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REVIEW

Retention, Fixation, and Loss of the [^{13}C] Label: A Review for the Understanding of Gastric Emptying Breath Tests

Masaki Sanaka · Takatsugu Yamamoto · Yasushi Kuyama

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Abstract A [^{13}C]-breath test is a promising method for measuring gastric emptying. The methodological relevance is based on a close correspondence between gastric emptying of [^{13}C]-acetate/octanoate (input) and pulmonary excretion of [$^{13}CO_2$] (output). Despite the close input-output correspondence, the pulmonary output is quite remote from the gastric input: the pulmonary output is delayed compared to the gastric input, and the total recovery of [$^{13}CO_2$] in the breath is incomplete. This review focuses on the kinetics of [^{13}C]-acetate/octanoate in the body and suggests that (1) the delayed pulmonary output results from temporal retention of [$^{13}CO_2$] in the well-perfused tissues (heart, brain, etc.), (2) the incomplete recovery results from incorporation of the label into metabolic products (ketone bodies, amino acids, etc.) or from fixation of [$^{13}CO_2$] in the low-perfused tissues (bone, skeletal muscle, etc.), and (3) knowledge on the retention is the key to appropriate interpretations of breath test results. Recognition of these kinetic aspects is essential for appropriate interpretations of these breath test results.

Keywords Breath test · Gastric emptying · Fixation · Retention · Turnover

M. Sanaka (✉)
Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital, Honkomagome, 3-18-22, Bunkyo-ku, Tokyo 113-8677, Japan
e-mail: sanaka.koma.@cick.jp

T. Yamamoto · Y. Kuyama
Department of Internal Medicine, School of Medicine, Teikyo University, Tokyo, Japan

Introduction

Gamma scintigraphy is regarded as the reference method for measuring gastric emptying, but the technique has the critical drawback of substantial irradiation [1]. As a non-radioactive alternative, a breath test using a stable isotope ([^{13}C]) is now widely used in research and clinical settings [2, 3]. In the breath tests, the [^{13}C] label incorporated in a free fatty acid (acetic acid or octanoic acid) is used as a tracer. Labeled acetate and octanoate are applied to studies on the liquid- and the solid-phase gastric emptying, respectively.

The breath tests are considered to be methodologically relevant based on the fact that the excretion of [$^{13}CO_2$] in the breath (output) immediately follows gastric emptying of [^{13}C]-acetate/octanoate (input) [2, 3]. Despite the immediate input-output correspondence, it has been well recognized that the gastric input is apparently remote from the pulmonary output as follows (Fig. 1).

(1) The excretion of [$^{13}CO_2$] in the breath (output) continues long after the end of gastric emptying (input) [4].
(2) The rate at which [$^{13}CO_2$] is recovered in the breath (output) is much slower than the rate at which the [^{13}C] label is emptied from the stomach (input) [2–4].
(3) Only ~40% dose of the [^{13}C] label is ultimately recovered in the breath (output), even though 100% dose of the label is emptied from the stomach (input) [4].

These input-output discrepancies are characterized as *delayed [$^{13}CO_2$] excretion* and *incomplete [$^{13}CO_2$] recovery*.

The human body is a "black box" that considerably warps the gastric input and then yields the pulmonary

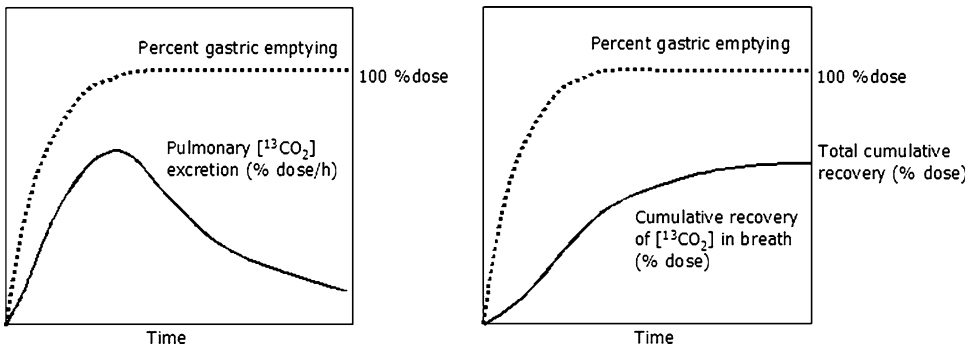

Fig. 1 Discrepancies between a time profile of gastric emptying and that of breath [$^{13}CO_2$]

output [5–7]. Some of the [^{13}C] label ingested is directly excreted in the breath as [$^{13}CO_2$], some is temporally stored in the body, and the remainder is irreversibly lost via nonrespiratory routes [5–7]. It is this storage and loss that generate the input-output discrepancies. To interpret test results properly and logically, it is essential to know how the human body handles the labeled fatty acids and consequently modifies the gastric input. The present review does not merely enumerate the past, established, well-known findings on [^{13}C]-breath tests, but also reports new theoretical insights into the breath tests based on the kinetics of the [^{13}C] label.

Overview of the Kinetics of [^{13}C]-Acetate and Octanoate in the Human Body

[^{13}C]-Acetate and octanoate are not absorbed from the stomach, whereas they are rapidly absorbed from the small intestine. Via the portal venous system, the labeled fatty acids are delivered to the liver, where the [^{13}C] label is liberated from the original compounds and is then incorporated into various metabolic products, such as CO_2, glucose, cholesterol, amino acids, and ketone bodies [8–12]. As a consequence, part of the [^{13}C] label is *fixed* in the metabolic pools as products that are not exhaled, and the remainder is converted into [$^{13}CO_2$] [8–12]. Following the quick hepatic metabolism, [$^{13}CO_2$] is released into the blood stream, forwarded to the pulmonary circulation, and then breathed out. Note that a substantial portion of the [$^{13}CO_2$] in the pulmonary circulation escapes from the respiratory elimination, and then flows in the systemic circulation. The circulating [$^{13}CO_2$] enters the large bicarbonate pools, where [$^{13}CO_2$] is temporarily *retained* or *fixed* before being excreted. The [^{13}C] label stored (= *fixed* or *retained*) in the metabolic or the bicarbonate pools returns into the systemic circulation later, and then the [^{13}C] label is redirected toward the pathway leading to pulmonary elimination (*turnover*) [12, 13]. As well as other exogenous substances, all of the [^{13}C] label ingested ultimately disappears from the body, being eliminated in breath, urine, faeces, sweat, and so on. It takes a long time for the [^{13}C] label to be completely eliminated from the body (>12 h) [14].

