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Urinary fructose: a potential biomarker for dietary fructose intake in children

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Running Title: Urinary fructose a biomarker for fructose intake

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Abstract (228 of 250 words)

Background/Objectives: Recently, urinary fructose and sucrose excretion in 24-h urine have been established experimentally as new biomarkers for dietary sugar intake in adults. Our objective was to investigate 1) whether the fructose biomarker is also applicable in free-living children and 2) for what kind of sugar it is standing for.

Subjects/Methods: Intakes of added and total sugar (including additional sugar from fruit and fruit juices) were assessed by 3-day weighed dietary records in 114 healthy prepubertal children, corresponding 24-h urinary fructose excretion was measured photometrically. The associations between dietary sugar intakes and urinary fructose excretion were examined by using linear regression models. To determine whether one of the two sugar variables may be better associated with the urinary biomarker, the statistical Pitman's test was used.

Results: Added and total sugar correlated significantly with urinary fructose, but the linear regression indicated a weak association between intake of added sugar and urinary log-fructose excretion ($\beta=0.0026$, $R^2=0.055$, $P=0.01$). The association between total sugar intake and log-urinary fructose ($\beta=0.0040$, $R^2=0.181$, $P<0.001$) showed a significantly better fit ($P<0.05$).

Conclusions: Urinary fructose excretion seems rather applicable for the estimation of total than of added dietary sugar intake in children. However, since excreted fructose stems almost exclusively from the diet (both from food-intrinsic and added intakes), it can be assumed that urinary fructose represents a potential biomarker for total dietary fructose intake, irrespective of its source.

Keywords: urine, fructose, biomarker, sugar intake, children

1. Introduction

Even though extensively researched since several years, the discussion persists, whether a high dietary sugar intake is (amongst other factors) responsible for the ongoing epidemic of obesity and related cardiovascular diseases (Johnson *et al.*, 2007). The term sugar comprises various mono- and disaccharides, that are considered to have different metabolic effects (Sigman-Grant *et al.*, 2003; Englyst *et al.*, 2007). Currently, the focus lies on the monosaccharide fructose as a potential risk factor, due to its proposed dysregulating effects on glucose and lipid metabolism
10 (Angelopoulos *et al.*, 2009; Jones, 2009; Libuda *et al.*, 2009; Stanhope *et al.*, 2009). Especially in the United States, this topic attracts rising attention, because of the constantly increasing fructose consumption (Vos *et al.*, 2008).

The major difficulty in estimating dietary sugar intake for the analysis of metabolic effects of sugar, is the lack of a precise assessment method. So far, typically epidemiologic assessments (interviews, questionnaires or dietary records) were used, which are known to be prone to measurement errors, like intentional underreporting, subjective estimations instead of objective measurements or simple
20 disremembering of foods (Biro *et al.*, 2002). To avoid these limitations the use of a biomarker for the estimation of dietary sugar intake would be generally preferred (Jenab *et al.*, 2009), especially one that can differentiate between the various types of sugar.

Recently, two new dietary biomarkers regarded as objective markers of sugar consumption in adults, have been introduced: urinary fructose and sucrose excretion (Tasevska *et al.*, 2005). As neither fructose nor sucrose are synthesized endogenously, their urinary excretion has to be of dietary origin. The mechanism by which they end up in urine is not exactly known. It is proposed that a small amount

of sucrose escapes from enzymatic hydrolysis in the small intestine, reaches general circulation and afterwards is excreted in the urine. Similarly, a small proportion of ingested fructose (derived from free fructose as well as from hydrolysis of sucrose) may escape from hepatic fructose metabolism and is excreted in the urine (Tasevska *et al.*, 2009). The excreted amount is very small and mostly proportional to dietary sugar consumption.

These newly developed biomarkers have been supposed to better reflect extrinsic (i.e. added sugar) than intrinsic sugar (naturally contained in food) (Tasevska *et al.*, 2009). This would allow to examine potential disease risks related to a high sugar consumption independently from the naturally occurring sugar in fruits.

