Urinary Sucrose and Fructose as Biomarkers for Sugar Consumption

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Abstract

The use of 24-hour urinary sucrose and fructose as potential biomarkers for sugars consumption was investigated in two studies of 21 healthy participants living in a volunteer suite where dietary intake was known and all specimens collected. The dose-response was assessed in 12 males using a randomized crossover design of three diets containing constant levels of 63, 143, and 264 g of sugars for 10 days each. Both sugars and sucrose intake were significantly correlated with the sum of sucrose and fructose concentration in urine (0.888; $P < 0.001$). To assess effects with volunteers consuming their habitual varying diets, seven males and six females were fed their usual diet (assessed beforehand from four consecutive self-completed 7-day food diaries) for 30 days under controlled conditions in the volunteer suite. The mean (±SD) calculated total sugars intake was 202 ± 69 g/d, 41% from sucrose. Mean (±SD) urinary sucrose and fructose were 36.6 ± 16.6 and 61.8 ± 61.3 mg/d, respectively. The sum of sucrose and fructose in urine was significantly correlated with sugars (0.841; $P < 0.001$) and sucrose intake (0.773; $P = 0.002$). In the regression, 200 g of sugars intake predicted ~100 mg of sucrose and fructose in urine. The correlation between individual means of randomized 16 days of sugars intake and 8 days of sugars excretion data (as used in validation studies) remained as high as that obtained with the means of 30-day measurements and the regression estimates were very similar. Twenty-four–hour urinary sucrose and fructose could be grouped into a new category of biomarkers, predictive biomarkers, that can be used in studies determining the structure of dietary measurement error in free living individuals and to relate sugars intake to disease risk. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1287–94)

Introduction

Some epidemiologic evidence suggests that diets high in refined sugars might be associated with the increased risk of colorectal cancer (1, 2), breast cancer (3, 4), pancreatic cancer (5), and endometrial cancer (6). One of the suggested possible mechanisms is through the glycemic effect of diets high in refined sugars that affect levels of insulin and insulin-like growth factors which can enhance tumor development by stimulating cell proliferation and by inhibiting apoptosis (7). Another potential mechanism may be the increased oxidative stress induced by postprandial hyperglycemia (8). Dyslipidemia induced by high sugar intake is suggested as an explanation for the possible association between high sugar intake and increased risk of gallbladder and biliary tract cancer (9). However, several studies have failed to show any association between different cancer sites and sugars intake (10-15). Furthermore, findings on the relation between sugars intake and obesity, which increases the risk of several cancers, have been largely inconclusive (16).

This inconsistency in the reported findings of epidemiologic studies directed at hypothesized relations between sugars and cancer may be due to ambiguity of dietary assessment methods employed in these studies. It has recently been shown, using biomarkers of intake, that epidemiologic methods to measure diet are associated with large correlated measurement error which affects the reliability and interpretation of the studies investigating the association between diet and disease (17, 18). Much of the evidence on which diet-disease relationships is based in self-reported intake, and there is substantial underreporting of foods perceived to be unhealthy (19). Sugars are major contributors to energy intake and their intake estimates are particularly associated with differential measurement error and selective underreporting (20).

One of the possible ways to enable more accurate findings to be gathered is to introduce a biomarker for sugars intake. One possibility is sucrose in urine, because early work showed that small amounts of sucrose cross the small intestine unchanged and are excreted in the urine (21). Most sucrose is absorbed in the small intestine as glucose and fructose and of the two cleavage products of sucrose, blood glucose is under tight insulin control and has an effective uptake mechanism in the renal tubule. Under normal conditions, glucose is not therefore expected to be found in urine samples. However, some of the second cleavage product, fructose, was detected in urine following an oral administration of sucrose (22). A study elsewhere has shown that the mean daily excretion of fructose and sucrose after a low-sucrose diet was significantly decreased compared with a basal diet and that sucrose intake was significantly correlated with urinary excretion of sucrose and fructose (23). To investigate the utility of urinary sucrose and fructose further as possible biomarkers for total sugar consumption, two studies were conducted in a residential volunteer suite, where dietary intake can be carefully controlled and all samples collected (24). The first study was aimed to assess whether there is a dose-response of sucrose and fructose in urine to increased levels in food, giving a predictive response to varying intakes independent of individual variations. In normal life, individuals do not consume constant diets; hence, the second study investigated the validity of the proposed biomarkers to estimate sugars intake while subjects were on their habitual varying diet, simulating dietary behavior of free-living subjects in a controlled environment.

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Materials and Methods

The experimental design and statistical methods are reported separately for study 1 and study 2. The remainder of this section refers to both studies.

Experimental Design

Study 1: Dose-Response Study

Subjects. Twelve healthy nonsmoking males ages 25 to 77 years (48.3 ± 16.7 years) from Cambridgeshire were recruited following local advertisements. They were of different social background and occupation (e.g., physicist, nurse, pub manager, welder, student, artist, etc.). Before the study, all were examined by a medical practitioner and none had a medical history of diabetes (fasting blood glucose, <6.1 mmol/L; if fasting blood glucose is 6.1-7 mmol/L then $HbA_{1c} < 6\%$). Upon entrance to the study, all subjects gave their fully informed written consent. The study was approved by the Cambridge Local Research Ethics Committee (LREC No. 01/421).

