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Comprehensive assessment of gastric emptying with a stable isotope breath test

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Abstract

Background *The [^{13}C]-Spirulina platensis gastric emptying breath test (GEBT) with five samples is accurate relative to scintigraphy. This study was primarily designed to further validate this GEBT using a slightly different process for incorporating [^{13}C] in Spirulina and to evaluate the utility of additional samples for assessing early gastric emptying.* ***Methods*** *After a 223 kcal, test meal labeled with ^{99m}Tc and [^{13}C]-S. platensis, scintigraphic images, and five breath samples (45, 90, 120, 180, and 240 min, GEBT5) were collected in 14 controls (Part A). In Part B, nine breath samples were collected at 15, 30, 45, 60, 90, 120, 150, 180, and 240 min (GEBT9) in 30 subjects (15 controls, 15 dyspepsia). Using correlation between [^{13}C] breath excretion and scintigraphic emptying, lag time (t_{10}, time for 10% emptying), emptying at 30 min (GE_{30}), and half time (t_{50}) were estimated for GEBT5 (Parts A and B) and GEBT9 (Part B).* ***Key Results*** *Half time values for scintigraphy, GEBT5, and GEBT9 were highly concordant. t_{10} by GEBT9 (90%CI, 6–15 min) was more strongly correlated [CCC 0.80 (95% CI, 0.63–0.90)] with scintigraphy (90% CI, 5–12 min), than GEBT5 [10–19 min, CCC 0.73 (95% CI, 0.54–0.85)]. The correlation between estimated values (GEBT9) and linearly interpolated values (GEBT5) was closer at 60 [CCC 0.95 (95% CI, 0.91–0.97)] than 30 min [CCC 0.81 (95% CI, 0.71–0.89)].* ***Conclusions & Inferences*** *The [^{13}C]-S. platensis GEBT can accurately measure GE. While 5- and 9-samples are equally accurate for measuring t_{50}, GEBT9 provides a more comprehensive assessment of early GE (t_{10} and GE_{30}).*

Keywords *Breath test, Dumping, Dyspepsia, Gastric emptying, Gastroparesis.*

Abbreviations: BT, Breath test; [^{13}C], 13-carbon; CCC, concordance correlation coefficient; CV, coefficient of variation; DOB, delta over baseline; GE, gastric emptying; GEBT, gastric emptying breath test; GEBT5, gastric emptying breath test with 5 breath samples; GEBT9, gastric emptying breath test with 9 breath samples; PCD, percent dose; kPCD, percent dose multiplied by 1000; ROI, region of interest; [^{99m}Tc], 99m- technetium; $t_{10,}$ time for 10% emptying; $GE_{30,}$ gastric emptying in 30 min; $t_{50,}$ gastric emptying half time.

INTRODUCTION

Compared with current scintigraphic methods, the measurement of gastric emptying (GE) by stable isotope gastric emptying breath tests (GEBT) has practical and safety advantages. In contrast with scintigraphy, GEBT does not require elaborate detection equipment and can be performed at the point of care, as in the office or bedside because the collected breath samples are collected simply with a straw and sealable container, and the excreted $^{13}CO_2$ is stable. Samples can be sent to a remote site for analysis. In addition, GEBT does not entail radiation exposure, and is safer than scintigraphy, particularly if repeated assessments are required, or, when GE needs to be assessed in pregnant or breast feeding women and in children. Our group has previously focused on developing an accurate mathematical analysis[1] and reducing the number of breath samples necessary, thereby reducing the cost of the test. Using five breath samples over 3 h

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(i.e., before as also 45, 90, 120, and 180 min after a meal), we showed that the intra- and interindividual coefficients of variation for gastric emptying half time (t_{50}) measured by [^{13}C]-octanoate and [^{13}C]-*Spirulina platensis* GEBT were comparable to corresponding values for scintigraphy.[2–6] The most recent version of the [^{13}C]-*S. platensis* GEBT uses a standardized test meal with shelf-stable components including [^{13}C]-*S. platensis*[7] that was validated against scintigraphy in healthy subjects, patients with accelerated and delayed gastric emptying, and healthy subjects with atropine-induced delayed gastric emptying.[6] The GEBT has been endorsed by consensus statements issued by the American and European Neurogastroenterology and Motility societies.[8]

There is increasing recognition that rapid gastric emptying may occur not only in diabetes mellitus or after fundoplication but also in patients with functional diarrhea, functional dyspepsia, and autonomic dysfunction.[9–17] We have observed that some patients with rapid early gastric emptying (e.g., at 30 or 60 min) have a normal gastric emptying t_{50} probably because the emptying rate slows after the initial rapid phase. It is conceivable that additional early (i.e., at 15, 30, and 60 min) and late (i.e., at 240 min) postprandial breath samples will increase the accuracy of the GEBT for identifying rapid and delayed gastric emptying relative to scintigraphy, respectively.

By growing *S. platensis* in a closed hydroponics chamber charged with a pure source of ^{13}C, the cellular content of ^{13}C is increased to 99%.[7] In comparison to a previous study validating the [^{13}C]-*S. platensis* GEBT,[6] this study utilized a slightly modified algal growth process to enhance the yield and process efficiency. Hence, the specific aims of this study were: to (i) to estimate normal ranges for scintigraphy with this test meal; (ii) to appraise the performance characteristics (interindividual coefficients of variation (CV) of both scintigraphy and [^{13}C]-*S. platensis* GEBT) in healthy volunteers; (iii) to assess the ability of the [^{13}C]-*S. platensis* GEBT breath kPCD (percent dose excreted *1000) values to predict scintigraphic GE proportions at the different times, and hence to measure GE t_{10}, GE at 30 min, and t_{50}; (iv) to categorize GE as delayed, normal or accelerated, and (v) to ascertain whether additional early postprandial breath samples increase the accuracy of characterizing the early phase of gastric emptying.

