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¹³C-Breath Tests for Sucrose Digestion in Congenital Sucrase Isomaltase Deficient and Sacrosidase Supplemented Patients

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Abstract

Congenital sucrase-isomaltase deficiency (CSID) is characterized by absence or deficiency of the mucosal sucrase-isomaltase enzyme. Specific diagnosis requires upper gastrointestinal biopsy with evidence of low to absent sucrase enzyme activity and normal histology. The hydrogen breath test (BT) is useful but is not specific for confirmation of CSID. We investigated a more specific ¹³C-sucrose labeled BT.

Objectives—were to determine if CSID can be detected with the ¹³C-sucrose BT without duodenal biopsy sucrase assay and if the ¹³C-sucrose BT can document restoration of sucrose digestion by CSID patients after oral supplementation with sacrosidase (Sucraid®).

Methods—Ten CSID patients were diagnosed by low biopsy sucrase activity. Ten controls were children who underwent endoscopy and biopsy because of dyspepsia or chronic diarrhea with

normal mucosal enzymes activity and histology. Uniformly-labeled ^{13}C -glucose and ^{13}C -sucrose loads were orally administered. $^{13}\text{CO}_2$ breath enrichments were assayed using an infrared spectrophotometer. In CSID patients the ^{13}C -sucrose load was repeated adding Sucraid®. Sucrose digestion and oxidation were calculated as a mean % coefficient of glucose oxidation (% CGO) averaged between 30 and 90 minutes.

Results—Classification of patients by ^{13}C -sucrose BT % CGO agreed with biopsy sucrase activity. The breath test also documented the return to normal of sucrose digestion and oxidation after supplementation of CSID patients with Sucraid®.

Conclusion— ^{13}C -sucrose BT is an accurate and specific non-invasive confirmatory test for CSID and for enzyme replacement management.

Keywords

^{13}C -breath test; glucose oxidation; congenital sucrase-isomaltase deficiency; sucrose digestion; sacrosidase supplementation

INTRODUCTION

Sucrose, also known as table sugar, is a disaccharide formed by glucose and fructose monosaccharide units. Sucrose is present in the human diet in fruits and is added to many prepared foods as refined beet or cane table sugar. Sucrase is the only brush border enzyme that digests sucrose. The membrane bound complex sucrase-isomaltase (SI) hydrolyzes disaccharide sucrose to free monosaccharides that are transported from the lumen by SGLT-1, GLUT-2, and GLUT-5 (2). A percentage of the absorbed glucose and fructose is quickly oxidized and exhaled as CO_2 and the remainder is metabolized or stored. SI has two maltase activities, which together with the two maltase activities of the maltase-glucoamylase (MGAM) complex, digest starch to free glucose. These four activities are better described as α -glucosidases. Approximately 60 to 80% of all mucosal α -glucosidase activity is accounted for by SI and the remainder of activity is due to MGAM (1). SI also has isomaltase and palatinase activities associated with the membrane bound isomaltase (I) portion of the enzyme complex.

Congenital sucrase-isomaltase deficiency (CSID) is an autosomal recessive intestinal disease caused by mutations of the SI gene (3–6). Duodenal mucosal histology is always normal. CSID patients have different phenotypes of enzymatic activities associated to SI, ranging from reductions of sucrase activity to total absence, as well as variable absence of isomaltase activity (7–10). Low sucrase activity leads to malabsorption of sucrose, resulting in dyspeptic-like symptoms such as diet-related chronic osmotic diarrhea and abdominal pain. Only rarely does CSID lead to failure to thrive (12). The severity of symptoms is related to the amount of sucrase activity and quantity of sucrose fed (11,12). A reduced maltase activity is expected to occur in patients with CSID because both subunits in the SI complex contribute to the total mucosal maltase activity (1). The low maltase activity can lead to malabsorption of starch products which may contribute to symptoms of dyspepsia and chronic abdominal pain (13). The prevalence of biopsy-assay proven CSID is 0.02% in individuals of European descent but is reported as high as 10% in indigenous Greenlanders (14). Frequency of heterozygous individuals carrying the CSID gene who have low but not deficient sucrase activity and normal small intestinal histology is reported to be from 2 to 9% in European Americans (7, 12). We found a frequency of isolated sucrase deficiency of 1% in our recent study of unselected clinically indicated duodenal biopsy enzyme assays (1)