Dietary Intervention. The study was a randomized 30-day crossover dietary intervention study in which the subjects lived in the volunteer suite of the Medical Research Council Dunn Human Nutrition Unit. They were placed on three 10d dietary periods of different total sugars (i.e., total sugars including monosaccharides and disaccharides, such as sucrose, maltose, and lactose) intake within the range of the habitual dietary intake of sugars in adult men in the United Kingdom (25). Dietary intakes were calculated from the U.K. food composition tables (26), using software Diet Plan 5 (27). Calculated total sugar intakes were as follows: low-sugar diet (63 ± 5 g/d, 9.5% of energy intake-EI), medium-sugar diet (143 ± 5 g/d, 21.8% EI), and high-sugar diet (264 ± 3 g/d, 40.2% EI). Sucrose contributed 34.4%, 37.4%, and 48.9% to the total sugars intake in the low-, medium-, and high-sugar diet, respectively. The intake of energy and other macronutrients was kept constant across the three dietary periods. Total energy intake was 10.3 ± 0.3 MJ/d; fat, protein, and carbohydrate intake were 102 ± 4, 92 ± 10, and 346 ± 10.0 g/d, respectively. The calculated intake of starch decreased as the total sugars content increased, 290 ± 6 g/d in the low-sugar diet, 190 ± 6 g/d in the medium-sugar diet, and 85 ± 3 g/d in the high-sugar diet. Subjects followed the same number of dietary periods but the order in which they received each intervention was randomized. The diets consisted of three meals and two snacks with three similar day rotating menus. The energy intake was matched to each participants' energy requirement and kept constant throughout the study. If a decrease in body weight was observed during the early part of the study, a standardized energy increment was provided. The subjects followed their normal life, but they were only allowed to take foods and drinks prepared for them in the suite. No other food was permitted. Tea and coffee, provided in the suite, were consumed freely, but subjects were asked to keep their intake consistent during the course of the study.

Urine Collections. On days 4 to 7 of each of the three dietary periods, the subjects collected 24-hour urine samples to be analyzed for sucrose and fructose. Twelve 24-hour urines were collected for each subject, or a total of 144 daily urines for the whole study. The completeness of the 24-hour urines was assessed by *p*-amino benzoic acid (PABA; see below).

Study 2: Habitual Varying Diet Study

Subjects. A total of 13 healthy subjects (7 males and 6 females) in the age range of 23 to 66 years (43.2 ± 15.9 years) were invited to participate in the second study. Four subjects were recruited from the first study and the rest were newly recruited. As before, they were of different social background and occupation (e.g., lab technician, retired civil servant, cleaner, research assistant, etc) and all were medically examined and gave their fully informed written consent before joining the study. The study was approved by the Cambridge Local Research Ethics Committee (LREC No. 02/323).

Dietary Intervention. This was a 30-day study in which the subjects lived in the volunteer suite of the Medical Research Council Dunn Human Nutrition Unit while consuming their usual diet. To assess their usual diet, the subjects were asked to keep 7-day estimated food diaries for four consecutive weeks while living at home. More detailed information, obtained at a weekly interview with one of the investigators, from the food diaries (including brand names) was then used to provide the subjects with their usual diet during the study period. During the study, each subject was given a choice of food daily, based on their food diaries. Approximately two and a half times the amount of food expected to be eaten by the subject was prepared and half of that kept for the preparation of duplicate meals. The other half of the prepared food was weighed to the nearest gram, labeled with the name and the day, and left in a separate refrigerator for each individual. During the day, subjects helped themselves and returned the uneaten food in the containers in the refrigerator. The next day, the uneaten food was weighed out and the amount of food eaten calculated. Duplicate diets of foods consumed the previous day were prepared daily for each subject and stored at −20°C for later analysis.

Dietary intake was calculated from the U.K. food composition tables using Data into Nutrients for Epidemiological Research (28). Tea and coffee were consumed freely during the course of the study but subjects were asked to keep their intake consistent. Five of the subjects occasionally consumed alcohol which is not permitted in the volunteer suite. These subjects were allowed to consume alcohol outside the premises but the amount and type had to be recorded in their study diary. The calculated dietary intake for alcoholic drinks was added into the consumption data obtained in the study.

Urine Collections. Continuous urine collections were made throughout the whole study. Thirty daily 24-hour urine samples verified for their completeness with PABA (see below) were collected from every subject.

Specimen Collection, Handling, and Storage. Upon entry to both studies, subjects were instructed on the technique of 24-hour urine collection. Subjects were asked to discard their first urine sample in the morning and from then on to collect all samples for 24 hours including the first sample of the following day. The subjects were given two 2-liter plastic containers each containing 3 g of boric acid to collect the urine and a rucksack for carrying the bottles when they were out of the suite. Some authors refer to instability of sucrose in urine samples kept at room temperature during the collection period (23). We conducted a range of experiments and found that both sucrose and fructose are stable in urine preserved with boric acid (pH ~6) and kept at room temperature during 24 hours ($t_{SUC} = -0.017$, $P = 0.987$; $t_{FRU} = -0.265$, $P = 0.795$). Every morning, soon after the subjects completed their 24-hour urine collection, daily urine collections were weighed and urine aliquots stored at −20°C for further analysis. The completeness of the 24-hour urine was assessed by urinary recovery of three 80 mg tablets of PABA (PABAcheck, Laboratories for Applied Biology, London, United Kingdom) given to the subjects to take with their meals (29). Urine collections with >85% recovery of the oral dose of PABA at the beginning of the collection period and >90% on succeeding days of the collection were considered as complete and included in the analyses. When there was confusion over two succeeding 24-hour collections (e.g., low marker recovery on one day followed by a high marker recovery the next day), the average of the two collections was used in the statistical analysis. Subjects recorded the time of taking PABA tablets or any missed urine collection in a diary, together with any medication taken.

Physical Activity and Body Weight Assessment. Physical activity was recorded in the study diary on a daily basis as time (minutes) engaged in different type of exercise. A four-level score (inactive, moderately inactive, moderately active, and active) was assigned by combining occupational physical activity together with time participating in higher-intensity physical activities such as cycling, aerobics, swimming, jogging, exercising at a gym on a regular basis, etc. (30). Subjects weighed themselves daily on an electric balance without shoes and in light clothing and recorded their body weight in the study diary.