METHODS

Experimental design

This is a report of two prospective, open-label comparison validation studies which were approved by the Mayo Clinic Institutional Review Board. In both studies, gastric emptying was simultaneously evaluated by the [^{13}C]-*S. platensis* GEBT and scintigraphy. In the first study labeled, Part A, breath samples and scintigraphic images were obtained at 5 time points in 14 healthy subjects. Thereafter, we were prompted, by increasing awareness of rapid gastric emptying in our clinical practice, to assess the potential utility of collecting additional samples after the GEBT5 study was completed. Hence, breath samples and scintigraphic images were obtained at 9 specific time points in the second study ('Part B'), in 30 participants – 15 healthy subjects and 15 patients with symptoms of dyspepsia.

Eligibility criteria for participants

Patients and healthy volunteers were recruited through public advertisement and a clinic. Participants (males and females) were aged 18–70 years and did not have clinically significant cardiovascular, respiratory, renal, hepatic, gastrointestinal, hematological, neurological, psychiatric, or other disease that may interfere with the study. Other exclusion criteria were a history of abdominal surgery other than appendectomy, cholecystectomy, tubal ligation, or hysterectomy; use of any medications that alter GI motility within 2 days of the study; any allergies to eggs, wheat, or milk or unwilling to consume such products; or receipt of an investigational drug within 30 days prior to the study. Although healthy subjects did not have symptoms of a functional GI disorder by questionnaire, patients had symptom criteria for functional dyspepsia.[18] Participants were excluded if they had severe nausea or vomiting precluding study assessments; any history of malabsorption due to mucosal disease, pancreatic disease, liver dysfunction, or other causes.

Procedures

All participants had an interview and physical examination and completed questionnaires. (i.e., Hospital Anxiety and Depression Questionnaire and gastrointestinal symptom questionnaires based on Rome III criteria).[18,19] Healthy subjects did not have symptom criteria for functional dyspepsia or a functional bowel disorder. Patients had Rome III symptom criteria for dyspepsia. In women of childbearing potential, a negative urine pregnancy test was required within 48 h of the gastric emptying test. After an overnight fast (minimum 8 h), the dual-label GE test was performed at the study center. Patients consumed the test meal containing ^{13}C-*Spirulina* and ^{99m}Tc-sulfur colloid in no more than 10 min. Scintigraphic images and breath samples were obtained upon completion of the meal and at 45, 90, 120, 150, and 180 min after the meal in Part A or at 15, 30, 45, 60, 90, 120, 150, 180, and 240 min after the meal in Part B. In both Parts A and B, a breath sample was also collected before the test meal. Gastric images were acquired with sequential 2-min anterior and posterior images in the standing position with a single-head camera. The breath samples were collected while the posterior image was being acquired.

Test meal

The test meal consisted of 27 g freeze dried egg mix, six saltine crackers, and 180 mL of water. The caloric content of the meal is 223 kcal, and the meal has a balanced composition of 19.2 g carbohydrates, 12 g protein, and 10.9 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. The meal was labeled with 0.5 mCi ^{99m}Tc-sulfur colloid and 100 mg of ^{13}C-*S. platensis*, with a ^{13}C content of 43%.

Substrate for $^{13}CO_2$ Breath Test (^{13}C-*S. platensis*)

Spirulina 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.[20,21] It contains 50–60% protein, 30% starch, and 10% lipid.[22] The natural level of ^{13}C in *S. platensis* and in all living things is about 1%.[23] 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] In an attempt to enhance the yield and process efficiency, this algal growth process was slightly modified from the previous study utilizing additional culture agitation and an abbreviated harvest procedure. The ^{13}C content and distribution of ^{13}C-labeled protein, carbohydrate, and lipids was comparable to prior lots.[6] 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 $^{13}CO_2$ during [^{13}C]-*S. platensis* GEBT

Breath samples were taken at baseline before the meal and thereafter on the same time schedule as the scintigraphic procedures. 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) 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, USA) by Gas 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, gender, height, and weight using the algorithms of Schofield *et al.*, as described by Klein *et al.*[24]

Analysis of GEBT and scintigraphy data

GEBT The currently preferred GEBT metric is the percent dose (abbreviated PCD) excreted at time *t* after consumption of the test meal.[25] To provide a more convenient scale, we multiply 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 = The measured difference in the ratio [$^{13}CO_2/^{12}CO_2$] between a postmeal breath specimen at any time (*t*-min) and the baseline breath specimen.

CO_2 PR = CO_2 Production Rate (mmol CO_2 min^{-1}) calculated using Schofield equations[26] which incorporate the patient's age, gender, height, and weight.

R_s = The ratio [$^{13}CO_2/^{12}CO_2$] in the reference standard (Pee Dee belemnite) for these measurements, Rs = 0.0112372.

13 = the atomic weight of Carbon-13.

