ABSTRACT

Background: The specific effects of fruit and vegetable (F&V) intake on water balance and consequently on 24-h hydration status (HS) are unknown.

Objectives: In a large observational cohort of German children, we examined whether a higher F&V intake per se is associated with improved HS and attempted to quantify the influence of greater consumption of F&Vs on HS.

Design: A total of 1286 complete 3-d weighed dietary records and 24-h urine samples for 442 children (4- to 10-y-olds) collected in 2000–2010 in the Dortmund Nutritional and Anthropometric Longitudinally Designed Study were analyzed. Free water reserve (FWR; urine volume [mL/24 h] minus obligatory urine volume [mL/24 h]) served as an HS biomarker. Median FWR and water balance variables were analyzed in different categories of solid-F&V intakes. Repeated-measures regression models (PROC MIXED; SAS Institute), adjusted for all other dietary water sources, were used to quantify the separate effects of solid-F&V and F&V-juice consumption on FWR.

Results: Negative FWR values, which indicated risk of hypohydration, were observed in 22% of children. FWR was significantly higher in solid-F&V consumers with high intakes than in those with low intakes (P < 0.0001). PROC MIXED models predicted an increase of 46 mL in FWR (average in boys and girls) when increasing solid-F&V intake by 100 g. Similar results were observed for F&V juice (β = 43, P < 0.0001). Drinking water and milk were the other significant dietary predictors of FWR. Solid F&Vs and F&V juices contributed 12% and 10%, respectively, to total water intake.

Conclusions: These data confirm that regular intake of F&Vs may relevantly improve HS in children. Dietary interventions to increase F&V intake may be a promising strategy to achieve positive water balance in this population.


INTRODUCTION

Hydration status (HS)4 is an important determinant of human health (1–3). Adequate hydration may reduce the risk of a range of physiologic disorders and diseases, such as headache, uricostasis, and constipation (3). HS is mainly determined by the balance of water intake (from foods and beverages) and water output (renal and nonrenal losses; eg, sweating, feces). The high and precise regulation of this balance in a range of ±0.2% of body weight over a 24-h period (4) is maintained by subtle hormonal changes, inducing thirst sensation and water reabsorption in the kidneys. How effective this regulation works was recently observed even in the elderly, a population at high risk of dehydration (5, 6).

The knowledge about the various variables that determine HS (water intake, water output, and dietary solute load) led to the concept of the “free water reserve” (FWR), introduced by Manz et al (7, 8) in the late 1990s. FWR is a physiologic concept to characterize 24-h HS in an individual and represents the balance between available body water (measured by urine volume) and water requirements (based on an individual’s solute load and the theoretical maximum urine osmolality); therefore, an FWR >0 mL indicates “euhydration.”

Although there is no universally accepted method for measuring HS to date, it is the biomarker most appropriate to characterize individual hydration in a 24-h period (8, 9).

The role of fruit and vegetables (F&Vs) as important food groups in the prevention of chronic diseases has been recognized (10–13), and the intake of F&Vs is part of dietary recommendations in many countries (12, 14, 15). As a result of the high water content of F&Vs (~70–95%, comparable to the 85–100% water content from beverages) (16), an increase in F&V intake can relevantly contribute to enhance water intake. However, to our knowledge, so far no study has investigated the impact of the latter on the physiologically well-regulated HS. This fact becomes relevant when it is hypothesized that HS might influence an individual’s food intake, ie, that a water deficit may induce an individual to prefer a diet with higher water content, potentially as a compensatory mechanism to counteract the deficit (17). To date, no study has investigated that hypothesis in free-living children.

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4 Abbreviations used: BMI-SDS, BMI SD score; DONALD Study, Dortmund Nutritional and Anthropometric Longitudinally Designed Study; F&V, fruit and vegetable; F&V juice, fruit and vegetable juice; F&V solid, solid fruit and vegetables; FWR, free water reserve; HS, hydration status; TWI, total water intake.

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We analyzed longitudinal data collected from 2000 to 2010 in 4- to 10-y-old free-living children as part of the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study to (1) examine whether a higher F&V intake per se leads to better HS or whether the higher water intake from F&Vs instead results in a compensational reduction in water intake from other sources, eventually keeping the water balance constant, and 2) to quantify the effect that adding F&Vs (solid or liquid) to the common diet has on the FWR as a measure of 24-h HS when other important water sources are controlled.

SUBJECTS AND METHODS

Study design

The DONALD Study is an ongoing open-cohort study that investigates relations between nutrition, growth, metabolism, and endocrine function in healthy subjects from infancy through early adulthood. The DONALD Study started in 1985 at the Research Institute of Child Nutrition, Germany. Participants are enrolled at the age of 3 mo and are followed with at least one visit per year to the institute until early adulthood. Details on the study design and methods are published elsewhere (18, 19). In brief, the assessments include 3-d weighed dietary records, anthropometric measurements, collections of 24-h urine samples, and interviews on lifestyle and medical assessments. To date, ~1070 children with at least one 24-h urine sample have participated (from 1985 to 2012). The DONALD Study is exclusively observational and noninvasive until the age of 18 y. All examinations are performed with parental, and later with the children’s, written consent. The study has been approved by the ethics committee of the Rheinische Friedrich-Wilhelms-University of Bonn (Germany).