Analytic Methods. PABA concentration in urine was measured by a colorimetric technique described elsewhere (29). Sucrose and fructose concentration in urine were measured with a kit specific for sucrose, glucose, and fructose (sucrose/D-glucose/D-fructose from Biochemica Mannheim, R-Biopharm, Roche) using Cecil CE 2041 2000 Series spectrophotometer to measure the absorbance (31). Urinary samples of each subject were analyzed in one run for the first study and as two batches for every subject in the second. On the day of the assay, urine samples were thawed and thoroughly mixed before the analysis. Standards in the range of expected values for sucrose (10, 20, and 30 mg/L) and fructose (5, 10, and 20 mg/L) were run in every assay. To ensure the consistency of the results and the assay conditions, an internal quality control was also included in each assay consisting of aliquots from a single source preserved with boric acid and stored at −20°C. Over 1.5 years of the study, values for this quality control remained consistent throughout ($mean_{SUC}$ = 12.36 ± 0.82 mg/L; $mean_{FRU}$ = 5.77 ± 0.24 mg/L).

Statistical Methods. SPSS version 11 for Windows was used for data analysis. Data are presented as means and SDs. Individuals' body weights at the beginning and at the end of the studies were compared by paired *t* test. Other statistical analyses employed are presented separately for the two studies.

Study 1: Dose-Response Study. Individuals' means of urinary sugars (sucrose, fructose, and the sum of both) were skewed; hence, they were transformed by square root transformation, whereas dietary sugars and sucrose data were normally distributed. The coefficient of variation (%CV; SD/mean × 100) was used to present the within- and between-subject variability in intake and excretion levels. Repeated measures two-way ANOVA was employed to assess the between-subjects effect and to compare the daily sugars excretion during different periods of sugars intake. Wilks' Lambda multivariate test and Eta squared were used to report the significance of the repeated-measures models and the effect size, respectively. Repeated-measures one-way ANOVA was used to compare the individuals' means of urinary sucrose and fructose excretion during different sugars intake periods. Spearman correlation coefficients were used to examine colinearity between dietary and urinary sugars. To further explore the association between urinary and dietary sugars, a linear regression model with the square root transformed individuals' mean sum of sucrose and fructose in urine as a dependent and mean total sugar intake as independent variable was fitted and adjusted R^2 was reported. No effect of age, body weight, or physical activity was detected. The regression equation was therefore derived by plotting unadjusted nontransformed individuals' means of sugars excretion against their mean total sugars intake.

Study 2: Habitual Varying Diet Study. Both daily measurements and individuals' means of urinary sugars were skewed; hence, they were log_{10} transformed, whereas dietary sugars and sucrose data were normally distributed. To compare body weight between men and women, an independent *t* test was used. Randomization was done for each subject separately; first, randomly selecting 16 of 30 days diet and second, randomizing 8 of the 16 days. A paired *t* test was used to compare levels of sugars excretion and intake between the means of 30-day and randomized measurements. An ANOVA random effect model was employed to quantify variance components in sugars excretion and intake within and between subjects to calculate the ratio of within- to between-subject variance ($\sigma^2_{WS}/\sigma^2_{BS}$). Reproducibility of the dietary intake and urinary excretion measurements was assessed by the intraclass correlation coefficient, calculated as the ratio of between-subject variance, and the sum of within- and between-subject variance [$\sigma^2_{BS}/(\sigma^2_{BS} + \sigma^2_{WS})$]. Spearman correlation coefficients were used to examine colinearity between dietary and urinary sugars. A hierarchical regression model with the log_{10} transformed individuals' mean sum of sucrose and fructose in urine as a dependent and mean total sugar intake as independent variable was fitted adjusted for sex, age, body weight, and physical activity. As there was little effect of these variables, the regression equation was derived by plotting nontransformed individuals' unadjusted means of sugars excretion against their mean total sugars intake.

Results

Study 1: Dose-Response Study

Study Compliance, Body Weight, and Physical Activity. All 12 subjects completed the three dietary periods. Of 144 24-hour

Table 1. Mean sucrose and fructose concentration in urine and daily within-subject variation by subject during different diets

Subject	Low-sugars diet (61 g/d)					Medium-sugars diet (144 g/d)					High-sugars diet (265 g/d)				
	n	Urinary sucrose (mg/d)		Urinary fructose (mg/d)		*n*	Urinary sucrose (mg/d)		Urinary fructose (mg/d)		*n*	Urinary sucrose (mg/d)		Urinary fructose (mg/d)	
		Mean	CV%	Mean	CV%		Mean	CV%	Mean	CV%		Mean	CV%	Mean	CV%
D1	4	6.04	45.2	2.58	30.2	4	18.19	32.1	13.89	14.2	4	36.97	36.0	21.92	16.8
D2	4	6.81	68.2	0	0	4	19.90	43.2	15.88	27.8	4	25.26	15.5	26.04	43.3
D3	4	10.98	55.4	2.34	78.2	4	20.01	33.9	13.09	16.1	4	39.77	15.4	23.03	26.7
D4	4	9.16	45.5	4.99	84.6	3	34.55	36.0	11.08	18.4	4	38.34	32.4	14.82	20.6
D5	4	7.61	41.4	1.48	71.6	4	24.76	26.3	19.40	22.4	4	27.79	18.7	25.26	19.5
D6	3	15.47	15.3	2.91	44.3	4	40.64	39.1	27.67	22.1	0	—	—	—	—
D7	4	16.36	56.6	1.85	25.9	4	34.13	14.1	7.37	4.3	2	47.90	33.1	9.82	7.2
D8	4	3.29	200.0	1.20	118.0	4	29.75	13.7	17.01	24.9	4	74.07	59.2	21.80	11.2
D9	3	5.93	62.0	0.72	87.5	4	8.13	21.0	4.89	53.0	4	62.76	76.5	14.35	15.0
D10	3	5.92	16.0	1.33	60.2	3	24.54	17.4	22.27	27.2	4	42.97	31.9	22.93	35.9
D11	3	5.21	72.9	0.50	92.6	3	29.14	61.6	17.80	36.5	3	28.19	19.8	24.83	22.9
D12	4	6.58	57.8	4.63	35.0	4	26.56	15.2	27.41	9.7	3	76.63	42.1	44.56	11.3
Mean	12	8.3	61.4	2.0	60.7	12	25.9	29.5	16.5	23.1	11	45.5	34.6	22.7	20.9