To develop more logical arguments hereafter, we clearly define the key terms *retention*, *fixation*, *loss*, and *turnover* as follows (Fig. 2).

(1) *Turnover*: Reentry of [^{13}C] label that has been stored in the metabolic and the bicarbonate pools to the pathway leading to pulmonary elimination as [$^{13}CO_2$]
(2) *Retention*: Temporal storage of [^{13}C] label in the body (metabolic and/or bicarbonate) pools, followed by its rapid turnover
(3) *Fixation*: Temporal storage of the [^{13}C] label in the body (metabolic and/or bicarbonate) pools, followed by its slow turnover
(4) *Loss*: Irreversible disposal of the [^{13}C] label outside the body via the nonpulmonary route

Conventionally, the term *retention* has been applied to the distribution of [$^{13}CO_2$] into the body bicarbonate pools, and the term *fixation* to the incorporation of the label into the intermediary metabolites. However, the two terms have often been used interchangeably [11, 13–17]. We think that *retention* is characterized by rapid turnover and *fixation* by slow turnover. On the other hand, *fixation* and *loss* have also been used interchangeably [11, 13–17]. We regard *fixation* as reversible and *loss* as irreversible. Since label that has been *retained* or *fixed* is recycled for pulmonary elimination as [$^{13}CO_2$] later (*turnover*) [11, 12, 15–19], *retention* and *fixation* are responsible for the delay in pulmonary [$^{13}CO_2$] excretion. According to the aforementioned definition, *loss* is responsible for the incomplete recovery of [$^{13}CO_2$] in the breath.

Detailed Kinetics of [^{13}C]-Acetate and Octanoate

The kinetics of [^{13}C]-acetate and octanoate in the human body is characterized by two distinct processes, namely post-gastric metabolism of free fatty acids (acetate/octanoate) and flow dynamics of bicarbonate/CO_2.

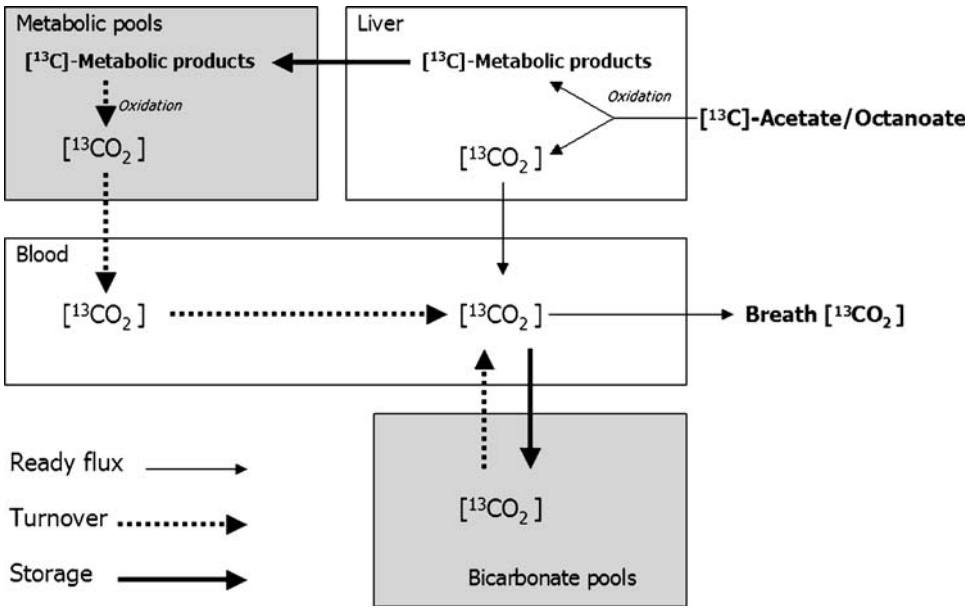

Fig. 2 Storage and turnover of [^{13}C] label in the body. The [^{13}C] label leaves the liver as [^{13}C]-metabolic products or as [$^{13}CO_2$], followed by the storage of the label in the metabolic pools, the storage of [$^{13}CO_2$] in the bicarbonate pools, or the excretion of [$^{13}CO_2$] in the breath. Later, the label stored turns over toward the pathway that leads to the respiratory elimination as [$^{13}CO_2$]

Postgastric Metabolism of Free Fatty Acids

The events that occur between the small intestine and the liver are described as the postgastric metabolism of free fatty acids. Free fatty acids are aliphatic carboxylic acids represented by the chemical formula R-COOH, where R stands for an alkyl chain composed of carbon and hydrogen atoms. The length of the carbon chain (number of carbons) is a major determinant of the fatty acid molecule. According to the chain length, free fatty acids are subdivided into short-chain (2–4 carbon atoms), medium-chain (6–10 carbon atoms), and long-chain (12–26 carbon atoms) fatty acids [20]. These structural differences determine molecular size and water solubility, and lead to differentiation between short-, medium-, and long-chain fatty acids during processes of absorption, transport, and hepatic metabolism [20]. Acetic acid is a short-chain free fatty acid with two carbon atoms, and octanoic acid is a medium-chain free fatty acid with eight carbon atoms.

