Review

Stable isotope breath tests for assessing gastric emptying: A comprehensive review

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Abstract

A stable isotope (^{13}C) breath test is a promising method for assessing gastric emptying, but it has not been pervasive yet in Japan. We think that there are some barriers to its popularization, including the uncertainty concerning its theoretical backgrounds, the ambiguity of analyzing and interpreting the data, and the lack of standard protocols for breath sampling. The aim of the present review is to break through these barriers. We hope this article could make the ^{13}C -gastric breath test more maneuverable for and more accessible to researchers and clinicians.

Key words: breath test, gastric emptying, the JSSMR standard protocol, loss, retention

Introduction

An array of techniques are currently available for assessing gastric emptying, including scintigraphy, ultrasonography, magnetic resonance imaging, acetaminophen absorption, and breath testing with a stable isotope (^{13}C) (Schmidt *et al.*, 2008; Parkman *et al.*, 2009). Of these techniques, the ^{13}C -breath test fulfills all the demand and desires for non-invasive investigations: it is free from radiation hazards, requires neither a skillful hand of the expert nor multiple venipuncture for blood sampling, and can be performed easily and repeatedly (Schmidt *et al.*, 2008; Parkman *et al.*, 2009). The accuracy of the breath test is well supported by a series of validation studies reporting a strong correlation between the breath test and the scintigraphy, which is the “gold standard” for measuring gastric emptying (Ghoos *et al.*, 1993; Braden *et al.*, 1995; Delbende *et al.*, 2000; Chey *et al.*, 2001; Bromer *et al.*, 2002; Chew *et al.*, 2003; Braden *et al.*, 2004). Regardless of its promising nature, however, the breath test is not so prevalent as might have been expected in Japan. We conjecture that there are some barriers to its popularization. The likely barriers that render one hesitant to use it are as follows. First, albeit the methodological principle of breath testing is apparently reasonable, it does not convincingly explain the reason why a real

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Fig. 1. Conventional model for explaining the breath test theory.

pattern of gastric emptying is not in agreement with a corresponding pattern of breath output (Sanaka *et al.*, 2005a; Sanaka *et al.*, 2008; Bluck *et al.*, 2009). The unconvincing explanation leaves behind some doubt on the credibility of the breath test. Second, the way of summarizing and interpreting breath-test results is ambiguous (Schommartz *et al.*, 1998; Bluck, 2009). The ambiguity has led to a longstanding debate. Last of all, since a myriad of protocols have been proposed, breath-test users are bothered in determining which to select (Nakada *et al.*, 2002). Development of a standard protocol is warranted.

The aim of the present review is to overhaul the $[^{13}\text{C}]$ -gastric breath test; special concern is placed on breaking through the above-mentioned barriers. We hope this article could make the $[^{13}\text{C}]$ -gastric breath test more maneuverable for and more accessible to researchers and clinicians.

Theoretical backgrounds of the $[^{13}\text{C}]$ -gastric breath test

Conventional model for explaining the breath test theory

A conventional scenario on which the $[^{13}\text{C}]$ -gastric breath test theoretically relies is as follows (Ghoos *et al.*, 1993; Perri *et al.*, 1998) (Fig. 1). After consumption of a test meal marked with $[^{13}\text{C}]$ -acetate for liquids or $[^{13}\text{C}]$ -octanoate for solids, the $[^{13}\text{C}]$ -substance remains intact in the stomach and is rapidly absorbed upon the passage of the pyloric ring. Subsequently, the labeled compound undergoes prompt and complete hepatic oxidation to $[^{13}\text{CO}_2]$, which is released into the venous blood and then is breathed out eventually. The sequence of events occurring between the stomach and the lung is assumed to be “a one-way direct road” (Fig. 1), suggesting that the pulmonary excretion of $[^{13}\text{CO}_2]$ corresponds to gastric emptying of $[^{13}\text{C}]$ -acetate/octanoate in a one-to-one manner. However, the one-to-one manner is incompatible with the fact that the breath output response is apart from the emptying input (Lee *et al.*, 2000; Sanaka *et al.*, 2008; Bluck, 2009). The truth is that the customary scenario is misleading albeit simple: the simplicity is attained only at the expense of overshadowing the more complex aspects of kinetics of the $[^{13}\text{C}]$ label (Sanaka *et al.*, 2008).

