Breath hydrogen testing as a physiology laboratory exercise for medical students

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Montes, Ramon G., Richard F. Gottal, Theodore M. Bayless, Thomas R. Hendrix, and Jay A. Perman. Breath hydrogen testing (BHT) is a simple and reliable method for identifying impaired carbohydrate absorption. We describe a laboratory exercise in physiology for medical students using BHT as the teaching tool. The students collect fasting samples of expired air from each other using a simple nasal prong technique. They then drink one of several different aqueous carbohydrate solutions. Additional samples of expired air are collected by the students at 90 and 120 min after substrate ingestion and are analyzed by gas chromatography. Between sampling periods, discussions of digestive physiology are provided by the faculty. Students tabulate their BHT results as well as recording any symptoms using a standard scoring system. A total of 460 students have participated. We found that the percentage of students who malabsorbed a given substrate was similar each year. The results obtained in these student exercises closely parallel those reported in the literature. We conclude that BHT is an excellent teaching tool for illustrating carbohydrate digestion and absorption, even when performed by minimally trained subjects.

BREATH HYDROGEN TESTING is the simplest and most reliable means of identifying carbohydrate malabsorption. This technique has been firmly established both as a noninvasive method for investigation of carbohydrate digestion and absorption and in adult and pediatric gastroenterology practice (6). Because of the simplicity and reproducibility of breath hydrogen testing and its wide use in clinical medicine, it seemed ideal for a laboratory exercise illustrating physiological principles that can be applied to patient management. Since 1987 we have been conducting a laboratory exercise in gastrointestinal physiology for first-year medical students at the Johns Hopkins University School of Medicine using breath hydrogen testing as the teaching tool. Our experience has shown that the technique of sample collection is easily learned. The data obtained following the ingestion of several different carbohydrates are consistent with previously published findings, supporting the educational value of this method for illustrating the physiology and pathophysiology of dietary carbohydrate digestion and absorption in the intestinal tract.

Principles. The breath hydrogen test is based on production of H₂ by colonic bacteria during metabolism of unabsorbed carbohydrate to short-chain organic acids. H₂ and other fermentation products, such as methane and carbon dioxide, readily diffuse into the portal circulation and eventually are exhaled (5). H₂ in expired air represents a portion of the total gas produced in the intestinal lumen when sugar is not absorbed in the small intestine.

Breath hydrogen measurement offers several advantages for the detection of carbohydrate malabsorption in adults and children. The breath is sampled noninvasively, which permits frequent repetition of measurements. No separation steps are required prior to analysis of the sample, thus permitting immediate measurement. Additionally, the breath samples can be stored in simple collection systems and analyzed at central facilities at a later time.

The test is generally performed after an overnight fast by obtaining samples of expired air before and at 30-min intervals for 3 h following ingestion of aqueous carbohydrate solutions. A conventional dose of carbohydrate for investigation of malabsorption is 2 g/kg (maximum 50 g) given as a 20% solution. Lower doses are used when a nonabsorbable carbohydrate, such as lactulose, is used as the test substrate. The breath is collected by a simple nasal prong system (Fig. 1) into which the patient breathes normally and that does not require active cooperation (7). The examiner aspirates small volumes of air into a plastic syringe with each expiration until a sufficient volume for analysis is collected.

Analysis of H₂ in breath samples is accomplished by gas chromatography. Instruments designed for this purpose are commercially available and easy to operate. Many gastroenterology departments have this equipment. Carbon dioxide concentration can be also measured in the sample as an internal standard for quality assurance in the sample as an internal standard for quality assurance in the sample. Carbon dioxide concentration can be also measured in the sample as an internal standard for quality assurance in the sample. Carbon dioxide concentration can be also measured in the sample as an internal standard for quality assurance in the sample. Carbon dioxide concentration can be also measured in the sample as an internal standard for quality assurance in the sample.

