Reliability of the time to maximal $[^{13}\text{CO}_2]$ excretion and the half-$[^{13}\text{CO}_2]$ excretion time as a gastric emptying parameter: Assessments using the Wagner-Nelson method

Masaki SANAKA¹, Takatsugu YAMAMOTO², Shin NAKAYAMA², Kunitaka NAGASAWA² and Yasushi KUYAMA²

¹Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677, Japan
²Department of Internal Medicine, School of Medicine, Teikyo University, Tokyo, Japan

Received August 5, 2007; Accepted September 10, 2007

Abstract

In the $[^{13}\text{C}]$-octanoate breath test, two popular parameters have been used to quantify gastric emptying rates, namely the time to the maximal $[^{13}\text{CO}_2]$ excretion ($T_{\text{max}}$) and the time to the half-$[^{13}\text{CO}_2]$ recovery ($T_{1/2b}$). Although each of $T_{\text{max}}$ and $T_{1/2b}$ is closely correlated with the scintigraphic half-emptying time, the two parameters occasionally indicate different judgments on a gastric emptying rate. In this study, to clarify which of the two parameters is more reliable, $T_{\text{max}}$ and $T_{1/2b}$ were compared to the “reference” parameters calculated using the Wagner-Nelson method, which allows accurate estimation of a time-course of gastric emptying from breath data. Ten healthy male volunteers underwent the breath test after ingestion of a muffin meal (320 kcal) containing 100 mg $[^{13}\text{C}]$-octanoate. Breath samples were collected at 15-min intervals for 6 h. According to the conventional analytical algorithm, $T_{\text{max}}$ and $T_{1/2b}$ were mathematically calculated. By applying Wagner-Nelson analysis to the breath test, the time-percent gastric retention curve was generated and the half-emptying time ($T_{1/2\text{WN}}$) was determined. $T_{1/2\text{WN}}$ was more closely correlated with $T_{\text{max}}$ ($r=0.954, P<0.0001$) than with $T_{1/2b}$ ($r=0.782, P=0.008$). $T_{\text{max}}$ was significantly correlated with the percent gastric retention value in the early ($t=0.25$ and 0.5 h), the middle ($t=1.0$ and 1.5 h), and the late ($t=2.0$ h) postprandial phase. $T_{1/2b}$ was significantly correlated with the gastric retention value in the middle and the late phase, but not with the gastric retention value in the early phase. The present results show that $T_{1/2b}$ has limited capability to reflect gastric emptying in the early postprandial period, suggesting that $T_{\text{max}}$ is more reliable than $T_{1/2b}$ as a gastric emptying parameter.

Key words: breath test, gastric emptying, Wagner-Nelson method

Introduction

The scintigraphy is the reference method for the assessment of gastric emptying, but use of
the technique is highly limited because of substantial irradiation. As a non-radioactive alternative, a breath test using a stable isotope (¹³C) is currently available. The [¹³C]-octanoate breath test is widely used for measuring gastric emptying of solid meals (Ghoos et al., 1993).

In the breath test, two popular parameters have been used to quantify gastric emptying rates, namely the time at which the pulmonary [¹³CO₂] excretion rate reaches the maximal level (Tₘₐₓ) and the time by which half of the total cumulative dose of the [¹³C] marker to be recovered as [¹³CO₂] in the breath (T₁/₂b) (Ghoos et al., 1993). Ghoos et al. (1993) reported that Tₘₐₓ and T₁/₂b were highly correlated with the scintigraphically measured half-gastric emptying time (T₁/₂s). However, interpretations of breath test results occasionally puzzle researchers, because Tₘₐₓ and T₁/₂b can indicate different judgments on a gastric emptying rate: Tₘₐₓ indicates a normal emptying while T₁/₂b indicates a delayed emptying, and vice versa (Shishido et al., 2002). Few reports have clearly shown which of Tₘₐₓ and T₁/₂b is more reliable as an emptying parameter. In contrast to the results of Ghoos et al. (1993), Choi et al. (1997) and Shirasaka et al. (2002) demonstrated a much weaker correlation between T₁/₂b and T₁/₂s. Nakada et al. (2002) and Shirasaka et al. (2002) seem to hold the view that T₁/₂b has no advantage over Tₘₐₓ in the gastric emptying breath test.

