A Stable Isotope Breath Test With a Standard Meal for Abnormal Gastric Emptying of Solids in the Clinic and in Research

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Background & Aims: **The aim of this study was to validate a [^{13}C]–*Spirulina platensis* gastric emptying (GE) breath test (GEBT) with a standardized meal.** ***Methods:*** **Thirty-eight healthy volunteers and 129 patients with clinically suspected delayed GE underwent measurements at 45, 90, 120, 150, 180, and 240 minutes after a 238 kcal meal labeled test with 100 mg [^{13}C]–*S platensis* and 0.5 mCi ^{99m}Tc. We established normal ranges for scintigraphy with this test meal, intraindividual and interindividual coefficients of variation (COVs), and the ability of the [^{13}C] GEBT breath percent dose excreted *1000 values to predict scintigraphic half-life and to categorize GE as delayed, normal, or accelerated.** ***Results:*** **In health, the 10th and 90th percentiles of half-life for scintigraphic GE with this meal were 52 and 86 minutes; intraindividual COVs for scintigraphy and the GEBT were, respectively, 31% and 27% at 45 minutes, 17% and 21% at 90 minutes, 13% and 16% at 120 minutes, 10% and 13% at 150 minutes, and 8% and 12% at 180 minutes. Interindividual COVs at each time for the [^{13}C] GEBT and scintigraphy were typically ~1%–4% lower than intraindividual COVs. Individual breath samples at 45, 150, and 180 minutes predicted GE category; at 80% specificity, 45- and 180-minute samples combined were 93% sensitive to identify accelerated GE, and 150- and 180-minute combined were 89% sensitive for delayed GE.** ***Conclusions:*** **[^{13}C]–*S platensis* GEBT is as reproducible as scintigraphy; imprecision with both tests reflects physiologic variation. With 4 breath samples, this method with an off-the-shelf meal is valid to assess GE in clinic and in research.**

The measurement of gastric emptying (GE) by stable isotope breath tests (GEBTs) has practical and safety advantages compared with current scintigraphic methods.[1] Unlike scintigraphy, which requires elaborate detection equipment and the patient to be located in the same setting, GEBT can be performed just about anywhere, including any office or bedside, because the collected breath samples are stable, and the samples can sent to a remote site for analysis. GEBT is safer than scintigraphy because it involves no radiation exposure, which is advantageous if repetitive assessments of GE are needed for research or clinical purposes, or if assessment of GE is needed in pregnant or breast-feeding women and in children.

In previous studies conducted in our laboratory,[2–5] we demonstrated that as compared with simultaneous scintigraphy, the 13-carbon ([^{13}C])-octanoate and [^{13}C]–*Spirulina platensis* GEBT provided an acceptable assessment of the GE of solids in humans, with acceptable coefficient of variation (COV) comparable to scintigraphy. [^{13}C]–*S platensis* GEBT was able to identify accelerated or delayed emptying induced pharmacologically with placebo, erythromycin, or atropine. Across the range of GE, the mean difference in half-life between the 2 methods was 0.15 minutes with standard deviation of 35.5 minutes.[6] The meals used in the prior studies were not completely standardized (eg, egg size and weight might differ) and were not shelf-stable.

To facilitate safe, point-of-care assessment of GE, a standardized test meal consisting entirely of shelf-stable components including 100 mg [^{13}C]–*S platensis* has been developed.[7] In the current prospective, validation study comparing [^{13}C]–*S platensis* GEBT by using a standardized, shelf-stable meal with simultaneous scintigraphic GE, our aims were (1) to establish normal ranges for scintigraphy with this test meal, (2) to appraise the performance characteristics (intraindividual and interindividual COVs of both scintigraphy and [^{13}C]–*S platensis* GEBT) in healthy volunteers, and (3) to assess the ability of the [^{13}C]–*S platensis* GEBT breath (percent dose excreted *1000 [kPCD]) values to predict scintigraphic half-life (t1/2) and to categorize GE as delayed, normal, or accelerated in patients with symptoms suggestive of abnormal GE.

Methods

Experimental Design

This prospective, open-label comparison validation study was conducted at the Mayo Clinic's Clinical Research Unit. All studies were approved by the Mayo Clinic Institutional Review Board.

In the first phase, we performed a calibration study in 38 healthy volunteers to estimate the reference range for scintigraphic results by using the standardized, shelf-stable test meal and to estimate the total variability (imprecision) of scintigraphy and [^{13}C]–*S platensis* GEBT measurements on healthy volunteers. Twenty-eight participants underwent studies on 2 occasions to study intraindividual variation.

Abbreviations used in this paper: AUC, area under the curve; BMI, body mass index; BT, breath test; [^{13}C], 13-carbon; COV, coefficient of variation; DOB, delta over baseline; GE, gastric emptying; GEBT, gastric emptying breath test; PCD, percent dose; kPCD, percent dose multiplied by 1000; ROC, receiver operating characteristic; ROI, region of interest; t1/2, half-life.

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In the second phase of this study, we validated the [^{13}C]–*S platensis* GEBT for use in the diagnosis of delayed GE by studying 124 participants who were referred for scintigraphy for clinically suspected abnormal GE and in 5 healthy subjects who received atropine (0.01 mg/kg IV bolus during a period of 10 minutes followed by 0.01 mg/kg infusion during a period of 50 minutes) to pharmacologically delay GE.

