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ORIGINAL ARTICLE

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

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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 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 to be rather applicable for the estimation of total sugar intake than for the estimation of added dietary sugar intake in children. However, as 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.
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Introduction

Even though extensively researched since several years, the discussion regarding whether a high dietary sugar intake is (among other factors) responsible for the ongoing epidemy of obesity and related cardiovascular diseases persists (Johnson *et al*., 2007). The term sugar comprises various mono- and disaccharides that are considered to have different metabolic effects (Sigman-Grant and Morita, 2003; Englyst *et al*., 2007). Currently, the focus lies on monosaccharide fructose as a potential risk factor, because of its proposed dysregulating effects on glucose and lipid metabolism (Angelopoulos *et al*., 2009; Jones, 2009; Libuda and Kersting, 2009; Stanhope *et al*., 2009). Especially in the United States, this topic attracts increasing 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 epidemiological assessments (interviews, questionnaires or dietary records) were used, which are known to be prone to measurement errors such as intentional underreporting, subjective estimations instead of objective measurements or simple 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

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introduced: urinary fructose and sucrose excretion (Tasevska *et al*., 2005). As neither fructose nor sucrose is synthesized endogenously, their urinary excretion has to be of dietary origin. The mechanism by which they end up in urine is not precisely known. It is proposed that a small amount of sucrose escapes from enzymatic hydrolysis in the small intestine, reaches general circulation and is afterwards excreted through urine. Similarly, a small proportion of ingested fructose (derived from free fructose and from hydrolysis of sucrose) may escape from hepatic fructose metabolism and is excreted through urine (Tasevska *et al*., 2009). The excreted amount is very small and mostly proportional to dietary sugar consumption.

These newly developed biomarkers have been surmised to better reflect extrinsic (that is, added sugar) than intrinsic sugar (naturally contained in food) (Tasevska *et al*., 2009). This would allow the examination of potential disease risks related to a high sugar consumption independently of 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 with fructose, which was the reason for focusing on the latter biomarker. Further, 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.

Materials and methods

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-day diet experiment with acute dietary sucrose loads (for fructose and sucrose analytics, see section urinary measurements). Nine healthy female adults (24–59 years, BMI: 18.9–28.4 kg/m^2) 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 on an average and 66.5 g, respectively, and held constant throughout the whole 5-day period. There was a 3-day 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. To maintain a constant energy intake, olive oil included in the basal dinner was isocalorically replaced with 40 or 80 g sucrose. Urine samples were collected over 24 h (each started immediately after lunch) 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 $<-20\,^{\circ}C$ 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 intraindividual variation of body weight-related 24-h creatinine excretion rates $<10\%$.

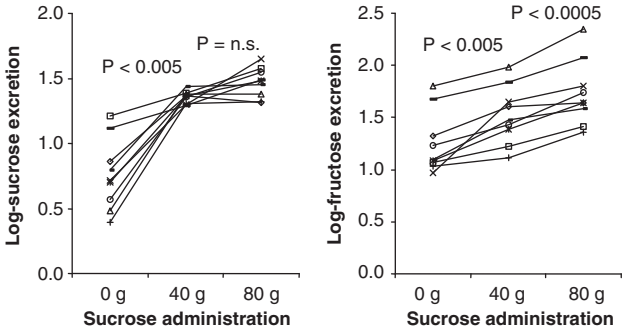

Figure 1 Urinary excretion of sucrose and fructose after varying sucrose intake during a constant and isocaloric diet in adults ($n=9$). Significance was analysed with paired *t*-tests.

Figure 1 shows the results of the interventions for both urinary fructose and sucrose excretions. There was a significant increase in log-sucrose excretion when comparing basal diet with 40 g-sucrose intervention; however no additional clear increase after 80 g sucrose intake was observed. The urinary fructose excretion, in contrast, increased significantly and almost gradually with increasing sucrose intake. Because of 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.

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 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 3-day weighted 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 conducted with parental, and later on with the children's written consent.