According to the conventional analysis of Ghoos et al. (1993), Tₘₐₓ is determined based on a time-profile of the pulmonary [¹³CO₂] excretion rate (% dose/h), and T₁/₂b is based on that of the cumulative amount of [¹³CO₂] recovered in the breath (% dose). Zai et al. (2002) showed that the [¹³CO₂] excretion rate begins to rise soon after meal ingestion whereas the cumulative [¹³CO₂] recovery substantially increases much later. This finding leads us to hypothesize that T₁/₂b can not reflect a change in gastric emptying in the early postprandial period, and thus T₁/₂b is less reliable than Tₘₐₓ. To test the hypothesis, in the present study, Tₘₐₓ and T₁/₂b were compared to the “reference” parameters calculated using the Wagner-Nelson analytical method, which makes the breath test as accurate as the scintigraphy (Sanaka et al., 2006; Sanaka et al., 2007).

**Materials and methods**

**Subjects**

Ten healthy male volunteers (29–39 years) were enrolled. At the time of this study, none of the subjects had a history or symptoms of digestive and pulmonary diseases, or received any medications. All the subjects gave written informed consents and the study was approved by the Ethics Committee of Teikyo University.

**Study protocol**

After an overnight fast, the subjects underwent the [¹³C]-octanoate breath test. A commercialized muffin (Moko-moko deluxe®, Nagatani-en, Tokyo, Japan) containing 100 mg [¹³C]-octanoate was used as a test meal. Each subject consumed the muffin meal spread with a piece of butter (total 320 kcal; 34.1 g carbohydrate, 7.0 g protein, and 17.0 g fat), followed by drinking 200 mL water. Results of *in vitro* studies confirm that the retention rate of [¹³C]-octanoate in a muffin meal is very high (>95%) (Bromer et al., 2002). After baseline breath samples were obtained, the test meal was consumed within 5 min: the time at which the subject
starts eating the meal was regarded as time 0. Following meal ingestion, breath samples were collected at 15-min intervals for 6 h. During the test period, the subjects were allowed to go to the toilet, and they otherwise remained seated. The $^{13}$CO$_2$ enrichment in the breath was determined using an isotope ratio mass spectrometer (UbiT-IR 300; Otsuka Electronics, Osaka, Japan). The results were converted to the percentage of $^{13}$CO$_2$ recovery in the breath per hour (% dose/h).

**Data analysis**

The breath data were analyzed according to the conventional method of Ghoos et al. (1993) and the Wagner-Nelson method (Sanaka et al., 2007). Gastric emptying parameters determined by Wagner-Nelson analysis were regarded as the “reference”, against which T$_{max}$ and T$_{1/2b}$ were compared.

The time-plot of pulmonary $^{13}$CO$_2$ excretion rate (% dose/h), C(t), was fitted to the following function.

$$C(t) = \frac{mk}{\beta} e^{-kt}(1-e^{-kt})^{\beta-1}$$

In the equation, $m$ is the cumulative $^{13}$CO$_2$ recovery at the infinite time (% dose), $t$ is in hour, and $k$ and $\beta$ are regression-estimated constants. T$_{1/2b}$ and T$_{max}$ were calculated as follows.

$$T_{1/2b} (h) = -\ln(1-2^{-1/\beta})/k$$
$$T_{max} (h) = (\ln\beta)/k$$

The Wagner-Nelson method allows accurate estimation of a fraction of the labeled test meal that has been emptied from the stomach by time t, F(t), as follows.

