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Urinary sucrose and fructose to validate self-reported sugar intake in children and adolescents: results from the I.Family study

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Abstract

Purpose: Excessive consumption of free sugar increases the risk for non-communicable diseases where a proper assessment of this intake is necessary to correctly estimate its association with certain diseases. Urinary sugars have been suggested as objective biomarkers for total and free sugar intake in adults but less is known about this marker in children and adolescents. Therefore, the aim of this exploratory study is to evaluate the relative validity of self-reported intake using urinary sugars in children and adolescents.
Methods: The study was conducted in a convenience subsample of 228 participants aged 5-18 years of the I.Family study that investigates the determinants of food choices, lifestyle and health in European families. Total, free and intrinsic sugar intake (g/day) and sugar density (g/1,000 kcal) were assessed using 24-hour dietary recalls (24HDRs). Urinary sucrose (USUC) and urinary fructose (UFRU) were measured in morning urine samples and corrected for creatinine excretion (USUC/Cr, UFRU/Cr). Correlation coefficients, the method of triads and linear regression models were used to investigate the relationship between intake of different types of sugar and urinary sugars.
Results: The correlation between usual sugar density calculated from multiple 24HDRs and the sum of USUC/Cr and UFRU/Cr (USUC/Cr+UFRU/Cr) was 0.38 ($p<0.001$). The method of triads revealed validity coefficients for the 24HDR from 0.64 to 0.87. Linear regression models showed statistically significant positive associations between USUC/Cr+UFRU/Cr and the intake of total and free sugar.
Conclusions: These findings support the relative validity of total and free sugar intake assessed by self-reported 24HDRs in children and adolescents.

Introduction

According to the World Health Organization (WHO) excessive consumption of free sugar is associated with poor diet, obesity and the risk for non-communicable diseases [1, 2]. Therefore, the WHO recommends the reduction of free sugar intake throughout the whole life-course, particularly focusing on the reduction of free sugar to less than 5-10% of the total energy intake [1]. Furthermore, in their regularly updated recommendations the World Cancer Research Fund and the American Institute for Cancer Research advised to avoid sugary drinks in order to prevent weight gain and cancer [3, 4]. Nevertheless, total sugar is one of the main contributors to energy in diet, with energy from total sugar being reported to exceed 20% of total energy intake in 2-9-year-old European children [5].

Although the assessment of dietary intake in epidemiological studies is difficult in all age groups, it is especially challenging in children, particularly because children have a highly variable diet and their food requirements are strongly age-specific [6]. Furthermore, recall methods such as the 24-hour dietary recall (24HDR) highly depend on the children's cognitive abilities to remember and to correctly estimate food quantities. If proxies report for the children 24HDRs will depend on the proxies' memory and their presence during children's meals [6]. Thus, on the one hand measurement error and misreporting are inherent problems of 24HDRs in children [7]. On the other hand, a proper assessment of dietary intake is an important prerequisite to correctly estimate the association of dietary intake with certain health outcomes. Therefore, appropriate validation methods such as biomarkers are

used to assess the correctness of individual reports. Even though it is well-known that the various types of biomarkers are not fully objective and not independent of the study subjects or assessment method, their use has been proven to reduce measurement error in nutritional epidemiological studies [8].

In the past we already successfully used the biomarkers urinary calcium and potassium and doubly labelled water in children to validate milk consumption frequencies assessed by food frequency questionnaires (FFQ) [9] and energy intake assessed by a 24HDR called SACINA (Self-Administered Children and Infants Nutrition Assessment) [10]. For our present study, concentration biomarkers that measure the concentration of specific compounds in urine or other tissues seem to be the only reasonable choice, where it has been shown in particular that urinary sucrose (USUC) and fructose (UFRU) are associated with dietary intake of simple sugars [11, 12]. In the 1970s some authors observed that small amounts of dietary fructose and sucrose are excreted in urine [11, 12]. Since fructose and sucrose cannot be synthesized in the human body UFRU and USUC have to originate from dietary intake. Although the process is not completely understood, it is known that dietary sucrose is decomposed into glucose and fructose in the duodenum. In addition, it was shown by Utter (1927) and Folin and Berglund (1922) that small amounts of sucrose pass intact through the intestinal wall and are then later excreted in urine [13]. Furthermore, Tasevska et al. assumed that a small amount of fructose escapes fructose metabolism in the liver and is excreted in urine afterwards [14, 15].

The beneficial use of USUC and UFRU measured in 24-hour urine as a biomarker for sugar intake was shown in two studies. In the first study Tasevska et al. showed that under controlled conditions, both, total sugar and sucrose intake were highly correlated with the sum of USUC and UFRU [14]. In the second study, it was shown that USUC and UFRU were strongly correlated with self-reported intake of extrinsic sugar over a period of 30 days [15]. Based on the findings of these studies [14, 15], the OPEN study used 24-hour urine sugar biomarkers as a new dietary reference instrument to evaluate total sugar intake (g/day) and sugar density (g/1,000 kcal) obtained from FFQ and 24HDR [16].

