Calcium Carbonate Breath Test for Non-Invasive Estimation of Gastric Acid Secretion

Hirohiko Shinkai,¹ Katsunori Iijima,¹ Tomoyuki Koike,¹ Kenichiro Nakagawa,¹ Ryuhei Maejima,¹ Hiroyuki Endo,¹ Nobuyuki Ara,¹ Naoki Asano,¹ Akira Imatani,¹ Shuichi Ohara² and Tooru Shimosegawa¹

¹Division of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan
²Department of Gastroenterology, Tohoku Rosai Hospital, Sendai, Miyagi, Japan

Gastric acid measurement is useful in assessing the effectiveness of antisecretory drugs, however, the conventional tests involve invasive nasogastric intubation. Orally administered ¹³C-labeled calcium carbonate (Ca¹³CO₃) reacts with gastric acid to produce ¹³C-labeled carbon dioxide (¹³CO₂), which is then excreted in the breath. The objective of this study was to evaluate the suitability of Ca¹³CO₃ breath test for estimating gastric acid secretion in human noninvasively. First, the Ca¹³CO₃ breath test and the measurement of pooled gastric acid under a fasting condition were performed in 6 healthy volunteers to evaluate the correlation between the two parameters. Next, endoscopic gastric acid collection and the Ca¹³CO₃ breath test were performed on different days after pentagastrin injection in 20 subjects to evaluate the correlation between the tests and the reproducibility. Finally, the same studies were repeated in 4 subjects before and after 1-week rabeprazole, a proton pump inhibitor, administration. The maximum ¹³CO₂ concentration (Cmax) correlated very well with the amount of pooled gastric acid (r = 0.95), suggesting that Ca¹³CO₃ breath test values well reflected the fasting intragastric acidity. The ¹³CO₂ concentration after pentagastrin injection correlated well with pentagastrin-stimulated maximal acid output (r = 0.79 at 20 min). The reproducibility of the Ca¹³CO₃ breath test under pentagastrin-stimulation was good (coefficient of variation = 0.11). Rabeprazole administration markedly reduced the values of the Ca¹³CO₃ breath test, suggesting that it can sensitively assess the efficacy of rabeprazole. The Ca¹³CO₃ breath test can potentially be a useful method for non-invasive estimation for gastric acid secretion in human.

Keywords: breath test; ¹³C-labeled calcium carbonate; gastric acid secretion; gastro-esophageal reflux disease; proton pump inhibitor


Introduction

Gastro-esophageal reflux disease (GERD) is a common disease in Western countries (Locke et al. 1997; Stanghellini et al. 1999; Diaz-Rubio et al. 2004), and the number of patients with GERD is increasing in Japan as well (Inamori et al. 2003; Fujimoto et al. 2003; Fujiwara et al. 2005). Gastric acid has an important role as the dominant injurious factor in the pathogenesis of GERD. The primary therapeutic approach is suppression of gastric acid secretion, and stronger and prompter gastric acid suppression is required (Bell et al. 1992). Proton pump inhibitors (PPIs) are commonly used as the first-line treatment because of the high effectiveness and prolonged duration of suppression of gastric acid secretion. Thus, the therapeutic effects of PPIs on GERD are associated with the gastric acidity. Gastric acidity is different in various disease states and their measurement may be useful in the diagnosis and treatment of these diseases. High levels of acidity are found in duodenal ulcers and patients with Zollinger-Ellison syndrome, whereas low or absent levels of acidity are found in pernicious anemia, atrophic gastritis and gastric carcinoma (McColl et al. 1997). Gastric acid measurement may also be useful in assessing the effectiveness of new antisecretory drugs.