$$F(t) = \frac{[AUC(t) + C(t)/K_{el}]}{AUC(\infty)}$$

In the equation, $K_{el}$ (1/h) is the first-order rate constant for total elimination of $^{13}$CO$_2$ from the human body, C(t) is the $^{13}$CO$_2$ excretion rate (%dose/h), AUC(t) is the area under the C(t) curve (%dose) (= the pulmonary recovery of $^{13}$CO$_2$), and AUC($\infty$) is the cumulative amount of $^{13}$CO$_2$ recovered in the breath at the infinite time (%dose). The $K_{el}$ value was estimated as a slope of the stable tail of a semi-logarithmic plot of C(t) between 3 and 6 h using the least squares algorithm. AUC(t) to each time point was calculated according to the trapezoidal rule. C(t)/$K_{el}$ was added to each corresponding AUC(t). AUC($\infty$) was computed as AUC(6.0) + C(6.0)/$K_{el}$. F(t) never exceeds the unity in theory, but it is occasionally above the unity in practice (Wang et al., 2002). This phenomenon is considered to be a random error. Thus, once F(t) exceeds 1.0, it is regarded as 1.0 thereafter. Percentage of the labeled meal retained in the stomach at each time, namely gastric retention, was calculated as GR(t) = 100 · [1 − F(t)] (%). The half-emptying time (T$_{1/2WN}$) was directly determined by interpolation from the GR(t) curve.

**Statistical analysis**

The linear association between two variables was assessed by a correlation coefficient (r). The level of significance was regarded as $P=0.05$ (two-sided probability).

**Results**

The time-profiles of C(t) and AUC(t) (Fig. 1) show that, during the first 1.0 h, the $^{13}$CO$_2$
excretion rate increased markedly, while the cumulative $[^{13}\text{C}]{\text{CO}}_2$ recovery increased only slightly. The time-profile of GR(t) generated by the Wagner-Nelson method (Fig. 1) demonstrates that about 50% of the test meal had been emptied from the stomach by 1.0 h postprandially, and about 90% had been emptied by 2.0 h postprandially.

As shown in Fig. 2, significant correlations were found between $T_{\text{max}}$ and $T_{1/2\text{WN}}$ ($r=0.954$, $P<0.0001$) and between $T_{1/2\text{b}}$ and $T_{1/2\text{WN}}$ ($r=0.782$, $P=0.008$). $T_{\text{max}}$ was more closely correlated with $T_{1/2\text{WN}}$ than $T_{1/2\text{b}}$.

Taking the pattern of gastric retention (Fig. 1) into consideration, GR(0.25) and GR(0.5) were regarded as the reference parameters in the early postprandial phase, GR(1.0) and GR(1.5)
Discussion

The present results support our view that $T_{1/2b}$ has limited capability to reflect gastric emptying in the early postprandial period and $T_{\text{max}}$ may be more reliable than $T_{1/2b}$ as a gastric emptying parameter.

Knowledge concerning the kinetics of the $^{13}$C label in the human body is essential to discuss the reliability of parameters obtained from breath data (Sanaka et al., 2004). $^{13}$C-Octanoate is rapidly absorbed once after it is emptied from the stomach. Though the portal venous system, $[^{13}C]$-octanoate is delivered to the liver, where it is quickly oxidized into $[^{13}CO_2]$. Following the quick hepatic metabolism, $[^{13}CO_2]$ is released into the blood stream, and is then forwarded to the pulmonary circulation to be exhaled. Not all the $[^{13}CO_2]$ that enters the pulmonary circulation is breathed out at once. A large portion of the $[^{13}CO_2]$ is not excreted, and then flows in the systemic circulation. The circulating $[^{13}CO_2]$ penetrates various body organs (the bicarbonate pools), where the $[^{13}CO_2]$ is temporarily retained (Sanaka et al., 2004). The $[^{13}CO_2]$ retained in the bicarbonate pools returns into the blood later, then reaches the lung, and is excreted in the breath at last. It is the retention of $[^{13}CO_2]$ in the bicarbonate pools that generates an apparent discrepancy between gastric emptying of $[^{13}C]$-octanoate and the pulmonary excretion of $[^{13}CO_2]$ (Sanaka et al., 2005a). The $[^{14}CO_2]$ retention is the key for understanding the phenomenon expressed in the excretion (% dose/h) and the recovery (% dose) curve (Fig. 3).