Furthermore, another study showed a good correlation between 24-hour urine collection and single urinary spot collections, the latter being easier to perform and less invasive [17]. Finally, [18] applied urinary sugar measured in single spot urine to compare dietary sucrose and USUC. Thus, measuring sugar biomarkers in spot morning urine seems to be a reasonable assessment method for the intake of total and free sugar to be used in free-living children and adolescents because of their limited compliance to 24-hour urine collection.

The present exploratory study aims to evaluate the relative validity of self-reported sugar intake by investigating the relationship between concentrations of USUC and UFRU measured in spot morning urine samples and the intake of total, free and intrinsic sugar and sugar density derived from self-reported 24HDRs in a subsample of children and adolescents participating in the I.Family study.

Materials and Methods

Sample

The I. Family study, which is an extension and a further follow-up of the IDEFICS cohort [19], investigates the determinants of food choices, lifestyle and health in European children, adolescents and their parents [20]. From March 2013 to June 2014, we obtained morning urine samples from all I.Family study centers located in

Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain, and Sweden. In addition, we got samples from a Polish center which joined the study at a later point in time. At the first visit at the study center a collection cup and instructions were given to the children and adolescents or their parents. The morning urine was collected at home and brought to the study center on the same day (94% of the samples were first morning urine). No preservative was used but parents were instructed to cool down the urine sample in the home fridge if the time span between collection and handing over at the study center exceeds two hours. At the study center urine samples were kept at 4°C until further processing and/or storage at -20°C or at -80°C on the same day of collection.

At least 19 children and adolescents per center, of both sexes, aged 5-18 years, agreed to recall their diet using a 24HDR and to provide a morning urine sample on the same day of the dietary recall. All children and adolescents underwent a comprehensive examination protocol. For the present analysis, only children and adolescents with complete information on age, sex, height, weight, urinary sugars and corresponding 24HDR were included, resulting in a sample size of 228. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, all procedures were approved by the local ethics committee in each center and written and oral informed consents were obtained from the parents, their children and adolescents, respectively, before participation.

Anthropometric measurements

Anthropometric measurements were taken by trained personnel following detailed standard operation procedures in accordance with international standards [21, 22]. Standing height (cm) was measured using a Seca 225 stadiometer (Seca GmbH & KG, Birmingham, UK), while body weight (kg) was assessed in fasting state in light clothing using a prototype of the TANITA BC 420 SMA digital scale for children and a TANITA BC 418 MA for adolescents (TANITA Europe GmbH, Sindelfingen, Germany).

Body mass index (BMI) was calculated by dividing body weight in kilograms by the squared body height in meters. Age- and sex-specific BMI z-scores were derived and categorized in groups (underweight, normal weight, overweight and obese) according to the extended IOTF criteria [23].

Dietary assessment

Food intake of the previous 24-hours was reported using a web-based 24HDR assessment program called SACANA (Self-Administered Children, Adolescents and Adult Nutrition Assessment), that is based on the previously validated offline version SACINA [24]. Children and adolescents were asked to recall their diet and to enter the type and amount (g) of all drinks and foods consumed during the previous day, starting with the first intake after waking up in the morning. Standardized photographs were provided to assist the accurate estimation of portion size. Children below 11 years were advised to ask their parents for help. In total 44.7% of the children were assisted by parents. The total sugar and energy values of all reported food items were derived from country-specific food composition tables as for instance from the Bundeslebensmittelschlüssel Release 2.3.1 for Germany, described in more detail by Börnhorst et al. [25]. Participants were requested to complete repeated 24HDRs on at least two workdays and one weekend day. If participants completed less than three 24HDRs they

were reminded repeatedly 3-7 days after their visit in the study center or after their previous recalled day by telephone or by e-mail to complete another 24HDR.

Further, participants were asked to complete an eating habits questionnaire that included an FFQ which was based on a validated FFQ described in detail elsewhere [9, 26] . For the FFQ, the previous month was chosen as the reference period and frequencies of consumption for 59 food items were offered in the form of a close-ended, mutually exclusive list of options: never/less than once a week; 1-3 times per week; 4-6 times per week; once per day; twice per day; three times per day or more, which were converted into weekly frequencies. To calculate the relative consumption frequency of sweet foods for participants, the sum of frequencies from all sweet food items (including sugar-sweetened drinks and juices) were divided by the sum of frequencies from all food items as described in [26, 27].

