Is the ^{13}C-Acetate Breath Test a Valid Procedure to Analyse Gastric Emptying in Children?

By C. Gatti, F. Federici di Abriola, L. Dall'Oglio, M. Villa, F. Franchini, and S. Amarri
Rome, Italy and Modena, Italy

Background/Purpose: Scintigraphy is regarded as the "gold standard" procedure in measuring gastric emptying (GE) rates. ^{13}C-acetate breath test (ABT), which already has been validated in adults, is a noninvasive and nonradioactive alternative method. The aim of the current study was to validate ABT against technetium Tc 99m scintigraphy in children affected by delayed GE.

Methods: Sixty children were recruited and divided into 2 groups: group A, 30 healthy controls; group B, 30 patients with gastroesophageal reflux, and scintigraphy-documented DGE (15 neurologically impaired). After an overnight fast, all of them underwent ABT using 25 to 150 mg ^{13}C-acetate. Breath samples were obtained at baseline and then every 10 minutes for 2 hours. The $^{13}CO_2$ to $^{12}CO_2$ ratio in breath samples was analysed by isotope ratio mass spectrometry. Data are expressed as follows: time of peak ^{13}C exhalation ($tP^{13}CO_{2b}$) and half emptying time in ABT ($t_{1/2b}$), and scintigraphy half emptying time ($t_{1/2s}$).

Results: In controls $tP^{13}CO_{2b}$ was 37 $\pm$ 13 minutes and $t_{1/2b}$ 74 $\pm$ 12 minutes. In patients $tP^{13}CO_{2b}$ and $t_{1/2b}$ were, respectively, 65 $\pm$ 26 minutes and 104 $\pm$ 18 minutes $t_{1/2s}$ was 91 $\pm$ 21 minutes. In group B $tP^{13}CO_{2b}$ and $t_{1/2b}$ were delayed significantly compared with controls, respectively, $P < .03$ and $P < .01$. In group B significant correlation between $t_{1/2s}$ and $t_{1/2b}$ was noted ($r_1 = 0.97$). A close correlation was also observed between $t_{1/2s}$ and $tP^{13}CO_{2b}$ ($r_2 = 0.95$).

Conclusion: The ^{13}C ABT is an easy, reliable, and less expensive procedure for measuring GE, and its results closely correlate with those of scintigraphy in a paediatric population.
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INDEX WORDS: Gastric scintigraphy, gastroesophageal reflux, delayed gastric emptying, breath test, ^{13}C.

CONCURRENT DELAY in gastric emptying (DGE) often can be observed in children with symptomatic gastroesophageal reflux (GER). The aetiology of such DGE is linked to a motility disorder of the antrum and occasionally of the entire stomach, and it is often accompanied by oesophageal dysmotility. The incidence of DGE may be higher than 50% in children with neurological impairment (NI), and it may significantly affect medical or surgical management.[1]

Radionuclide scintigraphy, first introduced by Griffith et al,[2] being noninvasive, quantitative, and physiological, has emerged as the "gold standard" procedure for measuring gastric emptying (GE) rates of solids and liquids. This radioisotope technique is simple and noninvasive but presents significant practical problems; it is relatively expensive (γ- camera with computer system), involves radiation exposure, and, especially with non co-operative subjects, may cause false detection of activity.

From the Digestive Surgery Unit, Bambino Gesú Children's Hospital, Rome, Italy, and the Department of Paediatrics, University of Modena, Italy.

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Address reprint requests to Claudia Gatti, MD, Via di Montefiore 3, 00156 Rome, Italy.

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In recent years, ^{13}C-stable isotope has been used as alternative method in measuring GE rates of liquid and solid meals in adults.[3-5] Parameters obtained from ^{13}C-labelled breath tests (BT) have shown a good correlation against results obtained with the scintigraphic method. ^{13}C octanoate and ^{13}C acetate BTs (ABT) have been used to measure the GE of solid and liquid phases with different test meals. BTs using ^{13}C labelled test meals are based on the principle that the GE of the tracer substance proceeds simultaneously with the emptying of the labelled phase of the test meal. After delivery to the stomach the tracer is absorbed rapidly and oxidised into labelled CO_2, which is then exhaled rapidly. The metabolism of the ingested ^{13}C-labelled substrate into labelled CO_2 and its exhalation in the breath are indirect parameters of GE.