To verify whether the potential biomarker fructose excretion also provides an adequate estimate of dietary sugar intake in free-living children, we studied recorded dietary sugar intake and its association with the 24-h excretion of fructose in a sample of **healthy** prepubertal German children. A preliminary diet experiment in our institute (results are shown in the method section) yielded less reasonable excretion rates for sucrose compared to fructose, which was the reason to focus on the latter biomarker. Further, in order to characterize the fructose biomarker, we examined in our study in children whether various sugar sources - added and total sugar - are associated with urinary fructose excretion differently.

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2. Methods and Materials

2.1 Preliminary diet experiment

To adapt the method of measuring urinary fructose and sucrose in our lab and to reproduce the applicability of these biomarkers for assessment of sugar intake we

- analysed fructose and sucrose excretion in 24-h urine samples collected in a 5-d diet experiment with acute dietary sucrose loads (for fructose and sucrose analytics see section urinary measurements). Nine healthy female adults (24 - 59 y, BMI: 18.9 - 28.4 kg/m²) participated and ingested a constant diet with all foods weighed and prepared in the institute's own kitchen. Energy and protein intake were 1962 kcal in average and 66.5 g respectively and held constant throughout the whole 5-d period. There was a 3-d run-in period to metabolically adapt to the lacto-vegetarian whole food basal study diet. The first intervention, consisting of 40 g sucrose, was administered with dinner on day 4, the second (80 g sucrose) with dinner on day 5.
- 10 To maintain a constant energy intake, olive oil included in the basal dinner was isocalorically replaced with 40 g or 80 g sucrose. 24-h urine collections (each started immediately after lunch) were performed on day 3 (basal diet), day 4 (40 g sucrose) and day 5 (80 g sucrose). All micturitions of the 24-h collection periods were immediately stored in preservative-free, Extran-cleaned (Extran, MA03; Merck, Darmstadt, Germany) 1-L plastic containers at <-20 °C before the samples were thawed for 24-h urine volume determination and further measurements. No specific precautions were taken to avoid possible sucrose hydrolysis. Compliance with 24-h urine collection was ensured by a mean intra-individual variation of body weight-related 24-h creatinine excretion rates <10%.
- 20 Figure 1 shows the results of the interventions for both urinary fructose and sucrose excretions. There was a significant increase in log-sucrose excretion from basal diet to 40 g sucrose intervention, however no additional clear increase after 80 g sucrose intake. The urinary fructose excretion, in contrast, increased significantly and almost gradually with increasing sucrose intake. Due to the obvious lack of a consistent dose-response relationship between sucrose intake and sucrose excretion, which is similarly observable in other diet studies (Luceri *et al.*, 1996; Tasevska *et al.*, 2005),

we decided to specifically focus on 24-h fructose excretion as a potential biomarker in our examinations in children.

2.2 Subjects and study design

The study population for the examinations in children was selected from the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study. The DONALD Study that started in 1985, is an ongoing longitudinal (open cohort) study gathering information about diet, development and metabolism between
10 infancy and adulthood in healthy subjects (Kroke *et al.*, 2004). The regular visits begin at 3 months of age and take place annually from the age of 2 years until the age of 18 years. The regular assessments include three day weighed dietary records, 24-h urine collections and measurements of anthropometric data as well as interviews on lifestyle and medical assessments. The DONALD Study is exclusively observational, non-invasive (until the age of 18) and approved by the Ethics Committee of the University of Bonn (Germany). All examinations are performed with parental, and later on with the children's written consent.

In the present evaluation only children were enrolled who had a completely collected 24-h urine and parallel three day weighed dietary record (including the day of urine
20 collection) one year before puberty initiation. Children and/or their parents were interviewed regarding completeness of urine collection. Subjects with reported unknown losses and/or with body weight-related 24-h creatinine excretion rates $<0.1 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ were excluded. Plausibility of dietary records was estimated by calculating the ratio of reported total energy intake (EI) to predicted individual basal metabolic rate (BMR, estimated by the method of Schofield (Schofield, 1985)). Dietary records with a ratio below the age- and sex-specific cut-off values (indicating

underreporting) (Sichert-Hellert *et al.*, 1998) were excluded. All EI:BMR ratios were below 2.0, indicating that no relevant overreporting existed (Johansson *et al.*, 1998).

