Urinary Excretion of Sucrose and Fructose as a Predictor of Sucrose Intake in Dietary Intervention Studies

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Abstract
The urinary excretion of sucrose, glucose, and fructose was measured in 9 healthy subjects consuming a common Italian diet and after 3 days of a low sucrose diet, in which the intake of sucrose was restricted but the other main nutrients were unmodified. After the low sucrose diet, we observed a significant drop in the average urinary excretion of sucrose, glucose, and fructose as a function of dietary changes such as the consumption of a diet in which nutrient intakes were restricted but the other main nutrients were unmodified. After the low sucrose diet, we observed a significant drop in the average urinary excretion of sucrose, glucose, and fructose determined at four different times (8:00 and 10:00 a.m.; 3:00 and 10:00 p.m.). The average urinary excretion of fructose in the four urine samples was significantly correlated with dietary sucrose intake. We also found a significant correlation between the average urinary excretion of sucrose and dietary sucrose intake. Urinary fructose can be used as a marker of sucrose intake in dietary intervention studies aimed at studying the effect of variation of carbohydrate intake on specific cancers.

Introduction
A large body of literature has been devoted to the analysis of correlations between diet and cancer; many studies have focused on the effects of fats, calories, and nonnutritive dietary components on the occurrence of cancer in both animals and humans (1–8).

Some epidemiological evidence suggests that diets poor in fiber and complex carbohydrates and rich in lipids may be associated with a higher risk of some types of cancer, such as breast and colon (3, 4, 7, 8). Among dietary carbohydrates, sucrose is used in widely different amounts in various countries, and several epidemiological and experimental studies have suggested an association between colon cancer risk and a high intake of sucrose-rich foods (9–14). Accordingly, in a recent study conducted in northern Italy, a strong correlation was demonstrated between the number of spoonfuls of sucrose added to coffee and colorectal cancer risk (10).

The mechanisms linking high sucrose consumption to colon cancer have not been clarified. However, sucrose, given as a bolus, has been shown to elevate colon mucosal proliferation and increase its sensitivity to carcinogens in mice (11).

A high level of sucrose in the diet has been indicated as a possible cofactor in several other diseases. In fact, serum cholesterol increases in humans on a high sucrose diet (15–17), and the lipemic response after a meal is greater after a breakfast rich in sucrose (18). A diet high in sucrose has been associated with an increased risk of gallstones (19), and a high sucrose diet might also play a role in irritable bowel syndrome and in alterations of intestinal function such as diarrhea and cramps (20, 21).

On this basis, a shift toward a more balanced diet richer in fibers and starches and lower in sucrose has been recommended with the aim of decreasing the risk of some types of cancer and coronary heart diseases (22).

The dietary intake of carbohydrates and other nutrients can be evaluated on the basis of dietary interviews or food records kept by subjects. However, the intake of carbohydrates is not easy to monitor because many different carbohydrates must be considered, and both the quantity initially present in foods and beverages and the amount added before consumption must be calculated. Besides, establishing nutrient intakes with dietary interviews or food records is inherently imprecise. It is, therefore, difficult to assess nutrient intake in humans and to verify the compliance to specific diets in intervention studies.

On the basis of these considerations, we were interested in finding new markers of carbohydrate consumption. With this aim, we studied the urinary excretion of sucrose, glucose, and fructose as a function of dietary changes such as the consumption of a diet in which sucrose, glucose, and fructose intake was restricted.

Materials and Methods
Subjects. Nine healthy volunteers of both sexes (2 males and 7 females, age 27–50 years) were recruited for the study among research workers and academic staff of the Department of Pharmacology, University of Florence. Because no damage or negative health effects were anticipated, we did not request approval for the study from the Ethical Committee on Human Experimentation of the General Hospital of Florence. None of these subjects had a clinical history of diabetes, and all had a fasting blood glucose concentration of <1.0 g/l. All subjects were nonhospitalized and followed a normal diet (defined in what follows as a “basal diet”) in which nutrient intakes were similar to those recommended by the Italian National Institute for Nutrition (23).