A positive test is indicated by a rise in H₂ concentration of ≥10 parts per million (ppm) above the baseline, defined as the lowest value obtained at any sampling time, occurring within 120 min of ingestion of the test carbohydrate (2). A high fasting H₂ concentration or an early H₂ rise in the first 30 min after substrate ingestion may indicate small bowel bacterial overgrowth. Fasting H₂ concentrations are particularly useful for this purpose if the pretest dinner meal is standardized to red meat and rice as the only source of...
carbohydrate (8). False negative tests attributable to inability of the colonic bacterial flora to produce H2 from large amounts of malabsorbed carbohydrate occur infrequently and sometimes may be associated with prior antibiotic usage (12).

MATERIALS AND METHODS

The nasal prongs were constructed as shown in Fig. 2. Alternatively, kits suitable for the laboratory exercise are available (Med-Care Home Health Resources, Baltimore, MD).

Carbohydrate solutions were prepared with boiling tap water the day before the test, placed in standard 8 oz. glass medicine bottles, and kept in a refrigerator until use. Boiling is necessary to dissolve the lactose and shorten the time needed to dissolve fructose and dextrose. Lactose USP (Mallinckrodt 6270) was given as a 20% solution (50 g in 250 ml water or 25 g in 125 ml). Dextrose (β-glucose) USP (Fischer Scientific D-15) and fructose USP (Sweet Lite-Batter-Lite Whitlock) were given in a dose of 50 g as a 20% solution. Lactulose (Cephulac, Merrell Dow Pharmaceuticals) was given in a dose of 10 g in 75 ml water. Breath samples were analyzed for H2 using the Quintron 12i or CM2 Microlyzer (Quintron Instrument), and the results are expressed as parts per million.

The first-year class of medical students is divided into sections of 30–40 students for participation in laboratory exercises during their physiology course. They are given a short handout on the physiology of carbohydrate absorption and the principles of breath hydrogen testing. The students are advised to fast for 12 h prior to the exercise and to inform their instructor if they have a known intolerance to milk or milk products. The laboratory section is divided into four groups, each of which is provided with a different carbohydrate solution (lactose, fructose, lactulose, and “unknown”). The students are expected to identify the unknown substrate as glucose based on the results of the exercise. Other easily absorbed sugars, such as sucrose, can be used as an alternative unknown if so desired.

A brief (<15 min) demonstration of the breath sampling technique is conducted by the instructor, and written instructions (Table 1) are reviewed. The exercise then begins with the students pairing up and obtaining fasting breath samples from their laboratory partners. The syringes are labeled time 0 and with the student’s name. The students then drink the test substrate in its entirety within 5 min and mark down the time they complete the ingestion of the solution on a 3 × 5-in. index card or worksheet. Subsequent breath samples at 90 and 120 min are measured from this time. Each syringe is labeled accordingly as 90 or 120 and with the student’s name. Samples are brought to the gas chromatograph immediately on completion of each sample for analysis. Two chromatographs operated by two technicians are sufficient to handle this volume of specimens in the time allotted for this portion of the exercise (2.5 h).

After ingestion of the test substrates and between samples the students listen to four 30 min discussions on relevant phases of digestive physiology by the medical staff. Throughout the exercise the students also score any subjective symptoms that they may have experienced after the test substrate is ingested, utilizing a standard scoring system (Table 2).

Following the 120-min breath sample, the students are provided with a lunch meal. After lunch the students tabulate the breath hydrogen data on a blackboard and compare their results with those of students in other groups. This part of the exercise lasts ~0.5 h. The instructor then closes the exercise by leading a discussion of the physiological and clinical implications of the results that were obtained. The total duration of the exercise is ~4 h.