In this evaluation, only those children were enroled who had a completely collected 24-h urine sample and parallel 3-day weighted dietary record (including the day of urine collection) 1 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 mmol/kg/ day were excluded. Plausibility of dietary records was estimated by calculating the ratio between reported total energy intake (EI) and predicted individual basal metabolic rate (BMR, estimated by the method by Schofield, (1985)). Dietary records with a ratio below age- and sex-specific cutoff 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. Age at takeoff was estimated on the basis of subjects' individual growth curves by using the parametric Preece and Baines model (Preece and Baines, 1978). This ensures that all investigated children were at the same biological age and possible effects of the start of puberty could be excluded. Age at takeoff 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 children remained for the present analysis.

Dietary records

Three-day weighted dietary records were used to estimate the individual food and nutrient intake. On 3 consecutive days, the weight of all consumed food and beverages was recorded using a digital food scale to the nearest 1 g. Out-of-home consumed food was estimated by semiquantitative recording (for example, 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 using our nutrient database LEBTAB (Sichert-Hellert *et al*., 2007).

In this analysis, we calculated the intake of added sugar and total sugar. Added sugar was defined as all refined sugar (for example, sucrose, maltose, lactose, glucose and 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 sections, we refer to sugar derived from fruit and fruit juices as intrinsic sugar.

Urinary measurements

The urine collection of the DONALD participants was generally carried out on the 3rd day of the 3-day weighted dietary records. Procedures for the 24-h urine collection have been described previously (Remer *et al*., 2006) The micturitions were immediately frozen at $<-20\,^{\circ}C$ (without preservative, in Extran-cleaned 1-l plastic containers) until transfer to the research institute by a dietitian (further storage at $<-20\,^{\circ}C$ until analysis). Creatinine concentration was measured by the Jaffé method using a creatinine analyser (Beckman-2; Beckman Instruments, Fullerton, CA, USA). Daily sucrose (diet experiment) and fructose (diet experiment and DONALD population) excretions were measured in 24-h urine samples with a kit for the enzymatic analysis of sucrose, glucose and fructose (saccharose/ D-Glucose/D-Fructose; Boehringer MANNHEIM, R-BIOPHARM, Darmstadt, Germany) using a spectrophotometer (PM2 DL, Zeiss, Oberkochen, Germany) 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 be 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 was 0.4 mg/l and 2 mg/l for fructose and sucrose, respectively. Intra- and interassay precision rates were below 4% (8%) and 10% (13%) for fructose and sucrose, respectively. All runs were performed as duplicates with a maximum deviation of duplicates of 15%.

Statistical analysis

As urinary fructose excretion was skewed, it was log10 transformed and presented as geometric mean with 95% confidence interval. All other investigated variables were normally distributed and presented as means ± 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 variables, 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 such as 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 in the regression model.

Pitman's test (Snedecor and Cochran, 1987) was used to determine which of the two dietary sugar variables (that is, 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). Thereafter, 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 a 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).

Results

Characteristics of the study population are presented in Table 1. The average age of the girls was about 1.5 years lower than that of the boys because of the physiological 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 an independent variable, instead of added sugar, R^2 and β values increased. The Pitman's 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, a 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 in absolute 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

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	P	
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, body mass index, urine volume and energy intake) were considered, but no further adjustment was required.

Figure 2 Association of dietary added sugar intake (g/day) and dietary total sugar intake (g/day) with urinary fructose excretion in 114 participants of the DONALD study.