The conventional tests of measuring gastric acid secretion involve nasogastric intubation into the stomach and continuous aspiration of gastric fluid for a few hours (Kay 1953; Johnston and Jepson 1967). The procedure of these invasive tests causes significant distress to the patients and is time consuming. To estimate gastric acid secretion easily and rapidly, we previously devised an endoscopic method of gastric acid secretory testing (endoscopic gastrin test:...
EGT, which is a simple modification of the conventional gastrin-stimulated maximal acid output test (Iijima et al. 1998). However, the endoscopic procedure still causes some discomfort in the subjects, hence repeating EGT is not always easy. Therefore, the development of a non-invasive method would be desirable.

$^{13}$C is a non-radioactive stable isotope of carbon and is widely used in clinical settings, such as in the $^{13}$C-urea breath test for the diagnosis of *Helicobacter pylori infection* (Graham et al. 1987). $^{13}$C can also be incorporated into calcium carbonate. When $^{13}$C-labeled calcium carbonate ($\text{Ca}^{13}\text{CO}_3$) is orally administered, it then reacts with hydrochloric acid in the stomach producing $^{13}$C-labeled carbon dioxide ($^{13}\text{CO}_2$).

$$\text{Ca}^{13}\text{CO}_3 + 2\text{HCl} \rightarrow \text{CaCl}_2 + \text{H}_2\text{O} + {^{13}\text{CO}_2}$$

The $^{13}$CO$_2$ is absorbed rapidly by the gastric wall and delivered through the bloodstream to the lungs, and finally excreted in the breath. Thus, the amount of exhaled $^{13}$CO$_2$ is expected to correlate with the amount of pooled gastric acid. Then, by measuring the ratio of $^{13}$CO$_2$ to $^{12}$CO$_2$ in the breath sample via an infrared spectrophotometer, the amount of gastric acid can be estimated.

The objective of this study was to evaluate the suitability of the Ca$^{13}$CO$_3$ breath test for measuring gastric acid secretion in human. First, we evaluated correlations between the breath response and gastric secretory parameters under fasting and pentagastrin stimulated conditions. In addition, we also evaluated the reproducibility of the breath test results under the same conditions. Then, we evaluated whether the Ca$^{13}$CO$_3$ breath test results reflected the change in gastric acid secretion induced by PPIs administration.

**Methods**

**Study protocols**

First, to evaluate the correlation between the results of the Ca$^{13}$CO$_3$ breath test and the total amount of pooled gastric acid under a fasting condition, 6 healthy volunteers (mean age 23.0 ± 1.3 years) underwent endoscopic examination after overnight fast, and the pooled gastric acid was collected. Two hours after endoscopy, the Ca$^{13}$CO$_3$ breath test was performed under a fasting condition. Then, the series of studies were repeated six times on different days with different doses of Ca$^{13}$CO$_3$ to determine the appropriate dose of Ca$^{13}$CO$_3$.

Next, to evaluate the correlation between Ca$^{13}$CO$_3$ breath test values and the amount of modified gastric acid output (EGT value) under pentagastrin-stimulation, 20 participants (mean age 39.8 ± 18.0 years) were enrolled. Of them, 15 were healthy volunteers, and the other 5 were diagnosed with atrophic gastritis. The subjects with atrophic gastritis did not receive acid suppressive drugs. They underwent EGT as mentioned below, in which gastric fluid was collected endoscopically after pentagastrin injection. On a different day, the Ca$^{13}$CO$_3$ breath test was performed 20 min after intramuscular injection of pentagastrin. The reproducibility of the Ca$^{13}$CO$_3$ breath test was estimated by repeating the same test on different days.

Finally, to evaluate whether the Ca$^{13}$CO$_3$ breath test results reflected the change in gastric acid secretion by PPIs, 4 healthy volunteers were given oral rabeprazole at a dose of 10 mg twice daily for a week. Then, endoscopic gastric acid collection (EGT) and Ca$^{13}$CO$_3$ breath test under pentagastrin stimulation were repeated before and after rabeprazole administration.

The study was performed in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of the Tohoku University Graduate School (2010-392). Subjects provided written informed consent before participating.

**Measurement of the amount of pooled gastric fluid**

After an overnight fast, pooled gastric fluid was obtained by aspiration through an endoscope. The H$^+$ concentration was determined by titration. The total amount of pooled gastric acid was calculated by multiplying the volume with the concentration, and was expressed as H$^+$ mEq.

