic of chronic diseases, many of which are related to unhealthy habits of consumption of different foods and beverages (6,7).

The objective of this paper was to compare the average daily consumption of fluids (water and other beverages) in selective samples

of Mexican, US and Spain population, evaluating in each case the quantity of fluid intake and understanding the contribution of each fluid type to the total fluid intake in order to draw conclusions about the adequacy of drinking habits (8). Furthermore, we examined if those populations reached AI values defined by the IOM, the EFSA or by the criterion of water density, a ratio between TWI (ml from food and beverages) and energy intake (kcal). This criterion suggests that water intake is inadequate when the ratio is lower than 1.

METHODS

CHARACTERISTICS OF THE THREE STUDIES COMPARED

1. From Mexico, the National Health and Nutrition Survey in 2012 (NHNS 2012) analyzed current patterns of beverage intake in adults and children. The NHNS 2012 was a nationally representative, cross-sectional, multistage, stratified survey whose main objective was to characterize the health and nutritional status of the Mexican population (9). It was conducted from October 2011 to May 2012 on a total of 10,343 individuals. Dietary intake was collected by trained interviewers using a single 24-h recall from both weekdays and weekend days (9,10). Respondents reported all foods and beverages consumed in the previous 24-h time period. Each interviewer was provided with a manual with photos of commonly consumed foods, a food scale, measuring cups, and serving spoons of various sizes to help in estimating the amount of food or beverages (in grams or milliliters) reported by each participant. Beverages were first grouped into 10 broad groups: a) water; b) *agua fresca* (beverages mixing water with small amounts of fresh natural fruits); c) coffee/tea; d) soda; e) fruit and vegetable beverages; f) milk and milk-based beverages; g) atole (cornstarch beverage); h) sports and energy drinks; i) alcoholic beverages; and j) other beverages. Sodas were further divided into "caloric" and "low calorie" groups. Other beverages, such as coffee/tea with milk and/or added sugars, were classified as caloric or low calorie using their energy density. Finally, all beverages were classified into 17 specific groups that represent all beverages consumed by the Mexican population. As a result, the mean per-capita consumption of beverages among Mexican adults aged 20-59 years was 382 kcal/day. All beverages represented 19.2% of the total daily energy intake. The top 3 most consumed beverages in these age groups were plain water (74.6%), caloric soda (42.0%), and caloric coffee/tea (37.6%). In terms of calories, caloric soda, caloric coffee/tea, and *agua fresca* were the top three major contributors to the total daily energy intake per capita. Compared with adult women, men had a significantly higher consumption of caloric soda and alcoholic beverages. Women were higher consumers of plain water compared with men (9).
2. From the US, the National Health and Nutrition Examination Surveys (NHANES) 2005-2010 were conducted using

a large and nationally representative database. Estimates of total dietary water from all sources (including plain water), from other beverages and from moisture in foods were compared to the IOM AI values. The analyses used data from three cycles of the study corresponding to years 2005-2006, 2007-2008 and 2009-2010 on a sample of 15,702 adults aged ≥ 20 years. The collection of data was assessed via two 24-h recalls for most respondents, allowing for estimation of usual intakes conducted by trained dietary interviewers in a mobile examination center while the second recall was conducted by telephone some days later (11-14).

Beverages were classified into nine broad groups: a) water (bottled or tap); b) milk (including flavored); c) fruit juice (100%); d) soda/soft drinks (regular and diet); e) fruit drinks; f) sports/energy drinks; g) coffee; h) tea; and i) alcoholic beverages. The NHANES 24-h recalls for each respondent provide information on the amount in grams of each food and beverage consumed. In the analyses of this database, results were all presented in ml of water content from selected beverages, not mean intakes by volume (e.g., ml of water in milk, not ml of milk consumed). Energy intakes from different beverages and foods were estimated for each respondent.

On average, American adults consumed 1.1 l (1,138 ml) of water as a beverage per day. Men and women consumed comparable amounts of water as a beverage. Overall, adults consumed 644 ml/d of tap water (about 56% of total water consumed as a beverage) and 502 ml/d of bottled water (44%). The principal beverage sources were plain water, soda, coffee, tea, milk, and alcohol, followed by fruit drinks and fruit juices. The contribution of plain water, soda (regular and diet), alcohol and fruit drinks to water intakes tended to decrease with age. By contrast, the contribution of coffee and tea to total water intake increased with age. Among adults aged 20-50 years the 83% of total water came from beverages, including 37% from plain water and 17% from moisture in foods. However, 42.7% of men and 40.6% of women adults failed to meet the IOM AI value for total water (3.7 l for men and 2.7 l for women). The contribution of beverages to energy intakes was 21.7% among younger adults (aged 20-50) and declined with the age. For this age group soda accounted for 5.7% of energy intakes (15).

3. From Spain, the ANIBES study was conducted using stratified multistage sampling (16,17). To guarantee better coverage and representativeness, the fieldwork was performed at 128 sampling points across Spain. The final sample comprised 2,007 individuals (1,011 men, 50.3%; 996 women, 49.7%). The fieldwork for the ANIBES study was conducted from mid-September 2013 to mid-November 2013. To equally represent all days of the week, study subjects participated during 2 weekdays and 1 weekend day. For food and beverage records, the study participants were provided with a tablet device (Samsung Galaxy Tab 2 7.0) and trained in recording information by taking photos of all food and drinks

consumed during the 3 days of the study, both at home and outside home. Photos were to be taken before starting to eat and drink, and again after finishing, so as to record the actual intake. Additionally, a brief description of meals, recipes, brands, and other information was recorded using the tablet device. Participants who declared or demonstrated that they were unable to use the tablet device were offered other options, such as digital camera and paper record and/or telephone interviews. In addition to details of what and how much was eaten, for each eating/drinking event, participants recorded where they were, who they were eating with, and whether they were watching television and/or sitting at a table. After each survey day, participants recorded if their intake was representative for that day (or the reason why if it was not), and details of any dietary supplements taken. The survey also contained a series of questions about participants' customary eating habits (e.g., the type of milk or fat spread usually consumed) to facilitate further coding. Food records were returned from the field in real time, to be coded by trained coders who were supervised by dietitians. An *ad hoc* central server software/database was developed for this purpose, to work in parallel with the codification and verification processes. A food photographic atlas was used to assist in assigning gram weights to portion sizes (16,17). Beverages were combined into eight categories for further analysis: a) hot beverages included hot tea and coffee (iced teas in cans or bottles were considered as caloric soft drinks); b) milk (all types of milk without separation by fat percentage); c) fruit and vegetable juices (including nectars and juice-milk blends); d) caloric soft drinks (including sports drinks such as isotonic drinks with mineral salts and caffeinated energy drinks, among others); e) diet soft drinks (with non-sugar sweeteners); f) alcoholic drinks, including two groups: low alcohol grade (mostly beer, wine, and cider) and high alcohol grade; g) water (including tap water and bottled water); and h) other beverages (including soy-based beverages and non-alcoholic beer, among others) (18).

On average, the TWI for adults' age range 18 to 64 years was 1.72 l/day for men and 1.61 l/day for women. Neither men nor women consumed sufficient amounts of water, according to EFSA AI reference values. Men consumed approximately 31% less than the AI and women nearly 20% less. The relative contribution to total EI from beverages was 12.5% for both, men and women. Furthermore, 68% of the TWI came from beverages and 32%, from food. Water was the most frequently consumed beverage followed by milk, for both sexes. Among men, the decreasing order of consumption was alcoholic drinks, caloric soft drinks, and hot beverages, with similar percentages (11.0%, 10.7%, and 10.5%, respectively). For women, the decreasing order was hot beverages (12.5%), caloric soft drinks (8.2%), and alcohol (5.3%). Fruit and vegetable juices and diet soft drinks were consumed in lower amounts by both sexes. In general, the contribution of water intake from food increased with age. This finding is possibly due to lower consumption

of fruits and vegetables, which are rich in water, for the youngest participants. Water contribution from beverages declined with age. For adults, the principal sources of total dietary water were plain water, followed by milk. Regarding alcoholic drinks, on average, consumption for men and women were 160 g/day (SE 9.05) and 71 g/day (SE 4.85), respectively. Caloric soft drinks contribution for the entire population was 2.3% of the total kcal per day. However, consumption was lower among adults than in adolescents and among women than in men (18).

RESULTS

The different categories of beverages were used to establish the pattern of energy intake for each of the three studied countries. Only the pattern of adults was selected. TWI of each study was compared with the EFSA, and IOM AI recommendation. Furthermore, the criterion of the water density was applied (ml/kcal) in order to provide a more comprehensive estimate of the proportion in which each country fulfilled the established aTWI. This criterion is based on the energy content per unit volume. The desirable water to energy ratio is considered as other index of AI (15). The value suggested was 1.0 l per 1000 kcal of energy intake. However, this value could be increased to 1.5 l/1,000 kcal depending on the activity level and water loss. TWI for adults should be no less than 1.0 l/1,000 kcal (15).

Table I shows the dietary sources of energy (%) from subgroups of beverages in the three studies included. In order to allow comparison between all three studies, some groups of beverages should be linked (i.e., in the Mexican study, "atole" was included as "other beverages"). Among the three studies selected, the

American study obtained the higher value of total kcal/day from food and beverages ($2,437 \pm 13$). Furthermore, the proportion of energy from beverages for average American adults was 21% (also the highest value in the three studies). Mexico was closely behind with 19%. Regarding Spain, the ANIBES study registered a value of 12%, very close to the recommendations of the EFSA, IOM and the panel of experts from Mexico. Milk was the beverage with the highest caloric intake in the Spanish study. It contributed 4.9% of the calories, followed by alcoholic beverages, with 2.9% of the calories. However, in the US study the percentages from the same drinks were higher than in Spain (6.6% and 6.1%, respectively). In Mexico, sugared soft drinks contributed with 6.8% of the calories, followed by coffee and herbal teas (4.1%). Surprisingly, juices and nectars and alcoholic drinks contributed the lowest amount of calories (0.9 and 2.2%, respectively) when compared with Spain and the US.

Table II shows the evaluation of adequacy between TWI value of each included study and the aTWI values provided by EFSA, IOM and the ratio ml/kcal. In the ANIBES study from Spain, only 12% of men and 21% of women fulfilled the EFSA recommendation of aTWI (data not shown) (18). Men reached only 69% of the 2.5 l/day recommended, which translates as a deficit of 0.78 l/day (or 31% below the daily recommendation). Women reached an 80% of the recommended aTWI of 2.0 l/day, meaning they had a deficit of 0.4 l/day (20% below the daily recommendation).

When IOM recommended values are taken into account, the percentages of both, men and women, in the Spanish population become even lower. Men reached only 46.5% of the recommended value of 3.7 l/day and women 60% of 2.7 l/day.

The ratio obtained between ml/kcal ingested was 0.87 for men and 0.97 for women, both values below 1, which is considered as insufficient intake.

Table I. Dietary sources of energy (%) from beverages subgroups in the Spanish, US and Mexican population

% Energy (kcal/day)	ANIBES study 2013 Spain	NHANES 2005-2010 US	NHNS 2012 MX
	<i>18 to 64 years</i>	<i>20 to 50 years</i>	<i>≥ 20 years</i>
n	1,587	8,389	3,272
Total kcal/day from food and beverages (mean)	1,816	2,437	2,010
% Energy from beverages only (mean)	12.1	21.7	19
Water	0.0	0.0	0.0
Milk	4.9	6.6	3.1
Sugared soft drink	2.1	5.7	6.8*
Energy and sports drink	0.1	0.6	0.1
Unsweetened soft drink	0.0	0.0	0.1
Juices and nectars	1.3	3.2	0.9
Others non alcoholic drinks	0.3	Nr	1.7^
Coffee and herbal teas	0.2	1.3~	4.1
Alcoholic drinks	2.9	6.1	2.2

*Included flavored water, fruit water, caloric soda. ^Included atole. ~Included coffee and tea. Nr: not reported.

Table II. Relationship between TWI of each study and the AI values provided by EFSA. IOM and the ratio ml/kcal

	ANIBES study 2013 Spain 18 to 64 years		NHANES 2005-2010 US 20 to 50 years		NHNS 2012 MX ≥ 20 years
% Energy (kcal/day)	<i>Men</i>	<i>Women</i>	<i>Men</i>	<i>Women</i>	
n	796	857	8389		3272
Total kcal/day from food and beverages (mean ± SE)	1957 (16.43)	1660 (13.52)	2437 (13)		2010 (28)
Total beverages consumption mL/day (mean ± SE)	1244.7 (21.86)	1169.24 (18.85)	2940 (13)		1500 (25)
Water from food mL/day (mean)	516	476	686	503	Nr*
Water from food + Beverages mL/day (mean)	1717	1608	3973	3016	24440
Water intake mL/day (mean)	582	598	1298	1274	626
EFSA TWI 2.5 L men, 2.0 L women	Population intake is 68.8% (1.7 L) of recommended value Deficit of 31.2% (0.8 L) Only 12% fulfill EFSA AI value for TWI (18)	Population intake is 80.5% (1.6 L) of recommended value Deficit of 19.5% (0.4 L) Only 21% fulfill EFSA AI value for TWI (18)	Population intake is 158.8% (3.9 L) of recommended value Excess of 58.8% (1.5 L)	Population intake is 151% (3.0 L) of recommended value Excess of 50% (1.0 L)	Population intake is estimated on 108% (2.4 L) of recommended value on average for both genders Excess estimated on 8% (0.1 L) ~
IOM TWI 3.7 L men, 2.7 L women	Population intake is 46.5% (1.7 L) of recommended value Deficit of 53.5% (1.98 L)	Population intake is 59.6% (1.6 L) of recommended value Deficit of 40.4% (1.09 L)	Population intake is 107.3% (3.9 L) of recommended value Excess of 7.3% (0.27 L) 57.3% fulfill the IOM AI value for TWI (15)	Population intake is 111.1% (3.0 L) of recommended value Excess of 11.1% (0.3 L) 59.4% fulfill the IOM AI value for TWI (15)	Population intake is estimated on 76.2% (2.4 L) of recommended value on average for both genders Deficit estimated on 23.7% (0.8 L) ~
Ratio mL/kcal > 1 or (1 L/1000 kcal)	0.87	0.97	1.63		1.22

*Not reported. ~Percentages related to the EFSA and IOM AI have been calculated as average (data of TWI by gender are not available).

In the NHANES study, the situation was literally the opposite to that of the Spanish study: 42.7% of men and 40% of women failed to meet the IOM aTWI (data not shown) (15). However, men reached an overall average of 159% of the 2.5 l/day that EFSA recommended as aTWI, which translates as an excess of 59% or 1.47 l/day over the daily recommendation. For women the excess was 1.0 l/day for the EFSA 2.0L recommendation.

Regarding IOM, there was also a surplus, but in this case, the amount was lower. For men, only a 7% excess (0.27 l/day) and for women the excess was only of 0.3 l/day.

The ratio obtained in this study was 1.63 for both genders. The value obtained was above the cut-point 1.

In the Mexican study, we were not able to make calculations separating men and women. Nonetheless, when considering a TWI of 2.4 l/day, on average the EFSA recommendation was nearly reached, but the IOM recommendation was not. Both sexes reached a 76% of this recommended aTWI, meaning they had a deficit of 0.8 l/day. The ratio obtained in this study was 1.22 for both genders.

In line with previous reports on beverage consumption in Mexico (19,20), the results of the selected study indicate that caloric sugar-sweet beverages (SSB) have been the top source of calories derived from beverages in the Mexican population. This current pattern of beverage consumption is also similar to the United States' beverage patterns. However, since the highest peak in SSB

intake in 2001-2002 there has been an important decline in caloric beverage consumption in both, the United States and Mexico (21,22). A great advantage of the NHNS 2012 is that this survey collected very detailed information on plain water consumption. However, this study had several limitations. It is a cross-sectional observational dataset, and the analysis used self-reported intake data, which may be affected by measurement error. Furthermore, the estimates were based on a single 24-h dietary recall; therefore, it may not reflect usual intake or represent the general beverage consumption patterns for the respondents. Despite these limitations, these datasets are the most comprehensive nationally representative data for studying dietary intake in the Mexican population in the last decade (9).

Regarding the US study, the analyses had some limitations. The NHANES data are based on self-reports and are subject to random and systematic reporting errors. Each of the two dietary recall days used different methods to collect the data, which may introduce bias into the estimate of water consumption (15). However, the large sample of the survey helps minimize potential biases of the study.

Concerning the Spain survey, the ANIBES study had several methodological strengths supporting its findings. The use of a 3-day consecutive period was continuously supported by a toll-free telephone number attended by call center-trained operators in order to answer any questions about the software, use of the device, food and beverage record, etc. This careful data collection method is more likely to capture the habitual ingestion of fluids than other methods used in previously published studies. In summary, the study included a careful design, protocol, methodology and employed the highest quality of trendy technology. We do recognize, however, that the study was also subject to few limitations. The possibility of bias in self-reported data is always present, but the large sample collected spreads across age, socio-economic level and geographic region, and greatly reduces the possibility of systematic biases across the whole sample. The study was carried out in the months that represent the transition from summer to autumn seasons (September to November). Season variability is one of the conditions to take into account when the evaluation of the hydration status is performed, so it is also the use of hydration biomarkers that would allow assessment of dietary beverage intake and hydration status without the bias of self-reported dietary intake as well as intra-individual variability (18).

DISCUSSION

The current analysis shows that, in general, American and Mexican population had higher values of energy intake from food and beverages. Results obtained from the Spanish study show significantly low values of aTWI for the overall population, but even more alarming in the case of males. Only 12% of men, in contrast with 21% of women, do satisfy the EFSA criterion. Even less reach the IOM criterion, with higher recommended values for daily intake (3.7 l/day for men and 2.7 l/day for women).

In the American study, almost 60% of the population reaches the recommended intake of the IOM criterion. However, available data does not allow to calculate which percentage reaches the EFSA criterion, it can only be assumed as a higher percentage. Data from the Mexican study neither allows calculations for the IOM nor for the EFSA, but it must be taken into account that the ratio of TWI as ml/kcal was higher than 1 in both, the American and Mexican studies, meaning intake was adequate in broad terms.

The present study raises some questions: a) firstly, how is it possible that the studies' results show such remarkable differences between the amount of food and beverage intake? Is it the methodology used in each study the cause of the different values obtained?; b) secondly, why the recommendations of the EFSA and the IOM are so different? Below these values a person becomes dehydrated?; and finally, c) does an "ideal" pattern of daily liquid intake really exist?

a) Dietary assessment methodologies have different limitations and advantages (23). One of the most frequent limitations is under-reporting. Even with currently available technologies which allow real-time tracking, until now, there is no single method capable of measuring with perfect accuracy the intake of any nutrient (24). In the case of water consumption there is an additional issue to take into account: it is well known that approximately 20% of the total water intake comes from food, while the remaining 80% comes from fluids. Then, it is necessary to calculate the amount of water that food and drinks contribute to daily water intake. Unfortunately, the amount of water in food is not always available at food composition tables. The focus on water intake is relatively new, and therefore, the science needed for robust methodologies are poorly developed (25). While there is no consensus on how to assess water intake in health, it is essential that surveys develop and use fluid-specific methodologies. Until recently, water intake was not always considered as important in population surveys. Undoubtedly, with increasing interest in intake of all fluids, including water, this is gradually changing so that all fluids, including tap water, are recorded. Data collection of intake and hydration status is an essential step in understanding the relationship between hydration and health. Biomarkers are increasingly being used in population surveys. Even though they are expensive and require ethical approval, biomarkers combined with intake data provide valuable information that is needed to set and evaluate public health policy (4,26).

b) If we consider that recommendations of water consumption or "adequate intake" is based on quantitative surveys of food and beverage consumption, it is easy to understand that they are not always sufficiently well suited for populations, even less if we consider different physiological states. The EFSA AI was based on the desirable values of urine osmolality and the observed intake data of population surveys from 13 European countries. On average, the intakes varied from 720 ml/day in Hungary to over 2,621 ml/day in Denmark (1). Countries of similar climate and cultural backgrounds showed very diverse TWI and patterns of the types of beverages consumed. This disparity throughout Europe cannot

be explained solely by the population characteristics or the environmental differences. A great part of these differences derived from the methodology used in the studies (4,27). The population intake data used by IOM are a single dataset that uses the same methodology. The current recommendation in the United States was established based on experimental criteria of electrolyte balance. Although they do not set a limit for daily intake, these proposed values could be excessive, so caution is advisable.