Fig. 2. Revised model for explaining the breath test theory.

Revised model for explaining the breath test theory

To give firm credence to the breath test, a more decent model for explaining its theory is presented, in which how the human body handles the ^{13}C label is meticulously illustrated (Fig. 2) (Sanaka *et al.*, 2008). The following points in the explanatory model should be stressed. First, although the intestinal absorption is considered to be immediate, the labeled octanoate in the chyme may take a longer time to be absorbed in the small intestine than the labeled acetate in the liquid phase (Sanaka *et al.*, 2007a). However, the magnitude of the time-difference would be minimal. Second, the liver extracts a portion of the ^{13}C label from its total dose absorbed by incorporating the ^{13}C label into non- $^{13}\text{CO}_2$ metabolites (*e.g.*, ketone bodies). This hepatic disposal of the label (metabolic loss) is substantial: about 50% of the ^{13}C label goes missing, thereby never being exhaled as available breath data (Sanaka *et al.*, 2008). Third, since the diffusion of carbon dioxide through the alveolar membrane is physiologically regulated, only a limited fraction of the $^{13}\text{CO}_2$ entering the pulmonary circulation is expired. In this sense, the lung actually serves as “a two-way forked road” that allocates the $^{13}\text{CO}_2$ either to the expired air or to the systemic circulation. Fourth, part of the $^{13}\text{CO}_2$ in the systemic circulation is eliminated via the non-pulmonary route (*e.g.*, feces and urine). The non-pulmonary loss is, however, so tiny as to be negligible (Sanaka *et al.*, 2008). Lastly, the $^{13}\text{CO}_2$ circulating around the body penetrates various organs (*e.g.*, heart and skeletal muscle) and stays there for a substantial period before being expelled. The organs (the bicarbonate pool) functionally work to reserve the $^{13}\text{CO}_2$, consequently delaying its recovery (*retention*) (van Nieuwenhoven *et al.*, 1999; Sanaka *et al.*, 2008).

We should pay heed to the *loss* and the *retention*, which are the bottom line for accounting for

Fig. 3. Difference between a real profile of gastric emptying and a time course of breath [$^{13}\text{CO}_2$] recovery. The cumulative [$^{13}\text{CO}_2$] recovery curve (CPDR[t]) is shaped as an inverse, blunted, reduced version of a true pattern of gastric emptying as a consequence of the incomplete total [$^{13}\text{CO}_2$] recovery due to the *loss* (CPDR[∞] < 100%) and the delayed [$^{13}\text{CO}_2$] recovery due to the *retention*.

the discrepancy between gastric emptying of the [^{13}C] label and the pulmonary recovery of [$^{13}\text{CO}_2$] (Sanaka *et al.*, 2008; Bluck, 2009). Independently of gastric emptying, the *retention* delays the pulmonary [$^{13}\text{CO}_2$] output and the *loss* makes the recovery of the output incomplete (Fig. 3). Nonetheless, the pulmonary [$^{13}\text{CO}_2$] output is considered to reflect gastric emptying, because the *loss* and the *retention* are much less variable between and within individuals than gastric emptying (Sanaka *et al.*, 2008). This fact ensures the methodological relevance of the [^{13}C]-acetate/octanoate breath test.

