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Original

Simultaneous $^{13}C/^{14}C$ dual isotope breath test measurement of gastric emptying of solid and liquid in normal subjects and patients: comparison with scintigraphy

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Abstract

BACKGROUND: To develop a simple method for simultaneous solid and liquid gastric emptying assessment using a dual isotope labelled breath test.

MATERIAL AND METHODS: 13 patients were given 100 g ground beef labelled with 25 MBq ^{99m}Tc sulphur colloid and 74 KBq ^{14}C octanoic acid, and 150 ml 10% glucose drink labelled with 8 MBq ^{67}Ga citrate and 150 mg ^{13}C acetate. 10 normal volunteers were given the same test meals but labelled with ^{14}C and ^{13}C only. Breath was collected at baseline and regularly for 4 hours. The $^{14}CO_2$ and $^{13}CO_2$ activity was measured with liquid scintillation counting and mass spectroscopy. The times to maximum $^{14}CO_2$ and $^{13}CO_2$, were determined. Comparison was made between times to maximum $^{14}CO_2$ with scintigraphic retention of ^{99m}Tc at 100 minutes and times to maximum $^{13}CO_2$ with the scintigraphic half-clearance time of ^{67}Ga.

RESULTS: For the solid meal, the times to maximum $^{14}CO_2$ were: 60–120 minutes in the 8 patients with normal gastric emptying of ^{99m}Tc; 75–145 minutes for the 10 healthy volunteers; 75–180 minutes for the remaining 5 patients with abnormal gastric emptying of ^{99m}Tc. There was a weak but significant correlation ($r = 0.56$, $p < 0.025$) between the time to maximum $^{14}CO_2$ and gastric retention of ^{99m}Tc at 100 minutes. For the liquid meal, times to maximum $^{13}CO_2$ were: 20–35 minutes for the 4 with normal gastric emptying of ^{67}Ga; 15–40 minutes for the 10 healthy volunteers; 20–75 minutes for the remaining 9 patients with abnormal gastric emptying of ^{67}Ga. There was a strong and significant correlation ($r = 0.88$, $p < 0.005$) between times to maximum $^{13}CO_2$ and gastric half-clearance time of ^{67}Ga.

CONCLUSIONS: Breath tests utilising test meals labelled with $^{*}C$ isotopes are valid alternatives to scintigraphic studies using ^{99m}Tc and ^{67}Ga for the simultaneous assessment of gastric emptying of solids and liquids.

Key words: ^{14}C octanoic acid, ^{13}C acetate, breath test, gastric emptying

Introduction

Delayed gastric emptying may result from a variety of motility disorders which include autonomic neuropathy associated with diabetes mellitus, the post gastric surgery state, and connective tissue disorders such as scleroderma. Whilst the associated symptoms maybe quite debilitating to some, the clinical presentation can be non-specific in others. An accurate and reproducible method of assessment of the rate of gastric emptying is thus desirable

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for the diagnosis of delayed gastric emptying in those with nebulous symptoms. In those with known or proven delayed gastric emptying, a reliable method of gastric emptying measurement serves as an objective tool for determining the patient's response to treatment. In addition it may be advantageous to have a technique available which does not require the patient's attendance in a nuclear medicine department.

Scintigraphic study using a radio-labelled meal is a well established method of assessing gastric emptying [1]. A dual isotope meal consistent of ground beef labelled with ^{99m}Tc sulphur colloid and a glucose drink labelled with ^{67}Ga citrate has been validated in our department [2], and is a regular clinical test.

Scrambled egg labelled with ^{14}C octanoic acid (which distributes in the lipid phase) has been used as the solid meal to assess gastric emptying by breath testing. After ingesting the test meal, the quantity of $^{14}CO_2$ in the patient's breath is measured. Maes and Ghoos et al. [3–5] have shown that the rate of gastric emptying of the test meal is the rate limiting step in the recovery of $^{14}CO_2$ in the breath. Mossi et al. [6] have shown that a glucose meal labelled with ^{13}C acetate is a technique that can be used to assess gastric emptying of liquids. After ingestion of the test meal, the rate of recovery of $^{13}CO_2$ was determined.