In addition, it is important to consider that both, the EFSA and IOM recommendations included water from food and beverages. In adults, food contribution to the TWI represents 20% to 30%. However, the overall percentage of water from foods varies between countries, seasons and food types or dietary patterns. Diets rich in fruits, vegetables and soups provide greater amounts of liquid than other types of diet. Furthermore, while the AI values established provide some benchmark in evaluating water intake, the proportions below this value should be interpreted cautiously. The use of biomarkers to evaluate hydration status at the population-level should be a priority (15).

- c) Overall, data Available at the literature on beverage consumption pattern is scarce. Although fluid requirements vary widely among individuals and population (2,6), the Spanish Society of Community Nutrition (SENC) (28) made a Healthy

Hydration Pyramid (Fig. 1) to be used as a guide for water intake to Spanish population. It is a practical and convenient tool that sets the recommendation in a simple and didactic way. They divide beverages into five groups: group 1 included mineral water, spring water or tap water with low salt content; group 2 included mineral or tap water with higher salt content, soft drinks non sugar/non-caloric, tea or coffee without sugar; group 3 included beverages with some calorie and nutrient content of interest, natural fruit juices, vegetable juices (tomato, *gazpacho*) and stews, milk or low-fat dairy products with or without sugar, alcohol-free beer, sports drinks, tea or coffee with sugar; group 4 included carbonated soft drinks not sweetened with sugar or fructose; and group 5 included low graduation alcoholic drinks. Although they are not included in the pyramid, moderate consumption is allowed.

CONCLUSION

The difference between EFSA and IOM recommendations is considerably large in terms of TWI. Although both of them include the water contained in food and both have their strengths and weaknesses, there is a notable difference of nearly 50% between European recommended values when compared to those of the

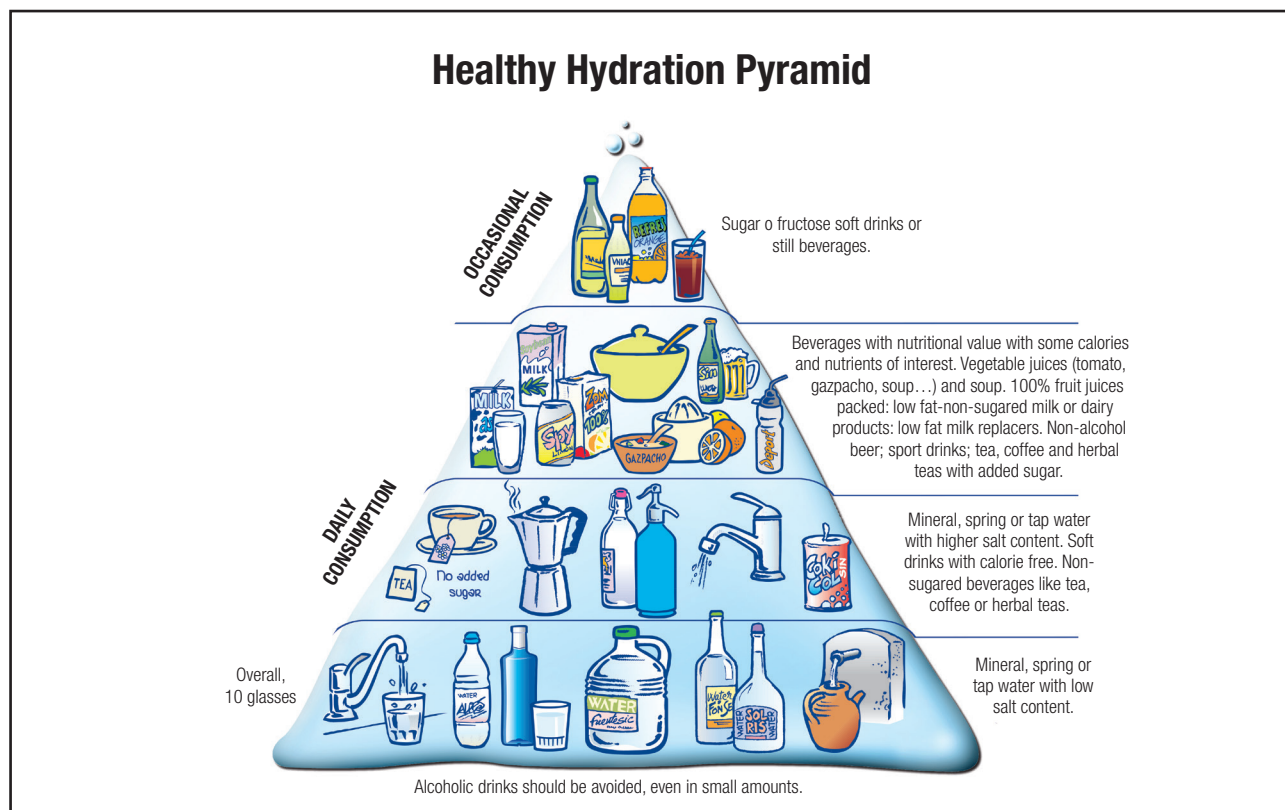


Figure 1.

US. Therefore, it is impossible that percentages of an individual or a given group or population can meet one criteria without under-achieving or, in due case, exceeding the other.

However, although there is great variability in global reference values, the recommendations for aTWI may help to establish public health policies and programs oriented to promote adoption of healthy life styles.

The human body is able to adapt to a wide variety of fluid losses, thanks to the wide ranges of urine osmolarity that the kidneys are able to achieve homeostatic regulation. However, unfortunately, so far there are not enough studies focused on the amount of water needed to prevent disease or improve health. More scientific evidence is required for the production of better defined water intake recommendations in the future as well as more studies looking at beverage consumption in different settings.

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Nutrición Hospitalaria



Urinary hydration biomarkers and dietary intake in children

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Abstract

Introduction: The importance of hydration is undoubtable but reliable data on hydration status and its relation with diet is lacking.

Objectives: We aimed to evaluate the hydration status and its relation to beverages and food intake in children.

Methods: A sample of 172 (50% male), 7-11 year-old children was included in this survey. Participants completed a 24 h urine collection; a 24 hours food recall corresponding to the day of urine collection was applied, weight and height were measured and parents/caregivers filled a lifestyle and socio-demographic questionnaire. The free water reserve was used to assess the hydration status. The intakes of food and beverages were compared according to hydration status using the t-test, Mann-Whitney test or unconditional regression models as appropriate.

Results: More than half of the participants were classified as hypohydrated or at risk of hypohydration (57% in girls and 58% in boys). Compared to hypohydrated children, a significant higher consumption of water (276.2 ± 208.4 vs 188.2 ± 187.4 g/day) and fruit juices (77.6 ± 139.4 vs 14.4 ± 57.2 g/day) was reported by euhydrated boys and girls, respectively. Lower consumers of water and fruit juices showed a higher risk of hypohydration (OR = 2.16, 95% CI: 1.02-4.58, $p = 0.045$), adjusting for confounders.

Conclusions: Most of the children included in this analysis were classified as at risk of hypohydration and those with higher water and fruit juices consumption showed a better hydration status.

Key words: Free water reserve. Children. Dietary intake. Hydration status.

Children are an especially vulnerable group to dehydration since they may have a limited renal excretion ability and difficulty in expressing thirst sensation. In addition, children are susceptible to voluntary dehydration defined as the lack of complete rehydration after a dehydrating stimulus because of inadequate or lack of prolonged thirst (3).

Although acute dehydration is relatively easy to recognize and its consequences are well documented, chronic mild dehydration may pass unnoticed but have adverse consequences for the long-term health (4). In addition, children seem to be particularly at risk of impaired cognitive function (concentration, alertness and short-term memory) due to insufficient hydration (3).

Although hydration status in breastfed infants seems to be similar all over the world, children and adults from different settings show notable differences regarding hydration biomarkers (5). Thus, accurate estimates of water intake and hydration status among populations are essential to identify groups at risk of dehydration.

Since water may be obtained from beverages and foods, it is important to distinguish water sources in populations in order to design strategies to prevent dehydration.

INTRODUCTION

Water is an essential nutrient for human life, the main constituent of the body, influencing the health status of individuals (1). The needs of this nutrient depend on its losses, which vary according to several factors (2).

Everyday water losses through the skin, lungs, stool and urine must be compensated with an appropriate intake. Losses through the skin and lungs vary with exercise, climate, clothing and other environmental conditions. The urinary losses are, in normal conditions, quantitatively the most important varying within a physiological range that depends on the load of solutes and fluid intake, in combination with the diluting and concentrating ability of the kidneys (2).

OBJECTIVES

We aimed to evaluate children's hydration status and its relation to beverages and food intake.

METHODS

A cross-sectional survey conducted between January and June 2014, included elementary school children (7-11 years old) from Portugal. Details about the aims and procedures involved in the study were explained to parents and caretakers

of 488 children, attending the 3rd and 4th grade. Participants were also informed that participation was voluntary and that they were able to withdraw at any time. All students and their parents received written information on the project. Of the 202 (41.4%) children who agreed to participate, all collected a 24-h urine sample, and 30 (16.3%) were excluded for incomplete 24-h urine collection according to the coefficient of creatinine (described in detail below), remaining a final sample of 172 participants (86 girls).

Prior to data collection, parents provided written informed consent and children gave oral assent. All schools where the study was carried out and the Ethical Committee of the University of Porto approved the protocol of study.

Trained researchers collected data through structured interviews. Dietary intake was assessed by a 24-h dietary recall; anthropometric measures were taken, and a 24-h urine samples were collected. Parents/caretakers provided information on socio-demographic characteristics, namely age and sex, parental education level, and children's physical activity. Body weight measurement was obtained using an electronic scale (TANITA® TBF-300A, capacity 200 kg, accuracy 100 g) and the height was obtained using an estadiometer (capacity 200 cm, accuracy 1 mm) with the head in the Frankfort plane. Participants wore lightweight clothing and no shoes. Body mass index (BMI) was computed as mass, weight (kg)/height² (m). After calculating the BMI, it was plotted on the WHO BMI-for-age growth charts and obtained a percentile ranking, classifying children as follows: underweight (less than the 3rd percentile), normal weight (3rd to less than the 85th percentile), overweight (85th to less than the 97th percentile) or obese (equal to or greater than the 97th percentile) (6). The physical activity questionnaire included the time spent watching TV/video during most days of the week (< 2 h/day; and ≥ 2 h/day); sleeping duration (≤ 8 h/day; 9 h/day; and ≥ 10 h/day); and practice of sports activities besides the physical education classes at school (≤ 1 time/week; 2-3 times/week; > 4 times/week) (7). Parents and caretakers were given verbal and written instructions in assisting children to collect a 24-h urine sample and received a standard sterilized urine collection bottle. On the first morning of the urine collection, instructions were given to discard the first specimen, and from then on to collect all specimens for up to 24 h, including the first specimen of the following day. The samples were analyzed by certified laboratories for 24-h creatinine (mg/day), 24-h urine volume (ml), and 24-h urine osmolality (mOsm/kg).

The 24-h urine collections were assessed for completeness using creatinine excretion in relation to weight (i.e., creatinine coefficient), calculated by the following formula: creatinine coefficient $\frac{1}{4}$ creatinine mg ÷ \bar{P} = day body weight kg ÷ \bar{P} . Creatinine coefficients above of 0.1 mmol · kg⁻¹ · day⁻¹ were classified as indicating an acceptable 24-h urine collection (8).

The hydration status was evaluated based on the free water reserve (FWR) parameter (ml/24 h) calculated by subtracting 24 hour urine volume to obligatory urine volume (Solute in urine 24 h [mOsm/day]/[830-3.4] × [age - 20]) and allows for the classification of the 24 hour hydration status (euhydrated vs hypohydrated subjects or at risk of hypohydration (9).

When participants delivered the 24-h urine collection, a 24-h dietary recall questionnaire was applied, taking into account the *Manual de Quantificação dos Alimentos* (10). Participants were questioned accurately about their food and drinks consumption, even reporting cooking methods, brands and consuming time and place. The software Food Processor® (ESHA Research, USA) was used to convert food into nutrients.

To identify under-reporters, Goldberg cut-offs were used as direct comparison of energy intake (EI) to energy expenditure (11). Goldberg cut-off values were applied to exclude under-reporters based on physical activity level (PAL) and compared with the ratio of EI to basal metabolic rate (BMR). BMR was calculated using the Schofield equations for children based on age, gender, height and weight (12).

Food records considered as implausible were found in 19 children. As there were no significant differences detected between the two groups for the hydration variables the total sample was included for the analysis.

Food and beverages groups were created in order to estimate their contribution to total water intake in water, fruit juices, soft drinks, milk/yogurt, tea/coffee, fruit, vegetables, and others (foods with reduced water content). We also considered high and low intake of these food groups based on, respectively, intakes at or above the median, and below the median. Statistical analysis was conducted using SPSS Statistical Package® 21.0 (IBM Corporation, 2012).

Continuous variables were presented as mean and standard deviation, and percentiles, and categorical variables were summarized as counts and percentages. Kolmogorov-Smirnov test was performed to test variables for normality. Independent samples t-test (parametric variables) and non-parametric test (Mann-Whitney U) were used to identify sex differences for urinary biomarkers. Categorical variables were tested using the Chi-squared test. For food/beverages groups whose contribution for water varied according to hydration status, unconditional logistic regression models were fitted in order to estimate the magnitude of the association between their contribution for water intake and the hydration status.

RESULTS

The mean age of the 172 children evaluated was 8.7 ± 0.8 years. The prevalence of overweight and obesity was 37.2% (36% overweight and 1.2% obesity) in girls and 33.7% (30.2% overweight and 3.5% obesity) in boys. The proportion of children engaged in physical exercise ≤ 1 time/week was 54.8% in girls and 37.2% in boys, whereas the proportion of children who sleep ≤ 8 h/day was 21.1% in girls and 29.1% in boys. The proportions of children who spent two or more hours watching TV/video during most days of the week were 9.3% in boys and 4.7% in girls. Nearly half of parents reported to have ≤ 9 schooling years (Table I).

Nutritional intake is described in table II. The contribution of protein (16.8% in boys and 17.1% in girls) and sugar (20% in

Table I. Characteristics of participants

		Boys (n = 86)	Girls (n = 86)	p
Age (years)	7	1 (1.2%)	0 (0%)	0.583
	8	39 (45.3%)	46 (53.5%)	
	9	31 (36.0%)	27 (31.3%)	
	10	12 (14.0%)	12 (14.0%)	
	11	3 (3.5%)	1 (1.2%)	
Mother's education* (years)	≤ 9	42 (48.8%)	35 (40.7%)	0.190
	< 9	32 (37.2%)	41 (47.7%)	
Fathers's education* (years)	≤ 9	41 (47.7%)	36 (41.9%)	0.261
	< 9	28 (32.6%)	36 (41.9%)	
BMI (Kg/m ²)	Under/Normal weight	57 (66.3%)	54 (62.7%)	0.468
	Overweight	26 (30.2%)	31 (36.0%)	
	Obesity	3 (3.5%)	1 (1.2%)	
Under and over reporters	Yes	11 (12.5%)	8 (9.3%)	0.466
	No	75 (87.2%)	78 (89.0%)	
Physical exercise* (times/week)	≤ 1	32 (37.2%)	47 (54.8%)	0.047
	2-3	33 (38.4%)	16 (18.6%)	
	≥ 4	8 (9.3%)	4 (4.7%)	
Sleeping (h/day)*	≤ 8	25 (29.1%)	18 (21.0%)	0.374
	9	27 (31.4%)	36 (41.3%)	
	≥ 10	22 (25.6%)	25 (29.1%)	
TV viewing (h/day)*	< 2	65 (75.5%)	64 (74.4%)	0.411
	≥ 2	8 (9.3%)	4 (4.7%)	

BMI: Body mass index. *Variables with missing values due to incomplete questionnaires.

boys and 19% in girls) for total energy intake was higher than the recommended by the World Health Organization (WHO). There were no significant differences between sexes, except for the mean intake of carbohydrates which was higher in boys (287 g vs 263 g, $p = 0.033$), with no statistically significant differences when carbohydrates expressed as percentage of total energy intake were compared (52% in boys vs 50% in girls, $p = 0.171$).

The overall mean energy intake was $2,245 \pm 560$ kcal for boys and $2,116 \pm 542$ kcal for girls ($p = 0.131$) and the average total water intake was $2,411 \pm 595$ g in boys and $2,286 \pm 649$ g ($p = 0.194$).

Table III presents the urinary biomarkers by sex. Compared to girls, boys showed higher mean 24 h osmolality (667 ± 158 mOsm/kg vs 585 ± 164 mOsm/Kg in girls, $p = 0.003$). The mean FWR was zero in both sexes (0 ± 0.5 ml in boys and girls) and the percentiles 25 and 50 were negative values both in boys (P25 = -239 ml, P50 = -32) and girls (P25 = -299 ml, P50 = -87). The prevalence of children with hypohydration or at risk of hypohydration was 58% in boys and 57% in girls.

Table IV shows the consumption of food and beverages according to hydration status. Euhydrated boys reported a higher water intake than hypohydrated/hypohydration risk ones (276 ± 208 ml

vs 188 ± 187 ml, $p = 0.041$). Among girls, those classified as euhydrated showed a higher intake of fruit juices, compared to hypohydrated/hypohydration risk (78 ± 139 ml vs 14 ± 57 ml, $p = 0.006$). No other significant differences were observed regarding the groups of food and beverages consumed according to hydration status.

Regarding nutritional intake according to hydration status, euhydrated boys showed higher average contribution of protein to total energy intake, compared to hypohydrated/hypohydration risk ones ($18 \pm 4\%$ vs $16 \pm 3\%$, $p = 0.034$). No other statistically significant differences were observed when comparing nutritional intake between euhydrated and hypohydrated or at hypohydration risk children.

A lower consumption of water and fruit juices was associated with a higher risk of hypohydration (OR = 2.16, 95% CI: 1.02-4.58, $p = 0.045$), adjusted for confounders (Table V).

DISCUSSION

Almost 60% of this sample of Portuguese children were classified as hypohydrated or at hypohydration risk according to the

Table II. Energy and nutritional intake by sex

	Boys				Girls				p
	Mean (SD)	Percentiles			Mean (SD)	Percentiles			
		25	50	75		25	50	75	
Energy (Kcal)	2,245 (560)	1,940	2,147	2,626	2,116 (542)	1,788	2,155	2,410	0.131
Water (g)	2,411 (595)	1,983	2,273	2,786	2,286 (649)	1,888	2,293	2,675	0.194
Protein (g)	94 (27)	73	88	116	95 (33)	71	87	110	0.835
Fat (g)	73 (29)	56	70	86	68 (24)	53	67	80	0.249
SFA	23 (11)	16	23	28	22 (10)	15	20	27	0.339
MFA	27 (12)	18	26	33	23 (10)	17	24	29	0.057
PFA	9 (5)	5	8	12	8 (4)	5	7	11	0.179
Carbohydrates	287 (74)	239	274	330	263 (74)	210	263	305	0.033
Sugar	109 (43)	81	103	127	98 (44)	65	91	124	0.107
Fiber	16 (6)	12	15	19	17 (8)	12	15	21	0.439
Protein (% total energy intake, TEI)	17 (4)	14	17	19	17 (5)	14	17	20	0.075
Fat (%TEI)	29 (7)	25	28	33	29 (6)	25	29	33	0.948
SFA (%TEI)	9 (3)	7	9	11	9 (3)	7	9	11	0.947
MFA (%TEI)	10 (3)	8	11	12	10 (3)	8	10	12	0.224
PFA (%TEI)	4 (2)	2	3	5	4 (2)	2	3	5	0.661
Carbohydrates (%TEI)	52 (7)	47	52	56	50 (8)	44	51	55	0.171
Sugar (%TEI)	20 (6)	14	19	22	19 (7)	13	18	23	0.365
Fiber (%TEI)	3 (0)	2	3	3	3 (1)	2	3	4	0.073

SD: Standard deviation; SFA: Saturated fatty acids; MFA: Monounsaturated fatty acids; PFA: Polyunsaturated fatty acids; TEI: Total energy intake.