The aim of the current study was to test ^{13}C ABT validity against technetium Tc 99m scintigraphy in children (also NI) affected by symptomatic GER and concurrent DGE.

MATERIALS AND METHODS

Subjects

In a period ranging from June to December 1998 60 children (mean age, 4.4 $\pm$ 3.5 years) were recruited for this study, and they were divided into 2 groups: group A, 30 symptom-free healthy controls (12 boys and 18 girls), assuming that healthy children have no DGE; group B, 30 patients (13 boys, 17 girls) with GER and scintigraphy-

documented DGE. Fifteen patients had NI. Average ages of the 2 groups were not significantly different. None of the healthy participants complained of gastrointestinal symptoms or had a history of previous abdominal surgery. Patients did not present any malabsorption or metabolism disorders or alteration of pulmonary function. NI was not so severe to compromise test execution and sample collection. Neither controls nor the patients received any medication with potential influence on gastrointestinal motility during the entire study. To evaluate day-to-day variability of gastric emptying, ABT was performed twice (at 2-day intervals) in all subjects. The study was performed according to the declaration of Helsinki, and written informed consent was obtained from parents.

Test Meal

All acetate BTs were carried out using 25 to 150 mg of ^{13}C acetate to the children at the time of their usual morning feeding, allowing for 3 to 8 hours of prior fasting according to age. A standard test meal was used. Up to 2 years of age milk amounts normalised per body weight; 200 mL of milk was given to those over 2 years of age. Total caloric content varied from 105 to a maximum of 160 kcal. The meal was consumed within 5 minutes in all subjects.

Acetate doses were established in accordance to CO_2 production linked to body surface area: 25 mg in neonates, 50 mg in infants, 100 mg in children, and 150 mg in adolescents. In patients (group B), the same meal used for BT was labelled with 0.5 MBq (children $<$5 years old) or 1 MBq (children $>$5 years old) ^{99m}Tc-albumin colloid and administered for a simultaneous scintigraphy.

^{13}C-Acetate BT

Double breath samples were collected at baseline (0 minutes) and at 10-minute intervals during the 2 hours after ingestion of the test meal. During the expiration phase, 20 mL of exhaled air were collected by a modified Ambu in all children. Each breath sample was collected in 2 vacutainers containing 10 mL each. $^{13}CO_2$ enrichment was measured using a gas chromatograph-isotope ratio mass spectrometer (Europa Scientific, Crewe, England; mod. ABCA-Automated ^{13}CO Carbon Analyser) and expressed as δ values. Carbon dioxide was first purified in a gas chromatograph before its measurement in an isotope ratio mass spectrometer. Total CO_2 production was assumed from the body surface area (5 mmol/m^2/min); body surface area was calculated according to the weight-height formula.[6] The results were presented as δ over baseline values, percentage of ^{13}C expired per minute, and cumulative ^{13}C recovery over a 2-hour period.

Scintigraphy Technique

A dual-headed gamma camera with a low energy collimator and interfaced with a computer was used for data acquisition. Data were recorded using a 20% window around the 140 keV ^{99m}Tc peak. The study was performed with the subject in a sitting or supine position. External ^{99m}Tc point sources were taped to the anterior and posterior thorax and abdomen to allow accurate repositioning of the subject between image acquisitions. Scanning scintigraphic information was obtained every 10 minutes up to 1 hour and every 15 minutes for another hour. The geometric mean value was calculated for each time interval and corrected for radioactive decay.