The aim of this study was to validate an alternative dual isotope breath test technique using ^{14}C octanoic acid labelled ground beef and ^{13}C acetate labelled glucose drink for the simultaneous assessment of gastric emptying of solid and liquid meals using our established current dual isotope ^{99m}Tc sulphur colloid/^{67}Ga citrate scintigraphic method, applying a unique quadruple isotope design.

Material and methods

Subjects

Patients. 13 patients (9 females, age 29–73) with suspected gastric emptying abnormality were recruited for scintigraphic study. After an overnight fast these patients were given 4 isotopes: a 100 g ground beef meal labelled with ^{99m}Tc sulphur colloid and ^{14}C octanoic acid, and a glucose drink labelled with ^{67}Ga citrate and ^{13}C acetate.

10 healthy volunteers (7 females) also agreed to participate. These subjects did not have a current or past medical history of gastrointestinal disorder, were not on medications that could potentially affect gastrointestinal motility and had not had previous gastrointestinal surgery. After an overnight fast, the volunteers were given 2 carbon isotopes: a ground beef meal labelled with ^{14}C octanoic acid, and a glucose drink labelled with ^{13}C acetate. To reduce their radiation exposure, scintigraphy was not performed in these subjects.

All participants gave written informed consent. The study protocol was approved by the Royal Adelaide Hospital Human Research Ethics Committee.

Test meal

The solid meal consisted of a 100 gm ground beef which was fried with 5 mls of egg yolk which was labelled with 75 MBq octanoic acid sodium salt [1 - ^{14}C] (ICN Pharmaceuticals, CA). For the 13 patients, their beef was also fried with 5 mls of egg white labelled with 25 MBq ^{99m}Tc Rhenium sulphide colloid (RAH Radiopharmacy, Adelaide Australia). This solid meal is estimated to contain 200 KCal.

The liquid meal consisted of 150 mls of water and 16 g D-glucose monohydride (10%), containing 100 mg acetate [1 - ^{13}C], (Cambridge Isotope Labs, Andover, MA). For the 13 patients, their drink was also labelled with 8 MBq ^{67}Ga citrate. This liquid meal is estimated to contain 64 KCal.

Scintigraphy

For the 13 patients who received ^{99m}Tc sulphur colloid and ^{67}Ga citrate in their meals, upper abdominal imaging was performed using a single headed gamma camera, (Siemens Basicam), with a medium energy all purpose collimator, 64 x 64 matrix. Posterior views were obtained with the patient sitting upright, regions of interest were drawn for the stomach and small intestines, and depth correction included. The data was processed as per Colins et al. [2]. As with the gastric emptying study protocol, 2 standard parameters were generated:

1. For the solid meal: the percentage scintigraphic gastric retention of ^{99m}Tc at 100 minutes (normal range between 4 and 61%).
2. For the liquid meal: the time taken for the stomach to clear half of the ^{67}Ga (normal range between 4 and 31 minutes).

Breath test

From all participants, breath samples were collected in 2 types of containers:

1. Glass vials containing a trapping solution — collecting 0.5 mmol CO_2. The quantity of breath $^{14}CO_2$ was measured by scintillation counting. The unit of $^{14}CO_2$ activity is disintegrations per minute or DPM.
2. Evacuated glass vials. The breath ^{13}C enrichment was determined by isotope ratio mass spectroscopy, and expressed as the difference (or delta) between the excess $^{13}CO_2$ recovered in the breath samples above the level of $^{13}CO_2$ in natural background CO_2.

Breath samples were collected at baseline and then at 15 minute intervals for 4 hours. The activity/quantity of $^{14}CO_2$/$^{13}CO_2$ were then plotted against time. The times to reach maximum breath $^{14}CO_2$ and $^{13}CO_2$ were then simply estimated from the plotted graphs of best fit for each subject without the use of non linear regression modelling on the data to determine the time of peak breath $^*CO_2$. For the 13 patients, the time to maximum breath $^{14}CO_2$ was then compared with the scintigraphic gastric retention of ^{99m}Tc, and the time to maximum breath $^{13}CO_2$ was compared with the gastric half clearance of ^{67}Ga. For the 10 healthy volunteers, the times to maximum breath $^{14}CO_2$ and $^{13}CO_2$ were used to determine the "healthy" or "normal" ranges. In addition to comparing the breath test results with our established scintigraphic method, we were also curious as to how our breath test data would compare when analysed using other published techniques. Thus we applied the mathematical model as proposed by Maes et al. [5] in determining the half clearance time. The scintigraphic and the breath test data were compared. Pearson's correlation coefficient was then determined for each comparison.