10 = A constant factor for converting units.

dose = the weight (mg) of Carbon-13 in the dose of [^{13}C]-*S. platensis* administered to the patient in the test meal. Since [^{13}C]-*S. platensis* is approximately 43% Carbon-13, a dose of 100 mg [^{13}C]-*S. platensis* corresponds to approximately 43 mg of Carbon-13.

Scintigraphy A region of interest (ROI) was drawn around the stomach on the anterior and posterior images for each time frame. Data were corrected for decay of ^{99m}Tc. To correct for depth or tissue attenuation, the counts of each anterior and posterior ROI were multiplied together and the square root of the product was taken to obtain the geometric mean. The scintigraphic GE metric, $Prop_t$, is the proportion of tracer emptied from the stomach at time, *t*. A linear interpolation was used to estimate the gastric emptying t_{50} values for each subject (i.e., linearly interpolate between the GE proportions around 0.5 to estimate the value corresponding to emptying of 50% of the meal.

Statistical methods The individual proportions of gastric emptying at each time point and the calculated t½ values obtained from scintigraphic data in 30 subjects were summarized. The 10th and 90th percentile values in healthy volunteers for five sample and separately nine sample data were used to define normal, delayed, and accelerated GE. Because there were only 15 healthy subjects for nine sample data, more accurate estimates of 10th and 90th percentiles can be obtained than for 5th and 95th percentiles. The pairwise correlations between scintigraphic GE proportions and GEBT kPCD values were estimated.

As the generalizability of discriminant models is limited by differences in study populations, a bootstrap validation approach was used to generate a multiple linear regression model predicting the scintigraphic GE proportion at each time point (dependent variable) from GEBT kPCD values; gender and BMI were covariates. A total of 200 bootstrap samples were used to obtain a final model to predict the individual scintigraphic GE proportions at each of 9 time points using the set of 9 kPCD values (i.e., Part B) and separately, at each of 5 time points (i.e., the corresponding 5 time points from Part A and the same 5 time points in Part B).[27] Including the baseline sample, a total of 6 and 10 breath samples were obtained. However, since only post baseline kPCD values were used in the models, these models are referred to as five sample and nine sample models, respectively. From the predicted GE proportions, a breath test estimate of the corresponding t_{50} values could be computed using the linear interpolation approach (as described above). The lag time which was estimated as the time required for 10% GE (t_{10}), could also be estimated from the scintgraphic proportions remaining and the corresponding GEBT estimated proportions again using linear interpolation. The agreement between the scintigraphic and GEBT estimated t_{50} and t_{10} values was then assessed [Lin's concordance correlation coefficient (CCC)][28] and a Bland–Altman plot generated to examine whether this would be a useful method to estimate gastric emptying t_{50} values for use in clinical practice or research. The GEBT predicted values obtained from the 9 time-point model (Part B) were compared with breath test predicted t_{50} values from the 5 time-point model (Parts A and B). The SAS/STAT® software package (version 9.2, SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

RESULTS

Clinical characteristics

There were 14 healthy subjects in Part A and 15 healthy subjects and 15 patients in Part B (Table 1). All patients had symptoms of dyspepsia by Rome III criteria; six had postprandial distress syndrome, two had epigastric pain syndrome, and seven had both. In addition, six had diabetes mellitus (DM; four of whom had type 2 DM), two had an autonomic neuropathy, and three had a cholecystectomy. Seven patients, including four with DM, had a history of delayed gastric emptying. Malabsorption and significant liver disease were excluded by reviewing the medical history and clinical records, respectively.

The results for scintigraphy *vs* GEBT5 and separately for scintigraphy *vs* GEBT9 are presented separately and followed by a comparison of GEBT5 *vs* GEBT9.

Table 1 Demographic features

	Males	Females
Part A (5-breath sample GEBT)		
N (Healthy subjects)	5	9
Median age (IQR) years	29.0 (26.0, 30.6)	31.9 (26.8, 41.1)
Median body mass index (IQR) kg m^{-2}	26.0 (25.1, 26.1)	24.2 (23.5, 27.0)
Part B (9-breath sample GEBT)		
N (Total, healthy subjects, patients)	10, 5, 5	20, 10, 10
Median age (IQR) years	41.5 (23.0, 52.0)	46.5 (29.9, 54.0)
Median body mass index (IQR) kg m^{-2}	24.3 (22.1, 29.5)	25.5 (22.8, 30.4)

Assessment of gastric emptying from healthy subjects & patients using the 5-breath sample model (Parts A and B)

In addition to all participants in Part A who had a 5-breath sample GEBT, the 5-breath sample model also incorporated the data from five selected breath samples (i.e., 45, 90, 120, 150, and 180 min) in all Part B participants, in whom nine breath samples were obtained.

Performance and correlations Interindividual CV% for scintigraphic GE proportions and the GEBT kPCD values were, respectively, 34.5% and 39.0% at 45 min; 28.5% and 34.8% at 90 min; 24.5% and 29.4% at 120 min; 20.0% and 25.9% at 150 min; and 17.4% and 22.9% at 180 min.

Tables 2 and 3 demonstrate the excellent correlation between GE parameters by scintigraphy and the corresponding GEBT values, which establishes the strong association between these variables. In each subject, equations derived from multiple linear regression models were used to predict GE proportions from breath test kPCD values at 45, 90, 120, 150, and 180 min, including gender and BMI as covariates (Table S1). The corresponding proportions remaining in the stomach averaged across all subjects for the scintigraphic proportions and separately the breath test-estimated proportions are shown in Fig. 1.