Absorption and Transport

Fatty acids are usually contained in dietary triglycerides. Pancreatic lipase is needed to liberate free fatty acids from dietary triglycerides before their intestinal absorption. The pancreatic digestion is unnecessary when free fatty acids per se are orally given. Short- and medium-chain free fatty acids instantly pass the intestinal mucosa, are bound to serum albumin in the portal blood, and travel directly to the liver quickly [8–10]. Fatty acids with shorter carbon chains are more absorbable [8, 20]. Thus, acetic acid is more rapidly absorbed than octanoic acid.

Hepatic Metabolism

The labeled carbon atom has a greater chance of *fixation* in the liver [11, 12] (Fig. 3). [^{13}C]-Acetate and octanoate undergo extensive uptake by the liver, whereas only a minor portion of them is directly discharged into the bloodstream, bypassing the liver for bioconversion in the peripheral tissues [9]. [^{13}C]-Acetate and octanoate that are taken in the liver rapidly cross the mitochondrial membrane [11, 12]. Once in the mitochondrial matrix, β-oxidation is the almost exclusive fate of all fatty acids, whatever the chain length [10]. Via β-oxidation, [^{13}C]-acetate and octanoate are converted into their acetyl-coenzyme A (CoA) derivatives. The acetyl-CoA is directed to ketone body production, or enters the tricarboxylic acid (TCA) cycle. Along the TCA cycle, the acetyl-CoA is degraded to various metabolic products (CO_2, oxaloacetate, and glutaminate) or is used to resynthesize lipids (long-chain fatty acids, cholesterol, etc.) de novo [8, 9]. Thus, a large portion of the [^{13}C] label can be incorporated into these metabolites, being *fixed* in the various metabolic pools [16]. The metabolites ultimately end up as ketone bodies, glucose (oxaloacetate), lipids, or amino acids (glutamate/glutamine). *Fixed* label will be oxidized into [$^{13}CO_2$] in various body tissues (liver, skeletal muscle, heart, and so on) much later, finally being excreted in the breath as [$^{13}CO_2$] (slow *turnover*) [12]. Different metabolic substrates have variable *turnover* times. Some (such as triglycerides and glutamate/glutamine) turn over particularly slowly [18]. The *fixation* of the label in the gluconeogenic pathway is much less important than the *fixation* in the ketogenic, lipogenic pathways, and glutamine/glutamate pools from a quantitative point of view [11, 12].

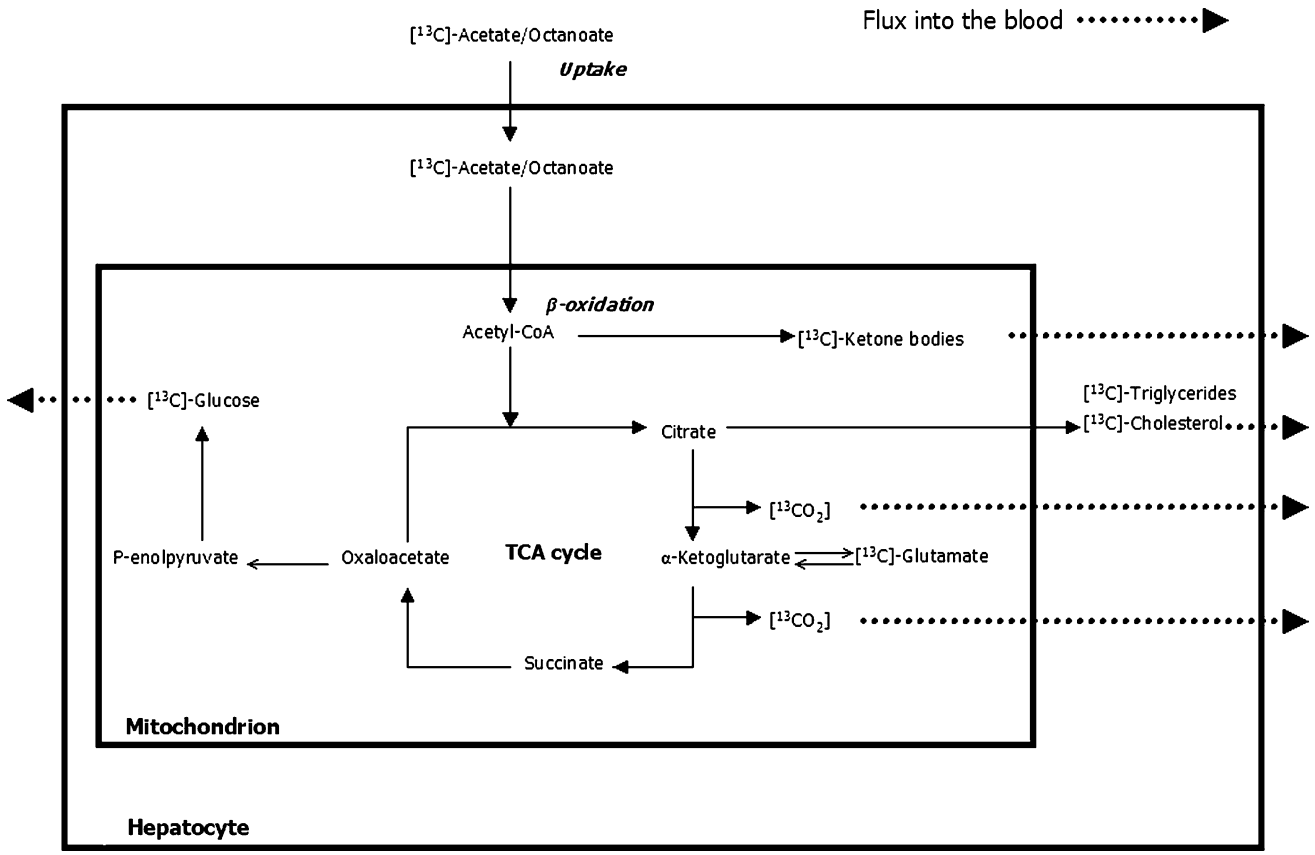

Fig. 3 Hepatic metabolism of [^{13}C]-acetate/octanoate. The [^{13}C] label is converted into [$^{13}CO_2$], or is incorporated into various metabolic products. A substantial portion of the label does not become [$^{13}CO_2$]