Mathematical and Statistical Analysis

Results were expressed as mean ± SD. GE of liquids was assessed using a nonlinear regression formula: $y = m\,(1 - e^{-kt})^{\beta}$ to fit the curve of the cumulative ^{13}C recovery when time is infinite. Nonlinear regression analysis was performed using commercial software programs (EXCEL 4.0 program; Microsoft Corp, Redmond, WA). Gastric half-emptying time of the breath test ($t_{1/2b}$) was determined by the function: $t_{1/2b} = -1/k\,\ln(1 - 2^{-1/\beta})$. The β value described the initial phase of emptying. Time of peak ^{13}C exhalation ($tP^{13}CO_{2b}$) also was obtained. Results were indicated as mean ± SD. Mean $tP^{13}CO_{2b}$ and mean $t_{1/2b}$ in healthy subjects versus B group were compared using paired *t* test. The estimated parameters from scintigrafically measured gastric emptying ($t_{1/2s}$) and by breath test assessment ($t_{1/2b}$, $tP^{13}CO_{2b}$) were plotted against each other to assess the association between the different approaches to measure gastric emptying. Correlation and linear regression was used.

The coefficient of intrasubject variation as a measure of reproducibility is described by the equation $CV_{intra} = SD_d/(mV2)$, where *m* is the mean value and SD_d is the SD of the differences between replicates.[7]

RESULTS

The half-emptying time of the controls was 74 ± 12 minutes. In the same group $tP^{13}CO_{2b}$ was 37 ± 13 minutes. In group B the time of peak $^{13}CO_2$ exhalation was 65 ± 26 minutes and half-emptying time was 104 ± 18 minutes.

Figure 1 illustrates the 2 GE parameters, $tP^{13}CO_{2b}$ and $t_{1/2b}$ in group A and B. Paired *t* test showed that the mean time of the $tP^{13}CO_{2b}$ and the mean half-emptying time were significantly delayed in patients compared with the 30 healthy controls ($P < .03$ and $P < .01$). Scintigraphic half-emptying time in group B was 91 ± 21 minutes.

Figure 2 shows a close correlation between $t_{1/2b}$ and the scintigraphic half-emptying time of GE ($r_1 = 0.97$). The regression model for $t_{1/2b}$ on $t_{1/2s}$ was estimated to be $y = 0.838 \times + 28.474$ ($R^2 = 0.957$). Similarly, when comparing $tP^{13}CO_{2b}$ to $t_{1/2s}$, we find a close linear relationship with an r_2 value of 0.95 (Fig 3). In this case the linear regression curve was $y = 1.22 \times - 45.61$ with $R^2 = 0.9073$.

The coefficients of variation for $t_{1/2b}$ and $tP^{13}CO_{2b}$ were 5.34% and 6.17%, respectively. Concerning $t_{1/2b}$ and $tP^{13}CO_{2b}$, there were no significant differences between the data obtained on 2 separate days, with a good degree of reproducibility both in controls and patients.

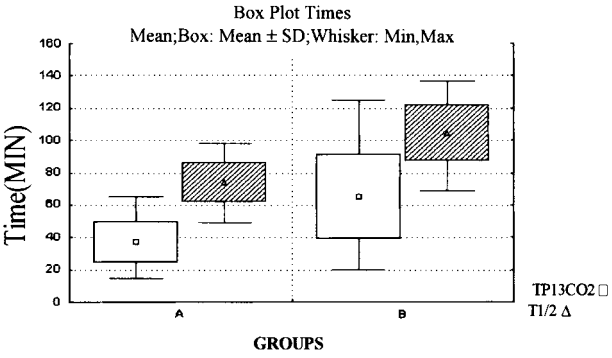

Fig 1. Plot shows the 2 GE parameters, $tP^{13}CO_2$ (□) and $t_{1/2b}$ (◁) in group A and B. Symbols indicate the mean in each group, boxes show the range, mean ± SD. Whiskers demonstrate extremes of the distribution. Paired *t* test shows that in patients the mean time of the $tP^{13}CO_2$ and the mean half-emptying times are delayed significantly compared with the 30 healthy controls ($P < .03$ and $P < .01$, respectively).