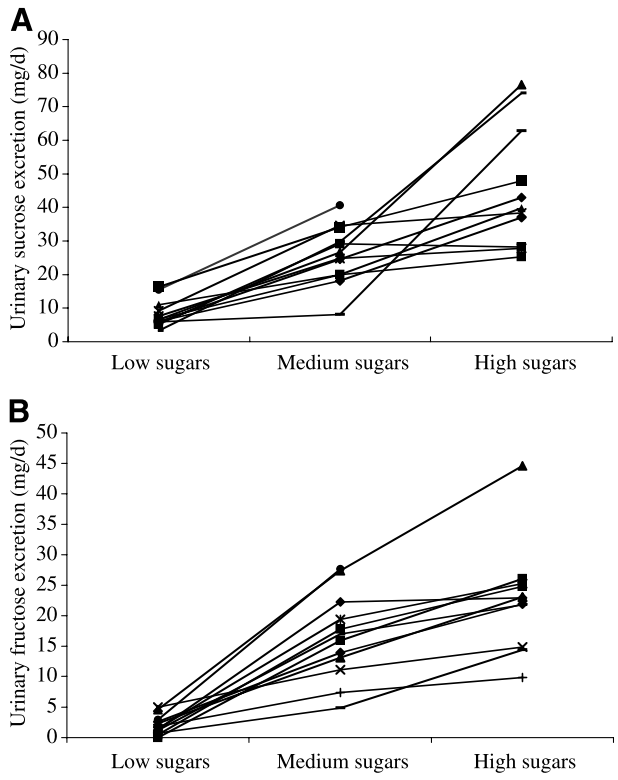

Figure 1. Urinary excretion of sucrose (**A**) and fructose (**B**) during low-, medium-, and high-sugars diet. *Points,* mean for each subject. (-●-, no data is available for the high-sucrose diet, because urines were excluded from the analysis due to ill health of the subject).

urine collections, 129 (89.6%) were complete as judged by PABA analysis. Eleven urine collections did not have satisfactory PABA recovery due to lost samples or spillage. One subject, due to ill health, had poor recovery for four consecutive days during the high sugars diet. These collections were excluded from the analysis.

There was a change in body weight to up to 1 kg in 10 of 12 subjects, with four of the subjects gaining weight and six losing weight. Overall, the mean body weight of the subjects did not change, being 76.7 ± 9.8 kg at the beginning and 76.4 ± 9.9 kg at the end of the study ($t = 1.561$; $P = 0.147$). Of 12 subjects, 4 were categorized as physically inactive, 1 as moderately inactive, 3 as moderately active, and 4 as active. They mostly practiced cycling, swimming, exercising at the gym, and jogging.

Urinary Sugars. Sucrose and fructose were measured in complete 24-hour urines collected on days 4 to 7 in each dietary period. Table 1 shows the mean and day-to-day within-subject variation of sucrose and fructose excretion during different diets by subject. The sum of the mean sucrose and fructose excretion in the urine was 0.02%, 0.03%, and 0.03% of the total sugars intake in the low-, medium-, and high-sugars diet, respectively. The daily within-subject variation of sucrose and fructose urinary outputs were high, especially while the subjects were on the low-sugars diet due to very low daily concentrations measured in urine for both sugars (CV_{suc} = 61.4%; CV_{fru} = 60.7%). The variability of sugars excretion was somewhat less during the medium- and high-sugars diets with CVs in the range of ~30% for sucrose and ~20% for fructose. However, despite this variability, as shown in Table 1, the mean urinary excretion of both sucrose and fructose in the 12 subjects increased with the increment of sugars consumption over the three dietary periods with a significant difference in the mean urinary excretion of both sucrose (Wilks' Lambda = 0.082, $F_{2,9}$ = 50.1, $P < 0.001$, Partial eta squared = 0.918) and fructose (Wilks' Lambda = 0.077, $F_{2,9}$ = 54.3, $P < 0.001$, Partial eta squared = 0.923) between the periods of different sugars intake.

A significant between-subjects difference in the effect of sugars intake on both sucrose ($F_{20,50} = 3.3$; $P < 0.001$, partial eta squared = 0.570) and fructose excretion ($F_{20,50} = 4.9$; $P < 0.001$; partial eta squared = 0.663) was detected when subjects were added as a factor. However, the differences between diets were greater, with an effect size of sugars intake on the excretion of both sucrose and fructose in the models that was almost double that of the appropriate interaction effect (partial eta squared = 0.925 versus partial eta squared = 0.570 for sucrose and partial eta squared = 0.975 versus partial eta squared = 0.663 for fructose). This showed that the level of sugars intake is a more significant determinant of sucrose and fructose concentrations in the urine than the individual variability. The individual sugars excretion mean values are shown in Fig. 1.

The correlation of the means between sugar intake and urinary sucrose was 0.864 ($P < 0.001$; individuals' coefficients range, 0.739-0.953). Sugars intake was also highly correlated with urinary fructose 0.803 ($P < 0.001$; range, 0.719-0.948) and urinary sucrose and fructose combined 0.888 ($P < 0.001$; range, 0.791-0.946). Correlation of urinary sugars to sucrose in diet was also investigated and the correlation coefficients were virtually the same for all three variables (data not presented). As the associations were improved by using the combined measure, the association between urinary and dietary sugars

Table 2. Thirty-day mean and within-subject variation of dietary intakes of energy and macronutrients in subjects on varying diet