One may consider that, if the hepatic oxidative capacity is limited, the conversion of [^{13}C] label to [$^{13}CO_2$] will depend on the rate at which the labeled fatty acid is emptied. In other words, if gastric emptying is very rapid, a large amount of the labeled fatty acids will reach the liver at once, then the oxidative capacity will be saturated, and the hepatic conversion will be slowed down. However, the metabolic saturation does not occur at a usual dose of the labeled acetate/octanoate (100 mg), even though gastric emptying is maximally rapid [21]. It seems that the rate of gastric emptying little alters the postgastric metabolism [22].

Flow Dynamics of Bicarbonate

Once the [^{13}C] label enters the systemic circulation as [$^{13}CO_2$], the fate of the label is governed by the flow dynamics of bicarbonates/CO_2, which are characterized by distribution and elimination [5]. In the human body, [$^{13}CO_2$] undergoes bidirectional exchanges with the large bicarbonate pools (distribution), is excreted in the breath (elimination), or is lost through nonrespiratory routes (elimination).

Distribution

Distribution is the serial process of reversible movement of [$^{13}CO_2$] between the blood and the tissue bicarbonate pools, operating at various rates and to various extents. Once the equilibrium is attained between the blood and the bicarbonate pools, a change of [$^{13}CO_2$] in the bicarbonate pools is proportional to that in the blood [6]. The bicarbonate pools are divided into two kinetically distinct groups according to the *turnover* rate, namely the rapid and the slow *turnover* pools [12] (Fig. 4). The distribution of [$^{13}CO_2$] is considered to occur in a perfusion-dependent manner. Since [$^{13}CO_2$] can rapidly penetrate the well-perfused, metabolically active tissues (heart, brain, kidney, etc.), the equilibrium of [$^{13}CO_2$] between the blood and these tissues is readily attained. The rapid equilibrium allows the ready return of [$^{13}CO_2$] to the blood (rapid *turnover*) [12]. Thus, the well-perfused tissues are

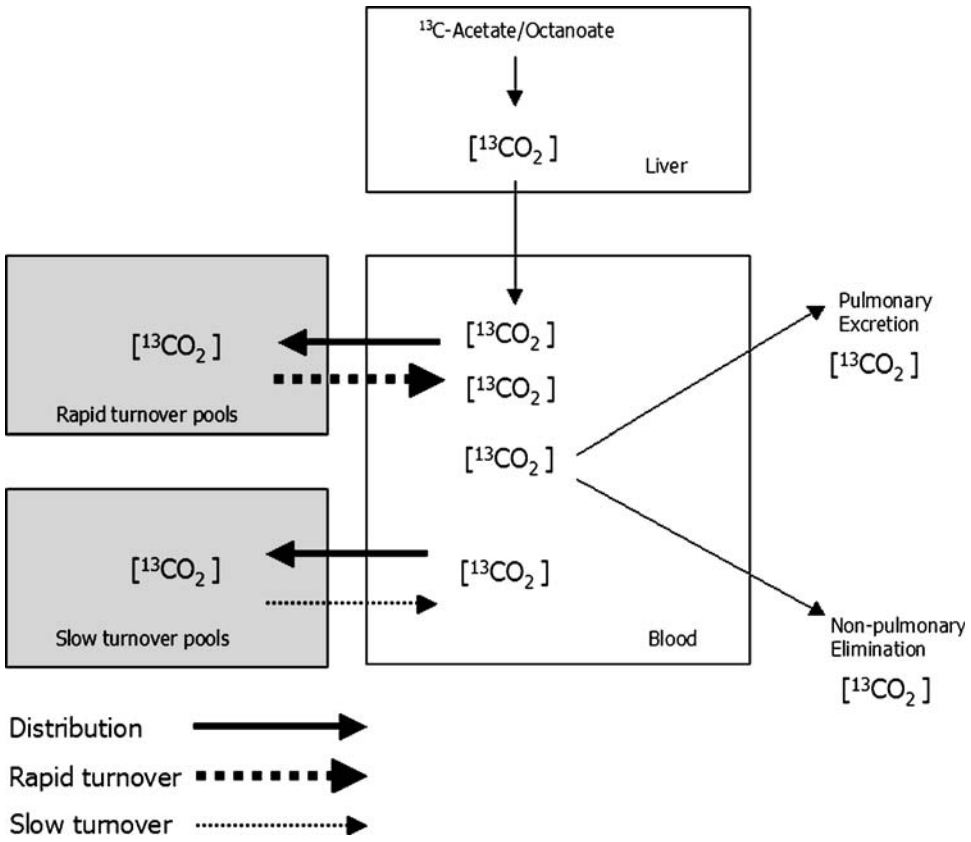

Fig. 4 Flow dynamics of [$^{13}CO_2$] in the body. [$^{13}CO_2$] is distributed into the bicarbonate pools via the blood. [$^{13}CO_2$] that enters the rapid turnover pools reemerges in the blood soon. In contrast, [$^{13}CO_2$] that enters the slow turnover pools returns to the blood much later

classified as the rapid *turnover* pools. In contrast, since [$^{13}CO_2$] slowly penetrates the low-perfused, metabolically inactive tissues (bone, resting skeletal muscle, etc.), [$^{13}CO_2$] is slowly accumulated in these tissues. Thereby, the equilibrium is barely attained, impeding the return of [$^{13}CO_2$] to the blood stream (slow *turnover*) [12]. Thus, the low-perfused tissues are classified as the slow *turnover* pools.