RESULTS

During a period of five years a total of 460 students have participated in this exercise. With the use of a rise of >10 ppm in H2 concentration as indicating carbohydrate malabsorption, the percentage of students testing positive for each of the test sugars is shown in Table 3. Figure 3 illustrates the proportion of positive tests in each class. The symptom scores were higher for students who malabsorbed lactose than for those who did not (mean scores

Table 1. Instructions for obtaining breath samples

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Open stopcock (white lever pointing toward ceiling)</td>
</tr>
<tr>
<td>2</td>
<td>Purge the syringe twice</td>
</tr>
<tr>
<td>3</td>
<td>Attach nasal prong to end of stopcock</td>
</tr>
<tr>
<td>4</td>
<td>Place nasal prong at tip of nose</td>
</tr>
<tr>
<td>5</td>
<td>Have subject breathe normally</td>
</tr>
<tr>
<td>6</td>
<td>As subject exhales and nasal prong fogs up, pull back on plunger 5 cc per expired breath and stop</td>
</tr>
<tr>
<td>7</td>
<td>Repeat step 6 until syringe is filled to 50 cc line</td>
</tr>
</tbody>
</table>
Table 2. Symptom scoring form

<table>
<thead>
<tr>
<th>Number of Loose Stools</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>No trouble 0</td>
<td>Slight or questionable 1</td>
</tr>
<tr>
<td>Mild 2</td>
<td>Moderate 3</td>
</tr>
<tr>
<td>Severe 4</td>
<td></td>
</tr>
</tbody>
</table>

- Cramps or abdominal pain
- Bloating or gas
- Borborygmi (rumbling noises in gut)
- Flatus (passage of gas)

Modified from Barr et al. (1).


<table>
<thead>
<tr>
<th>Test Carbohydrate</th>
<th>No. of Subjects</th>
<th>No. Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose (50 g)</td>
<td>113</td>
<td>82</td>
<td>72.6</td>
</tr>
<tr>
<td>Lactose (50 g)</td>
<td>117</td>
<td>63</td>
<td>53.8</td>
</tr>
<tr>
<td>Lactose (25 g)</td>
<td>41</td>
<td>19</td>
<td>46.3</td>
</tr>
<tr>
<td>Lactulose (10 g)</td>
<td>89</td>
<td>68</td>
<td>76.4</td>
</tr>
<tr>
<td>Glucose (50 g)</td>
<td>100</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Fig. 3. Comparison of breath hydrogen results from different years, showing percentage of positive tests indicating malabsorption of a particular substrate.

3.1 vs. 1.5, P < 0.05). The difference was even more marked for fructose (mean symptom scores 5.4 for malabsorbers vs. 1.0 for absorbers, P < 0.001). When the individual symptom scores were compared with the change in H2 concentration (defined as the difference between the baseline and the peak value) for those subjects who ingested fructose in whom symptoms were recorded (n = 24), there was a strong linear correlation (r = 0.62, P = 0.001). This correlation was not significant for lactose and lactulose.

D I S C U S S I O N

Most ingested carbohydrates are hydrolyzed to the monosaccharides glucose, galactose, and fructose prior to absorption and transport across the small intestinal mucosa. Starch is initially digested by the enzyme amylase secreted into the intestinal lumen by salivary and pancreatic glands, which hydrolyzes starch to shorter glucose polymers. Several distinct glycosidases are located in the microvillus membrane of the small intestinal absorptive cell. The most clinically relevant are the glucosamylase-maltase complex and the disaccharidases sucrase-isomaltase and lactase. The glucosamylase-maltase complex accomplishes the final hydrolysis of small glucose polymers derived from starches to free glucose. Branched glucose polymers resulting from the initial digestion of starch are completely hydrolyzed to glucose by the action of the dimer sucrase-isomaltase. The sucrase moiety of this dimer enzyme hydrolyzes sucrose to glucose and fructose. Lactase, which exists in dimeric form as lactase-phlorizin hydrolase, hydrolyzes lactose to its component monosaccharides glucose and galactose.

Primary lactase deficiency, also known as primary lactase nonpersistence or "adult onset" lactase deficiency, is the most important cause of carbohydrate malabsorption in normal individuals. This deficiency results from a genetically determined postweaning decline in intestinal lactase activity. The majority of the world's adult inhabitants are lactase deficient, but the incidence of this condition is low in certain Caucasian populations of northern and central European descent.