Dietary data analysis

For each participant, the daily intake of energy (kcal/day), total, free and intrinsic sugar (g/day), as well as the sugar density (g/1,000 kcal) calculated as the ratio of total sugar intake (g/day) to energy intake (1000kcal/day) were derived. In line with Tasevska et al., the intake of intrinsic sugar was calculated by summing the total sugar from bread, flour meals, pasta, rice, grains, fruits (excluding preserved fruit and fruit with dairy), vegetables, nuts, seeds, vegetarian burgers and mixed dishes based on meat, cereals, pasta, rice and vegetables [15]. The intake of milk sugar was calculated as the total sugar from dairy products (excluding chocolate and vanilla milk, sweet yoghurt, sweetened cheese, sweetened curd and milk-based ice-creams), milk-based dishes, cream, and mixed dishes based on dairy products. The sugar from the remaining food groups was considered as free sugar [15]. Based on the available recall days, individual mean energy, total, free and intrinsic sugar intake and sugar density were calculated.

Since it is known that the mean of repeated 24HDRs leads to variance inflation if the number of recalled days is small [25, 28], individual usual energy intake and usual intake of total, free and intrinsic sugar were estimated based on the National Cancer Institute (NCI) method [29, 30]. This method allows the inclusion of covariates such as age, accounts for different intakes on weekend vs. workdays, and corrects for the daily variation in diet. Usual intakes were estimated for participants stratified by sex and considering age as a covariate. Usual sugar density was calculated as the ratio of usual total sugar intake (g/day) to usual energy intake (1000kcal/day).

Age- and sex-specific Goldberg cut-offs were applied to classify each participant as plausible, under- or over-reporter, based on the estimated usual energy intake as described in [31].

Laboratory methods

Collected urine specimens (aliquots of 1.5 ml minimum) from the eight I.Family study centers were sent for analysis to the Dept. of Biochemistry, Biophysics and General Pathology of the 2nd University of Naples under standard shipping conditions (either at -20 C° or at -80°C which was recorded as part of the shipping history). USUC and UFRU concentrations were determined using an enzyme-based kit (sucrose/D-glucose/D-fructose from Boehringer Mannheim/R-Biopharm) and a Perkin Elmer Lambda Array spectrophotometer to measure the absorbance rate [32]. All determinations were run in triplicate. Detected concentrations were in the range of 1-150 mg/L. Within this detection range, linearity of measurements was observed. Assay control solutions in the

range of expected values for sucrose and fructose were provided in the enzyme-based kit. Values for this quality control were remarkably stable (glucose concentration: 100.6 ± 5.9 mg/L, mean ± standard deviation, coefficient of variation 5.9%). USUC and UFRU from the Polish participants were determined at the Center for Innovative Research in Medical and Natural Sciences of the University of Rzeszów using the same enzyme-based kit and spectrophotometer Evolution 300 (Thermo Scientific).

Concentrations of sucrose and fructose for all participants were expressed as mg/L of urine and also as mg/g of creatinine (USUC/Cr, UFRU/Cr) to correct for fluctuations in urine volume [17].

Statistical analyses

All descriptive analyses were performed stratified by sex where we report mean and standard deviation of urinary sugars and the intake on the previous day, the individual mean of repeated 24HDRs and estimated individual usual intake of energy, total, free and intrinsic sugar and of sugar density.

To assess the correlation between sugar intake (consumption on previous day, mean of repeated 24HDRs and usual intake of total, free and intrinsic sugar and sugar density) and urinary sugars (USUC/Cr, UFRU/Cr and the sum of both (USUC/Cr+UFRU/Cr)), unadjusted and partial Spearman's correlation coefficients were estimated. The partial correlation coefficients were estimated adjusting for age, sex and BMI z-score. Please note that the statistical tests were all conducted with a significance level of α=0.05, i.e. without adjusting for multiple testing. Therefore and because of the exploratory character of the analysis reported p-values should be interpreted with caution.

Additionally, the method of triads was used to estimate the so-called validity coefficients of total sugar intake and sugar density assessed by 24HDR (R), relative consumption frequency of sweet foods (Q) and urine biomarkers (B) [33]. Here, we assume that each of the three measurements of intake (R, Q and B) can be modeled as a linear regression with the unknown true intake as regressor. The error terms are assumed as mutually independent. Then, according to Kaaks [33], the validity coefficients, i.e. the correlations between the true intake and the measured intakes, can be calculated based on the observed correlations between R, Q and B. In contrast to correlation coefficients, validity coefficients are non-negative and can exceed +1. To increase comparability with other published studies we provide validation coefficients for log-transformed and untransformed biomarkers as well as for usual intakes and for intakes on the previous day.

By applying different linear regression models, the effects of sugar intake on previous day based on single 24HDRs (separately for total sugar intake (Model 1), free sugar intake (Model 2) and sugar density (Model 3)) and of usual sugar density based on multiple 24HDRs (Model 4) on USUC/Cr+UFRU/Cr were estimated. The models were adjusted for age, sex and BMI z-score. To account for skewness, log-transformed USUC/Cr+UFRU/Cr was used as dependent variable. Q-Q plots were used to check for normality of residuals. As sensitivity analyses, linear regression models were estimated (i) restricted to normal weight children and adolescents and (ii) to plausible reporters since Bingham et al. assumed that dietary reports of sucrose intake in obese individuals are less valid than in normal weight individuals [18]. In addition, further sensitivity analyses were conducted excluding the children and adolescents from the Polish center in order to prevent from bias due to overrepresentation of this subgroup. The adjusted R^2, the mean absolute percent error (MAPE) and the

221 percentage of participants whose true and predicted values differed less than 10% relative to the true value were
222 calculated to assess the model fit.