Subject	Energy (MJ)		Fat (g/d)		Protein (g/d)		Carbohydrate (g/d)		Total sugars (g/d)		Sucrose intake (g/d)		
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	% Total sugars
V1	10.9	15.2	105.8	32.3	99.1	15.4	339.7	12.8	179.7	14.9	88.90	14.8	49.6
V2	11.2	19.5	119.6	22.7	118.2	23.1	245.0	26.3	95.2	35.1	41.40	44.9	41.2
V3	10.8	23.2	116.8	28.6	77.1	21.3	321.8	25.2	151.9	34.4	79.10	44.0	51.2
V4	11.1	11.4	97.3	21.3	81.9	19.8	361.7	13.4	161.4	23.0	73.30	37.4	44.1
V5	10.3	16.1	55.2	38.4	82.4	31.9	429.2	12.1	323.0	7.7	132.30	8.9	41.0
V6	10.2	17.5	100.9	30.3	78.8	30.0	338.1	20.2	192.8	20.6	81.10	25.9	41.9
V7	15.6	19.0	178.1	27.8	110.0	29.9	433.6	19.6	302.5	17.2	93.70	25.2	31.1
V8	11.7	17.3	87.4	29.9	99.0	23.7	429.1	18.6	212.0	15.7	92.30	21.7	43.4
V9	11.6	25.2	89.7	42.9	110.6	42.2	402.0	25.1	245.7	25.3	92.30	40.5	37.1
V10	12.7	17.4	90.4	34.2	103.4	23.0	478.0	15.5	270.6	16.4	114.80	21.8	42.2
V11	14.1	21.7	126.9	36.8	120.5	24.4	462.1	19.6	209.9	18.8	83.80	24.6	39.8
V12	11.6	19.2	123.4	30.6	107.3	23.7	327.8	24.0	181.4	25.2	62.40	37.8	34.1
V13	9.4	15.9	92.7	23.6	84.8	23.0	278.6	17.3	104.9	25.4	35.50	40.3	33.1
Mean	11.6	18.4	106.5	30.7	97.9	25.5	372.8	19.2	202.4	21.5	82.4	29.8	40.8

Table 3. Variability estimates of dietary and urinary sugars in subject consuming their habitual diet

	CV_{WS}%*	CV_{BS}%†	$\sigma^2_{WS}/\sigma^2_{BS}$‡	ICC§
Dietary total sugars	21.5	34.2	0.36	0.74
Dietary sucrose	29.8	31.9	0.83	0.55
Urinary sucrose	50.5	45.5	1.26	0.44
Urinary fructose	38.6	99.1	0.23	0.81
Sum of urinary sucrose and fructose	36.9	67.0	0.49	0.67

Abbreviation: ICC, intraclass correlation coefficient.
*Within-subject coefficient of variation.
†Between-subject coefficient of variation.
‡Ratio of within- to between-subject variance.
§Intraclass correlation coefficient.

was further explored. The linear regression model with the sum of sucrose and fructose in urine as a dependent and total sugars intake as an independent variable explained 74% of the variability in urinary sugars (adjusted $R^2 = 0.741$; $F_{1,33} = 98.5$; $P < 0.001$). As total sugars intake was the main determinant of sugars excretion the individuals' means of sugars excretion were plotted against their total sugars intake. The unadjusted regression equation was $y = 0.28x - 3.45$, so that 200 g/d of total sugars in diet predicted a urinary output of 53 mg of sucrose and fructose per day.

Study 2: Habitual Varying Diet Study

Study Compliance, Body Weight, and Physical Activity. A total of 390 daily urines were collected, 30 for each subject. Only four urine collections were excluded from the analysis. One was incomplete, two were excluded due to spillage, and one subject failed to take the daily PABA dose.

All 13 subjects completed the study and remained healthy throughout. There was a change of body weight of about 1 kg in 7 of 13 subjects, with four gaining weight, two losing weight, and the weight fluctuating in one of the subjects. However, the mean body weight did not change in the group as a whole, being 76.2 ± 15.1 kg at the start and 76.3 ± 15.0 kg at the end of the study ($t = -0.611$; $P = 0.533$). There was no statistically significant difference in body weight between men and women ($t = 1.41$; $P = 0.185$). During the study, subjects continued their normal occupational and recreational engagements. Three of the subjects were physically inactive, four moderately inactive, five moderately active, and one active. The most frequent physical activities were cycling, swimming, going to the gym, badminton, and squash.

Dietary Sugars. As shown in Table 2, the mean calculated total sugars intake was 202 ± 69 g/d (29.2% EI), ranging from 95 ± 33 g/d (14.5% of EI) to as high as 323 ± 25 g/d (52.6% of EI). The mean sucrose intake for the group was 82 ± 25 g/d (11.9% EI) and between subjects, a similar proportion of the total sugars intake was due to sucrose ranging from 31.1% to 51.2% (see Table 2). There was no significant difference in the sugars intake between men and women ($t = -0.549$; $P = 0.594$). Energy, fat, protein, and carbohydrate intake are also given in Table 2. No difference in the intake of energy or other macronutrients was detected between men and women, hence, means are presented for the group as a whole. Day-to-day variability in sugars intake was lowest in the subject with the highest mean sugars intake (CV_{WS} = 7.7%) and greatest in the subject with the lowest intake (CV_{WS} = 35.1%; see Table 2). The average within-subject CV in total sugars intake for the group was 21.5%. The between-subjects variability in sugars intake (CV_{BS}) was 34.2% (see Table 3). The within-subject variance (σ^2_{WS}) was less than the between-subject variance (σ^2_{BS}) with a $\sigma^2_{WS}/\sigma^2_{BS}$ ratio of 0.36. The single measure intraclass correlation coefficient for calculated sugars intake was 0.737. Subjects who had the most variable total sugars intake also had the most variable sucrose intake but the between-subject variability was lower than that for sugars intake (CV_{BS} = 31.9%). The $\sigma^2_{WS}/\sigma^2_{BS}$ ratio was 0.83 and intraclass correlation coefficient for sucrose intake measurements was 0.55.

Urinary Sugars. Table 4 shows the means and CVs in urinary sucrose and fructose, and combined sucrose and fructose excretion in subjects fed their habitual varying diets; 0.05% of sugars dietary intake was excreted in urine as sucrose and fructose. A higher between-than within-subjects variance was detected for fructose ($\sigma^2_{WS}/\sigma^2_{BS}$ = 0.23), the sum of sucrose and fructose ($\sigma^2_{WS}/\sigma^2_{BS}$ = 0.49) but not for sucrose excretion ($\sigma^2_{WS}/\sigma^2_{BS}$ = 1.26; see Table 3). The $\sigma^2_{WS}/\sigma^2_{BS}$ ratio of fructose excretion was lower than the $\sigma^2_{WS}/\sigma^2_{BS}$ ratio for sugars intake. The intraclass correlation coefficient for urinary sucrose, fructose, and sum of the both was 0.44, 0.81, and 0.67, respectively.