Estimates of gastric emptying in health and dyspepsia The 10th and 90th percentiles for the GE t_{50} measured by scintigraphy in healthy volunteers were 48 and 85 min, respectively. The corresponding values for GEBT t_{50} values measured by the 5-point model were 51 and 91 min. The mean difference (10th, 90th percentile range) between GE t_{50} measured by scintigraphy and GEBT was −0.3 (−12, 14) min. For scintigraphy, the interindividual coefficient of variation for t_{50} was 42% (N = 44) overall, 22% in healthy subjects, and 35% in dyspepsia. The corresponding values for GEBT estimated t_{50} were 40%, 21%, and 36%, respectively.

Based on the scintigraphic normal values from the 5 time-point data, three patients had normal, 11 had delayed and one had rapid gastric emptying. Average

Table 2 Gastric emptying characteristics using 5-point model (Parts A and B)

	All subjects (N = 44)		Healthy subjects only (N = 29)	
	Scintigraphy	Breath test estimates	Scintigraphy	Breath test estimates
$T_{10\%}$, min	15.8 ± 7.6	15.01 ± 5.6	13.3 ± 4.0	12.8 ± 2.7
T ½, min	82.2 ± 34.6	82.5 ± 33.2	65.9 ± 14.7	67.3 ± 14.0
GE 45 min	0.330 ± 0.114	0.329 ± 0.091	0.365 ± 0.090	0.366 ± 0.067
GE 90 min	0.587 ± 0.167	0.588 ± 0.154	0.667 ± 0.104	0.659 ± 0.105
GE 120 min	0.719 ± 0.176	0.720 ± 0.165	0.809 ± 0.099	0.798 ± 0.104
GE 150 min	0.813 ± 0.163	0.813 ± 0.148	0.894 ± 0.086	0.882 ± 0.086
GE 180 min	0.869 ± 0.151	0.868 ± 0.133	0.942 ± 0.072	0.927 ± 0.073

Values are Mean ± SD.

Table 3 Correlations between proportion emptied from the stomach by scintigraphy and kPCD by [^{13}C]-*Spirulina platensis* GEBT at *a priori* chosen time points in all participants. 5-point model

Pearson correlation coefficients, n = 44

	GE45	GE 90	GE120	GE150	GE180
BT45	0.616*	0.673*	0.656*	0.638*	0.571*
BT90	0.649*	0.842*	0.844*	0.887*	0.782*
BT120	0.644*	0.846*	0.885*	0.898*	0.864*
BT150	0.524*	0.734*	0.790*	0.844*	0.841*
BT180	0.417†	0.610*	0.656*	0.752*	0.787*

All correlations marked '*' have a $P \le 0.0010$ and correlations marked '†' have a P > 0.0 10 and ≤0.0050.
BT = breath test kPCD; GE = proportion of gastric emptying at specified times.

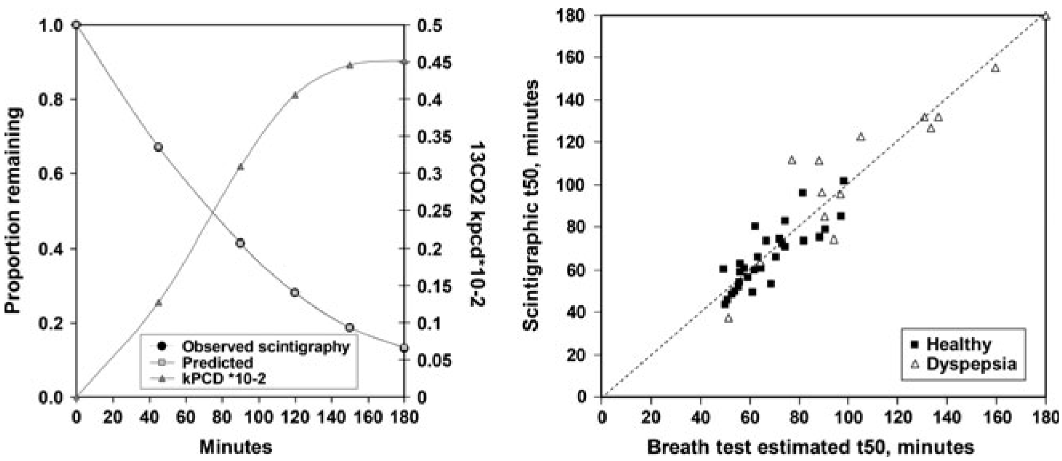

Figure 1 Summary of gastric emptying results from the 5 time-point model in 44 subjects. Left panel illustrates mean $^{13}CO_2$ enrichment of breath excreted over 3 h (right *Y*-axis κPCD × 10^{-2}), as well as the mean observed proportion emptied from the stomach by scintigraphy (left *Y*-axis) and the mean predicted GE based on the bootstrap regression model using the measured $^{13}CO_2$ excretion. The observed (scintigraphic) and predicted (GEBT) values are nearly identical; hence superimposed. Right panel illustrates scatterplot of the scintigraphic measured GE t_{50} values (*Y*-axis) *vs* the breath test-estimated t_{50} values (*X*-axis) obtained from linear interpolation of breath test predicted GE proportions based on the five time points model. Only data up to 180 min were used in this model. Hence, t_{50} was censored at 180 min in two subjects. The dotted line shows $X = Y$.

scintigraphic proportions emptied for these groups and for the 14 healthy subjects studied in Part A are provided in detail in Table S2.

