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Overview of Breath Testing in Clinical Practice in North America

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Abstract: Human breath is an easily, noninvasively obtained substance. It offers insight into metabolism and is used to diagnose disaccharide malabsorption, infection, small bowel bacterial over growth, and transit times. Herein, we discuss the readily available clinical breath tests, how they function, how they are administered and interpreted and some pitfalls in their use.

Key Words: bacterial overgrowth, breath hydrogen, breath methane, breath testing, fructose, lactose, pediatrics, stable isotopes, sucrose

What Is Known

- Breath testing is used clinically to identify disaccharidase deficiency.
- Breath testing is used clinically to diagnose small bowel bacterial overgrowth.
- Breath testing is used clinically to diagnose *Helicobacter pylori* infections.

What Is New

- Stable isotope breath testing offers a window into metabolism.
- Stable isotope breath testing can assess for gastric emptying.
- Stable isotope breath testing can assess for liver mitochondrial function at is known.

Human breath is a readily accessible, noninvasive window to the internal milieu that can be used to diagnose malabsorption of specific carbohydrates, infection, and motility. Some suggest that breath testing (BT) may offer an individual "breath print" or "metabolic signature." For the purpose of this discussion, we focus on the use of BT in clinical pediatric gastroenterology practice.

HYDROGEN AND METHANE BT FOR CARBOHYDRATE MALABSORPTION IN PEDIATRICS

The hydrogen measured in breath is the product of carbohydrate fermentation, the extraction of energy from carbohydrates in the absence of oxygen, and is produced by bacteria present in the colon (1). The H2 breath test was introduced 50 years ago by Calloway and Murphy (2) and Levitt and Ingelfinger (3). Approximately, 9% of children do not produce hydrogen (4). It is presumed either that the gut bacteria of these people do not produce hydrogen gas, or that the hydrogen is consumed by methanogens, micro-organisms that produce methane. The methane measured in breath is formed by Archaea, a group distinct from bacteria and eukaryotes, which is found in ruminants and humans. It is estimated that 54% of adults produce methane that is measurable in breath (5). Methane production in children increases from 0% to 44% at 9 years of age (6). Thus, the basis for hydrogen and methane BT is the premise that human tissues do not produce these gases, but the contents of the gastrointestinal tract do under anaerobic conditions. This means that the breath test can be used to assess for carbohydrate malabsorption. The carbohydrate that is malabsorbed traverses the small bowel and in the colon is fermented to yield hydrogen and/or methane gas. It is helpful to assess for both hydrogen and methane in the breath because Archaea use hydrogen to produce methane and, depending on the composition of the colonic ecosystem, may influence the hydrogen measurement.

The most common dietary carbohydrates of concern are lactose, sucrose, and fructose. Lactose is hydrolyzed by lactase in the small intestinal epithelium to glucose and galactose that are readily absorbed in the jejunum. If lactase is lacking, the unhydrolyzed disaccharide is malabsorbed and the microbes in the colon use it for metabolic functions producing the hydrogen and methane gas, which then enter the circulation and are exhaled in the breath. Sucrose is hydrolyzed to glucose and fructose that are also readily absorbed in the jejunum. Any unhydrolyzed sucrose is used by microbes in the colon for metabolic functions and to produce hydrogen and methane gas. Fructose is a dietary monosaccharide that is absorbed directly by the intestines, although the specific mechanism(s) is not clearly defined. Fructose is found in fruits, their juices and is added to many commercially prepared foods and snacks. In some patients with gastrointestinal symptoms, ingestion of fructose may cause or exacerbate symptoms (7,8) and a breath test might identify those patients for whom fructose causes symptoms. These patients should not be confused with those who have congenital fructose intolerance caused by a mutation in the ALDOB gene such that the aldolase B enzyme is either not made or is defective. Aldolase B metabolizes fructose to fructose-1-phosphate, glyceraldehyde and dihydroxyacetone phosphate. Hence, fructose should not be ingested in patients who have congenital fructose intolerance. There is no place for BT in suspected congenital fructose intolerance.

To administer a breath test for lactose, sucrose, or fructose malabsorption, it is important that the small bowel not contain carbohydrates that cause a high base line and make interpretation of the breath analysis difficult, if not impossible. Therefore, patients should

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eat a low-fiber diet the day before the test and fast overnight. In addition, antibiotics should be avoided for 4 weeks before the test and promotility agents and laxatives should be stopped 1 week before BT. Smoking should be avoided the day of the test because it increases the exhaled hydrogen and carbon dioxide and increases gastric transit time. Physical activity should be limited during the test because hyperventilation associated with exercise inversely affects hydrogen levels (9). It is suggested that it may not be necessary to stop proton pump inhibitors (10). An initial breath is collected. Then, the lactose, sucrose, or fructose is ingested at a dose of 1.0 g/kg body weight to a maximum dose of 25 g over a few minutes (10–12). Breath is then collected every half an hour for at least 3 hours.