223 All analyses were conducted using the statistical software SAS 9.3 (SAS Institute, Cary, NC, USA) (NCI method
224 and descriptive analyses) and R 3.2.3 (correlation analyses and linear regression models) [34].

225 **Results**

226 Description of study population

227 The number of participants ranged from 19 to 25 across all study centers apart from Poland (n=54) with slightly
228 more girls (n=120) than boys (n=108). Approximately two-thirds of the participants (65.8%) were normal weight
229 according to Cole and Lobstein [23] (Table 1). The number of recalled days varied from participant to
230 participant: 122 (53.5%) provided one recall, 32 (14.0%) two recalls and 74 (32.5%) three or more recalls. FFQ
231 information was available for 170 children and adolescents. We could not identify any over-reporters, but a total
232 of 124 plausible reporters (54.4%), where plausible reporters were underrepresented in Polish participants
233 (24.7%, data not shown).

234 Urinary sugar concentration and sugar intake

235 Higher levels of urinary sugar were found in girls compared to boys. Furthermore, sugar density (g/1,000 kcal)
236 was also higher in girls than in boys (Table 2), although boys reported a higher total intake of sugar compared to
237 girls. Standard deviations (SD) from intakes on a single day were higher than SD from individual mean intakes,
238 which in turn were higher than SD from estimated usual intakes. For instance, for total sugar intake the
239 corresponding SDs were 50.1 (intake on a single day), 44.3 (individual mean intake) and 20.2 (estimated usual
240 intake).

241 Correlation analyses

242 Table 3 presents Spearman correlation coefficients between sugar intake (total, free and intrinsic sugar intake
243 and sugar density) and urinary sugars (USUC/Cr, UFRU/Cr and USUC/Cr+UFRU/Cr). The highest raw
244 correlations with USUC/Cr were found for sugar density (previous day: 0.259; mean intake: 0.253; usual intake:
245 0.271). The same was true for UFRU/Cr (0.238; 0.295; 0.336) and for USUC/Cr+UFRU/Cr (0.32; 0.339; 0.38).
246 The correlation coefficients for intrinsic sugar were, however, lower than for free sugar. Compared to the
247 corresponding raw correlation coefficients, the partial correlation coefficients showed in general the same
248 patterns but were smaller.

249 Method of triads

250 The method of triads revealed higher validity coefficients for the 24HDR (0.64-0.87) than for the FFQ-based
251 relative consumption frequency of sweet foods and for USUC/Cr+UFRU/Cr (0.27-0.5). The highest validity
252 coefficient (0.87) was found for total sugar intake on the previous day where USUC/Cr+UFRU/Cr was used as
253 biomarker (Table 4).

Linear regression analyses

The results (β estimates and 95% confidence intervals (CI)) obtained from linear regression models are presented for the whole study population and for plausible reporters only, since sensitivity analyses did not reveal any deviations for the β estimates in the remaining subgroups (normal weight and non-Polish participants) (Table 5). For the whole study group we observed statistically significant associations of USUC/Cr+UFRU/Cr (log-transformed) with total sugar intake on previous day (100g) (β = 0.49, 95% CI = (0.25; 0.73)), free sugar intake on previous day (100g) (β = 0.44, 95% CI = (0.19; 0.70)), sugar density on previous day (100g/1000kcal) (β = 0.89, 95% CI = (0.42; 1.36)) and usual sugar density (100g/1000kcal) (β = 1.78, 95% CI = (0.53; 3.04)), respectively. We obtained similar results for plausible reporters. The Q-Q plot of residuals of Model 1 is shown in Figure 1. The remaining Q-Q plots showed a similar distribution of residuals (data not shown). For the whole study population the models explained 20.5% to 23.3% of variance. These values were slightly higher than in the three subgroups (normal weight, plausible reporters, non-Polish). In contrast, MAPE was lowest (~20%) in models only including plausible reporters. Further, for Model 1, 2 and 3, the percentages of participants whose true and predicted values differed by less than 10% were highest in the group of plausible reporters, ranging from 34.7% to 38.7%.

Discussion

The results showed moderate correlations between sugar density, free and total sugar intake and USUC/Cr+UFRU/Cr. The derived validity coefficients for sugar intakes obtained from 24HDRs were strong to very strong. Furthermore, there were statistically significant positive associations of log-transformed USUC/Cr+UFRU/Cr with intake of total and free sugar and sugar density.

