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¹³C-Labeled-Starch Breath Test in Congenital Sucrase-Isomaltase Deficiency

Claudia C Robayo-Torres, MD^{1,5}, Marisela Diaz-Sotomayor, MD¹, Bruce R Hamaker, PhD², Susan S. Baker, MD³, Bruno P Chumpitazi, MD⁴, Antone R. Opekun, PA⁴, and Buford L. Nichols, MD¹

¹Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX 77030

²Department of Food Sciences and Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, IN 47907

³Digestive Diseases and Nutrition Center, Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY 142021

⁴Baylor College of Medicine, Department of Pediatrics, Section of Gastroenterology, Hepatology, and Nutrition and Texas Children's Hospital, Houston, TX; 77030

⁵Pediatric Specialist Network P.A., Cypress, TX 77429

Abstract

Background—Human starch digestion is a multi-enzyme process involving six different enzymes: salivary and pancreatic α -amylase; sucrase and isomaltase (from sucrose-isomaltase; SI), and maltase and glucoamylase (from maltase-glucoamylase (MGAM)). Together these enzymes cleave starch to smaller molecules ultimately resulting in the absorbable monosaccharide glucose. Approximately 80% of all mucosal maltase activity is accounted for by SI and the remainder by MGAM. Clinical studies suggest that starch may be poorly digested in those with congenital sucrase-isomaltase deficiency (CSID).

Hypotheses—Poor starch digestion occurs in individuals with CSID and can be documented using a noninvasive ¹³C-breath test (BT).

Methods—¹³C-labeled starch was used as a test BT substrate in children with CSID. Sucrase deficiency was previously documented in study subjects by both duodenal biopsy enzyme assays and ¹³C-sucrose BT. Breath ¹³CO₂ was quantitated at intervals before and after serial ¹³C-substrate loads (glucose followed 75 minutes later by starch). Variations in metabolism were normalized against ¹³C-glucose BT (Coefficient of glucose absorption, CGO). Control subjects consisted of healthy family members and a group of children with functional abdominal pain with biopsy proven sucrase sufficiency.

Name and address for correspondence: Claudia C. Robayo-Torres, MD, 21212 Northwest Frwy, Suite 235, Cypress, TX 77429, Fax: 832-334-4005, Phone: 832-334-4004, crobayotorres@gmail.com.

Author Contributions

CRobayo-Torres wrote the manuscript in collaboration with BLNichols with contributions from MDiaz-Sotomayor BRHamaker contributed analytical and intellectual content
SSBaker contributed human homogenate characterizations
AROpekun and BPChumpitazi analyzed the breath-test results

Results—Children with CSID had a significant reduction of ^{13}C -starch digestion mirroring that of their duodenal sucrase and maltase activity and ^{13}C -sucrase BT.

Conclusion—In children with CSID, starch digestion may be impaired. In children with CSID, starch digestion correlates well with measures of sucrase activity.

Keywords

food starch; digestion; glucose production; Congenital Sucrase-isomaltase Deficiency (CSID)

Introduction

Congenital-sucrase isomaltase deficiency (CSID) is an autosomal recessive intestinal disorder produced by mutations on the intestinal brush border enzyme complex sucrase-isomaltase (SI) (1-3). CSID patients frequently experience symptoms such as chronic osmotic diarrhea, abdominal pain, and bloating. CSID symptoms rarely lead to failure to thrive. The severity of the resultant symptoms is related to multiple factors, such as the amount of sucrase activity, the amount of sucrose ingested, the rate of gastric emptying, colonic microbial diversity and fermentative and absorptive capacity of the colon (4).

CSID occurs in approximately 0.2% of individuals of European descent, and in more than 5% of indigenous Greenlanders (5). The SI mutation, c.273_274delAG, has been found to be responsible for the high prevalence of congenital sucrase-isomaltase deficiency among Inuit people (6). To date, more than 40 mutations to the SI gene have been identified that are variably expressed (7). Two to nine percent of Americans of European descent are heterozygous carriers, with normal small intestinal histology and sucrase/maltase activity levels below the lower limits for the general population (6,7). The pattern of inheritance of CSID is either based on single mutations on both alleles (homozygous pattern) or caused by compound heterozygosity, thus suggesting that CSID may be more common than previously thought (2). Seven different CSID phenotypes have been described, in which SI enzyme activities range from reduction of sucrase activity to total absence, and to variable absence of isomaltase and normal small intestinal histology (2).