A normal test, no increase in hydrogen or methane for the duration of the breath collection, indicates there is no malabsorption. It is unclear how to interpret a high base line breath hydrogen, >20 ppm, or methane, >10 ppm, because a high baseline may represent poor patient preparation or perhaps small bowel bacterial overgrowth. A positive test is characterized by a rise of more than 20 ppm of hydrogen and/or more than 10 ppm methane over the baseline. A BT that has a very low H2 and no methane production suggests the subject's colon ecosystem does not produce either gas and perhaps has an abundance of hydrogen sulfide producing bacteria (13).

H2 BT FOR SMALL INTESTINAL BACTERIAL OVERGROWTH

Small intestinal bacterial overgrowth (SIBO) is defined as a condition in which abnormally large numbers of bacteria are resident in the small intestines. The true prevalence and causal relationship to specific symptoms are unclear. The small bowel contains a small number of culturable bacteria that rarely exceeds 10^3 colony units/mL (CFU) in the jejunum. In about 33% of healthy individuals, no bacteria can be cultured. However, not all bacteria in the gastrointestinal tract can be cultured. The application of molecular techniques to the study of small bowel ecology may change current understanding of SIBO. In resource-rich countries, SIBO most often develops after surgery that creates a pouch or partial block, when the bowel is dysmotil, when it is short or when an ileocecal valve is lacking

BT is a simple, inexpensive, noninvasive diagnostic tool to assess for bacterial overgrowth. Hydrogen and methane breath tests are most commonly used and are based on the premise that proximally unabsorbed carbohydrate is fermented in the gut lumen. Two sugars are available to assess for SIBO, glucose, a monosaccharide, and lactulose, a disaccharide composed of fructose and galactose. Glucose is absorbed as a monosaccharide in the small bowel. The human organism has no mechanism to hydrolyze lactulose, so it arrives at the colon intact where bacteria and/or archaea can metabolize it producing hydrogen and methane that is measurable in the breath. The patient should be tested after consuming only a low-residue diet for 24 hours before the test, fasting for 8–12 hours, not smoking or exercising. An early rise in breath hydrogen and/or methane is indicative of bacteria present in the proximal bowel. For glucose, the dose is 1 g/kg (10) body weight to a maximum of 75 g; for lactulose the dose is 0.5 g/kg body weight to a maximum of 10 g. Breath is collected every 15 minutes for about 2 hours. An early rise in breath hydrogen or methane, defined as an increase over base line of ≥20 ppm hydrogen or ≥10 ppm methane within the first 90 minutes of the test is considered a positive test. A second peak in breath hydrogen and/or methane may also be seen when the substrate reaches the colon. The interpretation of BT for SIBO can be difficult because transit time varies and some contest that BT should only be performed when orocecal time is also measured as rapid transit time can give a false positive test (14,15). Similarly, the presence of an elevated fasting breath level of hydrogen or methane can indicate SIBO, or the failure of the patient to properly prepare for the study, that is, not fast or consume fiber rich foods the day before the test.

BT for SIBO has been questioned by some clinicians as the sensitivity for the glucose breath test varies from 20% to 93% and the specificity from 30% to 86% when compared to culture. Similarly, the sensitivity for the lactulose breath test is 31%–68% and the specificity 44%–100%, again when compared to culture (16). Some clinicians use empiric therapy for pediatric patients at high risk for small bowel bacterial overgrowth because of the lack of reliable diagnostic tests.

BT for carbohydrate malabsorption and bacterial overgrowth was reviewed in 2009 (17).