To the best of our knowledge, this is the first study comparing sugar biomarkers in spot morning urine with intake of different types of sugar in children and adolescents. We only found one further urinary sugar biomarker study that was conducted in children [35]. In this study the association between 24-hour urinary fructose and the intake of total and added sugar was investigated. The correlation of 24h urinary fructose with total sugar (0.43) was higher than with added sugar (0.23) [35]. We did not expect to find similar correlations because of the different methods applied in their and in our study (24-hour vs spot urine, Pearson vs Spearman correlation coefficient and added vs free sugar). Indeed, in our study the correlation of USUC/Cr+UFRU/Cr with total and free sugar was lower (0.22 and 0.17).

Due to the fact that we only found one comparable study in children, we searched for comparable studies in adults. In 12 healthy male volunteers who had to consume a mandatory 30-day diet with three meals and two snacks under strict conditions in their suites, Tasevska et al. found a correlation of 0.89 between 24-hour USUC+UFRU and total sugar intake. A correlation coefficient of 0.84 was found when the conditions were relaxed and seven male and six female volunteers were allowed to consume their habitual diet [14]. In a second study, Tasevska et al. observed this high correlation especially for extrinsic sugar intake (correlation coefficient of 0.84) whereas the correlation with intrinsic sugar intake was much lower (correlation coefficient of 0.43) [15]. Another study with controlled intake found similar correlations using for a timespan of three days four creatinine corrected spot urine samples per day [17]. We did not expect to observe such high correlation coefficients in our analysis since our study participants were not asked to follow any dietary requirements and the least invasive

morning spot urine was collected only once. Taking urine spot also leads to more variation in sugar urine data compared to other studies with repeated assessment of urinary spots on multiple days. Unfortunately, the only study that also used single spot urine in adults to compare dietary sucrose and USUC did not report any correlation coefficients, making it difficult to compare their results with those obtained in our study in this respect [18]. However, the study found statistically significant positive associations between sucrose intake and urinary sucrose in normal weight participants and between fructose intake and urinary fructose in obese participants.

There are only a few studies that compared total sugar intake and energy density assessed by both FFQ and 24HDR with true sugar intake in adults [16, 36]. In these studies measurement error models incorporating the information of repeated 24HDRs, FFQs and 24-hour sugar urine measurements were used to derive the correlation coefficients between true intake and these three measurements of intake [16, 36]. We applied the method of triads instead as repeated FFQ and sugar urine measurements were not available. The correlations between true intake and sugar intake/density assessed by 24HDR were on the one hand higher in our study than in the aforementioned study by Tasevska et al. [16]. On the other hand the correlations between true intake and FFQ in the present study were lower than the correlations found in males in the study of Tasevska et al. [16], but higher than those found in females. Beyond the use of 24-hour instead of spot urine, reasons for these differences could be that different statistical methods were used and different population groups were investigated. In particular, the validity coefficients of total sugar intake and of sugar density assessed by 24HDR were unexpectedly high in our analysis. This could be due to the fact that we used the method of triads, which possibly overestimates correlations. As suggested by Kaaks, the validity coefficients of the 24HDR should be interpreted as an upper limit of the correlation between true and reported intakes [33].

A clear strength of this study is that we were able to analyze data collected in nine European countries according to a highly standardized protocol where in addition numerous quality checks were performed. Furthermore, the use of the web-based 24HDR tool SACANA increased the accuracy of portion size estimation through the display of photos for different portion sizes of food items. The data were investigated extensively, including derivation of individual usual intake of different types of sugar, energy adjustment, differentiation between intrinsic and free sugar, creatinine correction and involvement of FFQ information as a third assessment instrument. Additionally, sensitivity analyses were conducted for three subgroups to prevent from bias due to misreporting, non-normal weights or overrepresentation of the Polish sample.

When comparing this study with others, the use of single morning spot urine instead of repeated spot urine or 24-hour urine could be seen as a limitation. It should nevertheless be noted that the use of morning spot urine lowers the burden for the participants and partly prevents selection bias. This approach seems to be more feasible in large scale population-based studies, in particular, if urinary sugar excretion is adjusted by urinary creatinine to account for the variability in the single urine measurement. In this context, it is important to mention that there are already biomarkers in spot urine which are good predictors for medium- and long-term endpoints, e.g. microalbumin-creatinine ratio for microalbuminuria and urinary sodium for blood pressure [37, 38]. In our data the correlations between medium-term sugar intake and urinary sugars were similar to and sometimes even higher than the correlation between the sugar intake on the previous day and urinary sugars. Two explanations may be possible: first, sugar consumption in children and adolescents is stable over time, or second, the small

amount of sucrose and fructose, which is excreted in the urine and escaped absorption, hepatic metabolism and re-uptake, is associated with the habitual consumption and is not just the immediate response to sugar over-consumption. Nevertheless we agree with Campbell et al. [39] that controlled feeding studies are necessary to further investigate the use of sugars from spot urine as biomarker for sugar consumption since short-term food intake probably influences the excretion.