In most of the subjects, the daily dietary and urinary sugars were significantly correlated (see Table 4), indicating rapid time-related response of urinary sugars excretion to their

Table 4. Thirty-day mean and within-subject variation of urinary sugars and their correlation with total sugars and sucrose intake in subjects on varying diet

Subject	*n*	Urinary sucrose (mg/d)				Urinary fructose (mg/d)				Sum urinary sucrose + fructose (mg/d)			
		Mean	CV%	*r**	*r*†	Mean	CV%	*r**	*r*†	Mean	CV%	*r**	*r*†
V1	30	29.4	37.1	0.565‡	0.528§	15.7	25.5	0.423§	0.536§	45.1	28.4	0.559‡	0.596‡
V2	30	12.8	60.9	0.381§	0.404§	12.6	68.3	0.670‡	0.681‡	25.4	48.4	0.681‡	0.679‡
V3	29	40.4	70.8	0.624‡	0.664‡	35.8	52.8	0.834‡	0.529§	76.2	50.6	0.869‡	0.713‡
V4	30	22.4	39.7	0.634‡	0.694‡	21.9	35.6	0.636‡	0.588‡	44.3	34.1	0.690‡	0.729‡
V5	30	41.2	73.3	0.049	0.133	228.2	50.5	−0.143	−0.131	267.5	46.6	−0.033	−0.043
V6	29	44.7	43.0	0.213	0.380§	106.4	28.9	0.535§	0.564‡	151.0	26.8	0.439§	0.550§
V7	28	43.7	24.3	0.380§	0.492§	56.5	44.6	0.112	0.318	100.2	31.7	0.275	0.481§
V8	29	37.4	42.8	0.288	0.342	103.0	28.9	0.503§	0.305	140.4	26.3	0.445§	0.326
V9	30	33.9	43.4	0.128	0.255	108.5	36.4	0.247	0.113	142.4	30.8	0.220	0.202
V10	30	59.4	32.7	0.179	0.322	34.0	23.5	0.308	0.210	94.3	22.7	0.372§	0.475§
V11	30	70.6	79.7	0.556‡	0.623‡	20.5	28.8	0.661‡	0.364§	91.0	64.6	0.616‡	0.642‡
V12	30	26.4	42.8	0.844‡	0.688‡	40.2	36.3	0.634‡	0.534§	66.6	33.2	0.794‡	0.671‡
V13	30	13.0	65.4	0.087	0.449§	20.5	41.5	0.386§	0.049	33.3	35.5	0.350	0.282
Mean	13	36.6	50.5	0.379		61.8	38.6	0.447		98.3	36.9	0.483	0.485
r Means				0.692§	0.674§			0.687§	0.550			0.841‡	0.773§

*Spearman correlation coefficient to total sugars intake.
†Spearman correlation coefficient to sucrose intake.
‡$P < 0.001$.
§$P < 0.05$.

intake. The between person correlations using individual's 30-day means of sugars intake and their 30-day mean excretion levels of both sucrose ($r = 0.692$; $P = 0.009$) and fructose ($r = 0.687$; $P = 0.01$) were also found to be significant. Individuals' means of sucrose intake were found to be significantly correlated with sucrose ($r = 0.674$; $P = 0.012$) but not with fructose in urine ($r = 0.550$; $P = 0.051$). The sum of sucrose and fructose concentration yielded a higher correlation with total sugars and sucrose intake than the two sugars alone ($r_{SUG} = 0.841$; $P < 0.001$; $r_{SUC} = 0.773$; $P = 0.002$).

In the hierarchical regression model, age, sex, body weight, and physical activity explained only 10% of the variability in sugars excretion, whereas adding sugars intake into the model contributed a further 72% to the prediction of the amount of urinary sugars (adjusted $R^2 = 0.82$; $F_{5,7} = 12.1$; $P = 0.002$). The unadjusted regression equation of the individuals' 30-day means of sugars excretion plotted against their 30-day means of total sugars intake was $y = 0.72x - 47.5$, where 200 g/d of sugars intake predicted 96.5 mg of sucrose and fructose in urine per day. Figure 2A shows the regression and 95% confidence interval of the individuals' means of 30-day total sugars intake and sugars urinary measurements.

Applicability of Urinary Sugars in Validation Protocols. In studies using the 24-hour nitrogen and potassium urinary markers to validate dietary assessments, the recommended number of 24-hour urines collected from each subject is eight for comparison with dietary information about usual diet obtained from diet history methods, or 16 days of dietary records (32, 33). Sixteen-days of sugars intake data and 8-days of corresponding fructose and sucrose excretion data were therefore randomly selected for each individual from the 30-day measurements for each of the variables. Table 5 shows the mean levels of dietary sugars compared with urinary sucrose, fructose, and the combined measure, and the correlation between dietary and urinary sugars in the randomized sample by subject. The means of both dietary ($t_{SUG} = -2.14$, $P = 0.054$; $t_{SUC} = -2.14$, $P = 0.053$) and urinary sugars ($t_{SUC} = -1.35$; $P = 0.201$; $t_{FRU} = -0.079$; $P = 0.938$; $t_{SUC + FRU} = -0.989$; $P = 0.342$) were very similar to the ones obtained by the 30-day measurements and correlations were almost as high as those obtained from the latter (see Tables 4 and 5). Furthermore, the regression equation of urinary sugars on dietary sugars for the randomized sample was very similar to the equation generated with the 30-day measurements ($y = 0.824x - 67.1$), where 200 g/d of sugars intake predicted 97.7 mg sucrose and fructose in urine per day. Figure 2B shows the regression and confidence intervals that can be expected in a validation study of individual diet history or 16-day recorded intakes of total sugars using 8-day urinary measurements.