Specific diagnosis of CSID presently requires duodenal biopsies with low to absent sucrase activity detected by enzyme assay and presence of normal histology to rule out secondary

deficiency. (12, 13, 15). Multiple genotypes make it impossible to establish a single molecular test suitable for the diagnosis of all CSID (7). The technique for diagnosis of SI deficiency by intestinal biopsy and assay of mucosal hydrolysis of sucrose was first described forty years ago by Charlotte M. Anderson et. al. (16). Presently the principles for diagnosis of SI deficiencies remain the same but the development of less invasive and less complex techniques is needed. The simplest treatment for CSID is dietary sucrose and occasionally starch restriction. Enzyme supplementation with liquid yeast sacrosidase (sucrase) enzyme derived from *Saccharomyces cerevisiae* relieves clinical symptoms and sucrose malabsorption in CSID patients. (17, 18, 19).

A hydrogen breath test (H_2 BT) for detecting carbohydrate malabsorption was introduced in the early 1970's creating the first clinical application for assessment of lactose malabsorption. The noninvasive nature of H_2 BT makes it particularly useful for application in pediatric clinical practice as an indirect test of carbohydrate malabsorption but it is not specific for the diagnosis of CSID (20). False-negative results may be obtained because of many factors affecting the H_2 production. The test requires absence of small bowel bacterial overgrowth and presence of colonic bacterial flora capable of fermenting proximally malabsorbed carbohydrate. There is great variability of fermentation by the colonic flora and no quantification of proximal carbohydrate malabsorption is possible. Failure to detect H_2 occurs in 2 to 40% of subjects. (21) A clinical problem arising from the H_2 BT is the large load of sucrose given to the patient. In CSID patients this load often precipitates severe symptoms of sucrose intolerance.

An evolution of the H_2 BT introduced in the early 1970's was the measurement of isotope-labeled CO_2 in breath using ^{13}C or ^{14}C (22). These tests depend on measurement of changes in isotope labeled breath CO_2 concentration; delta over baseline (ΔOB), detected by mass spectrometry or nuclear magnetic resonance (NMR) (23, 24). Isotope ratio ($^{13}C/^{12}C$) enrichment measured by mass spectrometry is the traditional method for BT and has high accuracy for low levels of enrichment (0.001 to 0.01 percent) (25–27). Most recently infrared mass-dispersion spectrophotometry has been introduced for breath $^{13}C/^{12}C$ isotope measurements and is clinically useful due to its simplicity and short turnaround time (28–30). Since the introduction of mass spectrometers for the detection of the stable isotope of ^{13}C in expired air the BT technique has been adapted for the study of malabsorption in the pediatric population with collection systems that are well-tolerated by infants and toddlers who can not actively cooperate (32, 33). The instruments required for measurement of ^{13}C -labeled CO_2 ($^{13}CO_2$) are less expensive now and naturally enriched and purified stable isotope labeled substrates are currently available (34, 35). The substrates most commonly used for $^{13}C/^{12}C$ BT include ^{13}C -labeled carbohydrates, starch, fatty acids, bile acids, amino acids and urea. Clinical applications include evaluation of the mucosal function, bacterial overgrowth, gastrointestinal motility, carbohydrate absorption, bile acid absorption, lipid absorption and lipase pancreatic activity, hepatic function, and protein absorption. (31). However the only test widely used in clinical practice is the ^{13}C urea BT for the diagnosis of *Helicobacter pylori* infection.