Restricting the regression analyses to the plausible reporters on the one hand led to very similar β estimates when compared to the results based on the whole sample, but on the other hand showed a substantially lower MAPE. The same was observed when restricting the analyses to normal weight children and adolescents. This suggests that the association between urinary sugar and reported intake of total and free sugar and sugar density may be more valid in this group than in overweight and/or misreporting children and adolescents.

In summary, this exploratory analysis showed that higher intake of total and free sugar resulted in an increased USUC/Cr+UFRU/Cr in children and adolescents. Correlations between sugar intake (total and free sugar and sugar density) and USUC/Cr+UFRU/Cr indicated the relative validity of SACANA as an instrument for assessing these intakes in children and adolescents. In particular, energy adjustment of total sugar intake and estimates of usual total sugar intake to correct for daily variation were statistically significantly associated with USUC/Cr+UFRU/Cr – which held true for the plausible reporters as well as for the whole study group. Nevertheless, the correlations were only moderate and the data included only single urinary sugar measurements which was not sufficient to evaluate the measurement error structure of the 24HDR. Therefore, further studies with repeated sugar measurements in spot urine are necessary to distinguish between person-specific bias and random error of intake of different types of sugar assessed by 24HDR in children and adolescents.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical Standards

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. The study was approved by the local ethics committee in each center and has been conducted according to the guidelines laid down in the 1964 Declaration of Helsinki and its later amendments. Study participants did not undergo any procedures unless they (and their parents) had given consent for examinations, collection of samples, subsequent analysis and storage of personal data and collected samples. Study subjects and their parents could consent to single components of the study while abstaining from others.

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Table 1: Main characteristics of the study population by sex (mean, standard deviation (SD) and total numbers)

	All		Boys		Girls	
	N	%	N	%	N	%
All	228	100	108	100	120	100
Underweight[a]	15	6.6	6	5.6	9	7.5
Normal weight[a]	150	65.8	69	63.9	81	67.5
Overweight[a]	44	19.3	21	19.4	23	19.2
Obese[a]	19	8.3	12	11.1	7	5.8
Italy	20	8.8	12	11.1	8	6.7
Estonia	25	11.0	14	13.0	11	9.2
Cyprus	19	8.3	9	8.3	10	8.3
Belgium	25	11.0	11	10.2	14	11.7
Poland	54	23.7	23	21.3	31	25.8
Sweden	22	9.7	10	9.3	12	10.0
Germany	21	9.2	9	8.3	12	10.0
Hungary	23	10.1	14	13.0	9	7.5
Spain	19	8.3	6	5.6	13	10.8
Plausible reporters	124	54.4	54	50.0	70	58.3
Underreporters	104	45.6	54	50.0	50	41.7
	Mean	SD	Mean	SD	Mean	SD
Age (y)	11.9	2.4	12	2.3	11.8	2.5
Height (cm)	152.4	14.7	154.5	15.5	150.5	13.6
Weight (kg)	47.6	16.4	50.1	17.9	45.4	14.7
BMI z-score[a]	0.6	1.1	0.8	1.2	0.5	1.1

485 [a]Cut-off and BMI z-score according to Cole and Lobstein (2012) [23].

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Table 2: Mean and standard deviation (SD) of urinary sugars and of energy and sugar intake by sex

	All (n=228)		Boys (n=108)		Girls (n=120)	
Urinary sugars	Mean	SD	Mean	SD	Mean	SD
Urinary sucrose (mg/L)	33.5	40.6	27.3	34	39.1	45.1
Urinary fructose (mg/L)	20.2	20.5	17.9	18.1	22.4	22.3
Urinary creatinine (g/L)	1.5	0.8	1.5	0.7	1.6	0.8
Urinary sucrose (mg/g creatinine)	24.7	35.5	22	38.7	27.1	32.3
Urinary fructose (mg/g creatinine)	17	19.7	16.5	20.4	17.5	19.1
Sum of urinary sucrose and fructose (mg/g creatinine)	41.7	46	38.5	50.4	44.6	41.7
Energy and sugar intake						
On the day before urinary morning spot						
Total energy intake (kcal/day)	1514.0	659.4	1652.2	701.2	1389.6	595.3
Total sugar intake (g/day)	73	50.1	79.3	54.3	67.3	45.6
Free sugar intake (g/day)	54.3	47.1	59	49.7	50	44.4
Intrinsic sugar intake (g/day)	11.5	12.8	12.1	14.3	11	11.3
Sugar density[a] (g/1000 kcal)	47.9	25.7	46.9	25.2	48.9	26.3
Mean intake[b]						
Total energy intake (kcal/day)	1522.2	584.0	1633.3	611.7	1422.3	541.1
Total sugar intake (g/day)	73.5	44.3	77	47.4	70.5	41.3
Free sugar intake (g/day)	54.5	41.6	57.2	43.5	52.1	39.8
Intrinsic sugar intake (g/day)	12	11.1	12.1	11.6	12	10.6
Sugar density[a] (g/1000 kcal)	48	22.6	46.4	22.5	49.5	22.6
Usual intake[c]						
Total energy intake (kcal/day)	1547.6	271.2	1673.1	197.3	1434.6	279.4
Total sugar intake (g/day)	75.7	20.2	79.6	20.1	72.2	19.7
Free sugar intake (g/day)	55.5	19	58.3	21.5	52.9	16.2
Intrinsic sugar intake (g/day)	12.6	5.4	12.5	5.8	12.7	5
Sugar density[a] (g/1000 kcal)	49.3	11.4	47.7	11.3	50.7	11.3

[a]The (mean/usual) sugar density was calculated as (mean/usual) total sugar intake per 1000 kcal of (mean/usual) total energy intake.