Study sample

The study sample for this analysis consisted of a subgroup of DONALD participants between 4 and 10 y of age who had at least one 24-h urine sample collected between January 2000 to December 2010 with a parallel dietary record (encompassing the day of urine collection) (n = 477 children providing n = 1589 urine samples). Because of the open-cohort design of the DONALD Study, the number of subjects varied each year, with new 4-y-olds entering and older 10-y-olds leaving the research module during the time period of 2000–2010. Within this period, each subject underwent a minimum of 1 and a maximum of 7 potential measurements. Twenty-four-hour urine samples were excluded from analysis when the body weight–related 24-h creatinine excretion rate was <0.1 mmol · kg⁻¹ · d⁻¹ (20), indicating an incomplete collection. Quality-control variables, eg, collection time <20 h, illness, or discontinuous cooling (leaving portions of the urine at room temperature for >3 h before storing refrigerated at <-12°C), were additional criteria for exclusion. Total energy intake was used to exclude potentially implausible records by relating it to the basal metabolic rate (21) applying age- and sex-specific cutoffs (22). This resulted in the rejection of 53 children (11%) and their corresponding urine samples and dietary records. The final sample consisted of 424 children who provided 1286 complete 24-h urine samples with corresponding plausible dietary records.

Urine sampling and analysis

The 24-h urine collection was generally carried out on the third day of dietary recording. Children and their caregivers received personal information and written instructions on how to collect 24-h urine samples. The time of the start (discard of the first micturition of the start day) and finish (the first micturition of the following day) of the urine collection were recorded in a questionnaire, including the time of any lost specimens or intake of medications during the urine collection period. The urine samples were immediately stored in preservative-free, Extran-cleaned (Extran, MA03; Merck), 1-L plastic containers at <-12°C before the transfer by a dietitian to the institute where they were stored at <-20°C until analyzed.

HS and FWR

As marker of HS, we used the FWR (mL/24 h). This concept was previously established and described elsewhere (6–8, 17, 23, 24). FWR was calculated for each child as the measured urine volume (mL/24 h) minus the obligatory urine volume (mL/24 h) for each child. The obligatory urine volume (mL/24 h) is 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 (~2 SDs) value of maximum urine osmolality of the renal concentrating test for healthy subjects in the respective age group. In other words, obligatory urine volume is the water volume necessary to excrete 24-h urine solutes at the lower limit of maximum urine osmolality. In children and young adults consuming a typical affluent Western diet, with a high intake of protein, fat, and sodium chloride and a relatively low intake of complex carbohydrates from starch- and fiber-containing foods, this value is ~830 mOsm/L (7). Positive values of FWR are defined as “euthydration”; negative values of FWR denote “risk of hypohydration” (7, 17). Furthermore, we estimated the Adequate Intake of total water (mL/d) for our population (7). On the basis of the definition of the Recommended Dietary Allowance ("the average daily dietary nutrient intake level sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a particular life stage and sex group" (25)), Adequate Intake was calculated as the difference of the observed median total water intake (TWI; in mL/d) and the third percentile value of the FWR, as previously applied (6, 7).

Dietary assessment

To estimate individual food and nutrient intake, 3-d weighed dietary records were used. Parents of the children or the older children themselves kept the weighed dietary records, in which they registered and weighed all of the foods and fluids consumed as well as leftovers during 3 consecutive days. An electronic food scale was used (~1 g). Recipes for meals prepared at home were recorded. The packaging of commercial food products was kept. Semiquantitative recording was accepted when exact weighing was not possible (eg, number of spoons, scoops). At the end of the 3-d recording period, a dietitian visited the family and checked the records for completeness and accuracy.

Energy and nutrient values for the analysis were obtained from the continuously updated in-house nutrient database LEBTAB, which incorporates information from standard nutrient tables,
product labels, or recipe simulation on the basis of the labeled ingredients and nutrients (26). Data on total daily energy (MJ/d) and nutrient intakes (g/d) were calculated for each participant by using 2 different approaches: 1) the mean of the 3-d recording and 2) the value only from the day of the urine collection.

Food group definition
All reported food items were classified into 12 food groups on the basis of nutrient profiles and usage, as described in Appendix A. Two main categories were distinguished: 1) beverages and 2) foods, according to the usual way of how foods are consumed. The nature of the LEBTAB database allows disaggregating each food item into its basic components when it comes from recipes. For example, the “fruit group” or “vegetable group” included the content of fruit, vegetables, or both of composite dishes with meat, fish, pasta, rice and eggs or pizza, breakfast cereals, yogurts, dairy desserts, soups, puddings, and fruit pies. Pulses and potatoes were analyzed in separated food groups. As a result of disaggregating all of the food items to the ingredient level, our category “drinking water” comprised plain water and the water component of other beverages, ie, water added as an ingredient in processed or homemade beverages (described in Appendix A).

The term TWI corresponds to the total available water from beverages and foods additionally including the water produced by the oxidative process of the body after ingesting the respective foods or beverages (metabolic water). Metabolic water was calculated with the following formula recommended to estimate the water from oxidation (17): fat intake (g/d) × 1.07 + carbohydrate intake (g/d) × 0.55 + protein intake (g/d) × 0.41.

