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ARÁN

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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.

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).

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.

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

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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)/height^2 (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 ¼ creatinine mg ð Þ = day body weight kg ð Þ. 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 (Solutes in urine 24 h [mOsm/day]/[830-3.4] x [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/m²)	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 ± 560 kcal for boys and 2,116 ± 542 kcal for girls (p = 0.131) and the average total water intake was 2,411 ± 595 g in boys and 2,286 ± 649 (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 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 ± 4% *vs* 16 ± 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)		
	Food and beverages intake (g)			*Food and beverages intake (g)*		
	Mean (SD)			*Mean (SD)*		
	Hypohydrated	*Euhydrated*	*p*	*Hypohydrated*	*Euhydrated*	*p*
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 oy 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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