Study Design. At the beginning of the study, each subject was requested to write down what he/she ate daily for 1 week. The 7th day urine samples were collected at 8:00 and 10:00 a.m. and at 3:00 and 10:00 p.m. for measurement of the basal urinary excretion of carbohydrates. The 8:00 a.m. sample was the first voided urine before breakfast. The 10:00 a.m. sample was...
collected to monitor the intake of carbohydrates with breakfast, whereas the 3:00 p.m. sample was collected to measure the effect of dinner. The 10:00 p.m. sample was taken to measure the effect of lunch (usually eaten between 8:00 and 9:00 p.m.). We collected, at the hours specified above, only a sample of ~150 ml, instructing the subjects to discard any other voided urine. The subjects were allowed to urinate between the indicated time points, but these samples were discarded.

After a week in which the basal diet was followed, the study was repeated; however, this time the subjects were instructed to follow for 3 days a diet (defined in what follows as "low sucrose" diet) in which sucrose, glucose, and fructose were reduced, eliminating foods such as candies, cakes, chocolate, soft drinks, and fruit juices. To facilitate compliance, we provided a list of foods to be avoided in this period because of high sucrose, fructose, or glucose content. Accordingly, we restricted fruit to only one apple for each main meal (lunch and dinner) because apples are easy to find and have a relatively low content of these carbohydrates. Sucrose in coffee, tea, or other beverages was substituted with saccharin. As specified for the basal diet, each subject was requested to write down what he/she ate in a daily diary during the low sucrose diet. Food portions were weighed by the participants. Urinary samples were collected at the end of the third day of the dietary intervention period as described before for the basal diet. The nutrient content of food recorded in the diaries was estimated from standard nutritional tables.

**Biochemical Analyses.** The concentrations of sucrose, glucose, and fructose in the urine during the basal and low sucrose diets were determined in the four urinary samples collected at different times as described above. We selected this procedure, and not the 24-h urine collection method, for a series of practical reasons. In fact, carbohydrates are not stable in alkaline urine without refrigeration, and we anticipated some difficulty in convincing nonhospitalized participants (in a future intervention study) to store large quantities of urine in their home refrigerators and to collect all the urine voided during the day.

To make the excretion study simpler, we also decided to express the excretion of carbohydrates not as concentration in urine (μg/ml) but as μg/mg creatinine. This procedure allowed us to collect only a small volume of urine at given times, considerably simplifying collection and storage. The concentration of urinary creatinine would correct for fluctuations of urinary volume. In fact, a high or low urinary creatinine concentration indicates a low or high volume of excreted urine, respectively. By expressing carbohydrate excretion as a function of urinary creatinine, we could avoid the collection of the 24-h voided samples. This approach is often used when studying the urinary excretion of drugs in humans.

To validate our simpler approach of testing urinary carbohydrates at four different times, we determined in nine subjects the correlation between the average excretion of glucose and fructose at the four different times specified earlier and the total 24-h urinary excretion.

The average urinary fructose excretion, expressed as μg/mg creatinine, was correlated \( r = 0.90; P < 0.001 \) with the excretion of fructose determined in a 24-h urine sample [Fig. 1; slope of the regression line, 0.55 ± 0.10 (SE)]. A linear correlation \( r = 0.77; P < 0.05 \) was also found between the glucose values calculated with the two methods (slope, 0.42 ± 0.13).

In the course of this study, the urinary samples (~150 ml) were collected in a 200-ml sterilized container containing a dry KOH crystal of ~0.5 g to increase pH and avoid sucrose hydrolysis. The urine samples were kept at 4°C during the collection period. The next day, an aliquot of urine was tested for creatinine concentration. The remaining urine was stored at −20°C for carbohydrate analysis, which was carried out within 1 week. For each subject, the urinary samples relative to the basal and low sucrose diets were analyzed together. Sucrose, glucose, and fructose concentrations in the urine were determined with a kit (Boehringer Mannheim, Mannheim, Germany; Ref. 25). With this method, sucrose, glucose, and fructose were detectable in the urine at a concentration range of 1–150 μg/ml. Standard curves were obtained by adding known amounts of the different carbohydrates to urine samples and calculating the difference in absorbance from the baseline absorbance. Straight calibration lines \( r = 0.99 \) in the described range were obtained. Creatinine concentration in the urine was measured spectrophotometrically with a kit from Boehringer Mannheim (Diagnostica Mannheim, Ref. 26).

**Statistical Analysis.** Data were analyzed with multifactor ANOVA and with multiple regression analysis using Statgraphic Statistical Package (Statistical Graphic Corp., Rockville, MD).