Anthropometric and additional variables
Anthropometric measurements of the DONALD participants were performed at each annual visit by trained personnel following standard procedures. Weight and height were obtained at the time of the dietary recording. Body weight was measured by using an electronic scale (Seca 753 E; Seca) to the nearest 0.1 kg. Height was measured in a standing position to the nearest 0.1 cm by using a digital telescopic stadiometer (Harpenden). BMI was calculated as body weight (in kg) divided by height (in m) squared. Sex-and age-independent BMI SD scores (BMI-SDSs) were calculated by using German national reference data (27).

On a child’s entry to the study, parents were asked to provide information about family characteristics (eg, education). The parents’ weight and height were measured by the same trained nurses who assessed the anthropometric characteristics of the participating children. BMI values were calculated, and maternal overweight was set to a BMI (in kg/m²) ≥25.

Physical activity (active, moderately active, and inactive) was assessed by a questionnaire on daily organized and unorganized activities, which is an adaptation of the Adolescent Physical Activity Recall Questionnaire (24).

Data handling and statistical analysis
An SAS statistical program (version 9.1.3; SAS Institute) was used for data analysis. A P value <0.05 was considered significant except for analyses of interactions, where P < 0.1 was considered statistically relevant. Outliers of TWI and FWR were defined as lying within 1.5 times the IQR below the first quartile or above the third quartile.

Each variable was tested for normality by the Shapiro-Wilk and Kolmogorov-Smirnov tests. Anthropometric, dietary, and urinary variables, stratified by sex and age group (4–10 y), are described as medians and IQRs as not normally distributed. Stratification by sex was based on the known sex differences in urinary osmolality (28); age groups were set according to the German reference values for nutrient intakes (29). Differences between age groups and sex were tested with unadjusted linear mixed-effects regression models (PROC MIXED in SAS) to account for the dependency between repeated measurements on the same child.

To examine the association between FWR and the corresponding water balance variables with solid-F&V (F&Vsolid) intakes, their distribution was grouped into sex-specific categories of consumption: quartiles of F&Vsolid intakes (g · d⁻¹ · MJ⁻¹) were assigned to low (<25th percentile), moderate (≥25th and ≤75th percentile), and high (>75th percentile) categories. Differences between categories of consumers were again tested with unadjusted PROC MIXED models.

The impact of F&Vsolid and F&V-juice (F&Vjuice) intakes on FWR was evaluated by using multivariable linear mixed-effects regression models (PROC MIXED), including both fixed and random effects. The random components of these models account for the nested nature of our data (children within families) and the lack of independence between repeated observations on the same person. Linear mixed-effects regression models consider all available measurements rather than using only participants with complete follow-up data (24). The basic longitudinal regression model included FWR as the dependent continuous variable and F&Vsolid and F&Vjuice intakes, chronological age, sex, chronological study years (2000–2010), and energy partition (description below) as principal independent fixed effects. The following nondiary variables potentially affecting the association between F&V solid or F&Vjuice intakes and FWR were considered: BMI-SDS, 24-h creatinine excretion (mmol/d), physical activity, seasonality, and maternal overweight and maternal level of education. Only those variables that 1) substantially modified the coefficient of F&Vsolid or F&Vjuice by 10%, 2) significantly predicted the FWR (P < 0.05), or 3) improved the fit statistic (Akaike’s information criterion) were additionally included as fixed effects to our model. To allow for the distinction of within-person and between-person effects of dietary F&V intakes on FWR, the main predictors (F&Vs as solids and juices) were centered on each individual’s mean of each individual predictor over time. Time-specific deviations from the person-specific means were used to test whether within-person changes in F&V intakes were associated with within-person changes on FWR. In addition, the person-specific, time-invariant means of the predictors were entered in the model to examine whether between-person differences in dietary F&V intakes are associated with differences in mean FWR between subjects (30).

The respective adjusted means of FWR in the categories of F&Vsolid or F&Vjuice consumers (low: <25th percentile of person-specific means; moderate: ≥25th to ≤75th percentile; high: >75th percentile) were the least-squares means predicted by the model when the other variables were held at their mean values. To quantify the changes in FWR when “adding” solid F&Vs or F&V juices to the diet, the PROC MIXED model was
Additionally adjusted for all other relevant dietary predictors of FWR. The following dietary variables were considered: drinking water (mL/d), intakes of other food groups (milk and whey-based milk products, cheese, meat/egg and cereals, potatoes, legumes, fat, and diverse listed in Appendix A); dietary fiber (g), and sodium excretion (as an estimate for salt intake). For energy adjustment of all PROC MIXED models, we used the energy partition model: ie, the dietary predictors (eg, F&Vsolid and F&Vjuice) were adjusted for energy intake from all other foods. The estimates of the F&V food group reflect the effect of “adding” the respective food group, comprising both its energy and nonenergy effect (31). Models for FWR contained a random statement for the family level and one for the person level with an unstructured covariance. The latter random statement considered individual differences in FWR at the first observation (intercept) and individual changes with increasing age (slope). On the basis of the known sex differences in urinary variables (28), the results of the models were stratified by sex.