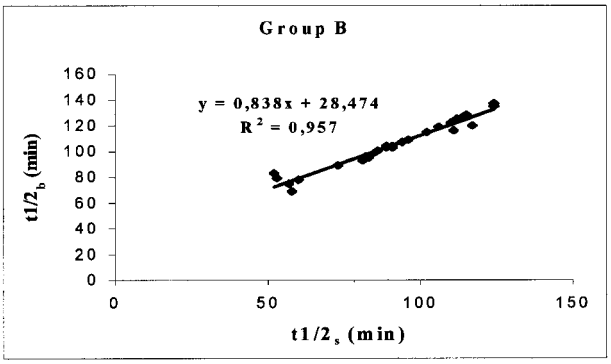

Fig 2. Comparison of $t_{1/2b}$ determined by ABT with $t_{1/2s}$ determined by scintigraphy for group B patients. A good correlation is seen ($r_1 = 0.97$).

DISCUSSION

GE is a complex physiological process that is linked to multiple factors. Factors such as food particle size, consistency, osmolality, viscosity, fibre, fat, or protein composition can influence the rate of emptying as can gastrointestinal hormone responses, diurnal variation, and intercurrent illness. Solid and liquid phases of a meal are delivered from the stomach in different patterns. Current thinking about the physiology of GE involves 2 separate and independent motor functions of the stomach: the fundus is considered to control the emptying rate of liquids, and the antrum is thought to control that of solids.

In patients with symptomatic GER, particularly those with NI, a high prevalence of autonomic neuropathy is documented in which oesophagogastric transit and GE frequently are delayed. In children affected by severe NI, a higher than 50% incidence of DGE is observed. In these patients, several mechanisms concur to define the GE process depending on the balance between propulsive forces and resistance to outflow: gastric accomodation and tone, antral contractivity, antroduodenal coordination, pyloric function, and entero-enteric reflexes.

The scintigraphic technique generally is accepted as the gold standard test for measuring GE, but it requires the availability of expensive equipment with specialised trained personnel and involves exposure to gamma radiation. Nonscintigraphic techniques have been developed to study GE, including applied potential tomography, acethaminophen[8,9] absorption, or ultrasonography. These tests do not expose patients to radiation, and they are most effective in evaluating the emptying of liquids. However, paracetamol should not be used in paediatric patients, especially not with infants because of possible toxic side effect.[10]

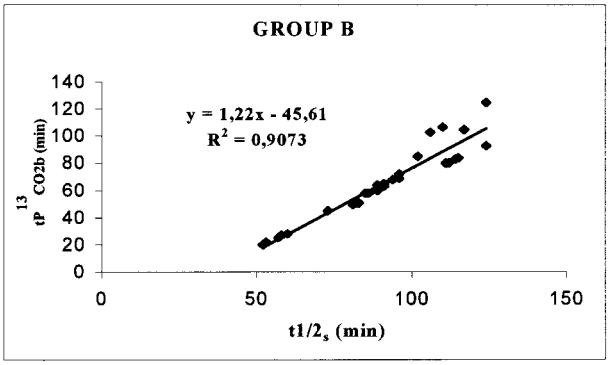

Fig 3. Comparison of $tP^{13}CO_2$ determined by ABT, with $t_{1/2s}$ determined by scintigraphy for group B patients. A close correlation is observed ($r_2 = 0.95$).

In recent years, ^{13}C-stable isotope has been reported as a possible substitute for scintigraphy in measuring GE rates of liquid and solid meals. ^{13}C octanoate and ^{13}C acetate are two carbon volatile fatty acids that have been used to measure GE of solid and liquid phases with different test meals. BTs using ^{13}C-labelled test meals are based on the principle that the marker is absorbed rapidly from the duodenum after GE. Then it is carried to the liver where it subsequently is oxidised into $^{13}CO_2$. More than 80% of the ^{13}C label appears as $^{13}CO_2$ in expired air.