The monosaccharides resulting from activity of the brush-border enzymes are absorbed and transported across the enterocyte. Glucose and galactose share an energy-dependent transport mechanism linked to sodium, which increases the affinity of the carrier for glucose. Fructose is thought to be absorbed by two separate transport mechanisms. When given alone it diffuses across the small bowel surface more efficiently than passively transported sugars (facilitated diffusion). The addition of glucose, however, stimulates fructose uptake in a dose-dependent fashion, and this effect appears to be additive to the transport of a saturating level of free fructose. This latter mechanism is thought to be active (against a concentration gradient) and, unlike the glucose-galactose carrier, it is sodium independent.

Recent interest has arisen in the clinical consequences of physiological malabsorption of fructose in otherwise healthy subjects. Several investigators have shown that ~70% of adults fail to completely absorb 50 g fructose (9-11, 13). This is the equivalent to the amount of fructose present in two 12 oz. cans of soda. Similar findings have been reported for children (3). Malabsorption of fructose in these subjects is usually associated with symptoms. When consumed in equivalent amounts with glucose, however, such as in sucrose, absorption is complete in most subjects (3, 9, 10, 13).

Lactulose is a synthetic disaccharide (fructose and galactose) that cannot be absorbed by humans. When given in sufficient doses it is associated with breath hydrogen...
excretion in the majority of individuals. The physiological consequences of malabsorbing this sugar, such as acidification of the colonic contents and osmotic diarrhea, are useful in clinical settings, including treatment of hepatic encephalopathy and constipation.

In this laboratory exercise we found that the percentage of students testing positive for each of the test sugars was remarkably constant from class to class (Fig. 3) and in accordance with previously published findings. The data on fructose malabsorption in particular represent the largest group studied to date and confirm the previously reported results cited above. The reproducibility of these numbers confirms the accuracy of this technique even when performed by minimally trained individuals. By comparing the breath hydrogen results following ingestion of different carbohydrates, the students in each session were able to integrate the concepts of normal carbohydrate digestion and absorption that were being taught, and they could easily identify the “unknown” test substrate as glucose.

Another useful part of the exercise is the students’ opportunity to correlate their breath hydrogen production with symptoms. In fact, the dose of lactulose used in this exercise is one-half of the standard clinical dose, and the dose of lactose was recently reduced to 25 g to minimize the discomfort that some students might experience. The difference in symptom scores between students who absorbed or malabsorbed certain sugars, such as lactose and fructose, was usually evident during the sessions. The strong correlation between H2 production and symptoms for fructose is greater than for the other sugars, such as lactose and fructose, was usually evident during the sessions. The strong correlation between H2 production and symptoms for fructose is greater than for the other sugars, such as lactose and fructose, was usually evident during the sessions. The strong correlation between H2 production and symptoms for fructose is greater than for the other sugars, such as lactose and fructose, was usually evident during the sessions. The strong correlation between H2 production and symptoms for fructose is greater than for the other sugars, such as lactose and fructose, was usually evident during the sessions. The strong correlation between H2 production and symptoms for fructose is greater than for the other sugars, such as lactose and fructose, was usually evident during the sessions. The strong correlation between H2 production and symptoms for fructose is greater than for the other sugars, such as lactose and fructose, was usually evident during the sessions. The strong correlation between H2 production and symptoms for fructose is greater than for the other sugars, such as lactose and fructose, was usually evident during the sessions. The strong correlation between H2 production and symptoms for fructose is greater than for the other sugars, such as lactose and fructose, was usually evident during the sessions.

Student acceptance of this exercise has been high, and the experience has been enjoyable for both instructors and participants. We conclude that this exercise is feasible as part of a medical school physiology curriculum and that it is an excellent teaching tool for this subject matter.

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REFERENCES