Because the analyses indicated no interactions of age group with the relation of F&Vsolid intakes and FWR (each \( P > 0.8 \)), data from both age groups were pooled for analysis.

Multivariate logistic regression was used to calculate ORs for the risk of hyponhydration (FWR <0) by the defined categories of F&Vsolid intakes (g · d⁻¹ · MJ⁻¹) (low, moderate, and high) adjusted for age. Only the first observation of each child was included in this analysis.

All analyses were performed twice: 1) with mean dietary intakes calculated from the individual means of all 3 recording days and 2) only from the one day parallel to the urine collection (see the “Dietary assessment” section). Because calculated means of energy intake, TWI and intakes of F&Vs (solid and juice), drinking water, and milk did not differ significantly (tested with the Wilcoxon Mann-Whitney test) and the results of the PROC MIXED model were comparable (similar \( \beta \)-values and identical significance levels; data not shown), we decided to present only the results for 3-d mean dietary intakes as more stable values reflecting the children’s usual dietary intakes.

RESULTS

Descriptive characteristics for anthropometric, urinary, dietary, and water variables of the study sample stratified by sex and age group are presented in Table 1. Median FWR values were positive in all age/sex groups; only in boys were negative values observed in the 25th percentile, denoting a risk of hyponhydration. FWR was significantly lower in boys than in girls; accordingly, positive FWR values (euhydration) were observed in 72% of the measurements in boys and in 84% of the measurements in girls. F&Vsolid intake (g/d) increased significantly with age but showed no differences between boys and girls. When related to energy, F&Vsolid intake (g · d⁻¹ · MJ⁻¹) was significantly lower in 7- to 10-y-old boys than in the younger boys and also lower than in their female counterparts. F&Vjuice intake contributed 10.7% to the TWI and 5.3% to total energy intake compared to the F&Vsolid intake, which contributed 12.0% and 5.6%, respectively.

Medians (25th, 75th percentiles) for TWI were 1380 (1195, 1625) mL/d in 4- to 6-y-old children (\( n = 533 \) observations) and 1700 (1465, 1975) mL/d in 7- to 10-y-old children (\( n = 753 \) observations) (\( P < 0.05 \)). Median (25th, 75th percentiles) water intake values (excluding metabolic water) stratified by age were 1215 (1040, 1450) mL/d for 4- to 6-y-old children and 1490 (1270, 1745) mL/d for 7- to 10-y-old children (\( P < 0.0001 \)). The median ratio between TWI and energy was 1.0 mL/kcal for all groups.

FWR and the general variables for water balance by categories of F&Vsolid intakes (low, moderate, and high) are presented in Table 2. Children with a high F&Vsolid intake had a significantly higher FWR than children with a low intake. Accordingly, TWI from solid F&Vs was significantly higher in children with high F&Vsolid intake; however, a significant decrease was observed in TWI from foods. Overall, TWI (from all foods and beverages) increased significantly with increasing F&Vsolid intake. With regard to the urinary variables, we observed a significantly higher urine volume in the high-F&Vsolid intake group, whereas the obligatory urine volume did not change (attributable to the constant solute load (mOsm/d)). Only boys in the high-F&Vsolid intake category had a significantly lower urine osmolality (mOsm/L).

The results of the multivariable linear mixed-effects regression models (PROC MIXED) adjusted for age, energy partition, chronological study years, BMI-SDS, and 24-h creatinine excretion to investigate the impact of F&Vsolid and F&Vjuice intake on FWR are presented in Figure 1.

Boys and girls with consistently high F&Vsolid and F&Vjuice intakes had significantly better FWR values; between-person differences and within-person changes (data on the latter not shown) were similar for F&Vsolid and F&Vjuice intakes.

The results from the PROC MIXED model, which was additionally adjusted for all other relevant dietary predictors of FWR (to quantify the changes in FWR when “adding” solid F&Vs or F&Vjuice to the diet), are presented in Table 3. The \( \beta \)-values for the between-person differences represent the difference in FWR in milliliters for 1-unit difference in the explanatory variable (F&Vsolid or F&Vjuice) between the individuals across time. The \( \beta \)-values for within-person change indicate the intra-individual change in FWR (mL) for 1-unit within-person change in the explanatory variable. F&Vsolid and F&Vjuice intakes both were significantly positively associated with FWR at the inter- and intra-individual level. A difference of 100 g in F&Vsolid intake between persons was related to a 56-mL between-person difference in FWR in boys and a 35-mL between-person difference in girls, whereas a within-person increase of 100 g in F&Vsolid intake predicted 39- and 40-mL within-person increases in FWR in boys and girls, respectively.

From the other food groups examined, as expected, drinking water and milk (milk and whey-based milk products) showed a significant effect on the FWR. According to our model, an intake of 100 mL of water from drinks increases the FWR by 44 mL in boys and 55 mL in girls. A lesser, but significant effect was observed for the milk group, which increased the FWR by 25 mL in boys and 33 mL in girls per 100 g of intake. The TWI from other food groups (ie, food groups not included in the model; see Appendix A) had no significant effect on the FWR (\( P > 0.5 \)).