^{13}CARBON BREATH TESTS IN PEDIATRICS

Isotopes are recognized by the variations in individual atomic elements within the periodic table location. Stable isotopes are identified by the variants in mass which can be identified by mass spectrometers. Spectrometers can test the ratio of ^{13}C to ^{12}C masses in substrates including breath CO_2. In the clinical BT the $^{13}CO_2$-to-$^{12}CO_2$ ratio in breath is measured as a product of biological processing and oxidation to ^{13}C-enriched breath over time. The use of ^{13}C-substrates for breath studies was introduced 40 years ago by Klein (18). The childhood uses of stable isotopes for investigation of developmental and of pathological states are more attractive for the ^{13}C BT then for H2 BT because of quantitative possibilities and well understood metabolic processes. The test subject experience and the technical resources can be roughly equal in both forms of breath tests. The clinical application of the two modes, however, is very different. The H2 BT equipment and maintenance are widely available to Pediatric GI clinicians and the US clinical resource laboratories. The H2 BT is an approved test for children by the US Food and Drug Administration (FDA) and the Current Procedural Terminology (CPT) codes are honored by most Health Insurance policies. The all or none responses of the H2 BT is a drawback. The required ^{13}C BT equipment and technical support are not available in most pediatric GI clinics and only in a few US clinical reference laboratories. Quantitation of response is possible to aid diagnostic and therapeutic management of digestion, absorption and oxidation with the ^{13}C BT but not the H2 BT. This may be very important as we enter the genomic age of personalized care. Instrument price and maintenance are not equal in cost. The original mass spectrophotometer has been approved by FDA for ^{13}C BT. The FDA has now approved an infrared $^{13}CO_2$ analyzer equivalent to the H2 BT analyzer.

Only two ^{13}C BT tests for clinical applications have been approved by the US FDA (19,20), but all the remainder must be approved as clinical research by local Institutional Review Boards. Because of the safety margin of the ^{13}C substrates, the clinical research applications of the ^{13}C BT are commonly approved. A much wider variety of approved clinical ^{13}C BT are available in Europe as reviewed elsewhere (21–24).

^{13}C UREA BT FOR *HELICOBACTER PYLORI* INFECTION

The FDA approved the ^{13}C Urea BT (UBT) in 2012 for children ages 3–17 years for qualitative detection of *Helicobacter pylori* infection and to monitor for treatment. The test is based the fact that *H. pylori* microorganisms produce urease enzyme that degrades urea and releases CO_2. The CO_2 is absorbed through the gastric mucosa and expired in the breath. For the UBT test, a fasting breath is collected, then the patients consume Pranactin-Citric, a solution containing aspartame, citric acid, mannitol, and a small amount of ^{13}C-labeled urea. Urease degrades urea, releasing $^{13}CO_2$, which enters the blood stream and is quantitated in a breath sample. The test result is detected as the difference between the ratio of $^{13}CO_2/^{12}CO_2$ in the postdose sample and the corresponding baseline sample, delta over baseline (DOB). The production of total CO_2 varies according to

age, gender, weight, and height. The Pediatric Urea Hydrolysis Rate (UHR) Calculation Application is available online to calculate the results for pediatric patients based on breath ^{13}C enrichment (DOB). Specifically, UHR (pg/min) = DOB × CO_2 Production Rate × 0.3427. Thus, a web-based application is provided to calculate the pediatric test result, where the DOB is converted into a UHR result. The UHR metric correlates to a test result as *H. pylori* positive (>10.0 gg/min) or *H. pylori* negative (<10.0 gg/min). The sensitivity is 95.8% and the specificity is 99.2% compared to composite tests of histology, culture and rapid urease test. For the test to be valid, the patient needs to fast, usually overnight, and for 1 hour before the baseline breath. Antibiotics, bismuth preparations, or proton pump inhibitors should not be taken for at least 14 days. In general, the ESPGHAN/NASPGHAN guidelines (25) recommend against testing for *H. pylori* for children and adolescents.

^{13}C SPIRULINA GASTRIC EMPTYING TEST

The US FDA has approved the Gastric Emptying Breath Test (GEBT) (20), a noninvasive test to aid in the diagnosis of delayed gastric emptying. A GEBT kit measures the rate of excretion of $^{13}CO_2$ in the patient's breath after a solid ^{13}C-meal. Patients eat a precisely formulated egg mixture containing pharmaceutical-grade Spirulina platensis, a ^{13}C-enriched blue green algae that is safe and edible. Quantitated feeding of the ^{13}C-enriched test meal, which is digested only in the small intestine, gives rise to breath $^{13}CO_2$. The rate of $^{13}CO_2$ excretion at any measurement time is proportional to the rate of gastric emptying. The kit contains the ^{13}C-meal and all components necessary to administer the test meal and collect breath samples is provided to the test administration site. Collected breath samples are returned to the clinical laboratory for analysis by mass spectrometry. The mass spectrometry platform is a validated, FDA-approved analytical system for conducting GEBT breath analyses. Baseline breath samples are collected before the administration of test meal. After an overnight fast, the patient consumes a ^{13}C-meal and postmeal breath samples are collected at multiple time points after meal ingestion. As the meal is digested, $^{13}CO_2$ is given off in the patient's breath. All premeal and postmeal breath samples are collected and analyzed by a gas isotope ratio mass spectrometer to determine the ratio of $^{13}CO_2/^{12}CO_2$ in each sample. GEBT test results are presented in a gastric emptying profile report provided to the clinician. The specificity for detecting delayed gastric emptying is 98.2% and sensitivity 54.4% at 120 minutes after feeding the test meal.