Accuracy assessed by concordance correlation The (linear) concordance correlation coefficient (CCC) between the scintigraphic and GEBT estimated t_{50} values was 0.95 (95% CI, 0.91–0.97) for all subjects, 0.83 (95% CI, 0.67–0.92) in healthy subjects, and 0.94 (95% CI, 0.83–0.98) in patients.

Assessment of gastric emptying from healthy individuals & dyspepsia using the 9-breath sample model (Part B only)

Performance and correlations Scintigraphic measurements and breath test samples were obtained at 9 time points (15, 30, 45, 60, 90, 120, 150, 180, and 240 min) in 30 subjects. Interindividual CV% for scintigraphic GE proportions and the GEBT9 kPCD values, respectively, were 53% and 56% at 15 min; 45% and 44% at 30 min; 38% and 42% at 45 min; 35% and 41% at 60 min; 32% and 38% at 90 min; 27% and 34% at 120 min; 22% and 31% at 150 min; 20% and 27% at 180 min; and 15% and 21% at 240 min.

Similar to the 5-sample analysis previously published,[7] a multiple linear regression model approach was used to estimate gastric emptying based on the breath test samples at all 9 time points (Table S3). As in Table 2, which is based on the 5-point model, Table 4 demonstrates the excellent correlations at individual time points for scintigraphic gastric emptying values and the corresponding time kPCD values based on the 9 time-point data.

Estimates of gastric emptying in health and dyspepsia The 10th and 90th percentiles in healthy subjects for breath test-estimated t_{10} values were 6 and 15 min and for breath test-estimated t_{50} values were 50 and 97 min. The 10th and 90th percentiles for scintigraphic GE t_{50} using all 9 time points in healthy volunteers were 46 and 86 min, respectively (Table 5). The mean difference (10–90th percentile range) between GE t_{50} measured by scintigraphy and GEBT (9-point model) was −0.7 (−13, 17) min. The interindividual CV(%) for the (9 time points) t_{50} values based on scintigraphy were: 53% overall, 25% in healthy volunteers, and 47% in dyspepsia. Corresponding values for GEBT9 were 46% overall, 27% in healthy subjects, and 41% in dyspepsia. Based on the scintigraphic normal values from the 9 time-point data, three patients had normal, 11 had delayed and one had rapid gastric emptying; these classifications were identical to the 5-point data.

Accuracy assessed by concordance correlation The (linear) CCC between the scintigraphic and breath test-estimated t_{50} values was 0.93 (95% CI, 0.86–0.96) overall, 0.94 (95% CI, 0.85–0.98) in healthy subjects, and 0.89 (95% CI, 0.72–0.96) in patients. Fig. 2 shows the plot of observed gastric emptying (mean proportions remaining in the stomach) and corresponding mean predicted proportions remaining. Also shown in Fig. 2 are the mean breath test kPCD values ($*10^{-2}$).

Comparison of accuracy of the 5 and 9 breath sample models

The relationship between 5- and 9-point model estimates (30 subjects in Part B) was assessed for t_{10}, GE 30 min, and GE 60 min, which represent early gastric emptying, and t_{50}, which summarizes the overall gastric emptying curve. Fig. 3 compares GEBT estimated t_{50} values from all 9 and the selected 5 time-point models (Part B). The concordance correlation between these two estimated t_{50} values was 0.96 (95% CI, 0.92–0.98). However, two individuals had prolonged GEBT estimated t_{50} values which the 5 time-point

Table 4 Correlations between proportion emptied from the stomach by scintigraphy and kPCD by [^{13}C]-*Spirulina platensis* GEBT: 9-point model

Pearson correlation coefficients, *n* = 30

	GE15	GE30	*GE45*	GE60	*GE 90*	*GE120*	*GE150*	*GE180*	GE240
BT15	0.432†	0.447†	0.411†	0.423†	0.375†	0.332	0.381†	0.317	0.328
BT30	0.437†	0.560*	0.548*	0.569*	0.5111*	0.494*	0.493*	0.416†	0.366†
BT45	0.392†	0.675*	0.664*	0.688*	0.639*	0.621*	0.598*	0.516*	0.455†
BT60	0.368†	0.740*	0.718*	0.746*	0.731*	0.720*	0.700*	0.623*	0.581*
BT90	0.416†	0.702*	0.690*	0.754*	0.820*	0.838*	0.838*	0.772*	0.691*
BT120	0.481*	0.693*	0.670*	0.731*	0.842*	0.893*	0.905*	0.859*	0.776*
BT150	0.439†	0.583*	0.541*	0.604*	0.746*	0.815*	0.868*	0.851*	0.791*
BT180	0.456†	0.504*	0.453†	0.511*	0.663*	0.725*	0.821*	0.835*	0.801*
BT240	0.257	0.276	0.166	0.213	0.347	0.418†	0.541*	0.601*	0.743*

All correlations marked '*' have a $P \leq 0.01$ and correlations marked '†' have a $P > 0.01$ and ≤0.05. All other correlations are >0.05. The canonical correlation analysis essentially jointly tests whether the correlations in the matrix below are simultaneously zero.
BT = breath test kPCD; GE = proportion of gastric emptying at specified times.