Elimination

Elimination means the irreversible removal of [$^{13}CO_2$] from the body, whether the removal takes place via the pulmonary or the nonpulmonary system (Fig. 4). The respiratory system is the main route for the elimination of [$^{13}CO_2$]. The nonrespiratory routes are minimally active: ~1% of [$^{13}CO_2$] is lost transcutaneously, <0.5% in faeces, and 1–3% in urine as urinary urea [14]. Since CO_2 cannot freely pass the alveolar semipermeable membrane, a substantial portion of [$^{13}CO_2$] survives pulmonary elimination, then circulates throughout the body, and is distributed to the body tissues [6]. A certain portion of [$^{13}CO_2$] is expired every time it enters the pulmonary circulation.

Kinetic Difference Between [^{13}C]-Acetate and [^{13}C]-Octanoate

In principle, the pathways along which [^{13}C]-acetate and octanoate go through the human body are regarded as the same [23]. However, a recent animal study demonstrated that the total recovery of [$^{13}CO_2$] was significantly higher following oral [^{13}C]-acetate than following oral [^{13}C]-octanoate [24]. The difference in [$^{13}CO_2$] recovery may be due to the difference in the absorption and the oxidation between the two fatty acids. [^{13}C]-Acetate is more absorbable from the small intestine than [^{13}C]-octanoate, because the former has a shorter carbon chain [8, 20]. In addition, an experimental study using isolated liver from fed rats showed that acetate was metabolized at lower energy than octanoate, suggesting that [^{13}C]-acetate is more effectively oxidized into [$^{13}CO_2$] than is [^{13}C]-octanoate [25]. The [^{13}C] label follows the same pathway once the label is converted into [$^{13}CO_2$], whether the [^{13}C] label is incorporated in acetate or octanoate.

The higher [$^{13}CO_2$] recovery from the labeled acetate supports the view that [^{13}C]-acetate is more sensitive for monitoring gastric emptying of liquids and solids than [^{13}C]-octanoate [24]. However, in practice, acetic acid is

unsuitable for solid-phase studies, since solid meals cannot firmly be labeled with [^{13}C]-acetate.

Kinetic Factors that Modulate the Pulmonary Excretion of [$^{13}CO_2$]

The label kinetics suggests that the following four processes are responsible for the discrepancies between the gastric [^{13}C] input and the respiratory [$^{13}CO_2$] output.

(1) Incorporation of the [^{13}C] label into metabolic products
(2) Distribution of [$^{13}CO_2$] into the rapid-turnover bicarbonate pools
(3) Distribution of [$^{13}CO_2$] into the slow-turnover bicarbonate pools
(4) Elimination of the [^{13}C] label via the nonpulmonary routes

Because *retention* is characterized by rapid *turnover* and *fixation* by slow *turnover*, *retention* is due to the distribution of [$^{13}CO_2$] into the rapid turnover pools and *fixation* is due to the incorporation of the [^{13}C] label into metabolic products or the distribution of [$^{13}CO_2$] into the slow turnover pools. In practice, however, *fixation* can effectively be regarded as irreversible *loss*, because the label fixed is rarely recycled within the timescale of a typical breath test (4–6 h) [5]. Hereafter, we regard *fixation* as *loss*.

As mentioned above, the input-output discrepancies results from the delayed excretion of [$^{13}CO_2$] in the breath and the incomplete pulmonary recovery of [$^{13}CO_2$]. *Retention* contributes to the delay of [$^{13}CO_2$] excretion, and *fixation* ($\approx$*loss*) and *loss* make the [$^{13}CO_2$] recovery incomplete. These terminological definitions lead us to assume that the delayed excretion of [$^{13}CO_2$] is associated with the rapid turnover pools (*retention*) while the incomplete recovery is related to metabolic incorporation (*fixation* $\approx$ *loss*), slow turnover pools (*fixation* $\approx$ *loss*), and nonpulmonary loss (*loss*) (Table 1, Fig. 5).

Characterization of Breath Test Parameters Based on the Label Kinetics

Gastric emptying is estimated from a time profile of breath [$^{13}CO_2$] data, including the pulmonary [$^{13}CO_2$]

Table 1 Determinants of the pulmonary excretion of [$^{13}CO_2$]

1. Gastric emptying (input)
2. Postgastric metabolism of free fatty acids
(1) Intestinal absorption
(2) Portal transport
(3) Hepatic metabolism
Incorporation of the [^{13}C] label into metabolic products (*fixation*)
3. Bicarbonate dynamics
(1) Distribution
Distribution of [$^{13}CO_2$] in the rapid-turnover bicarbonate pools (*retention*)
Distribution of [$^{13}CO_2$] in the slow-turnover bicarbonate pools (*fixation* $\approx$ *loss*)
(2) Elimination
Excretion of the [^{13}C] label via nonpulmonary routes (*loss*)
Excretion of [$^{13}CO_2$] via the pulmonary route (output)