The timing of the onset of the pubertal growth spurt (age at takeoff, ATO) was used as a marker for the initiation of puberty. ATO was estimated based on subjects' individual growth curves by using the parametric Preece and Baines model (Preece *et al.*, 1978). This ensures that all investigated children were at same biological age and possible effects of beginning puberty could be excluded. ATO could be calculated for 376 children. After excluding those, who had incompletely collected 24-h urines and/or no parallel plausible dietary records before puberty onset, 114
10 children remained for the present analysis.

2.3 Dietary records

Three day weighed dietary records were used to estimate the individual food and nutrient intake. On three consecutive days the weight of all consumed food and beverages was recorded using a digital food scale to the nearest 1g. Out-of-home consumed food was estimated by semi-quantitative recording (e.g. numbers of glasses, cups). Energy and nutrient intakes, including food fortification and nutrition supplements, were calculated as individual means of the three recorded days by
20 using our nutrient database LEBTAB (Sichert-Hellert *et al.*, 2007).

In the present analysis we calculated the intake of added sugar and total sugar. Added sugar was defined as all refined sugar (e.g. sucrose, maltose, lactose, glucose, dextrin), including honey, eaten separately at the table or used as ingredients in processed or prepared foods. Total sugar was defined as the sum of added sugar and sugar derived from fruits and fruit juices. In the following we referred to sugar derived from fruit and fruit juices as intrinsic sugar.

2.4 Urinary measurements

The urine collection of the DONALD participants was generally carried out on the third day of the three day weighed dietary records. Procedures for the 24-h urine collection have been described previously (Remer *et al.*, 2006) The micturitions were immediately frozen at <-20 °C (without preservative, in Extran-cleaned 1-L plastic containers) until transfer to the research institute by a dietitian (further storage at <-20 °C until analysis). Creatinine was measured by the Jaffé method with the use of a creatinine analyzer (Beckman-2; Beckman Instruments Inc, Fullerton, CA). Daily sucrose (diet experiment) and fructose (diet experiment and DONALD population) excretions were measured in 24-h urines with a kit for the enzymatic analysis of sucrose, glucose, and fructose (Saccharose/D-Glucose/D-Fructose; Boehringer MANNHEIM, R-BIOPHARM, Darmstadt) using a spectrophotometer (PM2 DL, Zeiss) according to the assay instructions. During all measurement runs, the analyses were checked for potential side (“creep”) reactions which – if occurred in the form of continuous unspecific absorbance increases – were considered as advised in the assay procedures. In case, the reactions did not stop in time, absorbances were continued to read until constant absorbance increases occurred and the corresponding readings were extrapolated to the time of addition of the respective enzyme suspensions. Limit of detection (LOD) was 0.4 mg/L and 2 mg/L for fructose and sucrose, respectively. Intra- and interassay precision were below 4% (8%) and 10% (13%), for fructose (sucrose), respectively. All runs were performed as duplicates with a maximum deviation of duplicates of 15%.

2.5 Statistical analysis

As urinary fructose excretion was skewed it was log 10 transformed and given as geometric mean with 95 % confidence interval. All other investigated variables were normally distributed and presented as means \pm S.D.. Sex differences were tested with an unpaired t test.

Analysis of the association between dietary sugar intake and urinary fructose excretion was performed by linear regression models. Log values of urinary fructose were included as dependent variable, and concomitant data of either added or total sugar, were investigated as independent variables. In a further regression model, added sugar and intrinsic sugar together were included as predictor variables for urinary fructose excretion. None of the potential confounding factors age, sex, BMI, urine volume and energy intake showed a significant association with the urinary fructose excretion. Thus, aside from the dietary sugar intake no other independent variables were included into the regression model.