**Results**

The urinary excretion of sucrose, glucose, and fructose in the basal diet is shown in Fig. 2. Around 10:00 a.m., ~2 hours after breakfast, the urinary excretion of sucrose and fructose increased from fasting values and then remained constant throughout the day. The variation between fasting and 10:00 a.m. fructose urinary excretion was statistically significant \( P < 0.01 \) when analyzed with multifactor ANOVA. We did not find any oscillation in the urinary excretion of glucose, a phenomenon to be expected given that glucose serum concentrations are tightly controlled by insulin and none of these subjects was diabetic.
In Table 1, we report sucrose, glucose, and fructose intake in the three main meals (breakfast, lunch, and dinner) on the urine collection days after the basal diet described in Fig. 2 and after the low-sucrose diet. Table 2 shows the mean daily intake of calories and nutrients in the basal and low sucrose diets. Most nutrients were similar in the two diets, with the exception of sucrose and glucose. The decrease of sucrose and glucose in the low sucrose diet was associated with a decrease in caloric content compared with the basal diet \((P < 0.01)\).

The average urinary excretion of sucrose, glucose, and fructose for each subject, during the basal and low sucrose diets, are shown in Fig. 3. After the low sucrose diet, we observed a drop in the average urinary excretion of sucrose.
Urinary Carbohydrates and Diet

Between the slopes of these two regression lines \( (P_{\text{sucrose}}) \) diets. Because no significant difference was found in the correlation times, in the basal and the low sucrose diets, was significantly correlated \( (P > 0.05) \), the final regression was calculated with a common slope \( b = 0.26 \pm 0.08 \text{ (SE)} \).

A similar approach was used for analyzing the correlation between sucrose intake and fructose excretion \( (P < 0.05) \), and the results indicated that this correlation was also significant \( (r = 0.82; P < 0.05) \). Also, in this case, the two slopes of the regression lines in the basal and the low sucrose diets did not differ \( (P > 0.05) \), and one common slope was utilized \( b = 0.15 \pm 0.05 \text{ (SE)} \).

On the contrary, using the same methods, we did not find any correlation between glucose intake and the average urinary excretion of glucose \( (r = 0.35; P > 0.05) \). Similarly, no correlation was found between urinary glucose and dietary sucrose intake \( (r = 0.38; P > 0.05) \).

We also tested whether the carbohydrate intake of the day of the urinary test after the basal and the low sucrose diets was correlated in each subject with intake of carbohydrates consumed during previous periods. In fact, there was a positive correlation between carbohydrate consumption the day of the test and the previous 7-day basal diet intake \( (r = 0.88, P < 0.01) \) for sucrose; \( r = 0.92, P < 0.001 \) for glucose; and \( r = 0.94, P < 0.001 \) for fructose). Similarly, during the low sucrose diet, we found a correlation between carbohydrate intake on the day of the urinary test and intake during the 3 days in which a low sucrose diet was consumed \( (r = 0.97, P < 0.0001) \) for sucrose; \( r = 0.86, P < 0.01 \) for glucose; and \( r = 0.94, P < 0.0001 \) for fructose).

Discussion

One of the dietary changes of industrialized nations in this century has been a dramatic increase in sucrose intake. For example, in the last 50 years in Italy, sucrose consumption has increased about 7-fold \( (27) \).

A shift to a diet containing high levels of sucrose is also associated with increased energy intake \( (28) \). Caloric intake and obesity have been, in turn, correlated with the incidence of colon and breast cancers \( (9, 29, 30) \). To define the correlation between levels of consumption of dietary sucrose and diseases is desirable to identify reliable markers of dietary intake. In our study, we measured the urinary excretion of sucrose, glucose, and fructose in healthy subjects consuming a common diet (referred as basal diet) and after 3 days of a low sucrose diet in which sucrose, glucose, and fructose were restricted.

The composition of the basal diet described in this paper reflects a common Italian diet. Fat represents 30.8% of total calories; protein, 15.5%; sucrose, glucose, and fructose, 10.8%; and starch, 34.1%. The remaining 8.8% of calories were accounted for by other dietary components such as alcohol and minor nutrients not included in the calculations. Similar percentages of nutrients were also measured in the low sucrose diet.