Analysis and interpretation of breath test results

Analysis of breath data

Breath [$^{13}\text{CO}_2$] data are usually expressed in percentage terms: the percent dose [^{13}C]-recovery per hour (PDR; % dose/h) and the cumulative percent dose [^{13}C]-recovery during a certain period (CPDR; % dose). The PDR (% dose/h) values are converted into the CPDR (% dose) values by means of the trapezoidal rule or mathematical integration; in reverse, the former are obtained by mathematical differentiation of the latter. The PDR and CPDR values are plotted against time (PDR[t] and CPDR[t] curve) for data analysis. Note that the breath output is modified by the *loss* and the *retention*, independently of gastric emptying (Sanaka *et al.*, 2008; Bluck, 2009) (Fig. 3).

A multitude of breath-test parameters have been proposed (Ghoos *et al.*, 1993; Braden *et al.*, 1995; Schommartz *et al.*, 1998; Kulik *et al.*, 2001; Sanaka *et al.*, 2004; Sanaka *et al.*, 2005; Bluck *et al.*, 2006; Sanaka *et al.*, 2006; Sanaka *et al.*, 2007a). Of these, the half-emptying time ($T_{1/2b}$), the lag phase (T_{lag}), and the gastric emptying coefficient (GEC) have been quoted frequently, all of which were first reported in the Ghoos's epoch-making paper (Ghoos *et al.*, 1993). By original definition,

$T_{1/2b}$ indicates the time by which half of the total cumulative recovery of the [^{13}C] label ($\text{CPDR}[\infty]/2$) is attained, and T_{lag} designates the time at which the [$^{13}\text{CO}_2$] excretion rate ($\text{PDR}[t]$) reaches its maximal level. GEC does not have a meaningful definition, only being described as “an overall index of gastric emptying” (Ghoos *et al.*, 1993). According to the Ghoos’s analytical procedure, GEC, T_{lag} , and $T_{1/2b}$ are computed by fitting the $\text{CPDR}[t]$ and the $\text{PDR}[t]$ plots onto the following power exponential model:

$$\text{CPDR}[t] = m(1 - e^{-kt})^\beta. \quad \text{Eq. 1}$$

Since the $\text{PRR}[t]$ function is expressed as the first derivative of the $\text{CPDR}[t]$ function, it is given by:

$$\text{PDR}[t] = mk\beta e^{-kt}(1 - e^{-kt})^{\beta-1}. \quad \text{Eq. 2}$$

Alternatively, the $\text{PDR}[t]$ function can be modeled on the χ^2 -distribution in statics as:

$$\text{PDR}[t] = at^b e^{-ct}. \quad \text{Eq. 3}$$

In all the equations, t is time in hours, and m, k, β, a, b , and c are all constants; the constant of m is an estimate of the ultimately cumulative recovery ($\text{CPDR}[\infty]$). These model functions are empirically deduced from the global appearance of actual [$^{13}\text{CO}_2$] data plot without any physiological relevance (Bluck *et al.*, 2006). It should be acknowledged that the constants of a and m determine the vertical scale (“height”) of the $\text{PDR}[t]$ and the $\text{CPDR}[t]$ curves, while the other constants determines the appearance (“shape”) of the curves (Bluck *et al.*, 2006). By the computerized curve-fitting algorithms, all the constants are extracted, and then the outcome parameters are calculated as:

$$\text{GEC} = \ln(a) \text{ (from Eq. 3),} \quad \text{Eq. 4}$$

$$T_{\text{lag}} \text{ (h)} = \{\ln(\beta)\}/k \text{ (from Eq. 1 or 2) or } b/c \text{ (from Eq. 3),} \quad \text{Eq. 5}$$

$$T_{1/2b} \text{ (h)} = (-1/k)\ln(1 - 2^{-1/\beta}) \text{ (from Eq. 1 or 2).} \quad \text{Eq. 6}$$

In case a computer is not at hand, T_{lag} can be determined directly by visual inspection of the “raw” PDR -data plot (Braden *et al.*, 1995).