Table III. Urinary biomarkers by sex

	Boys (n = 86)				Girls (n = 86)				p
24 h urine biomarkers	Mean (SD)	P25	P50	P75	Mean (SD)	P25	P50	P75	
Volume (ml)	763 (316)	588	795	980	739 (323)	528	740	983	0.642
Osmolality (mOsm/Kg)	667 (158)	552	672	796	585 (164)	458	583	712	0.003
FWR (ml)	0 (0.5)	239	32	153	0 (0.5)	-299	-87	156	0.642

SD: Standard deviation; FWR: Free water reserve.

FWR parameter, which is considered to reflect the 24 h hydration status of individuals. Compared to other biomarkers, FWR has the advantage of considering the maximum capacity of kidney concentration and, in addition, it takes into account a safety margin to ensure adequate hydration (9).

The prevalence of inadequate hydration status is higher than the one reported by Manz in German children, although the mean 24 h urine osmolality (667 mOsm/kg in boys and 585 mOsm/kg in girls), an indirect parameter of hydration status, was similar to those described for German children (13), probably reflecting higher non-renal water losses by Portuguese children. This is a relevant aspect, given the particular susceptibility of children to voluntary hydration (14), especially after exposure to physical exercise (15) and in a warm country such as Portugal.

Mean urine osmolalities from healthy subjects from different settings are surprisingly diverse, indicating the large cultural differences that influence the hydration status of different societies. Friedrich Manz, a brilliant German researcher who proposed the free water reserve algorithm, argued that it was not possible to define a physiological or natural narrow urine osmolality range as it is always influenced by cultural context, namely food habits. This author suggested that only after additionally considering 24 h urine volume, urine solute excretion and maximum urine osmolality, it would be possible to quantify the individual 24 h hydration status (9).

In line with urine biomarkers, data from 24 h intake showed a mean total water intake of 2,411 ml and 2,286 ml in boys and girls, respectively. Although these values are above those docu-

Table IV. Food and beverages intake according to hydration status by sex

	Boys (n = 86)			Girls (n = 86)		
	<i>Food and beverages intake (g)</i>			<i>Food and beverages intake (g)</i>		
	<i>Mean (SD)</i>			<i>Mean (SD)</i>		
	<i>Hypohydrated</i>	<i>Euhydrated</i>	<i>p</i>	<i>Hypohydrated</i>	<i>Euhydrated</i>	<i>p</i>
Water	188 (187)	276 (208)	0.041	332 (253)	281 (219)	0.359
Fruit juices	48 (122)	64 (134)	0.484	14 (57)	78 (139)	0.006
Soft drinks	357 (388)	248 (277)	0.186	161 (189)	212 (346)	0.844
Milk and yogurt	453 (277)	503 (271)	0.414	520 (278)	506 (242)	0.804
Tea/coffee	8 (41)	8 (31)	0.770	13 (53)	10 (46)	0.699
Fruit	195 (156)	238 (187)	0.353	219 (158)	166 (141)	0.137
Vegetables	428 (288)	517 (342)	0.198	439 (312)	370 (263)	0.287
Others	667 (198)	648 (189)	0.661	612 (210)	626 (207)	0.831

SD: Standard deviation.

Table V. Odds ratio (OR) for the hypohydration status according to the consumption of water and fruit juice

Water and fruit juice	OR*	CI 95%
High	1	
Low	2.16	(1.02-4.58)

OR: Odds ratio; CI: Confidence interval. *OR adjusted for sex, age, BMI categories, parental education, implausible energy intake and energy and protein intake.

mented in Germany, France and the USA (13,16,17), methodological differences, such as the methods used to assess dietary intake and the participants' age range, may contribute to explain the differences observed. Nevertheless, we found a very low proportion of children (18.6% of boys and 25.6% of girls) who met the European Food Safety Agency (EFSA) reference values for water (2), which ranged from 1.6 ml/day at the age of 4-8 and 2.1 ml/day at the age of 9-13.

The mean FWR was zero and the percentiles 25 and 50 were negative values on both sexes, emphasizing the need to intervene in this population in order to improve fluid intake and hydration status.

Euhydrated boys and girls reported a higher water and fruit juice consumption, respectively, than hypohydrated or at hypohydration risk children. Given the small sample size, for the logistic regression analysis we merged in one category water and fruit juice, and it was observed that a low (below the median) consumption of those beverages was associated with an increased odds of hypohydration or hypohydration risk, adjusting for confounders. No other food or beverage group showed a significant association with hydration status. Water and fruit juices were significantly associated with a better hydration status, which may indicate action tips for caregivers, educators and politicians who should

provide an environment where the access to beverages and food rich in water is encouraged.

In conclusion, a high proportion of this sample of Portuguese children were classified as hypohydrated or at risk of hypohydration, which reinforces the need to implement policies to increase the consumption of fluids and foods rich in water in children, an age group particularly vulnerable to the negative effects of hypohydration on cognitive performance (18). In addition, strategies promoting a good hydration status may be associated with the prevention of non-communicable diseases throughout life (1).

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DECLARATION OF INTEREST

P.P. was a member of the Scientific Board of the Portuguese Institute of Hydration and Health between 2008 and 2015.

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Nutrición Hospitalaria



Dietary intake according to hydration status in 9-10 year-old soccer players

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Abstract

Introduction: Children have an increased risk of voluntary dehydration especially during physical activity which may increase the risk of non-compensating water losses.

Objectives: The aim of this study was to assess the hydration status and its relation to food intake in a children group of soccer players.

Method: A sample of 36 boys aged 9-10 years was included in this study; 30 completed a 24 h urine collection. Participants completed a 24 h urine collection; a 24 hours food recall corresponding to the day of urine collection was applied, weight and height were measured and parents/caregivers filled a lifestyle and socio-demographic questionnaire. The free water reserve (FWR [ml/24 h] = urine volume [ml/24 h] - obligatory urine volume [ml/24 h]) was used to assess the hydration status. Food and beverage groups were created and models of unconditional logistic regression were fitted in order to estimate the magnitude of the association between the hydration status and diet.

Results: Forty three per cent of participants were classified as at risk of hypohydration. Children who reported a high fruit and vegetables intake (above the median) were at decreased risk of hypohydration (OR = 0.19, 95% CI 0.04-0.94, p = 0.041), compared to children who reported a low fruit and vegetables intake.

Conclusions: Almost half of the children were at risk of hypohydration. Our results suggested that water food sources such as fruit and vegetables may contribute to euhydration.

Key words: Free water reserve. Children. Dietary intake. Hydration status. Sport.

ological disturbances as headaches, obstipation, urolithiasis and renal malfunction (5). However, water consumption habits are complex and receive under-attention when evaluating eating habits (6).

Soccer is characterized as a high intensity exercise with a general intensity of 70-80% VO₂ max (7). The elevation of body temperature and the sweating response results from the high intensity of this sport and normally generate a loss of body water that needs to be replaced to avoid dehydration (8).

The amount of water in children is approximately 75% of their body weight (9), and in the case of young athletes, adequate fluid intake is important to maintain a satisfactory body hydration status and avoid the risk of low performance and heat injury (10).

As far as we are aware, very few studies addressed the hydration status in children using FWR as hydration marker and dietary intake, particularly fruit and vegetable intake (11) which favors hydration. New studies are needed focusing on dietary intake and hydration status (5,6) and the present study aims to contribute to fill this research gap in children soccer players.

INTRODUCTION

Water is essential to a wide variety of physiological functions in cell structure, metabolic transportation and temperature regulation, as well as in residual elimination (1). The human organism regulates body water content in order to maintain the equilibrium of fluid ingestion and excretion through the hypothalamic mechanism of thirst control, antidiuretic hormone regulation, renal function, breathing and perspiration (2,3).

The demand of water intake varies between subjects and depends on environmental factors such as climate conditions, lifestyle, physical activity and eating habits. The water intake should be sufficient to compensate daily losses (4). An appropriate hydration can reduce the risk of several diseases and physi-

OBJECTIVES

The aim of this research was the evaluation of the hydration status and its relation to food intake in a children group of soccer players.

METHODS

This study was performed in a convenience sample of 30 male 9-10 years soccer players from a club of the north of Portugal, which completed a 24 h urine collection. Additionally, a food record corresponding to the day of urine collection combined with a lifestyle and socio-demographic questionnaire was filled with their parents help.

URINE SAMPLING AND ANALYSIS

To estimate hydration status, urine samples were collected during 24 hours, on Sunday 23rd of November 2013. The mean temperature was 9 °C, with 59% humidity. All subjects and their parents received written instructions on the urine collection procedure. Only the first morning urine was discarded, while the first urine of the following day was considered. The urine collection was stored in individual containers that were maintained at low temperatures until analyzed. A certified laboratory analyzed the urine samples to determine urine volume (ml), creatinine (mg/d) and sodium content (mg/d) (12). Every child had a weight-related 24 h creatinine excretion rate equal or higher than 0.1 mol kg⁻¹ d⁻¹ (13,14), so the total sample was considered.

DATA COLLECTION

Trained interviewers from the Faculty of Nutrition and Food Science of the University of Porto performed data collection. A Tanita segmental BC-418 was used to evaluate the subject's weight (kg). Height (m) was obtained using a portable stadiometer and waist perimeter (cm) (29) was analyzed through a SECA® tape measure.

Body mass index (BMI) was calculated according to the following formula: BMI = (weight [kg])/(height² [m²])

BMI percentiles were classified using the OMS growth curves (5-18 year-olds): low BMI (< percentile 3), normal BMI (≥ percentile 3 and < percentile 85), overweight (≥ percentile 85 and < percentile 97) and obesity (≥ percentile 97) (15,16).

HYDRATION STATUS AND FWR

The free water reserve, calculated as FWR (ml/24 h) = (measured urine volume [ml/24 h] - obligatory urine volume [ml/24 h]) was used to assess hydration status. The obligatory urine volume (ml/24 h) was derived from the excreted 24 h urine solutes (mOsm/24 h; mainly determined by urinary concentrations of nitrogen, sodium, potassium, and phosphorus) divided by the mean value (2 standard deviation) of maximum urine osmolality of the renal concentrating test for healthy subjects in the respective age group. In children and young adults having a typical affluent Western diet, this value is 830 mOsm/l. Positive and negative FWR values correspond to "euhydrated state" and "risk of hypohydration", respectively (17).

DIETARY ASSESSMENT

To estimate individual food and nutrient intake one-day dietary record was used. Images of the Manual of Quantification of Foods (18) were also used to obtain a more accurate portion intake. To convert food into nutrients (including water composition of food), the Food Processor Plus® software was used (ESHA Research, USA). This software follows food composition guidelines of the

United States Department of Agriculture, however, typically Portuguese food were added to this database of nutritional content of foods, according to the Portuguese Food Composition Table (PFCT) (19,20).

Foods and beverages were grouped in four categories (Table I): "milk and dairy products", "fruit and vegetables", "coffee, barley, soft drinks, juices and water" and "others"; and then, each category was divided in two groups according the median of the ingested amount: "low consumption" (≤ 50th percentile) and "high consumption" (> 50th percentile). Models of unconditional logistic regression were fitted in order to estimate the magnitude of the association between the contribution of different food groups and the hydration status.

The total energy intake was used to reject potentially implausible records by relating it to the basal metabolic rate (21) considering age- and sex-specific cut-offs (22). Eight children presented implausible eating records, however, the data was analyzed considering the overall sample since non-significant differences in the hydration variables were detected between children with plausible and implausible eating records.

STATISTICAL ASSESSMENT

Data was analyzed with the statistical software IBM SPSS Inc. (Version 21.0). Kolmogorov-Smirnov test was used to test variables normality. Categorical variables were expressed in absolute value and percentage, and the continuous variables in mean ± standard deviation, median (P50) and percentiles (P25, P75). T-student's test was used for independent samples, and the Mann-Whitney U test was utilized to compare cardinal variables. Chi-squared test was used for categorical variables. Statistical significance was considered for a p < 0.05.

Table I. Categorization of dietary intake in food groups

	Foods included
Milk and dairy products	Natural milk, aromatized milk, yogurt, yogurt shake, buttermilk, cream, condensed milk and <i>cottage cheese</i>
Fruit and vegetables	Fresh, dried, canned (without syrup) and frozen fruit
	Soup and all of the vegetables: raw, cooked, canned and frozen
Coffee, barley, sodas, juices and water	Infusions, tea, coffee, barley, soft drinks including light/diet/zero versions (carbonated and non-carbonated), sugar drinks (lemonades and iced tea) aromatized drinks, sport drinks and 100% natural juices. Water (bottled or not)
Others	Meat, fish, eggs, cereals, cheeses non considered on the milk and dairy group, pastry, potato, legume, sweets, spices and foods containing fat

Fruit and vegetables groups were considered in an unconditional logistic regression model, in order to estimate the degree of association between food groups intake and hydration status of children.

RESULTS

The characteristics of children and their parents/legal guardians, according to hydration status, are presented in tables II and III; there are no significant differences between any of these descriptive variables with regard to hydration status. The majority of the children (16, 53.3%) had a normal weight. FWR was positive in 17 children (56.8%), presenting euhydrated status; however, negative FWR values with risk of hypohydration were observed in 13 children (43.3%). Energy intake was $1,683 \pm 566$ kcal, and it was not significantly different according to the hydration status.

In table IV, urine biomarkers of hydration are presented for each of the hydration status categories. Table V shows the intake of the different food groups (low and high intake) according to hydration status, and a significant higher prevalence of euhydrated children is found in the group with a higher intake of fruit and vegetables. This association was confirmed after logistic regression analysis, and children with higher intake of fruit and vegetables were at a lower risk of hypohydration (OR = 0.19, CI 95% 0.04-0.94).

DISCUSSION

This study provides evidence for higher intake of fruit and vegetables and lower risk of hypohydration, defined according

to FWR values, in children athletes aged 9-10. Although there is no fully accepted method to characterize hydration status, FWR may be recommended to define 24-h hydration status (23), and previous studies also revealed that FWR values may be affected by the quality of the diet (24,25). Montenegro-Bethancourt (2013) provided, for the first time, evidence for an association between fruit and vegetables intake and children hydration status. Although other authors (26,27) speculated that fruit and vegetables were an essential predictor of hydration status, we also assessed the association with other food groups such as "milk and dairy products", and "coffee, barley, sodas, juices and water". Nonetheless, our findings revealed that only high "fruit and vegetables" intake was significantly associated with a better hydration status. Therefore, a better hydration status could be achieved not only with water and other fluids but also with the ingestion of fruit and vegetables.

In the present study, urine collection was made on Sunday to facilitate the collection on a 24 h period with the help of the children's parents/legal guardians. Although this day may not represent a typical weekday, Sunday may offer the best logistic conditions to characterize familiar food and beverages intake pattern and avoid the influence of professionals at school, which we considered to be a strength of our study. On the other hand, the small size of the sample and the difficulty in obtaining precise data of the dietary intake were the bigger limitations in our study. Moreover, only one-day urine collection and one 24 h diet record made our results harder to generalize to normal daily behavior.

The results obtained in this research showed that the mean total intake of water was 1.5 l and when compared to the 2.1 l recommended by European Food Safety Authority (EFSA), only 71% of the children met EFSA recommendations. A large pro-

Table II. Age, life style characteristics and nutritional intake according to hydration status

	$\bar{x} \pm SD^a$	Percentile			Risk of hypohydration	Euhydration	<i>p</i>
		P25	P50	P75	$\bar{x} \pm SD^a$	$\bar{x} \pm SD^a$	
Age (years)	9.5 ± 0.5	9.5	9.5	10	9.6 ± 0.5	9.4 ± 0.5	0.285
Sleep (h/day)	9.3 ± 0.6	9.0	9.5	9.6	9.2 ± 0.8	9.3 ± 0.5	0.580
Physical Education classes (min/week)	114.0 ± 34.2	90	135	135	116.5 ± 45.1	112.1 ± 24.4	0.729
Sport activity (min/week)	30.1 ± 47.8	0	0	49	17.3 ± 34.6	39.88 ± 54.9	0.206
Energy (kcal)	$1,683 \pm 566$	1,357	1,552	1,887	$1,699 \pm 688$	$1,670 \pm 474$	0.889
Protein (g)	74.76 ± 20.0	62.5	73.9	85.9	77.8 ± 21.2	72.4 ± 19.5	0.487
Total CH (g)	215.7 ± 102.0	161.0	184.7	269.5	217.5 ± 120.6	214.4 ± 89.2	0.798
Total lipids (g)	54.9 ± 24.0	43.3	52.4	63.2	57.1 ± 21.8	53.2 ± 26.1	0.385
Protein (% TEV)	18.9 ± 6.8	14.9	17.0	21.1	19.8 ± 6.1	18.3 ± 7.3	0.325
Total CH (% TEV)	49.7 ± 11.0	44.5	49.8	55.5	48.9 ± 9.1	50.3 ± 12.5	0.735
Total lipids (% TEV)	29.1 ± 7.9	26.3	29.9	33.2	30.9 ± 4.7	27.9 ± 9.6	0.317
Fiber (g)	19.3 ± 26.1	11.2	14.3	20.5	12.6 ± 6.9	24.4 ± 33.7	0.053
Cholesterol (mg)	219.1 ± 127.5	117.9	188.9	296.9	246.5 ± 127.2	198.1 ± 127.4	0.312
Total water (g)	$1,473.0 \pm 526.2$	1,182.8	1,468.5	1,873.8	$1,444.2 \pm 548.9$	$1,495.1 \pm 524.1$	0.798

TEV: Total energy value; CH: Carbohydrate.

Table III. BMI, education and physical activity according to the hydration status

	n (%)	Risk of hypohydration	Euhydration	p
BMI for age				
Thinness	0 (0%)	0 (0%)	0 (0%)	0.823
Normal	16 (53.3%)	7 (53.8%)	9 (52.9%)	
Overweight	8 (26.7%)	4 (30.8%)	4 (23.5%)	
Obesity	6 (20.0%)	2 (15.4%)	4 (23.5%)	
Education				
3 and 4 year	13 (43,3%)	3 (10.0%)	10 (33.3%)	0.050
5 year	17 (56,7%)	10 (33.3%)	7 (23.3%)	
Education of the mother (years)				
≤ 12	17 (56.7%)	6 (20.0%)	11 (36,7%)	0.310
> 13	13 (43.3%)	7 (23.3%)	6 (20.03%)	
Education of the father (years)				
≤ 12	23 (76.7%)	10 (33.3%)	13 (43.3%)	1.000
> 13	7 (23.3%)	3 (10.0%)	4 (13.3%)	
Physical activity with shortness of breath (h/week)				
< 6	17 (56.7%)	8 (26.7%)	9 (30.0%)	0.638
≥ 6	13 (43.3%)	5 (16.7%)	8 (26.7%)	

BMI: body mass index.

Table IV. Association between food intake and hydration status

Food intake	Risk of hypohydration	Euhydrated	p
Tea, barley, juices, soft drinks and water			
Low intake	7 (46,7%)	8 (53,3%)	0,713
High intake	6 (40,0%)	9 (60,0%)	
Fruits and vegetables			
Low intake	8 (66,7%)	4 (33,3%)	0,035
High intake	5 (27,8%)	13 (72,2%)	
Milk and dairy products			
Low intake	7 (46,7%)	8 (53,3%)	0,713
High intake	6 (40,0%)	9 (60,0%)	
Other foods			
Low intake	6 (42,9%)	8 (57,1%)	0,961
High intake	7 (43,6%)	9 (56,3%)	

portion of hypohydrated children was also found in the study conducted by Phillips (28). However, according to the calculated minimal fluid intake recommendation (during and following an hour of exercise) of 945 ml (10) plus the daily water needs (2,1 l), children should consume a total of 3,045 ml of water in a single

day. Water intake below fluid intake guidelines, particularly considering sport activities, may result in increased risk of heat-related illnesses and increased physiological strain (29,30).

The possibility that inexperienced athletes, attending sports exercise sessions, could fail to drink properly is elevated and it might be explained by anxiety, distraction and diversion (10), typical in this age range. Therefore, coaches and parents should promote the fluid ingestion and educate in the importance of hydration and healthy eating practices in young athletes to improve the overall quality of children's diet and their hydration status (10), and increase sports performance.

CONCLUSIONS

In conclusion, almost half of the children were at risk of hypohydration. Our results suggested that a hydrated state could be better achieved in the group with a higher intake of fruits and vegetables.

DECLARATION OF INTEREST

P.P. was a member of the Scientific Board of the Institute of Hydration and Health between 2008 and 2015.