Results

For the solid meal, the time to maximum breath $^{14}CO_2$ for the 10 healthy volunteers ranged between 75 and 145 minutes (mean 100 ± 25.5 minutes). Among the 13 patients, 8 who had normal

Table 1. A summary of the results

SOLID: Times to maximum $^{14}CO_2$ [min] (number of subjects)			LIQUID: Times to maximum $^{13}CO_2$ [min] (number of subjects)		
	Patients (13)	Volunteers (10)		Patients (13)	Volunteers (10)
Normal ^{99m}Tc retention of at 100 min (4–61%)	60–120 min (8)	75–145 min mean: 100 ± 25.5	Normal scintigraphic half--clearance time of ^{67}Ga (4–31 min)	20–35 min (4)	15–40 min mean: 28.7 ± 8.6
Abnormal	75–180 min (5) r = 0.56		Abnormal	20 – 75 min (9) r = 0.88	

percentage gastric ^{99m}Tc residual activity at the 100 minute mark which ranged between 25 and 61% (our normal reference range for the 100th minute gastric residual is 4–61%), had times to maximum breath $^{14}CO_2$ ranging between 60 and 120 minutes. The remaining 5 had prolonged gastric retention of ^{99m}Tc of between 71 to 86%, had times to maximum breath $^{14}CO_2$ of between 75 to 180 minutes (Table 1).

There is a weak correlation, ($r = 0.56$, $p < 0.025$) between time to maximum breath $^{14}CO_2$ and scintigraphic gastric retention of ^{99m}Tc (Fig. 1).

For the liquid meal, the time to maximum breath $^{13}CO_2$ for the 10 healthy volunteers ranged between 15 and 40 minutes (mean 28.7 ± 8.6 minutes). Of the 13 patients, 4 had normal gastric half emptying times of ^{67}Ga which ranged between 17 and 28 minutes (our normal reference range for gastric half emptying time of ^{67}Ga is 4–31 minutes), had times to maximum breath $^{13}CO_2$ ranging between 20 and 35 minutes. The remaining 9 patients had delayed gastric half emptying times of ^{67}Ga of between 33 to 106 minutes, had times to maximum breath $^{13}CO_2$ of between 20 to 75 minutes (Table 1)

There is stronger correlation, $r = 0.88$ ($p < 0.005$), between the time to maximum breath $^{13}CO_2$ and the time to half clearance of ^{67}Ga (Fig. 2).

On comparing our established scintigraphic method with the breath test results using the mathematical model as proposed by Maes and Ghoos [1] in determining the half clearance time we found a non-statistically significant poor correlation for both the solid meal ($r = 0.54$, $p > 0.1$) and liquid meal ($r = 0.52$, $0.05 > p > 0.1$).

Discussion

Octanoic acid is a medium chain fatty acid which when ingested undergoes preferential hepatic metabolism and is not incorporated into triglyceride for storage. From the works of Maes et al. [3], the ^{14}C labelling of octanoic acid is quite robust and can withstand gastric acid retaining up to 87% of label at 180 minutes in *in vitro* experiments. They have also shown that ^{14}C octanoic acid binds well when baked with egg yolk. Using ^{14}C octanoic acid labelled scrambled eggs they have demonstrated that from the time of ingestion of the test meal to the appearance of ^{14}C as $^{14}CO_2$ in the breath, the rate limiting step is the gastric emptying