Since presently there are no practical and non-invasive methods for specific confirmation of SI deficiency conditions, we developed and validated a sucrose breath test for screening and confirmation of CSID using a novel non-invasive ^{13}C -sucrose labeled substrate. Our hypotheses were that primary sucrase deficiency can be confirmed using ^{13}C -sucrose breath test and that the effectiveness of sucrase replacement therapy can be evaluated by the same non-invasive method. The objectives of our investigation were to determine whether CSID can be detected with the ^{13}C -sucrose BT without duodenal biopsy sucrase assay and whether the ^{13}C -sucrose BT can document restoration of sucrose digestion in CSID patients after oral supplementation with yeast sucrase (Sucraid®).

METHODS

Clinical

After obtaining Institutional Review Board (IRB) approved informed consents under protocol H-10239, a total of 20 patients participated in this study. Ten CSID patients were diagnosed by intestinal enzyme activity determinations (5F: 5M, ages 1–15y) (Table 1). The CSID patients were recruited in three different ways: referral by Pediatric Gastroenterologists, direct self-referral by CSID families who called our study coordinator after reading an information letter about the study inserted in the Sucraid® package by QOL Medical Company; and families referred through the CSID website www.csidinfo.com. A control group of subjects was recruited from the Nutrition and Gastroenterology Service at Texas Children's Hospital (TCH). Ten controls (6F: 4M, ages 1–15 yrs) were patients who underwent endoscopy and biopsy because symptoms of dyspepsia or chronic diarrhea but with normal levels of mucosal enzymes measured according to the Dahlqvist method (36) and normal histology. The control group patients were participants of the IRB approved protocol H-1320 for recruiting children of both genders, 0–17 yrs with dyspepsia (ROME II criteria) and chronic diarrhea, pain or discomfort centered in the upper abdomen (37).

All CSID patients were biopsied and diagnosed by their primary GI physician before coming to the General Clinical Research Center (GCRC) at TCH for the BT study. In the control group the endoscopy procedures were performed for clinical indications by Pediatric Gastroenterologists at TCH. These biopsies were evaluated by the Pathology Department of TCH. Exclusion criteria for all subjects included villous atrophy on routine histology, fever, inability to cooperate with breath collections, failure to ingest the test ^{13}C -solution, diabetes, and chronic lung disease.

Biopsy enzyme assay and histology

The disaccharidase enzyme activity determinations for the control group and some of the CSID patients were done at the GI lab of Buffalo Women and Children's Hospital in N.Y (1). The remainder of the CSID patient's biopsies were assayed in other reference labs with the histology interpreted locally.

Breath tests

The $^{13}\text{CO}_2$ breath tests were done on 2 separate days for the control group and on 3 separate days for the CSID group at the GCRC at the TCH under protocol G-695. After overnight fasting, a 2.5 L reference breath sample was collected for comparison with the timed breath samples. Then 20 mg uniformly-labeled ^{13}C -glucose, (Isotec, Miamisberg, OH) was given using 10 gm unlabeled maltodextrins as carrier dissolved in water to a total volume of 100ml (Polycose® from Ross Division of Abbot Laboratories). Starting 15 minutes after the ^{13}C -glucose load 0.25 L breath samples were collected every 15 minutes for 120 minutes. After finishing the BT the subject was fed and released from the GCRC. The second day the procedure was the same but ^{13}C -sucrose was used. On the third day CSID patients had a repeat ^{13}C -sucrose load with addition of 22 drops of Sucraid® (8,500 IU of sacrosidase, provided by QOL Medical, Mooresville, NC) to the load solution.

Breath $^{13}\text{CO}_2$ enrichment analysis

After ^{13}C -labeled substrate loads were administered, breath collections and measurement of $^{13}\text{CO}_2$ enrichments were performed every 15 min $\times$ 9 using a $^{13}\text{CO}_2$ infrared spectrophotometer (POCone®, Otsuka Electronics, Tokyo, Japan). At each time point the total CO_2 concentration exceeded 2% in the breath sample and was thus in the $^{13}\text{CO}_2$ analytical range of the instrument. The BT results were recorded as total breath CO_2 concentration expressed as glucose- $\Delta\text{OB } ^{13}\text{CO}_2$ or sucrose- $\Delta\text{OB } ^{13}\text{CO}_2$.