ROLE OF ^{13}C BT IN HEALTH CARE OF CHILDREN

Despite the large margin of safety and the depths of understanding of the biological pathways the healthcare use of ^{13}C BT in North America is limited to the two above tests in older children. Klein (18) stated two decades ago that there were two challenges for the pediatrician wishing to use the ^{13}C BT in practice. The first is the requirement for FDA approval of each specific ^{13}C-substrate and the second is the absence of CPT codes for procedure reimbursements (26). These challenges have not yet been met. In contrast, the H2 BT is FDA approved and adequately covered by CPT codes.

INTERPRETING ^{13}C BT IN METABOLIC CONTEXT

The clinical interpretation of ^{13}C BT is more complex than the H2 BT. The ^{13}C concentration is referenced to the $^{13}C/^{12}C$ ratio, where ^{12}C is the denominator. The total ^{12}C excreted in breath is assumed to be proportional to the basal metabolic expenditure in calculating the quantity of ^{13}C-urea metabolized (26) and the rate of ^{13}C-spirulina emptied from the stomach (27). These calculations assume that the tracer has not impacted basal energy expenditure. Our approach has been to use the ^{13}C-glucose product as the denominator for testing ^{13}C-labeled carbohydrate precursor digestions (28).

ANALYTICAL RESOURCES FOR ^{13}C BT

The mass spectrophotometer has been approved as the gold standard for ^{13}C BT by FDA. A simpler infrared (IR) spectrophotometer which measures the $^{13}CO_2/^{12}CO_2$ ratio in breath samples is available, which is also approved by the FDA for the ^{13}C-UBT and is accepted by local ethical committees as $^{13}CO_2/^{12}CO_2$ isotope analyzer (29). Both types of spectrometers test the ratio of ^{13}C to ^{12}C masses in breath CO_2. The botanical source of the fed substrate greatly influences this ratio. The ^{13}C in cane sugar, the C4 photosynthetic process, is higher than beet sugar, a more discriminant C3 process. Differences in the photosynthetic pathway are important to design because cane and maize products are relatively enriched substrates for ^{13}C BT substrate (24). GCMS sensitivity and specificity is required when photosynthetic ^{13}C-substrates are fed in the ^{13}C BT. When more highly labeled substrates are used, either by biological enrichment with $^{13}CO_2$ as with ^{13}C Spirulina or synthetic ^{13}C Urea, the IR spectrometer analysis of ^{13}C BT is satisfactory. The IR $^{13}CO_2$ analyzer has economic and support requirements roughly equivalent to the H2 BT analyzer.

HEALTH CARE USES OF ^{13}C BT?

BT offers unlimited opportunity for future clinical research and possible clinical health care. The clear advantages of ^{13}C BT are that it is safe and noninvasive and, with the advent of infrared spectroscopy it offers rapid results (30). The commonality for most clinical research uses of BT is that a substrate labeled with a stable isotope, such as a ^{13}C-substrate, is administered and then the labeled isotope is recovered as $^{13}CO_2$ in exhaled breath. The ^{13}C is detected as $^{13}CO_2$ in expired breath by a central laboratory using mass spectrometers or locally using an infrared spectrophotometer. Out of the first 82 elements in the periodic table, 80 have stable isotopes. Many are not recoverable in exhaled breath, but the possibilities for use in clinical research are extensive. Among the many possibilities for future uses are, for the Pediatric Gastroenterologist, gastric emptying time (31), oral cecal transit time (32), energy balance (30), and fatty acid catabolism (33). For the Pediatric Hepatologist, there are the aminopyrine ^{13}C-propionate for liver microsomal function (34), the ^{13}C-keto-isocaproate (35) used to assess the mitochondrial function of patients with nonalcoholic liver disease, and the ^{13}C-metacetin breath tests (36) to identify acute liver injury. The ^{13}C-phenylalanine test identifies not only phenylketonuria but also perhaps schizophrenia (37). With all these potential clinical applications, comes a large amount of work to establish reliability and to standardize each test. Once completed, stable isotope tests will require support from NASPGHAN and working group interest to gain the US FDA approval and child-specific CPT codes for pediatric healthcare reimbursement.

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