Pitman's test (Snedecor *et al.*, 1987) was used to determine which of the two dietary sugar variables (i.e. added sugar or total sugar) provided a better estimation of urinary fructose excretion. Residuals of the regressions of the criterion variable (urinary fructose) with both estimation models (added and total sugar) were calculated (residuals A and B). Then the correlation of the sum (A + B) and the difference (A - B) was examined. If this correlation differed significantly from zero, the residual with the smaller S.D. was the model with better fit.

Significance was defined as $P < 0.05$. All calculations were performed with SAS procedures (version 9.1.3, SAS Institute, Cary, NC, USA).

3. Results

Characteristics of the study population are presented in Table 1. The average age of the girls was about 1.5 years lower than of the boys due to the physiologic earlier onset of puberty in females. Anthropometric values (except BMI) and energy intake differed significantly by sex, however no differences were observed in dietary sugar intake and urinary fructose excretion between boys and girls.

Dietary added sugar intake showed a significant association with urinary fructose excretion (Table 2), both R^2 and the correlation plot (Figure 2) indicated that the relationship was weak. When including total sugar intake as independent variable instead of added sugar, R^2 and β value increased. The Pitman test yielded significantly smaller S.D. of the residuals for the regression of total sugar intake on urinary fructose excretion when compared with added sugar intake. Therefore the association between total sugar intake and urinary fructose excretion has been proven as the one with a significantly better fit .

According to the regression equation, an 1-g increase of total sugar intake predicted an increase in log-fructose excretion by 0.004, which is equivalent to a 0.9 % increase of absolut fructose excretion.

In a separate regression model, the predictor variable total sugar intake for fructose excretion was divided into its two components added sugar and intrinsic sugar (Table 2). Both variables showed a significant association with urinary fructose excretion, whereby the intrinsic sugar intake explained a higher proportion of the variability of fructose excretion (part. $R^2 = 15.3\%$) than did added sugar intake (part. $R^2 = 5.5\%$).

4. Discussion

The results of our study in **healthy** children, extend the findings of Tasevska et al. and Luceri et al. (Luceri *et al.*, 1996; Tasevska *et al.*, 2005; Tasevska *et al.*, 2009) in adults, indicating that urinary fructose excretion may be used as a biomarker for sugar intake also during the growing years (at least until the begin of puberty). Furthermore, the data in children (who almost all consumed mixed, fruit-containing diets) show that urinary fructose can be significantly stronger associated with total sugar than with added sugar consumption. This and the facts that (1st) both
10 components of total dietary sugar intake, i.e., added sugar and natural sugar contain considerable amounts of fructose and (2nd) almost all of the urinary excreted fructose originates from diet, led us to the suggestion that 24-h urinary fructose excretion rather reflects total fructose consumption and may thus be an appropriate biomarker for it.

This is also indirectly confirmed by our preliminary diet experiment in adults, which shows a consistent dose response to the ingested amount of fructose administered in the form of sucrose. In this diet experiment the urinary sucrose excretion failed to turn out as a clear biomarker for sugar consumption which is somewhat in contrast to the suggestions of other authors who concluded that both sucrose and fructose are
20 excreted in the urine proportionally to dietary intake (Luceri *et al.*, 1996; Tasevska *et al.*, 2005; Joosen *et al.*, 2008; Tasevska *et al.*, 2009). However, also in some of these studies either a higher intra-individual variation (Tasevska *et al.*, 2005) or a less consistent association (Luceri *et al.*, 1996) was seen for urinary sucrose compared with fructose. **One limitation of our diet experiment, that could possibly explain the missing dose response, was the preservative-free refrigeration of the 24-h urines, hence hydrolysis of sucrose could not be fully excluded. Such a possible**

hydrolysis of sucrose would, however, query the successful application of urinary sucrose as a biomarker in large epidemiological studies in which urine samples are mostly stored without preservatives.