Our data show that breakfast induced a significant increase in the excretion of fructose in the urine with a lag time of about 2 hours. On the contrary, the urinary excretion of glucose was stable; a strong control of insulin over glucose concentration was demonstrated. Lunch and dinner did not seem to have much effect on urinary excretion of sucrose, glucose, and fructose.

These observations are not surprising, given that a typical Italian breakfast is quite high in sucrose (as shown in Table 1), usually consisting of sweet pastries and coffee with milk, to which sugar (sucrose) is added (in our basal diet, we calculated an average consumption of 22 g of sucrose at breakfast, accounting for almost one-half the daily sucrose consumption). Pasta and bread are the main carbohydrates eaten in the other meals and apparently do not alter fructose excretion.

The urinary excretion of sucrose, fructose, and glucose is

\( (P < 0.05; \text{Fig. 3a}) \), of glucose \( (P < 0.001; \text{Fig. 3b}) \) and of fructose \( (P < 0.001; \text{Fig. 3c}) \).

The average urinary excretion of sucrose in the four sampling times, in the basal and low sucrose diets, was significantly correlated \( (P < 0.01, r = 0.7) \) with the dietary sucrose intake of the collection day (Fig. 4). To perform this analysis, we used a model of multiple regression calculating two separate regression lines for the excretion data during the basal and the low sucrose diets. Because no significant difference was found between the slopes of these two regression lines \( (P > 0.05) \), the final regression was calculated with a common slope \( b = 0.26 \pm 0.08 \text{ (SE)} \).

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We also tested whether the carbohydrate intake of the day of the urinary test after the basal and the low sucrose diets was correlated in each subject with intake of carbohydrates consumed during previous periods. In fact, there was a positive correlation between carbohydrate consumption the day of the test and the previous 7-day basal diet intake \( (r = 0.88, P < 0.01) \) for sucrose; \( r = 0.92, P < 0.001 \) for glucose; and \( r = 0.94, P < 0.001 \) for fructose). Similarly, during the low sucrose diet, we found a correlation between carbohydrate intake on the day of the urinary test and intake during the 3 days in which a low sucrose diet was consumed \( (r = 0.97, P < 0.0001) \) for sucrose; \( r = 0.86, P < 0.01 \) for glucose; and \( r = 0.94, P < 0.0001 \) for fructose).
very low, and the mechanism by which these carbohydrates end up in the urine is not clear. Glucose has an effective uptake mechanism in the renal tubule that permits almost complete reabsorption. The renal transport mechanisms of sucrose and fructose are not well understood. It is likely that a fraction of these two sugars can escape renal reabsorption mechanisms. Most published data on the daily variations in the urinary concentration of glucose have been obtained in diabetic patients (31) and in subjects with renal diseases (32). Few studies have been published on sucrose or fructose excretion in the urine of control subjects, and no correlation has previously been attempted between dietary intake and urinary excretion of glucose or fructose (33, 34). A recent report (35) has shown that sucrose excretion in the urine is markedly increased after 100 g sucrose is administered to patients suffering from gastric ulcers and severe gastritis, as a result of the disruption of the absorption barrier at the level of the gastric mucosa (35).

We studied normal subjects in order to avoid possible biases due to pathological disturbances of sugar absorption and metabolism. The mean daily excretion of sucrose, glucose, and fructose after a low sucrose diet was significantly decreased compared with the basal diet. We also found that the intake of sucrose was significantly correlated with the urinary excretion of sucrose and fructose. On the contrary, we found no correlation between dietary intake of sucrose and urinary excretion of glucose. The present study has clear limitations given the small number of subjects analyzed and the absence of a control group for controlling variations of the parameters under study as a function of time. However, a time effect is unlikely because food consumption was stable in these subjects. In fact, we found a good correlation between the average consumption of sucrose, glucose, and fructose and the consumption of these carbohydrates on the day of the test.

Urinary fructose is derived from dietary fructose or sucrose, and urinary sucrose can only be accounted for by the fraction of dietary sucrose that enters the general circulation, escaping enzymatic hydrolysis by sucrose. On the basis of these premises, urinary fructose and sucrose could be used as indicators of dietary sucrose intake in normal subjects.

The methods described are simple and can assist in the validation of sucrose consumption measured indirectly using dietary interviews and questionnaires. We think, therefore, that this approach may be of use in studies aimed at investigating the correlation between dietary variation and the occurrence of cancer.

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References
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