Table 5 Gastric emptying characteristics using 9-point model (Part B)

	All subjects (*N* = 30)		Healthy subjects (*N* = 15)		Dyspepsia (*N* = 15)	
	Scintigraphy	Breath test estimates	Scintigraphy	Breath test estimates	Scintigraphy	Breath test estimates
$t_{10\%}$, min	13.7 ± 10.2	13.4 ± 9.1	8.8 ± 2.9	9.7 ± 3.6	18.5 ± 12.5	17.1 ± 11.3
t_{50}, min	93.6 ± 50.0	91.3 ± 42.3	65.9 ± 16.5	68.3 ± 18.2	121.4 ± 57.1	114.3 ± 47.3
GE 15 min	0.147 ± 0.078	0.145 ± 0.058	0.190 ± 0.065	0.171 ± 0.049	0.103 ± 0.067	0.120 ± 0.057
GE 30 min	0.230 ± 0.104	0.230 ± 0.095	0.282 ± 0.061	0.271 ± 0.069	0.179 ± 0.114	0.189 ± 0.101
GE 45 min	0.324 ± 0.123	0.323 ± 0.116	0.386 ± 0.086	0.375 ± 0.090	0.262 ± 0.126	0.271 ± 0.118
GE 60 min	0.393 ± 0.139	0.391 ± 0.135	0.467 ± 0.096	0.459 ± 0.103	0.318 ± 0.137	0.324 ± 0.131
GE 90 min	0.542 ± 0.171	0.540 ± 0.165	0.652 ± 0.101	0.641 ± 0.114	0.432 ± 0.157	0.439 ± 0.147
GE 120 min	0.665 ± 0.181	0.665 ± 0.174	0.784 ± 0.100	0.775 ± 0.111	0.545 ± 0.164	0.556 ± 0.158
GE 150 min	0.765 ± 0.172	0.763 ± 0.163	0.873 ± 0.096	0.864 ± 0.095	0.656 ± 0.163	0.663 ± 0.156
GE 180 min	0.827 ± 0.164	0.824 ± 0.151	0.925 ± 0.085	0.913 ± 0.084	0.729 ± 0.167	0.735 ± 0.151
GE 240 min	0.911 ± 0.138	0.903 ± 0.118	0.978 ± 0.083	0.958 ± 0.047	0.844 ± 0.167	0.847 ± 0.142

Values are Mean ± SD.

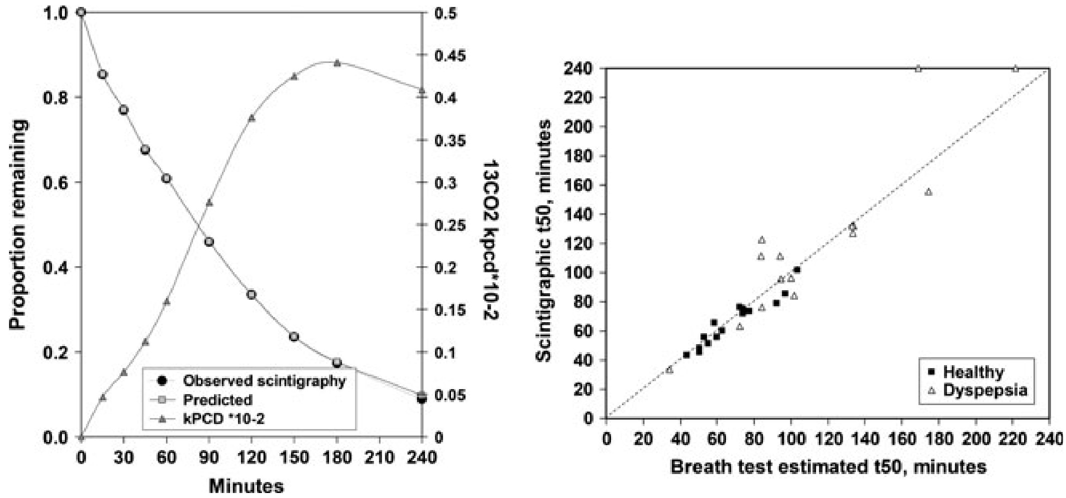

Figure 2 Summary of gastric emptying results from the 9 time-point model in 30 subjects. Data illustrate mean $^{13}CO_2$ enrichment of breath excreted over 4 h (right *Y*-axis $\kappa PCD \times 10^{-2}$), as well as the mean observed proportion emptied from the stomach by scintigraphy (left *Y*-axis) and the mean predicted GE based on the bootstrap regression model using the measured $^{13}CO_2$ excretion. The observed (scintigraphic) and predicted (GEBT) values are nearly identical; hence superimposed. Right panel shows scatterplot of the scintigraphic measured gastric emptying t_{50} values (*Y*-axis) *vs* the breath test-estimated t_{50} values (*X*-axis) obtained from linear interpolation of breath test predicted GE proportions in healthy subjects and patients with dyspepsia based on the nine time-points model. The dotted line shows *X* = *Y*.