**Calcium carbonate (Ca$^{13}$CO$_3$) breath test**

The Ca$^{13}$CO$_3$ breath test was performed six times with various doses of Ca$^{13}$CO$_3$ on different days. Subjects received a single oral dose of 20, 50, 100, 200, 500, and 1000 mg of Ca$^{13}$CO$_3$ suspended in 50 ml of carboxymethylcellulose sodium (CMC-Na) on each occasion. The participants were in an upright sitting position during the breath test. Breath samples were collected in breath collection bags before and 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, and 120 min after dosing. The $^{13}$CO$_2$ concentration in each breath sample was measured using an infrared spectrometer (POCone; Otsuka Electronics Co., Ltd., Hirakata, Japan).

**Endoscopic Gastrin Test (EGT)**

The details of the EGT have been reported previously (Iijima et al. 1998). Briefly, after an overnight fast, subjects were injected intramuscularly with pentagastrin at a dose of 6 $\mu$g/kg (pentagastrin; Sigma, St. Louis, MO, USA). 15 min after the injection, an endoscope was inserted into the stomach, and pooled gastric fluid was aspirated and discarded. Gastric fluid secreted between 20 and 30 min after the pentagastrin injection was then aspirated and collected under direct visualization during routine endoscopic examination of the stomach and duodenal bulb. The volume of the sample collected over the 10-min period was recorded, and the H$^+$ concentration was determined by titration. The acid output in the 10-min period was calculated by multiplying the volume by the H$^+$ concentration, and the EGT value was expressed as H$^+$ mEq/10 min.

**Calcium carbonate (Ca$^{13}$CO$_3$) breath test under pentagastrin-stimulated gastric acid secretion**

After an overnight fast, subjects were injected intramuscularly with pentagastrin at a dose of 6 $\mu$g/kg. A single oral dose of 500 mg of Ca$^{13}$CO$_3$ suspended in 0.5% CMC-Na was administered 20 min after the injection. A baseline breath sample was collected before injection. Breath samples were collected 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, 60, 80, 100, and 120 min after dosing. The participants were in an upright sitting position during the breath test. The $^{13}$CO$_2$ concentration in each breath sample was measured using an infrared spectral analyzer.

**Analysis of $^{13}$CO$_2$ in breath samples**

The $^{13}$CO$_2$ concentration in each breath sample was measured
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using an infrared spectral analyzer. The \( ^{13}\text{CO}_2/^{12}\text{CO}_2 \) ratio was expressed as the \( \delta^{13}C \) value (permil, ‰) relative to the Pee Dee Belemnite Limestone standard, and the \( \Delta^{13}C \) value (‰) was calculated using the following equations:

\[
\delta^{13}C (\text{‰}) = \left( \frac{[^{13}\text{CO}_2/^{12}\text{CO}_2]_{\text{sample}} - [^{13}\text{CO}_2/^{12}\text{CO}_2]_{\text{PDB}}}{[^{13}\text{CO}_2/^{12}\text{CO}_2]_{\text{PDB}}} \right) \times 1000
\]

\[
\Delta^{13}C (\text{‰}) = \delta^{13}C_t - \delta^{13}C_0
\]

Where \( \Delta^{13}C_t \) is the change in the \( \delta^{13}C \) value measured at the time \( t \) (\( \Delta^{13}C_t \)) from the baseline (\( \Delta^{13}C_0 \)) following the administration of \( ^{13}\text{C}-\text{product} \).

Statistical analyses

The data are expressed as mean values ± standard deviation (s.d.). The breath test results were defined as the maximum \( ^{13}\text{CO}_2 \) concentration during the 120 min sampling period (Cmax) or a value at a specific time point. A liner regression analysis was carried out to evaluate proportionality between the dose levels of \( ^{13}\text{C}-\text{Ca} \) and the breath-Cmax. Correlations between the \( ^{13}\text{C}-\text{Ca} \) breath test values and the EGT values were assessed using linear regression analysis. The reproducibility of the tests was assessed by the coefficient of variation (CV).