The $[^{13}C]$ label has been minimally recovered in the breath in the early postprandial period, during which a large portion of the $[^{13}C]$ label that has been emptied from the stomach is still retained in the body bicarbonate pools as $[^{13}CO_2]$ (Fig. 3). Thereby, the partial cumulative recovery of $[^{13}CO_2]$ in the early phase is very small compared to its total recovery. These findings can explain why $T_{1/2b}$ can not well reflects gastric emptying in the early phase. It is likely that $T_{1/2b}$ misses a significant change in initial gastric emptying.

---

**Table 1.** Correlations between gastric emptying parameters

<table>
<thead>
<tr>
<th></th>
<th>Early phase</th>
<th></th>
<th>Middle phase</th>
<th></th>
<th>Late phase</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GR (0.25)</td>
<td>GR (0.5)</td>
<td>GR (1.0)</td>
<td>GR (1.5)</td>
<td>GR (2.0)</td>
<td></td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>r=0.706</td>
<td>r=0.802</td>
<td>r=0.875</td>
<td>r=0.943</td>
<td>r=0.853</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=0.023</td>
<td>P=0.005</td>
<td>P=0.001</td>
<td>P=0.001</td>
<td>P=0.002</td>
<td></td>
</tr>
<tr>
<td>$T_{1/2b}$ (h)</td>
<td>r=0.450</td>
<td>r=0.531</td>
<td>r=0.639</td>
<td>r=0.834</td>
<td>r=0.800</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=0.197</td>
<td>P=0.115</td>
<td>P=0.047</td>
<td>P=0.003</td>
<td>P=0.006</td>
<td></td>
</tr>
</tbody>
</table>

$T_{\text{max}}$ (h): the time at which the $[^{13}CO_2]$ excretion rate reaches the maximal level; $T_{1/2b}$ (h): the time by which half of the total cumulative dose of the $[^{13}C]$ marker to be recovered as $[^{13}CO_2]$ in the breath; GR(t) (%): gastric retention at each time t (h).

as those in the middle phase, and GR(2.0) as that in the late phase. $T_{\text{max}}$ was significantly correlated with the gastric retention value in the early, the middle, and the late phase (Table 1). $T_{1/2b}$ was significantly correlated with the gastric retention values in the middle and the late phase, but not with the value in the early phase (Table 1).
What $T_{\text{max}}$ really means has poorly been understood. The first-order kinetics theory suggests that the pulmonary $[^{13}\text{CO}_2]$ excretion rate (\% dose/h) is proportional to the amount of $[^{13}\text{CO}_2]$ in the blood (\% dose) (Winchell et al., 1970; Pallikarakis et al., 1991; Saccomani et al., 1995): as $[^{13}\text{CO}_2]$ is more increased in the blood, more $[^{13}\text{CO}_2]$ is pushed out in the breath correspondingly (Fig. 3). This relationship is mathematically written as

\[
\text{Pulmonary }[^{13}\text{CO}_2]\text{ excretion rate (\% dose/h)} = k \text{ (1/h) } \cdot \text{Amount of }[^{13}\text{CO}_2]\text{ in the blood (\% dose)},
\]

where $k$ is a constant. It is therefore logical to consider that $T_{\text{max}}$ is the time at which the amount of $[^{13}\text{CO}_2]$ in the blood reaches the maximal level (Sanaka et al., 2005b) (Fig. 4). The amount of $[^{13}\text{CO}_2]$ in the blood is determined by the balance between gastric emptying of $[^{13}\text{C}]$-octanoate and the pulmonary $[^{13}\text{CO}_2]$ excretion (Fig. 3). As gastric emptying gets faster (slower), $[^{13}\text{CO}_2]$ is more rapidly (slowly) accumulated in the blood and consequently the amount of $[^{13}\text{CO}_2]$ in the blood is more rapidly (slowly) peaked. As a result, a faster (slower) emptying corresponds to a
shorter (longer) $T_{\text{max}}$. The response of the pulmonary $[^{13}\text{CO}_2]$ excretion rate to gastric emptying is very close. We think that $T_{\text{max}}$ reflects gastric emptying more directly than $T_{1/2b}$ (Fig. 3).