[b]Based on all available recall days (mean available days 2.1).

[c]Estimated based on the NCI method [30].

Table 3: Spearman's correlations between sugar intake (short term and medium term, g/day) and urinary sugars (mg/g creatinine)

Raw correlation coefficients		Urinary sucrose	p-value	Urinary fructose	p-value	Sum of urinary sucrose and fructose	p-value
Short-term intake: Previous day	Total sugar	0.215	0.001	0.219	0.001	0.285	<0.001
	Free sugar	0.225	0.001	0.174	0.008	0.268	0.000
	Intrinsic sugar	0.083	0.211	0.103	0.122	0.103	0.119
	Sugar density	0.259	0.000	0.238	<0.001	0.32	<0.001
Medium-term intake: Mean of repeated 24HDRs	Total sugar	0.162	0.015	0.263	<0.001	0.276	<0.001
	Free sugar	0.189	0.004	0.217	0.001	0.269	<0.001
	Intrinsic sugar	0.061	0.357	0.131	0.049	0.106	0.110
	Sugar density	0.253	0.000	0.295	<0.001	0.339	<0.001
Medium-term intake: Usual intake[a]	Total sugar	0.132	0.046	0.259	<0.001	0.252	<0.001
	Free sugar	0.167	0.011	0.226	0.001	0.257	<0.001
	Intrinsic sugar	0.031	0.646	0.125	0.060	0.087	0.193
	Sugar density	0.271	<0.001	0.336	<0.001	0.38	<0.001
Partial correlation coefficients							
Short-term intake: Previous day	Total sugar	0.198	0.003	0.196	0.003	0.267	<0.001
	Free sugar	0.205	0.002	0.148	0.026	0.246	<0.001
	Intrinsic sugar	0.072	0.284	0.093	0.166	0.091	0.172
	Sugar density	0.211	0.001	0.17	0.011	0.253	<0.001
Medium-term intake: Means of repeated 24HDRs	Total sugar	0.123	0.065	0.223	0.001	0.231	<0.001
	Free sugar	0.157	0.019	0.181	0.007	0.233	<0.001
	Intrinsic sugar	0.028	0.671	0.09	0.177	0.059	0.375
	Sugar density	0.183	0.006	0.204	0.002	0.24	<0.001
Medium-term intake: Usual intake[a]	Total sugar	0.093	0.163	0.194	0.003	0.189	0.004
	Free sugar	0.126	0.059	0.162	0.015	0.195	0.003
	Intrinsic sugar	-0.012	0.859	0.066	0.321	0.021	0.756
	Sugar density	0.167	0.012	0.181	0.006	0.216	0.001

491 [a]Estimated based on the NCI method [30].

Table 4: Validity coefficients of relative consumption frequency of sweet foods (Q), 24HDR reported intake (R), and biomarker (B) derived using the method of triads (N=170[a])

		r_{QT} [b]	r_{RT} [b]	r_{BT} [b]
B: Sum of creatinine corrected sucrose and fructose	Total sugar intake on previous day (R)	0.352	0.865	0.307
	Sugar density on previous day (R)	0.298	0.737	0.363
	Usual sugar intake (R)	0.397	0.778	0.272
	Usual sugar density (R)	0.304	0.789	0.356
B: Log-transf. sum of creatinine corrected sucrose and fructose	Total sugar intake on previous day (R)	0.408	0.746	0.411
	Sugar density on previous day (R)	0.341	0.643	0.491
	Usual sugar intake (R)	0.466	0.663	0.360
	Usual sugar density (R)	0.345	0.696	0.486

[a]The smaller sample size is due to missing FFQ data.

[b]The validity coefficient r_{RT} is the estimated correlation between true intake (T) and 24HDR reported intake (R) calculated using the method of triads [33] based on the observed correlations between R, Q and B. The same procedure was applied for the validity coefficients r_{QT} and r_{BT}.