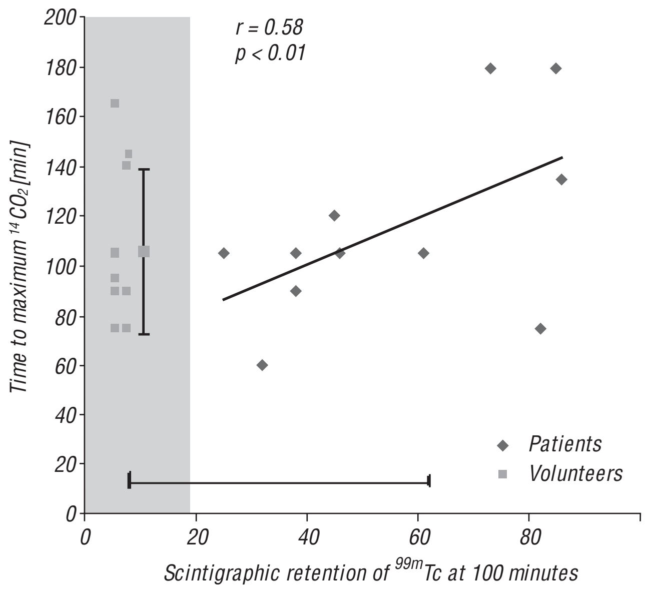

Figure 1. Solid meal: time to maximum breath $^{14}CO_2$ v. scintigraphic gastric retention of ^{99m}Tc at 100 minutes. For the healthy volunteers, the mean time to maximum breath $^{14}CO_2$ ± 1 SD is represented on the left (in the grey box).

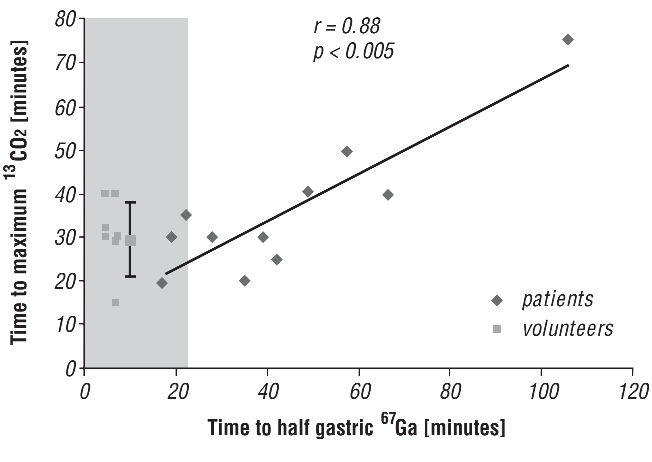

Figure 2. Liquid meal: time to maximum breath $^{13}CO_2$ v. scintigraphic gastric half emptying times of ^{67}Ga. For the healthy volunteers, the mean time to maximum breath $^{13}CO_2$ is represented on the left (in the grey box).

step [4]. Assuming that CO_2 production is 300 mmol per square meter body surface area per hour, the percentage recovery and the cumulative recovery of excreted $^{14}CO_2$ in the breath versus time were determined and characterised by non-linear regression formulae [3]. Constants derived from these formulae were then utilised to generate 3 parameters of gastric empting: gastric emptying coefficient, the half emptying time, and the lag phase [3]. For these 3 parameters they found good correlation with scintigraphic studies using ^{99m}Tc albumin colloid. Choi et al. [7] gave eggs labelled with ^{13}C octanoic acid and ^{99m}Tc pertechnetate to healthy volunteers and showed a weak but significant correlation between gastric half emptying times determined by scintigraphy and by breath test. They found no correlation between lag times determined by scintigraphy and by breath tests. The reason for the lack of validity for interindividual comparisons is purported by Lee et al. [8] to possibly be attributable to the non linear regression models used to fit the cumulative breath $^{13}CO_2$ recovered. Using an egg meal containing ^{13}C Spirula platensis and labelled with ^{99m}Tc sulphur colloid they compared breath test with $^{13}CO_2$ recovery versus scintigraphic study for the evaluation of gastric emptying in 30 healthy volunteers. Applying a generalised linear regression model to the $^{13}CO_2$ data they found comparable estimations of $t_{1/2}$ and t_{lag} as determined by scintigraphic data. Additionally, in 27 out of the 30 subjects, using a prepacked meal fg (^{13}C S platensis biscuit) the predicted $t_{1/2}$ was found to closely approximate the $t_{1/2}$ as determined by the breath test technique using the egg meal, the difference being consistent with normal variations in gastric emptying.