Interpretation of breath-test parameters

The physiological meaning of the three parameters has rarely been scrutinized (Sanaka *et al.*, 2008). Given the discrepancy between breath-test results and real emptying processes, it is hard to specify what process of gastric emptying corresponds to each of the three popular parameters. For instance, as discussed below, what T_{lag} exactly signifies is a state of flux. The lag phase originally means the time for the stomach to grind and mix ingested food before gastric emptying starts. In scintigraphic measurements, the lag phase is arbitrarily defined as the initial post-meal period during which only a small portion of food (5%–10%) leaves the stomach (Mariani *et al.*, 2004). In breath testing, on the other hand, more than 80% of the gastric content leaves the stomach by the time at which the [$^{13}\text{CO}_2$] excretion rate is maximized, namely T_{lag} (Nakada *et al.*, 2002). In this sense, a view that T_{lag} corresponds to the lag phase is unreasonable (Kitagawa *et al.*, 2002; Zai *et al.*, 2002). At present, we think it more pragmatic to consider that what the

Table 1. Mutual relationships among key concepts

Breath [$^{13}\text{CO}_2$] Recovery	Time-recovery curve	Constant in the model functions*	Confounding factor
Amount	Area under the PDR[t] CPDR[∞]	<i>a</i> value <i>m</i> value	Loss
Velocity	Shape of the curve	<i>b</i> and <i>c</i> values <i>k</i> and β values	Retention

PDR[t]: percent dose recovery (% dose/h); CPDR[t]: cumulative percent dose recovery (% dose). *PDR[t] = $at^b e^{-ct}$ and CPDR[t] = $m(1 - e^{-kt})^\beta$.

parameters indicate is merely whether gastric emptying is rapid, normal, or slow.

In pharmacokinetic models, the absorption of drugs is described by two different terms, the extent and the rate of absorption (Basson *et al.*, 1996). Following this way of thinking, it is quite operative to view the [$^{13}\text{CO}_2$] recovery from the two separate standpoints, the *amount* and the *velocity* of the recovery: the *amount* is represented by the area under the PDR[t] curve until the infinite time, namely CPDR[∞], and the *velocity* is represented by the shape of the PDR[t] and CPDR[t] curves (Table 1). A general rule for the *amount-velocity* concept is that: 1) as gastric emptying of the [^{13}C] label is enhanced (suppressed), the *amount* of [$^{13}\text{CO}_2$] recovery is increased (decreased) and the *velocity* of [$^{13}\text{CO}_2$] recovery is hastened (slowed) (Fig. 4), and 2) without any change in gastric emptying, the *loss* and the *retention* modify the *amount* and the *velocity*, respectively, thereby “contaminating” the breath output data (Table 1).

Needless to say, the rate of gastric emptying is the *velocity*, and therefore, the *velocity*-related parameter is more suitable for the breath test than the *amount*-related one. T_{lag} and $T_{1/2b}$ reflect the *velocity* but are unrelated to the *amount* of the label recovery (Ghoos *et al.*, 1993; Sanaka *et al.*, 2008). The interpretation of the two parameters is plain: a shorter (longer) T_{lag} or $T_{1/2b}$ indicates faster (slow) gastric emptying. In contrast to T_{lag} and $T_{1/2b}$, which are intuitively understandable, what GEC represents is somewhat vague. GEC is a direct function of the constant of *a* (Eq. 4), implying that it is more closely associated with the *amount* than with the *velocity* (Table 1) (Sanaka *et al.*, 2008). From the implication of the *amount*, a larger (smaller) GEC is considered to indicate faster (slower) gastric emptying. Even though a high correlation was documented between GEC and the half-emptying time measured by the scintigraphy ($T_{1/2s}$) (Ghoos *et al.*, 1993), GEC would theoretically be unsuitable as an emptying index because of its *amount*-related nature. Recently, GEC seems out of vogue (Shishido *et al.*, 2002).