Regarding the eligibility of 24-h urinary fructose as a biomarker for total sugar intake, it should be kept in mind that the corresponding R^2 that we found in children was rather small. The experimental data of Tasevska et al. (Tasevska *et al.*, 2005) and our corresponding data in adults indicate considerable inter-individual differences along with a relatively stable intra-individual excretion level for fructose, i.e. a relevant individual (at least partly genetic) component of urinary fructose excretion.

10 Therefore, the most feasible application of the biomarker fructose excretion might be in longitudinal surveys with repeated measurements for each subject, as the inter-individual variability can be mathematically accounted for using adequate statistical mixed effect models like proc mixed. Despite this, plausible results have been yielded in a cross-sectional study using urinary fructose as biomarker for sugar intake (Bingham *et al.*, 2007).

Our observation that intrinsic sugar explained almost the 3-fold of the variability of urinary fructose excretion compared to added sugar (Table 2), shows that sugar derived from fruits and fruit juices (intrinsic sugar) represents an important and not to be disregarded predictor of the urinary fructose excretion. In a recent paper
20 Tasevska et al. (Tasevska *et al.*, 2009) reported significant correlations for urinary sugar excretion with extrinsic sugar intake, but not with intrinsic sugar intake. Two reasons may account for the seemingly difference between the latter authors' and our findings. Firstly, as pointed out by Tasevska et al. their subjects consumed higher amounts of extrinsic than intrinsic sugar, and secondly the applied definition of extrinsic and intrinsic sugar differed from our definition, in that we differentiated between added and intrinsic sugar. Tasevska et al. classified sugar from fruit juices

(which in our definition is intrinsic) as extrinsic sugar. Actually, if we apply the definition of Tasevska et al. to our DONALD data (i.e., if we define extrinsic sugar as sum of added sugar and sugar from fruit juices), the association between this extrinsic sugar and fructose excretion turns more significant (showing a higher explained variability; $R^2=0.14$, $P<0.0001$) and the association between the remaining intrinsic sugar and fructose excretion loses significance ($R^2=0.06$, $P<0.01$).

A limitation of the present analysis is that the calculation of dietary sugar intake by a weighed dietary record could only provide estimates of the true sugar intake (e.g. due to recording errors or underreporting) (Biro *et al.*, 2002). However, this method has been deemed to be the most exact dietary assessment tool for a study population of this size (Bingham *et al.*, 1995; Gibson, 2005). Additionally, the quality of the investigated dietary records was improved by excluding potential underreporting: only such dietary records were considered that had plausible energy intake values. As the dietary sugar consumption is a major determinant of individual's energy intake, it can be assumed, that misreporting of dietary sugar could be limited by our plausibility checks.

Overall, our results suggest that urinary fructose excretion is clearly determined by the consumed amounts of intrinsic sugar sources, like fruits or fruit juices which are typical fructose providers (results in children). At the same time, renal fructose output has also been shown to vary clearly (and obviously dose-dependent) with added fructose (administered as sucrose; experimental diet results in adults, and reported data (Luceri *et al.*, 1996; Tasevska *et al.*, 2005)). Therefore, we suppose that urinary fructose excretion is rather applicable for the direct estimation of total dietary fructose intake (regardless of its origin) than for the estimation of total or added sugar intake. This possible field of application is of particular interest in light of the current discussion about dietary fructose intake and its impact on human health.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1 Study sample characteristics of 114 prepubertal healthy participants of the DONALD Study.