Results

The mean \( ^{13}\text{CO}_2 \) concentration (\( \Delta^{13}\text{CO}_2 \)) in the expired breath versus the time curves after various doses \( ^{13}\text{C}-\text{Ca} \) are shown in Fig. 1A. The magnitude of the response was related to the \( ^{13}\text{C}-\text{Ca} \) dose; the \( \Delta^{13}\text{CO}_2 \) increased with increases in the \( ^{13}\text{C}-\text{Ca} \) dose. When 20, 50, 100, 200, or 500 mg of \( ^{13}\text{C}-\text{Ca} \) was administered, the \( \Delta^{13}\text{CO}_2 \) rose sharply within 10 minutes after its oral administration and then decreased gradually. When 1,000 mg of \( ^{13}\text{C}-\text{Ca} \) was administered, the \( \Delta^{13}\text{CO}_2 \) rose sharply within 10 minutes, reached a plateau until 40 minutes and then increased gradually. The excessive amount of \( ^{13}\text{C}-\text{Ca} \) remaining in the stomach that did not react with gastric acid could be responsible for the delayed breath response to the dose of 1,000 mg of \( ^{13}\text{C}-\text{Ca} \).

The correlation between the Cmax and the \( ^{13}\text{C}-\text{Ca} \) dose is shown in Fig. 1B and C. Cmax increased linearly with increasing doses of \( ^{13}\text{C}-\text{Ca} \) up to 200 mg (Fig. 1B), and reached a plateau when 500 and 1,000 mg of \( ^{13}\text{C}-\text{Ca} \) were administered (Fig. 1C). The Cmax correlated very well with \( ^{13}\text{C}-\text{Ca} \) doses up to 200 mg (\( r = 0.87 \)). Hence, 200 mg was considered an appropriate dose of \( ^{13}\text{C}-\text{Ca} \) in this experimental condition. There was a very good correlation between the Cmax and the total amount of pooled gastric acid, when 200 mg of \( ^{13}\text{C}-\text{Ca} \) were administered (\( r = 0.947 \)). This correlation became even slightly higher when the \( ^{13}\text{CO}_2 \) values were modified by height and body weight, as shown in the appendix (\( r = 0.951 \), Fig. 1D). Therefore, the following results are presented using \( ^{13}\text{CO}_2 \) values modified by height and body weight, and are expressed as %dose/hr. It was clearly demonstrated that the \( ^{13}\text{C}-\text{Ca} \) breath test values well reflected the fasting intra-gastric acidity.

However, the reproducibility of the total amount of...
pooled gastric acid under a fasting state turned out to be poor in that the CV was 0.74 (data not shown). Because of the poor reproducibility, we could not expect satisfactory reproducibility for the Ca$^{13}$CO$_3$ breath test under a fasting state, as well. Hence, we employed pentagastrin as a gastric stimulant to make the Ca$^{13}$CO$_3$ breath test reproducible since high reproducibility of the maximal acid output after pentagastrin injection was reported (Card and Marks 1960). Then, we performed the Ca$^{13}$CO$_3$ breath test under pentagastrin-stimulated gastric secretion. In our previous study (Iijima et al. 1998), the mean EGT value representing the amount of gastric acid secreted between 20 and 30 min after the pentagastrin injection was 3.6 mEq. On the other hand, the mean amount of pooled gastric acid in this study was 1.4 mEq. Hence, we considered that an approximately 2.5 fold higher dose of Ca$^{13}$CO$_3$ is required under pentagastrin stimulation. Therefore, we used 500 mg of Ca$^{13}$CO$_3$, which was expected to maintain a linear correlation between the Ca$^{13}$CO$_3$ dose and the $\Delta^{13}$CO$_2$ under pentagastrin stimulation. Therefore, we used 500 mg of Ca$^{13}$CO$_3$, which was expected to maintain a linear correlation between the Ca$^{13}$CO$_3$ dose and the $\Delta^{13}$CO$_2$ of the breath test under pentagastrin stimulation.