As mentioned above, the *loss* and the *retention* have the delusive influence on the breath-test results, namely on GEC, T_{max} , and $T_{1/2b}$; the *loss* and the *retention* are the confounding factor for the gastric breath test. The susceptibility of the three parameters to the *loss* and the *retention* should be taken into account in interpreting the breath-test results (Fig. 5). A few research groups have devised more sophisticated strategies that can weed out the *loss* and *retention* factors from breath data (Sanaka *et al.*, 2004; Bluck *et al.*, 2006). However, the novel analytical strategies have not been widely endorsed yet.

Fig. 4. Changes in breath-test results in response to those in the *amount* and the *velocity* of the $^{13}\text{CO}_2$ recovery. PDR[t] is the percent dose recovery (% dose/h), and CPDR[t] is the cumulative recovery (% dose). The premise is that as gastric emptying of the ^{13}C label is enhanced (suppressed), the *amount* of $^{13}\text{CO}_2$ recovery is increased (decreased) and the *velocity* of $^{13}\text{CO}_2$ recovery is hastened (slowed). As the *amount* is increased (decreased), the PDR[t] and the CPDR[t] curves are heightened (lowered) without an essential change in the shape of the curves (A and B); a faster (slower) gastric emptying is indicated by the larger (smaller) vertical scale of the curves. As the *velocity* is accelerated (decelerated), the PDR[t] curve is steepened (flattened) without a change in its area under the curve (C) and the CPDR[t] curve is moved rightward (leftward) without a change in the ultimate cumulative recovery (CPDR[∞]) (D); a faster (slower) gastric emptying is indicated by the steepened (flattened) shape of the curves.

Debate on breath-test parameters

Since the introduction of the ^{13}C -octanoic acid breath test by Ghooos *et al.* in 1993, some issues on the breath-test parameters have been disputed. Weighing these debates is essential for a better understanding of the breath test.

Several authors uphold an opinion that the terms of “the half-emptying time” ($T_{1/2b}$) and “the lag phase” (T_{lag}) are used equivocally (Schommartz *et al.*, 1998; Nakada *et al.*, 2002). The two terms were originally applied to characterize a real pattern of gastric emptying, which is “directly” assessed by the intubation technique or the scintigraphy. Application of the two terms to the “indirect” breath test necessarily incurs confusion. The improper application would be attributed to use of the power exponential functions for curve-fitting analysis in the scintigraphy (Gastric retention = $1 - \{1 - (1 - e^{-kt})^\beta\}$) (Siegel *et al.*, 1988) and in the breath test ($\text{CPDR}[t] = m(1 - e^{-kt})^\beta$): the two functions are inherently the same; therefore, whichever equation is used, the half-

Fig. 5. Delusive effects of the *loss* and the *retention* of [^{13}C] label on breath-test results. PDR [t] is the percent dose recovery, CPDR [t] is the cumulative recovery, $T_{1/2b}$ is the half-recovery time, $T_{\max}$ is the time to the maximal recovery rate, and GEC is the gastric emptying coefficient. As the quantity of the *lost* label is increased, CPR [∞] (the *amount*) is decreased without an essential change in the shape of PER[t] and the CPR [t] curve (the *velocity*). As a result, the two curves are shifted downward vertically (A and B); if the *loss* increases, GEC becomes smaller even though $T_{1/2b}$ and $T_{\max}$ remain unchanged. On the other hand, as the time for the label to be *retained* in the bicarbonate pool is more prolonged, the shape of PER [t] and the CPR [t] curve (the *velocity*) is flattened without a change in CPR [∞] (the *amount*). As a result, the CPR [t] curve is tilted rightward, and the PER [t] curve is shifted toward the lower right side (C and D); if the *retention* is prolonged, GEC becomes smaller, $T_{1/2b}$ longer, and $T_{\max}$ longer.