Multivariate-adjusted ORs for the risk of being hypohydrated (FWR <0) showed that higher consumption of F&Vsolid was significantly associated with a reduced risk of hyponhydration. Boys in the low- and moderate-F&Vsolid intake categories showed a 2.6-fold (95% CI: 1.1-, 5.9-fold) and 1.1-fold (0.5-, 2.3-fold) higher risk, respectively, compared with the highest category (\( P \)-trend = 0.03). Girls had a 2.0-fold (0.8-, 4.8-fold) and 0.6-fold...
Anthropometric, urinary, and dietary variables for 424 (4- to 10-y-old) participants from the DONALD Study

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
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<tr>
<td>BMI-SDS</td>
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Dietary variables

<table>
<thead>
<tr>
<th>Food &amp; Vegetables (%)</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
</table>

Urinary variables

<table>
<thead>
<tr>
<th>FWR (mL/d)</th>
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<th></th>
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</thead>
</table>

Differences between age groups and sex were tested with linear mixed-effects regression models (PROC MIXED; SAS Institute) to account for the dependency between repeated measurements on the same child.

Significantly different from girls in the same age group.

Energy intake (MJ/d) = (energy intake from solid F&Vs + energy intake from liquid F&V juices + energy intake from beverages) / total energy consumed.

AI = median TWI

Water from beverages (%) = (water from beverages/TWI) × 100

Water from foods (%) = (water from foods/TWI) × 100

Metabolic water (%) = (0.41 × protein intake [g] + 0.55 × carbohydrate intake [g] + 1.07 × fat intake [g])/TWI × 100

Estimated as TWI/total energy consumed according to the European Food Safety Authority recommendation of 1 mL/kcal for total available water intake/energy (32).

AI = median TWI – FWR third percentile.

The fruit most consumed for this group of children was as follows: apples, bananas, and strawberries; in the vegetable category were these were cucumbers, carrots, and tomatoes. All F&Vs were consumed either raw or as part of preparations. With regard to fruit juices, the 3 most commonly consumed were apple, orange, and grape.

DISCUSSION

Although it is known that 24-h urinary osmolality and related FWR values are linked to the TWI from the diet (17, 23, 33, 34), studies on the direct effect of specific dietary factors on HS are limited. To the best of our knowledge, this study for the first time provides evidence and quantifies the association between F&V intake (solid and liquid) and HS in healthy children. Our analyses were based on the combination of dietary intakes, estimated from 3-d weighed records, and 24-h urine indexes to estimate the FWR; the latter to date is one of the recommended biomarkers to characterize 24-h hydration (35).

Our findings showed significantly higher absolute FWR values after a higher consumption of solid F&Vs in both boys and girls. This, at first glance, contrasts with the hypothesis that a higher F&V intake might act as a compensatory mechanism for a diminishing of water intake from other sources. In the expected direction, the higher FWR was accompanied by a higher overall TWI in children with higher F&V intakes; however, in parallel a decrease in TWI intake from other foods was observed. This indicates at least a partial compensatory effect of the TWI from solid F&Vs with regard to the TWI from other water sources. This also helps to explain why TWI from solid F&Vs increased by 230 mL from the low- to the high-F&Vsolid intake category, whereas the observed net change in FWR was only 130–140 mL (still indicating a more favorable FWR with higher F&Vsolid intakes). No differences were observed in the solute load (mOsm/d) from the diet; however, urine volume was significantly higher, which led to a significantly lower urine osmolality (mOsm/L) in the high-F&Vsolid intake group.

We chose to analyze total F&V intake and food groups in a conservative manner [i.e., total weight (g) including water and nonwater content] rather than just by their moisture content to

1 All values are medians; 25th, 75th percentiles in parentheses. Total of 1286 measurements. AI, Adequate Intake; BMI-SDS, BMI-SD score; DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; F&V, fruit and vegetable; F&Vjuice, fruit and vegetable juice; F&Vsolid, solid fruit and vegetable; FWR, free water reserve; TWI, total water intake.

2 Differences between age groups and sex were tested with linear mixed-effects regression models (PROC MIXED; SAS Institute) to account for the dependency between repeated measurements on the same child.

3 Significantly different from girls in the same age group.

4 TWI, estimated as the sum of water from beverages (plain and mineral water and water content from beverages and F&V juices), water from foods (including solid F&Vs, milk, dairy products, and solid foods), and metabolic water (from oxidation).

5 Water from beverages (%)/TWI × 100.

6 Water from foods (%) = (water from foods/TWI) × 100.

7 Metabolic water (%) = (0.41 × protein intake [g] + 0.55 × carbohydrate intake [g] + 1.07 × fat intake [g])/TWI × 100.

8 Estimated as TWI/total energy consumed according to the European Food Safety Authority recommendation of 1 mL/kcal for total available water intake/energy (32).

9 AI = median TWI – FWR third percentile.

(0.3- 1.5-fold) increased risk in the low and moderate categories compared with the highest category (P-trend = 0.02).