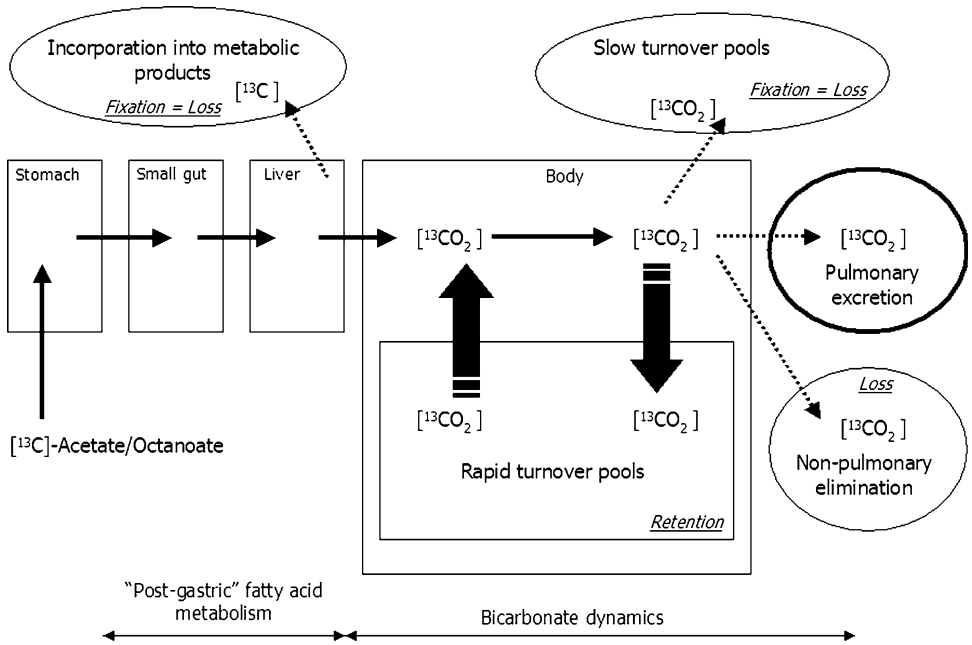

Fig. 5 Abstracted overview of the kinetics of the [^{13}C] label in the human body. After the label is emptied from the stomach, the kinetics of the label is described by postgastric metabolism of free fatty acids and bicarbonate dynamics. By definition, *fixation* is reversible, but is effectively regarded as irreversible ($\approx$ *loss*). Therefore, it is practical that label that is *fixed* or *lost* is not exhaled, being responsible for the incomplete total recovery. The amount (% dose) of the label that is *fixed*, *lost*, and breathed out (indicated by elliptical circles) totals 100%. The label that is *retained* is exhaled soon, being responsible for the delayed excretion

excretion rate (PER[t], % dose/h) and the cumulative pulmonary recovery of [$^{13}CO_2$] (CPR[t], % dose) [3]. The CPR[t] curve is generated by integrating the PER[t] curve, and the total recovery is expressed as CPR[∞]. An overall rate of gastric emptying is quantified as summary parameters, such as the gastric emptying coefficient (GEC), the time at which PER[t] attains the maximal rate (t_{max}), and the time by which half of CPR[∞] has been recovered in the breath ($t_{1/2b}$) [2, 3]. According to the conventional analytical method [3], GEC and t_{max} are derived from the PER[t] curve, and $t_{1/2b}$ from the CPR[t] curve. Currently, GEC seems to be going out of favor [26, 27], whereas t_{max} and $t_{1/2b}$ are frequently used.

Loss and *fixation* ($\approx$ *loss*) determine CPR[∞] whereas *retention* does not. Because neither $t_{1/2b}$ nor t_{max} depends on CPR[∞] [2, 3], neither is influenced by *fixation* or *loss*. In contrast, according to its mathematical definition, GEC is directly correlated with CPR[∞] [2], revealing that GEC is affected by *fixation* and *loss* (Fig. 6). On the other hand, because *retention* is related to the delayed excretion of [$^{13}CO_2$], *retention* determines the shape of the PER[t] and CPR[t] curves. Thus, *retention* affects all three of the parameters.

Despite the routine use of t_{max} and $t_{1/2b}$, the meanings of the two parameters have poorly been understood. *Retention* is the key to the understanding of t_{max} and $t_{1/2b}$. Based on the label kinetics, we have given a novel insight into the meanings of t_{max} and $t_{1/2b}$ as follows [28, 29]. The compartmental modeling theory reveals that PER[t] is proportional to the amount of [$^{13}CO_2$] in the blood ($A_{blood}[t]$, % dose) [30, 31]. In plain words, the more [$^{13}CO_2$] that is accumulated in the blood, the more [$^{13}CO_2$] that is pushed out in the breath. This relationship is mathematically written as:

$$\mathrm{PER}[t] = p \cdot A_{\mathrm{blood}}[t], \tag{1}$$

where p (1/h) is a constant. Once after the equilibrium is achieved between the blood and the rapid-turnover bicarbonate pools, $A_{blood}[t]$ is proportional to the amount of [$^{13}CO_2$] in the pools ($A_{pool}[t]$, % dose), so that:

$$A_{\mathrm{blood}}[t] = q \cdot A_{\mathrm{pool}}[t] \tag{2}$$

where q is a constant. From Eqs. 1 and 2, PER[t] is also given by:

$$\mathrm{PER}[t] = p \cdot q \cdot A_{\mathrm{pool}}[t] \tag{3}$$

Equation 3 discloses a direct relationship between PER[t] and the amount of [$^{13}CO_2$] in the rapid-turnover bicarbonate pools. From Eqs. 1–3, it is obvious that the [$^{13}CO_2$] *retained* "inside" the body ($A_{blood}[t] + A_{pool}[t]$) is proportional to PER[t] as follows.