	Boys (n=58)	Girls (n=56)	P ^b
Anthropometrics			
Age (y)	9,3 ± 0,8 ^a	7,9 ± 0,7	<0,0001
Weight (kg)	33,0 ± 6,1	27,2 ± 5,7	<0,0001
Height (cm)	140,3 ± 7,0	129,6 ± 5,9	<0,0001
BMI (kg/m ²)	16,7 ± 2,1	16,0 ± 2,2	n.s.
Dietary parameters			
Total energy intake (MJ/d)	7,5 ± 1,0	6,4 ± 1,0	<0,0001
Added sugar intake (g/d)	66,4 ± 33,1	56,9 ± 26,5	n.s.
Sugar from fruits (g/d)	16,3 ± 11,8	16,4 ± 11,0	n.s.
Sugar from fruit juices (g/d)	19,4 ± 19,1	16,1 ± 21,0	n.s.
Total sugar intake (g/d)	102,2 ± 39,2	89,4 ± 31,4	n.s.
Urinary parameters			
24-h urinary volume (ml)	848,3 ± 361,7	681,3 ± 251,6	0,005
24-h fructose excretion (mg/d)	19,8 (15,9; 24,5) ^c	20,7 (17,1; 25,0)	n.s.

^a All such values are arithmetic means ± S.D.

^b Sex differences were analyzed by using unpaired t test.

^c All such values are geometric means (95% confidence interval)

Table 2 Association between dietary sugar intake and urinary fructose excretion in 114 prepubertal children participating in the DONALD Study.

Predictor	Log values of fructose in urine		R ²
	β	<i>P</i>	
Regression 1			
Added sugar	0,0026	0,011	0,055
Regression 2			
Total sugar	0,0040	<0,001	0,181
Regression 3			
Added sugar	0,003	<0,005	0,055
Intrinsic sugar	0,006	<0,0001	0,153

Results of the linear regression models. Potential covariates (age, sex, BMI, urine volume and energy intake) were considered but no further adjustment was required.

Figure Legends

Fig. 1. Urinary excretion of sucrose and fructose after varying sucrose intake during a constant and isocaloric diet in adults (n = 9). Significance was analysed with paired t-tests.

Fig. 2. Association of dietary added sugar intake (g/d) and dietary total sugar intake (g/d) with urinary fructose excretion in 114 participants of the DONALD Study.

Figure 1

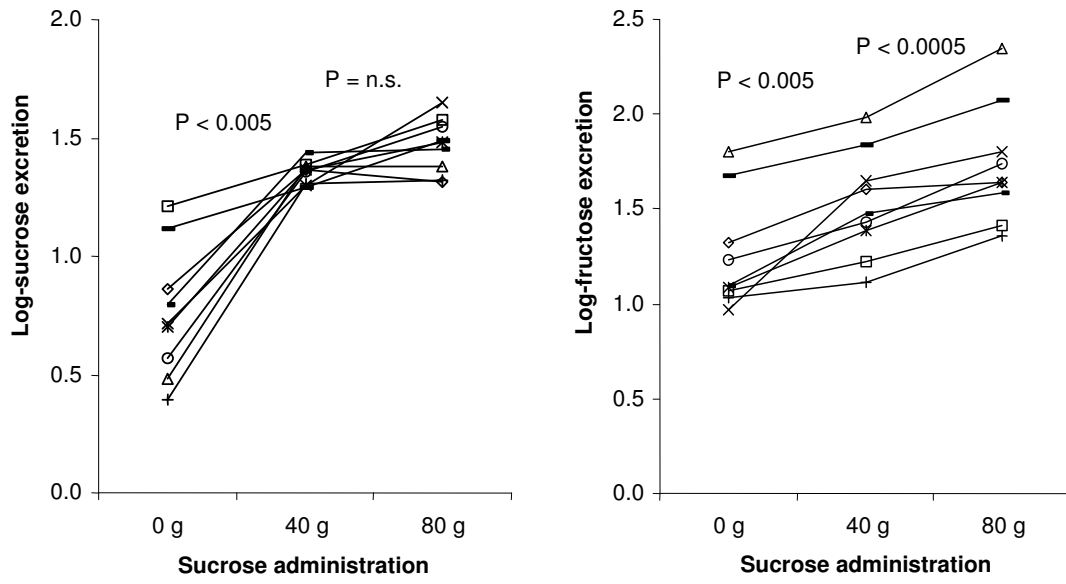


Figure 2