The fruit most consumed for this group of children was as follows: apples, bananas, and strawberries; in the vegetable category were these were cucumbers, carrots, and tomatoes. All F&Vs were consumed either raw or as part of preparations. With regard to fruit juices, the 3 most commonly consumed were apple, orange, and grape.
### TABLE 2
FWR and water balance in 4- to 10-y-old participants in the DONALD Study by categories of solid F&V intake

<table>
<thead>
<tr>
<th>F&amp;V solid categories</th>
<th>Low (n = 210; 633 measurements)</th>
<th>Moderate (n = 210; 633 measurements)</th>
<th>High (n = 210; 633 measurements)</th>
<th>P&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Low (n = 214; 653 measurements)</th>
<th>Moderate (n = 214; 653 measurements)</th>
<th>High (n = 214; 653 measurements)</th>
<th>P&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F&amp;V solid (g/d)</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>98 (60, 120)</td>
<td>196 (160, 240)</td>
<td>346 (290, 415)</td>
<td>&lt;0.0001</td>
<td>100 (80, 125)</td>
<td>210 (180, 254)</td>
<td>348 (300, 425)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>FWR (mL/d)</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>40 (60, 240)</td>
<td>160 (0, 350)</td>
<td>185 (60, 460)</td>
<td>&lt;0.0001</td>
<td>150 (25, 305)</td>
<td>200 (70, 400)</td>
<td>280 (125, 530)</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>0.6</td>
<td>7.2</td>
<td>7.0</td>
<td>7.0</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>BSA (m&lt;sup&gt;2&lt;/sup&gt;)</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.97 (0.8, 1.1)</td>
<td>0.94 (0.8, 1.1)</td>
<td>0.95 (0.8, 1.1)</td>
<td>0.93 (0.8, 1.1)</td>
<td>0.92 (0.8, 1.1)</td>
<td>0.92 (0.8, 1.1)</td>
<td>0.92 (0.8, 1.1)</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Water intake (mL/d)</strong>&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1600 (1300, 1870)</td>
<td>1610 (1345, 1870)</td>
<td>1720 (1440, 1985)</td>
<td>0.029</td>
<td>1420 (1205, 1610)</td>
<td>1520 (1300, 1750)</td>
<td>1640 (1350, 1970)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>From F&amp;V solid</strong></td>
<td>90 (55, 110)</td>
<td>180 (150, 220)</td>
<td>320 (270, 380)</td>
<td>&lt;0.0001</td>
<td>90 (70, 115)</td>
<td>195 (160, 235)</td>
<td>320 (275, 390)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>From F&amp;V juice</strong></td>
<td>190 (95, 345)</td>
<td>180 (85, 300)</td>
<td>170 (75, 305)</td>
<td>0.0005</td>
<td>150 (40, 240)</td>
<td>155 (60, 270)</td>
<td>115 (30, 220)</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>From drinking water</strong></td>
<td>700 (340, 720)</td>
<td>530 (380, 730)</td>
<td>560 (375, 800)</td>
<td>0.7</td>
<td>525 (405, 685)</td>
<td>550 (395, 735)</td>
<td>570 (405, 770)</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>From foods (except for F&amp;V solid)</strong></td>
<td>700 (540, 860)</td>
<td>655 (545, 785)</td>
<td>610 (490, 730)</td>
<td>0.003</td>
<td>620 (490, 740)</td>
<td>565 (485, 680)</td>
<td>550 (460, 660)</td>
<td>0.047</td>
</tr>
<tr>
<td><strong>Water excretion (mL/d)</strong>&lt;sup&gt;5&lt;/sup&gt;</td>
<td>900 (695, 1105)</td>
<td>840 (660, 1085)</td>
<td>880 (715, 1135)</td>
<td>0.3</td>
<td>725 (520, 910)</td>
<td>750 (565, 930)</td>
<td>775 (615, 990)</td>
<td>0.054</td>
</tr>
<tr>
<td><strong>Nonrenal water losses</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
<td>665 (460, 900)</td>
<td>710 (540, 960)</td>
<td>745 (580, 1040)</td>
<td>0.011</td>
<td>680 (490, 890)</td>
<td>720 (530, 950)</td>
<td>795 (605, 1075)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Urine volume</strong></td>
<td>565 (440, 695)</td>
<td>560 (460, 670)</td>
<td>535 (470, 670)</td>
<td>0.9</td>
<td>500 (415, 605)</td>
<td>485 (415, 590)</td>
<td>505 (410, 600)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Obligatory urine volume (mL/d)</strong></td>
<td>782 (600, 945)</td>
<td>652 (515, 833)</td>
<td>620 (470, 750)</td>
<td>&lt;0.0001</td>
<td>648 (502, 808)</td>
<td>580 (445, 720)</td>
<td>572 (400, 684)</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Urine osmolality (mOsm/L)</strong></td>
<td>470 (366, 575)</td>
<td>464 (380, 554)</td>
<td>445 (390, 554)</td>
<td>0.9</td>
<td>416 (342, 501)</td>
<td>405 (342, 488)</td>
<td>420 (340, 600)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<sup>1</sup> All values are medians; 25th, 75th percentiles in parentheses. Total n = 424 children (n = 1286 observations). BSA, body surface area; DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; F&V, fruit and vegetable; F&V<sub>juice</sub>, fruit and vegetable juice; F&V<sub>solid</sub>, solid fruit and vegetables; FWR, free water reserve; TWI, total water intake.