Table V. Characterization of the urinary variables according to the hydration status

	Mean \pm SD	Percentile			Risk of hypohydration	Euhydrated	
	$\bar{x} \pm SD$	P25	P50	P75	$\bar{x} \pm SD$	$\bar{x} \pm SD$	p
Urinary Volume (mL/24 h)	936,0 \pm 408,3	700,0	885,0	1092,5	643,8 \pm 178,5	1160,0 \pm 395,5	$\leq 0,001$
Osmolality (mosmol/l)	708,1 \pm 175,4	589,5	703,5	851,7	857,3 \pm 93,0	593,9 \pm 131,8	$\leq 0,001$
Urinary Density	1022,3 \pm 5,2	1019,0	1022,0	1026,0	1026,8 \pm 2,6		$\leq 0,001$
Obligatory Volume (mL/24 h)	853,1 \pm 211,4	710,0	847,6	1026,2	1033,0 \pm 112,0	715,6 \pm 158,7	$\leq 0,001$
FWR (mL/24 h)	83,2 \pm 574,5	-339,6	15,5	321,0	-389,1 \pm 198,5	444,9 \pm 498,9	$\leq 0,001$

FWR: free water reserve.

Table VI. Odds ratio hypohydration status according to fruit and vegetable intake

Intake of fruit and vegetable	OR	IC 95%	p
Low	1		
High	0,19	(0,04;0,94)	0,041

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Nutrición Hospitalaria



Fifty years of beverages consumption trends in Spanish households

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Abstract

Objectives: To describe the evolution of non-alcoholic and alcoholic beverages consumption in the Spanish households from the 60's to nowadays.

Methods: This study is based on beverages and food consumption in Spanish households; the data sample consisted of consumption and distribution data, obtained from the Household Budget Survey (HBS) since 1964 to 1991 and from the Food Consumption Survey (FCS) since 2000 to 2014, in collaboration with the Spanish Nutrition Foundation (FEN).

Results: In 2014 the average consumption of non-alcoholic beverages was 332 g/person/day, whereas alcoholic beverages consumption represented 72.6 g/person/day. Consumption of non-alcoholic beverages has increased 721% (1964: 46 g/person/day; 1991: 96 g/person/day; 2000: 240 g/person/day and 2014: 332 g/person/day), whereas alcoholic beverages consumption has decreased roughly a 50% (1964: 145 g/person/day; 1991: 113 g/person/day; 2000: 78.4 g/person/day and 2014: 72.6 g/person/day).

The most consumed alcoholic beverage in 2014 was beer (41.3 g/day), followed by wine (23.0 g/day). Regarding non-alcoholic beverages, the most consumed was water (144 g/day), followed by cola (ordinary: 30.7 g/day and diet: 20.5 g/day).

According to Spanish regions, in 2014 non-alcoholic beverages were the most consumed in the islands (Balearic Islands 521 grams/person/day; Canary Islands 515 grams/person/day), as it was in the nineties (Balearic Islands 148 grams/person/day and Canary Islands 281 grams/person/day). However in 1980-81 the largest consumption of alcoholic beverages was that of Galicia, 408 g/person per day, and the lower in the Canary Islands, 63 g/person per day. In 2014, Murcia and Andalucía represented the regions with the highest consumption of alcoholic beverages.

In 2014, alcoholic beverages provided roughly 1.89% of the total energy and 1.47% of sugars, whereas non-alcoholic beverages provided 3.28% of energy and 15.72% of sugars and, in 2000, alcoholic beverages contributed 2.29% of the energy and 1.47% of sugars and non-alcoholic drinks provided 3.76% of the energy and 22.7% of sugars.

Conclusion: There have been significant changes in the eating patterns at the Spanish homes, especially regarding beverages consumption, over the last five decades. In general, a higher consumption and variety for non-alcoholic drinks has occurred, especially in the islands. In parallel, a decline in alcoholic beverages consumption has been clearly observed.

Key words: Non-alcoholic beverages. Alcoholic beverages. Nutrition survey. Dietary habits.

INTRODUCTION

Many factors affect population health. Some of them, such as age, sex or race, cannot be modified, but others, like food

consumption, can be changed. The population nutritional status depends on the nutritional value of the diet, which varies according to food set; through an appropriate mix, we obtain a healthy diet. Eating habits are the result of a conscious behaviour, mainly collective and always repetitive, which leads to choosing, preparing and consuming a certain menu or food as additional part of their social, cultural and religious habits, and which is influenced by multiple factors (socio-economic, cultural, geographical, etc.). There is a continuous need for updating food and beverage consumption habits, dietary patterns and trends in Spain (1).

In Europe, national and regional lifestyle practices, including dietary habits, have been changing over the past 50 years. Spain has undergone a dramatic social and socioeconomic change since the 1960s, including a massive rural-urban migration and a rapid urbanization process (2).

A rapidly increasing number of people use catering services, restaurants and vending machines, both during weekdays and leisure time, which is also a key factor in understanding changes in diet (1). In addition, there has been a rapid increase the immigrant population, which now represents about 10% of the total population(3). In addition, the economic crisis has been a factor generating a new lifestyle (4).

These changes in dietary patterns and lifestyle appear to have had negative consequences for both, present and future populations, as overweight and/or obesity affect > 50% of the adult population and 25% of the infant/young population (5).

One the main objectives of the Spanish Nutrition Foundation (FEN) is to study, understand and improve the nutrition of the Spanish population, therefore, it has been working jointly with the National Institute of Statistics (INE) and lately with the Spanish Ministry of Agriculture, Food and Environment (MAGRAMA) in the Food Consumption Survey (FCS). This information is also essential in order to obtain information about nutritional parameters and diet quality which allows the identification of dietary patterns from the Spanish population.

The purposes of the present study are to analyze the evolution of beverages intake in Spanish households, according to

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the Household Budget Survey (HBS) and the Food Consumption Survey (FCS), from 1964 to 2014, in the context of the whole diet, as well as to evaluate alcoholic and non-alcoholic beverages intake, stratified by regions, and their contribution to total energy intake.

METHODS

The data sample is about shopping and product entrance into the home obtained from the Household Budget Survey (HBS) since 1964 to 1991 and from the Food Consumption Survey (FCS) from 2000 to 2014.

In both cases, the sample was the whole household and a two-stage sampling method was carried out (1964-2014). The sample size of the HBS was 24,000 randomly selected households and that of the PCA was 12,000 households representative of the Spanish habitat population. The differences between both surveys are listed in table I (6).

A "household" is considered as the person or group of people who occupy a family house together or part of it, and consume foods/beverages and other goods bought from the same budget. Products' data were registered by a scanner the same day the products were acquired, for seven consecutive days. Data from the households have also been considered according to geographical areas. The location of the study was Spain inland plus the Balearic and Canary Islands.

The purchase data obtained in both surveys have been transformed into energy and nutrients. Two different programs were used for that purpose: RSIGMA program, which includes DIETECA base data, for the HBS, and VD-FEN 2.1 program for the PCA. Both programs include the same food Spanish composition tables (7), containing over 600 foods and beverages, distributed

in 15 groups. The use of two different tools for converting data-bases was due to the evolution in time of computer systems.

In order to calculate the average of energy and nutrients intake, quantities were transformed to grams per person per day. The data were also compared to the most updated *Recommended Nutrient Intakes* for the Spanish population to evaluate the adequacy of the diet (7,8).

RESULTS

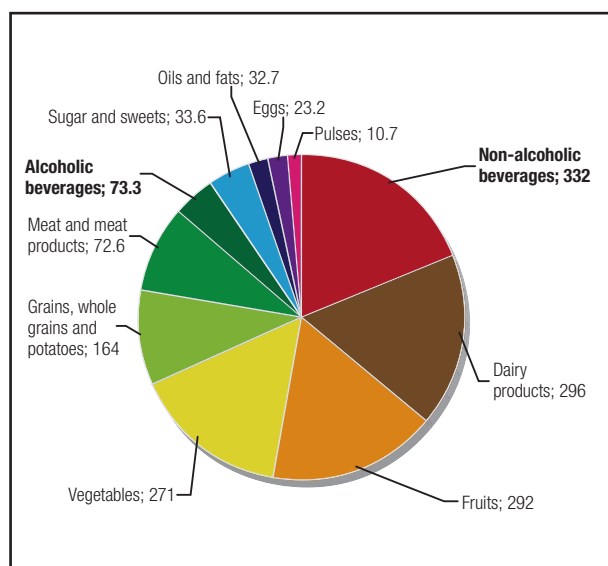
Analysis of food consumption data per capita availability based on the food surveys by the National Statistics Institute, over the period of 1964 to 1991, and the Ministry of Agriculture, Food and Environment over 2000 and 2014, allows estimation of the "average menu" in Spain and the associated distribution of the different food groups as shown in figure 1.

Therefore, as shown, in 2014 the average consumption of non-alcoholic beverages at home was 332 g/person/day, whereas alcoholic beverages consumption was 72.6 g/person/day. Non-alcoholic drinks consumption has increased gradually a 621 % from 1964 to 2014, whereas alcoholic beverages consumption has decreased roughly a 50% (Table II).

As already stated, the consumption of non-alcoholic beverages has largely increased; in 2014 the most consumed drink was bottled water (144 grams/person/day) followed by regular cola (30.7 grams/person/day) and diet cola (20.5 grams/person/day) as shown in table III. The consumption of soft drinks without calories has been steadily increasing in the last years. As for alcoholic drinks, the most consumed was beer (41.3 grams/person/day) followed by wine (23 grams/person/day) (Table IV). Alcoholic beverages consumption has undergone a slow decline during recent years. Within this group, wine, as a beverage traditionally included

Table I. Household Budget Survey (HBS) and Food Consumption Panel (FCS) characteristics

	Food Consumption Panel	The Household Budget Survey
Start	1987	1939
Frequency	Annual	Quarterly
Population scope	Households, hotel industry/restoration and institutions	Family homes, commercial areas and establishments (excluding institutions)
Geographic scope	Inland Spain and Islands (Balearic and Canary)	National territory
Sample size	12,000 households	24,000 households
Criteria breakdown	Geographic areas and autonomous Household socioeconomic status Habitat size Family size Age and activity of responsible for purchasing Presence of children	Autonomous Community Province Size of the municipality Household composition Household income level Educational level of the main breadwinner Quarter survey, etc.
Number of foods	130 food staples disaggregated in 416 inputs (quantified by weight)	187 foods 66 (quantified by weight)

**Figure 1.**

Distribution of the different food and beverages groups at Spanish households in 2014 (g/person per day).

Table II. Evolution of beverages consumption at homes in Spain: years 1964-2014 (g/person per day)

	Year	Alcoholic beverages	Non-alcoholic beverages
	Mean (2000-2014)	75 ± 3.6	298 ± 31.4
PCA	2014	72.6	332
	2013	73.1	326
	2012	81	316
	2011	70.2	328
	2010	68.9	330
	2009	70	338
	2008	77.2	305
	2007	76.1	297
	2006	76.6	291
	2005	75.3	283
	2004	77.4	283
	2003	79	276
	2002	76.3	262
	2001	76.6	256
	2000	78.4	240
EPF	1991	113	96
	1981	170	98
	1964	145	46

Table III. Availability of non-alcoholic beverages in 2014 (g/person day)

Non-alcoholic beverages	Quantity purchased
Bottled water	144
Regular cola	30.7
Diet cola	20.5
Other soft drinks	18.7
Soft drinks, orange	15.8
Nectars	12.3
Beverages juice + milk	10.7
Sodas	9.8
Soft drinks, lemon	8.6
Alcohol-free beer	8.4
Soft drinks, isotonic	7.2
Diet cola without caffeine	5.3
Concentrated others juice	4.3
Soft drinks tea/coffee	4.0
Fruit juice refrigerated/exp	3.7
Juices and nectars rest	2.4
Cola without caffeine	2.4
Concentrated peach juice and mixtures	2.3
Soft drinks, tonic	2.2
Concentrated orange juice and mixtures	1.8
Coffee beans or ground, mix	1.5
Coffee beans or ground, natural	1.4
Concentrated pineapple juice and mixtures	1.1
Coffee beans or ground, decaffeinated	0.7
Soluble coffee	0.7
Infusions	0.3
Vegetable juices	0.3

in the Mediterranean diet concept for the adult population, only represented a 31% of the total alcoholic beverages consumption in 2014, whereas it accounted for 62% of the total consumption in 1991. In the last few years, a gradual substitution of wine for beer has occurred, the latter representing almost 56% of the total alcoholic beverage consumption nowadays.

BEVERAGES CONSUMPTION BY THE AUTONOMOUS COMMUNITIES IN SPAIN

According to the Spanish regions, in 2014 non-alcoholic beverages were the most consumed in the islands (Balearics Islands 521 grams/person/day; Canary Islands 515 grams/person/day),

as it was in the nineties (Balearics Islands 148 grams/person/day and Canary Islands 281 grams/person/day).

Table V shows the data from purchases by regions in 1980-81 and 2014, and allows studying how the trends look like in our country.

Table IV. Availability of alcoholic beverages in 2014 (g/person day)

Alcoholic beverages	Quantity purchased
Beer	41.3
Wine	23
Other drinks with wine	3.6
Sparkling wines, champagne and cava	1.5
Cider	0.8
Other spirituous beverages	0.8
Brandy	0.4
Whiskey	0.4
Gin	0.4
Rum	0.3
Anise	0.2

Table V. Availability of beverages 1880-2014 (g/person day)

Spanish regions	Alcoholic beverages		Non-alcoholic beverages	
	2014	1980-81	2014	1980-81
Andalucía	79.3	123	345	105
Aragón	66.1	149	309	133
Asturias	55	166	272	118
Balears	83	144	521	148
Canarias	61.2	63	515	281
Cantabria	50.5	214	282	122
Castilla-La Mancha	65.5	168	310	148
Castilla y León	52.2	181	284	103
Cataluña	83.9	167	400	191
Extremadura	67.6	90	333	68.5
Galicia	52.7	408	245	132
La Rioja	54.8	221	255	119
Madrid	73.8	144	236	130
Murcia	94.3	237	343	100
Navarra	60.7	210	229	112
País Vasco	70.9	171	208	120
Valencia	75.3	145	380	141

The largest differences regarding availability of a food group between regions, in 1980-81, was observed in the group of alcoholic beverages, the largest purchases were made in Galicia, with 408 g/person per day, and the lowest in the Canary Islands, with 63 g/person per day. In 2014, Murcia and Andalucía were the regions with the highest consumption of alcoholic beverages.

ENERGY AND NUTRIENT INTAKE

Changes in eating and drinking patterns are reflected in energy and nutrient content of the diet in the last 50 years (Table VI). The mean energy intake for the Spanish population in 2014 was 2,219 kcal/person/day, which was much lower than in 1964 (3,008 kcal/person/day), a difference of 789 kcal/day.

In relation to macronutrients, major changes are shown in carbohydrates: in 1964, people consumed approximately 200 grams more than in 2014; lipids (1964: 108 g/person per day vs 2014: 103 g/person per day) and proteins (1964: 87 g/person per day vs 2014: 78.2 g/person per day) had, however, much lower variations in 50 years evolution. In 2014 fiber, consumption (16.6 g/day) was insufficient compared with the recommended amounts (25-30 g/day) while in 1964 the recommendations (27.5 g/day) were met. Finally, sugar consumption has increased 11 grams per day in just 14 years (2000-2014).

Energy contribution to total energy intake from non-alcoholic beverages was 4% in 2000-2006 and decreased 1% in 2014. Due to the increased consumption of sugar-free drinks, the percentage contribution of sugar from non-alcoholic beverages has decreased lately, representing in 2014 15.72% (Table VII).

DISCUSSION

Spanish society has undergone numerous changes in the second half of the twentieth century such as economic development, industrialization and a decrease in family members. We have changed from an almost universal family model to the coexistence of different types of families, so there is now a greater diversity of ways of living (9). Any pattern, however, seems to maintain the principle of "variety", even though the marked changes observed during the 1964-1980 period.

This change has been translated in lower consumption of certain food groups and significant increase of non-alcoholic beverages, being the main target of the present study.

Comparing the present results with those of other related countries, an increase in consumption of some food groups is also observed in other European countries as recorded by the European Nutrition and Health Report based on the balance sheets of FAO (10). Therefore, the Mediterranean countries have significantly reduced the consumption of some foods characteristics of the traditional Mediterranean diet, such as cereals, bread, potatoes, vegetables, olive oil and wine. Conversely, the consumption of meats, especially poultry, and dairy fats has increased significantly. In short, the diet is no longer predominantly vegetarian. From a nutritional point of view, this trend has resulted in an enrichment

Table VI. Evolution of energy intake, macronutrients and fiber in households

	Energy Kcal/day	Protein g/day	Lipids g/day	Carbohydrates g/day	Starch g/day	Sugar g/day	Fiber g/day
2014	2,219	78.2	103	228	128	100	16.6
2010	2,162	78.8	104	219	125	94.1	17.2
2005	2,065	76.1	90.9	225	134	90.8	15.7
2000	2,096	75.9	92	229	140	89.0	15.6
1991	2,634	93.5	121	294			20.6
1981	2,914	98	131	333			21.9
1964	3,008	87	108	423			27.5

Table VII. Energy and Sugar contribution from alcoholic and non-alcoholic beverages (%)

	Alcoholic beverages		Non-alcoholic beverages	
	<i>Energy</i>	<i>Sugars</i>	<i>Energy</i>	<i>Sugars</i>
2000	2.29%	1.47%	3.76%	22.68%
2006	2.04%	1.46%	4.17%	22.75%
2014	1.89%	1.56%	3.28%	15.72%

of dietary fat, saturated fat and cholesterol, salt and sugar at the expense of carbohydrates. That is, the current diet of Mediterranean countries is increasingly moving away from the pattern of prudent and healthy diet that it once represented (11,12). Comparing our results with other European and Spanish studies, we can observe that the main differences are due to the methodology used in each study (11,13,14).

All these changes have led to a deviation of the traditional Mediterranean diet in Spain, despite being a major producer and exporter of typical Mediterranean products (15).

The present study, conducted at national level, updates beverages and food habits and nutritional aspects of the Spanish population. In addition, trends emerging from other surveys mentioned above were considered.

Spanish beverages pattern, and consequently diet trends, may be still considered as varied and healthy, although some trends need to be corrected. More education and information of the large beverages market offered in Spain and their nutritional characterization is urgently needed.

ENERGY AND NUTRIENTS

These variations in the diet have been linked, however, to reduced energy consumption, from 3,008 kcal/person per day

in 1964 to 2,219 kcal/person per day in 2014. It is a very sharp drop, which has not been attached to obesity rates, as they are currently much higher, according to the National Health Survey in Spain: obesity has increased from 7.4% to 17.0% over the past 25 years (16), mainly indicating that obesity rates cannot be only related with energy consumption by population but needs to be considered as a multifactorial complex condition and disease. This decrease in the availability of energy can be explained by the decrease of carbohydrates intake, almost a 200 grams difference in the past 50 years. This led to a lower contribution of fiber intake (1964: 27.5 g/person per day; 2014: 10.9 g/person per day) which clearly indicates that the diet nowadays should include greater variety of foods such as whole grains, legumes, fruits and vegetables.

Regarding the contribution to energy intake from beverages, we can see that it has gradually declined since 2000. Similar results and trends were obtained on the ANIBES study (17).

In conclusion, in the last decades, there have been significant changes in drink intake patterns, with a remarkable increase of non-alcoholic beverage consumption, especially in some Spanish regions where a decline of alcoholic beverages consumption was also observed. The Spanish population diet has notably changed in the last 50 years, differing somehow from the traditional Mediterranean diet. This change resembles the one observed in other European countries (15). Regarding the intake of energy and nutrients, the results show a decrease in energy intake per person. It is necessary to design strategies that encourage a healthy diet allowing the recovery and maintenance of the traditional characteristics of the Mediterranean diet.

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Nutrición Hospitalaria



Macronutrients contribution from beverages according to sex and age: findings from the ANIBES Study in Spain

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Abstract

Methodologies and procedures used in dietary surveys have been widely developed with the aim of evaluating the nutritional status of a population. However, beverages are often either disregarded at national and international assessment of nutrients intake or poorly mentioned. Moreover, there is no standardized questionnaire developed as a research tool for the evaluation of beverages intake in the general population. Moreover, the contribution of different beverages to macronutrients intake is rarely provided. The latter in the context of a continuous expansion and innovation of the beverages market in Spain. Therefore, the main goal of the present study was to evaluate non-alcoholic and alcoholic beverages macronutrients contribution in the ANIBES study in Spain (9-75 years old).