Discussion

In these studies of subjects maintained in a volunteer suite where all dietary intake was measured and all urine specimens collected and validated, the combined measure of urinary sucrose and fructose was highly correlated with both total sugar ($r = 0.888$, dose-response study; $r = 0.841$, varying diet study) and sucrose intake ($r = 0.888$ dose-response study; $r = 0.773$, varying diet study). This means that urinary fructose and sucrose are potential biomarkers that can be used to estimate dietary intake of total sugars and sucrose.

The mechanism by which sucrose and fructose occurs in the urine is not well understood. Except in the case of endogenous sucrosuria (34), sucrose appearing in the urine is usually of dietary origin (21). In physiologic conditions very small amounts of sucrose may escape enzymatic hydrolysis by sucrase in the small intestine and enters the general circulation (34). The increased amount in urine has been attributed to either altered intestinal disaccharidase activity (35) or more often to gastric damage (36) and some of the individual variation seen here may relate to changed upper gastrointestinal permeability. Once in the blood stream, intact sucrose is very slowly metabolized, being almost quantitatively excreted in the urine (37). Fructose appearing in the urine is possibly a fraction derived from dietary fructose and hydrolysis of dietary sucrose that escapes fructose hepatic metabolism that passes on into the systematic circulation and is readily excreted in the urine not being subject to strict hormonal regulation as is glucose. After an oral administration of 50 g of sucrose, fructose was not detected in the blood but a considerable amount was recovered in the urine (22).

Calculated rather than analyzed intakes of sugars were used in these two studies to reflect usual practice in dietary surveys. Both total sugars and sucrose intake were studied rather than dietary sucrose only as the information on total sugars in food tables worldwide is more complete and far more reliable than that for separate sugars (38); 3% total sugars and 7% sucrose values are missing in the Data Into Nutrients for Epidemiological Research food database, most of them being in flour, bread, breakfast cereals and beverages

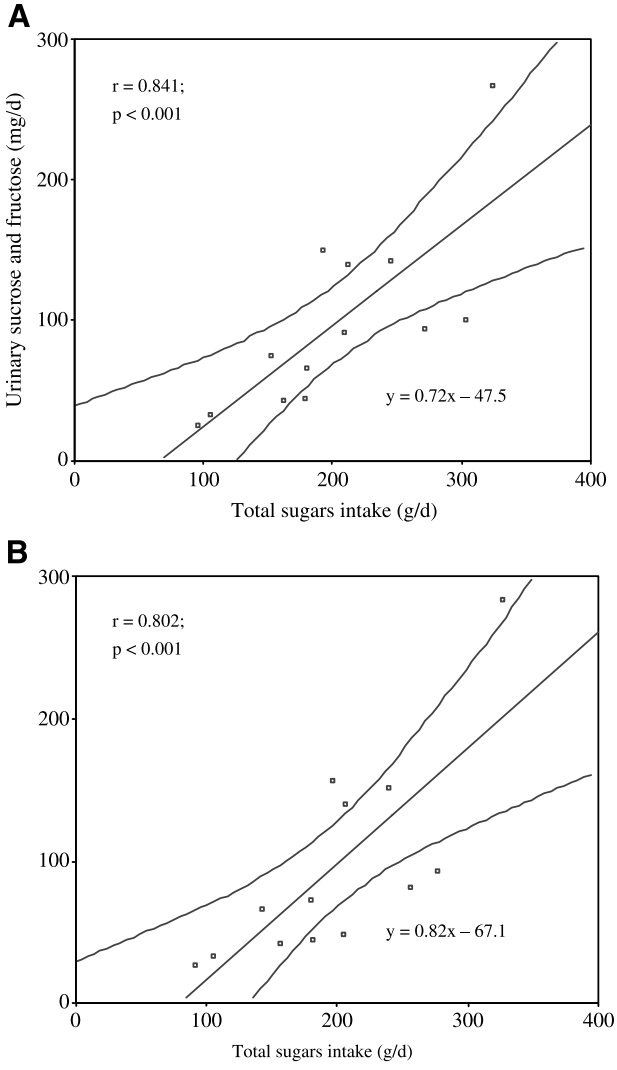

Figure 2. Regression and 95% confidence interval of the means of calculated dietary intake of total sugars intake and the sum of sucrose and fructose urinary excretion. **A.** Means of 30-day dietary and urinary sugar measurements. **B.** Means of 16-day dietary and 8-day urinary sugar measurements.

Table 5. Means and Spearman correlation coefficient of a randomized sample of 16 dietary and eight urinary sugars measurements by subject

Subject	Sugar intake (g/d)	Sucrose intake (g/d)	Urinary sucrose (mg/d)			Urinary fructose (mg/d)			Sum urinary sucrose + fructose (mg/d)		
			Mean	r^*	$r^\dagger$	Mean	r^*	$r^\dagger$	Mean	r^*	$r^\dagger$
V1	181.9	90.20	29.6	0.738‡	0.643	15.4	0.690	0.690	45.0	0.714‡	0.667
V2	91.9	40.40	12.5	0.738‡	0.762‡	15.0	0.857§	0.810‡	27.5	0.976§	0.952§
V3	143.1	74.10	36.2	0.595	0.548	31.2	0.714‡	0.667	67.4	0.833‡	0.738‡
V4	156.8	68.60	20.7	0.214	0.476	21.5	0.595	0.857‡	42.2	0.287	0.647
V5	325.7	131.60	46.2	0.262	0.262	237.0	0.262	0.167	283.2	0.524	0.452
V6	197.7	85.60	44.8	0.071	0.500	112.0	−0.167	−0.262	156.7	−0.286	−0.190
V7	276.4	87.80	41.2	0.286	0.143	52.0	0.929§	0.524	93.2	0.929§	0.524
V8	207.3	91.40	40.1	−0.071	−0.286	100.0	0.452	0.286	140.1	0.143	−0.143
V9	239.2	86.70	31.5	−0.048	−0.214	120.5	0.286	0.405	152.1	0.333	0.429
V10	256.0	111.00	49.8	−0.190	0.095	33.0	0.071	−0.024	82.8	−0.252	0.000
V11	204.9	79.40	32.7	0.071	0.262	16.4	0.476	0.214	49.1	0.333	0.286
V12	180.5	65.00	28.8	0.905§	0.643	44.9	0.857§	0.738‡	73.7	0.952§	0.738‡
V13	105.9	33.50	11.9	0.357	0.905‡	21.2	0.262	−0.071	33.2	0.571	0.548
Mean	197.48		32.8	0.302		63.01	0.480		95.86	0.470	0.434
r_{means}				0.802§	0.824§		0.698§	0.538		0.802§	0.703‡

*Correlation to total sugar intake.
†Correlation to sucrose intake.
‡ $P < 0.05$.
§ $P < 0.001$.