Table 5: Results of linear regression models: associations between sum of urinary sucrose and fructose [a] and sugar intake (total sugar intake on previous day, free sugar intake on previous day, sugar density on previous day and usual sugar density respectively) adjusted for sex, BMI z-score and age for the whole sample and for plausible reporters only

	All (N=228)			Plausible reporters (N=124)		
Model 1[a]: Covariates	β	95%CI		β	95%CI	
Total sugar intake on previous day (100g)	**0.49**	0.25	0.73	**0.47**	0.19	0.75
Sex[b]	**0.29**	0.05	0.53	0.25	-0.04	0.55
BMI z-score	**-0.20**	-0.30	-0.09	-0.08	-0.22	0.08
Age (y)	**-0.13**	-0.18	-0.08	**-0.14**	-0.2	-0.08
Adj. R^2	0.233			0.163		
MAPE[c]	36.6			19.7		
Percentage of participants whose true and predicted values differ less than 10% (relative to the biomarker value)	34.6			38.7		
Model 2[a]: Covariates	β	95%CI		β	95%CI	
Free sugar intake on previous day (100g)	**0.44**	0.19	0.70	**0.45**	0.17	0.74
Sex[b]	**0.27**	0.03	0.51	0.22	-0.07	0.52
BMI z-score	**-0.20**	-0.31	-0.09	-0.06	-0.21	0.09
Age (y)	**-0.13**	-0.18	-0.08	**-0.13**	-0.20	-0.07
Adj. R^2	0.220			0.150		
MAPE[c]	37.2			20.0		
Percentage of participants whose true and predicted values differ less than 10% (relative to the biomarker value)	31.1			34.7		
Model 3[a]: Covariates	β	95%CI		β	95%CI	
Sugar density on previous day (100g/1000kcal)	**0.89**	0.42	1.36	**0.75**	0.16	1.33
Sex[b]	0.21	-0.02	0.45	0.18	-0.11	0.48
BMI z-score	**-0.20**	-0.31	-0.09	-0.05	-0.21	0.10
Age (y)	**-0.12**	-0.17	-0.07	**-0.12**	-0.18	-0.06
Adj. R^2	0.225			0.131		
MAPE[c]	37.0			20.5		
Percentage of participants whose true and predicted values differ less than 10% (relative to the biomarker value)	30.7			35.5		
Model 4[a]: Covariates	β	95%CI		β	95%CI	
Usual sugar density (100g/1000kcal)	**1.78**	0.53	3.04	**1.9**	0.44	3.36

Sex[b]	0.18	-0.06	0.42	0.18	-0.12	0.48
BMI z-score	**-0.20**	-0.31	-0.09	-0.04	-0.2	0.11
Age (y)	**-0.09**	-0.15	-0.03	**-0.09**	-0.16	-0.02
Adj. R^2	0.205			0.132		
MAPE[c]	37.1			20.5		
Percentage of participants whose true and predicted values differ less than 10% (relative to the biomarker value)	29.8			32.3		

[a]The dependent variable was the log-transformed sum of creatinine corrected urinary sucrose and fructose (log(USUC/Cr+UFRU/Cr)) for every model. Each included covariates are listed below.

[b]Boys as reference category.

[c]Mean absolute percent error.

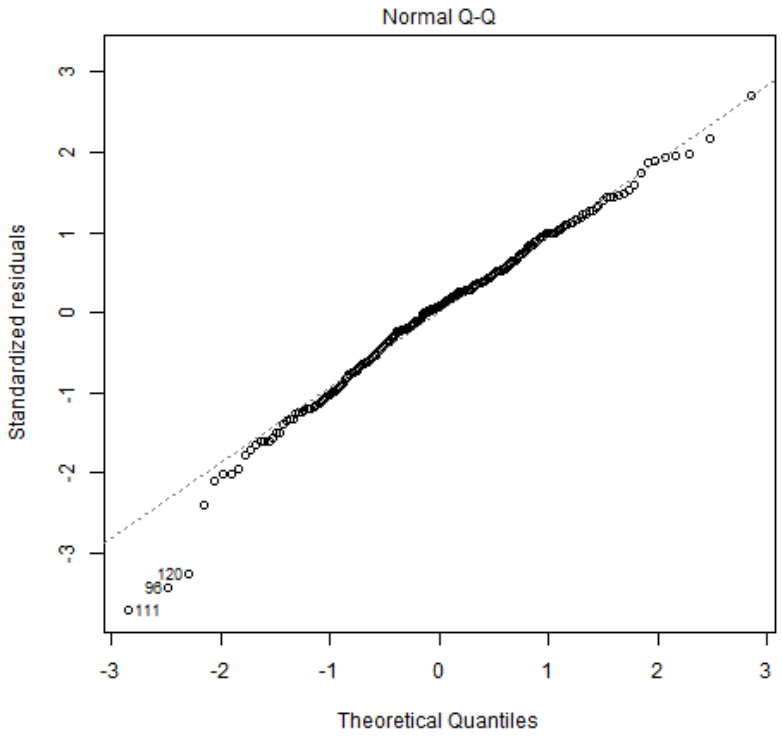

Fig.1 Q-Q-plot of linear regression Model 1 (whole study population): Log-transformed sum of creatinine corrected urinary sucrose and fructose against sugar intake on previous day adjusted for sex, BMI z-score and age.