As expected, those contributed to dietary macronutrient intake mainly as total carbohydrates and sugar. The contribution to other macronutrients (proteins and lipids) by the beverage groups was of much less importance. For non-alcoholic beverages, contribution to carbohydrates was much higher in younger populations (children: $10.91 \pm 9.49\%$, mean \pm SD for boys and $9.46 \pm 8.83\%$ for girls; adolescents: $11.97 \pm 11.26\%$ for men and $13.77 \pm 10.55\%$ in women) than in adults: $9.01 \pm 9.84\%$ for men and $7.77 \pm 8.73\%$ in women. Finally, a much lower contribution was observed in the elderly: $4.22 \pm 6.10\%$ for men and $4.46 \pm 6.56\%$ for women. No sex differences, however, across all age groups were found. Results for sugar contribution showed a similar trend: children ($23.14 \pm 19.00\%$ for boys and $19.77 \pm 17.35\%$ for girls); adolescents ($28.13 \pm 24.17\%$ for men and $29.83 \pm 21.82\%$ in women); adults $20.42 \pm 20.35\%$ for men and $16.95 \pm 17.76\%$ in women, $p \leq 0.01$; and elderly: $14.63\% \pm 9.97$ for men and $9.33 \pm 12.86\%$ in women. The main contribution corresponded to sugared soft drinks, juices and nectars, more relevant and significant in the younger populations.

As for alcoholic beverages, the contribution of macronutrients to the total diet is low for carbohydrates and sugar. The main contribution of this group, as expected, is alcohol, being higher from low alcohol content beverages, and in men vs women ($p \leq 0.001$).

Key words: ANIBES study. Nutrition surveys. Macronutrients intake. Beverages intake. Spain.

INTRODUCTION

The beverages groups are characterized by their great heterogeneity and innovation. In Spain, the non-alcoholic beverages segment has traditionally been composed of mineral water, soda, soft drinks, juices and nectars. At the same time, within the alcoholic

beverages category, beer has consolidated as one of the most demanded drinks by Spanish consumers (1). However, the most significant increase is the availability of non-alcoholic beverages: in 1964 roughly a drink was consumed per week (46 g/person per day), but in 2010 consumption increased tenfold (444 g/person per day). On the other hand, consumption of dairy products has also increased considerably from 1964 to nowadays, even though a marked decrease in the last years has occurred, mainly by replacing milk by other non-alcoholic drinks (the latter being the group with a higher growth in the recent years), which may lead to a poorer nutritional density, mainly of concern for children and young population (2). Moreover, the World Health Organization (WHO) acknowledges the high burden of disease caused by unhealthy dietary and lifestyle patterns in many countries of the Region; in particular, they are concerned about the rapid rise of overweight and obesity, especially in children. In their declarations, they support research of the population's nutritional status and behaviours, and accessibility of data to promote new research and better returns on investments (3).

In this regard, the methodologies and procedures used in dietary surveys have been widely developed with the aim of evaluating the nutritional status of a population (4-6). However, beverage is often either disregarded at national and international assessment of nutrients intake or poorly mentioned. Moreover, at present there is no standardized questionnaire developed as a research tool for the evaluation of beverage intake in the general population (7).

The Consensus Meeting on the Methodology of Dietary Surveys, Classification of Physical Activity and Healthy Lifestyles (8) in 2015 highlighted the aims of assessment of beverages as an area of scientific interest that is still in development and seems to be very promising for improving health research (9) and to extrapolate the results to practical recommendations at population level.

Many valuable dietary surveys have been previously conducted in Spain (10-13), although, to the best of our knowledge,

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no one has approached energy and nutrients intake and its determinants using new, more accurate technologies. To fill this gap, the ANIBES study (*Antropometría, Ingesta y Balance Energético en España*, that is, Anthropometry, Dietary Intake and Energy Balance in Spain) was specifically designed to focus on accurate and update collection on macronutrient intake in the Spanish diet, by analysing more detailed and accurate information of food and beverage sources that currently contribute dietary intake.

We have previously shown that beverages contribution to energy intake in the ANIBES study in Spain was 2.9% for non-alcoholic beverages and 2.3% for alcoholic beverages, showing a lower contribution to total energy intake. Sugared soft drinks contributed 2.0% (36 kcal/day out of 1,810 kcal/day) to total energy intake. A lower consumption compared with mean contribution was seen in children (1.9% of energy, 34 kcal/day) and the lowest corresponded to the elderly population (0.7% of energy, 13 kcal/day). Juices and nectars contributed 1.3%, more in children and adolescent than in adults and the elderly. Alcoholic beverages of lower/moderate alcohol content (beer, wine, cider) represented over 90% of energy contribution within this group (14).

OBJECTIVES

The main objective of the present study was to evaluate macronutrients contribution of non-alcoholic and alcoholic beverages in the ANIBES study in Spain.

METHODS

The design, protocol, and methodology of the ANIBES study have been already described in detail (15,16).

SAMPLE

The design of the ANIBES study aimed to define a sample size that is representative of all individuals living in Spain, aged 9 to 75 years. The initial potential sample consisted of 2,634 individuals, and the final sample comprised 2,009 individuals (1,013 men, 50.4%; 996 women, 49.6%). In addition, for the youngest age groups (9-12, 13-17, and 18-24 years), a boost sample was included to have at least $n = 200$ per age group (error $\pm 6.9\%$). Therefore, the random sample plus booster comprised 2,285 participants.

The fieldwork for the ANIBES study was conducted from mid-September 2013 to mid-November 2013, and two previous pilot studies were performed. To equally represent all days of the week, subjects participated during two weekdays and one weekend day, always consecutive days. The final protocol was approved by the Ethical Committee for Clinical Research of the Region of Madrid (Spain).

FOOD AND BEVERAGE RECORD

Study participants were provided with a tablet device (Samsung Galaxy Tab 2 7.0) and trained in how to record information by taking photos of all food and drinks consumed during the three days of the study, both at home and outside. Photos had to be taken before beginning to eat and drink, and again after finishing, so as to record the actual intake. Additionally, a brief description of meals, recipes, brands, and other data was also recorded using the device.

Food and beverages records were returned from the field at almost "real" time, to be coded by trained coders who were supervised by dietitians-nutritionists. The dietitians-nutritionists assigned appropriate codes for all flagged food and portion codes and checked any other queries raised by the coders.

Beverages and their contribution to energy and nutrient intakes were calculated from food consumption records using this software (VD-FEN 2.1), which was newly developed for the ANIBES study by the Spanish Nutrition Foundation and is based mainly on Spanish Food Composition Tables (17), with several expansions and updates. Data obtained from food manufacturers and nutritional information provided on food labels were also included. A food photographic atlas was used to assist in assigning gram weights to portion sizes.

STATISTICAL ANALYSIS

The intake data were grouped into 16 food groups, 38 subgroups and 754 ingredients for in-depth analysis. For beverages, data were grouped into 2 food groups, 10 subgroups and 64 ingredients. Once all dietary intake information had been transformed into energy and nutrient data, these data were processed using different statistical analysis tools and packages.

For those variables normally distributed, comparison between groups was performed by a Student's *t*-test for two independent samples and ANOVA test for more than two independent samples with a 95% confidence interval. Non parametric tests were performed to solve the lack of normality: a Mann-Whitney test for two independent samples and a Kruskal Wallis test for more than two independent samples with a 95% confidence interval. Bonferroni or Games Howell tests were used for non-equal variances to adjust multiple comparisons between groups. The Levene's test was used to check the equality of variances and the Kolmogorov-Smirnov normality test was used to test the normality of the distribution: random sample (2,009 participants) and random + booster sample (2,285). The random sample is used to show total sample data and to compare between sexes. To compare age groups and sex in age groups, a booster sample was included in order to expand those age groups less represented in the random sample. The following statistics were calculated to qualify each variable in the analysis: mean, standard deviation, and variance to measure dispersion in the values, minimum and maximum values, median, quartiles (as well as interquartile range), and deciles to describe the shape of the distribution.

RESULTS

Tables I-V show the dietary sources of macronutrients from the different beverages groups and subgroups by sex by age groups. Data are expressed as means.

Beverages groups mainly contributed to dietary macronutrients intake as total carbohydrates and sugar. The contribution of other macronutrients (proteins and lipids) by the beverages groups was almost irrelevant. Fiber was mostly provided by juices and nectars.

In relation to total carbohydrates and sugars, relevant differences with age were observed. For non-alcoholic beverages, in terms of age, the differences were of importance: a much higher contribution from the non-alcoholic beverage to carbohydrates in children (9-12 years): boys $10.91 \pm 9.49\%$ (mean \pm standard deviation) and girls $9.46 \pm 8.83\%$, and adolescents (13-17 years): men $11.97 \pm 11.26\%$ and women $13.77 \pm 10.55\%$, compared

to adults (men $9.01 \pm 9.84\%$ and women $7.77 \pm 8.73\%$) and the elderly (65-75 years): men $4.22 \pm 6.10\%$, and women $4.46 \pm 6.56\%$. No differences between sexes were found, however, for all age groups.

A similar trend was observed for sugar: children (boys $23.14 \pm 19.00\%$ and girls $19.77 \pm 17.35\%$); adolescents (men $28.13 \pm 24.17\%$ and women $29.83 \pm 21.82\%$); adults (men $20.42 \pm 20.35\%$ and women $16.95 \pm 17.76\%$, $p \leq 0.01$) and elders: (men $14.63\% \pm 9.97$ and women $9.33 \pm 12.86\%$).

Within the non-alcoholic beverages subgroups, for total population, sugared soft drinks contributed $5.07 \pm 8.19\%$ and $4.17 \pm 7.30\%$ ($p \leq 0.01$) of total carbohydrates intake, for men and women, respectively ($4.82 \pm 7.17\%$ - $3.19 \pm 5.06\%$ in children, $7.05 \pm 9.93\%$ - $7.68 \pm 7.97\%$ in adolescents, $5.13 \pm 8.16\%$ - $4.33 \pm 7.46\%$ [$p \leq 0.05$] in adults and $1.51 \pm 3.64\%$ - $1.60 \pm 4.5\%$ in elderly adults, also for men and women). Juices and

Table I. Macronutrients intake (%) from beverages food groups and subgroups in the ANIBES Spanish population by sex (total: 9-75 years)

	Proteins %	Lipids %	Carbohydrates %	Sugar %	Fiber %	Alcohol %
<i>Men (aged 9-75 years) (n = 1,013)</i>						
<i>Non-alcoholic beverages</i>	0.93	0.20	8.96	20.35	0.57	-
Water	-	-	-	-	-	-
Coffee and infusions	0.39	0.00	0.30	0.82	-	-
Sugar soft drinks	-	-	5.07	11.24	-	-
Non-sweetened soft drinks	0.00	0.04	0.01	0.06	-	-
Sports drinks	-	-	0.22	0.45	-	-
Energy drinks	0.23	0.16	0.21	0.46	0.04	-
Juices and nectars	0.31	0.01	3.06	7.10	0.53	-
Other drinks	-	-	0.10	0.22	-	-
<i>Alcoholic beverages</i>	0.49	-	1.78	4.96	-	100.00
Low alcohol content beverages	0.49	-	1.72	4.83	-	93.58
High alcohol content beverages	-	-	0.06	0.14	-	6.42
<i>Women (aged 9-75 years) (n = 996)</i>						
<i>Non-alcoholic beverages</i>	1.42	0.48	7.76	16.77	0.49	-
Water	-	-	-	-	-	-
Coffee and infusions	0.53	0.01	0.40	1.05	-	-
Sugar soft drinks	-	-	4.17	8.76	-	-
Non-sweetened soft drinks	0.01	0.06	0.03	0.10	-	-
Sports drinks	-	-	0.11	0.25	-	-
Energy drinks	0.59	0.40	0.23	0.52	0.05	-
Juices and nectars	0.30	0.01	2.76	6.00	0.44	-
Other drinks	-	-	0.05	0.09	-	-
<i>Alcoholic beverages</i>	0.23	-	0.89	2.21	-	100.00
Low alcohol content beverages	0.23	-	0.86	2.16	-	93.26
High alcohol content beverages	-	-	0.03	0.05	-	6.74

Table II. Macronutrients intake (%) from beverages food groups and subgroups in the ANIBES Spanish population by sex (children: 9-12 years)

	Proteins %	Lipids %	Carbohydrates %	Sugar %	Fiber %	Alcohol %
<i>Boys (aged 9-12 years) (n = 126)</i>						
<i>Non-alcoholic beverages</i>	0.56	0.06	10.91	23.14	0.65	-
Water	-	-	-	-	-	-
Coffee and infusions	0.02	-	0.01	0.02	-	-
Sugar soft drinks	-	-	4.82	10.05	-	-
Non-sweetened soft drinks	0.00	0.01	0.01	0.02	-	-
Sports drinks	-	-	0.41	0.87	-	-
Energy drinks	0.08	0.03	0.01	0.02	-	-
Juices and nectars	0.46	0.01	5.65	12.16	0.65	-
Other drinks	-	-	-	-	-	-
<i>Alcoholic beverages</i>	-	-	0.00	0.00	-	-
Low alcohol content beverages	-	-	-	-	-	-
High alcohol content beverages	-	-	0.00	0.00	-	-
<i>Girls (aged 9-12 years) (n = 87)</i>						
<i>Non-alcoholic beverages</i>	0.60	0.02	9.46	19.77	1.10	-
Water	-	-	-	-	-	-
Coffee and infusions	0.01	-	0.00	0.00	-	-
Sugar soft drinks	-	-	3.19	6.67	-	-
Non-sweetened soft drinks	0.00	0.01	0.00	0.01	-	-
Sports drinks	-	-	0.16	0.32	-	-
Energy drinks	-	-	-	-	-	-
Juices and nectars	0.59	0.01	6.11	12.77	1.10	-
Other drinks	-	-	-	-	-	-
<i>Alcoholic beverages</i>	-	-	0.00	0.00	-	-
Low alcohol content beverages	-	-	-	-	-	-
High alcohol content beverages	-	-	0.00	0.00	-	-

nectars were ranked second, contributing $3.06 \pm 5.71\%$ for men and $2.76 \pm 5.02\%$ in women, but differences were also observed according to age: $5.65 \pm 6.81\%/6.11 \pm 6.80\%$ in children; $4.49 \pm 6.27\%/5.83 \pm 8.66\%$ in adolescents; $2.93 \pm 5.63\%/2.55 \pm 4.53\%$ in adults and $2.01 \pm 4.91\%/1.98 \pm 4.50\%$ in the elderly, for men/women. A similar trend for sugar was shown. Lately, other types of drinks represented a much smaller contribution to carbohydrates and sugar intake. As for alcoholic beverages, the contribution of macronutrients to the total diet is low for carbohydrates and sugar. The main contribution of this group, as expected, is alcohol.

Fiber is basically contributed by juices and nectars (men $0.53 \pm 2.02\%$ and women $0.44 \pm 1.58\%$) and in a lower proportion by energy drinks (men $0.04 \pm 0.59\%$ and women $0.05 \pm 0.72\%$).

There were differences between the contribution of proteins and lipids by beverages as age and sex, but the amount contribut-

ed to the total diet for these groups is not very relevant. In relation to proteins, the group of non-alcoholic beverages, basically as low alcohol content beverage (beer, wine, etc.), was $0.93 \pm 1.96\%$ for men and $1.42 \pm 2.54\%$ for women ($p \leq 0.001$), and regarding high alcohol content beverages it was $0.49 \pm 1.06\%$ for men, and $0.23 \pm 0.62\%$ ($p \leq 0.001$) for women. As for lipids, the contribution of the group of non-alcoholic beverages to men intake was $0.20 \pm 1.38\%$ and $0.48 \pm 1.69\%$ to women intake ($p \leq 0.001$), whereas alcoholic beverages did not contribute to lipids intake.

DISCUSSION

The ANIBES study was designed to obtain accurate and updated information on the anthropometric data, macronutrients and micronutrients intake and expenditure, as well as the practice of physical

Table III. Macronutrients intake (%) from beverages food groups and subgroups in the ANIBES Spanish population by sex (adolescent 13-17 years)

	Proteins %	Lipids %	Carbohydrates %	Sugar %	Fiber %	Alcohol %
<i>Men (aged 13-17 years) (n = 137)</i>						
<i>Non-alcoholic beverages</i>	0.52	0.08	11.97	28.13	1.02	-
Water	-	-	-	-	-	-
Coffee and infusions	0.05	-	0.06	0.19	-	-
Sugar soft drinks	-	-	7.05	16.30	-	-
Non-sweetened soft drinks	0.00	0.02	0.01	0.03	-	-
Sports drinks	-	-	0.08	0.14	-	-
Energy drinks	0.06	0.04	0.04	0.09	0.03	-
Juices and nectars	0.41	0.01	4.49	10.81	0.99	-
Other drinks	-	-	0.24	0.57	-	-
<i>Alcoholic beverages</i>	0.00	-	0.01	0.04	-	-
Low alcohol content beverages	0.00	-	0.01	0.04	-	-
High alcohol content beverages	-	-	-	-	-	-
<i>Women (aged 13-17 years) (n = 74)</i>						
<i>Non-alcoholic beverages</i>	0.78	0.28	13.77	29.83	0.71	-
Water	-	-	-	-	-	-
Coffee and infusions	0.04	0.00	0.02	0.06	-	-
Sugar soft drinks	-	-	7.68	16.63	-	-
Non-sweetened soft drinks	0.00	0.02	0.01	0.02	-	-
Sports drinks	-	-	0.12	0.23	-	-
Energy drinks	0.28	0.22	0.12	0.25	0.13	-
Juices and nectars	0.46	0.03	5.83	12.65	0.59	-
Other drinks	-	-	-	-	-	-
<i>Alcoholic beverages</i>	0.02	-	0.02	0.09	-	-
Low alcohol content beverages	0.02	-	0.02	0.09	-	-
High alcohol content beverages	-	-	-	-	-	-

activity, socioeconomic data and lifestyles of the population for the first time in a study in Spain. For that purpose, a country representative survey has been approached, and new technologies for dietary food and beverage records and physical activity levels have been used.

An important goal within the ANIBES study was also to estimate beverages consumption accurately. Therefore, ANIBES represented the first population survey of the consumption and variety of beverages in Spain that used photography. Also it is important to stress that leftovers were also taken into account, a key factor for beverages where traditionally whole portions (glass, wineglass, can, etc.) are approached. Seasonality in the consumption of beverages is very important, thus, the fieldwork was performed from September to November as a period in which beverage consumption is usually more stable (18). Total energy intake (14) and macronutrients distribution for the other foods groups (19) was already shown in previous publications.

Beverage consumption certainly varies greatly according to age, sex, temperature, etc., and is also very different between countries (20). From the limited data available, the results show that the studies on beverage intake in European countries had very different design and methodology (7,12,21). Sometimes, studies from European countries had roughly similar beverage intakes, however, when taking into account different categories of beverages, the situation changes, and the composition of the subgroups of food is different (7).

In the ANIBES study, non-alcoholic beverages and alcoholic beverages showed a contribution to the total diet for proteins, lipids and fibre not very relevant in contrast to total carbohydrates and sugars, for which differences according to age were observed. Others recent studies have also reported differences between the age groups (22) and no differences in consumption patterns between genders in youngest groups (23), as well as in our case.