Calculations

Because of the age related variations of glucose oxidation to CO₂ described below, glucose-ΔOB¹³CO₂ was used as denominator to overcome the effect of age on sucrose-ΔOB¹³CO₂. ¹³C-sucrose digestion and oxidation was expressed as a % coefficient of glucose oxidation (% CGO) as calculated from ΔOB¹³CO₂ breath enrichments as follows:

$$\% \text{ CGO} = [\text{sucrose-}\Delta\text{OB}^{13}\text{CO}_2 / \text{glucose-}\Delta\text{OB}^{13}\text{CO}_2] \times 100$$

Since % CGO values were found relatively constant in the period of 30 to 90 minutes after the load these values were averaged for each individual. The individual subject mean % COG values were used to identify the lower reference limit of ¹³C-sucrose BT for controls and used to compare ¹³C-sucrose BT of CSID with duodenal sucrase activities (see below).

Statistical procedures

Agreement between duodenal sucrase activity and ¹³C-sucrose BT mean % CGO was tested with receiver operation analysis (ROC) using the statistics software SPSS. Additional subjects were recruited from the families of CSID patients for replicate ¹³C-glucose and ¹³C-sucrose BT to evaluate the within subject variations (Table 2) and to test the effect of age on glucose-ΔOB¹³CO₂ (Figure 1). General linear modeling techniques were used to assess possible effects of group age distribution differences on CGO% values and the ability of the breath test to discriminate between normal and CSID subjects. Two tail t-tests were used to compare groups; p values < 0.05 were interpreted as significant.

RESULTS

Clinical Description of CSID patients

Patients from the CSID group were referred by Pediatric Gastroenterologists. Their duodenal biopsy enzyme assays are shown in Table 1. Clinical histories varied but all CSID patients had duodenal biopsy sucrase activities below 6.5; all had maltase activities below 115; and 9 of 10 had palatinase activities below 5 U/g protein. None had villous atrophy.

Clinical Description of control subjects

Ten controls were children biopsied for clinical indications by the Pediatric Gastroenterology service at TCH because of the complaint of dyspepsia. All controls had levels of duodenal biopsy disaccharidase enzyme activities well above the reference levels (Table 1). None had mucosal histologic abnormalities.

Glucose oxidation with age

% CGO was used to normalize the sucrose-ΔOB¹³CO₂. The effect of age in months on glucose-ΔOB¹³CO₂ is shown in Figure 1. This analysis included 44 subjects by additional studies in CSID family members. 83% of the total variation of glucose-ΔOB¹³CO₂ was accounted for by the subject's age. (Figure 1, R² 83%).

Replicate ¹³C-glucose and ¹³C-sucrose BT

On replicate BT testing of the same subject, separated by 1–12 months, a mean % coefficient of variation (% CV) of 14% for the ¹³C-glucose BT and 9% for ¹³C-sucrose BT were observed (Table 2).

¹³C-sucrose oxidation in CSID and controls

In the control group an average of 146% ± 45.5 mean % CGO and for the CSID group an average of 25 ± 21 mean % CGO were observed (p<0.001)(Figure 2). The lowest mean %

CGO obtained was 0.7% and the highest was 56.5% in the CSID patients (Table 1). Analysis controlling for differences in group age distribution found no relationship between % CGO and age or any effect of age on the above group averages. Therefore age did not effect the assessment of the BT ability to discriminate.

Clinical utility of ^{13}C -sucrose BT mean % CGO

ROC analysis of mucosal biopsy sucrase activity vs. ^{13}C -sucrose mean % CGO established a cut-off value for ^{13}C -sucrose BT mean % CGO of 79% which yielded 100% sensitivity and 100% specificity (95% confidence interval 74% to 100% for both) for detection of low duodenal sucrase activity by ^{13}C -sucrose BT mean % CGO (Figure 2 and Figure 3).

Response of CSID patient's ^{13}C -sucrose BT to Sucraid® supplement

All CSID patients showed correction of sucrase deficiency with oral Sucraid® supplementation, responding to levels greater than their baseline ^{13}C -sucrose BT mean % CGO ($p = 0.001$) (Figure 3).