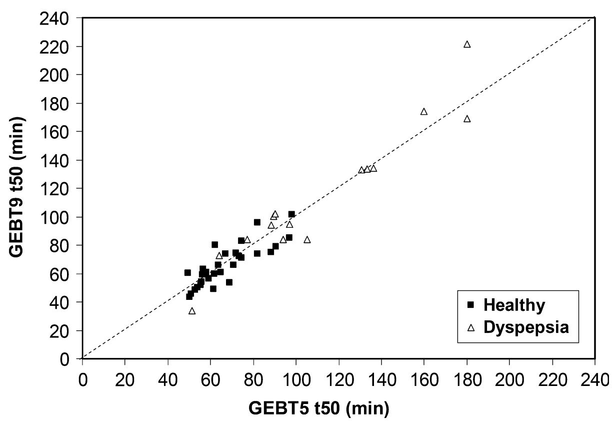

Figure 3 Comparison of breath test-estimated t_{50} values obtained from linear interpolation of breath test predicted GE proportions in healthy subjects and patients with dyspepsia based on the five time points model (*X*- axis) and nine time points model (*Y* axis). The dotted line shows *X* = *Y*.

model was unable to reproduce. For these two individuals, the scintigraphic proportions indicated a t_{50} of at least 240 min based on 9 time points and >180 min based on 5 time points. In these subjects, the estimated t_{50} was 169 and 222 min by GEBT9 and 180 by min GEBT5. At the other extreme, scintigraphy disclosed rapid gastric emptying, as defined by a GE t_{50} shorter than the 10th percentile value, in two healthy subjects and one patient. GEBT9 also demonstrated rapid emptying in this patient and in one of these two healthy subjects. However, none of these three subjects had rapid GE by GEBT5.

The Bland Altman plots demonstrate that the difference between GE t_{50} assessed by scintigraphy and 5 point (left panel) or 9 point (right panel) breath test models was not impacted by the average t_{50} for both tests in the range of GE tested in these cohorts (Fig. 4).

The 10th and 90th percentiles for t_{10} in healthy subjects were, respectively, 5 and 12 min by scintigraphy (9 time points), 6, and 15 min for GEBT 9-point model and 10 and 19 min for GEBT 5-point model. For t_{10}, the CCC for scintigraphy *vs* GEBT 5-point and 9-point models were 0.73 (95% CI, 0.54–0.85) and 0.80 (95% CI, 0.63–0.90), respectively.

Gastric emptying at 30 and 60 min were estimated by linear interpolation between 0 and 45 min and 45 and 90 min, respectively (Fig. 5). Actual (i.e., GEBT 9-point model) and estimated values (i.e., from GEBT 5-point model) were more closely correlated at 60 min [CCC 0.95 (95% CI, 0.91–0.97)] than at 30 min [CCC 0.81 (95% CI, 0.71–0.89)].

DISCUSSION

The primary goals of this study were to validate a different version of a standardized, shelf-stable breath test meal labeled with [^{13}C]-*S. platensis* to measure GE of solids in clinical practice, to appraise the performance characteristics of GEBT5 and GEBT9 *vs* scintigraphy, and to assess the accuracy of 9 compared to 5 breath samples, particularly for characterizing the early

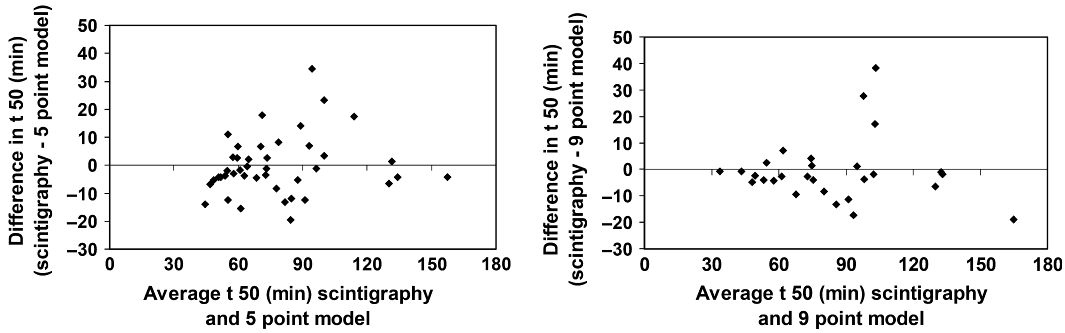

Figure 4 Bland Altman Plots for five point (left panel) and nine point (right panel) for t_{50} estimated by GEBT *vs* scintigraphy.