Table IV. Macronutrients intake (%) from beverages food groups and subgroups in the ANIBES Spanish population by sex (adult 18-64 years)

	Proteins %	Lipids %	Carbohydrates %	Sugar %	Fiber %	Alcohol %
<i>Men (aged 18-64 years) (n = 798)</i>						
<i>Non-alcoholic beverages</i>	0.99	0.24	9.01	20.42	0.54	-
Water	-	-	-	-	-	-
Coffee and infusions	0.42	0.00	0.34	0.94	-	-
Sugar soft drinks	-	-	5.13	11.36	-	-
Non-sweetened soft drinks	0.00	0.04	0.01	0.07	-	-
Sports drinks	-	-	0.26	0.55	-	-
Energy drinks	0.25	0.18	0.25	0.54	0.05	-
Juices and nectars	0.31	0.01	2.93	6.76	0.49	-
Other drinks	-	-	0.09	0.21	-	-
<i>Alcoholic beverages</i>	0.59	-	2.11	5.81	-	100.00
Low alcohol content beverages	0.59	-	2.04	5.66	-	93.52
High alcohol content beverages	-	-	0.07	0.15	-	6.48
<i>Women (aged 18-64 years) (n = 857)</i>						
<i>Non-alcoholic beverages</i>	1.51	0.55	7.77	16.95	0.44	-
Water	-	-	-	-	-	-
Coffee and infusions	0.53	0.01	0.40	1.08	-	-
Sugar soft drinks	-	-	4.33	9.15	-	-
Non-sweetened soft drinks	0.01	0.07	0.03	0.11	-	-
Sports drinks	-	-	0.14	0.30	-	-
Energy drinks	0.69	0.47	0.24	0.59	0.03	-
Juices and nectars	0.28	0.01	2.55	5.62	0.41	-
Other drinks	-	-	0.06	0.11	-	-
<i>Alcoholic beverages</i>	0.26	-	1.01	2.50	-	100.00
Low alcohol content beverages	0.26	-	0.97	2.43	-	92.13
High alcohol content beverages	-	-	0.04	0.07	-	7.87

The present results are lower to those obtained from the latest Spanish Food Consumption Survey (FCS), the latter performed in households (non-alcoholic beverages contributed 5.9% to carbohydrates, and 13.2% to sugar, whereas alcoholic beverages provided a 0.9% and 2.1%, of total carbohydrates and sugar, respectively) (24). Other studies in Spain such as ENUCAM (Nutrition Survey of Madrid) (25) also showed somewhat lower results for carbohydrates and sugar contribution from non-alcoholic beverages (5.6%-12.5%) and from alcoholic beverages (0.5%-1.4). For additional comparison, in the ENIDE dietary survey (*Encuesta Nacional de Ingesta Dietética*), non-dairy beverages (excluding alcoholic drinks) contributed roughly 6% of total carbohydrates in the Spanish population (26). All these mentioned surveys in Spain have been performed in the last five years.

The impact of sugared soft drink consumption on obesity and metabolic disorders has come under intense scrutiny and debate

worldwide in recent years (27-30), but large differences between countries have been observed. The present study showed that sugared soft drinks contributed $11.24 \pm 17.31\%$ - $8.76 \pm 14.81\%$ to total sugar intake, for men and women, respectively. A higher consumption compared with mean contribution was seen in adolescents ($16.30 \pm 20.71\%$ - $16.63 \pm 17.64\%$) whereas the lowest contribution was obtained for the elderly population ($3.43 \pm 8.59\%$ - $3.30 \pm 9.37\%$). Higher consumptions were found, however, for children ($10.05 \pm 14.91\%$ - $6.67 \pm 10.57\%$) and adults ($11.36 \pm 17.29\%$ - $9.15 \pm 15.07\%$; $p \leq 0.05$). More efforts are needed in order to improve the diet quality of the youngest, but also to inform about the increasing variety of non-caloric drinks that are offered at present in the Spanish food market.

Finally, we found a low to moderate contribution from alcoholic beverages (carbohydrates $1.78 \pm 3.41\%$ - $0.89 \pm 2.13\%$, $p \leq 0.001$, and sugar $4.96 \pm 9.51\%$ - $2.21 \pm 5.24\%$, $p \leq 0.001$, for

Table V. Macronutrients intake (%) from beverages food groups and subgroups in the ANIBES Spanish population by sex (seniors 65-75 years)

	Proteins %	Lipids %	Carbohydrates %	Sugar %	Fiber %	Alcohol %
<i>Men (aged 65-75 years) (n = 99)</i>						
<i>Non-alcoholic beverages</i>	1.65	0.41	4.22	9.97	0.39	-
Water	-	-	-	-	-	-
Coffee and infusions	0.64	0.01	0.39	0.92	-	-
Sugar soft drinks	-	-	1.51	3.43	-	-
Non-sweetened soft drinks	0.00	0.02	0.00	0.01	-	-
Sports drinks	-	-	0.07	0.15	-	-
Energy drinks	0.75	0.38	0.24	0.72	-	-
Juices and nectars	0.25	0.00	2.01	4.73	0.39	-
Other drinks	-	-	-	-	-	-
<i>Alcoholic beverages</i>	0.40	-	1.72	5.35	-	100.00
Low alcohol content beverages	0.40	-	1.63	5.11	-	93.65
High alcohol content beverages	-	-	0.09	0.24	-	6.35
<i>Women (aged 65-75 years) (n = 107)</i>						
<i>Non-alcoholic beverages</i>	1.39	0.28	4.46	9.33	0.34	-
Water	-	-	-	-	-	-
Coffee and infusions	0.83	0.03	0.62	1.47	-	-
Sugar soft drinks	-	-	1.60	3.30	-	-
Non-sweetened soft drinks	0.00	0.03	0.01	0.03	-	-
Sports drinks	-	-	0.02	0.11	-	-
Energy drinks	0.32	0.22	0.23	0.36	0.16	-
Juices and nectars	0.23	0.01	1.98	4.07	0.19	-
Other drinks	-	-	-	-	-	-
<i>Alcoholic beverages</i>	0.18	-	0.63	1.59	-	100.00
Low alcohol content beverages	0.18	-	0.63	1.59	-	98.72
High alcohol content beverages	-	-	0.00	0.00	-	1.28

men and women) in total population. Alcoholic beverages of lower alcohol content (beer, wine, cider) represented over 90% of alcohol contribution. In general, alcoholic beverage consumption has undergone a slow decline during recent years, according to FCS (259 g/person/day in year 2000 *versus* 208 g/person/day in year 2008) (31). Within this group, in the last few years, a gradual substitution of wine for beer has taken place, which represents almost 70% of the total alcoholic beverage consumption at present (31).

CONCLUSIONS

Current epidemiological studies in Europe focus on beverage intake and their contribution to nutrients intake are still scarce. From the limited data available and the diversity of the method-

ology used, the results showed that consumption of beverages is different between countries. Furthermore, it should be remembered that the contribution of different beverages on macronutrient intake is often not provided in the studies. Therefore, further research is needed to clarify the present role of the increasing beverages market on the dietary habits and subsequent nutritional status of the Spanish population. We, at the present study, have contributed by using the available ANIBES study innovative methodology.

Beverage groups mainly contributed to dietary macronutrients intake as total carbohydrates and sugar. For non-alcoholic beverages, in terms of age, the differences were very marked, showing a much higher contribution in younger populations than in seniors, and mainly as sugared soft drinks and juices and nectars. As for alcoholic beverages, the contribution of macronutrients is lower

for carbohydrates and sugar but is essentially by alcohol their contribution is higher as low alcohol content beverages in adult and elderly men.

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CONFLICTS OF INTEREST

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Nutrición Hospitalaria



Effects of maternal hydration status on the osmolality of maternal milk

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Abstract

Background: It has been demonstrated that human milk osmolality (Mosm) is regulated within an established range, typically 290 to 300 mOsm/kg, and appears to be resistant to effects of maternal dehydration, as reflected by high urinary osmolality (Uosm).

Objective: To determine the degree of association between Mosm and Uosm at a common point in time, as well as the reproducibility of both measures over a one-week interval of sampling.

Methods: Mosm and Uosm were measured with a Vogel Löser 450 osmometer on samples of the respective biological fluids collected concurrently in 31 lactating women, with infants aged between 30 and 340 days. In the first 15 women recruited, collections were repeated 7 days after the initial ones.

Results: The median Mosm for the 46 samples collected was 308 mOsm/kg with a range from 288 to 448 mOsm/kg. The corresponding values for Uosm were 598 mOsm/kg with a range from 93 to 1,678 mOsm/kg. The Spearman rank-order correlation coefficient for within-individual association of Mosm and Uosm was $r = 0.214$ ($p = 0.153$). The median Mosm for the 15 repeat-subjects was 309 mOsm/kg on both occasions, with a within-individual Spearman coefficient of $r = 0.326$ ($p = 0.118$). By contrast, for the Uosm, the within-subject association was much stronger, with $r = 0.699$ ($p = 0.002$).

Conclusions: The osmometry technique proved to be a highly stable and reproducible measurement technique. Mosm and Uosm are not significantly associated at a point in time. Intra-subject Mosm varies more across time than intra-subject Uosm.

Key words: Breastfeeding. Milk osmolality. Urine osmolality. Hydration status. Guatemala.

BACKGROUND

Adequate hydration is an unrecognized aspect of human nutrition and health (1-3). In theory, this would be a special concern among lactating women, who not only have their normal daily water losses from renal, intestinal, lung and skin, but also from a volume of milk oscillating normally around 780 ml (4). In a

certain way, additional water must be obtained from four sources: beverages, water in recipes, intrinsic moisture of foods, and metabolic water generated from macronutrients (5). Obviously, regarding water sources, it should be clarified that safe water (spring water, tap filtered and chlorinated water, etc.) as well as the liquid in beverages and that added to recipes would be the target sources for intervention to increase water intake.

Prentice et al. (6) imposed a 14.5-h total restriction of liquids on 10 lactating Gambian women, following plasma osmolality and total water balance. Women lost on average 7.6% of their body weight during abstinence and raised their plasma osmolality, but the turnover was twice as much as non-lactating controls. Water economy could not be explained by milk synthesis, which was not affected in the acute trial. Given the importance of this observation, subsequent literature on hydration status in lactating women was scarce in our literature search. A range of techniques can be used to estimate hydration status, from the color of the urine and its specific gravity, to daily liquid intake or urine output, to more refined laboratory techniques involving freezing-point depression osmometry of plasma or urine (7,8). The urine specimen can be derived from a causal spot sample or from a 24-h quantitative collection.

These different hydration indicators are neither mutually equivalent nor reflective of the same aspects of hydration status. Manz and Wentz (9) feel that urinary osmolality (Uosm) is the most integrative manner to assess human hydration, and they define normative "euhydration" as a Uosm in the range of 90-900 mOsm/kg. Plasma osmolality, by contrast, is tightly regulated in a narrow range around 300 mOsm/kg (9) and is generally only responsive to acute and profound hydration stress (7,10). Osmolality in human milk is also tightly regulated, and close to the range for plasma.

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How systemic hydration of lactating mothers might influence the osmolality of human milk remains unresolved so far. Experimental studies in cows, involving intravenous perfusion with saline, showed a temporal diluting effect on milk (11). There is a benefit to the infant from consuming beverages of lower solute loads, as the osmolality of fortified milk fed to premature infants can rise to unacceptably high levels (12,13). In Guatemala, the circumstances of availability of microbiologically-safe water (14) may be a disincentive to adequate consumption of beverages in lactating women in that nation.

OBJECTIVE

The availability of an osmometer in our laboratories in the Western Highlands allowed us to perform osmolality analyses on both urine and human milk. This enabled us to gather these background issues into a hypothesis-driven study to examine, in a cross-sectional manner, whether any significant association existed between maternal systemic hydration status, as represented by Uosm, and the osmolality of her breast milk. We present here the experience of that investigation.

METHODS

RECRUITMENT OF SUBJECTS

A total of 31 women, meeting the criteria for mother age (18 to 49 years) and of lactation age (days of life of the infant) varying from 30 to 365 days, were recruited into the study from the Quetzaltenango Health Center. Additional inclusion criteria included general good health of the dyad and absence of acute illness in either party on the day of study. It did not matter if the child was exclusively or predominantly breastfed or had already begun mixed feeding. The first 15 enrollees underwent collections twice at a one-week interval, whereas the remaining 16 subjects participated on a single occasion.

The study had been approved by the Human Studies Committee of the Center for Studies of Sensory Impairment Aging and Metabolism (CeSSIAM), and subjects gave informed written consent after the nature, purpose, inconveniences, risks and benefits had been described in Spanish. Subjects received the cost of their transportation, a snack and a meal, and set of dishes in compensation for their participation.

COLLECTION OF MILK SAMPLES

Milk samples were collected by the full-breast extraction method. One breast was reserved during at least 90 min, while the infant was allowed to nurse *ad libitum* from the contra-lateral breast. Using a breast-pump, the extractable volume was col-

lected from the reserved side while the infant suckled from the opposite side. The specimen was thoroughly homogenized and two samples of up to 15 ml were aliquoted into a 15 ml conical tube and frozen at -20 °C until analysis.

COLLECTION OF SPOT URINE SAMPLES

Either just before or just following the milk extraction, the subject evacuated the urine in her bladder into a receptacle. After thorough mixing, an aliquot of 250 ml volume was transferred into a 15 ml conical tube and froze at -20 °C until analysis.

MEASUREMENT OF OSMOLALITY IN BIOLOGICAL FLUIDS

Osmolality was measured on a calibrated Vogel Löser 450 osmometer (Giessen, Germany). The respective sample was thawed to room temperature and remixed for 3 min. A 100 µL volume of the biological fluid was transferred by pipette into the receiving vessel of the instrument, and the osmolality was measured by the freezing-point depression principle of Peltier. Results were expressed in milliosmoles per kilogram of urine or milk (mOsm/kg).

DATA HANDLING AND STATISTICAL ANALYSIS

The database and subsequent calculations were made using SPSS Version 20 (IBM, Chicago, IL, USA). Descriptive statistics of median, 95% confidence limits, and maximum and minimum values were displayed for all samples collected, as well as for appropriate sub-groupings. Repeated measures Friedman test was used to make comparisons.

The Spearman rank-order and Pearson product-moment correlation coefficients were generated to assess the association between paired values from the same subject. A probability level of 5% was accepted as the criterion for statistical significance.

RESULTS

CHARACTERISTICS OF THE MATERNAL-INFANT DYADS

A total of 31 lactating mothers were recruited for the donation of urine and milk samples. The characteristics of maternal age and offspring age are shown in the upper rows of table I; 26 were legally married or living in a partnership union and 5 were single and unaccompanied. Three were unschooled, 6 completed elementary school and 9 middle-school, 7 had completed high-school and 6 were university graduates.

DESCRIPTIVE STATISTICS OF MILK OSMOLALITY

Milk osmolality ranged from 291 to 465 mOsm/kg, with a median of 308 mOsm/kg ($n = 46$) (Table I). Although the distribution is somewhat right-shifted, 39 values (85%) fell between 292 and 324 mOsm/kg, which represents a $\pm 5\%$ boundary around the median. There were no differences in osmolality between milk fed to girl or boy babies ($p = 0.769$).

Table I. Descriptive statistics of the dyads and aggregated and disaggregated central tendency values for milk (Mosm) and urinary (Uosm) osmolality

Grouping	Variables	n	Median (min-max)
All subjects	Maternal age (y)	31	24 (18-41)
	Lactational age (d)	31	121 (30-340)
	Milk volume, all samples (ml)	46	13.5 (2-100)
			mOsm/kg
	Mosm all samples	46	308 (288-448)
	Mosm 1 st samples	31	307 (288-448)
All subjects by sex	Mosm 1 st sample, girls	14	306 (292-448)
	Mosm 1 st sample, boys	17	309 (288-415)
	p value (Mann-Whitney U test)		0.769
Duplicate specimens subsample	Mosm 1 st sample	15	309 (292-448)
	Mosm 2 nd sample	15	309 (300-329)
	p value (Mann-Whitney U test)		0.902
All subjects	Uosm all samples	46	598 (93-1,678)
	Uosm 1 st sample	31	606 (93-1,072)
Duplicate specimens subsample	Uosm 1 st sample	15	530 (93-1,072)
	Uosm 2 nd sample	15	586 (297-1,678)
	p value (Student t-test)		0.319

Mosm: Milk osmolality; Uosm: Urinary osmolality.

DESCRIPTIVE STATISTICS OF URINARY OSMOLALITY

Urinary osmolality ranged from 93 to 1,678 mOsm/kg, with a median of 598 mOsm/kg (Table I). If we accept 90 mOsm/kg as a convenient cut-off for overhydration (9), none of the lactating women fell into this category. With respect to the cut-off for hypohydration of 900 mOsm/kg (9,15), on 4 occasions (9% of all samplings) a participant had a Uosm in excess of that criterion on the day of sampling.

CORRELATES OF MILK OSMOLALITY

The Spearman rank-order correlation coefficient for the association of milk osmolality with urinary osmolality at the same time as the milk sample donation was $r = 0.214$ ($p = 0.153$) when all collected samples are considered (Table II). Equivalent and non-significant associations are seen when the correlations of women with duplicate sampling are considered for the first and second collections, separately ($p = 0.093$; $p = 0.939$). Moreover, Mosm was not related to the volume of milk accumulated in the breast ($p = 0.920$). Spearman's correlation coefficients for Mosm with maternal and offspring age, respectively ($p = 0.137$; $p = 0.194$).

CORRELATES OF URINARY OSMOLALITY

As shown in table II, Uosm similarly had no significant association with either maternal age ($p = 0.662$) or with offspring age ($p = 0.180$).

ASPECTS OF REPRODUCIBILITY OF MILK AND URINE OSMOLALITY

We have examined both biological and methodological aspects of the reproducibility of osmolality states. The median Mosm values for the 15 women providing two samples at a week's interval were identical at 309 mOsm ($p = 0.902$) (Table I), but the respective Pearson and Spearman correlation coefficients were not significant ($p = 0.233$; $p = 0.118$) (Table III).

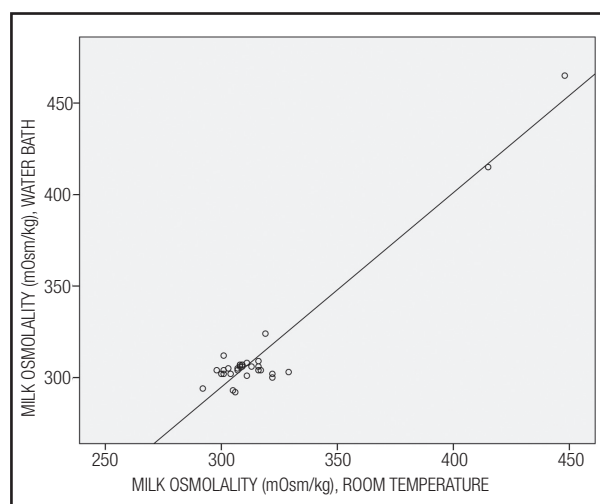
We also examined the stability of Mosm measurements if the milk was stored for different intervals and defrosted in different manners. In a subgroup of 23 samples, collected in August 2015, an initial Mosm measurement was made within 1 to 6 days of their being collected and stored at -20°C . For analysis, samples were simply thawed to room temperature prior to thorough mixing and injection into the osmometer. On a second occasion, in October 2014, with -20°C freezing intervals varying from 6 to 81 days, an identical defrosting and analysis protocol was conducted on a second aliquot of the same 23 samples. On yet a third aliquot, the preparatory phase included thawing to room temperature and then raising the temperature to 37°C in a warm-water bath, then cooling back to room temperature prior to thorough mixing for analysis. The Pearson correlation coefficients for the various paired comparisons were, however, not statistically significant (Table IV). When the October measurements for both the direct thawing and the water bath approaches were compared for the common 23 samples with the early measurements made in August as well, the median Mosms were 309, 305 and 288 mOsm/kg, respectively ($p = < 0.001$ by Friedman's test). Figure 1 shows the scattergram for the Mosm values with the two preparatory protocols for all 46 milk samplings in the study; the Pearson correlation coefficient was $r = 0.966$ ($p = < 0.001$).

Table II. Correlates of milk and urinary osmolality for all specimens and by subgroups, using the Spearman rank-order correlation coefficient

	Number of specimens and sampling frame	r-value	p-value
<i>Associations between milk and urinary osmolality</i>			
Mosm vs Uosm	all samples (n = 46)	0.214	0.153
Mosm vs Uosm	1 st subsample (n = 15)	0.449	0.093
Mosm vs Uosm	2 nd subsample (n = 15)	0.021	0.939
<i>Other associations with osmolality values</i>			
Mosm vs volume collected	all samples (n = 46)	0.015	0.920
Mosm vs maternal age	1 st samples (n = 31)	0.273	0.137
Uosm vs maternal age	1 st samples (n = 31)	-0.092	0.622
Mosm vs lactational age	1 st samples (n = 31)	-0.240	0.194
Uosm vs lactational age	1 st samples (n = 31)	-0.247	0.180

Table III. Within individual reproducibility of milk osmolality in duplicate subgroup

Statistic	n = 15 in each sub-sample	
	r-value	p-value
<i>Milk osmolality</i>		
Pearson correlation subsample 1 st vs 2 nd	0.204	0.233
Spearman correlation subsample 1 st vs 2 nd	0.326	0.118
<i>Urinary osmolality</i>		
Pearson correlation subsample 1 st vs 2 nd	0.642	0.005
Spearman correlation subsample 1 st vs 2 nd	0.699	0.002

**Figure 1.**

Scattergram of 46 paired values of milk osmolality for frozen milk aliquot thawed to room temperature prior to analysis (x axis) vs for a second aliquot of frozen milk aliquot thawed to room temperature then raised to 37 °C and cooled to room temperature prior to analysis (y axis). The Pearson correlation coefficient was $r = 0.966$ ($p < 0.001$).