Reduced maltase activity occurs in all CSID patients, since both subunits in the SI complex contribute to total mucosal maltase activity (8), which includes downstream α -amylase-derived soluble dextrin hydrolysates. This is consistent with previous clinical reviews that report some CSID patients may have starch maldigestion (9). Confirmation of starch maldigestion at the mucosal level is difficult, because there is no specific substrate for enzyme assays, and SI and MGAM contribute to both maltase and glucoamylase assay activities.

Stable isotope-labeled breath tests (BTs) provide a noninvasive method for evaluation of starch digestion (10-14). Previous investigations have shown that the integration of the multi-step starch digestion to the glucose pathway can be mapped both anatomically and physiologically by ^{13}C -substrates and H_2 production (11-14). Because the chemical structure of the starches affect the digestibility of the ^{13}C -substrate (12), we have chosen to use a universally labeled algal starch as the standard tracer to assess α -amylase, SI and

MGAM activity and eliminate the cramping symptoms commonly associated with large starch loads in H₂ BTs. When compared to maize starch (supplementary Figure 1), amylase treatment of algal starch produces 83% less maltotriose, thus a reduced “braking” effect (1), and longer branch chains (20), both features which specifically enhance MGAM activity.

This study was undertaken to prove our hypothesis, that sucrase deficiency results in decreased glucogenesis from starches and points to an unaddressed pathophysiologic concern.

Methods

We used a ¹³C-breath test to determine starch digestion in a group of CSID patients; ¹³C-labeled starch was used as the breath-test substrate. This group of patients was previously studied with a ¹³C-sucrose breath test showing correlation with mucosal sucrase activities as previously reported (15,16). Adults and children have different rates of glucose oxidation; thus we used a separate ¹³C-glucose breath test to normalize individual test substrate oxidation, called Coefficient of Glucose Oxidation (CGO%), to permit intergroup and between group comparisons to be performed. ¹³C-starch breath tests were performed on separate days to avoid erroneous attribution of recovered isotopic tracer indicative of the target or the reference substrate. Of note, ¹³C-glucose, derived from hydrolyzed ¹³C-starch theoretically undergoes the same oxidative fate as ¹³C-glucose, presented in monomer form, to the intestinal mucosal. This approach effectively eliminates the need to adjust oxidative outcome data using estimated carbon dioxide production rates or onerous calorimetry procedures. The control subjects were patients with normal mucosal enzyme activities ($M \pm 1$ SD); their histology had been biopsied for clinical indications.

Clinical

The study was approved by the Baylor College of Medicine Institutional Review Board. Informed consent was obtained from the parents of study participants; a total of 17 patients were studied under protocols, H-1320 and H-10239. Eleven patients with CSID, diagnosed by duodenal enzyme activity, with normal histology, were included in the study (6 females, 5 males; ages from 11 months to 15 years).

Six control patients underwent endoscopy and biopsy at the Nutrition and Gastroenterology Service at Texas Children’s Hospital (TCH) for symptoms of dyspepsia, abdominal pain, or chronic diarrhea (1 female, 5 males; ages 8 mo-10 years). All had normal disaccharidase levels according to results using the Dahlqvist method (16); all had normal histology. Six CSID family members were studied by administering a breath test, but without biopsy assays.

Characterization of algal starch substrate

Normal maize starch and UL ¹³C-algal starch samples were dissolved in deionized water (10 mg/mL, w/v), and boiled for 20 min with stirring. Starch solutions were combined with 100 mM sodium acetate buffer (100 μ L, pH 5.0). The mixture was incubated with pullulanase (0.72 units, Megazyme, Wicklow, Ireland) at 40°C for 48 h, resulting in the hydrolysis of the

α -1,6 linkages. Debranched linear chain-length distributions of the enzyme-treated starches were measured and molecular weight was determined using multi-angle laser-light scattering (MALS, Dawn Heleos-II, Wyatt Tech. Corp., Santa Barbara, CA). The collected data were analyzed using a Berry plot for curve fitting with Astra software version V (Wyatt Tech. Corp., Santa Barbara, CA) (18).