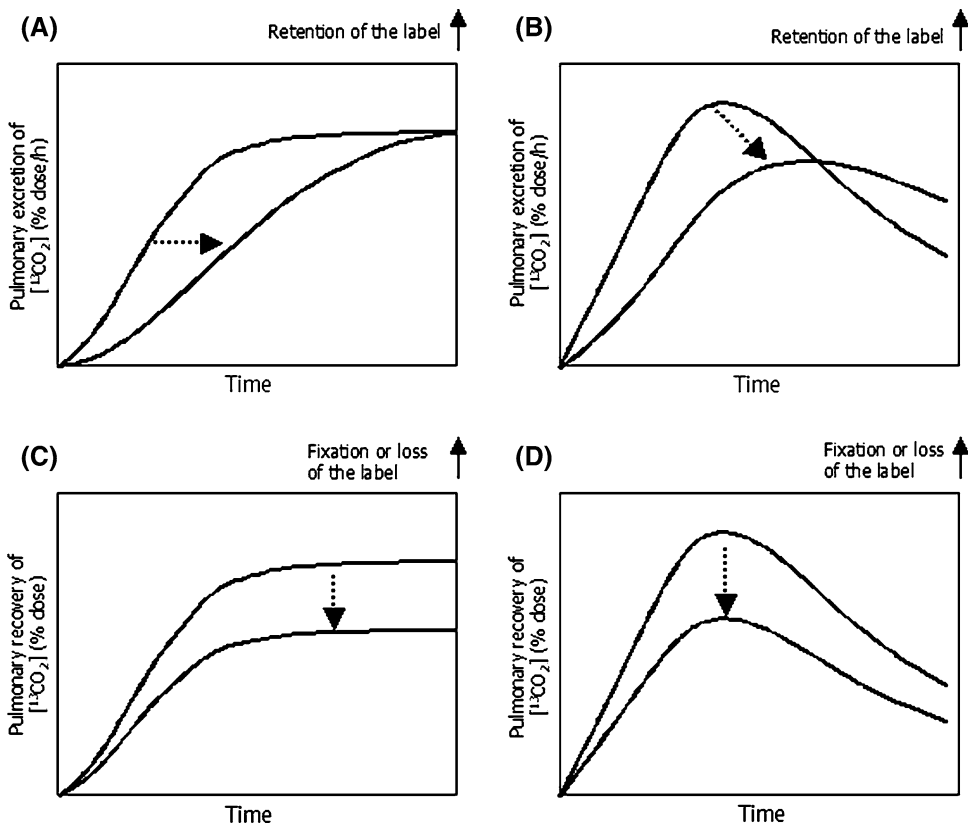

Fig. 6 Effects of *retention*, *fixation*, and *loss* of the [^{13}C] label on time profiles of breath [$^{13}CO_2$]. When *retention* of the label increases, recovery (**a**) and excretion (**b**) are delayed but the total recovery is unchanged. In contrast, when *fixation* and *loss* of the label increase, absolute values of the recovery (**c**) and the excretion (**d**) decrease in parallel with the absolute value of the total recovery

$$A_{\text{blood}}[t] + A_{\text{pool}}[t] = (1/p) \cdot \text{PER}[t] + (1/p \cdot q) \cdot \text{PER}[t] = \{(q+1)/p \cdot q\} \cdot \text{PER}[t] \quad (4)$$

As mentioned above, the [$^{13}CO_2$] *fixed* in the metabolic pools and the slow-turnover bicarbonate pools is considered to be *lost*, thus being neglected. Equation 4 implies that PER[t] becomes maximal when the [$^{13}CO_2$] *retained* inside the body becomes maximal, thus revealing that t_{max} agrees with the time at which the [$^{13}CO_2$] maximally increases in the body [28]. As gastric emptying becomes faster (slower), [$^{13}CO_2$] is more rapidly (slowly) accumulated inside the body and then the [$^{13}CO_2$] in the body more rapidly (slowly) reaches the maximal level. This is the reason why a faster (slower) emptying corresponds to a shorter (longer) t_{max}.

In sharp contrast to PER[t], CPR[t] represents the amount of [$^{13}CO_2$] that is eliminated "outside" the body. The notion of *retention* suggests that, immediately following gastric emptying, the majority of [$^{13}CO_2$] is first retained inside the body, and is then recovered outside the body (in the breath). From this point of view, the [$^{13}CO_2$] inside the body more directly responds to gastric emptying than the [$^{13}CO_2$] outside the body. This concept is supported by the fact that PER[t] evidently rises soon after ingestion of the labeled meal while CPR[t] apparently increases much later [2–4]. Considering that t_{max} is related to the [$^{13}CO_2$] inside the body (PER[t]) and $t_{1/2b}$ is to the [$^{13}CO_2$] outside the body (CPR[t]), t_{max} may be a more direct reflection of gastric emptying than $t_{1/2b}$ [29]. The theoretical superiority of t_{max} over $t_{1/2b}$ should be confirmed in practice.

Implications of the Label Kinetics for Interpretations of Test Results

Again, gastric emptying (input) is estimated from breath [$^{13}CO_2$] data (output) [2, 3]. However, the breath output is a function of not only the gastric input but also postgastric effects (*retention*, *fixation*, or *loss*) (Table 1). The postgastric processes have smaller variability within and between individuals than gastric emptying, indicating that the pulmonary output most likely depends on gastric emptying rather than on the postgastric processes. This notion ensures the methodological relevance of breath testing. However, the possibility remains that the postgastric variability substantially alters the pulmonary output independently of gastric emptying. If two subjects have the same profiles of gastric emptying but different profiles of fatty acid metabolism and bicarbonate dynamics, they will have different breath test results. This should be taken into considerations to appropriately interpret the breath data.

Inter- and Intra-individual Variances in the Label Kinetics

Experiments using [^{13}C]-bicarbonate and [^{13}C]-acetate provide much information about the kinetics of the [^{13}C] label. Since [$^{13}CO_2$] is instantly released from [^{13}C]-bicarbonate in the blood, breath [$^{13}CO_2$] data obtained after intravenous injection of [^{13}C]-bicarbonate represents a pure effect of the bicarbonate dynamics [12, 16]. On the other hand, [^{13}C]-acetate is readily converted to its acetyl-CoA derivative, which behaves as the acetyl-CoA originating from both of labeled acetate and octanoate [12, 16]. Thus, breath [$^{13}CO_2$] data obtained after ingestion of [^{13}C]-acetate dissolved in water represents the combined effects of fatty acid metabolism and bicarbonate kinetics.