emptying time is expressed as $(-1/k)\ln(1 - 2^{-1/\beta})\{\ln(\beta)\}/k$ and the lag phase as $(-1/k)\ln(1 - 2^{-1/\beta})$. Again, it should be noted that the pulmonary [$^{13}\text{CO}_2$] recovery curve is different from the scintigraphic emptying curve by nature. Some workers think it more appropriate to name $T_{1/2b}$ “the half-[$^{13}\text{CO}_2$] recovery (or excretion) time” (Kulik *et al.*, 2001; Zai *et al.*, 2009). Likewise, not a few authors feel that “the lag” in T_{lag} is a bit of misnomer (Clegg *et al.*, 2010) and the abbreviation of $T_{\max}$ is suitable for the time to the maximal [$^{13}\text{CO}_2$] excretion instead of T_{lag} (Takahashi *et al.*, 2006; Sanaka *et al.*, 2007b; Sanaka *et al.*, 2008; Iwase *et al.*, 2009; Shindo *et al.*, 2009; Mishima *et al.*, 2009). In this context, T_{lag} is replaced by $T_{\max}$ afterward in the present review.

Our revised explanatory model suggests that the difference between $T_{1/2b}$ and $T_{1/2s}$ is attributed to the time for the delay in pulmonary [$^{13}\text{CO}_2$] recovery due to the *retention* (Sanaka *et al.*, 2008) (Figs. 2 and 3). Formerly, the regression equation of $T_{1/2s}$ (min) = $T_{1/2b}$ (min) – 66 was preferably used to adjust $T_{1/2b}$ to $T_{1/2s}$, assuming that the difference of 66 min is constant between individuals (Ghoos *et al.*, 1993). However, the rationale for adjusting $T_{1/2b}$ has been challenged, because the

difference is more variable than constant (Choi *et al.*, 1997; Lee *et al.*, 2000), the way of deriving the equation is not adequate (Lee *et al.*, 2000; Sanaka *et al.*, 2003), and the adjustment procedure rarely yields additional diagnostic information (Schommartz *et al.*, 1998).

It is reasonable to consider that a high statistical correlation between $T_{\max}$ and $T_{1/2b}$ reported (Schommartz *et al.*, 1998; van Nieuwenhoven *et al.*, 1999; Shishido *et al.*, 2002) justifies the mutual exchange of the two parameters, either of which could be rejected according to researchers' preferences. Generally, $T_{1/2b}$ seems more popular than $T_{\max}$, despite a lack of tangible supportive evidence (Sanaka *et al.*, 2007b). Some investigators, however, are skeptical about the primacy of $T_{1/2}$: rather, they are confident that $T_{1/2b}$ is inferior to $T_{\max}$ as an emptying measure based on the following observations. First, $T_{1/2b}$ is less sensitive than $T_{\max}$ in detecting an early postprandial change in gastric emptying (Sanaka *et al.*, 2007b). This finding is quite understandable, considering that the CPDR[t] curve changes minimally while the PDR[t] curve does dynamically in the early phase (Zai *et al.*, 2009). Next, $T_{1/2b}$ would be prone to mistakenly judge a "normal" emptying to be "delayed" in case that the PDR[t] curve exhibits a very gentle descending slope (Shirasaka *et al.*, 2002; Shishido *et al.*, 2002; Lee *et al.*, 2000; Sanaka *et al.*, 2007b); in such a case, we speculate that the *retention*, rather than a real delay in the emptying, makes the pulmonary [$^{13}\text{CO}_2$] recovery drag on long after the emptying terminates. On the other hand, $T_{\max}$ has a disadvantage that its precise determination is difficult when sampling frequency is sparse or the PDR[t] curve is so flattened in shape that its peak is unclear.

Protocol for the [^{13}C]-breath test

Sampling frequency and duration

To date, a pile of studies have been conducted with breath testing under variable protocols. The difference in sampling frequency and test duration between the protocols are a matter of great concern (Clegg *et al.*, 2010), because the sampling schedule largely affects the precision and the feasibility of the breath test. A strict protocol with too many sampling points over a protracted period increases the precision, whereas it spoils the feasibility.