^{13}C-octanoate and ^{13}C ABTs have been used and validated against the scintigraphic technique in adults where an excellent correlation of the GE coefficients $tp^{13}CO_{2b}$ and the $t_{1/2b}$ with the scintigraphic half-emptying time has been reported.[3,4] There are few studies reporting on use of BTs in the paediatric population. A recent study[11] presents values for GE in healthy children obtained by the ^{13}C-octanoate BT. These values are in keeping with those for GE of solid in healthy adults. Veereman-Wauters et al[12] have utilised ^{13}C-octanoate BT in preterm infants, using a little soluble test meal with a high fatty composition, which can stimulate vomiting, being a contraindication in patients affected by GER disease (GERD). GERD is difficult to analyse if a solid meal is used. ABT analyses the liquid phase of GE, which is considered a specific aspect of GERD and requires a test meal that can be more easily standardised particularly in paediatric population; moreover, it permits the use of a meal with a lower fatty percentage than ^{13}C-octanoate BT.

Because DGE is one of the principle mechanisms responsible for GERD, it seems essential to develop an alternative diagnostic method for GE studies that, in the future, could replace conventional scintigraphy especially in paediatric population.

We know of no previous reports in literature that have tried to validate ^{13}C ABT against scintigraphy in paediatric population. We have applied ^{13}C ABT to evaluate DGE in children affected by GERD and particularly focusing this method on NI patients. Our findings suggest that BT parameters like $t_{1/2b}$ and $tP^{13}CO_{2b}$ are impaired in children with DGE; thus, they can be used to valuate a delay in GE also in children.

It is important to evidence that one of the major difficulties in interpreting the results of GE studies is intraindividual variability. Choi et al[13] have reported the

good reproducibility of BT technique, showing that their data represent the intrinsic variability in the normal GE rate. Our study on repeated breath testing has shown impressive reproducibility. Our data demonstrate a coefficient of variation for $t_{1/2b}$ and $tP^{13}CO_{2b}$ of 5.34% and 6.17%, respectively. The ^{13}C ABT presents several advantages over ^{99m}Tc scintigraphy. The ^{13}C ABT uses a stable carbon isotope and it avoids the risk associated with exposure to ionizing radiation.[14,15] Moreover, the test meal requires a lower fatty percentage than ^{13}C octanoate and can be used safely in paediatric patients with dyspeptic symptoms.

The test is easy to perform and is safe. Although there are few sites that have access to scintigraphy equipment, the ^{13}C ABT can be performed at any location, even at the bedside by a well-trained parent. Because it is totally noninvasive, the ^{13}C ABT can be repeated in the same subject on consecutive days and is a promising tool for clinicopharmacological studies.

It is finally important to stress the costs. Scintigraphy examinations can cost up to $400. The ^{13}C-acetate substrate costs approximately US$10 for each test. Each test costs approximately $192, which is about half the cost of scintigraphy. The investment for a gamma-camera and security conditions linked to radioactive isotopes are much more expensive than mass spectrometry equipment. Although gamma-cameras are more accessible than isotope ratio mass spectrometers, children are obliged to remain in front of a gamma-camera for the entire time of investigation. ABT is not restricted to the laboratory, where the analysis takes place. The specimens are mailed to a test centre. Additionally, the new low cost nondispersive infrared spectrometers[16] and the potential uses of isotope ratio mass spectrometry in gastroenterology (such as for urea and liver functions breath tests) may facilitate the clinical spread of ^{13}C.

We advocate a much more widespread use of such diagnostic modality; however, further studies are needed, before routine use, to overcome technical difficulties that may arise.

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Discussion

L. Kapila (Nottingham, England): It is a very elegant study, but I can imagine in a toddler it must be very difficult to put the mask on their face.

C. Gatti (response): Sometimes children are in their mother's lap. In case of older children, the test is like a joke; sometimes it is difficult to collect the samples, but we try. The results are good.

Question: Congratulations on your paper; it is really good. Does liver function affect the results of your tests?

C. Gatti (response): In our study we excluded all patients that had respiratory function problems and liver function problems. These are 2 contraindications to the test.

Professor Cozzi (Rome, Italy): This is a relatively new investigation so I found your presentation very interesting. Do you think that the severity of reflux can in anyway affect the results of this test?

C. Gatti (response): The aim of our study was to study delayed gastric emptying; we did not notice that results can be affected by the severity of GER.