DISCUSSION

Duodenal Enzyme Activities

In this $^{13}\text{CO}_2$ BT study we included 10 CSID patients with biopsy proven sucrase deficiency and normal histology (Table 1). The $^{13}\text{CO}_2$ BT 9–14% coefficient of variation (CV%) of replicate BTs compares favorably with the 27 CV% of sucrase activity assayed reported in replicate duodenal biopsies (1). All CSID duodenal sucrase enzyme levels fell below the 10th % reference value (27 U /gp) in a range from 0 to 6.5 U/gp, and palatinase (isomaltase) levels were from 0 to 4.9 U/gp. Patient 7 had normal isomaltase activity (6.7 U/gp) (1). All CSID patients had low maltase activities. Patient 1 and patient 8, the only two with glucoamylase enzyme determinations, were below the 10% reference value. For terminal starch digestion mucosal enzymes in the brush border are armed with 4 complimentary maltase activities, two from the SI complex and 2 from MGAM. SI accounts for 60–80% of the assayed maltase hydrolytic activity and the remainder is due to MGAM (1). From this we deduce that the CSID patients with mild reductions of maltase activities are retaining some hydrolytic activity from MGAM. In patient 7, where isomaltase was conserved, this also contributed to maintenance of maltase activity.

Glucose oxidation with age

Studies using combined gas chromatography-mass spectrometry (38) and neuroimaging techniques-positron emission tomography (PET) (39) have shown that fasting child endogenous glucose production and brain glucose oxidation are two-to-four fold greater than in the adult. In our study we confirmed that glucose oxidation was two to four times higher in children than adults (Figure 1). This may be due to the unique glucose needs for child brain development as reflected by our ^{13}C -glucose BT results in children. Central nervous system glucose consumption represents 60–80% of daily hepatic glucose output in the child, as it does in the adult (40), suggesting the importance of a good carbohydrate digestion and absorption in early child neurodevelopment. Because of the age dependence of glucose oxidation, % CGO is a necessary normalization for the digestion, absorption and oxidation of sucrose in children.

Gastric emptying

Using the ^{13}C -glucose BT we addressed the uniformity of liquid phase of gastric emptying for our study. We used 10% maltodextrin (Polycose ®) instead of water because maltodextrin made from corn is poorly isotopically enriched (0.2%) and provides a standard

osmotic and energy matrix for the uniformly enriched ^{13}C -labeled tracer substrate. The same dose of maltodextrins was used for each loading test to increase the uniformity of gastric emptying and the small amount of ^{13}C in the maltodextrin was thus blanked out in % CGO. The maltodextrin serves to standardize caloric load to mimic a meal and provide a trigger for liquid gastric emptying (41)

Test of Hypothesis 1

One of our objectives was to compare the less invasive ^{13}C -sucrose BT with duodenal biopsy sucrose assays obtained by endoscopy. A very strong relationship was observed and ROC analysis indicated that a reference value of 79 % mean % CGO discriminated between CSID and control populations, as confirmed by duodenal sucrose activities, with 100% sensitivity and 100% specificity (95% confidence interval 74% to 100% for both). This supports our first hypothesis that CSID can be confirmed with the ^{13}C -sucrose BT, however secondary sucrose deficiency cannot be excluded without clinical evaluation and biopsy.

Test of Hypothesis 2

We tested the ^{13}C -sucrose BT response to the enzyme supplement Sucraid® documenting a rise in mean % CGO for each CSID patient after the supplement to levels not different from controls ($P = 0.293$). The effectiveness of orally replacing sucrose was confirmed by the ^{13}C -sucrose BT. This response supports our second hypothesis that ^{13}C -sucrose BT quantitated the response of CSID patients to Sucraid® supplementation.