¹³CO₂-breath tests

The ¹³CO₂ breath tests were administered over 2 days for the control groups and over 3 days for the CSID group at the General Clinical Research Center (GCRC) at TCH (the 3rd day was a test of response to oral Sucraid® enzyme replacement). After overnight fasting, a 2.5 L-reference breath sample was collected for comparison with the timed breath samples. Then 20 mg uniformly labeled ¹³C-glucose (Isotec (Div. of Sigma-Aldrich), Miamisburg, OH) was given using 10 g-unlabeled maltodextrins, as a carrier, dissolved in water to a total volume of 100 mL (Polycose; Ross Division of Abbot Laboratories, Columbus, OH). Beginning 15 min after the ¹³C-glucose load, breath samples (0.25 L) were collected every 15 min for 120 min. After finishing the BT, the subject was fed and released from the GCRC. On the second day, the procedure was the same, but ¹³C-sucrose was used. For the ¹³C-starch BT, a uniformly labeled ¹³C-algal starch (Isotec, Miamisburg, OH) was used. After each ¹³C-labeled substrate load was administered, breath collections and measurements of breath ¹³CO₂ enrichments were performed using a ¹³CO₂ infrared spectrophotometer (POCone, Otsuka Electronics, Tokyo, Japan). The BT results were recorded as total breath CO₂ concentration expressed as delta over baseline of reference PeeDee Belamite 13C-standard (OB) for glucose- OB ¹³CO₂, sucrose- OB ¹³CO₂, or starch- OB ¹³CO₂. The sucrose and starch ¹³CO₂ concentrations were expressed as a ratio to the ¹³C-glucose concentrations, to normalize for the metabolism of age modification: % coefficient of glucose oxidation (% CGO) (18). The breath test results between 30 and 90 min were averaged to produce a simplified equivalent of approximate “area under curve” calculations.

Results

The debranched uniformly labeled ¹³C-algal-starch, produced by a proprietary culture of edible cyanobacteria grown within a ¹³CO₂ environment, was compared with debranched normal maize starch (Supplemental Figure 1). Compared to maize starch, the ¹³C-algal-starch spectrum reveals a 6-fold reduction of maltotriose, signifying a reduced maltotriose braking effect on glucoamylase activity (1) and a shift to longer linear oligomers that favor glucoamylase activity (20).

The ¹³CO₂ test substrate/¹³CO glucose (% CGO) was calculated for breath enrichments to adjust for individual glucose metabolic differences in our study subjects. No clinical symptoms or adverse events were observed from any of the ¹³C-substrates administered during the study. The ¹³CO₂ % CGO values from 30 to 90 minutes were averaged (Table 1). Controls defined a ¹³C-starch benchmark lower level as mean $\pm$ 1 SD (91.1 $\pm$ 25.7= lower level 65% CGO). All patients with CSID had deficient ¹³C-labeled starch digestion (19.4 $\pm$ 10.6 % CGO). The CSID group had starch digestion and oxidation below the levels of

the control group ($p=0.001$). There was a strong correlation between ^{13}C -starch and ^{13}C -sucrose BT-values in the combined groups (Figure 1, $R^2 = 71\%$) and a correlation between the ^{13}C -starch BT-values and the measured duodenal sucrase activity (Figure 2, $R^2 = 73\%$). These results were confirmed in supplementary Figures 2 and 3, after the ^{13}C -starch BT and sucrase activity values were correlated with assayed duodenal biopsy maltase activity values. The CSID family-member group had reduced ^{13}C -Starch BTs but not as low as the CSID group: (n , $M\pm\text{SD}$); CSID (11, 19.4 ± 10.6); CSID family-members (6, 54.5 ± 6.0); Controls (7, 94.6 ± 26.2); ANOVA $p=0.001$.

Discussion

Given the severity of clinical symptoms which may occur in children with CSID, identifying the dietary substrates which may be malabsorbed is important. Using a novel breath testing protocol with ^{13}C -starch we found that children with CSID who have impaired sucrase activity (and previously documented sucrose malabsorption) also have starch malabsorption. This technique may pave the way toward further noninvasive testing of mucosal starch capacity in other clinical populations. We also identified in children with CSID that starch malabsorption correlated well with the measures of sucrose malabsorption. If our findings are further supported, children with CSID who have severely affected sucrase activity are likely to benefit not only from sucrose avoidance but starch avoidance as well.