Table IV. Reproducibility expressions (of medians and of correlation) of milk osmolality measurements in relation early after collection and after prolonged freezing with two procedures for thawing

Statistic	n = 23 in each sub-analysis			
	Pearson's r value			p value
Original vs post-freezing with normal thaw	0.863			< 0.001
Original vs post-freezing with thaw, heat and cool	0.853			< 0.001
Post-freezing with normal thaw vs post-freezing with thaw, heat and cool	0.927			< 0.001
Median concentrations	Osmolality (mOsm/kg)			
	288 ^a	309 ^b	305 ^c	< 0.001 ^d

^aMeasured shortly after collection. ^bPost-freezing, with normal thaw. ^cPost-freezing with thawing, heating and cooling cycle. ^dRepeated measures Friedman test.

With respect to the reproducibility of Uosm on repeat collections in the subsample, table I shows the values for first and second samples; the difference in mean values was not significant ($p = 0.319$), and the respective Pearson and Spearman correlations showed significant reproducibility association ($p = 0.005$, $p = 0.002$). Our previous studies (16) demonstrated the stability of Uosm in urine samples during long-term frozen storage. This was confirmed with the August and October repeat measurements (data not shown).

DISCUSSION

Breastfeeding is the means to satisfy thirst and hunger of the infant and to provide the child with adequate hydration and nourishment. In the theory of lactation biology, it is the nursing demand of the child that determines the output of milk (17,18). Limitation of nutrients to the mother, however, may compromise the nutrient quality of the milk (19). It is logically sound to postulate that limited intake or retention of water will compromise the quantity, and similarly limit the flow of nutrients to an infant. Confirming a relationship of maternal hydration to the quality or quantity of her milk would have actionable consequences for public health programming.

COMPARATIVE PERSPECTIVE ON HYDRATION FINDINGS

The general tenor of hydration for these highland-dwelling lactating women may be that of adequate status. This conclusion comes from the evaluation of casual urine samples, however. It might be considered as preferable to collect formal 24-h urine samples to best assess hydration by Uosm measurement, but the logistics for such a tedious assignment were not reasonable, given our mode of subject recruitment. Many studies have employed spot urine samples for osmolality when 24-h collections were inconvenient or logistically impossible (15,20-24), but how they compare with concurrent full-day collections for rank-ordering of subjects' hydration status is not completely understood. Notwithstanding the caveats regarding a casual urine sample, lactating women, overall, have a Uosm that is ~ 600 mOsm/kg, which is some 75 mOsm/kg higher than the 530 mOsm/kg median for preschoolers of the same region (16). In addition, we can make comparison with a series of 15 casual samples collected from the female staff members and associates in Quetzaltenango at the same time of day, 09:00-11:00 am, as those of the lactating subjects (data not shown). The median was 492 mOsm/kg (110-1,020); this value is 106 mOsm/kg and 18% lower than the median for lactating women shown in table I.

Our findings from this small sample of women show, moreover, that, on any given day, lactating women can show hydration states at the extremes of the euhydration range (9). High values of Uosm, signifying hypohydration, are seen sporadically in our lactating women. At least in the few subjects in the repeated-collections

series, however, the altered hydration state had abated on the other measurement opportunity. A value of 93 mOsm/kg, just short of the hyperhydration criterion of Manz and Wentz (9), was seen on one occasion, but the same subject had a Uosm value of 321 mOsm/kg on the second collection. Without a larger series of lactating women, or a greater number of repeated values, no stable estimate for the frequency of Uosm values at the extremes of the hydration-status range in this population can be made.

COMPARATIVE PERSPECTIVE ON MILK OSMOLALITY FINDINGS

Typical osmolality for human milk is reported as 300 mOsm/kg, in a rounded-off fashion. This is coincidentally -or not coincidentally- identical to the 300 mOsm/kg, which is the value cited for human plasma osmolality (9). The median of 308 mOsm/kg for breast milk osmolality is close to that value. Although 85% of values were in a $\pm 5\%$ osmolality band of the median, strictly tight regulation cannot be inferred, as a residual 15% had a higher ($n = 6$) or lower ($n = 1$) value. Fortified milk or infant formula with an osmolality of > 400 conveys a risk of osmotic diarrhea in the baby (12). At least at the moment of collection, three samples of milk had a Mosm in this excessive range, but it was not constant in the mother on her other sampling.

THE ASSOCIATION OF SYSTEMIC HYDRATION STATUS AND MILK OSMOLALITY

The medullar finding in response to the main hypothesis of the present inquiry is the failure to detect any significant association between osmolality in the urine and the human milk at a common moment in time. In a mathematical sense, however, the narrow, core of distribution of Mosm, with an effective width of 32 osmolality unite, militates against any rank-order scaling that could reveal associations with another variable. Nevertheless, we can tentatively conclude that the maternal hydration status was adequate to maintain appropriate hydration in the nursing offspring. Brown et al. (25) found that all 40 exclusively breast-feeding Peruvian mothers maintained infants with adequate milk volumes as gauged by adequate infant urinary outputs with a 1.003 to 1.017 urine specific gravity.

INDIVIDUAL AND GROUP STABILITY OF OSMOLALITY

Uosm is well documented to be mutable and responsive to water balance (26,27). Likewise, as would be expected, Mosm is a state, not a trait, as the day-to-day fluctuation, even within the narrow range, was marked. On a group basis, for the constant group of 15 women with two collections separated by a week, however, the collective distribution remained very firm and solid. This suggests that a powerful intervention, such as a beverage-of-

fering intervention of a substantial magnitude, could shift the distribution of Mosm to the left in a detectable manner. Similarly, in a cohort of lactating mothers, drought conditions with severe thirst might shift this variable to the right.

Maternal age and lactation duration are factors known to affect other aspects of lactation and milk quality (28-30), so it was reasonable to probe our data for evidence of an association with osmolality. Likely, due to the intrinsically narrow distribution of Mosm it is not a readily scalable variable. Hence, the lack of association is not surprising.

EFFECT OF DURATION OF FROZEN STORAGE ON MILK OSMOLALITY

Freezing and storing of human milk has been shown to affect a host of analytes (31). Table IV shows a significant difference of a 7.2% increase in the median of analyzed samples. Neither median value for Mosm, nor that from timely or that from delayed analysis, is outside reported normative range, however. On the one hand, Neubauer et al. (32) found a range of 290-299 mOsm/kg in 33 control mothers in Connecticut; this is in line with the central tendency for the freshly analyzed samples, i.e. with a short duration of frozen preservation. On the other hand, breast milk from 84 healthy Viennese mothers had a median *osmolality* of 297 mOsm/l. If converted to osmolality by applying the specific gravity of breast milk of 1.031 (33), this converts to a median of 306 mOsm/kg, i.e., expressed as *osmolality* in terms of *kilograms* of sample weight. In many ways, however, most comparable in setting and population make-up with our Guatemalan women is a study from an urban settlement of Brasilia, published in 1988 by Dorea et al. (34); in breast-feeding women across a range of lactation age, the mean \pm SEM of human milk was 319 ± 4.5 mOsm/kg, with median values not reported.

These values are all in general accordance with the analysis of samples after up to 2.5 months of frozen storage. The additional water-bath treatment in thawing had no influence on assay results. It is worth mentioning that, despite a narrow intrinsic distribution making associations with extrinsic variables such as maternal or infant age difficult to discern (Table II), the stability of the osmometry methodology finds correlation coefficients of > 0.85 for repeated measurement of the same milk collection (Table IV). Specifically, as shown in figure 1, the correlation coefficient reached 0.97 for all 46 samples thawed in the two procedures. With the 23 samples measured shortly after collection and later after freezing, however, we see a significant increase in osmolality with the prolonged freezing.

STRENGTHS AND LIMITATIONS OF THE STUDY

The major strength was the nature of the primary hypothesis addressed. We found no evidence for or against an influence of

systemic hydration on milk osmolality using Uosm as the hydration status index. A limitation might be found in the number of participants and specimens. A sample-size of 31 women is not uncommon for a hydration study, as this was exactly the number of young women subjects of a comprehensive assessment of hydration markers by Anderson et al. (35). There are limitations in the statistical power to encounter associations. With 29 degrees of freedom for a two-tailed test of linear association, the lowest r value that would be significant at a 5% probability level would be 0.335. With a focus on analytical or short-term biological reproducibility, however, associations on the order of $> 0.80-0.90$ are generally anticipated, independent of the number of pairs. We can attribute the narrow range of distribution in Mosm for the limitation in detecting strong associations with extrinsic variables, whereas in the methodological domain, this distribution has little effect on high associations between split-sample repeated analyses.

CONCLUSIONS

Human milk has evolved to present an osmolality near to 300 mOsm/kg, which approximates to average plasma osmolality. Although Mosm values were narrowly clustered around the median, an occasional sample shows a sporadic high value exceeding 400 mOsm/kg. The hydration status of lactating mothers as a group is in the upper range of the euhydration range, and is inferior to local non-lactating professionals. Albeit as adequate in its nature, hydration status may not be optimal for lactation; a woman is occasionally seen to express under-hydration states on a given day. We find no significant association between hydration and osmolality in human milk, but recognize the poor capacity for stable rank-ordering within the condensed distribution of Mosm. Variation in the duration of frozen storage is small, but may add additional attenuation in efforts to associate Mosm with other variables.

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Nutrición Hospitalaria



A new educational tool to learn about hydration: taste workshops for children

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Abstract

Introduction: Nutrition education contributes to children's understanding and practice of healthy lifestyles behaviors. Having a well hydration status is an essential topic, especially since children are a vulnerable population who are much more prone to dehydration than adults are. The approval of the Report on the European Gastronomic Heritage: Cultural and Educational Aspects in 2014 served as starting point to work on innovative audio-visual and multimedia materials for children. The Spanish Nutrition Foundation (FEN) and the Royal Academy of Gastronomy (RAG), in collaboration with the Ministry of Education, Culture and Sport in Spain (MECD), developed educational videos for schoolchildren to learn about food, nutrition and gastronomy, specially, the importance of being hydrated.

Objectives: To develop a serial of videos for children between 3 and 9 years old with nutrition and cooking lessons to be used as educational resources in the official curricula.

Methods: Fourteen chapters related to food, nutrition, gastronomy, physical activity and hydration to be used to record videos were designed and tested. A nutritionist, a chef and two puppets were the main characters acting in the videos.

Results: The chapters were assembled in nine videos that included five sections: *introduction, video lesson, recipes* –in case of hydration, recipes with different water content foods were recorded–, *what have you learnt and check your knowledge*.

A summary of the new educational material was officially presented at the Spain Pavilion during the Expo Milano 2015. Moreover, they are included as education tool for teachers in the new PANGEl Programme (Food, Nutrition and Gastronomy for Infatle Education) jointly launched by FEN, RAG and MEDC.

Conclusion: Taste workshops are useful as innovative nutrition education tools to reinforce language, listening and motor skills as well as food and nutrition concepts, and specially, the importance of being well hydrated.

Key words: Beverages. Education. Children. Hydration.

INTRODUCTION

A good nutrition education promotes health through learning, adaptation and acceptance of healthy eating habits based on prior food culture and scientific knowledge (1). The role of food in health and well-being status is very important in all age states, especially during children growth and development (2). Exposure to a varied diet in early childhood has been linked to children's willingness to try new foods and drinks at a later age (3).

Therefore, nutrition education is recognized as a key part of the education of young population, either with cross-curricular links between the various subjects that make up the curriculum of the Elementary and Secondary Education in Spain or treated as content on its own (4).

The Spanish Nutrition Foundation (FEN) and the Royal Academy of Gastronomy (RAG) were pioneers to present the idea of including gastronomy and healthy eating habits into the European Education System. They advised the different European governments to take into consideration the possibility of asking the education system to teach students about nutrition, dietary habits and gastronomy. The result was the approval of the European Parliament report *European gastronomy heritage: cultural and educational aspects* in 2014 (5). The report asks the Member States "to include in education programmes, from early childhood, the study and sensory experience of food, nutritional health and dietary habits, including historical, geographical, cultural and experiential aspects" (5). Since then, and using as baseline the *Taste workshops* carried out by the Foundation for Science, Health and Education (SHE) (6) in collaboration with RAG and FEN, these latter institutions have been working and testing on innovative audio-visual and multimedia material for schoolchildren.

Water requirements in children and adolescents are changeable and depend on age, sex, energy consumption and physical activity level (7). One essential topic consists of having a well hydration status, moreover since children are considered as vulnerable population much more prone to dehydration than adults (8). Beverages are important for children, both for hydration and nutrition, and we must as well encourage their intake as a potential strategy to improve the nutrient density of children's diet (9).

Organizations and institutions perform different programmes and strategies for education in nutritional, gastronomic and sensorial aspects using face to face workshops with schoolchildren (3,10-12), but the use of audio-visual materials is still lacking

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even though “new technologies” are recognized as key tools to improve education and cognitive performance.

Therefore, the main goal was to develop videos for teaching schoolchildren about food, nutrition, gastronomy, physical activity and hydration, using a chef and a nutritionist as leading role and puppets as supportive material.

With these validated *Taste workshops* videos we have created a new educational tool for teachers of Infantile and Primary Education, which will enrich the school curricula with theoretical and practical knowledge through audio-visual materials. It is already included within the new strategy on Education in Nutrition and Gastronomy from the Ministry of Education, Culture and Sport in Spain.

OBJECTIVES

The objective of this study was to develop a series of videos for children aged 3 to 9 years old including lessons on cooking and nutrition and recipe demonstrations to be used as educational resources in the official Infantile and Primary curriculum of Spain.

METHODS

CONTENTS DEVELOPMENT

Due to the importance of including nutritional and gastronomic aspects in the school curriculum, FEN researcher team designed a list of topics related to this area. Members of RAG, FEN and teachers from the Nutrition Department of CEU San Pablo and Complutense University of Madrid wrote up 14 chapters related to food, nutrition, gastronomy, physical activity and hydration, thanks to the agreement with the Spanish Ministry of Education, Culture and Sport.

1. Why is learning how to eat important?
2. What is food? What do I eat?
3. What does food contain? Energy and nutrients.
4. How do I feed myself?
5. How much do I need to eat?
6. I feed myself for both: necessity and pleasure. Good habits in food.
7. Food as cultural aspect. Taste education.
8. The good use of food.
9. Food technology. Culinary techniques.
10. Food labelling.
11. School canteens.
12. Exercise and lifestyles.
13. Food related illnesses.
14. Myths, mistakes and realities.

These contents were adapted to language of targeted population (3 to 6 and 6 to 9 year old children) and used to record videos for nutrition and gastronomy education of schoolchildren.

VIDEO RECORDINGS

FEN team made contact with a film producer to design video scripts, chose main characters and record videos. The recordings occurred between May and September 2014.

RESULTS

VIDEO LESSONS: TOPICS AND STRUCTURE

The chapters were assembled in nine videos: *How we cook food?*, *Temperature in food*, *How much food is there?*, *Nutrients world*, *Eating with culture*, *Let's move on!*, *Don't forget beverages* and *Why can't I eat it?* The “characters” were a chef, 5 nutritionists and 2 puppets.

Each video contains 4 sections: *Introduction*, where the nutritionist and a puppet (Nutriñeco) introduce the topic of the video; *Video lesson*, taught by the nutritionist; *Recipes*, where the cook, the nutritionist and the chef puppet (Martin) elaborate different recipes, and *What have you learnt*, where nutritionist ask Nutriñeco about the lesson.

The video structure for 6-9 year-old pupils differed from 3-6 year-old pupils in language difficulty and in the section *Check your knowledge*, where the nutritionist asks different questions to check Nutriñeco knowledge improvement (Table I).

The video *Don't forget beverages* included the following script: for the *introduction*, the nutritionist explained to Nutriñeco that staying well hydrated helps to be alert in class and play sports (Fig. 1). In the *video lesson*, we reinforced the importance of a good hydration status to be healthy and the different water content in foods (Fig. 2). *Recipes* included foods with different water content. From the least to the most water content recipe we had: cereal, sandwich, soya, anchovies and watermelon soup (Fig. 3). In the section called *What have you learnt*, Nutriñeco explains to the nutritionist everything he has learnt about hydration. Finally, in *Check your knowledge*, the nutritionist asked Nutriñeco if he knew the water content of different foods.

VIDEO RECIPES

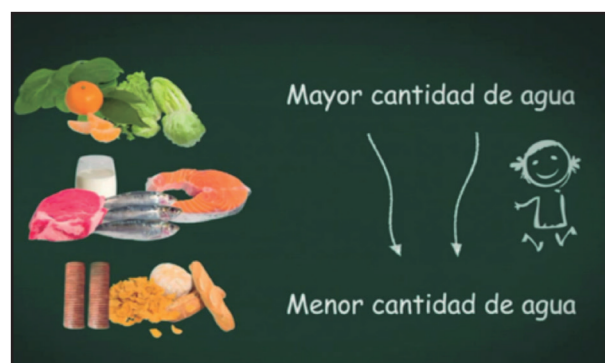
We included different recipes for each video/topic (Table II).

Table I. Video structure

Section number	3-6 year-old pupils	6-9 year-old pupils
1	Introduction	Introduction
2	Video lesson	Video lesson
3	Recipes	Recipes
4	What have you learnt	What have you learnt
5	-	Check your knowledge

**Figure 1.**

Nutritionist tells Nutriñeco the importance of drinking water (*Introduction*).

**Figure 2.**

Different water content in food (*Lesson* section).

Table II. Video recipes

Video number	Topic	Recipes
Video 1	How many flavours are there?	Orange and honey juice
		Fresh cheese and carrot sandwich
		Mango and pie cream
		Apple, cream and cookies compote
		Fresh tomato, oregano, black olives and artichoke pizza
Video 2	How we cook food?	Recipes with egg: boiled, poached, fried and omelette
		Tomato: salad, fried, breaded, microwave
		Ratatouille with poached egg
Video 3	Temperature in food	Creamy asparagus soup
		Andalusian <i>gazpacho</i>
		Roasted pineapple with coconut soup
Video 4	How much food is there?	Vegetables and tuna cannellone
		Rabbit with vegetables couscous
		Fried egg with rice and tomato
Video 5	Nutrients world	Breadcrumbs with meat slaughter
		Battered hake with potatoes
		Potatoes salad
Video 6	Eating with culture	3 delicious fried thai rice
		Cauliflower cream with egg and truffle
		Fresh sausage with mashed cinnamon potatoes
Video 7	Let's move on!	Pesto macaroni
		Pineapple, orange and banana juice
		Chicken sandwich
Video 8	Don't forget beverages	Watermelon soup
		Soya anchovies
		Cereal sandwich
Video 9	Why can't I eat it?	Soy crème caramel
		Fruits with mint soup
		Cornflour custard



Figure 3.

Martín speaks to the nutritionist and the chef about his favourite fruit (*Recipe* section).

VIDEO DISSEMINATION

A summary of the developed educational videos was shown in the Spanish Pavilion of the Expo Milano in summer 2015. In addition, they are included as supplementary material in the PANGEL Programme (Food, Nutrition and Gastronomy Programme for Infantile Education) that will be used in Spanish schools in the next academic year (13).

DISCUSSION

We found that *Taste workshops* development could easily serve as an education tool in schools. Teachers will use these audio-visual resources in class to educate through a new concept of nutrition, based on health and pleasure, gastronomy and sensorial education.

The last nutrition survey specifically targeted for children and adolescents (ENALIA survey) shows that liquid intake coming from beverages and milk is 876.9 g per day in children aged 3 to 9 years old (14). If we compare these data with the Scientific Opinion on Dietary Reference Values for water of EFSA Panel (15), they restate the necessity of working on education about the importance of being well hydrated.

Other institutions and food industries have recorded videos about hydration and focused in children and adolescents: The British Nutrition Foundation made an e-seminar about healthy hydration to students aged 8 to 14 year old in the frame of the Healthy Eating Week in 2013 (16). The Nestlé Healthy Kids Programme also includes four videos for Primary School students that highlight the importance of drinking water (17).