Eligibility Criteria for Participants

In the first phase, we recruited by public advertisement 38 normal participants, men and women between 18–75 years old. Women of childbearing potential were required to have negative pregnancy urine test within 48 hours of the dual-label GE test. Participants were excluded if they had any history, physical examination, or laboratory finding to suggest systemic diseases, history of abdominal surgery except appendectomy; clinically significant neurologic or psychiatric disorders, use of narcotics or anticholinergic agents within 2 days of the study, or receipt of an investigational drug within 4 weeks before the study.

In the second phase of the study, 124 participants were recruited from patients referred for GE by scintigraphy on the basis of clinical assessment by physicians at the Mayo Gastroenterology Motility Clinic. Participants were men and women, 18–75 years old, and had similar inclusion criteria, except concomitant general diseases or suspicion of delayed GE were not exclusion criteria, and in addition, there could be no history or suspicion of malabsorption caused by mucosal disease, pancreatic disease, or liver dysfunction. The 5 normal participants, who received atropine in the second phase of the study, were recruited by public advertisement and had the same inclusion/exclusion criteria as the normal participants used in the first phase of the study.

Procedures

For each potential participant, a screening visit was conducted in which consent was obtained, and a physical exam was performed. After an overnight fast (minimum 8 hours), the participants returned to the study center at approximately 7:00 AM, at which time the dual-label GE test was started. The patient consumed the test meal containing [^{13}C]–*Spirulina* and ^{99m}Tc sulfur colloid. Scintigraphic images were obtained on completion of the meal and at 45, 90, 120, 150, 180, and 240 minutes after the meal. Breath samples were collected at baseline before the test meal was started and simultaneously with scintigraphic image acquisitions after the ingestion of the test meal.

Test Meal

The test meal consisted of 1 mCi ^{99m}Tc-sulfur colloid, 100 mg [^{13}C]–*S platensis*, 27 g freeze-dried egg mix, 6 saltine crackers, and 180 mL of water. The caloric content of the meal is 238 kcal, and the meal has a balanced composition of 16.9 g carbohydrates, 14.4 g protein, and 11.2 g fat. The nature and size of the meal were selected to ensure stability at room temperature, palatability, and calorie content that would be consumed entirely, even by patients with suspected gastroparesis and upper abdominal symptoms.

*Substrate for 13–Carbon Dioxide Breath Test ([13-Carbon]–*Spirulina platensis*)*

S platensis is a protein-rich, blue-green algae eaten as a food source in many parts of the world and is sold as a dietary supplement in the United States.[8–10] It contains 50%–60% protein, 30% starch, and 10% lipid.[11] The natural level of ^{13}C in *S platensis* and in all living things is about 1%.[12] The *S platensis* used in this study was grown in a closed hydroponics chamber charged with pure ^{13}C-source, raising the level of ^{13}C in the resultant cells to 99%.[7] Because the contents of the algal cells are not freely diffusible, incorporation of ^{13}C-labeled *S platensis* into the egg mix provides a way to assess the emptying of the solid phase of the meal. ^{13}C can only be released from the algal cells after the egg mix is emptied from the stomach, the cells are digested, and the ^{13}C-labeled substrates (algal protein, fat, and carbohydrate) are absorbed and metabolized. In this way, [^{13}C]-*S platensis* gives rise to respiratory CO_2 that is enriched in ^{13}C.

*Measurement of Breath 13–Carbon Dioxide During [13-Carbon]–*Spirulina platensis *Gastric Emptying Breath Test*

Breath samples were taken at baseline before the meal and followed the same time schedule as the scintigraphic technique. End-tidal breath samples were collected while the participant's abdomen was being imaged by the gamma camera. Breath samples were stored in duplicate in glass screwcap Exetainer tubes (Labco Limited, High Wycombe, UK) by using a straw to blow into the bottom of the tube to displace contained air. After recapping the tubes, the $^{13}CO_2$ breath content was determined in a centralized laboratory (AB Diagnostics, Brentwood, TN) by isotope ratio mass spectrometry. The ^{13}C enrichment was expressed as the delta per mL difference between the $^{13}CO_2/^{12}CO_2$ ratio of the sample and the standard. To calculate the quantity of ^{13}C appearing in breath per unit time, delta over baseline (DOB) was used where 0.0112372 is the isotopic abundance of the limestone standard, Pee Dee belemnite, and CO_2 production was corrected for age, sex, height, and weight by using the algorithms of Schofield et al as described by Klein.[13]

Analysis of Gastric Emptying Breath Test and Scintigraphy Data

Gastric emptying breath test. The currently preferred GEBT metric is the percent dose (PCD) excreted at time t after consumption of the test meal.[14] To provide a more convenient scale, we multiplied PCD by 1000 to produce kPCD at any time, t.

$$kPCD_t = \left[\frac{DOB * CO_2PR * R_s * 13}{10 * dose}\right] * 1000$$

where DOB is the measured difference in the ratio [$^{13}CO_2/^{12}CO_2$] between a post-meal breath specimen at any time (t minutes) and the baseline breath specimen. CO_2 production rate (CO_2PR) (mmol CO_2/min) was calculated by using the equations of Schofield,[15] which incorporate the patient's age, gender, height, and weight. R_s is the ratio [$^{13}CO_2/^{12}CO_2$] in the reference standard (Pee Dee belemnite) for these measurements, Rs is 0.0112372, 13 is the atomic weight of carbon-13, 10 is a constant factor for converting units, and dose is the weight (mg) of carbon-13 in the dose of [^{13}C]-*S platensis* administered to