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

	Boys (n = 58)	*Girls (n = 56)*	P[a]
Anthropometrics			
Age (years)	9.3 ± 0.8[b]	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	NS
Dietary parameters			
Total energy intake (MJ/day)	7.5 ± 1.0	6.4 ± 1.0	<0.0001
Added sugar intake (g/day)	66.4 ± 33.1	56.9 ± 26.5	NS
Sugar from fruits (g/day)	16.3 ± 11.8	16.4 ± 11.0	NS
Sugar from fruit juices (g/day)	19.4 ± 19.1	16.1 ± 21.0	NS
Total sugar intake (g/day)	102.2 ± 39.2	89.4 ± 31.4	NS
Urinary parameters			
24-h urinary volume (ml)	848.3 ± 361.7	681.3 ± 251.6	0.005
24-h fructose excretion (mg/day)	19.8 (15.9; 24.5)[c]	20.7 (17.1; 25.0)	NS

Abbreviation: NS, not significant.
[a]Sex differences were analysed by using unpaired *t*-test.
[b]All such values are arithmetic means ± s.d.
[c]All such values are geometric means (95% confidence interval).

excretion (partial $R^2 = 15.3\%$) than did added sugar intake (partial $R^2 = 5.5\%$).

Discussion

The results of our study in healthy children extend the findings of Tasevska *et al*. (2005, 2009) and Luceri *et al*. (1996) in adults, indicating that urinary fructose excretion may be used as a biomarker for sugar intake during growing years as well (at least until the start of puberty). Furthermore, the data in children (who almost consumed all mixed, fruit-containing diets) show that urinary fructose can be significantly more strongly associated with total sugar than with added sugar consumption. This and the fact that (1) both components of total dietary sugar intake, that is, added sugar and natural sugar, contain considerable amounts of fructose and (2) that 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 excreted in the urine proportionally to dietary intake (Luceri *et al*., 1996; Tasevska *et al*., 2005, 2009; Joosen *et al*., 2008). However, in some of these studies, either a higher intraindividual 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 urine samples; 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*. (2005) and our corresponding data in adults indicate considerable interindividual differences, along with a relatively stable intraindividual excretion level for fructose, that is, a relevant individual (at least partly genetic) component of urinary fructose excretion. Therefore, the most feasible application of the biomarker fructose excretion might be in longitudinal surveys with repeated measurements for each subject, as the interindividual variability can be mathematically accounted for using adequate statistical mixed-effect models such as proc mixed. Despite this, plausible results have been obtained in a cross-sectional study using urinary fructose as a biomarker for sugar intake (Bingham *et al*., 2007).

Our observation that intrinsic sugar explained the almost threefold variability of urinary fructose excretion compared with 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, 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 apparent difference between the latter authors' and our findings. First, as pointed out by Tasevska *et al*. their subjects consumed higher amounts of extrinsic than intrinsic sugar, and second 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. In reality, if we apply the definition of Tasevska *et al*. to our DONALD data (that is, if we define extrinsic sugar as the sum of added sugar and sugar from fruit juices), the association between this extrinsic sugar and fructose excretion becomes 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 this analysis is that the calculation of dietary sugar intake by a weighted dietary record could only provide estimates of the true sugar intake (for example, because of 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). In addition, 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 the 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, such as 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 surmise 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.

Conflict of interest

The authors declare no conflict of interest.

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References

Angelopoulos TJ, Lowndes J, Zukley L, Melanson KJ, Nguyen V, Huffman A *et al.* (2009). The effect of high-fructose corn syrup consumption on triglycerides and uric acid. *J Nutr* **139**, S1242–S1245.

Bingham S, Luben R, Welch A, Tasevska N, Wareham N, Khaw KT (2007). Epidemiologic assessment of sugars consumption using biomarkers: comparisons of obese and nonobese individuals in the European prospective investigation of cancer Norfolk. *Cancer Epidemiol Biomarkers Prev* **16**, 1651–1654.

Bingham SA, Cassidy A, Cole TJ, Welch A, Runswick SA, Black AE *et al.* (1995). Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. *Br J Nutr* **73**, 531–550.

Biro G, Hulshof KF, Ovesen L, Amorim Cruz JA (2002). Selection of methodology to assess food intake. *Eur J Clin Nutr* **56** (Suppl 2), S25–S32.