<sup>2</sup> Categories of solid F&V intake were derived by quartiles of mean intakes per energy (MJ) combining the second and third quartile. Categories were defined as follows: low (<25th percentile), moderate (25th to 75th percentiles), and high (>75th percentile).

<sup>3</sup> Differences between categories of intake were tested with linear mixed-effects regression models (PROC MIXED; SAS Institute) to account for the dependency between repeated measurements on the same child.

<sup>4</sup> Estimated as the total water from beverages (plain and mineral water and water content from beverages and F&V juices), water from foods (including solid F&Vs, milk, dairy products, and solid foods), and metabolic water (from oxidation).

<sup>5</sup> Estimated as the difference between TWI (mL/d) and 24-h urine volume (mL/d).
Nonetheless, different authors have previously speculated that F&V content of the diet may be an important predictor of HS (28, 33). With the present study, we were able to confirm this assumption and quantify the isolated effect of the F&V food group on FWR.

Girls in our study showed a better HS than boys (especially in the younger age group). Interestingly, we could not confirm that the better HS observed in girls than in boys can be exclusively explained by a preference of girls for foods with a high water content such as F&Vs, as different authors have suggested (24, 28, 33, 36, 37). F&Vsolid (MJ/d) intakes were significantly higher only in the older age group, and TWI (related to energy) was the same in girls as in boys. Our data suggest that the lower nonrenal water losses in girls and the lower solute load (determining the obligatory urine volume) are 2 of the various explanations for the better HS observed in girls.

With regard to our second aim, the longitudinal mixed-effects regression model allowed a quantitative understanding of the relations of F&V intake (F&Vsolid and F&Vjuice) and FWR when all other water-supplying sources were kept constant. The effects on FWR were comparable between F&V solids and juices. Intentionally, F&V juice was included separately as a drink in the model and controlled as another water source that predisposes children to better hydration. Yet, a specific effect of F&Vsolid intake persisted after adjusting for F&Vjuice and other water sources.

Not surprisingly, the only other significant dietary contributors to FWR were drinking water and milk (milk and whey-based milk products). In theory, our findings can be illustrated with a simple example (derived from the within-person changing β-values): for a boy with 1600 mL TWI/d (from drinking water, solid F&Vs, F&Vjuice, milk, and water from other foods), an extra intake of approximately half a glass of plain water (115 mL) would increase the FWR by 50 mL. The same effect would be observed when approximately three-quarters of a glass of orange juice (156 mL) or one medium-sized apple (≈125 g) is additionally consumed. By contrast, drinking one glass of milk (200 mL) would be necessary to improve the FWR by the same volume.

Logistic regression analysis showed that the risk of hypohydration can be markedly influenced by F&Vsolid intake. An ~250-g higher intake of solid F&Vs (~2 portions) could reduce the risk of having a negative FWR by 2.6-fold in boys and 2.0-fold in girls. However, these risk effects should be interpreted with caution because of the rather wide 95% CIs.

The median intake of F&Vs (solids and juices taken together) in this cohort of highly selected children was 390 g/d, nearly meeting the current dietary recommendations for children “to eat 400 g/d of combined items limiting the 100% F&V juice to one portion (150 mL)” (14, 15, 38). Caution should be noted with regard to the definition of F&Vs, because we did not limit F&V juice portions and excluded foods such as legumes and potatoes, which are allowed in the recommendations. The data on the investigated children reflect the importance of solid F&Vs and F&V juice as dietary water contributors (23% of the TWI) and their relatively low energy contribution to the diet (~10%).

Current German recommendations for water intake in children, including metabolic water (4- to <7-y-olds: 1600 mL/d; 7- to <10-y-olds: 1800 mL/d) (29), are slightly higher than the median TWI values observed in our children (1380 and 1700 mL/d, respectively). Accordingly, we observed 22% measurements with negative FWR values. As described in our population, an additional water intake of 110–140 mL/24 h in boys better represent the typical form in which food is generally consumed.

The lack of studies relating diet and urinary markers to assess HS was a disadvantage in comparing our results. To our knowledge, to date only 2 studies in children have associated dietary characteristics and FWR as an HS biomarker. In both studies it was suggested that FWR values were affected by the quality of the diet; however, neither of the studies systematically examined the dissociated food group effect on FWR (17, 24).
and an extra 80–100 mL/24 h in girls would guarantee euhydration (ie, minimizing a potential risk of hypohydration).

Strengths of our analysis were its longitudinal design, ie, the availability of repeated measurements for >75% (n = 324) of the children, and the ability to quantify all foods consumed in 3 d by weighed dietary records encompassing a 24-h urine collection for assessing HS. A limitation of our analysis as well for other analyses of F&V intake quantification is the inconsistent universal definition and classification of F&Vs (38–41). However, the advantages of having the data disaggregated into basic food items, as was applied in this analysis, helped us to reduce the chance of misclassification of foods (especially composite foods) and therefore to have a very accurate estimate of total F&V intake. A priori, we decided to group the F&Vs following a classification similar to the “5-A-Day” definition (38) but excluded potatoes and legumes. These are 2 culturally important foods) and therefore to have a very accurate estimate of total F&Vs in children aged 4–10 y may lead to a better HS estimated by the FWR values from 24-h urine samples. The hypothesis that a higher intake of F&Vs may lead to a compensatory reduction in water intake because of effective regulatory mechanisms proved to be only partially true. Our findings suggest that adding solid F&Vs to the diet could in fact improve the hydration status. On the basis of these findings, dietary water recommendations should focus not just on water from fluids to meet needs but should also consider the promotion and availability of F&Vs in schools.