Future studies evaluating *Taste workshops* videos are needed to check the effectiveness of the use of audio-visual material in classroom teaching and the efficacy of improving the hydration concept and status in the youngest.

CONCLUSIONS

Taste workshops can be used as an innovative nutrition education tool to reinforce language, listening and motor skills as well as food and nutrition concepts, specially, the importance of being hydrated.

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Nutrición Hospitalaria



The use of moderated mediated analysis to study the influence of hypo-hydration on working memory

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Abstract

Introduction: To date, dehydration has been typically reported to influence psychological parameters when there has been at least a 2% loss of body mass, although there has been little examination of those going about their everyday lives, those who have lost less than 1% of body mass. In such situations factors such as the initial hydration status and individual differences in the response to a reduced fluid intake are likely to be influential. Yet to study the complexity added by such additional variables novel methods of statistical analysis are required.

Objectives: The present study describes the use of moderated mediation, an approach that asks various questions: firstly, is drinking influential?; secondly, does a mediator (*e.g.*, thirst) sit between an independent and dependent variable?; and thirdly, does an effect only occur under certain conditions such as initial osmolality?

Method: In the study, 118 subjects were exposed to 30 °C for four hours during which they half drank 300 ml water. The serial sevens test of working memory was performed before and at the end of the procedure.

Results: A 0.6% loss of body mass reduced the efficiency of working memory. Those who consumed water had better working memory; working memory was worse in participants who lost more body mass or became thirstier, but only in those with higher levels of baseline osmolality.

Conclusions: Small variations in hydration status influenced cognitive functioning although there were individual differences in the response. The parameters that influence an adverse response to hypo-hydration need to be established to allow giving appropriate advice.

Key words: Hypo-hydration. Moderated mediation analysis. Water. Working memory.

OBJECTIVES

In the popular press there is often the suggestion that dehydration is a common and largely unrecognized problem, with advice such as we should “drink eight 8-ounce glasses of water a day” or we should examine the color of our urine to monitor to what extent we are well hydrated. In fact, there is very little evidence that hypo-hydration is an everyday problem. Changes in mood and cognitive functioning are often the first symptoms of minor nutritional deficiencies (1), yet a review of the influence of dehydration on psychological parameters concluded that any evidence of an adverse effect occurred when there had been at least a 2% loss of

body mass (2). However, as it has also been suggested that those going about their everyday lives are not likely to lose 1% of their body mass (2), it might be argued that hypo-hydration will rarely be a concern. However, with minor differences in hydration status, factors such as the initial hydration status and individual differences in the response to a reduced fluid intake may be influential. The present study describes a novel approach in this area: the use of moderated mediation (3). The way in which this approach allows the study of the interaction between relevant variables is illustrated. It is reported that a 0.6% body mass loss reduced the efficiency of working memory.

Moderated mediation (3) can be conceived as a series of questions (Fig. 1). First, we have the “what question”: for example, does drinking as opposed to not drinking influence a dependent variable such as mood? Secondly, the “how question”: does a mediator sit between an independent and dependent variable. Thus an independent variable, such as drinking, may influence a mediator variable, for example thirst. In turn, the mediator influences the dependent variable mood. This is termed an indirect effect. In this example, drinking as such does not influence mood. Drinking rather influences thirst and thirst then influences mood. Finally, the “when question”: moderated mediation implies that a mediator is only influential at certain levels of another variable, the moderator. This is termed a conditional indirect effect, as the influence of a mediator depends on another variable. With the present hypothetical example, thirst may mediate the effect of drinking but this may only occur under certain conditions; for example, it may depend on an individual's initial osmolality.

This suggested statistical approach will be illustrated, and the findings in this area extended, by examining a test of working memory, using regression equations and moderated mediated analysis. It is important to distinguish working memory from short-term memory. Short-term memory involves storing information for a short period but, critically, it does involve the manipulation of that information or organizing how it is held in memory. In

contrast, working memory holds, but also processes, information while reasoning or calculating takes place. Thus, working memory involves the temporary storage and manipulation of information.

In the present context, the choice of working memory as the dependent variable offers the advantage that it is a suitable measure to consider whether there is a need to look at individual differences when considering hypo-hydration. If there exist sub-groups who are susceptible to hypo-hydration, how should they be characterized? Although there are reports stating that a loss of body mass greater than 2% disrupts working memory (4,5), this finding has not always been replicated (6-8). Working memory has never been reported to be disrupted by a loss of 1-2% of body mass (4,8-10). As such, if the use of moderated mediation can demonstrate that a small difference in hydration can be disruptive under some conditions, the value of this novel statistical approach would be demonstrated.

METHODS

PROCEDURE

The procedure was approved by the local Ethics Committee and participants gave written informed consent. From an under-graduate population, 118 (61 male) subjects were recruited by a circular email. Applicants were excluded if they reported a major neurological or psychological disorder or had, in the last week, used a sleeping tablet, anti-histamines, a decongestant or a painkiller. Among the females, 70% were taking an oral contraceptive. Participants were non-smokers aged 18 to 30 years with an average age of 20.4 years.

They were exposed to a temperature of 30 °C for four hours, after randomly being allocated to groups that either did or did not drink 300 mL of pure water. At baseline, those who subsequently did or did not drink were well matched: they did not differ in urine osmolality (no water condition 688.5 milliosmoles/kg [SD 51.6]) or BMI (no water 24.0 [16.7-33.6] v water consumed 23.6 [16.4-33.3]). They were blind as to the nature of the experiment (they were informed it was a study of the effect of heat) and unaware of the fact that others did or did not consume water. To capture the full range of habitual hydration status on the day of testing participants arrived having consumed their usual breakfast.

Upon arrival, body temperature and body mass were measured and a urine sample was collected. Subjects remained in a room at 30 °C for four hours, during which on two occasions they performed a battery of tests (Table I). The temperature of the room varied from 30 to 31 °C, with an average humidity of 53% (depending on testing day it ranged from 43% to 62%). Half of the participants received two 150 ml glasses of water. At the end of the procedure, body temperature and body mass were measured again and a second urine sample was collected.

WORKING MEMORY - SERIAL SEVENS

A computerized version of the serial sevens task was used, in which subjects were required, from a starting number between 800

Table I. Outline of experimental procedure

0 min	Urine sample, body temperature, body weight
15 min	Working memory
1 h 30 min	150 mL of water for those who drank
3 h	150 mL of water for those who drank
3 h 45 min	Working memory
3 h 50 min	Temperature, body weight (before urination)
4 h	Urine sample, body weight (after urination)

and 999, to say whether a second number was exactly seven less. One of two buttons that corresponded to yes or no was pressed. The test is scored as the time taken, in milliseconds, to perform each subtraction. A change in functioning was obtained by subtracting the time taken at baseline from the time taken at the end of the procedure.

BODY MASS

Body mass was measured using an electronic scale (Kern KMS-TM, Kenr and Sohn GmbH, Germany) that, to avoid problems associated with movement, took 50 assessments over a 5 second period and produced an average value. It was sensitive enough to weigh to within 5 grams (17% of an ounce). Subjects were weighed on arrival (after urinating) and after having emptied the bladder for a final time after 240 minutes. Changes in mass from baseline to 240 minutes reflected water loss due to perspiration, breathing and urine excretion. The total body mass lost due to urination, perspiration and breathing was calculated as the change percentage in body mass from the beginning to end of the study. The data presented were obtained wearing the same light clothing throughout the procedure, as preliminary studies found that these data were virtually identical to when measured naked.

OSMOLALITY

Urine osmolality was assessed using an Osmomat 3000 freezing point osmometer (Gonotec GmbH, Berlin, Germany).

BODY TEMPERATURE

Body temperature was measured using a TH8 Infrared Ear Thermometer (Radiant Innovation, Taiwan).

STATISTICAL ANALYSIS

Cook's distance: detection of possible outliers

As with regression analysis, a particular observation may exert undue influence, Cook's distance (11) was calculated, establishing

the extent to which model residuals would change if a particular data point was excluded. Larger Cook's distance values indicate a greater influence. The threshold for determining influential observations was set as $4/N$ in line with previous recommendations (12). On the occasions that a case had a Cook's distance that exceeded this threshold, it was excluded and the data was re-analyzed. On no occasion did this affect the outcome: therefore as there was no reason to suspect that these cases were unusual, the reported results included all cases.

Mediation analysis

A number of possible mediators of the effect of hydration on cognition have been suggested, including changes in thirst, fluid loss, osmolality and body temperature. In order to consider these mechanisms a moderated multiple mediation analysis was carried out, using the SPSS PROCESS macro (model 8) (3), using bootstrapped sampling to estimate the indirect mediation effect. In the present analysis, 5,000 bootstrapped samples were drawn with replacement from the dataset, to estimate a sampling distribution for the indirect mediation pathway. A conceptual diagram of the model is presented in figure 1. A dummy variable was created for the dichotomous independent variable, drinking vs not drinking (X), with the change in speed of working memory as the dependant variable (Y). Change in thirst, change in body temperature, amount of fluid lost and change in osmolality were parallel mediators (M). The total effect of X on Y (denoted by c in figure 1), can be expressed as the sum of the direct effect (denoted by c') and indirect effects, that is the product of the a and b paths (denoted by ab), such that $c = c' + ab$. Indirect effects and 95% confidence

intervals are reported. Given the large variation in participants urine osmolality (and hence hydration status) upon arrival at the laboratory, this factor was entered as a potential moderator (W) of both the direct and indirect effects (Fig. 1).

RESULTS

The effect of drinking water on each of the mediators was considered (path a) and also whether the effect of drinking on each of the mediators depended on baseline osmolality.

PERCENTAGE OF WEIGHT LOST

As expected, those who consumed water lost significantly less weight than those who did not ($\beta = .235$ 95% CI LL -.822, UL -.653); on average, 0.6% of their body mass. Those who arrived with the highest osmolality lost less weight, irrespective of whether they had drunk ($\beta = .445$ 95% CI LL -.001, UL -.002). The interaction drink X osmolality was also significant ($\beta = .227$ 95% CI LL .001, UL .002); when no drink was consumed those who arrived with a higher osmolality lost less weight.

THIRST

Participants reported greater thirst if they had not drunk water ($\beta = -.245$ 95% CI LL -18.624, UL -4.477), but this did not depend on baseline osmolality.

CHANGE IN OSMOLALITY

As expected, participants who consumed water had a lower increase in osmolality ($\beta = -.369$ 95% CI LL -.242.049, UL -.17.775). In addition, those who arrived with the highest osmolality had the smallest increase ($\beta = -.464$ 95% CI LL -.524, UL -.298) but there was no interaction between drinking and baseline osmolality.

BODY TEMPERATURE

Those who arrived with the highest osmolality tended to have an increase in body temperature ($\beta = .153$ 95% CI LL .001, UL .002), however, drinking did not influence body temperature ($\beta = -.095$ 95% CI LL -.237, UL -.055) and this did not depend on baseline osmolality.

EFFECTS ON WORKING MEMORY

Next, the effect of drinking water on working memory was considered (path c) and then the influence of each mediator (path b).

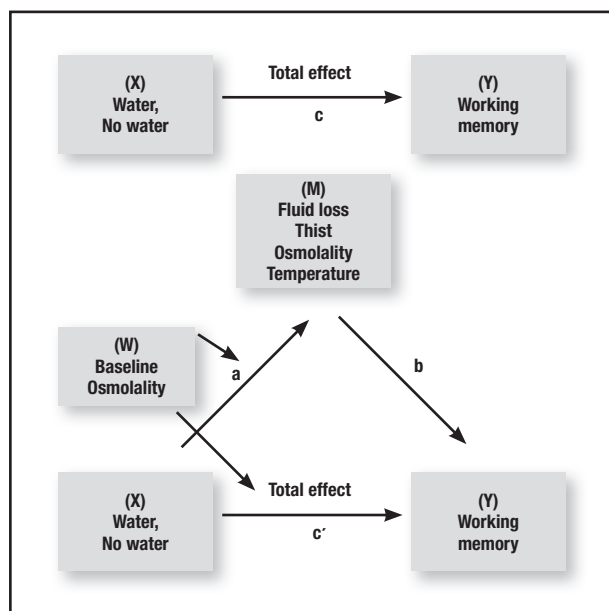


Figure 1.

Schematic of the moderated mediation analysis.

Those who consumed water performed the task more quickly at the end of the morning ($\beta = -.265$ 95% CI LL -497.932, UL -132.416) (Fig. 2). In addition, reaction times became longer in those who lost the greatest percentage of body weight ($\beta = -.237$ 95% CI LL -117.760, UL -45.824) and those who became the more thirsty ($\beta = -.226$ 95% CI LL -14.269, UL -2.449). There were no effect of changes in body temperature or osmolality on working memory.

CONDITIONAL DIRECT AND INDIRECT EFFECTS

Finally, the direct (path c') and indirect effect of drinking water on working memory, through each mediator, was considered. Whether these effects were conditional upon baseline osmolality (Fig. 1) was also examined.

The direct effect of drinking on working memory was not significant and baseline osmolality did not moderate this effect (Table I). Neither change in osmolality nor body temperature mediated the effect of drinking on working memory. However, both thirst and loss of body mass mediated the effect but only in those with higher levels of baseline osmolality. Those who lost more body mass had a poorer working memory, as did those who became most thirsty; these effects were prevented by drinking water but only in those with higher levels of osmolality at baseline (Table I). For both fluid loss (-.301, 95% CI LL -.688, UL -.091) and thirst (.163, 95% CI LL .0149, UL .424) the index of moderated mediation (the indirect effect of highest order product) (3) was significant.

SUMMARY

- Those with a higher baseline osmolality tended to have a greater increase in body temperature and lost less weight, irrespective of whether they drank or not.
- Those who consumed water had better working memory.
- Working memory was worse in those who lost more body mass or became more thirsty, but only in those with the higher levels of baseline osmolality.

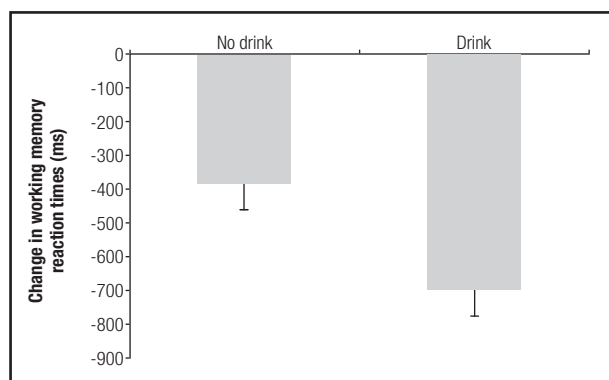


Figure 2.

The effect of drinking water on working memory.

DISCUSSION

There is a parallel between drinking water preventing a decline in working memory (Fig. 2) and previous findings: using the same paradigm, drinking prevented a decline in episodic memory and focussed attention (13). Yet the present report contrasts with previous reports in that working memory was not disrupted by a loss of 1-2% of body mass (4,8-10) and that even a loss of more than 2% has only sometimes (4-8) been found to be disruptive.

Various factors may account for present positive response to drinking. Firstly, the sample size was by far the largest in this area and previous studies may have used a sample that was too small to pick up a change of the size that is likely to result (2).

Sample size is likely to be fundamental, as both psychological and physiological measures in this area are subject to considerable individual variability with the consequence that large samples are needed to achieve the required statistical power. Rather than trying to study subjects who had been treated identically, the present study considered subjects who had consumed their normal breakfast and whose hydration status reflected the habitual fluid intake.

In the event baseline osmolality varied from 115 to 1,168 milliosmoles/kg. In those who had not drunk, the identical hypo-hydration inducing situation produced a loss of body mass that ranged from -1.52% to -0.24%. In those who had drunk, the change in body mass varied from -1.51% to +0.54%. Those with a higher baseline osmolality tended to have a greater increase in body temperature; they also lost less weight, irrespective of whether they drank water.

A difference in response, depending on baseline osmolality, suggested the possibility that physiological adaptations may have occurred to the level of habitual water intake: that is, those used to a low level of intake responded differently. Similarly, with both attention and episodic memory the loss of a greater percentage of body mass resulted in poorer memory and attention (13). If the basic phenomenon is an interaction between hypo-hydration, and individual differences in physiological status, this is a reason to use a moderated mediation analysis.

It is possible that previous negative findings reflect attempts to experimentally produce a similar physiological state, when there is a need to consider the interaction between drinking and individual differences in physiological status. Alternatively, if physiological parameters had not been controlled, then large individual differences would greatly decrease the chance of statistical significance because of a greater variability in response. The finding that working memory was worse in those who lost more body mass, and became more thirsty, but only in those with the higher levels of baseline osmolality (Table II), illustrated the need to consider hypo-hydration in the context of individual physiological differences. The results demonstrate the value of using moderated mediation when considering such interactions.

A major problem when researching hydration is that it is inevitable that you know whether you have or have not drunk. As such, there is potentially a role for a placebo response; a problem as there is evidence that many factors associated with

Table II. Moderated mediation analysis of the effect of drinking on working memory

Baseline osmolality mOsm/kg	Conditional direct path c' (B, 95% CI)	Conditional indirect path ab (body mass) (B, 95% CI)	Conditional indirect path ab (thirst) (B, 95% CI)	Conditional indirect path ab (osmolality) (B, 95% CI)	Conditional indirect path ab (body temp) (B, 95% CI)
388.03	-266.24 (-550.90, 18.43)	-37.56 (-170.17, 37.32)	19.19 (-2.78, 106.40)	-47.30 (-201.76, 85.65)	17.62 (-4.30, 102.39)
669.92	-218.97 (-449.31, 11.44)	<i>-122.60</i> (-266.72, -34.66)	<i>65.32</i> (14.94, 153.40)	-35.633 (-148.07, 70.44)	-10.21 (-70.80, 7.59)
951.80	-171.70 (-484.71, 141.31)	-207.64 (-423.51, -73.71)	60.17 (35.58, 252.60)	-23.97 (-134.86, 37.63)	-38.03 (-173.69, 9.056)

Data are B and 95% CI for the direct effect of drinking and the indirect effects of drinking through the parallel mediators; fluid loss, change in thirst, osmolality and body temperature, moderated by baseline osmolality at +/- 1SD from the mean. Italics indicate that the path was significant.

drinking, rather than the actual drink, influence cognitive functioning. Amongst many other effects, telling the subject that drink improved performance resulted in a better outcome and even paying a discounted price for an energy drink decreased the ability to problem solve (14). Given the many widespread pre-existing assumptions about the benefits of remaining hydrated, it is to be expected that drinking, as such, would be beneficial. Benton and Young (13) suggested that one approach is not to study groups, who have or have not drunk, but rather to examine individual differences. As there is a limited ability of individuals to know their hydration status or to understand individual differences in the ability to deal with hypo-hydration, where objective measures such as osmolality can be obtained, these can be considered without the worry of a placebo response. The use of regression equations and moderated mediation considers continuous variables that are not simply related to whether a drink has been consumed. In this manner, the use of regression equations can prevent aspects of a placebo response driving the results.

The present and related findings (13) suggest that small variations in hydration status influence cognitive functioning. However, it cannot be assumed that when faced with the same hypo-hydration inducing situation that everybody will respond in the same way. To be able to offer appropriate advice, parameters that influence an adverse response to hypo-hydration need to be established and convenient methods of measurement developed. For example, three questions related to the frequency of drinking were found to predict the baseline osmolality of urine (15). Using the same paradigm as the present study, individuals who reported habitually drinking more fluid at the end of the morning had a lower urine osmolality and had lost more body mass (15).

A final question is whether the phenomena reported, although statistically significant, have practical significance? An important consideration is individual differences in habitual water intake and the ability to deal with hypo-hydration: these vary greatly to the extent that the benefit for some individuals is likely to be small, whereas in others it will be significant. A task that emerges is to establish the habitual pattern of fluid intake that prevents an acute negative response to a subsequent low fluid intake. Similarly, the

optimal pattern of consumption when faced with a hypo-hydration inducing situation needs to be established: how much should be consumed and how frequently? Another response is to accept that human behavior reflects the influence of a vast range of factors, all of which have a small effect. Although it is unrealistic to expect that a change in a single variable is going to have a major impact, a philosophy of looking for many "marginal gains" may make a difference: ensuring adequate hydration offers one such gain.

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