Under the curve-fitting approach, the reliability of the breath-test parameters highly depends on how well a plot of actual [$^{13}\text{CO}_2$] data fits each of the model functions. In general, the shorter the test duration, the poorer the curve fit; this phenomenon is more manifest as gastric emptying is more delayed. In the [^{13}C]-octanoate breath test, $T_{1/2b}$ and $T_{\max}$ (T_{lag}) values are more variable and more erratic as a sampling period is shorter (Choi *et al.*, 1997; Delbende *et al.*, 2000; Lee *et al.*, 2000; Gonlachanvit *et al.*, 2001). Concerning the duration, some authors believe that a 4-h study is sufficient to keep its reliability (Ghoos *et al.*, 1993; Bromer *et al.*, 2002; Yamamoto *et al.*, 2004), while others have claimed that a shorter timeline of < 6 h is suboptimal (Choi *et al.*, 1997; Clegg *et al.*, 2010). Regarding the frequency, a number of investigators deem that sampling at 15-min intervals is appropriate (Maes *et al.*, 1998; Clegg *et al.*, 2010). In order to guarantee the precision of curve-fitting analysis, a criterion has been advocated that any breath test with poor curve-fit (the r^2 coefficient < 0.9) should be rejected (Perri *et al.*, 1998). Under this strict criterion, however, the feasibility of the test would dwindle considerably, especially in case of the delayed emptying. On the other hand, in the [^{13}C]-acetate breath test, few studies have investigated the optimal test

Table 2. Protocol for the [^{13}C]-acetate breath test standardized by the Japan Society of Smooth Muscle Research (The JSSMR protocol)

Test meal: a commercialized liquid meal (200 kcal/200 mL) (<i>e.g.</i> , RACOL [®] , Otsuka, Tokyo)
[^{13}C]-compound: 100 mg [^{13}C]-sodium acetate
Sampling frequency and duration: at time 0 (preprandial baseline), 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165, 180, 210, and 240 min
Parameter: $T_{1/2b}$, $T_{\text{max-calc}}$, T_{max}
Reference range: $T_{1/2b}$ (59.8 – 122.6 min), $T_{\text{max-calc}}$ (32.8 – 74.4 min), T_{max} (23.3 – 64.5 min)
Note
1) The reference ranges are derived from 63 healthy volunteers (19 – 51 years, 34 males and 29 females). The mean (SD) values are 91.2 (15.7) min for $T_{1/2b}$, 53.6 (10.4) min for $T_{\text{max-calc}}$, and 43.9 (10.3) min for T_{max} .
2) $T_{\text{max-calc}}$ is calculated by the Ghoos's curve-fitting technique, and T_{max} is determined by directly inspecting the [$^{13}\text{CO}_2$] % dose/h- curve.
3) Liquid test meals from different manufacturers are applicable to this protocol if the meals contain 200 kcal/200 mL.
$T_{1/2b}$: The half-[$^{13}\text{CO}_2$] recovery time; T_{max} : The time to maximal [$^{13}\text{CO}_2$] excretion.

duration. After all, a scheme for sample collection is still more optional, but we consider that the most practical and prevalent schedule at present is a 4-h timeframe with breath samples being collected at 15-min intervals for solid-meal studies (Perri *et al.*, 2010) and more frequently (*e.g.*, at 5-min intervals during the early postprandial phase) for liquid-meal studies (Nakada *et al.*, 2002). If subjects are highly suspicious of having delayed gastric emptying or higher-caloric meals are used for the test, a 6-h timeframe would be preferable.