Shishido et al. (2002) reported that $T_{\text{max}}$ and $T_{1/2b}$ often provided a different judgment on gastric emptying rate and in that case, a combination of normal $T_{\text{max}}$ and delayed $T_{1/2b}$ was much more frequent than a combination of delayed $T_{\text{max}}$ and normal $T_{1/2b}$. The observation suggests that $T_{1/2b}$ is likely to judge gastric emptying to be delayed whether gastric emptying is really delayed or not. We think that the biased judgment by $T_{1/2b}$ toward the delayed emptying is an inherent drawback of $T_{1/2b}$. As mentioned above, $T_{1/2b}$ is determined based on the cumulative $[^{13}\text{CO}_2]$ recovery (% dose), which is an area under the time-$[^{13}\text{CO}_2]$ excretion rate (% dose/h) curve. The $[^{13}\text{CO}_2]$ recovery involves the extensive area that is not related to gastric emptying. In case the pulmonary excretion of $[^{13}\text{CO}_2]$ drags on after the end of gastric emptying, the cumulative $[^{13}\text{CO}_2]$ recovery becomes large independently of gastric emptying. As a result, $T_{1/2b}$ is “erroneously” prolonged. This is a likely explanation for the biased judgment by $T_{1/2b}$ (Shishido et al., 2002; Shirasaka et al., 2002). It may be relevant to rely upon $T_{\text{max}}$ if $T_{\text{max}}$ and $T_{1/2b}$ indicate different results.

The present study has two methodological limitations. The first one is that $T_{\text{max}}$ and $T_{1/2b}$ were not compared against scintigraphically measured parameters, such as $T_{1/2s}$. Regardless of the widespread use of the $[^{13}\text{C}]$-octanoate breath test, the discrepancy between the scintigraphic
and the breath test measurements has been challenged (Choi et al., 1997). The Wagner-Nelson method can diminish the discrepancy by adjusting the time for \( [\text{13CO}_2] \) to be retained in the bicarbonate pools (Sanaka et al., 2007). We have recently shown that the gastric retention curve created using the Wagner-Nelson method is almost completely superimposed on the scintigraphic retention curve (Sanaka et al., 2007). A critical shortcoming of the Wagner-Nelson method is that a period for breath sampling should be prolonged so that the \( K_{el} \) value can accurately be estimated (Bluck and Coward, 2006; Sanaka et al., 2007). In the current study, the sampling period was extended up to 6 h to allow the accurate estimation of the \( K_{el} \) value. We therefore think that the gastric emptying profiles assessed by Wagner-Nelson analysis (Fig. 1) are as accurate as those by the scintigraphy.

The second limitation is the small sample size \((n=10)\). To firmly establish the superiority of \( T_{\text{max}} \) to \( T_{1/2b} \), further studies are necessary in a large numbers of subjects with a broad range of gastric emptying rates, including patients with delayed emptying (e.g., diabetic gastroparesis) or rapid emptying (e.g., post-gastrectomy status).

References


Zai, H. and Miwa, T. (2002). The variant results in gastric emptying measurement by $^{13}\text{C}$ breath test, in case of using the same solid test meal labeled with $^{13}\text{C}$-acetic acid or $^{13}\text{C}$-octanoic acid. *J. Smooth Muscle Res. (Jpn. Sec.)* **6**: 115–119 (in Japanese with English abstract).