The mean $\Delta^{13}$CO$_2$ versus the time curves after oral administration of 500 mg of Ca$^{13}$CO$_3$ after pentagastrin injection is shown in Fig. 2. The $\Delta^{13}$CO$_2$ under pentagastrin-stimulation rose sharply around 10 min. after oral administration of Ca$^{13}$CO$_3$ and then decreased gradually. In 4 participants who underwent the Ca$^{13}$CO$_3$ breath tests with and without pentagastrin, the $\Delta^{13}$CO$_2$ under pentagastrin-stimulation was consistently higher than that without pentagastrin-stimulation at any measuring time point, and the Cmax under pentagastrin-stimulation was 2.7 times higher than that without pentagastrin-stimulation (data was not shown). The Cmax thus acquired in the stimulated Ca$^{13}$CO$_3$ breath tests well correlated with the EGT value (Fig. 3A, $r = 0.72$). The high correlation between the stimulated Ca$^{13}$CO$_3$ breath test and the EGT value was evident even when a one point sampling value of the breath test was applied in place of the Cmax. In particular, the $\Delta^{13}$CO$_2$ at 20 min after dosing showed the highest correlation with the EGT value ($r = 0.79$), suggesting that the Ca$^{13}$CO$_3$ breath test can be performed as a simple and reliable procedure with one-point sampling for the measurement of gastric acid secretion in human (Fig. 3B). In addition, the stimulated Ca$^{13}$CO$_3$ breath tests were repeated in 4 participants to evaluate the reproducibility. Consequently, the reproducibility of the breath test was good with a CV of 0.11, as shown in Fig. 4.

Fig. 5 shows the $\Delta^{13}$CO$_2$ versus the time curves of the stimulated Ca$^{13}$CO$_3$ breath test before and after rabeprazole administration at a dose of 10 mg twice daily for a week. Compared with $\Delta^{13}$CO$_2$ without rabeprazole, $\Delta^{13}$CO$_2$ after rabeprazole administration dramatically decreased and nearly flattened at each measuring time point, and the Cmax decreased from 61.6 ± 7.5 %dose/hr to 8.1 ± 5.8 %dose/hr. Meanwhile, rabeprazole administration reduced the mean EGT value from 3.7 ± 1.5 mEq/10 min to 0.17 ± 0.25 mEq/10 min in the same participants.

Discussion

This study shows the usefulness of the Ca$^{13}$CO$_3$ breath test for estimating the total amount of gastric acid secretion. The Ca$^{13}$CO$_3$ breath test well reflected the amount of fasting pooled intragastric acid. The Ca$^{13}$CO$_3$ breath test under pentagastrin stimulation had high reproducibility and correlated well with the modified maximal acid output (EGT). Moreover, the stimulated Ca$^{13}$CO$_3$ breath test could sensitively detect changes in the intragastric acidity induced by PPIs administration.
There was a very good correlation between the Cmax and the total amount of fasting pooled gastric acid \((r = 0.95)\), suggesting that the Ca\(^{13}\)CO\(_3\) breath test results well reflected the fasting intragastric acidity at any given time. The chemical property of Ca\(^{13}\)CO\(_3\), which is poorly watersoluble, but reacts immediately with hydrochloric acid in the stomach producing \(^{13}\)CO\(_2\), which is excreted in the breath at a high rate, would be responsible for the high correlation between the breath test and the amount of pooled intragastric acid. These results are consistent with those of a study in rats reported by Inada et al. (2012), in which the Ca\(^{13}\)CO\(_3\) breath test with or without proton pump inhibitors or pentagastrin showed a high correlation between the breath-Cmax and the total amount of gastric acid \((r = 0.994)\). Thus, the Ca\(^{13}\)CO\(_3\) breath test can be a potentially promising candidate as a noninvasive test for measuring gastric acid secretion in human.