We have a suspicion about the validity of the model-dependent analytical strategy, which forces biological variability in the breath output pattern (namely, gastric emptying) to be fitted into a “ready-made” non-biological mold. In real, there exist pathological patterns of gastric emptying that are never fitted to the available model functions (Zai *et al.*, 2009). In this context, the model-independent T_{max} , which is determined visually, would play a more important role. The accuracy of T_{max} would be equivalent either based on the curve-fitting or based on the visual inspection, if only breath samples are collected frequently (Braden *et al.*, 1995; Nakada *et al.*, 2002). In addition, the test duration could effectively be shortened using the visually determined T_{max} , because most of the descending phase of the plot is unnecessary for the determination (Nakada *et al.*, 2002; Shishido *et al.*, 2002). There seems no particular reason for use of the model-dependent T_{max} over the more convenient, model-independent one.

Standardized protocol for breath testing

In the face of confusion about the sampling scheme, the standardization of the breath test protocol has been necessitated. To the best of our knowledge, two standardize protocols for breath testing have been reported; one has been proposed by the Japan Society of Smooth Muscle Research (JSSMR) for the [^{13}C]-acetate breath test (Nakada *et al.*, 2002) and the other by the Italian Group for Gastric Emptying Testing (IGGET) for the [^{13}C]-octanoate breath test (Perri *et al.*, 2010). These standard protocols are expected to settle the problem originating from non-uniformity of protocols across institutes.

Table 3. Protocol for the [^{13}C]-octanoate breath test standardized by the Italian Group for Gastric Emptying (The IGGET protocol)

Test meal: a manufactured muffin containing 378 kcal (EXPIROGer®, Sofar, SpA Milan)
[^{13}C]-compound: 100 mg [^{13}C]-ctanoic acid
Sampling frequency and duration: at time 0 (preprandial baseline) and at 15-min intervals for 240 min
Parameter: $T_{1/2b}$
Reference range: $T_{1/2b}$ (upper limit; 146 min)
Note
1) The reference ranges are derived from 131 healthy volunteers (47 ± 18 years, 58 males and 73 females). The mean (SD) value is 89 (29) min.
2) $T_{1/2b}$ is calculated by the Ghoo's curve-fitting technique.
3) Age and gender do not influence the reference value.

The JSSMR protocol is made as a consensus guideline for the [^{13}C]-acetate breath test (Table 2). In 2001, the working party, which comprises JSSMR expert members, was convened from nine different institutions to discuss how to develop a standardized protocol for breath testing, setting the primary goal of making the breath test more accessible not only to researchers but also to clinicians. Over the one-year thorough discussion, the JSSMR protocol was yielded and was then promulgated at the JSSMR 44th annual meeting (Sendai, Miyagi, July 2002). The hallmark of the JSSMR protocol is its simplicity: the test meal is a ready-made liquid, which is sanitary and easy to prepare; in busy clinical settings, the foreshortened 90-min version is also available, by which clinicians can discriminate between normal and abnormal gastric emptying based on the visually determined $T_{\max}$ and its reference values (23.3–64.5 min). Recently, clinical and experimental studies using the JSSMR protocol have increasingly been published (Takahashi *et al.*, 2006; Iwase *et al.*, 2009; Shindo *et al.*, 2009; Mishima *et al.*, 2009). The working party also dedicated to standardizing the protocol for the [^{13}C]-octanoate breath test. Regrettably, however, this attempt ended up coming at a standstill, because the consensus regarding a standard solid meal could not be reached.

More recently, the IGGET protocol has been elaborated for the [^{13}C]-octanoate breath test (Table 3). The attempt to standardize a solid test meal has resulted in admirable success by adopting a manufactured muffin meal, which can be prepared with minimally complicated procedures. The reference range for $T_{1/2b}$ has been determined from a large database, therefore being highly reliable.

The two standard protocols would make the breath test more viable and maneuverable. However, both protocols are still conditional, and further augments are crucial in search of more optimal ones.

Conclusion

The [^{13}C]-breath test is a promising method, although some problems remain unsolved. We expect that more clinicians and researchers use it in their practical and experimental work.

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