CSID patients have very low to a total deficiency of duodenal assay sucrase, although some retain residual isomaltase activity at a variable level. The main importance is that in CSID, the symptoms are attributed to sucrose intolerance leading to aforementioned symptoms, but the importance of starch maldigestion has not been generally appreciated. The reason for this may be as simple the avoidance of discussion of what could not be easily measured or assessed. As such, the ^{13}C -glucose-paired ^{13}C -starch breath test addresses this diagnostic void. In addition, some patients present with fermentative diarrhea caused by low starch digestion; a condition that occurs to a lesser degree, however, than that caused by ingestion of sucrose. This observation was made first by Aurichio et al. in 1963 (9). We have demonstrated in our study of CSID patients that all had low ^{13}C -starch % CGO for starch digestion. Our test hypothesis that sucrase deficiency results in decreased glucogenesis from starches was confirmed. Although first reported in animal studies (20,21), our findings provide clinical confirmation of the leading role of small intestinal sucrase in normal human starch. Given that Sucraid® is a specific sacrosidase used for treating CSID that is without starch digesting activity, further research is suggested to test other oral glucosidase enzymes that can correct their reduced starch digestion. We envision a potentially broader role for a simplified noninvasive ^{13}C -Starch Breath Test as quantitative clinical measurements throughout recovery from secondary small intestinal mucosa diseases such as malnutrition, environmental enteropathy, celiac disease, Crohn's disease, ulcerative colitis, chemotherapy mucositis, or transplant rejection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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What is known

- Children with congenital sucrase isomaltase deficiency (CSID) have both sucrase and maltase deficiencies.
- Identifying children with CSID with starch malabsorption is currently challenging.
- Breath testing may provide a non-invasive method to determine the potential for starch malabsorption.

What is new

- In children with CSID, ^{13}C -labeled-starch breath testing provides valuable information re: starch absorption
- Sucrase deficiency in children with CSID correlates well with decreased glucogenesis from starches.

Figure 1.

^{13}C -labeled starch vs ^{13}C -labeled sucrose breath tests (BTs). The two BT substrates were tested on different days. The breath $^{13}\text{CO}_2$ enrichments between 30 and 90 minutes following substrate ingestion were averaged and expressed in ratio to ^{13}C -glucose (expressed as % CGO) tested on a third day. The linear equation fit is $^{13}\text{C}\text{-Starch} = 6.53 + 0.564 \text{ } ^{13}\text{C}\text{-Sucrose}$ ($R^2 = 71\%$). Note that the ^{13}C -starch BT response is driven by the ^{13}C -sucrose response suggesting that sucrose digestion accounts for about 70% of starch digestion.

Figure 2.

Relationship of starch digestion to duodenal biopsy sucrase activity. The starch breath test results were compared with biopsy sucrase activity. The linear equation is fit by ^{13}C -Starch (%CGO) = $18.89 + 1.51$ Sucrase specific activity. The significant correlation ($R^2 = 73\%$) confirms Figure 1 by suggesting that sucrase activity drives about 70% of starch digestion to glucose. ^{13}C -starch BT is described sucrase activity. Note that the y intercept is positive at almost 20 SA, suggesting that sucrase is not the only enzyme activity participating in starch glucogenesis.

TABLE 1

Descriptive statistics of patient group's mean mucosa enzyme activity (U/g protein) and ^{13}C -substrate mean breath % CGO.

Variable	Group	N	Mean	SD	Minimum	Maximum
Sucrase, U/g protein	Control	6	44.8	11.4	26.90	60.10
	CSID	11	2.1	2.8	0.0	7.80
Maltase, U/g protein	Control	6	139.6	21.8	117.4	174.9
	CSID	9	41.3	26.1	0.0	93.9
Palatinase, U/g protein	Control	6	24.9	27.2	5.7	77.9
	CSID	11	1.9	2.2	0.0	6.7
^{13}C -Starch, %CGO	Control	6	91.1	25.8	46.4	115.2
	CSID	11	19.4	10.6	6.8	46.4
	CSID Kin	6	59.60	17.2	47.3	93.4
^{13}C -Sucrose, %CGO	Control	6	137.7	24.6	101.2	175.3
	CSID	11	29.6	24.2	0.70	72.3
	CSID Kin	6	172.1	26.4	138.5	208.4

%CGO: % coefficient of glucose oxidation; CSID: congenital sucrase isomaltase deficiency; SD: standard deviation.