Variance in the Fixation and the Loss of the Label

The total [$^{13}CO_2$] recovery (CPR[∞]) directly depends on *fixation* and *loss*. Various results have been reported about [$^{13}CO_2$] recovery following administration of labeled bicarbonate or acetate. The variability among the results of these studies may be due to the differing lengths of time over which breath samples were collected. The label recovery increases as the sampling time is prolonged, because the recycling of the [^{13}C] label continues for a long period [14]. After intravenous [^{13}C]-bicarbonate, the cumulative [$^{13}CO_2$] recovery reaches about 80% over 6 h and about >90% over 12–36 h post dose [14]. On the other hand, following oral [^{13}C]-acetate, the recovery is about 50% over 6 h and about 55% over 10 h post dose [32]. These recovery data indicate that, up to 10 h post dose, the recovery is nearly complete after [^{13}C]-bicarbonate injection while it is far from complete after [^{13}C]-acetate ingestion. It is therefore reasonable to consider that fatty acid metabolism (*fixation* due to the metabolic pools) contributes more to incomplete recovery than does bicarbonate dynamics (*fixation* due to the slow-turnover bicarbonate pools) [11]. Results of experiments using [^{13}C]-acetate show that about half of the label is missing during the [^{13}C]-gastric breath test [32].

Several studies have shown that the total recovery of [$^{13}CO_2$] following oral [^{13}C]-acetate is more variable between than within subjects: coefficients of variation were ~4.0% within subjects and ~8.0% between subjects [11]. This finding suggests that fatty acid metabolism and bicarbonate dynamics are more variable between than within subjects. Thus, the [^{13}C]-breath tests are more reliable in a crossover than in a cross-sectional comparison [33].

Variance in the Retention of the Label

Retention is determined by the capacity of the rapid-turnover pools to hold [$^{13}CO_2$] and the capability of the lung to eliminate [$^{13}CO_2$]. In subjects with normal respiratory function, the pool capacity is the sole determinant of *retention*. The rapid-turnover bicarbonate pools with the a larger capacity hold a larger amount of [$^{13}CO_2$], and consequently take longer to release [$^{13}CO_2$] completely into the blood stream for pulmonary excretion. Larger pools *retain* the label for longer. The pool capacity can vary between individuals, and thus so can *retention*.

The extent of *retention* is assessed from the pulmonary elimination of [$^{13}CO_2$] following administration of the labeled bicarbonate [12]. *Retention* is partly quantified by the first-order rate constant for the total elimination of [$^{13}CO_2$] (K_{el}, 1/h), which is determined as the slope of the stable tail of a semilogarithmic plot of the PER[t] curve [34]. A human study showed that coefficients of variation for K_{el} were 4.5% following oral dose of 50 mg [^{13}C]-bicarbonate and 8.6% following 100 mg [^{13}C]-bicarbonate [35], suggesting that *retention* is substantially variable between subjects. Data on the intra-individual variability in the K_{el} value seem to be scanty.

Impacts of the Postgastric Variability on Breath Test Parameters

Impacts of the postgastric variability on the three parameters are visualized in Fig. 6. As *fixation* or *loss* increases, CPR[∞] decreases without any change in the shape of PER[t] curve [3]: the CPR[t] curve (Fig. 6c) and the PER[t] curves are shifted downward. As a consequence, if *loss* and *fixation* increase, GEC becomes smaller but $t_{1/2b}$ and t_{max} are unchanged. This finding suggests that GEC is less specific to gastric emptying than $t_{1/2b}$ and t_{max}. On the other hand, as *retention* is more prolonged, PER[t] is more delayed but CPR[∞] is unchanged: the CPR[t] curve is shifted to the right (Fig. 6a), and the PER[t] curve is shifted downwards and to the right (Fig. 6b). As a result, if *retention* is prolonged, GEC becomes smaller, $t_{1/2b}$ becomes longer, and t_{max} becomes longer.

Novel Strategy for Analyzing Breath Data Based on the Label Kinetics

In order to adjust *retention*, *fixation*, and *loss*, we have recently introduced a novel analytical strategy, the Wagner-Nelson method, for breath tests [34]. This method was proposed to accurately estimate the rate of drug absorption from blood and urine data, and the method has been in widespread use in pharmacokinetic studies. The Wagner-Nelson equation for breath data incorporates the aforementioned functions of CPR[t] and PER[t] and the constants K_{el} and CPR[∞]. The fraction of the labeled test meal that has been emptied from the stomach by time t, F[t], is given by:

$$F(t) = [\mathrm{CPR}[t] + \mathrm{PER}[t]/K_{el}]/\mathrm{CPR}[\infty] \quad (5)$$

In the Wagner–Nelson equation, the second term of the numerator (PER[t]/K_{el}) adjusts the *retention*, and the denominator (CPR[∞]) adjusts *fixation* and *loss*. Plotting $100 \cdot \{1-F[t]\}$ against time t yields a time-percent gastric retention curve, which is an analogue of the scintigraphic retention curve. Recent small-sample studies have shown that, both for liquid- and solid-phase gastric emptying, the Wagner-Nelson method allows creation of the gastric retention curves that are almost superimposed on the scintigraphic retention curves [34]. However, the utility of the Wagner-Nelson method applied to breath tests deserves further validation in a large numbers of subjects with a broad range of gastric emptying rates.

Conclusions

The methodological relevance of breath testing is based on a close correspondence between gastric emptying and pulmonary excretion of [$^{13}CO_2$]. However, gastric data are obviously remote from breath data. The remoteness is associated with the passage of the [^{13}C] label through the metabolic and the bicarbonate pools. Recognition of these mechanisms is essential for appropriate interpretations of the breath test results.

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