Englyst KN, Liu S, Englyst HN (2007). Nutritional characterization and measurement of dietary carbohydrates. *Eur J Clin Nutr* **61** (Suppl 1), S19–S39.

Gibson R (2005). Validity in dietary assessment methods. In: R. Gibson (ed). *Principles of Nutritional Assessment*. Oxford University Press: New York, pp 161–187.

Jenab M, Slimani N, Bictash M, Ferrari P, Bingham SA (2009). Biomarkers in nutritional epidemiology: applications, needs and new horizons. *Hum Genet* **125**, 507–525.

Johansson L, Solvoll K, Bjorneboe GE, Drevon CA (1998). Under- and overreporting of energy intake related to weight status and lifestyle in a nationwide sample. *Am J Clin Nutr* **68**, 266–274.

Johnson RJ, Segal MS, Sautin Y, Nakagawa T, Feig DI, Kang DH *et al.* (2007). Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *Am J Clin Nutr* **86**, 899–906.

Jones JM (2009). Dietary sweeteners containing fructose: overview of a workshop on the state of the science. *J Nutr* **139**, S1210–S1213.

Joosen AM, Kuhnle GG, Runswick SA, Bingham SA (2008). Urinary sucrose and fructose as biomarkers of sugar consumption: comparison of normal weight and obese volunteers. *Int J Obes (Lond)* **32**, 1736–1740.

Kroke A, Manz F, Kersting M, Remer T, Sichert-Hellert W, Alexy U *et al.* (2004). The DONALD Study. History, current status and future perspectives. *Eur J Nutr* **43**, 45–54.

Libuda L, Kersting M (2009). Soft drinks and body weight development in childhood: is there a relationship? *Curr Opin Clin Nutr Metab Care* **12**, 596–600.

Luceri C, Caderni G, Lodovici M, Spagnesi MT, Monserrat C, Lancioni L *et al.* (1996). Urinary excretion of sucrose and fructose as a predictor of sucrose intake in dietary intervention studies. *Cancer Epidemiol Biomarkers Prev* **5**, 167–171.

Preece MA, Baines MJ (1978). A new family of mathematical models describing the human growth curve. *Ann Hum Biol* **5**, 1–24.

Remer T, Fonteyn N, Alexy U, Berkemeyer S (2006). Longitudinal examination of 24-h urinary iodine excretion in schoolchildren as a sensitive, hydration status-independent research tool for studying iodine status. *Am J Clin Nutr* **83**, 639–646.

Schofield WN (1985). Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr Clin Nutr* **39** (Suppl 1), 5–41.

Sichert-Hellert W, Kersting M, Chahda C, Schaefer R, Kroke A (2007). German food composition database for dietary evaluations in children and adolescents. *J Food Comp Anal* **20**, 63–70.

Sichert-Hellert W, Kersting M, Schoch G (1998). Underreporting of energy intake in 1–18 year old German children and adolescents. *Z Ernahrungswiss* **37**, 242–251.

Sigman-Grant M, Morita J (2003). Defining and interpreting intakes of sugars. *Am J Clin Nutr* **78**, S815–S826.

Snedecor GW, Cochran WG (1987). *Statistical Methods*. Iowa State University Press: Ames, IA.

Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL *et al.* (2009). Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest* **119**, 1322–1334.

Tasevska N, Runswick SA, McTaggart A, Bingham SA (2005). Urinary sucrose and fructose as biomarkers for sugar consumption. *Cancer Epidemiol Biomarkers Prev* **14**, 1287–1294.

Tasevska N, Runswick SA, Welch AA, McTaggart A, Bingham SA (2009). Urinary sugars biomarker relates better to extrinsic than to intrinsic sugars intake in a metabolic study with volunteers consuming their normal diet. *Eur J Clin Nutr* **63**, 653–659.

Vos MB, Kimmons JE, Gillespie C, Welsh J, Blanck HM (2008). Dietary fructose consumption among US children and adults: the Third National Health and Nutrition Examination Survey. *Medscape J Med* **10**, 160.