(26, 28). Nevertheless, as mentioned, dietary sucrose was included in the analysis to check how this biomarker would correlate to calculated sucrose intake.

In the dose-response study using constant diets, the CVs of calculated intake were 7.6%, 3.4%, and 1.2% when subject were consuming 63, 143, and 264 g of total sugars, respectively. An increase of both sucrose and fructose excretion across periods of higher sugar intake was observed in all subjects. Although the within-subject day-to-day variability in sugar excretion was high, the average urinary excretion levels of sucrose and fructose were distinguishable on an individual basis between different periods of intake, showing a dose-related response between dietary intake of sugars and both sucrose and fructose in the urine (Fig. 1). The diet outweighed any individual variability and in the multiple regression model, the intake level was shown the only significant predictor of the sugar excretion independent of individual variation.

In normal life, dietary intake is highly variable, and the findings that intake and output were highly correlated from a controlled study of constant diets may not be applicable to biomarker and dietary studies of free-living individuals. In the second study of habitual varying intake reported here, the range of intake was from 95 to 323 g sugar/d and the mean day-to-day variation in intake was 21.5%, compared with 4.1% when diet was kept constant. The mean intake of 202 g/d (29.5% EI) total sugars was somewhat higher than in the representative dietary and nutrition survey of British adults, where the average daily intake of sugars was 118 g (20.4% EI) in males and 88 g (21.6% EI) in females (39). However, the fact that no weight was lost or gained on average over the study period of 1 month suggests that the intake achieved in this study was a valid reflection of usual dietary habits in these volunteers. No significant differences in intake were shown between men and women, but the study size was not designed to investigate population differences.

In subjects consuming their normal diet, the sum of sucrose and fructose in urine was highly correlated with both sugars ($r = 0.841$) and sucrose intake ($r = 0.773$). Correlations between the daily measurements were highly significant in most of the subjects, suggesting rather rapid response and sensitivity of urinary excretion to intake. In the final regression model, total sugar intake explained 70% of the variance in sugar excretion. Although high day-to-day variation in both sugar intake and excretion was evident, the higher between-than within-subject variation indicated that fewer observations would be needed to discriminate between subjects (40). The reproducibility of sugar intake has been shown the one of the highest when compared with other nutrients (41), with the fewest number of days required to rank dietary sugar intake with desired precision. In our study, reproducibility of urinary sugar measurements was even higher than reproducibility of the dietary measure, suggesting that less urinary measurements are needed to characterize individuals with respect to their urinary sugar excretion. When only eight urinary measurements were randomized from the 30-day data, the means of all three observed urinary variables were similar to the means obtained from the 30-day measurements (see Tables 4 and 5). Moreover, their correlation with estimated intake from 16-day was almost as high as the correlation obtained from the 30-day measurements of diet and urinary sugar, showing that this biomarker can be used within the current validation protocol. Regression lines of the means of 30-day measurements and the means of the randomized sample gave similar prediction of about 100 mg sucrose and fructose in urine per 200 g of total sugars in the diet (see Fig. 2A and B). However, the first study predicted only half of this amount, and this nonagreement between the regression estimates may well be due to the small sample size involved. Larger studies would therefore be needed to verify this prediction estimate.

Three main types of biomarkers have been described (24, 42). Recovery biomarkers are based on a balance between intake and output over certain time period and can be translated into estimates of absolute intake levels over the same period, such as 24-hour urine nitrogen (32) and potassium (33) and doubly labeled water (43) as biomarkers for 24-hour protein, potassium and energy intake, respectively. Concentration biomarkers cannot be translated into absolute levels of intake but concentrations in blood, urine, or other biological materials correlate with intake levels of corresponding foods and nutrients. Where data base values for food constituents are unreliable or nonexistent, replacement biomarkers are required (24).

In the present study, overall recovery of urinary sugars was low (0.05%) compared with intake so that these markers cannot be used as recovery markers to assess the overall validity of intake assessed by a particular method nor can they be used to assess underreporting of food intake, unlike urinary nitrogen and potassium which account for about 80% of dietary intake (24). On the other hand, this biomarker exhibits much higher correlation with intake than the concentration biomarkers and, unlike those, is time-related and sensitive to intake in a dose-response manner. The high correlation with intake, high reproducibility, and high predictive potential

suggest that urinary sugars could qualify as a biomarker for sugar intake and could be grouped into a new fourth category of dietary biomarker, a predictive biomarker.

No special conditions are needed for sample collection and urine sugars are stable for up to at least 1.5 years of storage at −20°C. The method of analysis is a simple colorimetric method which can be easily set up in most laboratories. Provided the 24-hour urine collections are shown complete, we believe that urinary sucrose and fructose have great potential as a predictive biomarker of sugar intake. Correlation coefficients of >0.6 are needed for biomarker studies of measurement error (42), and the correlation of at least 0.8 expected when eight urines are collected and ''usual'' diet is assessed indicates that urinary sugars will have a useful role in studies of measurement error together with other dietary biomarkers (17, 18). Larger studies in which dietary assessments have been validated with recovery markers such as, doubly labeled water and 24 urine N (44), will be needed to verify this assumption and that the regression estimates given here are appropriate for population estimates. Furthermore, this predictive biomarker may be a useful independent qualitative index of sugar consumption to investigate diet in relation to cancer and to obesity and diabetes which have themselves been shown related to cancer (45, 46).

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