Non-invasive BT

One of the advantages of ^{13}C -sucrose BT which we and parents observed was that many CSID patients who had previous hydrogen BT experienced severe symptoms, passage of watery stools, bloating abdomen, and cramps from the 2 g/Kg sucrose load. We did not observe this symptomatic response in any CSID patient because the load of sucrose ingested was only 0.02 g for the ^{13}C -sucrose BT. As previously noted; the H_2 BT is not specific for sucrose malabsorption. With ^{13}C -sucrose BT we demonstrated a sensitivity and specificity of 100% (95% confidence interval 74% to 100% for both) in CSID patients and suggest that this diagnostic tool can be used as a non-invasive method for the confirmation and management of CSID.

SUMMARY

^{13}C -sucrose BT was evaluated as a non-invasive method for the confirmation of CSID. The results of sucrose digestion and oxidation were expressed as percentage of glucose oxidation (% CGO) and averaged between 30 and 90 minutes after the ^{13}C -substrate loads (mean % CGO). In controls and patients ^{13}C -sucrose BT mean % CGO agreed with duodenal sucrose enzyme activity determinations with 100% sensitivity and 100% specificity (95% confidence interval 74% to 100% for both). All CSID patients tested had ^{13}C -sucrose BT mean % CGO lower than 79%. Supplementation of CSID patients with sacrosidase enzyme corrected ^{13}C -sucrose BT mean % CGO to control levels.

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Figure 1. Effects of age on oral glucose breath test CO₂ enrichment

Effects of age in months on individual mean breath ¹³CO₂ ΔOB enrichments after a 20 mg ¹³C-glucose load to controls, CSID patients and their family members. Breath enrichments of $^{13}\text{CO}_2 \Delta\text{OB} = 1/(\text{Age} * 3.38 \times 10^{-3} + 2.24 \times 10^{-2})$; $R^2 = 0.83$, $n = 44$. Predicted mean ¹³CO₂ ΔOB is shown as heavy black line ± 95% CI thin lines.

Figure 2. Effects of CSID on oral sucrose breath test mean % CGO

Mean % CGO of individual subjects after a 20 mg ^{13}C -sucrose BT load and group means of all control and CSID subjects. The solid bar depicts the group average $\pm$ SD of controls. Individual values are shown as filled circles. The open bar depicts the average $\pm$ SD of the CSID patients. Individual values are shown as open circles. The dashed line is the 79 % mean CGO reference value for discriminating between control and CSID subjects (see text).

Figure 3. Effects of oral sacrosidase supplementation of CSID patients on sucrose breath test mean % CGO

Mean % CGO of individual CSID patients untreated (Left) and treated (Right) with 22 drops of oral sacrosidase supplement added to the sucrose load ($p = 0.001$, $n = 9$). The dashed line is the 79 % mean CGO reference value for discriminating between normal and untreated CSID subjects (see text).

Table 1

Duodenal biopsy disaccharidase activities (U g/protein)

Reference Values (1)	Age	6.5	26	89	5	32
Patient		Lactase	Sucrase	Maltase	Palatinase	Glucosaminylase
CSID 1	11m	91.5	0.3	28.1	2.3	5.4
CSID 2	15y	30.1	0	37.3	0	-
CSID 3	3y	43.1	0	39.4	0	-
CSID 4	2y	23.7	1.4	60.5	0	-
CSID 5	4y	126.4	3.6	93.9	1.8	-
CSID 6	4y	33.9	0.7	0	0.5	-
CSID 7	4y	23	0	0	6.7	-
CSID 8	23m	58.3	0	-	2	10.4
CSID 9	13m	53.3	6.5	50.9	4.9	-
CSID 10	11m	37.8	2.7	22.9	0	-
* Control	10 (2–15) yr	(23–126)	(35.5–96)	(115–268)	(5–16.5)	(95–110)

* Range of control group age and activities for each substrate

Table 2

Within individual ^{13}C -glucose and ^{13}C -sucrose BT mean $^{13}\text{CO}_2$ ΔOB replicate variations (% CV) after 20 mg ^{13}C -substrate oral loads.

	^{13}C-glucose BT % CV	^{13}C-sucrose BT % CV
Average $\pm$ SD	13.5 $\pm$ 11.4	9.4 $\pm$ 7.1
Range	0–30	0–20
n	7	8