High reproducibility is essential to establishing a new biological test, and is especially true for gastric secretory testing because it is well-known that the gastric secretory state shows wide-variation in daily life. Nonetheless, the reproducibility of the total amount of pooled gastric acid without any stimulants was poor in the present study \((CV = 0.74)\), consistent with a previous study showing poor reproducibility of a gastric fluid analysis in the basal state (White and Juniper 1973). Therefore, the Ca\(^{13}\)CO\(_3\) breath test value under fasting could fluctuate in repeating tests, albeit each test value could reflect the fasting intragastric acidity at a
In addition, using this stimulated $\text{Ca}^{13}\text{CO}_3$ breath test, a retrospective study found a good correlation with the EGT values, which is a conventional test, and the procedure was somewhat bothersome and time-consuming. Moreover, the extensive use of costly $\text{Ca}^{13}\text{CO}_3$ lacks versatility for daily clinical practice. Compared to this test, the present $\text{Ca}^{13}\text{CO}_3$ breath test could simply and reliably assess the gastric acid output in a shorter time, although our test requires pentagastrin injection. However, the test was performed in a single subject and was not compared with a conventional test, and the procedure was somewhat bothersome and time-consuming. Moreover, the extensive use of costly $\text{Ca}^{13}\text{CO}_3$ lacks versatility for daily clinical practice. Compared to this test, the present $\text{Ca}^{13}\text{CO}_3$ breath test could simply and reliably assess the gastric acid output in a shorter time, although our test requires pentagastrin injection.

There may be a limitation of this study. Although the overall correlation between the stimulated $\text{Ca}^{13}\text{CO}_3$ breath test and EGT value was good, the regression line did not pass the origin of the coordinates and was relatively gentle (Fig. 3A and B). This suggests that the breath test is not a quantitative test of acid secretion over the range studied although it is useful as a qualitative test to pick up severe hypochlorhydria. Further studies are required to investigate appropriate test conditions to improve the ability of quantitative measurement of wide range of gastric acid secretion level. In addition, gastric motility such as gastric emptying may affect the breath test results by loss of acid secretion through the pylorus prior to the $\text{Ca}^{13}\text{CO}_3$ administration. However, once $\text{Ca}^{13}\text{CO}_3$ is administered into the stomach, gastric emptying should rarely affect the rate of the reaction occurring in the stomach since chemical reaction between $\text{Ca}^{13}\text{CO}_3$ and hydrochloric acid proceeds promptly.

In conclusion, the $\text{Ca}^{13}\text{CO}_3$ breath test could be a simple, noninvasive method for estimating gastric acid secretion in human, and the test may be suitable for the clinical management of acid-related diseases. The development of ideal test meals as an alternative to pentagastrin injection...
for achieving high reproducibility of the Ca$^{13}$CO$_3$ breath test will broadly enhance the clinical suitability of the test.

**Conflict of Interest**
The authors declare no conflict of interest.

**References**


**Appendix**

$^{13}$CO$_2$ values modified by height and weight body (The $^{13}$C-excretion rate)

The $^{13}$C-excretion rate (%Dose/hr) against the dose is obtained by the following equation (Ghoos et al. 1993).

$$\frac{\text{%Dose / hr}}{\text{A}} = \frac{\Delta^{13}\text{C} \times V_{\text{CO}_2} \times R_{\text{PDB}} \times MW \times 10}{\# \times APE}$$

$\Delta^{13}\text{C}$: Change in δ$^{13}$C value (%)

$V_{\text{CO}_2}$: Velocity of CO$_2$ formation (mmol / hr)

$R_{\text{PDB}}$: $^{13}$C abundance ratio in PDB standard gas = 0.01123726

MW: Molecular weight of reagent (g/mol)

A: Dose of reagent (mg)

#: Number of labeled carbons in the molecule of reagent

APE: $^{13}$C-enrichment in reagent (atom%)