We acknowledge the participation of all children and their families from the DONALD Study. We thank the staff from the Research Institute of Child Nutrition for collecting anthropometric and dietary data and for urine laboratory analyses.

The authors’ responsibilities were as follows—TR and GM-B: designed the research; TR: was responsible for the urine analysis; GM-B and SAJ: performed the statistical analysis; and GM-B: wrote the manuscript and had primary responsibility for the final content. All authors were involved in data interpretation and in reviewing the manuscript and made contributions to and approved the final manuscript. None of the authors had any personal or financial conflicts of interest.

REFERENCES

# Table 3

<table>
<thead>
<tr>
<th></th>
<th>Boys (n = 210; 633 measurements)</th>
<th>Girls (n = 214; 653 measurements)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>F&amp;V_{solid} (g/d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between-person difference</td>
<td>0.56 (0.33, 0.79)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Within-person change</td>
<td>0.39 (0.14, 0.64)</td>
<td>0.0025</td>
</tr>
<tr>
<td>F&amp;V_{juice} (mL/d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between-person difference</td>
<td>0.41 (0.27, 0.55)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Within-person change</td>
<td>0.32 (0.17, 0.46)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Drinking water² (mL/d)</td>
<td>0.44 (0.36, 0.51)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Milk³ (g/d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between-person difference</td>
<td>0.25 (0.14, 0.36)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

¹Values are the results of the longitudinal mixed model with a random intercept adjusted for age, chronological study years (2000–2010), BMI-SD score, 24-h creatinine excretion, total water intake from other foods (total water intake minus the total water intake from the food groups included in the model, ie, solid F&Vs and F&V juices, drinking water, and milk), and energy from other foods (total energy minus the energy contribution from the food groups included in the model). DONALD: Dortmund Nutritional and Anthropometric Longitudinally Designed; F&V, fruit and vegetable; F&V_{juice}, fruit and vegetable juice; F&V_{solid}, solid fruit and vegetables; FWR, free water reserve; β, estimated change of the dependent variable per unit change of the independent variable.
²Comprises plain water and water component from other beverages.
³Milk and whey-based milk products.
<table>
<thead>
<tr>
<th>Category</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beverages</td>
<td><strong>1) Drinking water</strong> Plain water (tap water, bottled noncarbonated and mineral water) and the water component from infusions (coffee, tea), regular and diet soft drinks (carbonated and noncarbonated), sugar-sweetened drinks (lemonades, iced tea), fruit-flavored drinks, sports drinks, and energy drinks</td>
</tr>
<tr>
<td></td>
<td><strong>2) Fruit and vegetable juices</strong> 100% fruit and/or vegetable juices, homemade or commercial</td>
</tr>
<tr>
<td>Foods</td>
<td><strong>3) Milk and whey-based milk products</strong> Whole fluid milk, flavored milk, partly skimmed and skimmed milk, yogurt, yogurt shake, buttermilk, quark, sour cream, condensed milk, and cottage cheese</td>
</tr>
<tr>
<td></td>
<td><strong>4) Fruit</strong> All fruit: fresh, dried, canned (without syrup), and frozen</td>
</tr>
<tr>
<td></td>
<td><strong>5) Vegetables</strong> All vegetables: raw, boiled, canned, and frozen</td>
</tr>
<tr>
<td></td>
<td><strong>6) Cheese</strong> All types of processed cheese: cheddar type, cream cheese, and creamy cheese bases</td>
</tr>
<tr>
<td></td>
<td><strong>7) Meats, poultry, seafood, and eggs</strong> Beef, lamb, pork, chicken, fish and seafood, eggs, meat substitutes (soy), all processed meats (sausages, ham, paté, etc)</td>
</tr>
<tr>
<td></td>
<td><strong>8) Fats</strong> All types of vegetable oils, margarine, butter and animal fat, spreadable butter, lard, and nuts and seeds</td>
</tr>
<tr>
<td></td>
<td><strong>9) Cereals</strong> All types of bread, flour and flour products, crackers, biscuits, fortified and nonfortified hot or cold cereals, rice (all types), pasta (all types), couscous, and cereal bars</td>
</tr>
<tr>
<td></td>
<td><strong>10) Potatoes</strong> Boiled, roasted, and flour</td>
</tr>
<tr>
<td></td>
<td><strong>11) Legumes</strong> Beans, chickpeas, lentils, and peas</td>
</tr>
<tr>
<td></td>
<td><strong>12) Diverse</strong> Sugar (reported as table sugar or sugar from beverages and recipes), sweets (hard candy), chocolate, cocoa, crisps, and condiments</td>
</tr>
</tbody>
</table>

1 Food groups not included in the final PROC MIXED (SAS Institute) regression model.