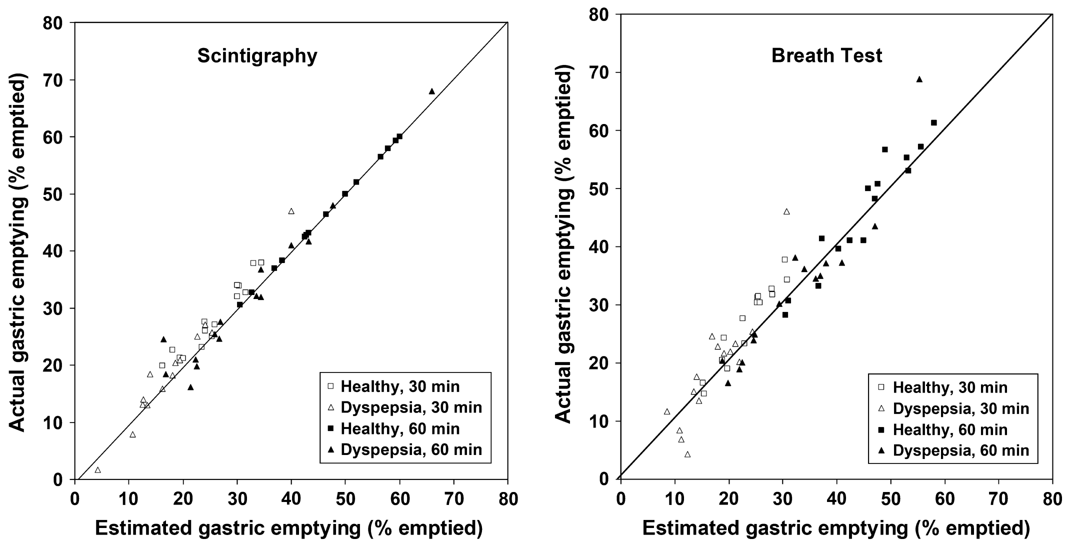

Figure 5 Comparison of actual (nine time-point data) and linearly interpolated (five time-point data) gastric emptying at 30 and 60 min by scintigraphy (left panel) and GEBT estimated proportions (right panel). The linear interpolation estimates were obtained by linear interpolation between 0 and 45 min for gastric emptying at 30 min and between 45 and 90 min for gastric emptying at 60 min.

phase of GE. The study confirms previous observations obtained with a whole, fresh egg meal and with ^{13}C GEBT in our prior studies.[2–6,29] There was excellent concordance between breath excretion of $^{13}CO_2$ and the proportion of isotope-labeled meal emptied from the stomach at specified time points for the 5 and separately for the 9 time-point assessments. The results confirm that the breath test provides a very valid estimate of the GE t_{50}, which is widely used to make clinical decisions and in pharmacodynamic studies. This is supported by the observation that the concordance correlation coefficient for the GE t_{50} measured by scintigraphy and GEBT 5-and 9-point models averaged 0.9 and the average difference between the GE t_{50} calculated by scintigraphy and GEBT was less than 1 min, with a 10–90th percentile range of approximately ±15 min. Likewise, actual and linearly interpolated GE at 60 min was very similar probably because emptying is generally approximately linear between 45 and 90 min.

However, these data suggest that a GEBT with 9 time points may be preferable to a GEBT with five points for identifying rapid GE and for characterizing early GE (i.e., t_{10} and GE 30 min). For example, two of three subjects (one healthy subject, two patients) with rapid GE by scintigraphy also had rapid GE by GEBT9 but none had rapid GE by GEBT5. The correlation between actual (i.e., GEBT 9-point model) and estimated values (i.e., from GEBT 5-point model) for GE at 30 min was modest [CCC 0.81 (95% CI, 0.71–0.89)]. Likewise, for $t_{10,}$ the CCC for scintigraphy *vs* GEBT 5-point model was 0.73 (95% CI, 0.54–0.85) and lower than scintigraphy *vs* the GEBT 9-point model [0.80 (95% CI, 0.63–0.90)]. In our previous study, breath samples at 45 and 180 min had 93% sensitivity at 80% specificity for identifying rapid GE as defined by the scintigraphic t_{50}.[6] However, it is conceivable that an assessment of GE at 30 min may be more useful for identifying some patients with accelerated GE, i.e., those in whom GE is accelerated at 30 min but plateaus later, resulting in a normal t_{50}. Because few patients had rapid GE in this study, further studies are necessary to evaluate the utility of measuring t_{10}, and GE at 30 min by the 9 time-point model in patients with rapid GE.

Moreover, although the vast majority of asymptomatic individuals emptied the meal from the stomach before 80 min, the 240-min observation may be useful, particularly when GE is delayed. For example, when the 5 time-point model suggests a $t_{50} > 180$ min, the 240-min sample is necessary to clarify whether the t_{50} is closer to 180 min or even >240 min, as was observed in two patients.

In summary, the current data confirm that the stable isotope technology developed in earlier studies is also applicable with a meal in which a slightly modified process was used to enrich *S. platensis* with ^{13}C. The [^{13}C]-*S. platensis* GEBT has high reproducibility, external validity and excellent performance characteristics. The statistical models previously proposed, based on linear regression, continue to demonstrate that they are robust to estimate GE in health and disease, and offer further support for the use of this GEBT in clinical practice, epidemiological studies, or clinical research studies. A 5-sample test is as accurate as a 9-point test for identifying normal GE in symptomatic patients. The 9-breath sample test is more accurate for evaluating early GE (e.g., t_{10} and GE at 30 min), which is accelerated in patients with rapid GE, and provides a more comprehensive assessment in some patients with delayed GE. Further studies need to fully address the relative accuracy of the 5- and 9-point models in patients with rapid GE.

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DISCLOSURE

No conflicts of interest exist for all of the authors.

AUTHOR CONTRIBUTIONS

Adil E. Bharucha was principal investigator for the study and contributed to study design and concept, data analysis and interpretation, and manuscript preparation, critical revision, and final approval. Michael Camilleri contributed to study design and concept and critical revision of manuscript. Erica Veil and Duane Burton contributed to study conduct. Alan R. Zinsmeister conducted data analysis and critically revised the manuscript. All authors approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Predicted gastric emptying proportions at different time points based on gender, BMI, and $^{13}CO_2$ excretion at specified times for 5-point model.

Table S2. Summary of gastric emptying results by t_{50} (scintigraphy) in 44 subjects (29 healthy, 15 dyspepsia).

Table S3. Predicted gastric emptying proportions at different time points based on gender, BMI, and $^{13}CO_2$ excretion at specified times for 9-point model.