Acetate is a 2 carbon molecule which when ingested is readily absorbed and metabolised. Mossi et al. [6] showed that after ingestion of ^{13}C acetate, > 80% appear in the breath as $^{13}CO_2$. In 8 healthy volunteers, a multiluminal tube was placed in the 1st part of the duodenum, infused with a non absorbable indicator phenol red, and then aspirates analysed to determine the duodenal flow rate. The volunteers were then fed 4 different test meals (glucose, amino acid, fat and Ensure) labelled with ^{13}C acetate and another non absorbable indicator (0.6% Polyethylene glycol 4000, PEG 4000). An index of gastric emptying was then determined for each meal as the product of the duodenal flow rate (as derived from the first non absorbable indicator) and the duodenal aspirate concentration of the PEG 4000. The appearance of $^{13}CO_2$ in the breath was found to closely reflect the gastric emptying of the 4 test meals in this "double-indicator technique".

While scintigraphy is a well established and reproducible method of assessing gastric emptying rates [1, 2], there are some drawbacks. An alternative method using breath test offers numerous potential advantages. The first advantage is the lower radiation burden to the patient. With 75 KBq ^{14}C-octanoic acid (^{13}C-acetate being non radioactive), the effective dose is about 2.3 μSv, compared with an effective dose of 2690 μSv with 25 MBq ^{99m}Tc-sulphur colloid and 8MBq ^{67}Ga citrate [9]. The breath test technique without needing a gamma camera for imaging is far more portable, which thus allows testing patients in remote sites. The improved portability of the test also translates into improved patient comfort as patients need not sit upright against the gamma camera as they consume the test meal. The analysis of the scintigraphic data can be complicated when there is overlap of the image of the small intestines over the stomach

Hence we sought to validate a breath test technique against our established scintigraphic method. Unlike previous authors we chose to use our own test meal of egg burger and also to compare our breath test results with our usual scintigraphic parameters of gastric emptying. We also simultaneously assessed gastric emptying for solids and liquids with participating patients being test cases for a direct comparison of ^{14}C-octanoic acid with ^{99m}Tc-sulphur colloid, and ^{13}C-acetate with ^{67}Ga citrate. The $^{13}CO_2/^{14}CO_2$ results of healthy volunteers served to determine the "normal" ranges.

Also unlike previous authors [1–3, 5] we did not use complex formulae which apply non linear regression models to determine the parameters of $t_{1/2}$ and t_{lag}. Instead we applied a much more simplistic approach of determining the time to maximum recovery of breath $^{14}CO_2$ for solids and $^{13}CO_2$ for liquids.

In our study of the solid meal, we found a weak but statistically significant correlation between time to peak $^{14}CO_2$ and gastric retention of ^{99m}Tc at 100 minutes. Factoring in all patients with normal gastric emptying of solids by scintigraphic analysis and all healthy volunteers, the "normal range" (mean ± 2 SD) should be 55.2–142.6 minutes.

In our study of the liquid meal, we found a strong correlation between the peak $^{13}CO_2$ and the gastric half emptying times of ^{67}Ga. Factoring in all patients with normal gastric emptying of liquids by scintigraphic analysis and all healthy volunteers, the "normal range" (mean ± 2 SD) should be 13.2–44.2 minutes. Of interest our results closely approximates the results by Gatti et al. [10] who also measured the time to peak $^{13}CO_2$ excreted following ^{13}C acetate liquid (milk) meals given to children with gastro-oesophageal reflux and healthy controls. Among the healthy children, the time to peak $^{13}CO_2$ measured was found to have a mean of 37 ± 13 minutes.

Conclusions

Breath test technique using ^{14}C-octanoic acid labelled egg burger and ^{13}C acetate labelled glucose drink correlates with scintigraphic method using the same test meal labelled with ^{99m}Tc sulphur colloid and ^{67}Ga citrate respectively, and thus is a valid alternative method for the simultaneous assessment of gastric emptying of solid and liquid meals.

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